Adherence to the Mediterranean diet and its association with glycaemic control in Type 1 diabetes

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Declaration

I declare that I have composed this thesis myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others included in the thesis.

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Abstract

Introduction: The Mediterranean diet (Mdiet) is defined as the dietary patterns of people living around the Mediterranean regions during the 1950s and 1960s. This thesis aimed to investigate the adherence to the Mdiet by Cypriot and Greek populations, and its association with glycaemic control in people with Type 1 diabetes (T1DM) in Cyprus.

Methods: Longitudinal adherence to the Mdiet in Cyprus and Greece was explored in a systematic review. The cumulative adherence, stratified by Mdiet scoring systems, was explored alongside the potential age and gender differences. Adherence to Mdiet, glycaemic control and their association (using linear regression models) were investigated in a cross-sectional study among people with T1DM in Limassol, Cyprus. The methodology of this study was tested in a pilot study.

Results: The systematic review included 15 independent studies (18 papers). The adherence to the Mdiet was graded as moderate. The KIDMED and the MedDietScore were the most used scores and indicated cumulative mean adherence of 51.6% (4.3 points) and 52.5% (28.9 points), respectively. There was a suggestion of lower adherence in younger ages and a reducing trend over time; no gender difference was observed.

For the cross-sectional study, 103 participants were recruited through random sample selection. The mean adherence was 57.6% (31.7 points); 80% and 19% of the participants had a moderate and high adherence, respectively. The median HbA1c and fasting glucose was 65 mmol/mol and 10.3 mmol/l, respectively. Most participants had suboptimal glycaemic control. Mdiet adherence and glycaemic control were poorer in younger ages; no gender difference was observed. The Mdiet was statistically significantly associated with HbA1c but not with fasting glucose, after adjusting for potential confounders. The fully adjusted model predicted a reduction in HbA1c (mmol/mol) by 1.5% for every additional point in the MedDietScore.

Conclusion: Mdiet is associated with a clinically and statistically significant reduction of HbA1c in T1DM.

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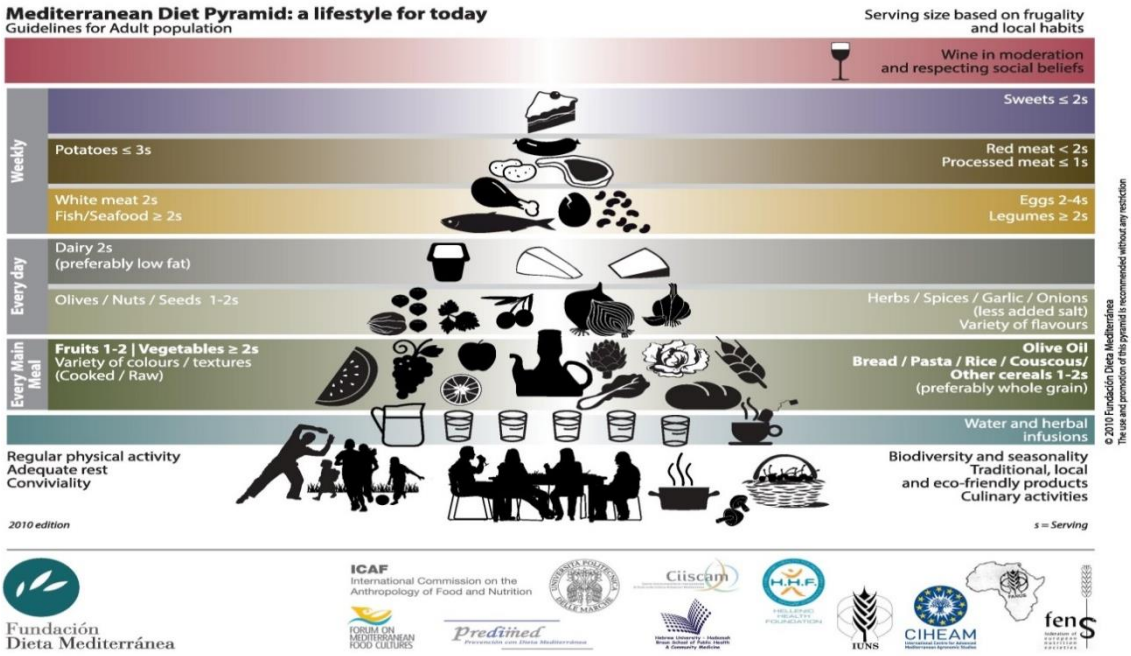
Chapter 1: Introduction

Mediterranean diet

Definition and health

The Mediterranean diet is a collective term used to describe the dietary patterns of the people who live around the Mediterranean Sea. The definition reflects the dietary patterns of the olive-oil-growing Mediterranean regions before the invasion of fast food culture had influenced the countries of the Mediterranean basin, but after the hardships of the Second World War: chronologically, the late 1950s and early 1960s (Trichopoulou *et al.*, 2000). The Mediterranean diet is a collection of diets that share some common features but have their own unique characteristics, varying between Mediterranean regions and countries. Some of these common features are that the diet is based on wholegrain, minimally processed foods; and there is a high intake of fruits, sometimes eaten as desserts, and vegetables. Furthermore, most meals are accompanied by a salad; the main fat source is olive oil; fish is consumed regularly throughout the week; milk and dairy products are consumed daily but in small portions; and the consumption of meat, especially red meat, is minimal (Trichopoulou *et al.*, 2000; Bach-Faig *et al.*, 2011; Romagnolo and Selmin, 2016). A pictorial representation of the Mediterranean diet (one of the most widely used in the literature) is shown in *Figure 1.1* (Bach-Faig et al., 2011).

Figure 1.1: Mediterranean diet pyramid



Interest in the Mediterranean diet, as related to health, was first raised after the completion of the *Seven Countries Study*, which suggested that this diet occupies a cardio-protective role compared to other dietary patterns (Keys, 1980; Toshima *et al.*, 1994). The Mediterranean diet has been widely studied since then and has been found to have important health benefits (Dinu *et al.*, 2018), including a reduction in all-cause mortality (Trichopoulou *et al.*, 2003; Sofi *et al.*, 2010; Dinu *et al.*, 2018), a reduction in incidence and mortality related to cardiovascular disease (CVD) (Trichopoulou *et al.*, 2003; Sofi *et al.*, 2010; Bach-Faig *et al.*, 2011; Dinu *et al.*, 2018) and cancer (Trichopoulou *et al.*, 2003; Sofi *et al.*, 2010; Bach-Faig *et al.*, 2011; Schwingshackl *et al.*, 2017; Dinu *et al.*, 2018), and a reduction in the incidence of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (Sofi *et al.*, 2010; Dinu *et al.*, 2018), and of type 2 diabetes (Koloverou *et al.*, 2014; Schwingshackl *et al.*, 2015; Dinu *et al.*, 2018).

Mediterranean lifestyle

In recent years, there has been a discussion among academics, researchers and clinicians regarding the need to consider the traditional Mediterranean lifestyle, rather than just simply advocating for the Mediterranean diet (Bach-Faig *et al.*, 2011; Grosso *et al.*, 2017; Diolintzi, Panagiotakos and Sidossis, 2019). The Mediterranean lifestyle encompasses the Mediterranean diet, but also includes a wide range of additional features, such as moderation, locality and seasonality of food, participation in culinary activities, physical activity, rest, relaxation and sleep, social involvement, and spiritual, intellectual, emotional, occupational and financial wellness (Bach-Faig *et al.*, 2011; Diolintzi, Panagiotakos and Sidossis, 2019). However, no universally agreed definition of Mediterranean lifestyle currently exists (Diolintzi, Panagiotakos and Sidossis, 2019). Nevertheless, several studies have tried to capture some of the aspects of the Mediterranean lifestyle by means of a scoring system and have related this to health and disease, such as all-cause mortality and premature death (Van Den Brandt, 2011; Behrens *et al.*, 2013; Prinelli *et al.*, 2015), primary and secondary prevention of CVD and CVD-related mortality (Booth *et al.*, 2014; Hoevenaar-Blom *et al.*, 2014), with very encouraging results; while other studies are ongoing (Grosso *et al.*, 2017).

Mediterranean diet in Cyprus: definition and epidemiology

Cyprus is an island in the eastern Mediterranean basin. By definition, the diet of the Cypriot population during the 1950s and 60s was defined as a traditional Mediterranean dietary pattern. The traditional Mediterranean diet in Cyprus consisted mainly of grains,

greens, all kind of vegetables, legumes mixed with olive oil, cheese (halloumi), and plenty of fruits, and was accompanied with moderate amounts of wine (Matalas, 2001; Simopoulos and Visioli, 2007; Hoffman and Gerber, 2012).

An important question is whether the Cypriot population still follows the dietary patterns that define the traditional Mediterranean diet or whether they have moved to a more Westernised diet. Evidence from the analysis of data from the Food and Agriculture Organisation's (FAO) food datasheets (Balanza *et al.*, 2007; da Silva *et al.*, 2009; Vareiro *et al.*, 2009) suggests that there may have been a change in dietary habits and a reduction in uptake of the Mediterranean diet in Cyprus, Greece and elsewhere in the Southern European Mediterranean countries since the 1960s. Most worrying are the results that compare the dietary habits of the discussed Mediterranean populations, showing lower adherence to the Mediterranean diet when compared to non-Mediterranean populations (Dedoussis *et al.*, 2008; Van Diepen *et al.*, 2011; Tognon *et al.*, 2014). Furthermore, this reported transition from the traditional Mediterranean diet to a more Westernised diet appears to be particularly prominent in children, adolescents and young adults (Lazarou *et al.*, 2009; Lazarou, Panagiotakos and Matalas, 2009; Van Diepen *et al.*, 2011; Tognon *et al.*, 2014; Iaccarino Idelson, Scalfi and Valerio, 2017) – this may suggest a change in dietary habits across ages, but, more importantly, a worrisome trend over time.

Diabetes (Type 1 Diabetes)

Background

Diabetes is a disorder of glucose metabolism, due to partial or complete absence of insulin and/or peripheral insulin resistance, which results in an abnormally raised blood glucose concentration known as hyperglycaemia. The most prevalent forms of diabetes are Type 1, Type 2 and gestational diabetes. Other types of diabetes include the monogenic diabetes syndromes, such as maturity onset diabetes of the young (MODY) and neonatal diabetes, drug-induced diabetes, such as glucocorticoid-induced diabetes, and secondary diabetes, for example, that related to pancreatic disease or endocrinopathies (Pearson, 2014; Bonora and DeFronzo, 2018; American Diabetes Association, 2019a).

Type 1 diabetes is an autoimmune-mediated condition resulting in the destruction of β-pancreatic cells and eventually in the absolute absence of endogenous insulin production and secretion. The aetiology is complex and not well understood; genetic and environmental factors have been implicated, while suggested triggers have been

proposed, including infections and dietary habits (Katsarou *et al.*, 2017; Regnell and Lernmark, 2017; DiMeglio, Evans-Molina and Oram, 2018). Untreated diabetes can lead to hyperglycaemia and diabetic symptoms in the short term, such as polyuria and polydipsia, and eventually to diabetic ketoacidosis (DKA), coma and death. In the long term, hyperglycaemia can lead to micro- and macro-vascular complications, such as cardiovascular disease (CVD), cerebrovascular accident (CVA), neuropathy, nephropathy and retinopathy (Melmed *et al.*, 2016; DiMeglio, Evans-Molina and Oram, 2018; American Diabetes Association, 2019b).

The management of Type 1 diabetes includes appropriate lifestyle management, that is: dietary advice and exercise; pharmacotherapy, which consists of multiple daily insulin injections (MDI) or continuous subcutaneous insulin infusion (CSII); and, when possible, appropriate structured education programmes. The dietary management includes healthy eating advice and education, including how to match carbohydrates to insulin. Management targets are individualised, although they ideally include glycosylated haemoglobin (HbA1c) of less than 48 mmol/mol (6.5 %) and minimum variation in daily blood glucose measurements, intended for the prevention of future diabetes-related complications, avoidance of hypoglycaemic episodes, especially severe hypoglycaemia, and a normal pregnancy for women (NICE, 2015a, 2015b; Dyson *et al.*, 2018; American Diabetes Association, 2019c, 2019d).

Dietetic management: Good glycaemic control is achieved through the appropriate matching of insulin to food (carbohydrate) intake, while taking other factors into account, such as exercise, alcohol intake, non-diabetes medications (such as cortisol) usage, illness, insulin delivery method (insulin pen or pump) and others. Structured education programmes are available, such as DAFNE (Dose Adjustment for Normal Eating) in the UK, for the education of patients on how to appropriately adjust their insulin dosage. These programmes have good evidence to suggest that they improve glycaemic control (NICE, 2015a, 2015b; Dyson et al., 2018; American Diabetes Association, 2019a, 2019b) and other parameters, such as lifestyle flexibility and quality of life (Cooke *et al.*, 2013). On the other hand, following a healthy lifestyle, such as healthy eating (as well as regular physical activity and avoidance of smoking), is also considered to be important for the reduction of risk of diabetes-related complications (and co-morbidities). Nevertheless, healthy eating (and healthy lifestyle) is regarded as a distinct subject from good glycaemic control, and in agreement with the advice given for the rest of the population (Piłaciński

and Zozulińska-Ziółkiewicz, 2014; NICE, 2015a, 2015b; Dyson et al., 2018; American Diabetes Association, 2019c). In theory, it is considered feasible that a person who follows a healthy lifestyle can have poor glycaemic control and vice versa, although, quite often, in clinical practice, good glycaemic control and having a healthy lifestyle seem to go together.

Diabetes (Type 1 Diabetes) in Cyprus

In order to explore the published literature and available evidence in regard to Type 1 diabetes in patients residing in Cyprus, a database search was conducted. The databases consulted were PubMed and MEDLINE (via EBSCOhost); the search terms were 'diabetes' (all fields) AND 'Cyprus' (all fields), with no restrictions. The search was conducted up to 28 January 2017 and resulted in 126 and 117 published papers in PubMed and MEDLINE, respectively, each of which were reviewed for relevance.

Type 1 diabetes in the Cypriot population is a poorly researched subject and published data are limited – confined solely to four studies discussing incidence (Skordis and Hadjiloizou, 1997; Skordis *et al.*, 2002, 2012; Toumba *et al.*, 2007) and a single study in the field of genetics (HLA-G 14-bp polymorphism and age of onset) (Gerasimou *et al.*, 2016). Updating the PubMed search resulted in the identification of 121 additional published papers to 12 August 2019. Of these, one paper was identified as being relevant. This was a further genetic analysis of HLA-phenotype in the same cohort of the genetic study (Gerasimou *et al.*, 2018) reported in the previous search.

In more detail, the incidence of Type 1 diabetes, up to the age of fifteen, was 12.5 per 100,000 people, during a twenty-year period (1990 – 2009), with a statistically significant increase observed during the second half of the studied period (14.4 / 100,000) compared to the first decade (10.8 / 100,000; $p < 0.001$) (Skordis and Hadjiloizou, 1997; Skordis *et al.*, 2002, 2012; Toumba *et al.*, 2007). A number of methods were used to capture all Type 1 diabetes cases in the pre-mentioned studies, as there is no central systematic register similar to, for example, the 'The Scottish Care Information – Diabetes' (SCI-diabetes) in Scotland or the 'National Diabetes Registry' (NDR) in Sweden. The methods used to gather the data were the patient records from a state hospital, private sector physicians and the database of the Cyprus Diabetes Association (CDA).

Mediterranean diet and Diabetes (Type 1 diabetes)

Background

The published literature and available evidence, in regard to the relationship between Mediterranean diet and Type 1 diabetes, was also explored through a database search. The databases consulted were PubMed and MEDLINE (via EBSCOhost); the search terms were 'Mediterranean diet' (all fields) AND 'diabetes' (all fields), with no restrictions. The search was conducted up to 28 January 2017. The search resulted in 693 and 570 published papers in PubMed and MEDLINE, respectively, each of which were reviewed for relevance.

The majority of studies that have investigated the relationship between the Mediterranean diet and diabetes are confined to the area of Type 2 diabetes. There is good evidence, including systematic reviews and meta-analysis, that show a reduction of HbA1c and fasting blood glucose with better adherence to the Mediterranean diet in Type 2 diabetes (Esposito *et al.*, 2010; Ajala, English and Pinkney, 2013; Carter *et al.*, 2014; Huo *et al.*, 2015).

The evidence for establishing a relationship between a Mediterranean diet and diabetes in patients with Type 1 diabetes is considerably more scarce. An interventional study in an Italian paediatric population showed that structured training in relation to the Mediterranean diet resulted in decreased HbA1c in males but not in females (Cadario *et al.*, 2012). This study did not measure the participants' adherence to a Mediterranean diet at baseline or after the intervention and thus a definite cause for the reduction in HbA1c (e.g., education and more regular contact with the dietitian vs improved adherence to the Mediterranean diet) or in the effect size (increase in adherence to the Mediterranean vs reduction in HbA1c) is difficult to assert. An older randomised, cross-over trial attempted to investigate the Mediterranean diet against a normal diet in combination with two different types of insulins for one year (Provenzano *et al.*, 2001). The small size of the study, that is to say, 12 patients, the change of insulin over the trial period, that is to say, lispro versus normal human insulin (normal human insulin is not regularly used nowadays in Type 1 diabetes) and the regular change of diet/insulin combination (every three months; four times in one year for each group) makes the extrapolation of any results difficult.

Two more recent studies in non-Mediterranean populations, one longitudinal study in children (Zhong *et al.*, 2016) and one cross-sectional in adults (Gingras *et al.*, 2015) examined the relationship between the Mediterranean diet and glycaemic control in patients with Type 1 diabetes. Both studies showed a small decrease in HbA1c with better adherence to the Mediterranean diet (Gingras *et al.*, 2015; Zhong *et al.*, 2016), but only the cross-sectional results of the study in children were statistically significant (Zhong *et al.*, 2016). The uptake of the Mediterranean diet, as would be expected in the North American populations studied (USA and Canada), was considerably low in both studies and may have affected the results.

An updated PubMed search to 12 August 2019 resulted in 334 additional published papers. Of these, three additional papers (Costacou *et al.*, 2018; Fortin *et al.*, 2018; Mouslech *et al.*, 2018) were identified as being relevant. The first paper (Costacou *et al.*, 2018) reports the results of a longitudinal study, the same study with USA children and young adults that examined the results of Mediterranean diet and HbA1c discussed above (Zhong *et al.*, 2016). In the more recent paper (Costacou *et al.*, 2018), the association between Mediterranean diet and microalbuminuria was the primary focus, and this was reported as not being statistically significant. The authors admit that their participants' adherence to the Mediterranean diet was low and acknowledge that this may have confounded the results – as discussed in the introduction to this section. Furthermore, in regard to the Mediterranean diet, the study was not designed to examine the Mediterranean diet specifically, as this analysis was post-hoc, but, more importantly, the Mediterranean Diet Quality Index (KIDMED) score used was significantly modified to match the data that authors collected. Nevertheless, as reported in the introduction to this section, this study found a statistically significant association between the Mediterranean diet and reduction in HbA1c when baseline data were examined, although not in the longitudinal data.

The second paper (Mouslech *et al.*, 2018) is an intervention study conducted with 62 Greek adults. The study examined a 12-month education programme that motivated patients to follow a Mediterranean diet, exercise regularly and to adjust carbohydrate intake and insulin dose. The results were encouraging, resulting in a drop in HbA1c, hypoglycaemic episodes and glucose fluctuation. Unfortunately, the study did not measure the participants' adherence to the Mediterranean diet before and after the completion of the study – similar to another study in children reported in the introduction

to this section (Cadario *et al.*, 2012). Therefore, it is difficult to point to the Mediterranean diet as a cause of this change; especially bearing in mind that there is good evidence from research involving DAFNE in the UK that structured education programmes, those that include carbohydrate counting and flexible insulin dosage, improve glycaemic control and reduce hypoglycaemic episodes (Owen and Woodward, 2014; NICE, 2015b; Dyson *et al.*, 2018).

The third study (Fortin *et al.*, 2018) is a small un-blinded RCT study that randomised 14 patients to a low-fat diet group and 14 patients to a Mediterranean diet group, each of which included 9 dietitian-led education sessions delivered over a period of six months. The reduction in HbA1c was not statistically significant in either group despite the increase in adherence to the Mediterranean diet. Note that, the power analysis for the study was reported only in regard to the waist circumference.

Despite the limitations identified in the studies identified here, the results seem to suggest that there is a possible positive association between the Mediterranean diet and glycaemic control in Type 1 diabetes.

Mediterranean diet and Diabetes (Type 1 diabetes) in Cyprus

Finally, in order to explore the published literature and available evidence in regard to Type 1 diabetes in patients residing in Cyprus, a further database search was conducted. The databases consulted again included PubMed and MEDLINE (via EBSCOhost); the search terms were 'Mediterranean diet' (all fields) AND 'Cyprus' (all fields), with no restrictions. The search was conducted up to 28 January 2017. The Search resulted in 46 and 34 published papers in PubMed and MEDLINE, respectively, each of which were reviewed. Neither this search, nor an updated search (to 12 August 2019), yielding 18 further papers, revealed any studies of interest.

Rationale for a study that investigates the association between Mediterranean diet and glycaemic control

The current guidelines (NICE, 2015b; Dyson et al., 2018; ADA, 2019b) for the management of the Type 1 diabetes emphasize diabetes education and appropriate matching of insulin to carbohydrate intake. This is based on good evidence that such structured education programmes improve glycaemic control and other parameters (Owen and Woodward, 2014; NICE, 2015b; Dyson et al., 2018). At the same time, the current guidelines consider the issue of glycaemic control and healthy eating patterns,

such as the Mediterranean diet, as two unrelated subjects in the management of Type 1 diabetes. Nevertheless, the following issues arise from this proposed disassociation of the two subjects:

i. The evidence presented above, suggests a beneficial effect of Mediterranean diet on glycaemic control, primarily on HbA1c. Unfortunately, these studies have some important limitations, such as the recruitment of subjects in countries with very low adherence to the Mediterranean diet, the parallel delivery of education including carb-to-insulin matching and healthy eating, and a small sample size. Nevertheless, these studies in combination with the good evidence in favour of the adoption of the Mediterranean diet in Type 2 diabetes, allows us to speculate a beneficial effect of Mediterranean diet on glycaemic control in Type 1 diabetes but which is not proven beyond doubt. Overall, one can argue that the guidelines reflect the absence of strong evidence (in combination with the good evidence for diabetes education) rather the presence of evidence of no (or detrimental) effect of Mediterranean diet on glycaemic control.

ii. The Mediterranean diet encompasses food aspects, which may be beneficial for glycaemic control and there is a biological basis to this as well as a modest amount of evidence. Possible explanations for such beneficial effects include the following: low glycaemic index (GI) and load (GL) of the diet, high fibre content, small amounts of alcohol consumption with foods, an emphasis on less refined foods, such as the wholegrain foods, significant amount of fruit and vegetable consumed, minimal consumption of highly processed foods and a central theme of Mediterranean diet (and lifestyle) that is moderation. Therefore, it is not unreasonable to expect that the combination of these factors will have an effect on blood glucose levels, such as better post-prandial glucose levels through better matching of insulin and food (i.e., by better matching absorption and insulin action), less rapid glucose peaks and consequently more time in range and less glucose variation. Nevertheless, Mediterranean diet is not unique in influencing the blood glucose; other dietary habits e.g., highly processed foods (usually fast foods), require special attention to be matched with insulin action, such as by the use of dual function in insulin pumps (that is a combination of bolus insulin and square insulin i.e. insulin given in a particular space of time).

iii. The Mediterranean diet is strongly recommended by all guidelines (NICE, 2015b; Dyson et al., 2018; ADA, 2020) for prevention of hard endpoint outcomes (e.g., CVD)

and risk factors (e.g., hyperlipidaemia) in Type 1 diabetes (irrespective of glycaemic control). Consequently, although in theory these two subjects (healthy eating and glucose control) are considered as two independent entities, in practise, both are tackled simultaneously and have similar aims (e.g., prevention of CVD). Therefore, it is prudent to understand any potential interaction and inter-relationship of Mediterranean diet and glycaemic control to better inform clinical practice.

iv. Finally, in clinical practice it is common for people that have good glycaemic control to also have a healthy lifestyle, including following a healthy eating pattern; at the same time, good glycaemic control with a poor diet is less often observed. Given that this is just empirical evidence and do not prove an association, it is a subject worth investigating further.

In conclusion, the current theoretical, empirical and research evidence supports the hypothesis of an association between the uptake of Mediterranean diet and glycaemic control. At the same time, the current research evidence is limited and suffers from significant methodological issues. This is also reflected in the current guidelines, which although they recommend the Mediterranean diet for cardiovascular health, they fall short of suggesting the Mediterranean diet, or in fact any other eating pattern, for glycaemic control. For these reasons there is an urgent need for a study that investigates the interaction of the two sets of co-existing advice and more specifically a potential effect of Mediterranean diet on glycaemic control in the adult population with Type 1 diabetes. Furthermore, this study should try to overcome some of the methodological limitations of the previous studies, e.g., by adjusting for parameters that are known to improve the glycaemic control, such as carbohydrate counting and diabetes education. Any such study should be adequately powered to measure such an association and recruit subjects that (at least traditionally) follow the Mediterranean diet in order to increase the likelihood of finding an association if there is indeed one.

Summary

In this section, the evidence on adherence to the Mediterranean diet and its association with glycaemic control in people with a diagnosis Type 1 diabetes were reviewed. There is a suggestion that the adherence in the Mediterranean diet in the Cypriot (and Greek) population is reduced but the evidence have not been studied in a systematic manner. The association of the Mediterranean diet with the glycaemic control in Type 1 diabetes has

not been well-studied; the available studies, overall, suggest a positive association but often are confounded by significant limitations.

Chapter 2: Methodology

Aims & Objectives

The overall aim of this PhD thesis was to explore adherence to the Mediterranean diet in Cyprus and to investigate the associations between adherence to the Mediterranean diet and glycaemic control in an adult population with a diagnosis of Type 1 diabetes in Cyprus. The choice to focus on Type 1 diabetes was based on several practical and theoretical factors, including that, as described above, although there is strong evidence for Type 2 diabetes that better adherence to the Mediterranean diet translates to a lower glycaemic control, as measured by HbA1c and fasting glucose, the evidence for Type 1 diabetes is scarce.

Aims

The thesis had two main aims:

- To conduct a systematic review that presents the adherence to the Mediterranean diet in the Cypriot and the Greek population.
- To examine associations between adherence to the Mediterranean diet and glycaemic control in patients with Type 1 diabetes in Cyprus.

These two aims were addressed within three studies: a systematic review, a pilot study, and a large cross-sectional study.

Objectives

In facilitating these aims, the following objectives were determined.

To systematically review the available evidence regarding the Mediterranean diet, as measured by Mediterranean diet scoring systems, and quantify the following:

- adherence to the Mediterranean diet by the Cypriot and Greek populations.
- the difference in adherence between males and females.
- the difference in adherence between age groups.
- trends in changes in adherence to the Mediterranean diet over time.

To test the following methods and measures in a pilot study:

- the feasibility of the recruitment procedure – both the participation rate and the practical aspects of the process.
- the adequacy of the information gathered, so as to examine the primary and secondary aims of the study.
- the newly developed or adapted, *food frequency questionnaire*, *medical and diabetes* questionnaire and the *demographics characteristics* questionnaire.
- the electronic delivery of questionnaires, i.e., online using self-developed computer software rather than on paper; and the resulting 'export' of the results of the questionnaires to MS Excel files.
- the accuracy of the self-developed software and the algorithms used to calculate the Mediterranean diet score.
- the co-ordination of all the people (the research team and the secretarial staff) needed for the current study.

To conduct a cross-sectional study, in people diagnosed with Type 1 diabetes in Cyprus, to measure the following primary and secondary outcomes:

- the association between adhering to the Mediterranean diet, as measured by a Mediterranean diet score, and glycaemic control, as measured by HbA1c and fasting glucose [primary outcome].
- adherence to the Mediterranean diet [primary outcome].
- glycaemic control [primary outcome].
- the effect of the demographic characteristics of the participants on the Mediterranean diet and glycaemic control [secondary outcomes].

Methodology

This section briefly discusses various methods available on i. reviewing the evidence (literature) and ii. collecting data (study designs), with an emphasis on the systematic review and cross-sectional design, methodologies that were employed in the current thesis. The primary outcomes measures, namely the various Mediterranean diet scoring systems, and the HbA1c and fasting glucose tests, that were used for the quantifying of adherence to the Mediterranean diet and glycaemic control, respectively, are also discussed here.

Research type: quantitative and qualitative paradigm

Quantitative research has a well-defined methodology and the resulting data typically possess a numerical structure and are analysed using mathematics and statistics. In contrast, qualitative research aims to acquire more in-depth insight on topics through narrative, usually informed by a less-rigid methodology (Kumar, 2011; Bairagi and Munot, 2019). In the thesis, a quantitative approach was employed as it was more appropriate for measuring the outcomes of interest (adherence to the Mediterranean diet and glycaemic control, as well as the other secondary outcomes) and in answering the set research questions and hypotheses. Nevertheless, on a limited number of occasions, qualitative measures were also employed, as appropriate, in the analysis, for example, in the pilot study, the participants were encouraged to comment and discuss their experience of the study in an unstructured manner while all of their comments were recorded.

Literature review: narrative and systematic review, and meta-analysis

Literature reviews aim to summarise the existing evidence on a topic that is available in the literature and can take the format of either a narrative or systematic review. A narrative review has a more descriptive format and employs an unspecified methodology, which often portrays the (expert) opinion of the author. Consequently, this type of review could suffer from evidence selection bias and may draw misleading conclusions, and its results are not able to be reproduced to either confirm or refute such bias. In contrast, systematic reviews have a pre-planned, well-defined and consequently reproducible methodology, including pre-defined inclusion criteria and a search strategy that aims to capture as much available literature on the topic as possible. Furthermore, systematic reviews can encompass a meta-analysis component, which is the analysis of the collective evidence through statistical methods in order to produce a (single) summary effect size (Uman, 2011; Higgins *et al.*, 2019). In this thesis, I present a systematic review that aimed to capture all available evidence on the topic while minimising the risk of bias. Although, strictly speaking, a meta-analysis component was not included in the systematic review due to the nature of the data (i.e. the presence of only one variable, namely the adherence to the Mediterranean diet), I have combined the results through statistical methods, so as to allow for their better interpretation.

Study design

Clinical studies can either have an interventional or observational design. The former has an interventional component and can be (singly or doubly) blinded and usually includes

a control group. Alternatively, an observational study has no interventional component and could take place at a particular point in time, known as a cross-sectional study, or run over a pre-determined period of time (either retrospective or prospectively longitudinal study) or can have a control group (case-control study). Intervention trials and longitudinal studies tend to be more time-consuming and require more resources (Supino and Borer, 2012; Lovegrove, Sharma and Hodson, 2015). In the thesis, I present a cross-sectional study. An interventional study or a longitudinal study was overall deemed unnecessary, as the available evidence at the time did not justify such a study design. Furthermore, an interventional or longitudinal study design would have placed more pressure on our already restricted time frame and stretched resources.

Primary outcomes measures

Mediterranean diet scoring systems

In an attempt to measure the adherence to the Mediterranean diet, several diet scoring systems have been developed. In this section, three widely used Mediterranean diet scores are described. A more thorough discussion of the various Mediterranean diet scoring systems is available in the literature, including descriptions of their differences and limitations (Bach *et al.*, 2006; Arvaniti and Panagiotakos, 2008; Kourlaba *et al.*, 2009; Panagiotakos, 2009; Zaragoza-Martí *et al.*, 2018) and is beyond the scope of the thesis. Note that the scoring systems described here are the ones utilised by the studies included in the systematic review, while the MedDietScore scoring system (Panagiotakos *et al.*, 2007) was selected for the cross-sectional study. The choice to utilise the MedDietScore was based on its attractive theoretical framework, such as the relatively accurate representation of the Mediterranean diet's food groups and the *a priori* scoring of the food servings, in addition to the fact that is widely adopted by other researchers who have shown that it correlates well with various health outcomes.

MDS: The MDS (Trichopoulou *et al.*, 1995, 2003, 2005) – one of the first Mediterranean diet scoring systems developed – consists of ten components, categorised into beneficial components, namely vegetables, legumes, fruits and nuts, cereals, fish, and monounsaturated to saturated fat ratio (MUFA: PUFA); and the detrimental components, namely meat, and poultry and dairy products; and alcohol. The scoring cut-off points are not pre-defined (apart for alcohol), but rather, the sex-specific median is used, that is to say, one point is allocated for each beneficial component above the median and one detrimental component below the median. The total score can range between zero and

nine, and higher values indicate a better adherence to the Mediterranean diet. The MDS has been widely used in the adult population, including in a well-known study that revealed a reduction in all-cause mortality, coronary heart disease (CHD) and cancer with better adherence to the Mediterranean diet, conducted in over 22,000 adults from Greece (Trichopoulou *et al.*, 2003); and also in the European Prospective Investigation into Cancer and Nutrition (EPIC) study (Trichopoulou *et al.*, 2005, 2007).

MedDietScore: The MedDietScore (Panagiotakos, Milias, *et al.*, 2006; Panagiotakos, Pitsavos and Stefanadis, 2006; Panagiotakos *et al.*, 2007) has also been widely used in the adult population. It comprises 11 components, which are confined to three food-groups categories; the beneficial food groups, namely non-refined cereals, potatoes, fruits, vegetables, fish, and olive oil; and the detrimental food group, namely red meat and products, poultry and full-fat dairy products; and alcohol. The MedDietScore has a wider scoring range, that is to say, each component score ranges from zero to five – a positive monotonic score for beneficial foods, a negative monotonic score for detrimental foods and a polytonic score for alcohol – based on the reported servings consumption. The scoring of servings is pre-defined, that is to say, fixed; however, note that there are two versions of the MedDietScore where these cut-off points (for scoring) are different. Finally, the total score can range between zero and 55, and higher values indicate better adherence to the Mediterranean diet.

KIDMED: The KIDMED (Serra-Majem *et al.*, 2004) scoring system has been validated for use in research with children and adolescents and has mostly been used in these age categories. The KIDMED has 16 components that reflect not only food groups but also individual foods and eating patterns. The score of the beneficial components ranges from zero to one, while the score of the detrimental components ranges from minus one to zero. The KIDMED scoring system can range from -4 to 12, with higher values indicating better adherence.

Glycaemic control

Glycaemic control is defined as the glucose levels and their variation in the blood and, in people with diabetes, it reflects their control of the disease. It can be measured directly through glucose measurements or indirectly by measuring the glycosylated haemoglobin (HbA1c) levels, which are discussed below. In the cross-sectional study, I utilised HbA1c and fasting glucose tests, which were measured in a clinical laboratory from serum blood.

Glucose: The blood glucose measurement is the measurement of glucose in the blood of a person at a particular point in time and is the cornerstone of diabetes diagnosis and management. The blood glucose measurement can identify normoglycemia, hyperglycaemia and hypoglycaemia (normal, high and low blood glucose levels, respectively) at the time of measurement. The blood glucose (of a person with or without diabetes) fluctuates continuously, and, therefore, analysing a series of glucose measurements can provide information on glucose variation over a period of time and can identify patterns at particular times, such as pre- and post-prandial, and in the fasting state. Blood glucose levels can be measured in a clinical laboratory using venous plasma or through blood glucose meters (used by patients with diabetes) using a capillary blood sample. Blood glucose is measured in mg/dl and mmol/l, where one mmol/l is equal to 18 mg/dl (Monnier, Colette and Owens, 2008; NICE, 2015b; Melmed *et al.*, 2016; American Diabetes Association, 2019d). In the cross-sectional study, I utilised a single fasted measurement due to its practicality (i.e., it can be combined with other tests that require fasting) and clinical significance.

HbA1c: The HbA1c measurement reflects the average blood glucose levels of a person over the last three months. It has been widely studied, and suboptimal (i.e., high and possibly very low) HbA1c has been correlated with adverse health outcomes. It is widely used in clinical practice for the diagnosis and management of people diagnosed with diabetes. In contrast to blood glucose testing, the HbA1c is not affected by food intake prior the measurement; however, at the same time, it does not provide any information (at least directly) on glucose variation, and hypoglycaemia and hyperglycaemia frequency. The HbA1c is measured in mmol/mol units (using the International Federation of Clinical Chemistry (IFCC) method) and these readings can be converted to a percentage (diabetes control and complications trial (DCCT)) using the formula *DCCT unit (%) = 0.09148 x IFCC unit (mmol/mol) + 2.152* (Heinemann and Freckmann, 2015; NICE, 2015b; Melmed *et al.*, 2016; American Diabetes Association, 2019d).

Sampling

Target population: The main cross-sectional study aimed to study a Mediterranean adult population which was traditionally considered to follow the Mediterranean diet and which was diagnosed with Type 1 diabetes. More precisely, the target population was the people with Type 1 diabetes currently residing in Limassol, Cyprus.

Available frame: The CDA database was to be used to capture a sample of the target population. The CDA database was the most complete database to our knowledge and likely to reflect the target population, as all people with Type 1 diabetes in Cyprus are required to register with the CDA in order to obtain medications and devices free of charge (Skordis *et al.*, 2012). Nevertheless, the exact prevalence of Type 1 diabetes (in Cyprus) is unknown as there are no epidemiologic studies of this sort, albeit some evidence exists regarding the incidence of the disease.

Sample: The sample size was to be defined based on sample size calculations for the association of the Mediterranean diet and glycaemic control. The sample size recruitment procedure was based on a random sample selection procedure to allow for better representation of the epidemiological outcomes, which were the adherence to Mediterranean diet and the glycaemic control. To allow for a more homogenous group but also for ethical reasons, people with severe physiological or psychological conditions (for example, patients with terminal illness) and pregnant women, which potentially could alter their food intake and physiology (consequently the glycaemic control), were excluded.

Errors of Inclusion: The most significant way of potential misclassification of participants identified, was the inclusion of other types of diabetes. For this reason, the diagnosis of Type 1 diabetes was established by a rigorous methodology that integrated both clinical and laboratory parameters and measurements. The place of residency, which was defined as currently residing in Limassol so to allow us to capture the current population of Limassol, was another potential (although arguably less significant) source of misclassification.

Errors of exclusion: The CDA database may not capture the full population with type 1 diabetes of Limassol, Cyprus. In such a case, the epidemiological results may not reflect the full picture of the target population (i.e., of Limassol, Cyprus) but the results of the association between Mediterranean diet and glycaemic control are less likely to be affected by such an event.

Summary

A quantitative approach was primarily employed in the thesis. The adherence to the Mediterranean diet was assessed through a systematic review and the association of

Mediterranean diet and glycaemic control through a cross-sectional study. Adherence to the Mediterranean diet was measured using a Mediterranean diet score. Glycaemic control was measured using HbA1c and fasting glucose. The target population were adults with Type 1 diabetes residing in Limassol, Cyprus and this was captured through the CDA database.

Chapter 3: Adherence to the Mediterranean diet by the Greek and Cypriot population: a systematic review

Introduction

This chapter presents a report of a systematic review conducted to gather existing evidence on the adherence to the Mediterranean diet by the Greek and Cypriot population to obtain a more comprehensive understanding of the context and to provide a background and rationale for the main study reported in this thesis. The chapter has previously been published in the *European Journal of Public Health* – available at https://doi.org/10.1093/eurpub/ckv124 (Kyriacou *et al*., 2015, *Appendix: Chapter 3*) and is reproduced here in part with permission from the publisher.

The Mediterranean diet is a collective term used to describe the dietary patterns of the people living around the Mediterranean Sea who share some common features but at the same time have their own unique characteristics between the Mediterranean regions and countries (Trichopoulou et al., 2000; Bach-Faig et al., 2011; Romagnolo and Selmin, 2016). This review concentrated on the dietary patterns and the traditional Mediterranean diet of the countries of Cyprus and Greece. While Cyprus is the country of interest for the thesis, the number of studies was expected to be limited, and, given that Cyprus and Greece share very similar dietary habits, largely owing to their close geographical proximity, shared language and religion, and the close cultural ties, it was considered appropriate to combine the literature of the two countries to provide more meaningful results. Furthermore, Greece is considered to be the origin of Mediterranean diet, as described in the widely cited *Seven Countries Study* (Keys, 1980).

There is debate as to whether adherence to the Mediterranean diet in modern Greek and Cypriot society has remained constant or whether people have moved towards a more Westernised/Americanised diet, and as to the extent of the reduction in adherence if the latter holds true. For example, ecological studies using the food balance sheets of the United Nations' Food and Agriculture Organization have shown a significant drop in the Mediterranean dietary pattern among the Mediterranean countries over the last decades, with Greece experiencing the biggest drop and Cyprus also having a considerable negative change (Balanza *et al.*, 2007; da Silva *et al.*, 2009; Vareiro *et al.*, 2009).

In this review, I aimed to answer this question by systematically reviewing the evidence for adherence to the Mediterranean diet of the Greek and Cypriot population. More precisely, the primary outcomes of the systematic review were as follows:

i. To measure the adherence to the Mediterranean diet by the Cypriot and Greek population, as measured by Mediterranean diet scoring systems
ii. To quantify the difference in adherence between males and females, and between age groups
iii. To explore potential trends in the change of adherence to the Mediterranean diet over time

A further (post-hoc) outcome was to describe the populations that were included, especially in regard to their health; for example, the general population or populations with a specific health problem, such as Type 1 or Type 2 diabetes, heart disease, etc.

Methods

Search strategy

A MEDLINE search was conducted, up to 15 July 2013, using the free terms 'adheren* or prevelan*'(topic) and 'Mediterranean diet*'(topic) and 'gre* or cypr*'(topic). The search was conducted independently by two researchers (AK and NE), and any disagreement was settled after discussion between the two. The papers were reviewed progressively in stages on the basis of the title, abstract and, finally, the full text. One of the authors (AK) hand-searched the references in all of the identified papers (*Figure 3.1*). The search was not limited to language, date or any other limitations.
However, to be included in the review, the study had to fulfil the following criteria:

- The study was conducted in a Cypriot or Greek population, or with people of both nationalities who were permanently residing in Cyprus or Greece or both.
- Adherence to the Mediterranean diet was measured as a primary or secondary outcome of the total study population and/or of the total population of each gender of the study and/or of different age groups.
- A Mediterranean diet score was used.
- A direct method of data collection, such as questionnaires, interviews, self-reported individual methods and surveys, was used.

- The data were not collected after an intervention; i.e., the data must have originated from an observational cross-sectional or longitudinal study or at baseline of an intervention study (before the intervention commenced).

Studies which were not original (e.g., reviews and meta-analysis) or that used an indirect method of measurement of data collection (e.g., the World Health Organization/Food and Agriculture Organization data or household food surveys) were excluded from the review, as were studies that collected data after an intervention (but not the baseline data). When the same study population or a partially overlapping population was described in several papers, then only the study with the highest sample size was included in the review. The author of the study was consulted when unsure about the population used or when it was thought that there might be overlap.

Data extraction

The data extraction process was conducted by AK and reviewed by NE. Information extracted included the author(s), title, publication media, date of publication, study design, year of survey, population characteristics, sample size, age range, Mediterranean diet scoring system used, health status, Mediterranean diet score (Mdiet score) by country and/or gender and/or age group and/or of the total population.

Statistics

Mean score and standard deviation

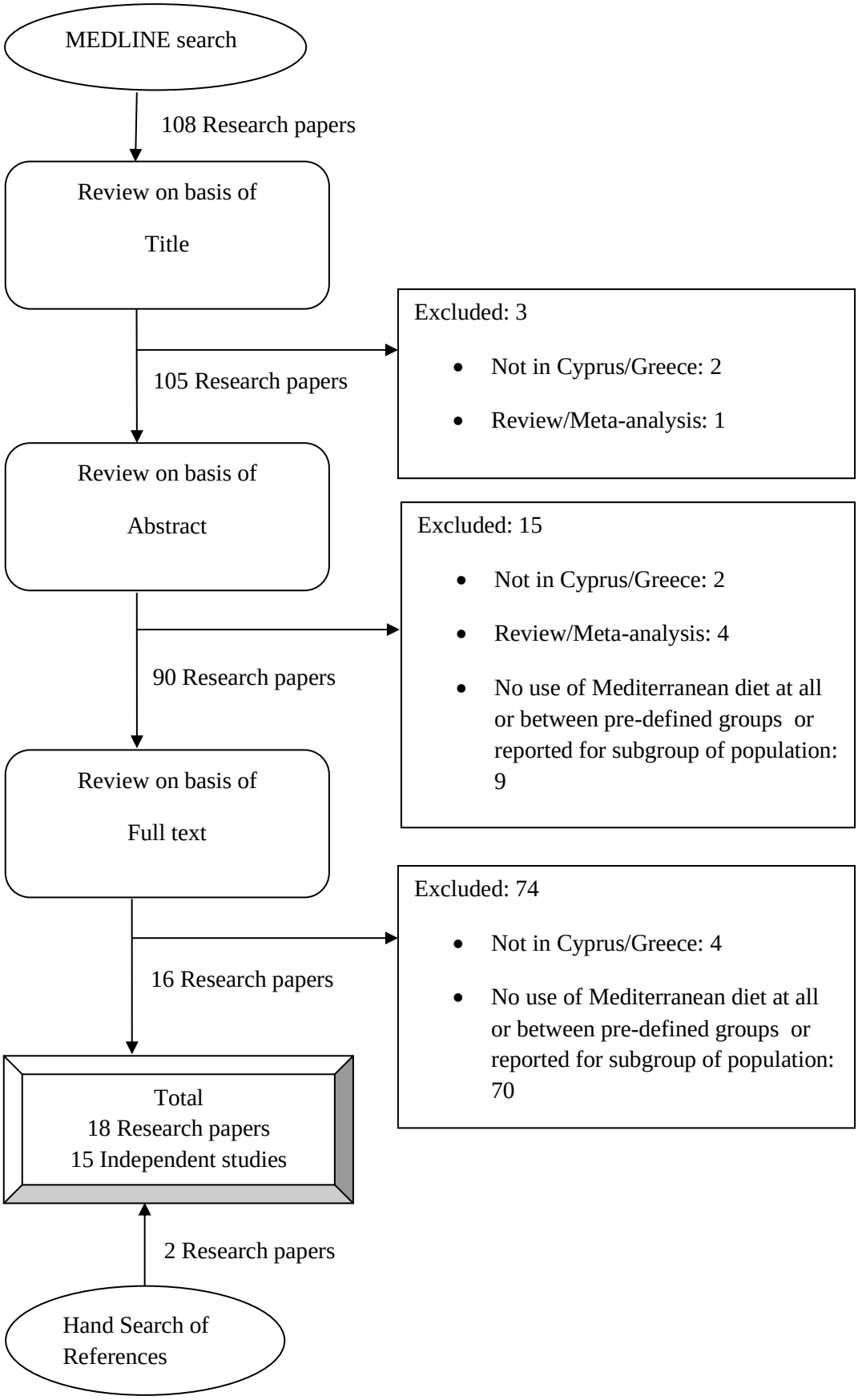
The total mean score and standard deviation of the Mdiet scores were noted, but, if they were reported for subgroups only, the total population's mean score and standard deviation were calculated using appropriate formulae. In the same way, the mean and standard deviation, for all studies using the same Mdiet score, was calculated and is reported as 'Total' (the equations used are available in *Appendix: Chapter 3*).

Percentages of adherence to the Mediterranean diet

There are a number of scoring systems that can be used to measure adherence to the Mediterranean diet. These scoring systems have several differences, e.g., different maximum and minimum possible scores and different definitions of low, medium and high adherence. This makes inter-study comparison difficult. In addition to considering the definitions presented in the original research papers, I calculated a measure of percentage adherence to enable comparison between studies that used different scoring systems. This was defined as the minimum Mdiet score subtracted from the Mdiet score

of the total population, then divided by the difference of the maximum and minimum Mdiet score of the particular scoring system and multiplied by 100% (Romaguera *et al.*, 2009; Tyrovolas *et al.*, 2009, 2011; Chrysohoou *et al.*, 2011). However, for the KIDMED scoring system, the lowest possible score was not zero, but -4. Therefore, the total maximum score was 16, which is the highest possible score (12) minus the lowest possible score (-4). Furthermore, the percentage adherence to Mediterranean diet was categorised by dividing the (maximum) score into three equal parts. Below the first cut-off point, or 33.33% adherence, participants were considered to have low adherence, and between the second and third cut-off points (33.33 – 66.67%), they were considered to have moderate adherence. Finally, above the 66.67% cut-off point, participants were considered to have good adherence to the Mediterranean diet.

Figure 3.1: MEDLINE database and other search procedures



Results

The initial MEDLINE search resulted in 108 papers. After the three-stage reviewing process, 92 papers were excluded. A hand search of references generated a further two papers. The final total of 18 papers represented 15 independent studies (*Figure 3.1*).

All 15 included studies were cross-sectional. They were all conducted in Greece, apart from one study (Tyrovolas *et al.*, 2009, 2011), which had a mixture of Cypriot and Greek participants. The demographic characteristics of the study populations are presented in *Table 3.1* and represent a diverse range of groups, such as general adult, paediatric and elderly, and school and university students. No study that represented a group of people with a common health background, such as cardiovascular problems or diabetes, met the criteria for inclusion.

The results of the 15 independent cross-sectional studies are shown in *Table 3.2* and in *Figure 3.2* in a separate graph for each scoring system. The cut-off points used in *Figure 3.2* to define low, moderate or high adherence were those defined in the original research papers. Note that different studies used different terms for 'moderate' adherence (e.g., average and medium). I use the term 'moderate' throughout this review.

The most commonly used scoring system was the MedDietScore, used by eight independent studies (Arvaniti *et al.*, 2006; Panagiotakos, Chrysohoou, *et al.*, 2006; Panagiotakos *et al.*, 2007; Dedoussis *et al.*, 2008; Kanoni and Dedoussis, 2008; Doupis *et al.*, 2009; Tyrovolas *et al.*, 2009, 2011; Chrysohoou *et al.*, 2011; Van Diepen *et al.*, 2011; Katsiardanis *et al.*, 2013). The point range of the score was 0 to +55 and the cut-off points used to define adherence were the 33.33% and 66.67% (represented by scores of 18.3 and 36.7; *Figure 3.2b*). All five paediatric studies (Kontogianni *et al.*, 2008; Arvaniti *et al.*, 2011; Farajian *et al.*, 2011; Lydakis *et al.*, 2012; Costarelli, Koretsi and Georgitsogianni, 2013) used the KIDMED scoring system, which has a possible score range of between -4 and +12 and cut-off points at 46.88% and 71.88% (representing scores of +3.5 and +7.5, respectively; *Figure 3.2a*).The MDS (Psaltopoulou *et al.*, 2004) and 44-point scoring system (Filippidis *et al.*, 2011) were less widely used (*Figure 3.2c* and *Figure 3.2d*). *Figure 3.3* presents the results for percentage adherence with the corresponding cut-off points (33.33% and 66.67%), for all studies.

The total adherence for the KIDMED was 51.6% (4.3 points) and for the MedDietScore was 52.5% (28.9 points). No total adherence was calculated for the MDS and 44-points score, which consisted of a single study (48.8% and 60.4%, respectively). The results show consistent moderate adherence to the Mediterranean diet, whether the original Mdiet score system and cut-off points are used (*Figure 3.2*) or whether they were converted to percentages, and the corresponding cut-off points are used (*Figure 3.3*), regardless of the study population.

Most studies found no statistically significant difference between genders (Tyrovolas *et al.*, 2009, 2011; Arvaniti *et al.*, 2011; Chrysohoou *et al.*, 2011; Farajian *et al.*, 2011; Van Diepen *et al.*, 2011; Lydakis *et al.*, 2012). Four studies showed statistically significant higher adherence to the Mediterranean diet among males (Psaltopoulou *et al.*, 2004; Dedoussis *et al.*, 2008; Kanoni and Dedoussis, 2008; Filippidis *et al.*, 2011) and only one for females (Arvaniti *et al.*, 2006; Panagiotakos, Chrysohoou, *et al.*, 2006; Panagiotakos *et al.*, 2007), with four studies not reporting the *p*-value (Kontogianni *et al.*, 2008; Costarelli, Koretsi and Georgitsogianni, 2013; Katsiardanis *et al.*, 2013) or the gender adherence Mdiet scores (Doupis *et al.*, 2009).

Furthermore, although a visual comparison of *Figure 3.2a* and *Figure 3.2b* might suggest general lower adherence in the younger population (using the KIDMED score) than the adult population (using the MedDietScore), this difference becomes less apparent in *Figure 3.3*. Only two studies investigated adherence by age. One study in the adult population showed a statistically significant increase with age (Filippidis *et al.*, 2011). One in the paediatric population showed higher adherence among younger children, but no statistical test was reported (Kontogianni *et al.*, 2008).

Table 3.1: Demographic characteristics of the included studies

(Reference)	Year of data collection	Country; Area; Representative	Sample size	Age range (years)	Health status
(Costarelli, Koretsi and Georgitsogianni, 2013)	Not available	Greece; Athens and Dodecanese	359	13 – 16	General population
(Katsiardanis *et al.*, 2013)	2005 – 2006	Greece; Valestino	557	> 65	General population
(Lydakis *et al.*, 2012)	2011	Greece; Heraklion, Crete	277	12	No clinical history of cardiac (congenital) or renal conditions, diabetes mellitus, receiving immunosuppression or cytotoxic drugs
(Chrysohoou *et al.*, 2011)	2009	Greece; Ikaria island	538	> 65	No clinical history of CVD, other atherosclerotic disease and use of diuretic drugs
(Farajian *et al.*, 2011)	2009	Greece; 10 regions; Representative	4786	10 – 12	General school population
(Filippidis *et al.*, 2011)	2006	Greece; Representative	1005	18 – 99	General population
(Van Diepen *et al.*, 2011)	2008	Thessaloniki; Greece	85	21.6 ± 3.2[a]	Healthy university students
(Tyrovolas *et al.*, 2009) (Tyrovolas *et al.*, 2011)	2005 – 2007	Greece and Cyprus; 7 islands in Greece and the Republic of Cyprus; Representative	1190	> 65	No clinical history of CVD, cancer or institutionalised
(Arvaniti *et al.*, 2011)	2005 – 2006	Greece; Athens	700	10 – 12	School boys and pre-menstrual girls
(Kontogianni *et al.*, 2008)	2007	Greece; Representative	1305	3 – 18	General population
(Kanoni and Dedoussis, 2008)	Not available	Greece; Athens	782	> 60	General population
(Dedoussis *et al.*, 2008)	Not available	Greece	163	> 60	Healthy, non-institutionalized, free of medications and chronic conditions
(Arvaniti *et al.*, 2006; Panagiotakos, Chrysohoou, *et al.*, 2006; Panagiotakos *et al.*, 2007)	2001 – 2002	Greece; Attica	3042	18 – 89	No clinical history of CVD, other atherosclerotic disease, chronic viral disease and surgery the week before the data collection
(Psaltopoulou *et al.*, 2004)	1994 – 1999	Greece	20343	20 – 86	No diagnosis of hypertension; volunteers
(Doupis *et al.*, 2009)	Not available	Greece	832	17 – 39	General navy recruits

[a]mean ± standard deviation when range is not available.
CVD, cardiovascular disease.

Table 3.2: Mdiet score of the included studies

(reference) [figure no.]	Mean Mdiet score of all population	Mean Mdiet score of female population	Mean Mdiet score of male population	*p*-value	Mean Mdiet score of age group categories (years)	*p*-value
KIDMED						
(Costarelli, Koretsi and Georgitsogianni, 2013) [10]	6.3 ± 2.4	6.2 ± 2.5	6.3 ± 2.5	*p* = DNS	DNS	
(Lydakis *et al.*, 2012) [11]	6.6 ± 2.20	6.82 ± 2.17	6.48 ± 2.22	*p* = 0.197	DNS	
(Farajian *et al.*, 2011) [12]	3.65 ± 2.27	3.66 ± 2.24	3.64 ± 2.29	*p* = 0.86	DNS	
(Arvaniti *et al.*, 2011) [13]	4.8 ± 2.0[a]	4.8 ± 2.0	4.8 ± 1.9	*p* = 0.87	DNS	
(Kontogianni *et al.*, 2008) [14]	5.13 ± 1.9[a]	5.17 ± 1.9[a]	5.09 ± 2.0[a]		5.4 ± 1.8 (3 – 12) 4.8 ± 2.1 (13 – 18)	*p* = DNS
MedDietScore						
(Katsiardanis *et al.*, 2013) [15]	34.7 ± 2.87[a]	35.1 ± 2.48	34.1 ± 3.25	*p* = DNS	DNS	
(Chrysohoou *et al.*, 2011) [16]	35 ± 2	35 ± 3	34 ± 2	*p* = 0.26	DNS	
(Van Diepen *et al.*, 2011) [17]	26.1 ± 3.4	DNS	DNS	*p* = NS	DNS	
(Tyrovolas *et al.*, 2009, 2011) [18,19]	33.5 ± 4.0	33.7 ± 3.8	33.3 ± 4.3	*p* = 0.10	DNS	
(Kanoni and Dedoussis, 2008) [20]	30.0 ± 3.2[a]	29.7 ± 3.0	30.7 ± 3.4	*p* < 0.001	DNS	
(Dedoussis *et al.*, 2008) [21]	28.2 ± 3.8 [a]	27.7 ± 3.4	29.0 ± 4.2	*p* = SS[b]	DNS	
(Arvaniti *et al.*, 2006; Panagiotakos, Chrysohoou, *et al.*, 2006; Panagiotakos *et al.*, 2007) [22 – 24]	26 ± 3	27.18 ± 3.21	25.46 ± 2.94	*p* < 0.001	DNS	
(Doupis *et al.*, 2009) [25]	24.5 ± 3.68	DNS	DNS		DNS	
MDS						
(Psaltopoulou *et al.*, 2004) [26]	4.4 ± 1.69[a]	4.3 ± 1.67	4.5 ± 1.70	*p* < 0.001	DNS	
44-point score						
(Filippidis *et al.*, 2011) [27]	26.59 ± 6.23	26.14 ± 6.17	27.09 ± 6.25	*p* = 0.017	25.84 ± 6.58 (18 – 36) 26.92 ± 5.93 (37 – 56) 27.05 ± 6.08 (57 – 99)	*p* = 0.011

[a]Value not available, but calculated using the formulas reported in *Appendix: Chapter 3*; [b]after age adjustment.
Mdiet score, Mediterranean diet score; DNS, data/value was not shown; NS, not statistically significant (but *p*-value not given); SS, statistically significant, $p < 0.05$ (but *p*-value not given)

Figure 3.2: Results using different scoring systems with original definitions of adherence levels

Figure 3.2a: KIDMED scoring system: mean (standard deviation)

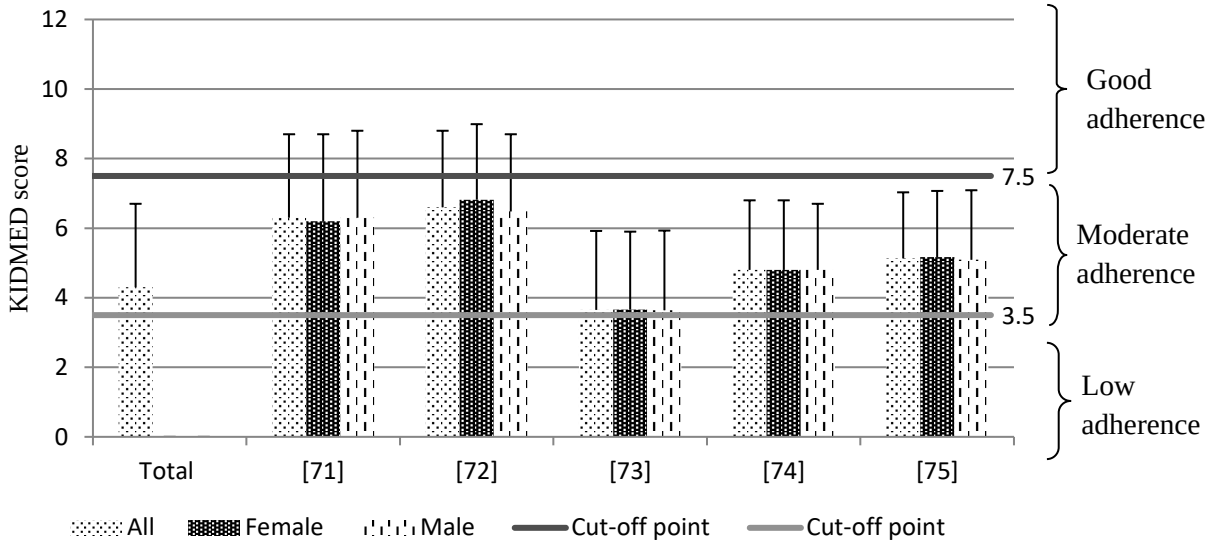


Figure 3.2b: MedDietScore scoring system: mean (standard deviation)

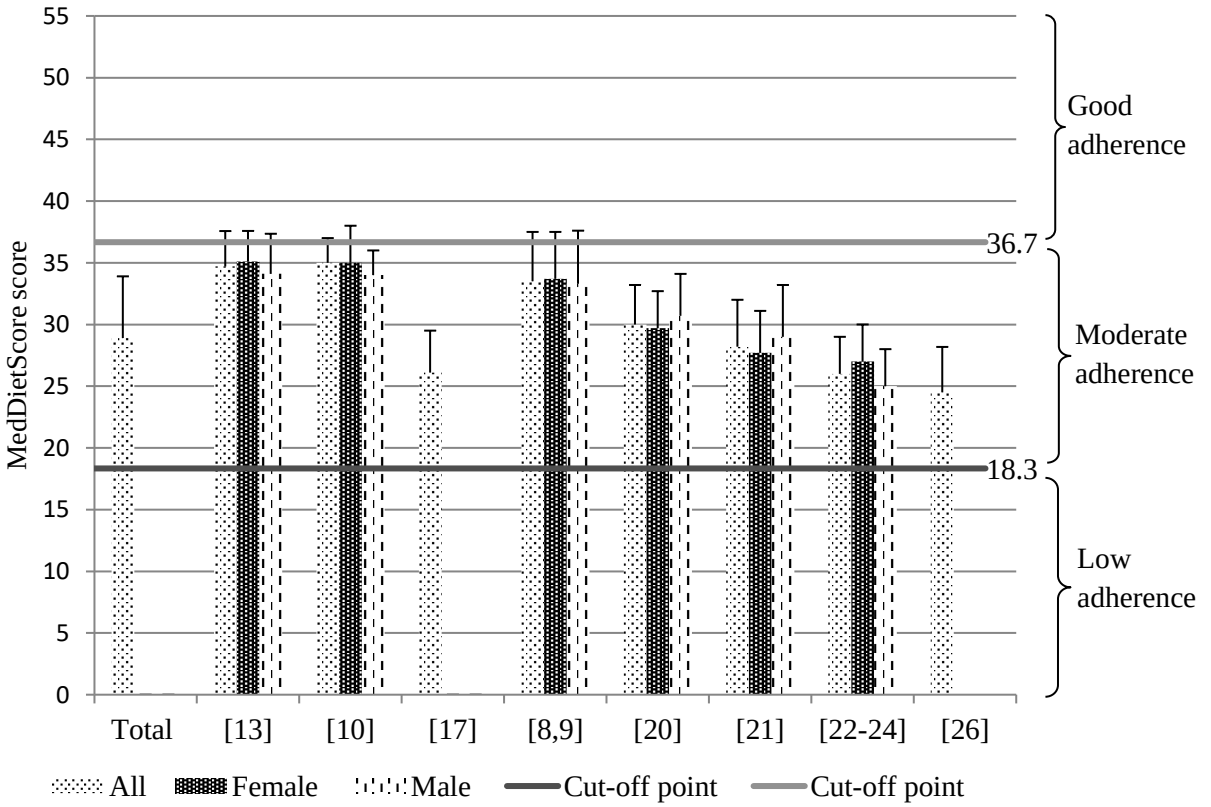


Figure 3.2c: MDS scoring system: mean (standard deviation)

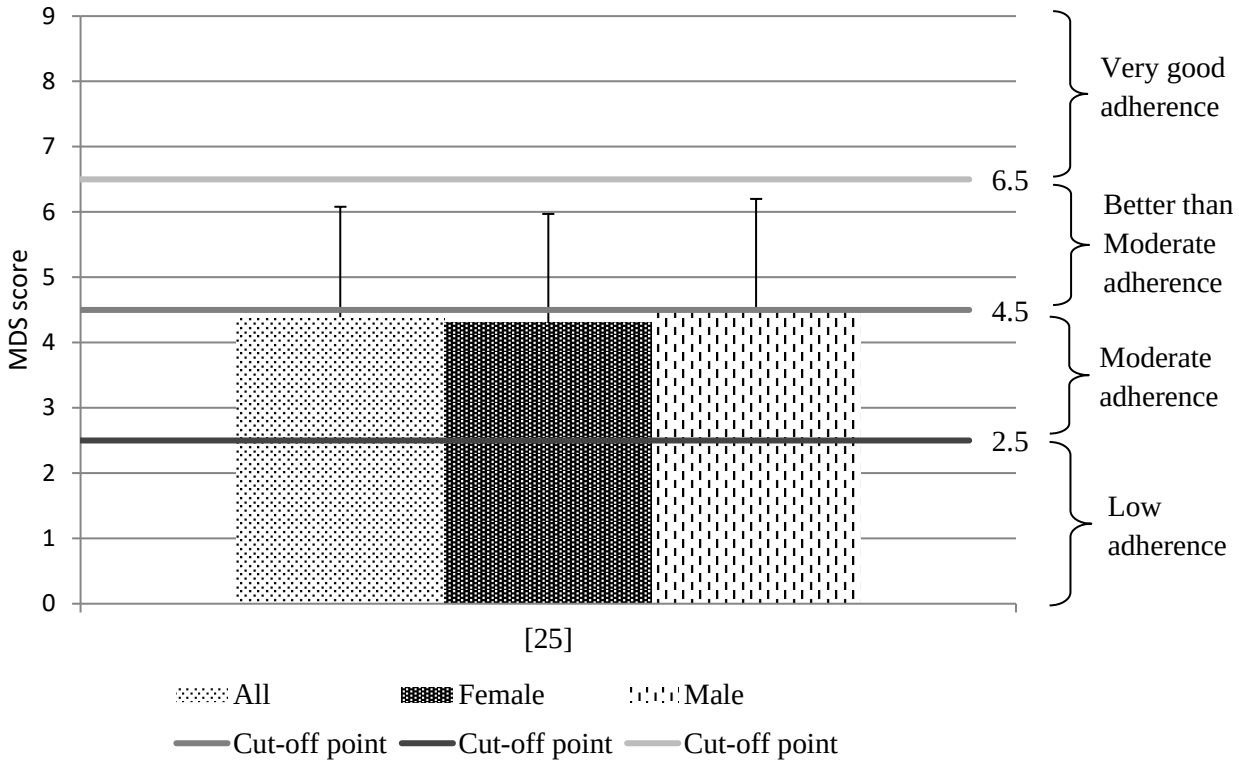


Figure 3.2d: 44-point scoring system: mean (standard deviation)

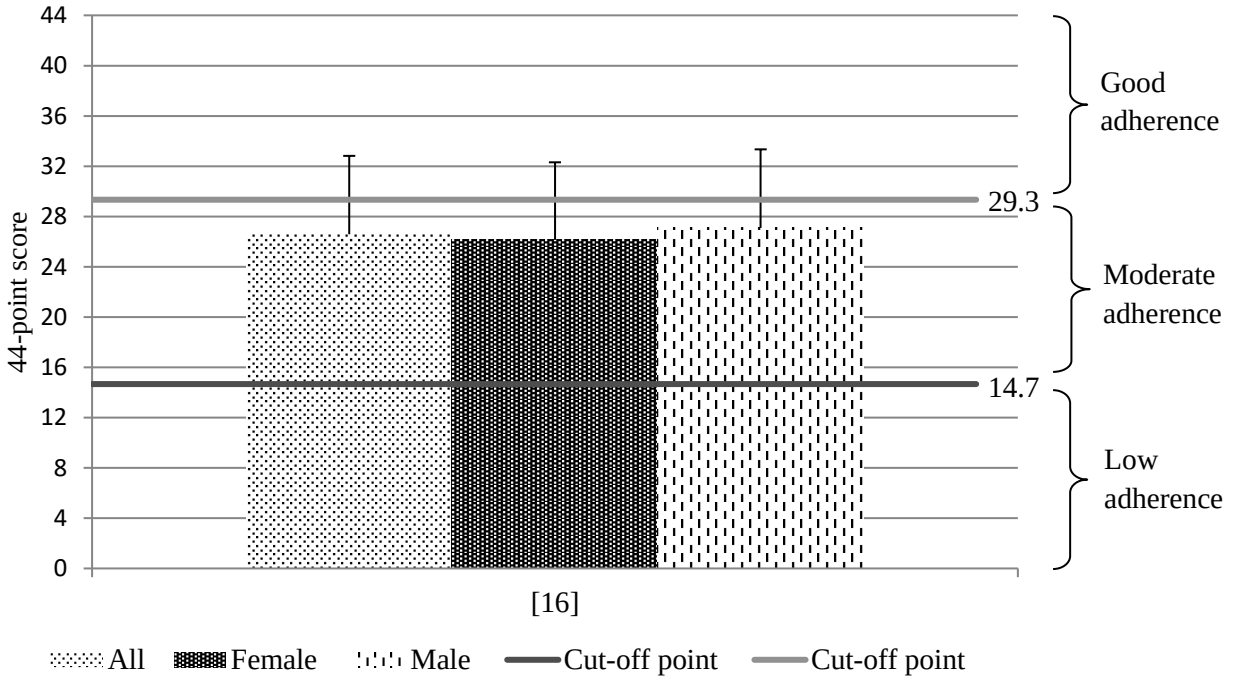
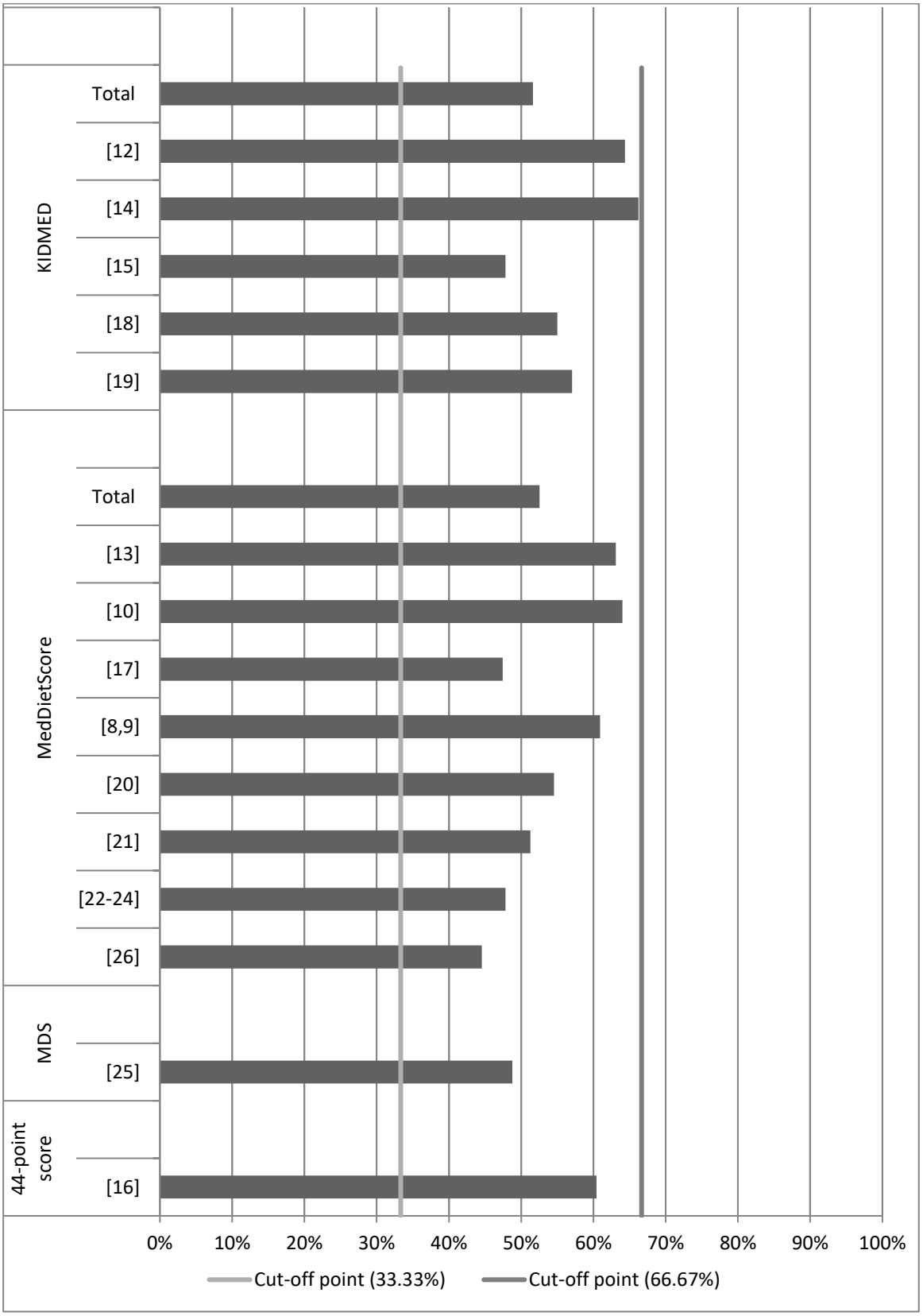


Figure 3.3: Percentage adherence to the Mediterranean diet



Discussion

The current review has investigated adherence to the Mediterranean diet of two related Greek-speaking Mediterranean populations, namely the Greek and Cypriot populations. All studies show consistent results regarding adherence to the Mediterranean diet: widespread moderate adherence. These results were seen independently, whether the original Mdiet score and cut-off points were used (*Figure 3.2*) or whether results were converted to percentages and the corresponding cut-off points (33.33% and 66.67%) were used (*Figure 3.3*). Similar results, that is to say, moderate adherence, have been observed when using a Mdiet scoring system in several other countries of the Mediterranean region such as Spain (León, Henríquez and Serra-Majem, 2006), Italy (Sofi *et al.*, 2007; Pelucchi *et al.*, 2010) and France (Dedoussis *et al.*, 2008) but also elsewhere in Europe, such as the Netherlands (Van Diepen *et al.*, 2011), Germany and Poland (Dedoussis *et al.*, 2008).

This review suggests that adherence to the Mediterranean diet has decreased since the 1950s and 1960s, when it was high by definition (Trichopoulou *et al.*, 2000). More precisely, this reduction was estimated to be almost 50% as shown by the two most used scoring systems; the total adherence decrease for KIDMED score was 48.4% and for MedDietScore was 47.5%. Almost all studies included in this review were conducted after 2000. This may be because interest has changed from single nutrients to whole diets in the last decade (Hu, 2002); with a corresponding development of the various Mdiet scores. The MDS scoring system was developed in the decade between 1990 and 2000, while the rest followed after the year 2000. This finding is backed up by data that used the food balance sheets of the Food and Agriculture Organization of the United Nations of the early 1960s and just before (Balanza *et al.*, 2007) or after 2000 (da Silva *et al.*, 2009; Vareiro *et al.*, 2009). These data showed a statistically significant large decrease (56.3%) in the Mediterranean Adequacy Index (MAI) in Mediterranean Europe, including Cyprus and Greece (3.39 ± 1.12 to 1.48 ± 0.39, $p = 0.0009$, MAI); in contrast, a non-statistically significant increase was observed in Northern countries and a decrease in Eastern European countries (Balanza *et al.*, 2007). While Greece ranked first between 1961 and 1965 using the MAI score, among the 41 countries studied, it ranked only 10th between 2000 and 2003. Cyprus dropped from 20th place to 27th place during the period (da Silva *et al.*, 2009). Although this study, in line with the previous studies (Balanza *et al.*, 2007; da Silva *et al.*, 2009; Vareiro *et al.*, 2009), reports a decrease in adherence to

the Mediterranean diet, it is not possible to determine with certainty when exactly this happened; that is, whether there was regular decline over time or whether there were periods of rapid change, or, alternatively, whether this phenomenon was limited to isolated time intervals. There is insufficient data on this topic between the 1960s and the 2000s to determine this.

Although moderate adherence seems to be the norm in most countries studied, some variation does exist. Van Diepen *et al.* (2011), who compared the Mdiet score of Greek and Dutch university students, found an unexpected higher statistically significant adherence in favour of the non-Mediterranean origin Dutch students (26.1 ± 3.4, 27.5 ± 3.9, $p \leq 0.05$, MedDietScore). Dedoussis *et al.* (2008), who studied older people from five different countries, showed that Greece had a statistically significant lower Mdiet score when compared with Italy, a higher one than Poland, but no discernible difference from Germany and France. Comparison between Greek and Cypriot populations in the present review was not possible as there was a very limited number of data on Cyprus; only one independent study was found, which had a mixture of Cypriot and Greek subjects (Tyrovolas *et al.*, 2009, 2011). The adherence to Mediterranean diet in the current review ranged from 48.8% to 60.4% (*Figure 3.3*).

The majority of studies included in the current review reported no statistically significant difference between genders (*Figure 3.2*). Only four studies showed a gender difference, of which three independent studies (Psaltopoulou *et al.*, 2004; Dedoussis *et al.*, 2008; Kanoni and Dedoussis, 2008) reported a significant higher Mdiet score for males, and one independent study (Arvaniti *et al.*, 2006; Panagiotakos, Chrysohoou, *et al.*, 2006; Panagiotakos *et al.*, 2007) reported a higher Mdiet score for females. Two studies in the Italian population (Sofi *et al.*, 2007; Dedoussis *et al.*, 2008) reported a statistically significant higher score for males, while, in the other populations studied, namely the French, German and Polish, there were no statistically significant differences between male and female participants (Dedoussis *et al.*, 2008).

There is a question as to whether adherence to the Mediterranean diet is lower in younger populations. Results from inter-study comparison show a probable lower but small difference in adherence of the younger population against the older population (51.6% versus 52.5%, respectively; *Figure 3.3*). This difference in adherence was more obvious when using the original Mediterranean diet scoring systems (*Figure 3.2*). This

discrepancy may be explained by the considerably different cut-off points used by the MedDietScore (33.33% and 66.67%; *Figure 3.2b*), which was used for the study of adult population, and the KIDMED score (46.88% and 84.38%; *Figure 3.2a*), which was used for the study of the paediatric population. Only two studies investigated the Mdiet score between various age groups within the same study population. The first study among adults showed similar results to the inter-study comparison, that is, a statistically significant increase with age ($p = 0.011$) (Filippidis *et al.*, 2011). The second study in a paediatric sample reported higher adherence among the younger group (3 – 12 years) than the older age group (13 – 18 years) (Kontogianni *et al.*, 2008). The heterogeneity of the results for the second study may reflect the fact that the diets of younger-aged children are likely to be influenced by other parameters, such as food provided by the parents (Birch, Savage and Ventura, 2007).

The aforementioned change in the diet of Greek-speaking populations in the South Eastern Mediterranean basin reflects a transition from the traditional Mediterranean diet to a more Westernised diet and may (at least partially) explain the observed deterioration in the health of these populations. Indeed, in recent decades, these populations have experienced an inexorable increase in the incidence of diet-related disease, such as obesity, diabetes, cardiovascular disease, some cancers, dyslipidaemia and hypertension (Kafatos *et al.*, 1997). The beneficial association between the Mediterranean diet pattern and health, and the reduction in diet-related diseases, is supported by several studies (Sofi *et al.*, 2010; Kastorini *et al.*, 2011; Koloverou *et al.*, 2014). Other contributing non-diet, modified lifestyle-related risk factors may include the high prevalence of smoking (Vardavas and Kafatos, 2007) and reduced physical activity observed in the Greek population (Cavill *et al.*, 2006). In an effort to take these factors into account, Polychronopoulos, Panagiotakos and Polystipioti (2005) have proposed the Mediterranean lifestyle scoring system. This Mediterranean lifestyle scoring system combines the Mediterranean diet, alcohol consumption, physical activity and smoking to create a score with range from 0 to 4. This scoring system is not yet used extensively and, even more importantly, its results need to be shown to be related to hard point clinical outcomes such as mortality and morbidity in order to prove its usefulness.

The KIDMED diet score is the Mediterranean diet scoring system of choice for all five studies (Kontogianni *et al.*, 2008; Arvaniti *et al.*, 2011; Farajian *et al.*, 2011; Lydakis *et al.*, 2012; Costarelli, Koretsi and Georgitsogianni, 2013), each of which investigated a

paediatric population. In comparison, a number of diet scores were used for the adult population. The most popular of the adult Mediterranean scoring systems was the MedDietScore (*Table 3.2*). The different types of scoring systems used to measure the adherence to the Mediterranean diet makes inter-study comparison more difficult (Buckland, Bach and Serra-Majem, 2008), and, for this reason, I decided to use the percentage of adherence to the Mediterranean diet (*Figure 3.3*), although I am aware of its limitations. Each Mdiet scoring system has its own advantages and limitations, which are explored elsewhere (Arvaniti and Panagiotakos, 2008; Kourlaba *et al.*, 2009; Panagiotakos, 2009) and are beyond the aims of this study.

In conclusion, this review has brought together evidence on adherence to the Mediterranean diet in Greece and Cyprus. The included studies have been carried out in a diverse range of study populations, and I consider that the moderate adherence observed, which was estimated to be just above 50%, to likely be a valid picture. Given the well-documented health benefits of the Mediterranean diet, evidence that adherence is following a downward path with time and may be lower in younger populations is worrisome. Public health and education measures need to be implemented if this decrease in adherence to the Mediterranean diets is to be reversed.

Conclusion

The average adherence to the Mediterranean diet of the population in Greece, and possibly for Cyprus, is moderate or just above 50%, as shown by the most used scoring systems (total adherence decrease for KIDMED score was 48.4% and for MedDietScore was 47.5%), when compared to the high adherence, by definition, of the 1950s and 1960s. The majority of studies showed no statistically significant difference between genders in adherence to the Mediterranean diet. Although moderate adherence seems to be widespread, some variation seems to exist within countries but also in between countries (range of adherence of studies includes was 48.8% to 60.4%). Public health and education measures need to be implemented to reverse this worrisome decrease in adherence to the Mediterranean diet.

Chapter 4: Mediterranean diet and glycaemic control in Type 1 diabetes: a pilot study

Introduction

In the previous chapter, I showed that adherence to the Mediterranean diet in two related populations, namely the Greek and Cypriot population, reduced considerably, by around 50%, when compared to the 1950s and 1960s when adherence was 100% by definition. Furthermore, this reduction seemed to be more prominent in the younger generations with a likely worrisome trend over the years towards a more Westernised/Americanised diet.

A second aim of the thesis was to investigate adherence to the Mediterranean diet and glycaemic control in the population of interest, namely, in patients with a diagnosis of Type 1 diabetes in Cyprus; and to explore associations between the two or more precisely, the power of the Mediterranean diet to predict glycaemic control. Furthermore, a secondary aim was to gather more detailed epidemiological information – such as data on lifestyle, medical, physical activity, anthropometric and other related epidemiological evidence – that are not widely available for this population.

This section therefore focuses on the main obstacles and challenges identified in running a study to investigate the Mediterranean diet and glycaemic control in patients with a diagnosis of Type 1 diabetes in Cyprus. I aimed to make use of technological advances – despite the limited financial capacity, resources and support available – so that I could provide methods that were both convenient for the participants and environmentally friendly (less paper used); but, more importantly, the time and financial cost for the research team (taking into account that these methods will hopefully be used in the future) was reduced considerably, while the possibility of error during data cleaning (and analysis) was reduced. At the same time, I had to ensure that my chosen methods ran smoothly (while collecting a large amount and various types of data), that a larger study was feasible using the same methodology and that any obstacles were avoided before the main study was executed. Therefore, a pilot study was needed to address the following issues:

Absence of official centralised database: Cyprus does not have a centralised diabetes database – such as, for example, the Scottish Care Information – diabetes (SCI-diabetes) in Scotland or the National Diabetes Registry (NDR) in Sweden. Therefore, I needed to

identify and pilot a method that would provide a representative sample of the population and, at the same time, an adequate sample size for this research. I decided to use the Cyprus Diabetes Association (CDA) database, which is the most complete database to my knowledge in Cyprus, after discussing this with members of the CDA. This database, as reported in the introduction to this thesis, has previously been used to estimate the incidence of Type 1 diabetes in children, but it has not been used for recruitment purposes, therefore it was difficult to predict what the response (and study completion rate) would be. Furthermore, the method of contacting the patients also had to be tested; the CDA database was first accessed by members of its clerical staff to initially contact its members with a diagnosis of Type 1 diabetes and, if patients gave their consent, I contacted them. The database in question was reported to have some misclassification of diabetes patients – for example, patients with a diagnosis of Type 2 diabetes, in the past, when initiated with insulin were wrongly misclassified as having Type 1 diabetes and therefore I needed to test the adequacy of the measures put forward to identify the correct patients (i.e. other types of diabetes versus Type 1 diabetes). Finally, given that the contact details of the patients in the CDA database had been infrequently updated in the past, the pilot study was required to provide an estimate of the number of patients who had correct contact details available.

Absence of validated measures for the Cypriot population: There are few questionnaires (diet and medical-related) that have been formally validated in the Cypriot population, including patients diagnosed with Type 1 diabetes in Cyprus. There was therefore a need to either construct these measures from scratch or significantly adapt other measures that have previously been validated in different populations and countries. These measures included a demographic questionnaire which was based on various other questionnaires available in the literature; a food frequency questionnaire (FFQ), comprising a semi-structured FFQ with the foods included very loosely based on a previous validated questionnaire in Greece (Katsouyanni, 1997); a medical and diabetes questionnaire that was constructed completely from scratch as none was available in the literature for Cyprus and considerable differences exist in relation to other countries, including in the health care system *per se*; and a physical activity questionnaire, which was the short version of the IPAQ (international physical activity questionnaire) questionnaire (Craig *et al.*, 2003) and was used to measure the physical activity levels of the participants – the Greek language version, validated in a Greek population (Papathanasiou *et al.*, 2009), of the

IPAQ was preferred. In addition to the questionnaires, a number of data collection methods were used, including clinical examination, anthropometry, and bioelectrical impedance analysis (BIA); there was a need to evaluate their adequacy to answer the primary and secondary questions of the study.

Electronic delivery of questionnaires: The current study aimed to deliver the questionnaires electronically (i.e., through a laptop or desktop PC) in one or more participants simultaneously. For this purpose, we developed software that included the four questionnaires, and the pilot study provided an opportunity to pilot this software for the first time and in real-world conditions, albeit it had been extensively tested before with co-workers and others during the development phase, in a controlled environment.

The process of examining the feasibility of delivering the questionnaires electronically included assessing the reactions of the participants, the number of participants who would need help and to what extent, and the time needed to complete each questionnaire through this method. Furthermore, the technical aspects of the software required testing, including the addition of medications (through browsing and manually) and of date of birth (through the calendar and manually – and the automated calculation of age), and that the questions were properly individualised (based on previous answers) and allowed the appropriate number of fixed answers to be selected.

Saving and displaying the questionnaire results: Through the electronic delivery of questionnaires, we aimed to improve the experience for the participants but also to create the tools that would reduce the time and resources needed for ourselves to transfer data from paper to statistical software. The software was therefore programmed to produce, on request, an MS Excel file that included the results (one for each questionnaire) in a format that could be exported to statistical software after applying only minimal adjustments.

In regard to storage, when each questionnaire was saved, the data needed to be stored in a secure and safe manner. For this reason, when a participant saved a completed questionnaire, the data were not saved on the PC or laptop that the participant was using, but instead, the data were transferred through the internal network to the CEDM (CEDM Centre of Endocrinology, Diabetes & Metabolism) server in a password-protected file. Furthermore, participant names were not saved; only the number of the participant – numbers and matching names were available only in paper format, stored in a secure

place. Because the actual questionnaires were not saved this way, only the data, and because the data could not be edited after the questionnaire had been saved, it was of paramount importance to ensure that all the processes described above worked correctly – from data insertion and saving to data transformation and re-production in five MS Excel files.

Algorithms used in the calculation of the Mediterranean diet: The methods used for the calculation of adherence to the Mediterranean diet are described in detail elsewhere, including their origin, adaptions and the algorithm used for this purpose. In short, the data from the semi-structured food frequency questionnaire were used to calculate adherence to the 11 components that define the Mediterranean diet score; depending on the amount eaten, the foods were equally weighted – as there is no available information on servings, therefore, the portion described in the food frequency questionnaire was the one used as indicating one serving. For example, one medium tomato and one medium aubergine, of approximately 80g and 150g weight, respectively – as used in the FFQ – are equally weighted as one serving of vegetables for the calculation. Furthermore, servings of fruits, due to large variability in consumption – from empirical evidence – and due to significant seasonal variability, were not added up but rather an additional question was asked for the average consumption of fruits across the year. When the total consumption of each component was calculated, that is, the unweighted addition of foods comprising each food category or component, the components were then scored according to an *a priori* adapted Mediterranean diet scoring system. This scoring was conducted either through positive or negative monotonic, or polytonic functions and resulted in a score that ranged from zero to five for each component (and each participant). Finally, unweighted addition of the 11 components resulted in the calculation of a Mediterranean diet score that ranged from zero to 55 points for each participant. I decided that it was preferable for all these processes to be completed electronically through the custom-made software and specifically via the use of a basic algorithm (which I derived, based on the available literature). The output was a total Mediterranean score, the score for each component, and also the number of servings used for the calculation, to being electronically calculated and re-produced in a fifth Excel file. Through the pilot study, I aimed to assess the algorithm used, including the unweighted addition of foods for the calculation of Mediterranean diet components score, for the assessment of adherence to the

Mediterranean diet. Overall, I aimed to ensure that the whole process worked correctly, in a real-world research environment.

Coordination: For the successful completion of this project, a number of people were involved; firstly, the secretarial staff of the CDA, who had the responsibility for initial contact of their members, organising their written consent and providing the research team with their contact details; I was responsible for making the initial telephone contact with the patients – in addition to providing the secretarial staff with random numbers to contact; the secretarial staff of the CEDM who were responsible for booking appointments that required the presence of both the dietitian and the endocrinologist (most often in between clinical appointments); myself and (to lesser extent) the endocrinologist (DrAK) were responsible for providing the participants with information about the research, the signing of the consent form and the data collection process, with the help of the secretarial staff of the CEDM (and, in the main study, the additional assistance of a podiatrist). Finally, I had to ensure the availability of the IT consultant in case any major problems arose. Furthermore, for a more effective use of time, quite often two or three participants were recruited at the same time in a 'rotation' process, or they completed questionnaires simultaneously on different computers. Although, for a dedicated research team, all these duties may not sound like huge undertakings, for our small research team, with its limited resources and the need to run a (full-time) busy clinic, it was of paramount importance that all the research steps worked effectively and that there was excellent coordination, whilst any problems in regard to these processes were identified and solved during the pilot study.

Aims of the pilot study

The aim of the pilot study was to test the methods chosen to conduct a cross-sectional study that aimed to investigate the association between the Mediterranean diet and glycaemic control in patients diagnosed with Type 1 diabetes in Limassol, Cyprus.

Research questions

Participation rate

- Is it feasible to use the Cyprus Diabetic Association database to recruit patients diagnosed with Type 1 diabetes?
- How many patients need to be contacted in order to recruit 20 patients?

- Is the use of a free blood and urine test in a private laboratory an adequate incentive for the patients to participate?

Suitability of the questionnaires used and adequacy of data*

- Are the data sufficient (i.e., food frequency questionnaire) to calculate the adherence to the Mediterranean diet of the participants, using a Mediterranean diet score?
- Are the data satisfactory (i.e., blood tests) to investigate the diabetic control of the participants, including biochemical measures and the presence of complications?
- Are the data adequate (including the measurement of covariates) to investigate the relationship between Mediterranean diet and diabetic control?

* This requires that all steps – data collection, storage, processing and presentation – work correctly.

Methods

Study design

This is the pilot component of a study that eventually aimed to recruit patients diagnosed with Type 1 diabetes in Cyprus so as to investigate adherence to the Mediterranean diet, glycaemic control, as by measured by HbA1c and fasting glucose, and the association between following a Mediterranean diet and glycaemic control. The pilot study was designed to investigate the feasibility of the recruitment process and of the methodology. This study adopted a cross-sectional approach; the participants were recruited by means of a randomised procedure from the database of the CDA. The pilot study involved data collection by means of clinical examination, anthropometric and other measurements, and electronic-delivered questionnaires; and blood and urine tests in a clinical laboratory. These methods are described below – additional emphasis is given to the more practical aspects of the study which were relevant to the pilot study – and were simultaneously run until the desired sample was achieved or the aims and objectives of the pilot study were met.

Population characteristics

Type 1 diabetes: Patients with a diagnosis of Type 1 diabetes were selected as the study group, due to the fact that dietary intervention is crucial for the management of Type 1 diabetes, but also due to an absence of research in the relevant fields of this thesis.

Limassol, Cyprus: Limassol is a city located on the south coast of Cyprus; an island country in the eastern Mediterranean Basin. Limassol is the second most populous city in Cyprus, with an estimated population of 240,000 according to the 2011 official population census (Statistical Service of Republic of Cyprus, 2018) (note that it is likely that the population of Limassol has increased substantially since then due to inbound migration from other countries, such as the EU's eastern countries and Russia). The city of Limassol was preferred as the recruitment site for practical reasons; specifically, access to a database of patients with a diagnosis of Type 1 diabetes and the availability of sufficient resources to conduct the study.

Others: Other characteristics of the participants included the age (adults) and the absence (exclusion) of patients with a diagnosis of a terminal illness or other advanced health problems, and women that were pregnant (see *inclusion and exclusion criteria* for a detailed list).

Population recruitment methodology

Recruitment pool: Cyprus currently lacks a database of all cases of diabetes, including Type 1 diabetes. The most complete pool of diabetes patients, to our knowledge, is the database of the members of the CDA. This database has been used previously to estimate the incidence of Type 1 diabetes but has not been directly used to recruit patients for a study. Therefore, one of the challenges of the current study was to investigate the feasibility of recruiting from the CDA database. The challenges anticipated were primarily related to the recruitment rate – whether this recruitment method is adequate to support a larger study; and the accuracy of the database – to our knowledge, this database has rarely been updated, for example, we were expecting that it would include people who were no longer residing in Limassol, Cyprus. Furthermore, diabetes type misclassification was also expected to be prevalent, for example, we were told that patients with Type 2 diabetes had (wrongly) been classified as Type 1 diabetes, in the past.

Sample Size

To achieve the aims of the pilot study, it was determined that a maximum number of 20 patients was adequate.

Inclusion and exclusion criteria

The inclusion and exclusion criteria used for the pilot study were defined as follows:

Inclusion criteria: The prospective participants were defined as those who were: adults, between the ages of 17 and 70 years, had a diagnosis of Type 1 diabetes, currently residing in Limassol, Cyprus. Although only the data (collected) of the participants who had adequate information for analysis of the primary outcomes were to be included in the main study – that is, their Mediterranean diet score (derived from the FFQ) and glycaemic control (blood tests) – in the pilot study, all available data were processed in order to gather feedback and other valuable information.

Exclusion criteria: Patients were excluded if they were housebound, had a terminal illness diagnosis, such as cancer, with a poor prognosis defined as an expectant survival of less than six months, had a significant cognitive impairment and other related advanced stage conditions, or were pregnant.

Recruitment rate

Adequacy of recruitment pool: The sample of the current study was randomly sourced from the CDA (members) database. Through the recruitment rate, described below, I sought to obtain an estimate of the number of patients who had to be approached before achieving the number of participants that I aimed for.

Recruitment rate: Through the current pilot study, there was the intention to estimate the recruitment rate at different stages of the recruitment process, for example, the number of patients that the secretarial staff of the CDA had been able to contact (i.e., they had correct contact information); the number of patients who had given consent for their contact details to be provided to the research team; the number of patients who attended their appointment at CEDM, signed a consent form, and completed the data collection stage successfully; and, finally, the number of participants who had also attended the clinical laboratory and consequently completed the study successfully. The purpose of gathering these results was to provide valuable information on the feasibility of completing a larger study with a similar recruitment process.

Recruitment process acceptability: The recruitment process was complex, not only from the research team's point of view, but also for the potential participants. Therefore, the current pilot study was also intended to explore the acceptability of the discussed recruitment process – by recording any difficulties that the potential participants might have faced through this process as well as any suggestions they might have for improvement.

Recruitment process

Population sample and random sample selection: The CDA database was used as the sample pool for recruiting patients with a diagnosis of Type 1 diabetes, from Limassol, Cyprus. For the sample to be as representative as possible, random patients from the database were identified to be contacted. More specifically, I produced 20 random numbers through an online random number generator (RNG), available at https://www.random.org/. This process was repeated until the desired sample was achieved (i.e., 20 participants) or the aims and objectives of the pilot study were met (i.e., the data for the primary and other outcomes were collected without further changes to the process been required).

CDA database and consent to contact: The generated random numbers were provided to the secretarial staff of the CDA who were responsible for contacting the CDA's members (by phone), as defined by the random numbers. The research team had no access to the CDA database, and the prospective participants were required to provide a signed consent form to the CDA, in order for the research team to gain access to their contact information, that is, their name, surname and phone number(s). If the prospective participants did not sign the *consent to contact* form, either because they did not want to participate or the CDA was unable to contact them for whatever reason, then the CDA provided anonymised demographic characteristics; namely, age and gender.

Contact by research team and appointment arrangements: The CDA provided the research team with the contact details (on a daily basis) of the prospective participants who had signed the *consent to contact* form. I then contacted them (by phone), provided them with a short description of the study, and, if the patients were still interested, they were transferred to the secretarial staff of the CEDM who were responsible for booking the appointment (they also provided other practical information, such as directions etc.). The appointment time had to be both convenient for the prospective participant and also for the research team who were responsible for the data collection (me and DrAK). The secretarial staff were also responsible for sending a reminder text to the prospective participants the day before the appointment, arranging the PC or laptop that was to be used for data collection and making available all of the necessary paperwork, such as the *consent form*, needed for the study.

Consent form: At the appointment, the prospective participants were initially briefed (individually) about the study by a member of the research team (me), provided with the *consent form* (including the *information sheet*), and allowed time to read it and ask questions. If a prospective participant was still interested in participating, then all pages of the *consent form* (and the *information sheet*) were initialled and signed by the participant, as requested by the ethics committee in Cyprus. If a patient did not want to sign the *consent form* or did not wish to continue with the study for whatever reason, they were free to leave without any further questions asked.

Data Collection and other related issues

Data collection: The signing of the *consent form* was followed by the data collection. The data collection process was conducted at the *CEDM* by AK and DrAK. Data collection included questionnaires, anthropometrics and clinical examination.

The questionnaires were delivered by using the pre-designed survey loaded on a computer and included a demographic, a semi-structured food frequency, a medical and diabetes, and a physical activity questionnaire (as described above). The questionnaires could be self-administered or completed with the help of a member of the research team (usually me) depending on the IT abilities and the desire of the participant. If the questionnaires were self-administered, an initial brief explanation was provided, and a member of the team was available at all times to answer any questions and provide help when needed. When a participant completed all four questionnaires, we double-checked that all the relevant information was indeed saved.

The anthropometric data were collected by me and included weight, height and waist circumference. Furthermore, body composition was estimated by bioelectrical impedance analysis with information collected on body fat, lean mass and fluid composition. The clinical examination included the inspection for the presence or absence of lipohypertrophy (or lipoatrophy) at the injection sites and the measurement of blood pressure.

The entire data collection process was estimated to last for about 30 minutes. To confirm this estimation, in order to provide accurate information in the primary study, the time taken for the completion of the data collection was (manually) recorded for each participant – in addition, the time needed for each questionnaire to be completed was

(electronically) recorded by the software (i.e., from the time that the questionnaire was commenced to the time it was completed and saved).

Laboratory (blood and urine) tests: The participants were provided with a sealed sterile urine container after the questionnaire and anthropometric data collection. The laboratory (blood) tests were completed at a time convenient to the participant, within a few days or weeks. The private clinical laboratory where the tests took place was pre-specified and a business card that included the contact details and a map to the clinical laboratory was provided at the end of the data collection appointment. No appointment was required for the clinical laboratory test. Nevertheless, patients were asked to attend in the morning in a fasting state (at least ten hours without food) for the blood tests. Regarding the urine tests, the first urine of the day (on which they attended the clinical laboratory) was requested to be collected by the participants and provided to the clinical laboratory. The bloods tests were collected by an experienced consultant biochemist and no major problems were expected.

Results collection (by participants): The results of the anthropometric data, BIA and of clinical examination were reported to the participants verbally. These were to be provided in written form only if the participants had requested. The blood and urine test results were sent from the clinical laboratory to the CEDM by fax, usually within one to two working days. Once received, the secretarial staff were responsible for contacting the participant to inform them regarding the availability of the results and the participants could specify their method of preference for receiving the results, including by email, post or fax, or they could collect a hard copy of the results from the front office of the CEDM at any time, at their convenience. Although the reason for conducting each test was specified both in the *consent form* and the *information sheet* and explained verbally, no further information was provided regarding the actual results for each participant – particularly the blood and urine results. Rather, they were encouraged to attend the doctor who was responsible for their diabetes management to discuss the results; nevertheless, this decision was left to the discretion of the participants due to the complexity of the health care system in Cyprus. However, a ‘safety net’ was put in place for concerning results (defined in the protocol submitted for ethics – data not shown) where the participants were explicitly told, by a member of the research team (me), to visit their current doctor within days or weeks.

Participant feedback: The current study was a pilot with the main objective of providing the necessary information for and identifying the main challenges of conducting a larger well-designed study. Therefore, receiving feedback from the participants in the current pilot study was important to achieve this objective. Participants were encouraged to comment on anything they thought to be important, such as a difficulty in the process that they faced or their perceptions on anything they thought could be done differently. Furthermore, at the end of the data collection period, they were promptly encouraged to comment on the whole experience of the study and their experience in the clinical laboratory. These comments, in combination with the questions asked by the participants and difficulties that they may have faced during the study, etc., were recorded.

It is also worth noting that we conducted a patient and public involvement (PPI) exercise before the pilot study started, as requested by the ethics committee of the University of Stirling. Through this process we provided the questionnaire, protocol and other related information to the central committee of CDA in Limassol and asked them to comment. Of note, the central committee consists of people who have a diabetes diagnosis, the majority having Type 1 diabetes. The PPI process resulted in minor changes being made to the study materials, with mostly small additions to the questionnaire, including the suggestion to include in the *medical and diabetes* component a question regarding the sexual health of the participants and a question allowing participants to report how they perceived the cost of food for people with Type 1 diabetes as compared to people without Type 1 diabetes. Furthermore, other people, such as doctors and other health care professionals, including dietitians, and people diagnosed with Type 1 diabetes, also commented on the questionnaires before the pilot study, as part of the development stage, and this led to some mostly minor but useful changes and amendments.

Data storage, transformation and re-production

Storage: During the study we collected a large volume of data, mostly in an electronic format, that had to be stored both in a safe manner and in a convenient format for statistical analysis. The electronic data were stored on the server of the CEDM. This server is accessible only through the local area network, that is, the CEDM internal computers (connected through *remote desktop services*). The data were anonymised and saved in a password-protected file but this was not encrypted. The limited information available in hard copy format, which included the *consent forms* (and *information sheet*)

and a sheet listing the participants' allocated numbers and their corresponding names, was saved in a locked filing cabinet.

The data from the questionnaires were not saved at any time in the computers on which the participants were working, but rather, the software was programmed to save this data directly on the server. Similarly, the rest of the data collected were manually added to MS Excel files available on the server and remotely accessed through the computer of a member of the research team (me or DrAK). The blood and urine tests were saved on the local area network of the clinical laboratory (as per usual practice) and they were sent from the clinical laboratory to the CEDM by fax. The faxes received at the CEDM were automatically converted to pdf documents that were saved to the server (and not printed). The secretarial staff were responsible for checking the available faxes (PDF documents), informing the participants of their availability and providing them at their convenience, whilst I was responsible for scanning the results to identify any substantially abnormal results, as previously defined. Once the blood and urine tests were provided to the participants, the PDF documents were transferred to the secure password-protected file location on the server that contained the rest of the study documents.

Transformation and Presentation: The data had to be stored in a safe manner, as described above, but also in a manner that required the least possible processing (before exporting to statistical software). This meant that the data were coded, when possible, either automatically or manually, and saved in the same manner.

Data collection and other derived data

Feedback and adverse events

Feedback: The study used number of new measures (questionnaires), or adapted others, and computerised some of the methods. These methods of data collection are described below, but we emphasise that feedback from participants was an integral part of the pilot study (as previously described). Any type of comments and suggestions were welcomed and were recorded in a separate MS Word document, anonymised. These comments and suggestion were assessed through the pilot study and (if considered appropriate) the necessary adjustments were executed throughout (or at the end of) the pilot study.

Adverse events: The study was cross-sectional non-interventional study and therefore no major adverse events were expected. Nevertheless, adverse events could occur – not necessarily due to involvement in the study *per se* – when dealing with participants with

health problems such as our participants who were all receiving insulin treatment. Hypoglycaemia was the main (and solely) expected adverse event expected through the current study. Therefore, we planned to record the frequency of such events but, more importantly, to assess the practical aspects of the current management, if such an event occurred; and the reaction of the participants in regard to their willingness to complete the study (for mild hypoglycaemia).

Questionnaires (and questionnaire-software)

A large portion of the data collected at the current pilot study was gathered through questionnaires delivered through a computer using self-developed software for the main study. Through the current pilot study we aimed to test these methods; both the practical aspect (that the software at all levels – from presentation to export – worked well), and also the reaction of the participants to the mode of delivery and the content of the questionnaires; and, finally, the feasibility and convenience of the methods for the research team.

Delivery mode (presentation to participants): The questionnaires were delivered electronically. The versions of the questionnaires used in the pilot study are not presented here, but the final versions used in the main study are available in *Appendix: Chapter 5* and the appropriate sections are included in the description of each questionnaire.

Storage mode: Once the questionnaires were completed and saved, the data were transferred (automatically) – in an appropriate format for convenient presentation – to the CEDM server through the internal network. No data were saved at any point on the laptop or desktop used for the completion of data collection or transfer. The data were saved in a password-protected file, in an anonymised format, on the server, although the data were not encrypted.

Demographic questionnaire

The demographic questionnaire was designed to collect data on the demographic characteristics of the participants, such as age, gender, nationality and socioeconomic status (*Appendix: Chapter 5: Questionnaires: Demographic characteristics questionnaire*). It is loosely based on various questionnaires available in the literature while taking into account the characteristics of the population of interest (people with a diagnosis of Type 1 diabetes residing in Cyprus).

The first version of the demographic questionnaire consisted of 11 questions. The first was the age of the participant – the participant could find the date of birth through a drop-down calendar or write it down manually; when the date of birth was selected by the participant, their age (that is the date of birth minus date that the questionnaire is saved) appeared in a box next to the date of birth. When the questionnaire was saved, only the age was saved on the server; this minimised the risk of identifying a participant whilst it resulted in the gathering of more meaningful data for the research team that needed less processing before being transferred to the statistical software. The next three questions related to the gender, ethnicity and nationality of the participants – with all having two available possible answers. For nationality and ethnicity, the options were limited, as the numbers of non-Cypriot (and non-White Caucasians, respectively) expected to participate was small; if that was not the case in the pilot study, the options were to be increased. The remaining questions referred to the socio-economic status of the participants, including marital status, co-habitants, number of children, highest education level and occupation. The number of children was the only question that was not a close-ended question, but the participant had to add a number with the default answer being zero. Finally, the last question about occupation consisted of two close-ended questions with the second one appearing if the participant selected an answer other than *unemployed* in the first one.

Food Frequency Questionnaire

The food questionnaire was a semi-structured FFQ (with minimal extra questions) evaluating mainly food intake and cooking methods (*Appendix: Chapter 5: Questionnaires: Food frequency questionnaire*). The foods used to construct the FFQ were drawn from a previously validated FFQ in the Greek population (Katsouyanni, 1997), as described above. Despite many similarities between the two countries, some of the names of the foods had to be adapted and some foods were removed completely, whereas others needed to be added.

The first page of the FFQ element included the instructions, a further 11 pages were devoted to foods and food categories, and the last page was about cooking methods plus one close-ended question. The 196 foods included in the FFQ were broadly categorised into 15 food or drink categories; namely: starchy foods; nuts and dried fruits; ready meals and fast foods; potatoes; bakery foods; vegetables and salads; fruits; legumes, peas and ladies' fingers; fish and fish products; meat, egg and related products; milk and dairy products; soups; sugar and sugary foods; alcoholic beverages; non-alcoholic beverages;

and fats and oils. The foods were also accompanied by a typical food portion size for each food, expressed (most of the time) in both a household measure and in grams – for foods that are eaten cooked, the cooked portion was mentioned. The participants were asked to report the number of servings that they consumed for each food; in practice, the participants were asked to add a number next to each food in the column for daily or weekly or monthly, indicating the number of servings that they consumed. Furthermore, the participants were instructed to either leave blank or add an *X* in the rarely/never column, for foods, that they consumed less than once a month. For example, if a participant consumed two pieces of white bread three times a week, they were instructed to add the number *6* in the weekly column for the *white bread – 1 medium slice (30g)* item. Also included was a bracket next those foods mostly eaten seasonally in Cyprus (this applies mostly to fruits) and participants were asked to report how many they ate when they were seasonally available. All of these instructions were available on the first (introductory) page but we also explained these, verbally, to the participants. The (exported) results presented in the MS Excel document displayed the participant number, the question number (food number), the value added by the participant, the frequency reported (zero indicating that this was left blank, and one to four to indicate daily, weekly, monthly, and rarely/never options, respectively), the total amount consumed (automatically calculated) and the date of completion. The total amount consumed was automatically calculated (by the software) and presented the portions consumed monthly; in practice, the amount in the daily field was multiplied by 30, for example, if a participant reported consuming two portion of white bread each day, that would result in a value of 60 (2 x 30) for the total amount; the amount reported for weekly was multiplied by four; if a monthly consumption was reported then it was multiplied by one; and, finally, the rare/never was multiplied by zero (resulting always in zero), and fields left blank were also reported as zero. An exception to the above rule of frequency categories was for the oils and fat category, where there was a need to distinguish between never and rarely – for the use of olive oil in the Mediterranean diet scoring system.

The final page of the FFQ element of the questionnaire included a closed-ended question and questions about their cooking methods. The participants were asked to report (in the same method as before) how often they used a cooking method. In regard to the single question, the participants were asked to report whether they thought that food cost is higher for people with a diagnosis of Type 1 diabetes, with available answers being *Yes*,

No and *I don't know*. The question regarding food cost was added after suggestion by the members of the CDA during the PPI process. The results of the question were coded and reported in the usual manner in the resulting MS Excel file.

Mediterranean diet

In the current study, an adaptation of the *a priori* Mediterranean diet scoring system *MedDietScore* (Panagiotakos *et al.*, 2007) was used, which is an updated and validated (in the Greek population) version – mainly in regard to the servings – of the previously published *MedDietScore* (Panagiotakos, Milias, *et al.*, 2006; Panagiotakos, Pitsavos and Stefanadis, 2006). The FFQ data were used to feed the algorithm used by the software in order produce a score – for each participant – for each component of *MedDietScore*, and, consequently a total score for the *MedDietScore* for each participant. This process has been computerised and is described below, with the benefit of considerably reducing the data processing time and the possibility of a human error; nevertheless, for the purpose of this pilot study, a manual calculation was also completed so as to compare the results with the computerised ones and to make any necessary amendments to the process, algorithm, etc.

MedDietScore (components)

The adherence to the Mediterranean diet is measured using the newest version of the MedDietScore scoring system, *MedDietScore* (Panagiotakos *et al.*, 2007); *Table 4.1*. The *MedDietScore* consists of 11 components or food groups; namely, non-refined cereals, potatoes, fruits, vegetables, legumes, fish, red meat and products, poultry, full fat dairy products, olive oil, and alcoholic beverages, that resemble the Mediterranean diet pyramid. The individual components or food groups – for each participant – are scored, from zero to five, according to the servings of the particular food group reported as being usual consumption, as shown in *Table 4.1*. For example, if a participant reports a usual consumption of 5 servings of non-refined cereals, then this falls within the category of one to six servings and is scored as one point. The *MedDietScore* has components that have a positive monotonic function – as the servings consumed increases the resulting score for the component increases – namely, non-refined cereals, potatoes, fruits, vegetables, legumes, fish, and olive oil; these are considered to be desirable foods in the traditional Mediterranean diet. In contrast, the food groups that are considered undesirable in the traditional Mediterranean diet are scored using a negative monotonic

function – as the servings consumed increases, the score decreases – namely, red meat and products, poultry, and full-fat dairy products. For alcohol, a polytonic function is used, reflecting the regular consumption of alcohol in the traditional Mediterranean diet – mainly accompanying the lunch – but mostly in small amounts. The *MedDietScor*e scoring system was adapted – minor changes – to correct some ambiguous values; *Table 4.2*. Based on this adapted scoring system, an algorithm was produced (presented in *Appendix: Chapter 4*) so that the software could automatically calculate the *MedDietScore* for each component, and then the total *MedDietScore* for each participant.

Table 4.1: MedDietScore scoring system

Servings*/week / Food category	Points					
	0	1	2	3	4	5
Non-refined cereals (whole grain bread, pasta, rice, etc.)	Never	1 – 6	7 – 12	13 – 18	19 – 31	> 32
Potatoes	Never	1 – 4	5 – 8	9 – 12	13 – 18	> 18
Fruits	Never	1 – 4	5 – 8	9 – 15	16 – 21	> 22
Vegetables	Never	1 – 6	7 – 12	13 – 20	21 – 32	> 33
Legumes	Never	< 1	1 – 2	3 – 4	5 – 6	> 6
Fish	Never	< 1	1 – 2	3 – 4	5 – 6	> 6
Red meat and products	> 10	8 – 10	6 – 7	4 – 5	2 – 3	≤ 1
Poultry	> 10	9 – 10	7 – 8	5 – 6	4 – 5	≤ 3
Full fat dairy products (cheese, yoghurt, milk)	> 30	29 – 30	21 – 28	16 – 20	11 – 15	≤ 10
Use of olive oil in cooking (times/week)	Never	Rare	< 1	1 – 3	3 – 5	Daily
Alcoholic beverages (ml/day, 100 ml = 12 g ethanol)	0 or > 700	600	500	400	300	< 300

*Portions not specified apart from alcoholic beverages, therefore alcohol presented in portion size consumed.

Table 4.2: Adapted MedDietScore scoring system

Servings*/week / Food category	Points					
	0	1	2	3	4	5
Non-refined cereals (whole grain bread, pasta, rice, etc.)	0	1 – 6	7 – 12	13 – 18	19 – 31	> 32
Potatoes	**0**	1 – 4	5 – 8	9 – 12	13 – 18	> 18
Fruits	**0**	1 – 4	5 – 8	9 – 15	16 – 21	> 22
Vegetables	**0**	1 – 6	7 – 12	13 – 20	21 – 32	> 33
Legumes	**0**	< 1	1 – 2	3 – 4	5 – 6	> 6
Fish	**0**	< 1	1 – 2	3 – 4	5 – 6	> 6
Red meat and products	> 10	8 – 10	6 – 7	4 – 5	2 – 3	≤ 1
Poultry	> 10	9 – 10	7 – 8	5 – 6	4	≤ 3
Full fat dairy products (cheese, yoghurt, milk)	> 30	29 – 30	21 – 28	16 – 20	11 – 15	≤ 10
Use of olive oil in cooking (times/week)	Never	Rare	< 1	1 – 3	4 – 6	≥ 7
Alcoholic beverages (ml/day, 100 ml = 12 g ethanol)	> 700 or 0	600 – 700	500 – 599	400 – 499	300 – 399	< 300

*Portions not specified apart from alcoholic beverages, therefore alcohol presented in portion size consumed.; Red colour indicates an amendment

Note: The term *portion* (or *portion size*) and *servings* are often used interchangeably in the available literature; in contrast, in the current report these terms had strict definitions. Through the term *portion* we meant the actual quantity of a food; for example, 1 apple portion was equal to 110 g of apple or 1 medium apple (as defined at the FFQ). In contrast, by using the term *serving* we referred to the number of portions; for example, 5 servings of apple implied 5 apple portions or 5 x 110 g of apple or 5 medium apples. Therefore, we had modified the *MedDietScore* to reflect the terminology that we used in the current report.

MedDietScore (total)

The total *MedDietScore* score is calculated by adding the score of each component – for each participant – in an unweighted manner; as follows, for the i^{th} participant

Total *MeDietScore* score_i (range, 0, 55) = *non-refined cereals* score_i (range, 0, 5) + *potatoes* score_i (range, 0, 5) + *fruits* score_i (range, 0, 5) + *vegetables* score_i (range, 0, 5) + *legumes* score_i (range, 0, 5) + *fish* score_i (range, 0, 5) + *red meat and products* score_i (range, 0, 5) + *poultry* score_i (range, 0, 5) + *full fat dairy products* score_i (range, 0, 5) + *olive oil* score_i (range, 0, 5) + *alcoholic beverages* score_i (range, 0, 5)

Portion sizes of food groups

The *MedDietScore* score was calculated by scoring the servings reported (consumed) by the participants, as explained previously. The portion size, of each serving, was based on the portions used in the FFQ. During the construction of the algorithms, I was faced with a few difficulties and these are discussed below, along with the methods/solutions that were used to overcome them.

Quantifying portion sizes: Currently, there is no international or regional (e.g., within the EU) or other widely accepted definition of a portion size (Kirwan *et al.*, 2016). While there are suggested portion sizes by organisations, such as by WHO, for the Eastern Mediterranean region (World Health Organization, 2012), and by countries, such as the Ministry of Health in Greece (Ministry of Health and Welfare, 1999), to my knowledge, no such guidelines exist for Cyprus. Furthermore, those available are often too imprecise for research purposes, for example, 2 – 3 cups, 120 – 200 g, one medium etc., and are incomplete, that is to say, not all foods and food groups are covered. Additionally, in the published papers of research applying the *MedDietScore* scoring method (Panagiotakos, Milias, *et al.*, 2006; Panagiotakos, Pitsavos and Stefanadis, 2006) – although they report a scoring method using servings that is the number (consumed) of a specific quantity defined as a portion of a food – these portion sizes are not defined. Similarly, the published research for the FFQ (Katsouyanni, 1997) reports solely the foods and not their portion sizes. This is also reflected in the published consensuses on the Mediterranean diet (pyramid), which, while it recommends the consumption of an amount of servings for each foods group, goes on to say that portion sizes should be individualised depending on local habits and socioeconomic factors (for the Mediterranean diet pyramid and the corresponding recommended servings, see *Chapter 1*).

For these reasons we decided to use the portions as they were defined in the FFQ (to constitute a serving) and these were based on the available published research, guidelines and public health advice; as well on the clinical experience and acumen of the research team as a whole.

Presentation of portion sizes: The presentation (or description) of portion sizes – for easier conceptualisation by the participants – can be accomplished through written or/and pictorial methods (Lee and Nieman, 2013). The pictorial method, although it would have been useful, was not utilised. The first reason for not doing so was that it would have been extremely time-consuming (and costly) to prepare the 200 foods (often complex foods), photograph them at appropriate portions, incorporate them in the questionnaire and then conduct additional testing of the software. A second reason was that we were struggling to incorporate the photos in the electronic questionnaire in such a way that completion would not become more difficult and time-consuming. Consequently, the description of the portion sizes was provided in written format, in two different measures – that is, in grams and in common household measures, such as cups and teaspoons (for example, *sugar: 1 teaspoon – 5 g*) or everyday measures (for example, *white bread: 1 medium slice – 30 g*).

Measuring the food groups directly versus indirectly: The consumption of food groups can be assessed directly, for example *how many dairy products do you consume?* and indirectly by adding up the reported consumption of milk, cheese, yoghurt and perhaps other complex foods that contain significant amounts of dairy products. While this is easier with dairy products, it becomes more complicated for other food groups, such as fruits, due to their large variety in Cyprus but also due to their considerable seasonality in their consumption. In contrast, if participants are to ask about food groups in general, for example, *how many dairy products do you consume?* they are more likely to forget about specific foods, for example, feta cheese, which they may add to their salad. Therefore, it was decided that, for food groups that have significant seasonality or inadequate data through the existing FFQ, a question would be used that would address the food group directly, while, for the rest of the food groups, simple unweighted additive algorithms would be used. More precisely, we preferred a direct measurement (that is, directly asking about the food group) for fruits due to their large seasonality (and, to a lesser extent, larger variety), and for fish, given that the FFQ may not adequately cover the variety of fish available in the market.

Definition of servings (or food categories): Another consideration when measuring foods indirectly is which foods constitute a food group. Often there are hundreds of foods that are available that may constitute a category, for example the vegetable category. Obviously, although not all foods that are available could be covered by a FFQ, we strongly believe that the foods that are more often consumed in Cyprus were covered by the FFQ that we developed and used. Nevertheless, during the questionnaire development, the PPI and the current pilot study, participants were explicitly asked to report any foods that they often consumed and that were not included in the FFQ. Furthermore, through the data analysis in this pilot study, especially through the data analysis of foods of the FFQ, we tried to identify any foods that are consumed in significant amounts, especially complex foods, that needed to be included in a food group. Therefore, for the construction of food groups and the relevant algorithms, we used the foods that were available in the FFQ that we considered more relevant. In regard to the more complex foods, such foods were counted (in the total food group) if we considered that they contained adequate amounts of that food group and at the same time are consumed often enough and in adequate amounts (in the average population) to affect the relevant food group. This helped to keep the algorithm from becoming even larger and more complex with all of the related consequences.

Algorithm

The algorithm was sub-divided into four parts, namely; *standardisation of servings (of individual foods)*, *total number of servings of (individual) MedDietScore components*, *scoring of servings*, and *total MedDietScore score*, which are presented in *Appendix: Chapter 4*.

Software

The software was programmed to run the above algorithm so that the participants added the data and the research team obtained the results (in the form of an export file in MS Excel format), namely, the total servings for each participant and component, the score for each participant and component, and the total *MedDietScore* for each participant. Although, overall, this was a worthwhile undertaking to save time and resources during data processing and analysis, nevertheless it was, in practice, a complex process which required close cooperation between the members of the research team (led by me) and the IT personnel. Verbal mathematical advice was also sought by NE.

Medical and diabetes questionnaire

The *medical and diabetes* questionnaire was used to collect data on the epidemiological characteristics, the use of insulin and other medications and the clinical history, including the presence of micro- and macro-vascular complications and other co-morbidities (*Appendix: Chapter 5: Questionnaires: Medical and diabetes questionnaire*). The questionnaire was not based on another (specific) questionnaire, but it was constructed after discussion between the members of the research team (drafted by me after discussion and advice from DrAK; and further reviewed by DrJE and DrAK). The construction of a questionnaire from scratch was compelled by the uniqueness of the health system in Cyprus – although, whenever possible, questions were based on international guidelines, while clinical experience was also taken into account. In more detail, the questionnaire initially explored participants' diabetes diagnosis and family history of diabetes; this was followed by questions on insulin delivery methods and devices, and insulin type and dosage; the next dozen questions explored diabetes management and lifestyle choices such as smoking; the next section related to the (possible) presence of complications, including sexual health complications in men and women; finally, the questionnaire asked about the presence of co-morbidities or other diseases for which people with Type 1 diabetes are at increased risk, while (free-text) space was available for manual entry of additional clinical history and medication history.

The *medical and diabetes* questionnaire had a mixed, more complex structure. More specifically, it included closed-ended questions with one, two or more possible answers, with the software allowing only the correct number of answers to be chosen by the participants; and also filter questions. Therefore, this pilot study was needed to confirm that the participants could understand and complete the questionnaire (without major problems); and for the research team, that the results were saved and presented correctly and in a manner that required only limited processing before exporting to statistical software.

Physical activity questionnaire (IPAQ)

The physical activity questionnaire used was the short version of the *IPAQ* questionnaire (Craig *et al.*, 2003) and, more specifically, the Greek language version of the *IPAQ*, which was validated in a Greek population (Papathanasiou *et al.*, 2009). To our knowledge, no such questionnaire has ever been validated in the Cypriot population. Only the

participants' insertions were saved, and the results were presented in an MS Excel file, in a similar way as the other questionnaires. The results were analysed according to the *IPAQ* guidelines for data processing (IPAQ, 2005), which are not presented here. The calculations were not converted to algorithms and computerised, but instead, were executed manually due to time and resource constraints.

Anthropometric data

The anthropometric data were collected by me; the anthropometric measurements performed were the participant's weight, height and waist circumference. BMI was calculated from the weight and height measurements. The collected data were recorded, as continuous data, in a pre-designed Ms Excel document; data were anonymised (coded) and saved in the *CEDM* server. The methods applied for each measurement are described below.

Weight: The participants' weight was measured using an electronic weighing scale (SECA 703; Hamburg, Germany). The participants were weighed in light indoor clothing, without shoes, to the nearest 0.1 Kg.

Height: The participants' stature was measured using a stadiometer (SECA 217; Hamburg, Germany). Each participant's height was measured without shoes, in light indoor clothing; and standing with their head placed on a Frankfurt plane, shoulder blades, buttocks and heels in contact with the vertical surface of the stadiometer (but not necessarily the head), feet together, knees straight and arms at the side (Gibson, 2005; Lee and Nieman, 2013). The height measurement was recorded to the nearest 0.001 m (0.1 cm).

Waist circumference: The waist circumference was measured using a measuring tape (SECA 201; Hamburg, Germany) at the natural waist; that is, the mid-distance between the tenth rib and the ileac crest. The two points were located and marked with a washable marker, and then the mid-point was also located and marked on each side. Then, the measuring tape was passed through these two points, without the tape compressing the skin and parallel to the ground; the participants were asked to breathe normally during the measurement (Akram *et al.*, 2000; Gibson, 2005). The waist circumference measurement was recorded to the nearest 0.1 cm. The waist circumference is a good indicator of visceral adiposity and of health and disease (including risk of CVD, mortality and morbidity) and is recommended by different clinical guidelines. It is overall

considered a more practical, a more clinically meaningful and a less invasive measurement than the waist-to-hip ratio measurement (Akram *et al.*, 2000; Dobbelsteyn *et al.*, 2001).

Body Mass Index (BMI): The BMI is calculated by dividing the weight (kg) by the square of the height (m^2). The BMI was automatically calculated in the MS Excel file by pre-specifying the equation, after the weight and height was added for each participant.

Bioelectrical Impendence Analysis (BIA)

The BIA measurement was conducted by me. This measurement provides an estimation of the body composition of the subject.

The BIA essentially measures the impedance to current flow through the body – based on the principle that different body components, that is, fat and lean mass, have different conductivity (lean tissue, which is rich in water and electrolytes, has minimal impedance, whereas fat mass acts as a capacitor thus increasing the impedance). The measurement of impedance is then used to estimate the total body water and, consequently, the values of interest through equations based on parameters such as gender, age and ethnicity (Dehghan and Merchant, 2008; Preedy, 2012; Lee and Nieman, 2013).

In practice, the participants were asked to lie flat on their back on the examination bed (a non-conductive surface) and to remain still, with legs flat and apart (from each other), hands apart (from the body) and palms flat away from the body. Then, two electrode pads (jewell disposable electrode pads; Rheine, Germany) were placed on the right foot and two on the right hand, as per the manufacturer's instructions (Maltron International, 1999). The measurement was conducted using a single frequency (50 KHz) hand-to-food BIA device (Maltron BF-907; Essex, UK). The results recorded were the estimated body fat and lean mass, and water (measured in % and Kg or L), suggested targets (in %) and the resting metabolic rate (RMR); these results are recorded as continuous data, in a pre-designed Ms Excel document, and saved in an anonymised (coded) manner on the CEDM server.

Finally, several factors have previously been reported to affect the results, such as the hydration status, medications such as diuretics, the point in the menstrual cycle, exercise (over the last 12 h), the consumption of alcohol and caffeine-containing drinks (over the last 12 h), and the time of last meal and urination (Maltron International, 1999; Dehghan

and Merchant, 2008; Preedy, 2012). For practical reasons we did not control for all these factors; the extent to which these factors have affected our results is difficult to determine.

Clinical examination

The clinical examination was limited to the measurement of blood pressure and the inspection of injection sites, for the presence of lipohypertrophy and lipoatrophy. The clinical examination was conducted by DrAK, a Consultant in Diabetes and Endocrinology. The results were stored in a pre-designed Ms Excel document and saved in an anonymised (coded) manner on the CEDM server.

Blood pressure: The blood pressure measurement was taken while the participant was in a sitting position; with the arm uncovered and supported at the level of the heart. The blood pressure was measured using a validated manual sphygmomanometer (Riester big ben; Jungingen, Germany) and a stethoscope. Two measurements were taken (within a few minutes from each other) and the average systolic and diastolic blood pressure were recorded, as continuous data, to the nearest mmHg.

Presence of lipohypertrophy in the injection sites: The injection sites were observed and palpated for the presence of lipohypertrophy and lipoatrophy. Then, the injection sites and the presence or absence of lipohypertrophy and lipoatrophy were recorded, as categorical and coded data.

Blood and urine tests

The venous blood sample was drawn in a pre-specified private clinical laboratory by an experienced consultant biochemist. The participants were asked to visit the clinical laboratory during the morning hours in a fasting state; regarding the urine sample, they were asked to fill the sealed sterilised container, which was provided during the appointment at the CEDM, with the first void of the day, after waking.

The laboratory tests and other estimated measurements directly calculated from these data included the following (unless otherwise stated, the laboratory measurements are blood tests):

- glycaemic control: glucose, glycated haemoglobin (HbA1c) and estimated glucose disposal rate (eGDR)
- β-cell function: C-peptide

- lipid profile: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (Tg)
- thyroid function tests (TFTs): thyroid stimulating hormone (TSH) and free T4 (fT4)
- liver function tests (LFTs): alanine transaminase (ALT) and aspartate transaminase (AST)
- renal function: urea, creatinine, albumin (*urine*), creatinine (*urine*), estimated glomerular filtration rate (eGFR) and albumin to creatinine ratio (ACR; *urine*)
- inflammatory markers: C-reactive protein (CRP)

The fT4, TSH, and the C-peptide tests were measured using ECL (Electrochemiluminescence; Roche Cobas e 411 analyzer; Rotkreuz, Switzerland; Roche Diagnostics, 2009), the glucose, TC, LDL-C, HDL-C, Tg, urea, albumin, creatinine, ALT and AST using absorbance photometry (Cobas Integra® 400 plus; Rotkreuz, Switzerland); and the HbA1c and the CRP using turbidimetry (Cobas Integra® 400 plus; Rotkreuz, Switzerland; Roche Diagnostics, 2009b). eGDR and eGFR were calculated based on the available equations in the literature (that were converted to executable commands in *STATA* to calculate and produce the new variables – commands not shown, but available on request as a *STATA do-file*). The estimation equations for eGDR (Williams *et al.*, 2000; Epstein *et al.*, 2013; Nyström *et al.*, 2017) and eGFR (Levey *et al.*, 2009) are as follows:

$$eGDR = 21.158 - (0.09 * wc) - (3.407 * [if\ hypertensive]) - (0.551 * HbA1c)$$

where: eGDR (mg / kg / min) is a validated tool for estimating insulin sensitivity in patients with Type 1 diabetes; wc = waist circumference (cm); hypertension was defined as systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg or the use of antihypertensive medications, and if hypertension was present then *[if hypertensive]* = 1 and if hypertension was absent then *[if hypertensive]* = 0; HbA1c = glycated haemoglobin (%)

For the calculation of eGFR (mL / min / 1.73 m^2) the CKD-EPI equation was used as recommended (Levey *et al.*, 2009; Levey and Stevens, 2010; Florkowski and Chew-Harris, 2011; NICE, 2014b).

$$eGFR = 141 * min(Scr/\kappa, 1)^{\alpha} * max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018\ [if\ female] * 1.159\ [if\ black]$$

where: Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1; if sex is female then *[if female]* = 1, otherwise *[if female]* = 0; if race is black then *[if black]* = 1, otherwise *[if black]* = 0

Data analysis and statistics

Data Processing: The data were partially processed using the self-developed software, then further processed manually (e.g., coding of string data) by the research team (mainly by me), when appropriate, before being entered into the statistical software.

Presentation: The data were not checked for normality due to the small number of participants and given that it was unlikely to offer any additional information for the purpose of the pilot study. Instead, the data were presented in mean values, standard deviation (sd), and minimum (min) and maximum (max) values. Furthermore, continuous data were presented both as continuous and categorical data; in addition, when appropriate, the number of participants who were included in the analysis of the FFQ data, and the number of participants who reported consuming a specific food, were reported.

Participants: All data gathered were considered as being valuable (for the purpose of the pilot study) and therefore we included the data from all participants who had completed the study, whether partially or in full.

Statistical analysis, tests and software: The sole statistical analysis that was pre-planned for the pilot study was the association between the Mediterranean diet, measured through the *MedDietScore* and glycaemic control. In a post-hoc analysis we also compared the *MedDietScore* and the BMI of the participants; and the adherence to the Mediterranean diet by gender. The non-parametric tests of Spearman-rank correlation and Wilcoxon rank-sum test were used for statistical comparisons for continuous and categorical data, respectively. The statistical tests *per se* and reasoning of use are explained, in detail, in the main study (*Chapter 5: Methods: Statistics*). The statistical analysis was performed using STATA 11.2 (StataCorp LP, College station, TX, USA).

Ethics and practical safety consideration

Ethics: The pilot study received ethics approval from committees in both Cyprus and the UK. In Cyprus, ethics approval was granted by the *Committee for Review Bioethics*

*Biomedical Research on Human Beings and Their Biological Substance*s of The Cyprus National Bioethics Committee of The Republic of Cyprus (ΕΕΒΚ/ΕΠ/2016/09). Similarly, in the UK, ethical approval was given by the *School of Health Sciences Ethics Committee* of the University of Stirling, UK (SREC 14/15 – Paper No.26 – Version 2).

Although the pilot study did not involve any intervention, we considered precautions to avoid any adverse events, and procedures to deal with adverse events.

Hypoglycaemia: Hypoglycaemia is an adverse effect of diabetes treatment – caused by the use of insulin, the solely pharmacological treatment of all people with a diagnosis of Type 1 diabetes. Hypoglycaemia is not an unusual occurrence in people who use insulin as a treatment, including people with Type 1 diabetes, either in everyday life or during clinical research (Elliott *et al.*, 2016). For this reason, hypoglycaemia was considered a potential adverse event during the current study and all necessary precautions were put in place to tackle it. Note that hypoglycaemia occurrence is unrelated to the methods used during the current study and, therefore, no increased risk of experiencing a hypoglycaemic episode was expected. Firstly, blood glucose meters were available in different areas of the CEDM in order to check for hypoglycaemia if need be. Hypoglycaemia was defined as blood glucose levels of below 70 mg/dl (3.9 mmol/l) based on the ADA (American Diabetes Association, 2019d) guidelines and the presence of hypoglycaemia indicated that action should be taken. The action was based on the severity of the hypoglycaemia experienced; if non-severe hypoglycaemia was experienced (which meant that the participant could self-manage the hypoglycaemia), patients were offered fruit juice (250 ml cartons were available at CEDM that contain 22.5 g of simple carbohydrates) or four to five glucose tables to chew (Glucotabs® are used, which contain 4 g of simple carbohydrates per tablet). The participant was then left to rest for 5 – 10 minutes, and thereafter the blood glucose was re-measured. If the blood glucose levels returned to a normoglycaemic level, the participant was offered 20 g of a slow-absorbed carbohydrate-containing food (biscuits were available that contained 4 g of carbohydrates in each biscuit). Where blood glucose levels remained within the hypoglycaemic range, the process for correction of hypoglycaemia was to be repeated, that is, the rapid-absorbed carbohydrate was provided to the participant, until the blood glucose levels returned to normal. In the less likely case of severe hypoglycaemia, that is, when a patient is unable to treat the hypoglycaemia without third-party assistance, injectable glucagon was readily available to be administered (the GlucaGen® Hypokit,

which contains 1 mg of intramuscular injectable glucagon hydrochloride) – this was to be injected by the endocrinologist (DrAK) or the dietitian (me) if required; followed by 20 g of rapid-absorbed carbohydrates and thereafter 40 g of slower-acting carbohydrates. In the case of non-severe hypoglycaemia, the patient was asked whether s/he would like to continue with the study or, otherwise, discontinue, while if severe hypoglycaemia occurred, then the participant was to be automatically withdrawn from the study, as a safety precaution. Furthermore, patients who experienced a hypoglycaemic episode and were required to drive after completing the tests were encouraged to re-check their blood glucose levels and to confirm that they were in a non-hypoglycaemic range (ideally blood glucose levels above 90 mg/dl [5.0 mmol/l]) before driving.

These measures were no different to the measures that are used in our everyday clinical practice for patients who are insulin-treated and experience a hypoglycaemic episode. Furthermore, given that hypoglycaemia is not an uncommon occurrence in this insulin-treated population, patients themselves are invariably well-aware of how to recognise and treat hypoglycaemia independently and quite often they carry carbohydrate-containing drinks and foods for that particular purpose.

Bioelectrical impedance analysis: The estimation of the body composition was achieved through the bioelectrical impedance analysis technique; that is, the measurement of impedance of a current passed through electrodes attached to the foot and hand of an individual (Gibson, 2005). The manual for the device (Maltron International, 1999) used and available guidelines (Kyle *et al.*, 2004) state that this test should be avoided in people with artificial pacemakers or other cardiac implanted devices and this is based on the theoretical possibility that the induced field of current (from the BIA device) may alter the potential electromagnetic interferences of these devices (Kyle *et al.*, 2004). Despite the current advice from BIA device manufacturers and relevant guidelines, the available research on the topic disputes this advice and indicates that BIA can be safely applied with patients with artificial pacemakers or other cardiac implanted devices (Meyer *et al.*, 2017; Pinto *et al.*, 2017), in addition to the fact that there are no reported adverse events with such devices in the literature (Kyle *et al.*, 2004). Nevertheless, participants were asked before the procedure for the presence of such devices and, if present *in situ*, they were excluded as a precaution.

Results

Participation rate

Recruitment: The CDA was given 20 random numbers (by me) to select the corresponding patients from the database of the CDA. These 20 potential participants are described in *Table 4.3*. Of these 20 patients contacted by the CDA secretarial staff, one refused to participate (*Patient 11*). This patient was a female in her twenties who was 'not interested'. The names and telephone numbers of the remaining 19 were provided to me. These were patients who were registered with the CDA as having Type 1 diabetes and residing permanently in Limassol.

I contacted the 19 patients and invited them to participate in the current study. Two of the patients were actually university students studying in Nicosia, Cyprus and London, UK (*Patients 8* and *19*). Similarly, one patient was unlikely to have had Type 1 diabetes and hence was not eligible for the study (*Patient 9*). More precisely, the patient mentioned on the phone to me that he had 'necrotic pancreatitis after an accident' and then had to take insulin and consequently had 'Type 1 diabetes'. Although the clinical management of necrotic pancreatitis may be similar to that of Type 1 diabetes, this patient did not meet the inclusion criteria of autoimmune Type 1 diabetes. However, the most likely diagnosis was that of secondary diabetes due to necrotic pancreatitis, which inflicted destruction of the β-cells and hence the need for insulin. Of the remaining 16 patients, 12 of them agreed to arrange an appointment time. Two refused participation as they were 'too busy with work' (*Patient 16*) or 'not interested' (*Patient 4*). One patient 'was too busy but maybe could have made it in the following week' (*Patient 2*) and was not re-contacted, and, for one patient, we were in fact given the father's telephone number, who refused to provide us with his daughter's number and he was supposed to let his daughter know about the study (*Patient 14*). Of the 12 remaining patients, one, during our initial chat (during the initial appointment), mentioned that he was diagnosed with diabetes about two to three years ago, and was initially on insulin but then switched to oral medications only (*Patient 17*). We therefore suspected that this patient had Type 2 diabetes based on the fact that he was obese (BMI > 40 Kg/m^2 on inspection), had good glycaemic control with oral medications and was diagnosed with diabetes later in life in his 50s. The patient was therefore excluded before he signed the consent form. Of the 11 patients for whom an appointment was made, two did not attend.

Data collection: Nine patients completed all measurements during the first appointment. None refused to sign the consent form or to have a particular measurement. One patient (the first patient to be recruited) was in a hurry and completed the questionnaires in an unsatisfactory manner, as she admitted on leaving (*Patient 5*).

Blood and urine tests: Although none of the nine patients refused to have a blood or urine test, only six patients actually attended the clinical laboratory and had their blood and urine tests completed. All patients who went to the clinical laboratory had both the blood and the urine tests. The three patients who did not have the lab tests were contacted and reminded and, although they appeared to be keen to have them, each of them failed to do so.

Table 4.3: Prospective participants

No.	Gender	Appointment	Data collection	Lab tests	Comments
1	M	Yes	DONE	DONE	
2	F	No*			*too busy
3	F	Yes	DONE	X	
4	F	No*			*rejected – not interested
5	F	Yes	DONE*	X	*in a hurry (esp. FFQ)
6	M	Yes	DONE	DONE	
7	F	Yes	DONE	DONE	
8	M	No*			* not in Limassol (Nicosia, Cy)
9	M	No*			* excluded (necrotic pancreatitis)
10	M	Yes	DONE	X	
11	F	No*			* rejected at CDA call
12	F	Yes	DONE	DONE	
13	F	Yes	DNA		
14	F	No*			*spoke only to a relative
15	M	Yes	DONE	DONE	
16	F	No*			*rejected – too busy
17	M	Yes	excluded*		*likely Type 2 diabetes
18	F	Yes	DONE	DONE	
19	F	No*			* not in Limassol (London, UK)
20	F	Yes	DNA		

CDA, Cyprus Diabetic Association; DNA, did not attend; FFQ, food frequency questionnaire

Recruitment (study) early termination: The study was stopped prematurely, number-wise, that is, before 20 patients were recruited. This decision was taken as all members of the research team agreed that the data collected fulfilled the intended aims and objectives of the current pilot study, and further recruitment was unlikely to provide any significant additional information.

Other notes on recruitment: The CDA secretarial staff, who are familiar with the CDA database, insisted that we should expect a significant number of the patients available in the CDA database to have incorrect or outdated contact details. Fortunately, this was not the case for those selected to take part in the pilot study.

Acceptability and feasibility of the delivery methods of the study measures and questionnaires

This pilot study recruited nine participants, of which six had blood and urine tests available, from the twenty potential participants initially contacted (*Table 4.3*). No major problems were faced with the chosen methodology, including the delivery methods, the data collection process, the coordination of the study and the export of the results. Nevertheless, some minor but important changes were implemented before the main study. The most significant findings on the process (of the acceptability and feasibility of the delivery methods of the study measures) included the following:

- none of the patients refused any of the measurements during the data collection process
- patients, in general, appeared satisfied with the data collection process
- no major problems with completing the questionnaires were observed and we tried to be available and answer any questions that the participants had
- patients who struggled with computers had to receive help with the questionnaires but seemed to be satisfied with the process
- feedback was received from all participants
- the export process of the results worked well
- adverse events were recorded (*Results: Adverse events*)
- the collection process of the blood and urine tests result ran smoothly
- the patients provided positive feedback regarding the clinical laboratory experience
- the provision of free blood and urine tests in a private clinical laboratory were perceived by the majority of patients as a strong motivational factor

Results of data collected

Demographic characteristics

The demographic characteristics of the participants of the pilot study are presented in *Table 4.4*.

Table 4.4: Demographic characteristics of participants

Characteristics[1] (units)	Total ***n***	Variables[2]	***n***	%	Mean	sd	Min	Max
Age (years)	7[3]				34.6	10.7	22	53
Gender	9	Male	4	44				
		Female	5	56				
Ethnicity	9	Caucasian[4]	9	100				
Marital status	9	Cyprus	6	66.7				
		Other	3	33.3				
Cohabitants[5]	9	Alone	2	22.2				
		Spouse & Children only	6	66.7				
		Parents only	1	11.1				
No of children	9				0.9	0.8	0	2
Household income	9	0 – 10.000	2	22.2				
		10.001 – 20.000	4	44.4				
		20.001 – 30.000	1	11.1				
		30.001 – 60.000	1	11.1				
		> 60.001	1	11.1				
Educational level[6]	9	University (Doctorate)	3	33.3				
		University (Master)	4	44.4				
		University (Bachelor)	1	11.1				
		Lyceum (high school)	1	11.1				
Employment[7]	9	Full-time	8	88.9				
		Unemployed	1	11.1				
Employment sector	7[8]	Public sector	3	33.3				
		Private sector	4	44.4				
Duration(min)[9]	9				1.7	1.3	0.42	4.17

[1]Obtained from *demographic characteristics* questionnaire. [2]Only options that received an answered appear on the table. [3]Two participants did not answer. [4]Other available option was *other*. [5]Any combination of the answers *spouse*, *children*, *parents alone*, *other* was possible. [6]Other options available were *Dimotiko (primary school)* and *gymnasium (secondary school)*. [7]Other option available was *part-time*. [8]This was not applicable for one participant (as unemployed) and one participant did not answer. [9]Time needed to complete questionnaire.

Anthropometric and clinical examination data

The measurements collected through the anthropometry and the clinical examination are available in *Appendix: Chapter 4*.

Mediterranean diet

The current section presents the Mediterranean diet score, one of the main data outcomes of interest, and other related data that were used for calculation of the total Mediterranean diet score, such as the food groups (or components) reported consumption and scoring, and a categorisation of the Mediterranean diet score. The results are presented in *Table 4.5* and *Table 4.6*.

Table 4.5: Adherence to the Mediterranean diet

MedDietScore[1]	***n***	MedDietScore (0 – 55 points)				MedDietScore (category)		
		Mean	sd	Min	Max	Category[2]	%	***n***
MedDietScore	8[1]	28.9	5.2	22	35	Low	0	0
						Medium	100	8
						High	0	0
Gender								
Male	4	27.5	4.9	22	33	Medium	100	4
Female	4	30.3	5.7	22	35	Medium	100	4
Nationality								
Cypriot	6	28.5	5.2	22	33	Medium	100	6
Other	2	30.0	7.1	25	35	Medium	100	2
Marital status[3]								
Married	4	29.5	5.2	22	32	Medium	100	4
Single	3	30.3	5.0	25	35	Medium	100	3

[1]The adherence to the Mediterranean diet was measured using the *MedDietScore* scoring systems as described at *Methods*. [2]The categories of the adherence to the Mediterranean diet score, namely *low*, *medium* and *high adherence* are described at *Chapter 3*. [3]One patient was excluded from the current analysis as she has completed the FFQ inadequately, as discussed in *Results: Participation rate* section.

Table 4.6: Mediterranean diet score components

MedDietScore components[1]	MedDietScore (0 – 5 points)				Serving per week[2]			
	Mean	sd	Min	Max	Mean	sd	Min	Max
Non-refined cereals	1.4	0.9	0	3	5.5	5.6	0	15.5
Potatoes	1.4	0.7	1	3	3.6	2.9	1.5	10.3
Fruits	2.4	1.4	1	5	10.1	7.7	2.0	22.5
Vegetables	4.1	1.5	1	5	37.4	24.8	4.3	85.3
Legumes	3.1	1.4	2	5	4.3	3.0	1.5	9
Fish	1.8	0.9	0	3	1.7	1.2	0	4
Red meat and products	2.4	2.4	0	5	6.9	6.5	0	16
Poultry	2.5	2.3	0	5	12.0	16.1	0	49.5
Full fat dairy products	3.8	1.9	0	5	14.1	13.7	0	40.5
Use of olive oil in cooking	4.8	0.7	3	5	16.3	7.7	3.0	22.5
Alcoholic beverages	1.4	2.3	0	5	8.2	7.6	0	21.3

[1]The calculation of the Mediterranean diet components of the *MedDietScore* scoring systems as described at *Methods*. [2]The servings represent the number of a food group of pre-specified portion that is reported to be consumed.

Food and beverages consumption

The food and beverages intake reported by the participants of the foods available in the FFQ, and the frequency in which they used various cooking methods are available in *Appendix: Chapter 4*. The current section of the questionnaire (in combination with the preceding section of the *Mediterranean diet*) was crucial to help us identify significant issues and irregularities in the calculation of the adherence to the Mediterranean diet. This included identifying foods and beverage that were consumed in a quantity that overly affected the measurement of adherence to the Mediterranean diet or the opposite, that is, the foods and beverages that are underrepresented due to the large portion size used (i.e., the portion defined in the FFQ); and complex foods, which the participants reported to consume often enough and in such quantities that this may affect a food group (or component) and which were not included in the algorithm for that specific component. For this reason, the foods and beverages are reported (and were scrutinized), in detail.

Bioelectrical impedance analysis data

The results of the body composition analysis measurements collected via the BIA method are available in *Appendix: Chapter 4*.

Medical and Diabetes characteristics

The results of the *medical and diabetes* questionnaire are available in *Appendix: Chapter 4*. The electronic version of the *medical and diabetes* questionnaire had some functions which required that we test them in real-world conditions and verify that they functioned as they should.

Blood and Urine tests

The results of the blood and urine tests' results collected at a private clinical laboratory are presented in *Table 4.7*. The blood tests are from a spot fasting morning venous sample; the urine tests, namely albumin, creatinine and the derived ACR (data of albumin and creatinine *per se* not shown) are from a spot fasting morning sample taken from the first void of the day i.e. after waking up. Six participants had the blood and urine tests done.

Table 4.7: Blood and urine tests

Blood & urine tests	Units	Mean	sd	Min	Max	Within normal range[a] ***n***[%]		
						Yes	No	
							↑	↓
Glycaemic control								
Glucose	mg/dl	227.7	26.0	160.7	294.6	0	6[100]	0
	mmol/l	12.6	3.5	7.5	17.6	0	6[100]	0
HbA1c	mmol/mol	63.5	2.3	57.5	69.5	0	6[100]	0
	%	7.9	0.5	7.5	8.9	0	6[100]	0
Male	mmol/mol	66	7.5	59	74	0	6[100]	0
Female	mmol/mol	61	2.6	29	64	0	6[100]	0
eGDR[b]	mg/kg/min	5.5	2.5	2.3	9.3	n/a	n/a	n/a
Lipids								
TC	mg/dl	221.7	64.4	157	332	2[66.7]	4[33.3]	n/a
LDL-C	mg/dl	140.8	56.9	79	226	0	6[100]	n/a
HDL-C	mg/dl	67.7	10.8	56	80	6[100]	n/a	0
Tg	mg/dl	65.5	31.9	39	127	6[100]	0	n/a
LFTs								
ALT	U/l	17.2	3.5	12	22	6[100]	n/a	0
AST	U/l	19.7	4.7	14	28	6[100]	0	0
TFTs								
fT4	pmol/l	16.4	1.6	14.7	18.4	6[100]	0	0
TSH	µIU/ml	1.5	0.5	0.9	1.9	6[100]	0	0
Inflammation markers								
CRP	mg/dl	0.3	0.2	0.05	0.5	5[83.3]	1[16.7]	n/a

Blood & urine tests	Units	Mean	sd	Min	Max	Within normal range[a] ***n***[%]		
						Yes	No	
							↑	↓
β-cell function								
							Detectable	Undetectable
C-peptide	ng/ml	-	-	-	-	0	0	6[100]
Renal function								
Urea	mg/dl	25.7	8.0	15	38	6[100]	0	0
Creatinine (blood)	mg/dl	0.7	0.15	0.6	0.9	5[83.3]	0	1[16.7]
						Yes	Moderately ↓	Severely ↓
eGFR[b]	ml/min/1.73m^2	114.3	10.9	102	133	6[100]	0	0
						Yes	Moderately ↑	Severely ↑
ACR[b]	mg/g	6.3	2.4	1.7	8.8	1[16.7]	5[83.3]	0

[a]The normal ranges used for HbA1c and glucose were based on the NICE guidelines and were defined as below 48 mmol/mol (6.5 %) for HbA1c and between 90 – 126 mg/dl (5.0 – 7.0 mmol/L) for fasting glucose (NICE, 2015b). The eGDR had not defined normal range, to our best of knowledge, and although the tertiles had been used before (Epstein *et al.*, 2013) to categorised the results, were not used here due to the small population of the pilot study. The normal ranges for lipids were based on the guidelines of the *American association of clinical endocrinologists* and were defined as TC < 200 mg/dl, LDL-C < 70 mg/dl (very high risk), HDL > 40 mg/dl for men and > 50 mg/dl for women, and Tg < 150 mg/dl (Jellinger *et al.*, 2017). The C-peptide is reduced in patients with Type 1 diabetes (Jones and Hattersley, 2013; Leighton, Sainsbury and Jones, 2017) and considering the small sample of the pilot study, we had decided not to define a normal range (or other cut-off points) of the C-peptide but the cut-off point of C-peptide were placed *ad hoc* using the detectable level of the essay used which is ≥ 0.010 ng/dl. The eGFR and ACR cut-off points were based on the NICE guidelines (NICE, 2014a); ACR was defined as normal (to mildly increased) if ACR < 3 mg/mmol, as moderately increased if ACR was between 3 – 30 mg/mmol and severely increased if > 30 mg/mmol; eGFR was defined as normal if eGFR was > 90 mL/min/1.73m^2, as mildly decreased if eGFR was between 60 – 90 mL/min/1.73m^2, as moderately decreased if eGFR was between 30 – 60 mL/min/1.73m^2 and severely decreases if eGFR was < 30 mL/min/1.73m^2 (severely decreased not shown on the table – none of the participants had severely decreased eGFR). For the rest of the blood tests, the normal ranges were based on the cut-off points provided by the clinical laboratory and were < 35 U/l for ALT, between 6 – 38 U/l for AST, 12 – 22 pmol/l for fT4 and 0.27 – 4.2 μIU/ml for TSH, < 0.5 mg/dl for CRP, between 14 – 45 mg/dl for urea and 0.6 – 1.2 mg/dl for creatinine.
[b]eGDR, eGFR and ACR were calculated based on the available equations in the literature, described in *Methods*.
HbA1c, glycated haemoglobin; eGDR, estimated glucose disposal rate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Tg, triglycerides; TFTs, thyroid function tests; TSH, thyroid stimulating hormone; fT4, free T4; LFTs, liver function tests; ALT, alanine transaminase; AST, and aspartate transaminase; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio; CRP, C-reactive protein.

Physical activity

The physical activity data collected was adequate (i.e., there was no missing data and all required data was collected). The results are not presented here, given that the available questionnaire and protocol of data processing (of the *IPAQ* questionnaire) are well studied and no changes were required for the main study.

Results from statistical analysis

Demographics of interest:

- Eight patients completed the data collection, of which six also had a blood and urine test.
- Mean age was 34.6 ± 10.7 years.
- 50% were female.

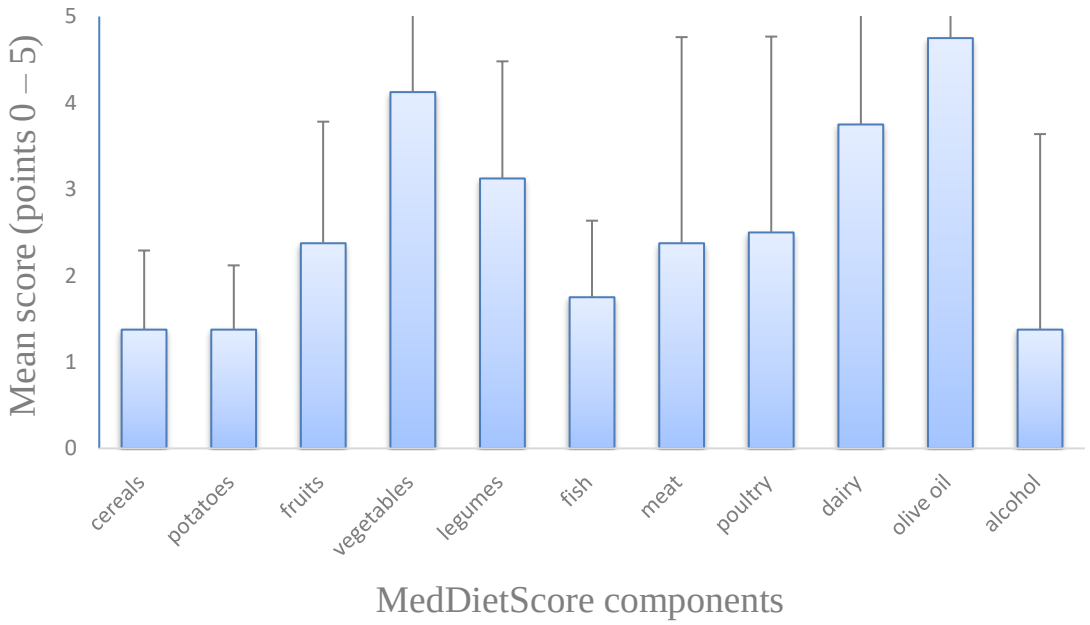
Epidemiological outcomes:

- All patients had a moderate adherence to the Mediterranean diet (n = 8; mean *MedDietScore* = 28.9 ± 5.2 points; range: 0 – 50 points).
- All patients had a suboptimal glycaemic control i.e. HbA1c > 53mmol/mol (n = 6; mean HbA1c = 63.5 ± 5.8 mmol/mol; fasting blood glucose = 227.7 ± 64.8 mg/dl).
- BMI was 27.0 ± 5.5 kg/m^2 (n = 8); six patients were overweight or obese.
- Olive oil was the better adhered while non-refined cereals, potatoes and alcohol the least (*Figure 4.1*).
- C-peptide levels were undetectable i.e. < 0.10 ng/ml (n = 8).

Statistical comparisons:

- The Mediterranean diet score was non-statistically significantly correlated with glycaemic control, that was HbA1c (r_s = -0.65; p = 0.16) and fasting glucose (r_s = -0.66; p = 0.15).
- Females had a non-statistically significant better adherence to the Mediterranean diet (p = 0.38; females: 30.3 ± 5.7; males: 27.5 ± 4.9).
- BMI was marginally related to HbA1c (r_s = 0.8117; p = 0.0499).

Figure 4.1: MedDietScore scoring system components scoring: mean (standard deviation)



Adverse events

There were no significant adverse events observed during the current study, other than mild hypoglycaemia in two participants. The participants were treated according to protocol, without any significant complications. Once capillary blood glucose levels returned to normal range, the participants completed the study as normal.

Discussion and Recommendations

Recruitment process

Database – identifying and recruiting the correct participants

The pilot study confirmed that the CDA database has significant misclassification, such as the presence of patients with other types of diabetes, given that patients with Type 2 diabetes and necrotic pancreatitis were the actual diagnoses in patients recorded as having Type 1 diabetes. Taking into account the limited research resources, the wish to reduce inconvenience for potential participants and the need to recruit eligible participants to ensure validity of the study, I decided to implement the following measures:

1. That the individual (i.e., me) who was responsible for contacting the patients after the potential participants provided the *consent to contact*, should ensure that participants were informed that the study is for people diagnosed with Type 1 diabetes and would ask confirmatory questions, i.e. '*Can I, please, confirm that you have Type 1 diabetes?'*, '*Are you currently on insulin treatment?'*, '*Are you currently on diabetes*

treatment other than insulin?', '*What was your age at diagnosis?'*, and '*Initial diabetes treatment following the diagnosis of diabetes?*' etc. Not all questions would be necessary for all participants, whereas a different set of clarifying questions may, on occasion, be required for some other potential participants. Nevertheless, because I have a non-medical background and am not trained to diagnose diabetes type (especially difficult cases), for this reason, only clear-cut cases of non-Type 1 diabetes would be excluded through this manner.

2. Similar processes to those described above would need to take place at the first meeting before the data collection process and the signing of the *consent form*, for the prospective participants who we are unsure about their diagnosis. Advice from DrAK, who is appropriately trained and highly experienced in diabetes diagnosis, would be sought in such instances.
3. Finally, if, despite the above measures, the diagnosis of Type 1 diabetes is still in doubt, then a C-peptide test would be utilised in combination with medical history. The suggested cut-off point is at 0.2 nmol/l (or 0.6 ng/ml). Although various numbers are available in the literature, ranging from a fasting C-peptide of 0.075 nmol/l to 0.25 nmol/l (Gjessing *et al.*, 1989; Jones and Hattersley, 2013; Leighton, Sainsbury and Jones, 2017), no consensus exist to our knowledge, and the current guidelines fail to report a cut-off point (NICE, 2015b; American Diabetes Association, 2019a). Those participants with a C-peptide above the pre-defined C-peptide cut-off point were discussed amongst the members of the research team (in consideration of the medical history, such as age, duration of diabetes, presentation of diabetes, BMI, etc.) and were excluded (or included) after mutual agreement between DrAK and me. The C-peptide test result, in combination with the medical history, was also added to the list of inclusion and exclusion criteria.

Random sample generation

The process of selecting a random sample for the current pilot study was achieved through using a true RNG. One of the features of using a true versus a pseudo RNG is reproducibility. In this thesis, after completing the pilot study, the reproducibility (which is often considered a negative aspect) was perceived as being more important than true randomness. The reproducibility of random numbers would help with practicalities; whereas true randomness was less of a priority as it was expected that there would be a need to contact a large portion of the patients available in the database. Therefore, the

method of generating random numbers was changed to one of the random function tests available in STATA; nevertheless, this pseudo RNG still passes many tests for randomness (StataCorp LLC, 2019a, 2019b).

Data collection methodology

Questionnaires

The questionnaire was well-received by the participants. Minor suggestions, made by the participants, were discussed between the members of the research team and, when accepted as reasonable amendments, they were applied as such (minor edits to the questionnaires are not reported here). No major problems were identified in relation to the electronic delivery method of questionnaires, other than a need to add a reminder to the participants to avoid entering characters other than numbers (such as letters, dashes, etc.) on the FFQ, which, in that case, would fail to save.

Duration of the data collection period

The time needed to complete the data collection for each participant was estimated to be around 40 minutes (not taking into account travel time and the clinical laboratory visit), in contrast to the estimate of 30 minutes that was initially expected. This, in addition to the fact that one of the participants completed the process (mainly the questionnaires) inadequately as she was in a hurry, made it necessary to make it clear to future potential participants that the data collection (including the completion of the consent form and podiatric screening) would last around 50 minutes, so that prospective participants would allow adequate time for their appointments.

Data processing (electronic and manual)

The electronic processing of the data, as described previously, ran smoothly. Nevertheless, some adjustments were recommended, outlined below, regarding the calculation of the Mediterranean diet score and, more precisely, to the algorithm used.

Mediterranean diet calculation

- Through an analysis of the results of the individual foods of the FFQ, it was revealed that certain foods were highly influential on the final *MedDietScore* score of individual participants, primarily owing to their small portion size, or the opposite. In order to standardise (as much as possible) our data with the already published literature on *MedDietScor*e, it was considered prudent to attempt to contact the authors of the *MedDietScore* and request clarification on the portion sizes used for

the calculation of the score. If the portion sizes they used differed significantly, the algorithm would need to be weighted accordingly (based on these portions).

- Another significant drawback was identified in the algorithm used. In an attempt to fill the gaps between scores for the published MedDietScore, the total servings were rounded to the nearest integer number. For example, the non-refined cereals score is zero points for zero servings, one point for one to six servings, two points for seven to twelve portions, etc., therefore, if the total score of a participant is 0.35, then the algorithm will fail as it does not match any of the available scores. Subsequently, the total score was rounded to 0. Similarly, if the score of a participant was 6.56, then it was rounded to seven to match one of the available scores. Through this process, although we prevented our algorithm from collapsing, we identified two consequent problems. Firstly, some scores became mathematically impossible to achieve; for example, when scoring 1 point for legumes or fish that was allocated as $0 >$ servings < 1, i.e., if the servings consumed are less than 0.5 ($\neq 0$), then a participant scores zero points for that food, and, if a participant reports servings of more than 0.5 (up to one), then the score is rounded up to one and, consequently, the participant scores two. Apart from the fact that some scores became mathematically impossible to achieve, someone could legitimately also argue about misclassification; in the latter example the correct score, really, is 0.5 or a score of less than one but more than zero. Similarly, it could also be argued in the first example that, with non-refined cereals (although mathematically correct), the participant that reported 6.56 servings, and thus it is more appropriate for this food to be placed in the lower category of one to six portions (and consequently receive one point) than the higher category of seven to twelve (two points). To overcome this problem, the MedDietScore scoring system will need to be adapted further to cover these gaps with a suggestion to move the higher bound of the lower number up to lower bound of the higher number (when appropriate); for example, regarding the non-refined cereals, the score will become < 1 serving (one point), ≥ 1 and < 7 (two points) and ≥ 7 and < 13 (in constant, to previously, 0, 1 – 6 and 7 – 12). This will eliminate the need for rounding numbers, but instead allow the actual full number (i.e., including the decimal places) could be utilised without the risk of the algorithm failing. Furthermore, this will solve the problems of scores that are mathematically impossible to achieve and the misclassification of scoring for total servings (while the actual scoring used will become better defined and the algorithm steps will need to be reduced, resulting in a

reduced time processing). Nevertheless, this would require the re-writing some of the parts of the algorithms (and re-testing) before the main study.

- The portions of the alcoholic beverages were modified at the start of the pilot study to match the 12 g ethanol or 1 unit of alcohol (note that this value differs from the accepted definition of 1 unit of alcohol in the UK, which is 8 g), therefore, the algorithm presented for the pilot study is the algorithm used after this modification. The decision to adjust the portion sizes, rather than weigh the servings reported, was a prudent choice, taking into account that the participants reported small amounts of alcohol consumption; therefore, no further changes were recommended. What, importantly, had slipped our attention, was that the servings were per day (defined at the *MedDietScore*), and not per week as the other food groups, therefore, the servings would need to be divided by the appropriate number (that is, 28 and not four).
- The number of servings of a (food or a) food group was interchanged between daily, weekly and monthly depending on the need; for example, initially all servings were converted to monthly (at exported results), then for the *MedDietScore* needs were converted to weekly and daily. The conversion factors used in the pilot study (one month equals 30 days or four weeks, and one week equals seven days) did not produce consistent (and comparable) results. Therefore, there was a need for better standardisation. To achieve that, strictly speaking, we could have divided the 30 days per month (average days per month is about 30.4) by four, which will result in a number with several decimal places (4.34285… weeks). Instead, we favoured a simpler method that would result in an integer number; that is, 28 days, and, consequently, four weeks; this is easy to apply and understand, and would always result in the same number, independent of how many times the servings are converted to daily, weekly or monthly.

Data adequacy

Two major areas were identified that would supplement the results: the addition of a podiatry screening and having a clearer definition of what is a healthy (normal) range for the BIA test results.

Podiatrist and podiatric screening

A foot screening by a qualified podiatrist would significantly enhance the data collection procedure; there would be no need for participants to attend an extra research site and minimum consumables are required for the foot screening.

BIA – healthy range of results

The results obtained through BIA measurements for body fat, lean mass and water estimations were compared to normal values provided by the BIA device that was used (Maltron BF-907; Essex, UK). These values change depending on the age of the participants (and probably on other factors), but they are not widely available. The company would need to be contacted to provide us with the actual ranges used in each case. If the company failed to do so, for whatever reason, or the healthy ranges used are not based on solid evidence, then the use of healthy range values available in the literature could be considered (as an alternative option). Through this process, we should be able to better define and clarify what is a normal range.

Coordination – completion check list

The main study necessitated a reasonable amount of coordination; from coordinating all three people who were involved in the data collection – with one of the research members (the podiatrist) requiring to do some travelling from his own clinical site to the research site – to coordinating the clinics of each of the research members (mine and that of DrAK); coordinating participants attending the research site in attending a different data collection site (one could be doing the questionnaires, while the other could be going through the clinical examination and other the anthropometric measurements – in different rooms/on different sites); and making sure that the patients have completed all of the data collection and that all items were provided for the clinical laboratory (that is, a prescription and a urine container), etc. To make sure that all this was done properly, the secretarial staff of the CEDM had to contribute considerably. They needed to make sure that all available materials were made available before the arrival of patients (such as printed consent forms, completed prescriptions for the clinical laboratory, etc.) to avoid delays. A simple checklist was constructed that included all process that were necessary to complete for each participant; for example, the signing of the consent form, that all anthropometric data were collected, the clinical examination was completed, the foot screening was completed, the clinical laboratory items (prescription, urine container, contact details) were provided, etc. This checklist was completed by the secretarial staff, under my supervision.

Conclusion

The pilot study provided valuable information in addressing the research questions. It demonstrated the feasibility of a larger study with the current methodology, with few modifications. Furthermore, it provided me with an insight into the adaptation and changes needed to the chosen methodology, including the population recruitment, the adequacy of the data collection process recording, and analysis, and on practical aspects, such as project coordination. Such modifications were expected to improve the efficiency of the recruitment and data collection processes, improve the robustness of the study results and help with the more efficient allocation of the limited resources.

Chapter 5: Mediterranean diet and glycaemic control in Type 1 diabetes: a cross-sectional study

Introduction

This main study aimed to investigate the association between a healthy eating pattern, more specifically, the Mediterranean diet, and glycaemic control. Previous studies investigating this association provided encouraging results, although each had significant methodological limitations (*Chapter 1*) (Provenzano *et al.*, 2001; Cadario *et al.*, 2012; Gingras *et al.*, 2015; Zhong *et al.*, 2016; Fortin *et al.*, 2018; Mouslech *et al.*, 2018). However, no relevant studies, to our knowledge, have yet been conducted in Cyprus.

In addition, I aimed to quantify the degree of adherence to the Mediterranean diet and the glycaemic control in the chosen population. I have shown that there is moderate adherence in the Cypriot population (*Chapter 3*). Similarly, studies conducted in North America (USA and Canada), have shown that patients with a diagnosis of Type 1 diabetes in those countries had (very) low adherence to the Mediterranean diet, and this was in line with the general population of those countries (Gingras *et al.*, 2015; Zhong *et al.*, 2016). Nevertheless, adherence to the Mediterranean diet of patients with Type 1 diabetes in Cyprus, and in general in the Mediterranean region, still remains unexplored. At the same time, while glycaemic control is well-studied in some populations with a diagnosis of Type 1 diabetes, there is an absence of data for patients with a diagnosis of Type 1 diabetes in Cyprus (*Chapter 1*).

The implications of the above questions are profound, as they might provide evidence of a need (or not) to restructure education programmes for patients with Type 1 diabetes, and, in general, of a need for change in the persisting attitude towards the association between glycaemic control and healthy eating or eating patterns such as the Mediterranean diet. Furthermore, such data could have public health implications if this high-risk population for cardiovascular disease (CVD) was found to benefit from increased Mediterranean diet adherence (in terms of glycaemic control).

The current methodology was explored extensively during the pilot study (*Chapter 4*). The current chapter focuses on the primary outcomes. While some secondary outcomes were set, they were (for the most part) beyond the scope of the current thesis.

Methods

Study design

Outline

The present study was a cross-sectional study that explored adherence to the Mediterranean diet, glycaemic control and the association between the two in a population with a diagnosis of Type 1 diabetes, residing in Limassol, Cyprus. The design, methods and measures of the current study were assessed in a pilot study (*Chapter 4*).

Sample: The targeted population were the adults residing in Limassol, Cyprus with a diagnosis of Type 1 diabetes. The CDA database was to be used to capture a sample of the target population. The sample recruitment procedure was based on a random sample selection procedure and the sample size of 100 participants was based on sample size calculations for the association of the Mediterranean diet and glycaemic control.

Primary outcomes: The primary outcomes were the measurement of adherence to the Mediterranean diet, glycaemic control and the association between the two, that were defined *a priori*. Adherence to the Mediterranean diet was measured through the *MedDietScore* scoring system. The glycaemic control was assessed through HbA1c and glucose independently, through a collected spot venous blood sample. Adherence to the Mediterranean diet was investigated for its predictive power of glycaemic control through OLS models that were defined *a priori*. More precisely, three OLS models were run with HbA1c and glucose as the dependent variables (independently) and Mediterranean diet as the independent variable; a simple OLS model, and two multivariable models with additional covariates.

Secondary outcomes: Further to the primary outcomes, several secondary outcomes were set. The secondary outcomes were either predefined or were planned post-hoc. One of the main purposes of the secondary outcomes was to provide epidemiological information, including medical, dietary and lifestyle data on patients living with Type 1 diabetes in Cyprus, which to date has not been compiled. These results (where currently available) are not included in this thesis.

Recruitment process

The recruitment process is graphically shown at *Figure 5.1* and was stopped once we had collected at least 100 participants who had valid information on adherence to the

Mediterranean diet and glycaemic control and were confirmed to have had a diagnosis of Type 1 diabetes. The recruitment process was as described for the pilot study (*Chapter 4*), using the database of the Cypriot Diabetes Association (CDA), with the following changes:

I. Inclusion criteria

It was necessary to confirm the presence of Type 1 diabetes, both clinically and biochemically. Therefore, specific measures were set and implemented during the main study in an effort to recruit and include only the participants who had a diagnosis of Type 1 diabetes, as follows:

i. During the initial contact with the potential participants via telephone, they were explicitly told that the study only recruits patients with a diagnosis of Type 1 diabetes. At the same time, I identified clear-cut cases of non-Type 1 diabetes by means of asking simple questions, such as ''*Can I, please, confirm that you have Type 1 diabetes?'*, '*Are you currently on insulin treatment?'*, '*Are you currently on any diabetes treatment other than insulin?*', '*What was your age at diagnosis?'*, '*Initial diabetes treatment following the diagnosis of diabetes?*' etc.
ii. In doubtful cases, I consulted with DrAK to discuss the diabetes type diagnosis during the data collection appointment and before the *consent form* was signed by the potential participant.
iii. Finally, the diagnosis of Type 1 diabetes was confirmed through the C-peptide blood test. More specifically, an unstimulated (fasting) serum C-peptide below the cut-off point of 0.2 nmol/l (or 0.6 ng/ml) was considered confirmation of the diagnosis of Type 1 diabetes and required no further action. In contrast, a C-peptide above this level required further consideration, that is to say, the results of the C-peptide and the medical history, for example, age, duration of diabetes, presentation of diabetes and BMI, were considered (by DrAK and me) before deciding on whether the corresponding participant should be included or excluded.

II. Random sample selection process

During the pilot study, the random selection was made based on a true RNG, whereas the process in the main study was instead based on a pseudo RNG, that is, on a random number function. The random sample selection process is described below and resembles

the process of dealing cards, that is to say, shuffling the cards and then picking the top card (Gould, 2012).

Derive a function seed: The seed is a number that the function uses to be initialised and consequently, generate the pseudo random number sequence. The seed can take any value between 0 and $2^{31} - 1$ (or 2,147,483,647) and the STATA manual states that any randomly selected number will work equally well (StataCorp LLC, 2019a). Therefore, the *www.random.org* website was used for the selection process of the seed, which provides a true RNG engine (through measurements of the atmospheric noise) (Haahr, 2019). Initially, a random number was selected between the number one and ten, in order to generate the number of the digits of the seed, which resulted in eight digits. Thereafter, the digits themselves were randomly selected in an order of ab,cde,fgh, (where each letter represents a number – digit – and where 'a' is selected first, followed by 'b', etc.), which resulted in the random seed number *43505540*.

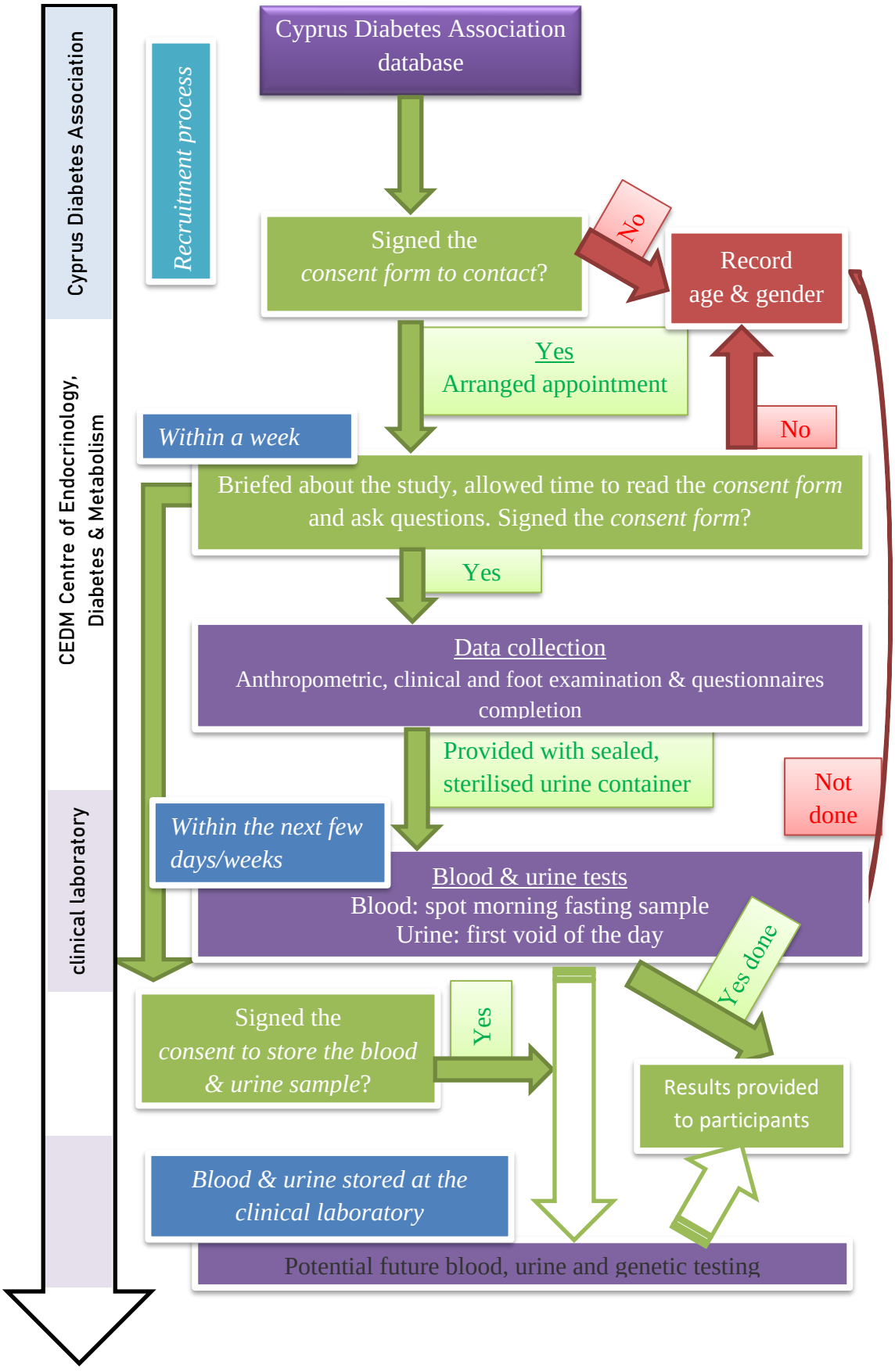
Allocation of the random numbers: The 389 potential participants (which essentially were represented by the CDA database codes) were allocated a random number (*u1*) generated through a mathematical function with the seed *43505540*; the uniform (rectangular) function and the default 64-bit Mersenne Twister (mt64) RNG (method) were implemented. Thereafter, these individuals were allocated a second random number (*u2*) to allow for ties – STATA uses a RNG to order ties, and, while this does not affect the randomness of the results, it does affect reproducibility (Gould, 2012; StataCorp LLC, 2019b). Furthermore, the random allocated *u1* and *u2* numbers were stored in STATA as *double* data type, which prevented number rounding (or decimal figures loss) and hence reducing further the probability of ties (Gould, 2012; StataCorp LLC, 2019a). Although the probability of ties (of both numbers, *u1* and *u2*, on the same participant) was eventually infinitesimal (Gould, 2012), nevertheless, the values were still examined for ties. No *u1* and *u2* ties were observed. The STATA commands are stored in a do-file and are available in request.

Order the random numbers: The participants were ordered by means of the random allocated number *u1* (if a tie, then followed by *u2*); with the smallest *u1* number ordered first, followed by the second smallest number etc., up to the largest allocated *u1* random numbers. The first two potential participants contacted were the 89 (*u1* = 0.00453378)

and the 39 (*u1* = 0.00513178) participants, whereas the two highest *u1* random numbers were allocated to the 27 (*u1* = 0.99899467) and the 213 (*u1* = 0.99932542) participants.

Provision of numbers to CDA: Once the process of allocating random numbers was completed, the first 50 participant numbers were provided to the secretarial staff of the CDA. When these 50 individuals (corresponding to the random numbers provided) were contacted by the CDA secretarial staff, then a further batch of 50 numbers were provided and so on, up to the point that the desirable sample was achieved.

Figure 5.1: Graphic outline of the study



Recruitment pool

The CDA database pool consisted of 389 patients who potentially met the eligibility criteria; while (potentially) a minority of the patients with a diagnosis of Type 1 diabetes who were not on this database, were not given the chance to participate. The main issue identified during the pilot study was that of diabetes misclassification and has been addressed previously.

Sample size

The target sample size was set to 100 participants who fulfilled the inclusion criteria and who had adequate data for their inclusion in the primary outcomes analysis (i.e. completed the FFQ and had blood tests done) and was based on theoretical and practical considerations. The power analysis (based on the association of Mediterranean diet and glycaemic control) indicated that the 100-participant sample size provided sufficient power (π) to detect the predefined smallest clinically significant effect size, in a simple or a multiple regression model ($\hat{\pi}$ = 76% and $\hat{\pi}$ = 81%, respectively). The technical details of how we derived to the aimed sample size of 100 participants, are described below.

Sample size ceiling: Initially, the sample size ceiling (or the maximum number of participants to aim for) was estimated based on the available number of potential participants in the CDA database and the pilot study's participation rate. Note that the estimated sample size ceiling was defined as the maximum number of participants forecast to participate in the study while at the same time provide sufficient data for their inclusion in the data analysis of the primary outcomes. The sample size ceiling was estimated to be 116 participants and was calculated as follows:

1. The number of participants for whom data was used in the statistical analysis of the association between the Mediterranean diet and the glycaemic control in the pilot study was six from a pool of 20 potential participants, resulting in a 30% participation rate.
2. The size of the recruitment-database pool, when restricted to adult patients (i.e., at or above the age of 17 years) who have a recorded diagnosis of Type 1 diabetes and a place of residence as the municipality of Limassol, was 389 patients.
3. Therefore, based on points 1 and 2, the estimated sample size was calculated to be as 389 x 30% ≈ 116 participants.

Modelling: The estimated parameters (or the degrees of freedom spend) in the regression model (the independent variables in this case) should be accompanied by an adequate number of observations so to avoid overfitting. More precisely, (at least) ten observations should be available for each independent variable in a linear regression model (Babyak, 2004; Harrell, 2015). In this main study, the most complex linear regression model for the primary outcomes had one predictor and eight covariates, or a total of nine independent variables. Consequently, the smallest number of observations (i.e., participants) should be ten observations for each of the nine independent variables (10 x 9 = 90) and hence, a total number of observations or a sample size of 90 participants.

Power and sample size analysis: Taking into consideration the sample size ceiling and the degrees of freedom, as described above, the sample size could range from 90 to 116 participants. Therefore, a power and sample size (PSS) statistical analysis was run, thereafter, in order to confirm that this sample size (range) yields sufficiently powered models and that it can detect an adequately small, but clinically significant, difference. The desirable power for the current study was placed at 80% and, similarly, the smallest clinically significant effect difference was defined as b = -0.015 ($\delta \approx -0.27$) or as R^2 = 15% ($\delta \approx 0.18$)[i]. Note that, a larger, less conservative $|\delta|$ is acceptable or clinically meaningful for the glucose, while a smaller $|\delta|$ (b = -0.01) could be observed for the HbA1c based on previous available research (Zhong *et al.*, 2016) although this would have less clinical value. The PSS analysis was executed by implementing a test of the slope in a simple linear regression (b test, where H_0: b = 0, H_a: b ≠ 0) and an R^2 test in a multiple linear regression (R^2 test, where H_0: R^2 = 0, H_a: $R^2 \neq 0$), which reflected the planned regression models of the primary outcomes. In regard to the (expected) standard deviations (sd) required for the computation of the b test, the values of 0.3 for the dependent variable sd_y and 5.2 for the independent variable sd_x were inputted. These values are based on the pilot study results and, more specifically, the sd_y on the $sd_{ln(glucose)}$ = 0.3 and the $sd_{ln(HbA1c)}$ = 0.1 and the sd_x on the $sd_{MedDietScore}$ = 5.2, which were calculated from the six observations available and rounded to one decimal figure. Note that the (more

[i]δ is the effect size or the standardised difference; for the b test can be calculated using the formula $\delta = \frac{(b_a - b_0)\sigma_x}{\sigma}$, and for R^2 test using the formula $\delta = \frac{R^2}{1-R^2}$, where b_a is the slope of the alternative hypothesis, b_0 is the slope of the null hypothesis, R^2 is the *R*-squared of the model, σ_x is the standard deviation of the independent variable and σ is the standard deviation of the dependent variable (Cohen, 1988; StataCorp LLC, 2019c).

conservative) value of $sd_{ln(glucose)}$ was considered to be a better representation of the true value of the sd_y over that of $sd_{ln(HbA1c)}$ and, therefore, it was favoured as the inputted value of the sd_y (the values of 0.2 and 0.4 for sd_y, were also considered during the PSS analysis – data not shown). Furthermore, the PSS analysis for linear regression models assumes that the error terms (ε) are independently and normally distributed with a mean of zero and a constant standard deviation σ (Lenth, 2001; StataCorp LLC, 2019c). For this reason, and in combination with the fact that HbA1c and glucose variables were expected to be skewed towards the right, we decided to use the sd of their natural logarithm instead; the assumption of positive skewness for the HbA1c and the glucose variables is based on our clinical experience and the published data (Menke *et al.*, 2014). In contrast, no major skewness was expected for the independent variable (Kyriacou *et al.*, 2015) and, therefore, the value of the sd of the *MedDietScore per se* was inputted. At the same time, the number of independent variables inputted in the R^2 test, was nine, based on the more complex linear regression model of the primary outcomes, which had a predictor and eight covariates, or a total of nine independent variables. Finally the α value was set to 0.05 and STATA default iterations' settings for computation of the PSS models were used (StataCorp LLC, 2019c).

The PSS analysis, using the imputed parameters described above, indicated that the sample size range of 90 to 116 participants provided satisfactory power to detect the predefined smallest clinically significant effect size (*Figure 5.2* and *Figure 5.3*). More precisely, the estimated sample size that was required to meet all the set parameters was as follows:

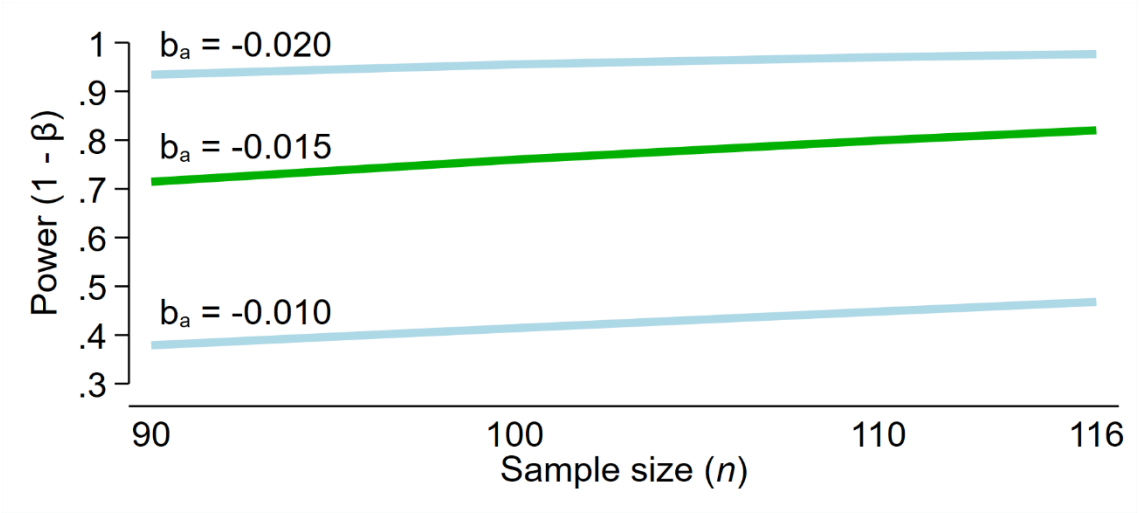
i. For the b test: if $b_a = -0.015 \mid sd_y = 0.3$, $sd_x = 5.2$, $\pi = 0.8$ and $\alpha = 0.05$ then $\tilde{n} \approx 111$ participants, where b_a is the alternative slope, sd_y is the standard deviation of the dependent variable, sd_x is the standard deviation of the independent variable, π is the power, α is the alpha value and ñ is the estimated sample size. Note that, as discussed before, the true value of sd_y for HbA1c could be smaller than the applied value of 0.3 and the clinically meaningful $| b_a |$ for the glucose could arguably be higher than the inputted value of - 0.015; both of which could indicate that the estimated sample size, $\tilde{n} \approx 111$, is higher than the true one, *n*, required.
ii. For the R^2 test: if $R^2 = 15\% \mid n_x = 9$, $\pi = 0.8$ and $\alpha = 0.05$ then $\tilde{n} \approx 98$ participants, where n_x is the number of independent variables, π is the power, α is the alpha value and $\tilde{n}$ is the estimated sample size.

The aimed sample size: After discussion between the members of the research team, and based on the above theoretical and practical considerations, it was agreed that the sample size that should be aimed for was that of 100 participants. At the same time, it was clearly stated that these 100 participants should fulfil the inclusion criteria and should have adequate data for their inclusion in the primary outcomes analysis. In practice, this meant that there was a chance to surpass the aimed sample size (n = 100 participants) by a small number of additional participants before we were able to verify these set criteria (specifically of that of C-peptide) for 100 participants and, consequently, the final recruited sample size could be slightly larger than that of the target.

Finally, the PSS analysis, using the imputed parameters described above, indicated that the agreed sample size of 100 participants provided satisfactory power to detect the predefined smallest clinically significant effect size (*Figure 5.4* and *Figure 5.5)*. More precisely, the estimated power, given the parameters set, was as follows:

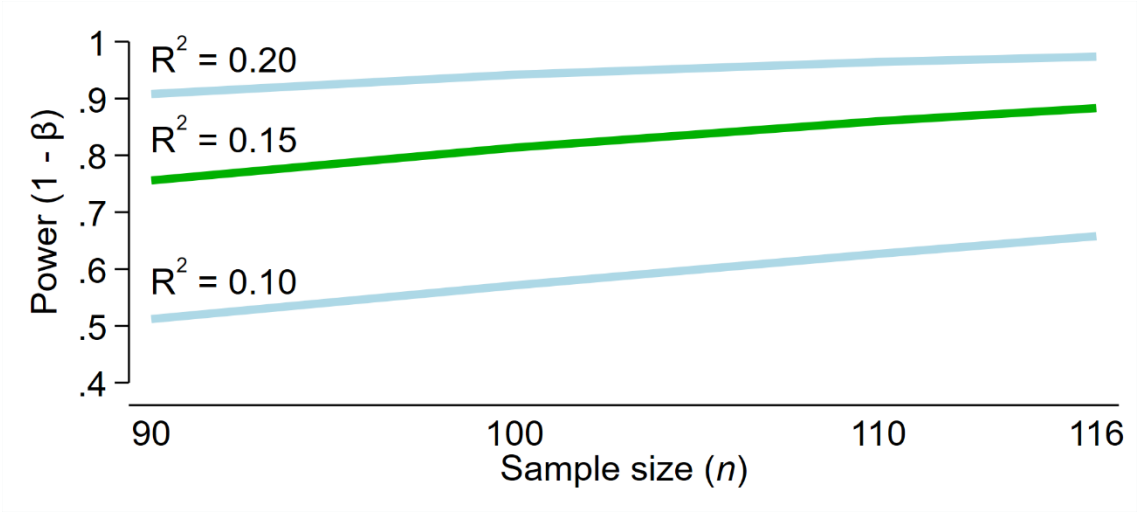
i. For the b test: if n = 100 participants | sd_y = 0.3, sd_x = 5.2, b_a = -0.015, α = 5%, then $\hat{\pi}$ = 76%, where n is the sample size, sd_y is the standard deviation of the dependent variable, sd_x is the standard deviation of the independent variable, b_a is the alternative slope, α is the alpha value and $\hat{\pi}$ is the estimated power. Similarly, as discussed above, the true value of sd_y for HbA1c could be smaller than the used value of 0.3 and the clinically meaningful | b_a | for the glucose could be arguable higher than the inputted value of - 0.015; both of which could indicate that estimated power, $\hat{\pi}$ = 76%, is lower than the true power, π, of the model.
ii. For the R^2 test: if n = 100 participants | n_x = 9, R^2 = 15% and α = 0.05 then $\hat{\pi} \approx 81\%$, where n is the sample size, n_x is the number of independent variables, α is the alpha value and $\hat{\pi}$ is the estimated power.

Figure 5.2: The power of a simple linear regression model for three given b_a within a sample size range



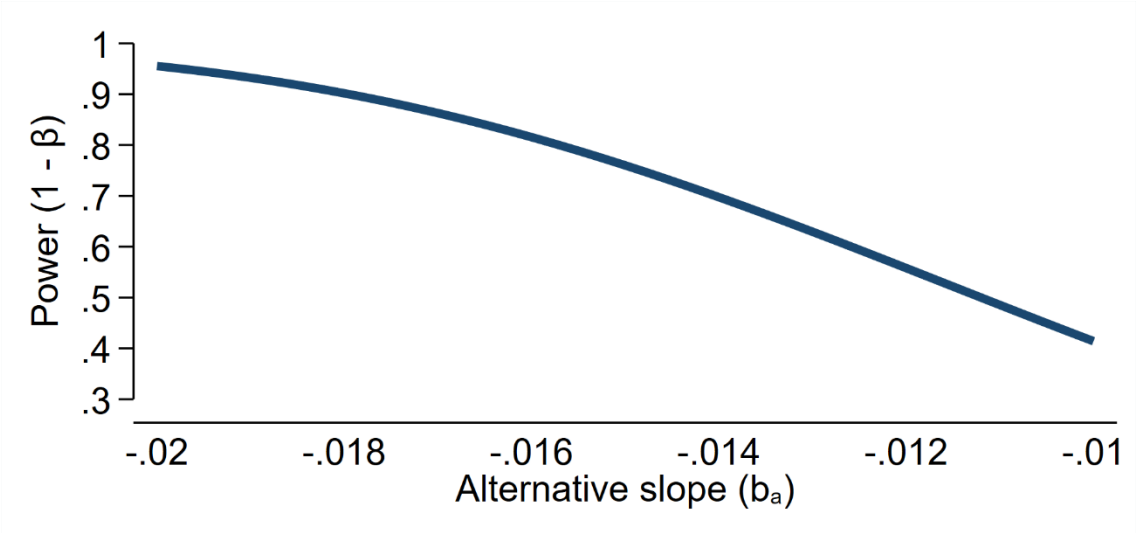
†Power for a two-sided test of H_0 : b = 0 versus H_a : b ≠ 0 with null slope b_0 = 0, alternative slopes b_a = -0.01 (δ ≈ -0.18), b_a = -0.015 (δ ≈ -0.27) and b_a = -0.02 (δ ≈ -0.37), covariate standard deviation $s_{MedDietScore}$ = 5.2, dependent variable standard deviation of $s_{ln(HbA1c)}$ = 0.3 or $s_{ln(glucose)}$ = 0.3, within a sample size range n = 90 to n = 116 and a significance level α = 0.05.

Figure 5.3: The power of a multiple linear regression model for three given R^2 within a sample size range



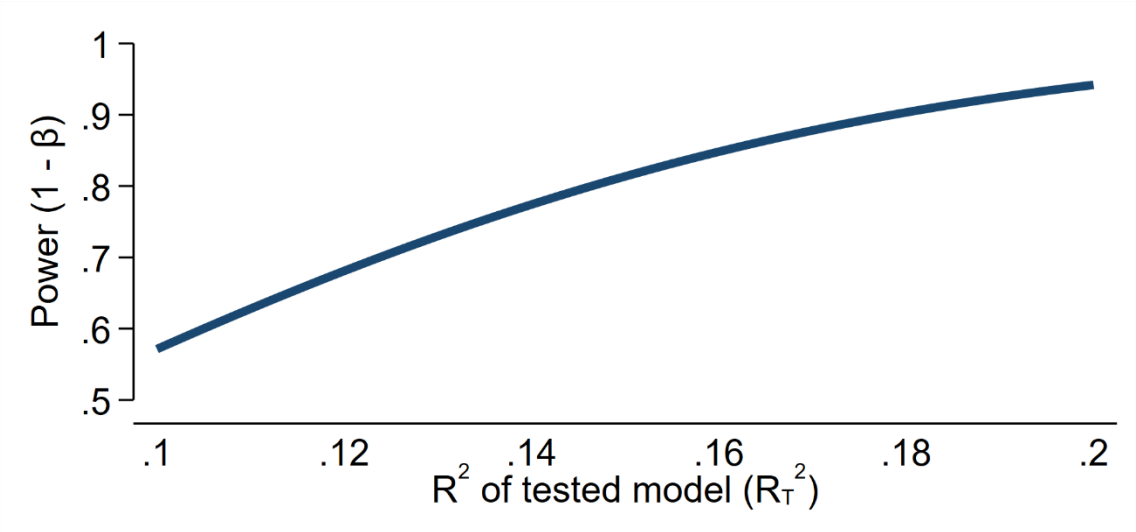
†Power for a test of H_0: R^2 = 0 versus H_a: R^2 ≠ 0 given alternative R^2 of 0.10 (δ ≈ 0.11), 0.15 (δ ≈ 0.18) and 0.20 (δ = 0.25) and nine tested covariates within a sample size range n = 90 to 116 and a significance level α = 0.05.

Figure 5.4: The power of a simple linear regression model for a given n = 100 within an alternative slope range



†Power for a two-sided test of H_0: b = 0 versus H_a: b ≠ 0 with null slope b_0 = 0, sample size *n* = 100, covariate standard deviation $s_{MedDietScore}$ = 5.2 and dependent variable standard deviation of $s_{ln(HbA1c)}$ = 0.3 or $s_{ln(glucose)}$ = 0.3, within an alternative slope range b_a = -0.01 (δ ≈ -0.18) to -0.02 (δ ≈ -0.37) and a significance level α = 0.05.

Figure 5.5: The power of a simple linear regression model for a given n = 100 within an R^2 range



†Power for a test of H_0 : R^2 = 0 versus H_a: R^2 ≠ 0 given a sample size *n* = 100 and nine tested covariates within an R^2 range *n* = 0.10 (δ ≈ 0.11) to 0.20 (δ = 0.25) and a significance level α = 0.05.

Data collection overview

This section provides a brief description of the data collected. The data were collected at CEDM (CEDM Centre of Endocrinology, Diabetes and Metabolism) by me as a dietitian (AK), an endocrinologist (DrAK) and a podiatrist (NG), straight after the *consent form* was signed. At a later date, the blood sample was collected in a pre-specified private clinical laboratory by a biochemist, while for the urine sample, the participants were provided with a sealed sterilised container. The participants were asked to visit the clinical laboratory during the morning hours in a fasting state; regarding the urine sample they were asked to fill the container with the first void of the day after waking up on the day they were visiting the clinical laboratory. The stored samples may further be analysed on a different date but, as yet, we have not carried out any analysis.

Clinical examination & measurements overview

Anthropometric data: The anthropometric data were collected by me (AK) at the CEDM, and included weight, height, waist circumference and body composition analysis, as described in the pilot study (*Chapter 4*).

Clinical examination: The clinical examination was undertaken by the endocrinologist (DrAK) at the CEDM and included blood pressure and presence or absence of lipohypertrophy and lipotrophy, as described in the pilot study (*Chapter 4*).

Foot screening: The foot screening was undertaken by the podiatrist (NG) at the CEDM. The foot screening consisted of (the following tests were executed for both the left and the right foot):

- pulse palpitation of dorsalis pedis artery
- pulse palpitation of tibialis posterior artery
- doppler ultrasound of dorsalis pedis artery
- doppler ultrasound of tibialis posterior artery
- feet sensation – using 10g monofilament
- claudication (presence or absence) – self-reported after prompt asking
- rest pain (presence or absence) – self-reported after prompt asking
- clinical examination (i.e., through feet inspection) including for the presence of atrophy, corns, calluses, nail dystrophy, blisters, swelling, tinea pedis, Charcot's foot, ulcers, cysts, other wounds, ingrown toenails, numbness, onychomycosis (OM), hallux abducto valgus (HAV) and other

Questionnaires

Questionnaires were delivered electronically (i.e., completed on a computer) at the CEDM; they were either self-administrated or help was provided by me if the participants were struggling with completing them, for example, if they had any difficulty using computers, or understanding the Greek language, etc. Further information on these questionnaires is presented in *Chapter 4* and the full set of questions are presented in *Appendix: Chapter 5*.

Blood and urine tests

The venous blood sample was drawn in a pre-specified private clinical laboratory by a qualified and highly experienced biochemist, and the following measures were taken:

- glycaemic control: glucose, glycated haemoglobin (HbA1c) and estimated glucose disposal rate (eGDR)
- β-cell function: C-peptide
- lipid profile: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (Tg)
- thyroid function tests (TFTs): thyroid stimulating hormone (TSH) and free T4 (fT4)
- liver function tests (LFTs): alanine transaminase (ALT) and aspartate transaminase (AST)
- renal function: urea, creatinine, albumin (*urine*), creatinine (*urine*), estimated glomerular filtration rate (eGFR) and albumin to creatinine ratio (ACR; *urine*)
- inflammatory markers: C-reactive protein (CRP)

Communication of results: The participants were provided with the clinical laboratory results in written format by the secretarial staff of CEDM and were encouraged to discuss them with their usual physician in charge of their diabetes management. Although no further explanation was provided, exception was given to the results that were particularly high, in which case the participants were urged to seek medical advice more urgently, that is, within days (high results were defined in the study protocol, data not shown).

Ethical considerations

Ethics approvals: This project received ethics approval from committees in both Cyprus and the UK. The ethics approval in Cyprus was granted by the Review Bioethics Committee for Biomedical Research on Human Beings and Their Biological Substances of The Cyprus National Bioethics Committee of The Republic of Cyprus

(*EEBK/EΠ/2016/09*). In the UK, the ethics approval was granted by the NHS, Invasive or Clinical Research Committee (NICR) of the University of Stirling, UK (*NICR 16/17 - Paper No.44*). Note that these ethics approvals are distinct from the ethics approval received for the pilot study of the main research project (*Chapter 4*).

Consent form: All participants signed the *consent form*, as approved by the ethics committees, after this was verbally explained by me and after being given adequate time to read the *information sheet* and ask questions. The process for data collection started strictly only after the participant had signed the *consent form*.

Blood and urine storage: The participants signed a distinct consent form providing permission for us to store the spot blood and urine collected at the clinical laboratory for this study. The blood and urine samples are currently locked in secure storage in a clinical laboratory that has appropriate facilities, and these may be used for future further testing, as specified on the *consent form* and as approved by the ethics committees.

Compensation: The participants received no compensation, financial or otherwise, for participating in the study. In practice, the free blood and urine tests executed in a private clinical laboratory were perceived by many of the participants as a further motive to contribute to the current study.

Disclosure summary: This research was self-funded by the CEDM in Cyprus with an additional grant from the University of Stirling, UK (e.g., laboratory tests, Cyprus ethics approval fee, software development by IT and other resources needed). The team members who assisted (e.g., the endocrinologist, podiatrist, etc.) were not compensated, financially or in any other way, but rather, they contributed by investing their own valuable time and resources.

Outcomes

The outcomes were sub-grouped into primary outcomes, which were strictly predefined including their methodology, and secondary outcomes, which were either predefined or specified *post hoc*.

Primary outcomes

This main study aimed to investigate adherence to the Mediterranean diet and glycaemic control and the association between the two. The primary outcomes and hypotheses of the current project were defined as follows:

1. The null hypothesis that the level of adherence to the Mediterranean diet, as measured by *a priori* Mediterranean diet score, does not affect (positively or negatively) glycaemic control, as measured by HbA1c and fasting blood glucose, independently.
2. To estimate the level of adherence to the Mediterranean diet as measured by *a priori* Mediterranean diet score.
3. To assess glycaemic control, as measured by the HbA1c and fasting blood glucose.

A priori Mediterranean diet score: The Mediterranean diet score used was the *MedDietScore* scoring system. Data used for the calculation of the *MedDietScore* score were collected through the FFQ component of the questionnaire.

Glycaemic control: Glycaemic control can, theoretically, be measured independently through the related tests of HbA1c and that of blood glucose. Although both measurements are useful indicators of glycaemic control, in practice, they provide somewhat different information, especially as here we are concerned with single fasting measurements performed in a clinical laboratory. The HbA1c provides an overall picture of glycaemic control over the last three months, whereas fasting glucose is more likely to give a picture of its variability (Makris and Spanou, 2011; American Diabetes Association, 2019d).

Statistical methodology: The statistical calculations were predefined, including the regression models and covariates.

Secondary outcomes

The large amount of data collected in this study allowed its potential use for purposes other than for the investigation of the primary outcomes; namely, the secondary outcomes. The examined secondary outcomes are reported below and were either predefined or detailed after the current study was completed. Note that, although further secondary outcomes were considered, these results (where available) are not reported in the current thesis as they are beyond its purpose.

Secondary Outcomes considered in the current thesis

Predefined:

- Gender (male or female) versus adherence to the Mediterranean diet (*MedDeitScore*, points).
- Gender versus glycaemic control (HbA1c, mmol/mol; glucose, mg/dl).

- Age (years) versus adherence to the Mediterranean diet.
- Age versus glycaemic control.

Post-hoc:

- Demographic characteristics (as measured by the *Demographic characteristics questionnaire*) versus adherence to the Mediterranean diet
- Demographic characteristics versus glycaemic control

Data collection, processing and presentation

The data collection process and related methodology was described in *Chapter 4*. Only changes to these methods and parameters of interest are described here.

Primary outcomes processing and presentation

Adherence to Mediterranean diet

Adherence to the Mediterranean diet was measured using the *a priori MedDietScore* scoring system by analysing data collected through the FFQ component of the questionnaire. The *MedDietScore* score could range from zero to 55 with a higher score indicating better adherence to the Mediterranean diet. These data (of the adherence to the Mediterranean diet) are presented as continuous and categorical variable in tables, and graphically. The continuous variable is presented using various descriptive statistics – mean and standard deviation, median and interquartile range, min and max – and, in a *post hoc* analysis, the tolerance limits were also reported, irrelevant of normality, to allow for a more detailed presentation of this epidemiological outcome. The participants' adherence to the Mediterranean diet was also presented as a percentage, for example, a *MedDietScore* score of 55 points indicated perfect adherence to the Mediterranean diet (100%) and a *MedDietScore* score of 0 points indicated a perfect deviation from the Mediterranean diet with 0% adherence, calculated using the following equation:

$$MedDietScore\ (\%) = \frac{MedDietScore\ (points)}{55} \times 100\ (\%)$$

The score was also categorised to low, moderate and high adherence by dividing the (maximum) score into three equal parts as shown in *Table 5.1a* and resembles the cut-off points used in Chapter 3 (for presentation purposes only). Finally, adherence to the Mediterranean diet is graphically presented as a spike plot – a frequency plot in which

the frequencies are depicted as vertical lines from zero – with relevant cut-off and reference points.

Table 5.1: Categorisation of MedDietScore score and glycaemic control

Table 5.11: Categorisation of MedDietScore score

Adherence to Mediterranean diet	Cut-off points	*MedietScore* score	
		Points*	%*
Low	< 1st	< 18.33	< 33.33
Moderate	≥ 1st and ≤ 2nd	≥ 18.33 and ≤ 36.67	≥ 33.33 and ≤ 66.67
High	> 3rd	> 36.67	> 66.67

*rounded to 2 decimal places

Glycaemic control

Glycaemic control was quantified through HbA1c (mmol/mol) and fasting plasma glucose (mg/dl) measurements. These data (for HbA1c and glucose) are presented as continuous and categorical variables, and graphically. The continuous variable is presented using various descriptive statistics – as described for the *MedDietScore* – irrelevant of normality, to allow for a more detailed presentation of this epidemiological outcome. These variables are reported in units as provided by the clinical laboratory, mg/dl for glucose and mmol/mol (IFCC unit), but also in mmol/l for glucose and % for HbA1c (DCCT unit). The data were also categorised based on the relevant NICE (NICE, 2015b) and ADA guidelines (American Diabetes Association, 2019d) as shown in *Table 5.1b* and *Table 5.1b* (for presentation purposes only). Finally, the glucose and HbA1c results are graphically presented as a histogram and a kernel density estimate plot with relevant cut-off and reference points, and the smoothing parameters are also reported (starting point, width and number of bins for histogram, and kernel function and bandwidth, and number of points evaluated for kernel density estimate plot).

Table 5.12: Categorisation of glucose, fasting

Glucose category	Reference	*Glucose levels*	
		mg/dl	mmol/l
Hypoglycaemic	ADA, 2019b	< 70	< 3.9
Normoglycaemia	ADA, 2019b	≥ 70 and ≤ 130	≥ 3.9 and ≤ 7.2
Hyperglycaemia	ADA, 2019b	> 130	> 7.2

Table 5.13: Categorisation of HbA1c

HbA1c category	Reference	*HbA1c levels*	
		mmol/mol	%
Optimal	NICE, 2015b	< 48	< 6.5
Optimal	ADA, 2019b	< 53	< 7.0
Suboptimal	NICE, 2015b	≥ 48	≥ 6.5
Suboptimal	ADA, 2019b	≥ 53	≥ 7.0
Poor control	(Scottish Diabetes Data Group, 2018)	> 75	> 9.0

Adherence to Mediterranean diet and glycaemic control modelling

The association between adherence to the Mediterranean diet, as measured by the *MedDietScore*, and glycaemic control, HbA1c and glucose, was investigated through three *a priori* OLS models. The three models are presented in *Table 5.2* and are described as follows:

Model 1: This was a simple OLS model with *MedDietScore* as the predictor and the HbA1c or glucose as the dependent variable. No covariates were added to this model.

Model 2: This multivariable model had the same predictor and dependent variable as Model 1 but with the addition of two covariates; gender and age.

Model 3: The second multivariable model was as Model 2, but with the addition of six additional covariates, that of BMI, C-peptide, household income, injection method, smoking status, and insulin adjustment to carbohydrate intake.

The covariates for each model were pre-specified before the study commenced. The selection process was challenging, due to the large amount of data collected during the study and consequently the large number of potential influential factors that could be added in the model. The final list of covariates (*Table 5.2*) was agreed after reaching consensus between Dr. AK and me – this was based on clinical experience of significant factors that influence the medical, endocrinological and dietetic aspect of the discussed relationship, on factors that other researchers have used as covariates in related published work, on the results of the pilot study (*Chapter 4*), and also on appropriate modelling. The latter was the obstacle of using a large number of covariates, which would have reduced the degrees of freedom preserved so that the models could no longer be generalised. Previous studies, using Monte-Carlo simulations, have suggested that, for linear models, a minimum of 10 to 15 observations per independent variable are generally needed so as to allow good estimates of the true values (Babyak, 2004; Harrell, 2015); therefore, we tried to adhere to this principle. Approaches used to achieve this included methods such as giving preference to variables that may indirectly (or directly) be a combination of other factors, for example, the BMI was assumed to be a projection of physical activity, sedentary lifestyle, energy intake and insulin dosage; for categorical variables, categories that are clinically meaningful and were expected to have sizable size (observations per cell) were chosen, for example, for household income, the categories were reduced from five to two (i.e., 4 dummy variables to 1 variable). It is fair to note that, through these methods, specific information about the components of the index are lost, however, degrees of freedom are preserved (Babyak, 2004; Harrell, 2015) and, unfortunately, this is a trade-off that is often needed for the models to produce results that can be confidently relied upon. Furthermore the covariates were pre-specified, as this method is often considered superior by many statisticians and other experts (Babyak, 2004; Harrell, 2015). Other methods, such as the stepwise approach or univariate pre-screening of predictors or pulling variables in and out of a model to see which produce the best fit, were avoided because they are not recommended (Babyak, 2004; Harrell, 2015), whereas more sophisticated methods, such as LASSO and ridge regression where considered as being too complex for the needs of the current models and, as mentioned before, these methods are considered to be inferior to the method of defining *a priori* the dependent variables.

Table 5.2: Prediction of glycaemic control by adherence to Mediterranean diet: Models and variables

Model variables	**Name of variable**	**continuous *or* categorical**	**units [continuous] *or* categories [categorical]**	**Model 1**	**Model 2**	**Model 3**	**Comments**
Dependent	Glycaemic control						
	HbA1c	continuous	mmol/mol (%)	+	+	+	
	or glucose, fasting	continuous	mg/dl (mmol/l)	+	+	+	conditional on glucose ≥ 70 mg/dl (3.9 mmol/l) on clinical grounds
Predictor	Mediterranean diet	continuous	points	+	+	+	measured by the *MedDietScore* scoring system
Covariate 1	gender	categorical	male | female		+	+	
Covariate 2	age	continuous	years		+	+	
Covariate 3	BMI	continuous	kg/m^2			+	BMI = weight [kg] / (height)^2 [m^2]
Covariate 4	C-peptide	categorical	undetectable | detectable			+	C-peptide assay lower limit of detection = 0.010 ng/ml
Covariate 5	household income	categorical	≤ 2000€ | > 2000€			+	includes that of the spouse if applicable
Covariate 6	injection method	categorical	MDI | CSII			+	MDI: Multiple Daily Insulin Injections; CSII: Continuous Subcutaneous Insulin Infusion
Covariate 7	smoking status	categorical	current | other			+	other = ex-smoker, non-smoker, occasional smoker that is less than 1 cigarette per day
Covariate 8	insulin adjustment to carbohydrate intake	categorical	no | yes			+	self-reported and does not necessarily reflect an appropriate adjustment

Questionnaires and questionnaire derived parameters

Four questionnaires were completed by the participants *(Appendix: Chapter 5)*, namely, the *demographic characteristics questionnaire*, *medical and diabetes questionnaire*, food frequency questionnaire (FFQ) and the *International physical activity questionnaire* (IPAQ). These questionnaires were delivered electronically, where the data were automatically transformed, stored in a convenient format and fed to algorithms to compute new variables of interests, such as the *MedDietScore* score.

Demographic questionnaire

The variables of *gender* and *age* were inputted in the multivariable *Model 2* and *Model 3* as covariates. *Age* was available in years rounded to the nearest integer. The variable of *household income* was also inputted in the multivariable *Model 3* as a covariate. The household income was defined as the income of the participant and of their spouse, if applicable. It comprised five categories (*0 – 10,000*; *10,001 – 20,000*; *20,001 – 30,000*; *30,001 – 60,000*; and ≥ *60,001* euros per year) which were reduced to two (≤ *20,000* and > *20,000* euros per year) for the purposes of the regression model. The cut-off point of the new dichotomous variable was based on the results of the pilot study (*Chapter 4*), personal experience and official data on the average earnings in Cyprus (Statistical Servvice of Republic of Cyprus, 2019); for all other purposes, the non-reduced version of the variable was used. Finally, in the case of *ethnicity*, the term of *White-Caucasian* was used, although is fair to note that this term is ill-defined and has long been debated for its usefulness (Bhopal, 2004; Braun *et al.*, 2007), though widely used in science, including medicine and health sciences (Bhopal, 2004).

Food frequency questionnaire

The food (and drink) intake and the utilisation of cooking methods were quantified using a semi-structured FFQ, a modified version of a previously validated FFQ in the Greek population (Katsouyanni et al., 1997), as discussed above.

MedDietScore scoring system (adherence to the Mediterranean diet)

The FFQ data were fed to an algorithm that was run by the software in order to produce a score for each component of *MedDietScore*, and, consequently, a total score for the *MedDietScore* was calculated for each participant. Changes made after the pilot study were:

I. The conversion factors between the number of servings per day, week and month were modified so as to allow for interchangeability while these remained integer numbers (for simplicity) as shown in the box below and in the consequent algorithm (*Appendix: Chapter 5*).

$$n\ (serving\ per\ day) = \mathbf{7}\ n\ (serving\ per\ week)$$

$$n\ (serving\ per\ day) = \mathbf{28}\ n\ (serving\ per\ month)$$

$$n\ (serving\ per\ week) = \mathbf{4}\ n\ (serving\ per\ month)$$

where: *n* is the number of serving and in bold the conversion factors

II. The authors of the *MedDietScore* scoring system kindly provided us with an unpublished protocol (Panagiotakos D, 2018, personal communication, 19 November) that provided a slightly more rigid definition of the food groups (protocol not shown) in terms of portions and individual foods, although, again, it was not always clear cut. Therefore, the algorithm was modified to better reflect this protocol to a weighted summation of (an updated list of) individual foods and this is shown in the consequent algorithm (*Appendix: Chapter 5*).

III. The scoring system used previously (*Table 4.1* and *Table 4.2*) required that food group (or components of the *MedDietScore*) servings were rounded to the nearest integer number to prevent the algorithm from collapsing. This had led to two noticeable consequences – some scores became mathematically impossible to achieve and there was a suggestion of misclassification. To overcome this, the *MedDietScore* scoring system was further adapted in order to cover the gaps, by moving the higher bound of the lower number up to lower bound of the higher number. This newly adapted *MedDietScore* is shown in *Table 5.3* and in the consequent algorithm (*Appendix: Chapter 5*).

IV. The alcoholic beverages component was converted to servings per day (and not servings per week) as in the consequent algorithm (*Appendix: Chapter 5*).

Table 5.3: Further adapted MedDietScore scoring system

Servings / week / Food category	Points					
	0	1	2	3	4	5
Non-refined cereals	< 1	≥ 1, < 7	≥ 7, < 13	≥ 13, < 19	≥ 19, ≤ 32	> 32
Potatoes	< 1	≥ 1, < 5	≥ 5, < 9	≥ 9, < 13	≥ 13, ≤ 18	> 18
Fruits	< 1	≥ 1, < 5	≥ 5, < 9	≥ 9, < 16	≥ 16, ≤ 22	> 22
Vegetables	< 1	≥ 1, < 7	≥ 7, < 13	≥ 13, < 21	≥ 21, ≤ 33	> 33
Legumes	= 0	> 0, < 1	≥ 1, < 3	≥ 3, < 5	≥ 5, ≤ 6	> 6
Fish	= 0	> 0, < 1	≥ 1, < 3	≥ 3, < 5	≥ 5, ≤ 6	> 6
Red meat and products	> 10	≥ 8, ≤ 10	≥ 6, < 8	≥ 4, < 6	≥ 2, < 4	< 2
Poultry	> 10	≥ 9, ≤ 10	≥ 7, < 9	≥ 5, < 7	≥ 4, < 5	< 4
Full fat dairy products	> 30	≥ 29, ≤ 30	≥ 21, < 29	≥ 16, < 21	≥ 11, < 16	< 11
Use of olive oil in cooking[a]	= 0, Never	= 0, Rare	> 0, < 1	≥ 1, ≤ 3	> 3, < 7	≥ 7
Alcoholic beverages[b]	> 7 or = 0	≥ 6, ≤ 7	≥ 5, < 6	≥ 4, < 5	≥ 3, < 4	< 3, > 0

[a]Time / week or 1 serving = 1 time [b]Serving / day, where 1 serving = 100 ml of wine or 12 g ethanol for other alcoholic beverages.

Estimated energy and nutrient intake

The use of an FFQ has allowed us to estimate the intake of energy and selected nutrients of the participants, as named in the box below. These were calculated using information available from composition tables.

Energy

Macronutrients: fat, protein, carbohydrates, dietary fibre, total sugars, saturated, mono- and polyunsaturated fatty acids, and ethanol.

Micronutrients: vitamin C, sodium and iodine.

Nevertheless, these were beyond the scope of the thesis and the results are not presented here. Despite this, the calculations and results are available on request.

Medical and diabetes questionnaire

The *medical and diabetes* questionnaire was used to collect data on the participants' epidemiological characteristics, their use of insulin and other medications and their clinical history, including the presence of micro- and macro-vascular complications and other co-morbidities.

The dichotomous variables of *MDI versus CSII*, *smoker versus non-smoker*, and *adjusting versus not-adjusting insulin dose to carbohydrate intake* were inputted in the

multivariable *Model 3* as covariates and were based on the answers of questions five, 15 and six, respectively. The question related to smoking allowed for four answers; namely, *smoking*, *never smoked*, *ex-smoker*, and *occasional smoke*r (where each category was well defined in the questionnaire), which were reduced to two, namely *smoking* and *not-smoking* for the purpose of the regression model. In the two variables, the *smoking* category was identical, whereas the *non-smoking* category in the reduced variable was the sum of the participants who reported that they had *never smoked*, were *ex-smokers* and *occasional smokers*. More precisely, the new variable (based on the definition of these terms) distinguished the participants who were smoking at least one cigarette per day, at or up to a year before, at the time of completion of the questionnaire versus the participants who did not. In regard to the *dose adjustment* variable, a *Yes* answer has the limitation that it does not necessarily reflect an appropriate adjustment of insulin dose to carbohydrate intake and other factors. In addition, note the absence of formal structured education programmes, such as the DAFNE in the UK, and of local guidelines on structured education, dose adjustment and in general on nutrition for Type 1 diabetes, in Cyprus. Furthermore, it is worth noting the variable dietary advice provided by doctors and health care professional in Cyprus. Nevertheless, to my knowledge the attempt to correct the examined relationship for this potentially significant confounding factor is a novelty of our study.

Anthropometry

The anthropometric data were the weight, the height and the waist circumference. The methodology was the same as described in the pilot study (*Chapter 4*).

BMI is an obesity index and was inputted in the multivariable Model 3 as a covariate. The BMI was calculated as follows:

$$BMI = \frac{Weight}{Height^2}$$

where the weight is measured in Kg and the height in metres and, consequently, the units of BMI is in Kg/m^2. BMI is a widely used tool and is categorised as shown in *Table 5.4* (Akram *et al.*, 2000; Gibson, 2005; NICE, 2014c; Lovegrove, Sharma and Hodson, 2015).

Table 5.4: BMI classification

BMI (Kg/m^2)	Classification	Risk of comorbidities
< 18.50	Underweight	Low*
18.50 – 24.99	Healthy weight	Average
25.00 – 29.99	Overweight	Increased
≥ 30.00	Obese:	
30.00 – 34.99	Obese class I	Moderate
35.00 – 39.99	Obese class II	Severe
≥ 40.00	Obese class III	Very severe

*but the risk of other clinical problems is increased.
BMI, body mass index.

Bioelectrical Impendence Analysis (BIA)

The BIA measurement provides an estimation of the body composition of the tested participant. The results recorded were the estimated body fat and lean mass, and water (measured in % and Kg or L), suggested targets (in %) and the resting metabolic rate (RMR). The suggested targets (or healthy range) provided by the BIA device used (Maltron BF-907; Essex, UK) change depending on the age, gender and ethnicity (and on other factors) of the tested participant, but they are not widely available. Although I contacted the company (Maltron International) on several occasions through different means (by phone and email) and, despite their promise to provide us with these suggested target ranges, unfortunately, they failed to do so.

The *American association of clinical endocrinologists and American college of endocrinology* (AACE/ACE) and others suggest cut-off points for body fat of 20 – 25% for men and 30 – 35% for women (Mizrahi-Lehrer, Cepeda-Valery and Romero-Corral, 2012). However, there is not enough scientific evidence to justify these cut-off points, and, to my best knowledge, there are no internationally accepted cut-off points (Gallagher *et al.*, 2000; Ho-Pham, Campbell and Nguyen, 2011; Mizrahi-Lehrer, Cepeda-Valery and Romero-Corral, 2012). This is reflected in most guidelines, which refrain from providing any recommendations (Mizrahi-Lehrer, Cepeda-Valery and Romero-Corral, 2012; Lovegrove, Sharma and Hodson, 2015), such as the NICE guidelines (NICE, 2014c).

Based on all of the above, the measurement of body fat is preferable to be considered as a sex-specific continuous variable in future statistical analysis and, therefore, eliminating

the need for such cut-off points. Nevertheless, the cut-off points suggested by the AACE/ACE could be used to supplement the presentation of the BIA results (but not their statistical analysis) as their limitations are clearly stated.

Blood and urine tests

The participants were asked to visit the clinical laboratory during the morning hours in a fasting state. The methodology was the same as that used in the pilot study (*Chapter 4*). Furthermore, these spot blood and urine samples (in contrast to the pilot study) were stored in the clinical laboratory, provided that the participant consented by signing the c*onsent to store the blood and urine sample* form. These samples may be used in the future for further testing, as described in *Table 5.5*.

HbA1c and glucose are measures of glycaemic control. More importantly, it is well-established in Type 1 diabetes (and generally in diabetes care) that a sub-optimal glycaemic control correlates well with diabetes-related complications and adverse health outcomes, such as quality of life and mortality (Lind *et al.*, 2014; Nathan, 2014; NICE, 2015b; Gubitosi-Klug *et al.*, 2016). Therefore, the HbA1c and glucose measurements were inputted as the dependent (or outcome) variables in distinct OLS models, which have been described previously. Furthermore, they were used to provide epidemiological evidence of diabetes control of the studied population; for this reason, they were also presented as categorical variables, also as defined previously. Note that, in the OLS models of glucose, it was pre-specified that only the participants with a glucose measurement above the hypoglycaemic range (glucose ≥ 70 mg/dl [3.9 mmol/l]) would be considered – the rationale was that a fasting glucose in the hypoglycaemic range more likely reflects the incorrect use of insulin (i.e., excess dose of long-acting insulin), instead of reflecting glycaemic control as such, although, on occasion, it may be susceptible to other variables, for example, late evening exercise and missed meals. Moreover, as glucose increases above 70 mg/dl, we can roughly say that glycaemic control deteriorates but this principle is not valid below 70 mg/dl.

The dichotomous variable of *detectable* versus *undetectable C-peptide* was inputted in the multivariable Model 3. The C-peptide was a special case of variable, which, although it was measured as a continuous variable, in the OLS model was inputted as a categorical variable. Although, it is often considered inappropriate to categorise continuous variables (Babyak, 2004; Harrell, 2015), in the case of C-peptide, this was considered unavoidable,

as a number of blood samples with undetectable levels of C-peptide were expected; this was based on the pilot study results – where C-peptide was undetectable in all six of the samples provided – and the relevant literature (Wang, Lovejoy and Faustman, 2012), but also from a clinical perspective, it makes sense to break down C-peptide to *very low*, indicating severe insulin deficiency, and *low but detectable*, indicating some degree of residual secretion. More precisely, if these values were to be treated as zero, this could cause the following problems: i. the expected skewed data, when logged-transformed, similarly to previous studies (Oram *et al.*, 2015; Shields *et al.*, 2018) a significant number of observations would be lost (the natural logarithm of zero is undefined); and ii. based on research where more sensitive methods (more sensitive assays and stimulation methods) were used (Wang, Lovejoy and Faustman, 2012; Oram *et al.*, 2014, 2015), these values, although below the detection level, were more likely not zero, even for participants with long-standing Type 1 diabetes. Therefore, to overcome these problems, this variable was categorised with a cut-off point of the C-peptide assay lower limit of detection, which was equal to 0.010 ng/ml; consequently, a detectable C-peptide indicated a C-peptide $\geq$ 0.010 ng/ml and an undetectable C-peptide a C-peptide $<$ 0.010 ng/ml.

Table 5.5: Potential blood, urine and genetic tests on stored blood and urine samples

Sample category	Test name	Clinical significance*
Blood sample	Lp(a)	Lipoprotein, CVD risk
	apoB	Lipoprotein, CVD risk
	hs-CRP	Inflammation, CVD risk
	cortisol	Addison's disease, Cushing's syndrome
	ICA	Autoantibodies, Type 1 diabetes
	IA-2A	Autoantibodies, Type 1 diabetes
	GAD	Autoantibodies, Type 1 diabetes
	Zn T8	Autoantibodies, Type 1 diabetes
	anti-TPO	Thyroid autoimmune disease
	ATAs	Thyroid autoimmune disease
	Tg	Thyroid cancer marker
	ATA	Coeliac disease
Urine sample	UIC	Iodine status
Genetic testing	HLA	Autoimmune disease, Type 1 diabetes
	GCK	Monogenic diabetes
	HNF1a	Monogenic diabetes
	HNF4a	Monogenic diabetes
	SIRT1	Monogenic diabetes

*this is not intended to be a full list.

hs-CRP, high-sensitivity C-reactive protein; CVD, cardiovascular disease; apoB, apolipoprotein B; Lp(a), lipoprotein(a); Tg, thyroglobulin; ATAs, anti-thyroglobulin antibodies; ICA, islet cell cytoplasmic autoantibodies; IA-2A, insulinoma associated-2 autoantibodies; GAD, glutamic acid decarboxylase; ZnT8 antibodies, zinc transporter 8 antibodies; anti-TPO, anti-thyroid peroxidase antibodies; ATA, anti-transglutaminase antibodies; UIC, urinary iodine concentration; HLA, human leukocyte antigen; GCK, glucokinase; HNF1a, hepatocyte nuclear factor 1-alpha; HNF4a, hepatocyte nuclear factor 4-alpha; SIRT1, silent mating type information regulation 2 homolog.

Podiatric screening

The foot screening was an addition to the main study and aimed to detect the present (or absence) of diabetic neuropathy, peripheral artery disease (PAD) and of other foot problems. The results were recorded in a pro forma (pro forma not shown).

Pulse palpitation: The dorsalis pedis (DP; between the 1st and 2nd metatarsals and lateral to the extensor halluces longus tendon) and posterior tibial (PT; behind the medial malleolus, and about halfway between the malleolus and the Achilles tendon) artery pulses (Deery and Guzman, 2018) in each leg were palpated. The presence or absence of DP and PT pulse (in each leg) was then recorded.

Doppler ultrasound: The pattern of the waveform of DP and PT was assessed using a Doppler ultrasound (Huntleigh Dopplex D900; Cardiff, UK) in audible form. The presence of monophasic, biphasic or triphasic Doppler waveform of DP and PT (in each leg) was recorded.

10g monofilament: The 10g monofilament (Bailey 10-g Retractable Monofilament; Manchester, UK) was applied to ten different points on the plantar and dorsal surface of the foot, while the participant's eyes were closed (the foot sites and methodology followed was as per Mishra *et al.*, 2017). The number of times that the monofilament was felt (participants responded with a *yes* each time) in each foot was then recorded.

Claudication and rest pain: The participants were asked for a history of intermittent claudication and rest pain. The presence or absence of claudication and/or rest pain was recorded.

Feet inspection: The foot was inspected for the presence of atrophy, corns, calluses, nail dystrophy, blisters, swelling, tinea pedis, Charcot's foot, ulcers, cysts, other wounds, ingrown toenails, numbness, onychomycosis (OM), hallux abducto valgus (HAV) or any other visible abnormality/pathology. The presence of any of the above was recorded in the pro-forma.

Statistics

Distribution and normality

A combination of visual methods, descriptive statistics and more robust statistical tests were considered and combined to examine the distribution of variables and residuals (e). The distribution was initially evaluated by means of the descriptive statistics of skewness

and excess kurtosis, and by comparing the values of median and 10% trimmed mean. Furthermore, the visual method of histogram and the more robust Shapiro-Wilk W test for normality were employed. Thereafter, if an approximately normal distribution could not be assumed then the distribution was further assessed using the skewness and kurtosis test. In the case of the e, the diagnostic plots, namely the Quantile-normal (Q-Q) plot (i.e., the quantiles of the variable were plotted against the quantiles of the normal distribution) and the normal-probability (P-P) plot (i.e., a standardized normal probability plot), were additionally used. In the case of variables or e that could not be assumed to have a normal distribution, some form of action was required, which was similar to those described in the *outliers* section. These methods and the steps taken if a normal distribution could not be assumed, are described in more detail in *Appendix: Chapter 5*.

Outliers

All data were inspected for the presence of outliers, univariate and bivariate as required. The variables were examined for outliers using a boxplot graph and the STATA community-contributed *iqr* command. Once the outliers were identified, they were classified into mild or severe outliers. Furthermore, the data were inspected for the bivariate presence of extreme observation (i.e., outliers and leverage points) through a scatterplot of the two variables, and the community-contributed *BACON* command. Mild outliers are not uncommon in data samples and thus action was considered only if at least two mild outliers were present. The presence of any severe outlier should be sufficient evidence to reject normality at a 5% significance level as they lie far out enough to have a substantial effect on the mean, standard deviation, and other classical statistics and thus action was always considered necessary. In such a case, a treatment method was considered and included the options: i. to transform the variable, primarily through the use of the natural logarithm (ln), ii. to perform the data analysis (or the statistical inference test) with and without the outlier(s), and iii. the use of a non-parametric method or a different estimator that was less sensitive to outliers; if any of these options was used, the method and the results were clearly stated. These outliers-related methods (including the use of variable transformations) are described in more detail in *Appendix: Chapter 5*.

Statistical significance

The level of significance used through this project for a statistical inference of a hypothesis on a parameter (θ), including that of testing for normality, was 5%. Therefore,

a *p*-value < 0.05 indicated that the null hypothesis (H_0) could be rejected, in favour of the alternative hypothesis (H_a), while if at a *p*-value ≥ 0.05 the H_0 could not be rejected.

Statistical tests

Regression

Ordinary least-squares (OLS) linear regression

The OLS regression can be used to estimate the linear association between the dependent variable (Y) and the independent variables (X; or, essentially, to obtain estimates (b) of the population coefficients (β)); thereafter, the hypotheses with a H_0: $b_1 = b_2 = \ldots = b_n = 0$ and an H_a: at least one $b \neq 0$ (F statistic), and with a H_0: $b_p = 0$ and an H_a: $b_p \neq 0$ (t statistic) can be tested (Peacock and Peacock, 2011; Gareth *et al.*, 2014). The linear regression with the OLS estimator was used when the examined outcome was a continuous variable (i.e., HbA1c and glucose) and the assumptions of an OLS model were met. Initially, a simple linear regression model was fitted, $E(Y|X = x) = \beta_0 + \beta_1 x_1 + \varepsilon$, that was followed by a multiple regression model, $E(Y|X = x) = \beta_0 + \beta_1 x_1, \ldots, \beta_n x_n + \varepsilon$. The b_p and the corresponding 95% confidence interval (CI) and t-statistic, and the model's R^2, adjusted R^2 and *F* statistic were reported. The OLS linear regression comes with several assumptions that if are not met, the estimated b_p and test statistics (standard errors ($SE(b_p)$)) become unreliable (Harrell, 2015). This, in addition to the OLS regression model being utilized in the primary outcome hypothesis, meant that considerable attention was given on predefining the methods of investigating and meeting the assumptions and other model requirements, and also in using sound modeling techniques. These (assumptions) are discussed in *Appendix: Chapter 5*.

Robust Methods

Robust standard errors

Homoscedasticity is one of the main assumptions of the OLS regression. An OLS model suffers from heteroscedasticity if the $Var(e_i|X) \neq \sigma^2$ (variance of the residuals given X), where σ^2 is a constant (Gareth *et al.*, 2014; Wooldridge, 2015). If the model is diagnosed with heteroscedasticity, the estimation of the $SE(b_p)$ becomes unreliable although the b_p itself remains unaffected (Mendenhall and Sincich, 2012). When other methods, for example transforming variables, had failed to correct the problem, then the robust standard errors (or White-corrected standard errors) method with the Huber-White sandwich estimator, was used. In simple words, in robust standard error regression, the residuals e_i are calculated for each observation and then added to the variance-covariance

matrix, instead of the σ^2. Then, through this matrix, the heteroscedastic-robust Var(b_p), SE(b_p) and relative test statistics are calculated (Wooldridge, 2015).

Robust-to-outliers estimator

In regression analysis, the presence of outliers, leverage points and influential observations in the dataset can strongly distort the OLS estimator and lead to unreliable results. To deal with this, several robust-to-outliers estimators have been proposed in the literature. In this main study, the MM-estimator of regression was preferred if the data were contaminated with extreme values, due (among other reasons) to its high breakdown point, high efficiency, low bias and high resistance to all types of unusual outliers, compared to other methods (Verardi and Croux, 2009; Susanti *et al.*, 2014). An MM-estimator of regression is a robust fitting approach that minimizes a ρ function – in this case, the Tukey biweight function – of the regression residuals, which is less increasing than the square function (Verardi and Croux, 2009; Susanti *et al.*, 2014). The default settings were used, including the Gaussian efficiency of 70% – although it can take higher efficacy at the same time bias increases. As the inference is concerned, standard errors robust to heteroscedasticity are used. Furthermore, the *dummies* option was specified when variables were dichotomous (categorical). Note that the MM-estimator is not an official STATA command, but is a STATA community-contributed package available through the *mmregress* command (*Appendix: Chapter 5*).

Inference Statistics

One-tailed and two tailed-statistics

Two-tailed statistic tests (H_0: $\theta = \theta_0$; H_1: $\theta \neq \theta_0$) were mostly used, but, in some cases, one-tailed test statistics (H_0: $\theta = \theta_0$; H_1: $\theta < \theta_0$ or H_1: $\theta > \theta_0$) were employed. In the thesis, a notation for one-sided test statistics was added, whereas an absence of notation indicates a two-tailed test statistic.

Continuous variables

The Pearson product-moment correlation test was used for testing the linear correlation between two continuous variables (H_0: $p = 0$; H_1: $p \neq 0$) when a bivariate normal distribution could be assumed. Otherwise, the non-parametric Spearman-rank correlation (H_0: $p_s = 0$; H_1: $p_s \neq 0$) test was preferred, which examines the monotonic correlation between the two variables. Finally, for data that suffered several tied ranks (especially for discrete data), the Kendall's rank (τ_b) coefficient correlation (H_0: $\tau_b = 0$; H_1: $\tau_b \neq 0$) was

used, which also quantifies a monotonic relationship. A positive or a negative value of $r_{(s)}$ and τ_b indicates a positive or a negative correlation, respectively (Pagano and Gauvreau, 2018; Peacock and Peacock, 2011; Sheskin, 2004; Conover, 1999) while, Cohen (1988) described the correlation as weak if $| 0.1 | \leq r_{(s)} < | 0.3 |$, moderate if $| 0.3 | \leq r_{(s)} < | 0.5 |$, and strong if $r_{(s)} \geq | 0.5 |$, but, in practice, even a weak correlation may indicate a meaningful relationship (Peck, Olsen and Devore, 2016).

Continuous dependent variable and level independent variable

One sample

In this case, a continuous dependent variable (y) was compared to a reference value (θ_0). The one-sample t-test (H_0: $\mu = \mu_0$; H_1: $\mu \neq \mu_0$) was used when the variable was assumed to be approximately normal. If normality could not be assumed, then the exact Wilcoxon signed-rank test (if the distribution could be assumed to be approximately symmetrical) or the sign rank test (H_0: $m = m_0$; H_1: $m \neq m_0$) were employed (Daniel and Cross, 2013).

Two levels

The two-sample t-test (H_0: $\mu_1 = \mu_2$; H_1: $\mu_1 \neq \mu_2$) was employed if the assumption of normality and homogeneity of variance could be assumed to be true. If the variances were unequal ($p < 0.05$ using the F-test of equality of variances), then the t-test for unequal variances (Satterthwaite approximation method) was performed. The non-parametric Wilcoxon rank-sum test was employed when the assumption of normal distribution could not be assumed, while the exact p-value was reported (Bergmann, Ludbrook and Spooren, 2000; Harris and Hardin, 2013; StataCorp LLC, 2019a). Note that, when the Wilcoxon rank-sum test was used, the assumption that the distributions of the populations compared have an equal shape was not checked (as this may convey further clinically interesting information). Therefore, a statistically significant result could be translated into a difference in the median value for the populations (H_0: $m = m_0$; H_1: $m \neq m_0$) or/and a difference in shape and variance (H_0: $s^2_1 = s^2_2$; H_1: $s^2_1 \neq s^2_2$) (Rosner, 2016; Hart, 2001).

Three or more levels

The one-way analysis of variance (one-way ANOVA) was used (H_0: $\mu_1 = \mu_2 = \ldots = \mu_k$; H_1: not all μ are the same) when the assumptions of normal distribution for each of the populations of y (or more precisely, of the residuals), and of homogeneity of variance (checked through residuals-against-the-fitted-values plot and the Bartlett's test), were true. If the result of the ANOVA was statistically significant ($p < 0.05$), a *post-hoc* analysis for multiple comparison test was applied to identify significant difference(s)

between the groups and, at the same, it was adjusted for multiple comparisons (Tukey's HSD and Tukey–Kramer adjustment for equal and unequal sample sizes, respectively). When the assumption of normality or homogeneity of variance was not met, the non-parametric test Kruskal–Wallis test was preferred (H_0: $m_1 = m_2 = \ldots = m_k$; H_1: not all m are the same). The Kruskal–Wallis test is an extension of Wilcoxon sank-rum test and, therefore, because the assumption for distributions was not controlled, a statistically significant result can indicate not only a difference in means, but also a stochastic dominance, as discussed before. If the *p*-value from the Kruskal–Wallis test was statistically significant, it was followed by the Dunn's Pairwise Comparison, corrected for multiple-comparison (Holm-Šidák) (Bewick, Cheek and Ball, 2004; Brown and Forsythe, 1974; Wilcox, Charlin and Thompson, 1986; Dinno, 2015; Abdi, 2007). Finally, groups with small sample size (i.e., ≤ 5) were merged with a second group for statistical analysis purposes (Bland and Altam, 2009); the choice of the second group was somewhat arbitrary but chosen on clinical grounds and clearly stated.

Categorical variables

The Pearson's chi-squared test (χ^2) for independence was used when exploring the relationship between two categorical variables (H_0: *X*, *Y* are independent H_1: *X*, Y are dependent). If > 20% of the expected values in the contingency table were less than five then the Fisher's exact test (H_0: *X*, *Y* are independent H_1: *X*, *Y* are dependent) was preferred (Rosner, 2016). When considered of interest, the effect size was also estimated using the Cramér's V; this effect size V could range from $-1 \leq V \leq 1$ (or φ) for two by two contingency tables and from $0 \leq V \leq 1$, otherwise (Cramér, 1946; Howell, 2013).

Data collection and statistical analysis software

Statistical analysis: STATA 16.0 MP edition (2019; StataCorp, College Station, TX, USA) was used for all data analysis, test statistics and graphs (for methodological and presentation purposes). Further to the official STATA commands and functions, a number of STATA community-contributed ado-files (commands) and packages were employed – we present these in *Appendix: Chapter 5*. Finally, when considered appropriate, the name of the *command* was reported in the appropriate section for replicability purposes.

Questionnaire-data collection: For the purpose of the data collection and appropriate data export, self-developed software was used. This has been described and presented in the appropriate sections.

Results

Recruitment process and recruited sample

Recruitment process

The recruitment process overall ran smoothly, and no major problems were observed. In regard to the random number provision, eventually, we had to provide the CDA secretarial staff with all random numbers (corresponding to potential participants in the CDA database) while the majority of these numbers were used. The study sample (those participants who fulfilled the inclusion criteria) included 103 participants and reflects a significant chunk of the available CDA database (26.5%); a graphical outline of the potential study sample at different stages of the recruitment process is shown in *Figure 5.6.* and is described below.

The potential study sample at different stages of the recruitment process

The CDA database encompassed 389 patients with Type 1 diabetes. Of those, we had to attempt to contact 353 patients in order to achieve the aimed sample of 100 participants (who met the inclusion criteria, including having adequate data to be included in the statistical analysis of the primary outcomes). We were unable to contact 117 out of the 353 patients (33.1%) due to incorrect contact details (phone number) being available (*n* = 112, 31.7%) or them having deceased (*n* = 5, 1.4%). Although this issue was not identified during the pilot study, it was of no surprise as the CDA secretarial staff had verbally warned me about the presence of outdated contact details. The number of patients we were able to contact was 236, and 75 (31.8%) of them were not interested in participating in this study. This number includes the participants who reported that they were not interested in participating at any stage of the recruitment process, such as when contacted (through phone) by the CDA secretarial staff or by me, and, therefore, they were not necessarily eligible potential participants. Of the remaining 161 patients who showed an interested in the study, 45 (28.0%) were not eligible to participate due to a diagnosis of diabetes other than Type 1 diabetes (*n* = 27, 16.8%, of whom 26 had Type 2 diabetes), their place of residence (*n* = 13, 8.1%) and the presence of health-related concerns as defined in the exclusion criteria (*n* = 5, 3.1%, of whom 4 had a significant learning difficulty). The remaining potentially eligible participants were 116, of whom 113 completed the data collection stage and 106 provided us with blood and urine samples – reflecting a high completion rate. Finally, three patients were excluded (after they had completed the study) based on their C-peptide results and medical history.

Study sample

The study sample size of 103 participants translated to a participation rate of 55%, that is, 103 participants out of 188 potentially eligible participants who we were able to contact; or 29.2% of the total number of patients that we tried to contact (n = 353). This is similar to the participation rate of 30% in the pilot study. All 103 participants provided consent for their blood and urine tests to be stored. This reflects the high acceptability of the study methodology by the participants. Finally, the study sample surpassed the aimed sample size of 100 participants due to the complexity of the recruitment procedure, that is, the blood tests and, more specifically, the C-peptide results had to be reviewed before we could include a participant in the final sample (and consequently recognise that we had reached our sample size target) while the recruitment process was still running.

Demographics

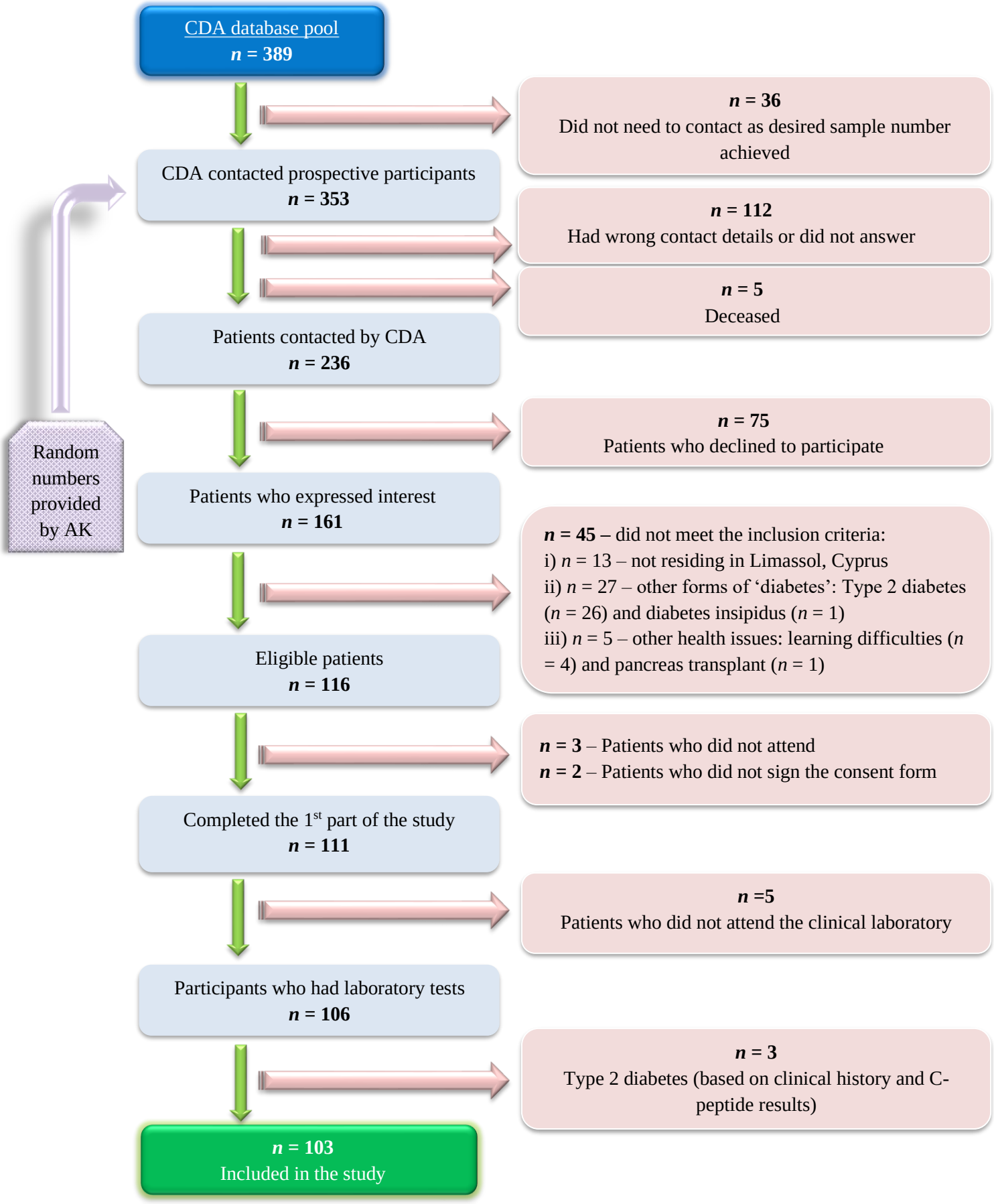
The demographics of gender and age were made available to the research team for all of the patients registered in the CDA database. Therefore, this information was used to identify any potential biases in the recruitment process through comparison of the participants to the (various groups of) non-participants (for gender and age). These are presented below; the Pearson's chi-squared test for independence and the Wilcoxon rank-sum test were employed for gender and age, respectively (as the expected values were above five and the age sub-categories could not be assumed to be normally distributed) and age is reported as median and IQR.

Gender: The study sample (male = 51, female = 52) was not different in terms of gender to those who declined to enrol to the study (male = 42, female = 33, p = 0.392); to the potentially eligible participants, that is, the patients who had no blood tests available, declined to enrol to the study, were not contacted, had incorrect contact details and did not attend their appointments (male = 114, female = 119, p = 0.921); and to the total number of the non-recruited sample, that is, all patients in the database other than the study sample (male = 150, female = 136, p = 0.610). Of interest is the non-statistically significant (but with a trend towards statistical significance) larger number of males in the excluded sample of patients with Type 2 diabetes and deceased (male = 24, female = 11) when compared to the study participants (p = 0.051); nevertheless, the effect size of this is small (V = - 0.1665). Finally, the gender of the patients contacted (male = 185, female = 168) was not statistically different when compared to the gender of the patients

who were not contacted by the CDA secretarial staff (male = 16, female = 20, $p = 0.362$), possibly reflecting the effectiveness of the randomised selection process (at least in terms of gender).

Age: The study sample ($n = 103$, m = 33 [26 – 43]) was younger when compared to the sample of patients who declined to enrol to the study ($n = 75$, m = 38 [28 – 54], $p = 0.0308$), the total number of potentially eligible participants ($n = 233$, m = 37 [28 – 49], $p = 0.0225$) and the total number of non-recruited patients ($n = 286$, m = 38 [29 – 53], $p = 0.0012$). This difference in age was present in the medians but also in the distributions, with an observable smaller skewness to the right of the distribution of the study sample over the other groups, which was also reflected in the IQR (the 75th percentile is lower in the study sample, while the 25th percentile is largely unaffected). We hypothesized that this difference in age could be at least partially explained by the fact that these other groups (in contrast to the study sample) included potentially eligible patients, which does not definitely preclude the presence of ineligible patients, such as patients diagnosed with Type 2 diabetes (and those who were deceased) who could have further skewed the age towards the right (taking into account the relatively large portion of registered patients other than those having a diagnosis of Type 1 diabetes and deceased individuals excluded in the study). In support of this hypothesis was the fact that the age of the patients who expressed an interest in participating (which was irrelevant if they participated or were excluded at a later stage) was not statistically different ($n = 161$, m = 35 [26 – 52], $p = 0.5468$) to the age of those who declined to participate ($n = 75$, m = 38 [28 – 54]). Furthermore, statistical evidence that the assumption that the presence of patients with a diagnosis of Type 2 diabetes and deceased individuals (through using potentially eligible patients group samples) would have skewed the age towards the right is the fact that the age of the excluded sample of patients with a diagnosis of Type 2 diabetes (at any stage) and deceased individuals ($n = 35$, m = 61 [51 – 64]) was statistically older than the study sample ($n = 103$, m = 33 [26 – 43], $p < 0.00005$). Finally, the random selection process probably did not affect this discrepancy in age, as the sample of patients who were not contacted was not statistically different in regard to age ($n = 36$, m = 35.5 [24 – 47], $p = 0.2142$) to the sample of patients who were contacted by the CDA secretarial staff ($n = 353$, m = 37 [28 – 51]). Despite the above evidence, it is still possible that there was some degree of selection or recruitment bias towards a younger population.

Figure 5.6: Flow chart of the potential study sample: from the CDA database to the study sample



Demographic characteristics

Description

The data were collected through the *Demographic characteristics questionnaire*. There were no missing data (n = 103) and the results are presented in *Table 5.6*. The demographic characteristics were also compared to the primary parameters of the study, namely, adherence to the Mediterranean diet and glycaemic control; these results are also presented in *Table 5.7*, with graphical representations of the relationship between the primary parameters and age and gender presented in scatterplots and box plots, illustrated in *Figure 5.7* and *Figure 5.8*. The mean values and standard deviations are rounded to one decimal place, whereas the mean values and the IQRs are rounded to the nearest integer figure. The comparisons of demographics with primary parameters were not corrected for confounding or for multiple comparisons, although the pairwise comparisons after one-way ANOVA were always adjusted for multiple comparisons. Note that only the (exact) p-values of the results of multiple pairwise comparisons, after one-way ANOVA, that were statistically significant are presented (otherwise reported as $p \geq 0.05$). For age and gender, if parametric tests were used and a mild outlier was detected, then the analysis was repeated again, excluding the outlier, and the results are reported in the relevant sections, although not for the rest of the demographic characteristics. Furthermore, if the results for fasting blood glucose were significant, the statistical analysis was repeated, this time excluding the patients with hypoglycaemia, which was defined for the purposes of this study as a value of less than 70 mg/dl.

Age

The median age of the participants was 33 years (range 17 – 68 years). The associations between age, as a continuous variable, and the primary outcomes, namely, Mediterranean diet, HbA1c and fasting glucose, and the results are presented in *Table 5.7* and graphically in *Figure 5.7*. The associations between age and Mediterranean diet and HbA1c were compared using Pearson's moment-correlation test [ln(age) vs MedDietScore and ln(age) vs ln(HbA1c)] while, against the fasting glucose, the Spearman-rank correlation test was preferred. Age was statistically significantly correlated with all primary outcomes so that a positive moderate association was observed with adherence to the Mediterranean diet (n = 103, $p < 0.00005$, r = 0.4386), while a weak negative relationship was seen with glycaemic control (HbA1c n = 103, p = 0.0056, r = -0.2712; glucose n = 103, p = 0.0028, r_s = -0.2915). In the scatterplot of ln(age) with MedDietScore (*Figure 5.7*) and the

BACON test, a mild lower-bound outlier was observed and, because the Pearson's moment-correlation test is sensitive to outliers, the test was repeated without the discussed outlier observation (n = 1). The results did not change when the outlier was excluded (n = 102, r = 0.4491, p < 0.00005), probably due to its mild nature and since as a single observation (out of 103 observations) carries very little weight overall. Furthermore, the statistical analysis for age and fasting glucose was repeated after excluding the participants (n =11) who had hypoglycaemia (for graphical representation with cut-off line at defined point of hypoglycaemia see *Figure 5.7*), with the results remaining statistically significant (n = 92, r_s = -0.2279, p = 0.0289).

Gender

The number of male and female patients recruited was approximately equal (male = 51, female = 52). The adherence to the Mediterranean diet (n = 103, p = 0.8603) and the glycaemic control (HbA1c n = 103, p = 0.1987; glucose n = 103, p = 0.9777) results were not different between the two genders when compared with the Wilcoxon sum-rank test (*Table 5.7*), while the mean values, IQRs and sample distributions were very similar between the two groups (*Figure 5.8*). The statistical comparison of Mediterranean diet between males and females was repeated after dropping the lower bound outlier observation (n = 1) present in the male group (*Figure 5.8*), and after assuming an approximately normal distribution for the discussed group, and therefore this time using the t statistic. The results did not change with the association remaining non-significant (n = 102, p = 0.8207).

Other demographic characteristics

Ethnicity and Nationality: The sample of the study was homogenous, with all 103 participants identifying themselves as white-Caucasians. The majority of the participants were Cypriots with a small number having a nationality other than Cypriot, such as Greek, Russian and British. The small size number of 'other' nationality or 'non-Cypriot' group (n = 6) made the use of non-parametric tests more relevant, although, for the same reason, the results should be interpreted with caution. Adherence to the Mediterranean diet was statistically not different between the Cypriot and non-Cypriot group (n = 103, p = 0.6565). Similarly, the difference observed in the mean of HbA1c between Cypriots and non-Cypriots was not statistically significant (n = 103, p = 0.3222). In contrast, the difference observed in fasting glucose between the non-Cypriot group and the Cypriot

group was statistically significant, with non-Cypriots having a lower fasting glucose when compared to the Cypriot group (m = 99, 175 mg/dl, n = 6, 97, p = 0.0208). Further analysis, by excluding the participants with hypoglycaemia, was considered redundant, owing to the small number of remaining participants in the 'other' group (n = 3).

Marital status and co-habitants: The majority of the participants reported married or single as their marital status, with a smaller number in the remaining groups. No statistically significant difference was observed between the different groups in their adherence to the Mediterranean diet (n = 103, p = 0.0886) or their HbA1c levels (n = 103, p = 0.4877). In contrast, the difference in the fasting blood glucose between groups reached statistical significance (n = 103, p = 0.0127). When the association was explored further using the Dunn's Pairwise Comparison with the Holm-Sidák adjustment, the pairwise relationships 'married' versus 'single' (m = 154, 185mg/dl, n = 50, 37, p = 0.0498), 'single' versus 'divorced' (m = 185, 116 mg/dl, n = 37, 8, p = 0.0449) and 'divorced' vs 'in a relationship' (m = 116, 262 mg/dl, n = 8, 8, p = 0.0437) were statistically significant, with all other pairwise group combinations not significant (data not shown, $p \geq 0.05$). The comparison was repeated, this time excluding the participants (n =11) who had hypoglycaemia, with the association remaining statistical significant (n = 92, p = 0.0308), although only the pairwise comparisons 'single' versus 'divorced' (m = 185, 116 mg/dl, n = 35, 7 p = 0.0301) and 'divorced' versus 'in a relationship' (m = 116, 262 mg/dl, n= 7, 8, p = 0.0437) remained statistically significant (data not shown, $p \geq 0.05$). The groups of 'divorced' (n = 8, n = 7) and 'in a relationship' (n = 8, n = 8) are small and the results involving these two groups should be interpreted with caution. The participants mainly reported living with their spouse and/or children, while a relatively significant number also reported leaving with their parents, with less reporting living 'alone'. The remaining two groups 'children only' (n = 3) and 'other' (n = 1) were merged with the groups 'spouse only' (n = 14) and 'alone' (n = 15) respectively, due to their small number of observations. The groups of 'co-habitants' variable reached statistical significance when comparing the difference in adherence to the Mediterranean diet (n = 103, p = 0.0352), although not in glycaemic control (HbA1c n = 103, p = 0.9603; glucose n = 103, p = 0.2125). When the relationship between *MedDietScore* scoring and cohabitants was further explored, the adjusted pairwise comparison showed a statistically significant difference between living with 'parents' versus living with 'spouse &

children' (m= 29, 32 points, n = 29, 41, p = 0.0137) with all other pairwise comparisons were not statistically significant (data not shown; $p \geq 0.05$).

Children: Approximately half of the participants reported having no children (n = 49, 48%), while for those who reported that they have, having one (n = 16, 16%) or two children (n = 28, 27%) was the most common, with maximum of five being reported. The number of children reported seems to have a positive relationship with adherence to the Mediterranean diet (n = 103, t_b = 0.2564, p = 0.0009) and a negative relationship with fasting blood glucose (n = 103, t_b = -0.1876, p = 0.0133) although not with HbA1c (n = 103, p = 0.1909). Note that when the Kendall's test was repeated after excluding the patients with hypoglycaemia, the relationship between number of children and fasting blood glucose ceased to be significant (n = 92, p = 0.0759).

Household income and educational level: The annual household income (including that of the spouse) was mainly confined in the categories to between 0 to 30,000 euros, with fewer participants reporting more than 30,000 euros and, consequently, the groups of '30,001 to 60,000' (n = 13) and '> 60,000' (n = 2) were merged into one group. The participants in the income variable groups were statistically different (n = 103, p = 0.0306) in their adherence to the Mediterranean diet, but not in their glycaemic control (HbA1c n = 103, p = 0.6769; glucose n = 103, p = 0.4556). Further analysis revealed that the difference was statistically significant between the groups with income '0 – 10,000' vs '20,001 – 30,000' (m = 29, 30, n = 25, 35, p = 0.0176) and '10,001 – 20.000' versus '20,001 – 30,000' (m = 30, 35, n = 35, 28, p = 0.0421) with all other pairwise comparisons non-significant (data not shown; $p \geq 0.05$). The participants were well educated with more than half having a university degree(s), and the majority having at least a high school degree. Two of the groups were small, 'primary school' (n = 1) and 'university – doctorate or higher' (n = 3) and therefore were merged with the most relevant group, that is, 'secondary school' (n = 5) and 'university – Master' (n=19) groups, respectively. The difference between adherence to the Mediterranean diet (n= 103, p = 0.7755) and HbA1c (n= 103, p = 0.1786) among different 'educational level' variable groups was not statistically significant. In contrast, fasting blood glucose reached statistical significance (n =103, p = 0.0261), although the pairwise comparison revealed only one borderline significant difference between the groups of 'high school' and 'university – Bachelor' (m =151, 214 mg/dl, n = 38, 37, p = 0.0446) with all other groups not being significant (data not shown; $p \geq 0.05$). Furthermore, this significance between the various education

groups disappeared if the analysis was repeated without the participants who had hypoglycaemia (n = 92, p = 0.1252).

Employment: More than half of the participants were working in full-time jobs, whereas about one in three reported not working, that is, unemployed, school and university students, and retired individuals. The groups were not statistically different in terms of adherence to the Mediterranean diet (n = 103, p = 0.9945) or the HbA1c (n = 103, p = 0.5051) but were statistically different in terms of fasting blood glucose (n = 103, p = 0.0163) and this remained significant after excluding the patients with hypoglycaemia (n = 92, p = 0.0230). The pairwise comparison showed that the significant association was between participants working 'full-time' versus 'part-time' (m = 147, 268 mg/dl, n = 67, 8, p = 0.0114), which persisted after excluding participants who had hypoglycaemia (m = 154, 268 mg/dl, n = 59, 8, IQR = 102 – 246, 210 – 345, p = 0.0230). Then, the people who reported working (full-time or part-time; n = 75) were also grouped by type of employment, that is, public, private and self-employed, with the most being employees in the private sector. Type of employment was not related to adherence to the Mediterranean diet or glycaemic control (MedDietScore n = 75, p = 0.1397; HbA1c n = 75, p = 0.9619; glucose n = 75, p = 0.8298).

Table 5.6: Demographic characteristics of participants

Characteristics (units)	***n***	**%**	**Mediterranean diet**		**HbA1c (mmol/mol)**		**fasting glucose (mg/dl)**	
			Mean ± sd or Median (IQR)[1]	*p*-value *r* or r_s or t_b[2]	Mean ± sd Median ± IQR[1]	*p*-value *r* or r_s or t_b[2]	Mean ± sd Median ± IQR[1]	*p*-value *r* or r_s or t_b[2]
Age (years)	103	100	Age 33 (26 – 43)	***p* < 0.00005** ***r* = 0.4386**	continuous variable	***p* = 0.0056** **r = -0.2712**	continuous variable	***p* = 0.0028** **r_s = -0.2915**
Gender								
Male	51	50	32 (29 – 35)	*p* = 0.8603	58 (50 – 74)	*p* = 0.1987	164 (101 – 247)	*p* = 0.9777
Female	52	50	31 (28 – 36)		64 (54 – 74)		172 (105 – 248)	
Ethnicity								
White-Caucasian	103	100	n/a		n/a		n/a	
Other	0	0	n/a		n/a		n/a	
Nationality								
Cypriot	97	94	31 (29 – 36)	*p* = 0.6565	61 (51 – 74)	*p* = 0.3222	175 (109 – 249)	**p = 0.0208**
Other	6	6	31 (23 – 36)		53 (50 – 61)		99 (63 – 159)	
Marital status								
Married	50	49	33.1 ± 4.7	*p* = 0.0886	58 (50 – 70)	*p* = 0.4877	154 (90 – 217)	***p* = 0.0127**
Single	37	36	30.6 ± 6.9		62 (52 – 74)		185 (143 – 270)	
Divorced	8	8	30.9 ± 4.4		59 (54 – 78)		116 (93 – 143)	
Widowed	0	0	n/a		n/a		n/a	
In a relationship	8	8	28.9 ± 4.7		66 (57 – 85)		262 (166 – 301)	
Co-habitants[3]								
Spouse only	14	14	Cells merged 31 (27 – 35)	***p* = 0.0352**	Cells merged 57 (50 – 77)	*p* = 0.6903	Cells merged 153 (107 – 190)	*p* = 0.2125
Children only	3	3						
Spouse & children	41	40	32 (30 – 36)		60 (52 – 70)		161 (87 – 247)	
Parents	29	28	29 (26 – 33)		60 (51 – 74)		185 (145 – 246)	
Alone	15	15	Cells merged 32 (30 – 36)		Cells merged 64 (59 – 75)		Cells merged 157 (133 – 323)	
Other	1	1						

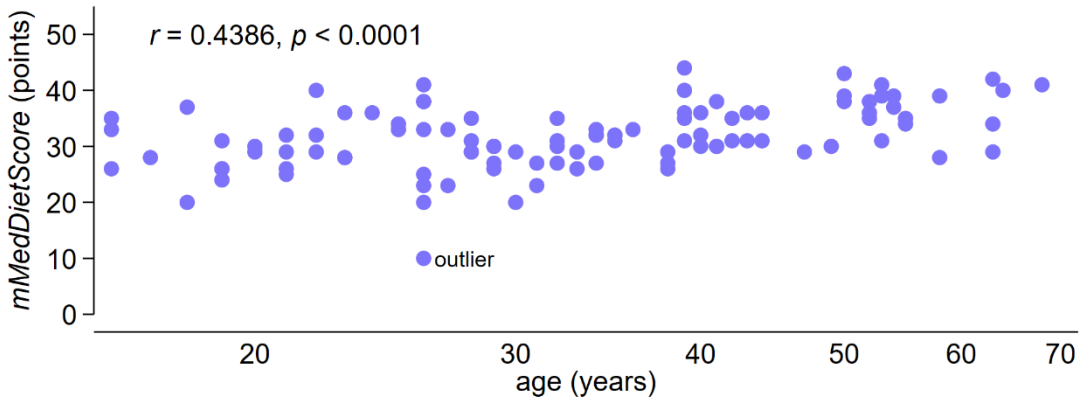
Number of children	103	100	Children 1 (0 – 2)	**t_b = 0.2564** **p = 0.0009**	continuous variable	t_b = - 0.0991 p = 0.1909	continuous variable	**t_b = -0.1876** **p = 0.0133**
Average annual household income (€)[4]								
0 – 10.000	25	24	29 (26 – 33)	**p = 0.0306**	64 (57 – 78)	p = 0.6769	190 (141 – 270)	p = 0.4556
10.001 – 20.000	35	34	30 (27 – 36)		60 (51 – 74)		148 (86 – 254)	
20.001 – 30.000	28	27	35 (32 – 37)		57 (50 – 68)		163 (121 – 241)	
30.001 – 60.000	13	13	Cells merged		Cells merged		Cells merged	
> 60.000	2	2	31 (29 – 35)		64 (49 – 74)		171 (87 – 214)	
Highest educational level								
Primary school	1	1	Cells merged	p = 0.7755	Cells merged	p = 0.1786	Cells merged	**p = 0.0261**
Secondary school	5	5	32 (29 – 33)		75 (59 – 78)		127 (81 – 160)	
High school	38	37	32 (29 – 36)		60 (51 – 71)		151 (95 – 235)	
University (level)								
Bachelor	37	36	30 (28 – 38)		64 (54 – 74)		214 (143 – 271)	
Master	19	18	Cells merged		Cells merged		Cells merged	
Doctorate or higher	3	3	34 (29 – 36)		56 (49 – 63)		150 (87 – 217)	
Employment time								
Full-time	67	65	31.7 ± 5.6	p = 0.9945	58 (50 – 74)	p = 0.5051	147 (91 – 227)	**p = 0.0163**
Part-time	8	8	31.9 ± 5.3		65 (57 – 79)		268 (210 – 305)	
Not working[5]	28	27	31.7 ± 6.1		65 (51 – 73)		188 (141 – 244)	
Employment type[6]								
Self-employed	8	11	30 (26 – 35)	p = 0.1397	62 (51 – 74)	p = 0.9619	164 (145 – 214)	p = 0.8298
Employee								
Public sector	11	15	35 (30 – 38)		60 (52 – 77)		157 (99 – 268)	
Private sector	56	75	31 (29 – 36)		59 (51 – 73)		96 (82 – 301)	

[1]Descriptive statistics of adherence to the Mediterranean diet and HbA1c and fasting blood glucose levels by group, if categorical variable. For continuous variables, the descriptive statistics of the discussed variables are shown. [2]If relevant – for continuous variables. [3]Any combination of the answers 'spouse', 'children', 'parents' 'alone' 'other' was possible; combinations are presented as separate answers. [4]includes the income of spouse. [5]This category includes unemployed, school and university students and retired individuals. [6]It was not applicable for participants reported not working in 'Employment time' question.

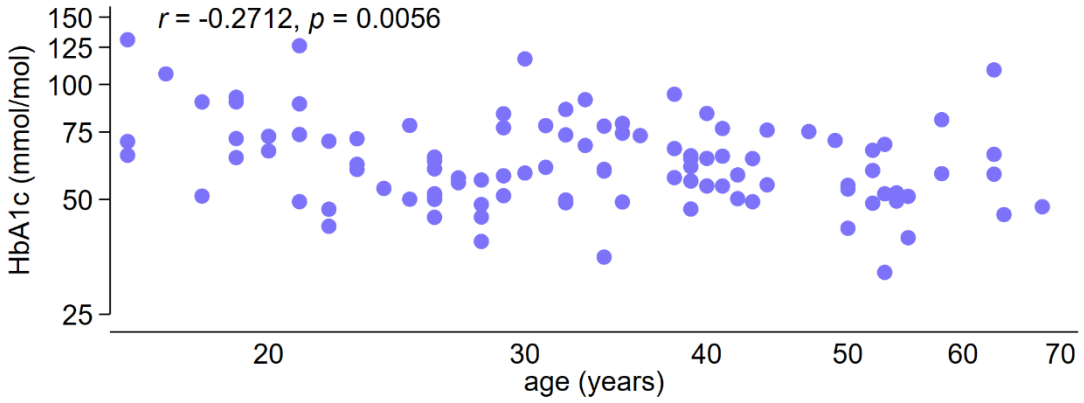
sd, standard deviation; IQR, interquartile range; n/a, not applicable; r, Pearson's correlation coefficient; r_s, Spearman's correlation coefficient; t_b, Kendall's tau-b.

Figure 5.7: Scatterplots of age against the primary outcomes

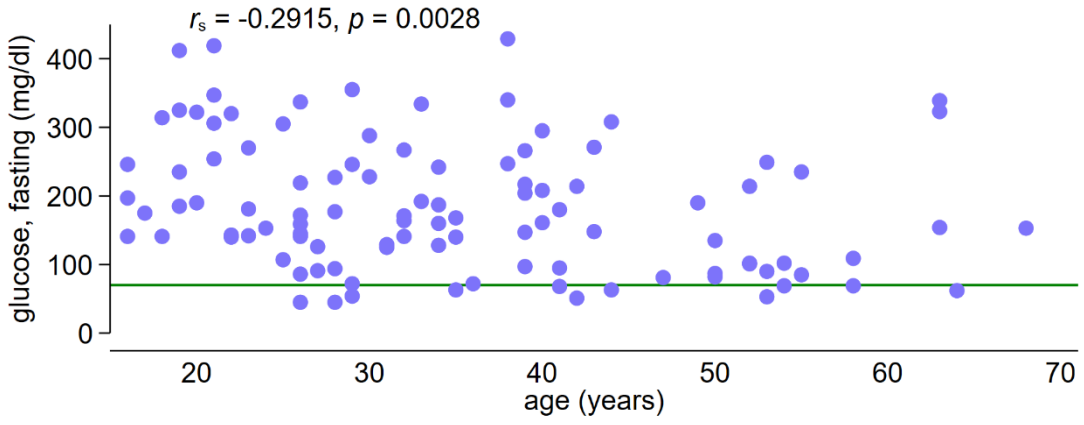
a) Scatterplot of Mediterranean diet and age with log-transformed x-axis



b) Scatterplot of HbA1c and age with log-transformed y-axis and x-axis



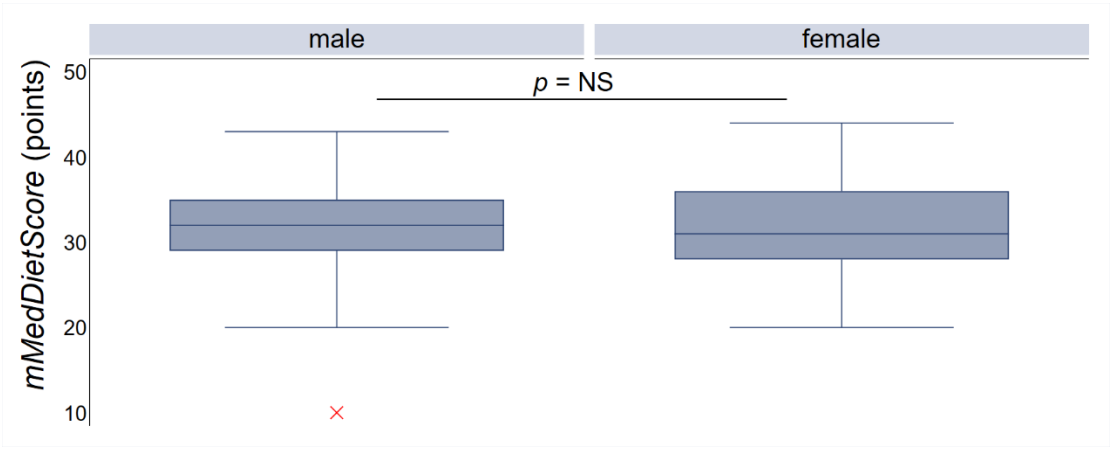
c) Scatterplot of glucose and age with y-axis cut-off1 line



[1]Cut-off line = 70mg/dl, below which was defined as hypoglycaemia.

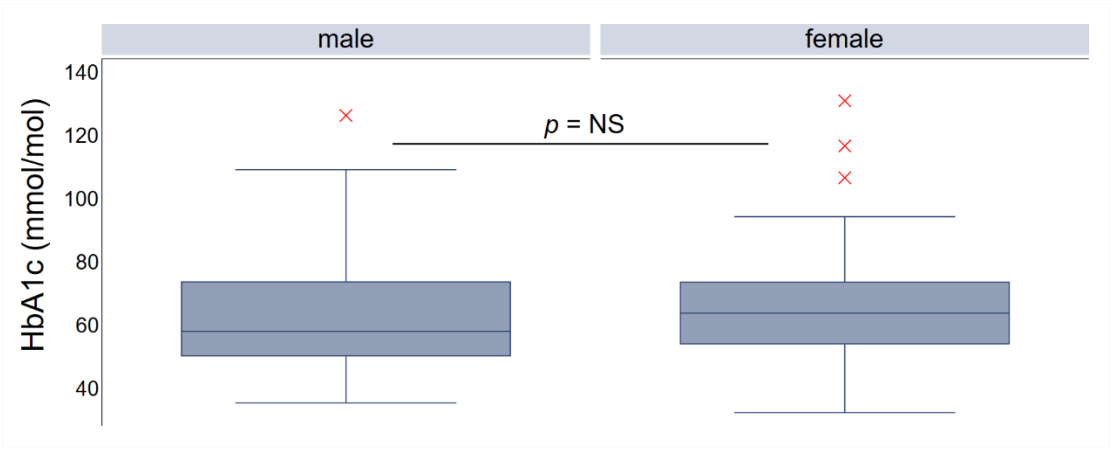
Figure 5.8: Box plots of the primary outcomes group by gender

a) Box plot of Mediterranean diet by gender



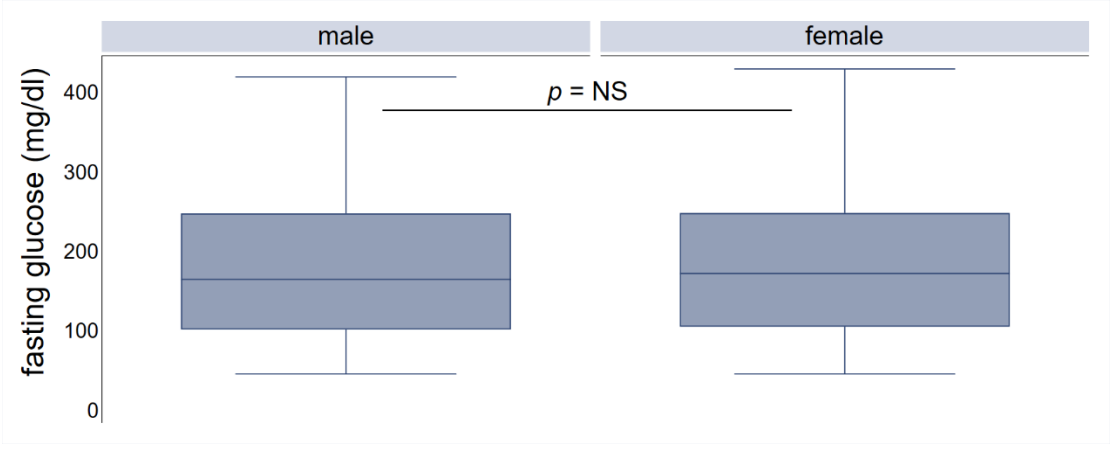
NS, not significant

b) Box plot of HbA1c by gender



NS, not significant

c) Box plot of glucose by gender



NS, not significant

Primary Outcomes:

The current study was designed and executed to investigate the pre-specified primary outcomes: current adherence to the Mediterranean diet and glycaemic control, and the association of the two through univariate and multivariate regression models.

Adherence to the Mediterranean diet

The participants' adherence to the Mediterranean diet was quantified using the *a priori MedDietScore* scoring system based on the aggregate score of eleven pre-defined food categories. Food intake was measured by means of an FFQ delivered electronically, and algorithms were used to estimate the *MedDietScore*. A higher *MedDietScore* score indicates better adherence to the Mediterranean diet, with a maximum possible score of 55 (or 5 for each category), and a lower score indicates a more Westernised/Americanised diet, with a minimum possible score of 0. The adherence to the Mediterranean diet is also presented in percentage, such as a *MedDietScore* score of 55 indicating perfect adherence to the Mediterranean diet of 100% and a *MedDietScore* score of 0 indicating deviation from the Mediterranean diet with 0% adherence. Finally, the score was also categorised to low, moderate and high adherence (*Table 5.1a*), resembling the categories used in *Chapter 3*. Descriptive characteristics of adherence to the Mediterranean diet are reported in the *Table 5.7* and graphically in *Figure 5.9*. Medians, IQR, minimum and maximum values are rounded to nearest integer figure, whereas mean values and standard deviation are presented with one decimal figure.

MedDietsScore: There were no missing data (n = 103). The mean adherence was 31.7 points, corresponding to a 57.6% adherence and falling within the moderate adherence category. The range was between 10 to 44 points, with the participant scoring 10 points being a mild outlier and the only participant falling with low adherence category. Of the remaining participants, 80% had a moderate adherence and 19% a high adherence to the Mediterranean diet. Finally, a *post-hoc* computation of the 95% tolerance interval yielded: [T̃0.99, T̰0.99] = [18.3, 45.1]. Thus, it can be inferred with 95% confidence (95% CL) that at least 99% of the population (99% coverage) lies within these limits (in points).[2]

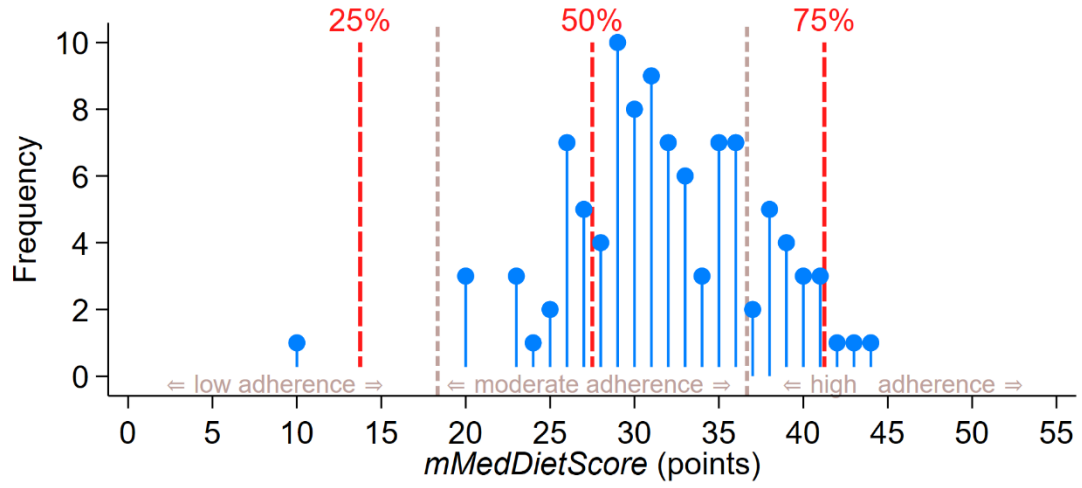
[2]Tolerance intervals = $\bar{x} \pm k_2$ s, where $\bar{x}$ is the mean, k_2 is a multiplier for two sided tolerance intervals and s is the standard deviation (Krishnamoorthy and Mathew, 2009; Meeker, Hahn and Escobar, 2017). This is calculated using the *STATA* community-contributed command *tolerance*, which computes the tolerance limits of a normal distributed variable. CL: confidence level.

Table 5.7: Mediterranean Diet - Descriptive characteristics

Adherence to the Mediterranean diet – MedDietScore scoring system					
Descriptive statistics	points	%	Adherence category	*n*	%
Mean ± sd	31.7 ± 5.7	57.6 ± 18.1	Low	1	1
Median (IQR)	31 (28 – 41)	56 (51 – 65)	Moderate	82	80
Min - Max	10 – 44	18 – 78	High	20	19

sd, standard deviation; IQR, interquartile range; Min, minimum; Max, maximum

Figure 5.9: Spike plot of adherence to the Mediterranean Diet frequency with cut-off points



Represents the percentage adherence, the cut-off points of 25%, 50% and 75% are presented – – –
Represents the cut-off points of low, moderate and high adherence -----

Glycaemic control

Glycaemic control was measured independently through the related tests of HbA1c and that of blood glucose. Although both measurements are useful indicators of glycaemic control, in practice, they provide different information, especially in this case, as we are concerned with single fasting measurements performed in a clinical laboratory. The HbA1c provides an overall picture of glycaemic control over the last 3 months, whereas fasting glucose provides a spot measurement and is more likely to give a picture of its variability. In the current section, HbA1c is reported in mmol/mol and % units, and glucose in mg/dl and mmol/l. The cut-off points used are based primarily on the NICE (NICE, 2015b) and ADA (American Diabetes Association, 2019d) guidelines (*Table 5.1b* and *Table 5.1c*). Descriptive characteristics of HbA1c and glucose are reported in *Table 5.8* and *Table 5.9*, respectively; the results are also presented graphically in *Figure 5.10* and *Figure 5.11*. Descriptive characteristic values are presented with one decimal figure for % (HbA1c) and mmol/l (glucose), whereas mmol/mol (HbA1c) and mg/dl (glucose) are rounder to the nearest integer.

HbA1c: There were no missing data (n = 103). The mean and median HbA1c were 65 mmol/l (8 %) and 60 mmol/l (7.7 %), respectively; both values are higher than the optimal HbA1c recommended by the NICE (< 48 mmol/mol (6.5 %)) or the ADA (< 3 mmol/mol (7.0 %)) guidelines. Based on ADA guidelines, only 30% (n = 31) had good control, while using the more strict NICE guidelines, a mere 12% (n = 12) were within optimal control. The HbA1c values are positively skewed (skewness = 1.27) with several outliers presented on the right-hand side, such as, the 23% (n = 22) of the participants had an HbA1c higher than 75 mmol/l (9.0 %) with a further 5% (n = 5) of the participants having an HbA1c higher than 105 mmol/l (11.8 %). This deviation from the clinical guidelines targets was statistically significant. More precisely, the computation of one-sided one-sample sign test (Ha: m - b_0 > 0) yielded p-values of < 0.00005 against the b_0 = 48 mmol/mol (6.5 %; NICE, 2015b) and b_0 = 53 mmol/mol (7.0 %; American Diabetes Association, 2019b). Note that the sign test was preferred over the Wilcoxon sign rank test due to the presence of significant skewness of the data (*Figure 5.10*); nevertheless, the Wilcoxon sign rank test produces almost identical results (data not shown). Furthermore, the 95% tolerance interval was computed, yielding: $[\tilde{T}_{0.967}, \tilde{T}_{0.967}]$ = [32.2, 130.8]. Thus, it can be inferred with 95% confidence (95% CL) that at least 97% of the population (97% coverage) lies within these limits (in mmol/mol)[3].

Glucose, fasting: There were no missing data (n = 103). The mean and median fasting blood glucose are 185 mg/dl (10.3 mmol/l) and 168 mg/dl (9.3 mmol/l), well above the ADA (80 – 130 mg/dl (4.4 – 7.2 mmol/l)) or the NICE guidelines (90 – 126 mg/dl (5 – 7 mmol/l)) recommendations for optimal fasting glucose levels. A mere 20% (n = 19) of the participants were within the ADA recommendations for fasting glucose levels, while the majority (n = 69, 71%) of participants having a higher glucose measurement than the recommendations; a significant number of participants (n = 11, 11%) were hypoglycaemic during their visit to the clinical laboratory and, among them, worryingly, 4% (n = 4) been on level 2 hypoglycaemia (< 54 mg/dl [3 mmol/l] as defined by ADA) with two participants (2%) having blood sugar levels as low as 45 mg/dl (2.5 mmol/mol). The fasting glucose levels were skewed to the right (skewness = 0.56), that is, 38% (n =

[3]In oversimplified terms, the sample space of a continuous random variable is partitioned into blocks and then, the side-most blocks are removed (Tukey, 1947; Murphy, 1948; Krishnamoorthy and Mathew, 2009; Meeker, Hahn and Escobar, 2017). This was calculated using the STATA community-contributed command *dftol*, which computes the distribution-free tolerance limits of a variable. A two-sided higher coverage (for example, the 99% used for *MedDietScore*) was not mathematically possible with the number of available observations, as the discarded number of blocks will be only one. CL: confidence level.

39) of the participants having blood sugar levels above 200 mg/dl (11.1 mmol/l), 17% above 300 mg/dl (16.7 mmol/l) with three participants supressing blood sugars of 400 mg/dl (22.2 mmol/l). Finally, the 95% tolerance interval was computed, yielding: [$\tilde{T}_{0.967}$, $\underset{\sim}{T}_{0.967}$] = [45, 429]. Thus, it can be predicted with 95% confidence (95% CL) that at least 97% of the study population (97% coverage) lies within these limits (in mg/dl)[3].

Table 5.8: HbA1c - Descriptive characteristics

Glycaemic control – HbA1c					
Descriptive statistics	mmol/mol	%	Cut-off points[1]	*n*	%
Mean ± sd	65 ± 18	8.0 ± 1.7	≤ 48 (6.5)	12	12
Median (IQR)	60 (51 – 74)	7.7 (6.8 – 8.9)	≤ 53 (7.0)	31	30
Min - Max	32 – 131	5.1 – 14.1	> 75 (9.0)	23	22

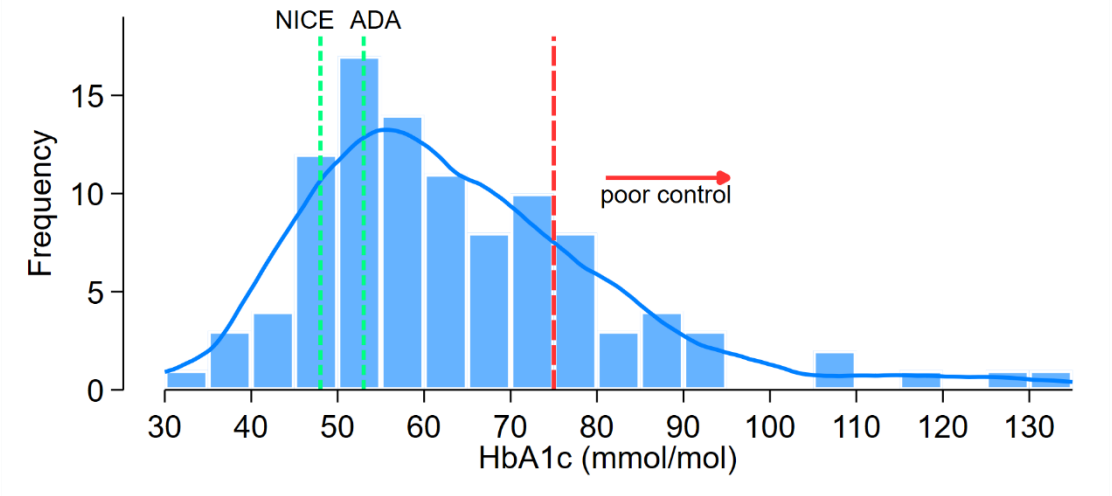
[1]Cut-off points in mmol/mol (%).
sd, standard deviation; IQR, interquartile range; Min, minimum; Max, maximum.

Table 5.9: Glucose - Descriptive characteristics

Glycaemic control – Glucose, fasting					
Descriptive statistics	mg/dl	mmol/l	Cut-off points[1]	*n*	%
Mean ± sd	185 ± 94	10.3 ± 5.2	< 70 (3.9)	11	11
Median (IQR)	168 (102 - 247)	9.3 (5.7 – 13.7)	70 – 130 (3.9 – 7.2)	19	20
Min - Max	45 - 429	2.5 – 23.8	> 130 (7.2)	71	69

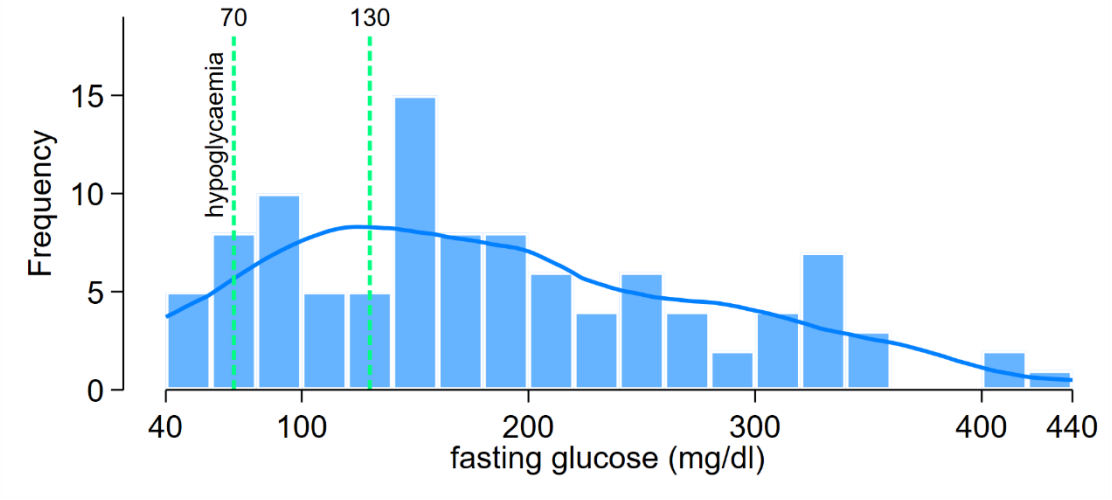
[1]Cut-off points in mg/dl (mmol/l) indicating hypoglycaemia, good control and sub-optimal control.
sd: standard deviation; IQR, interquartile range; Min, minimum; Max, maximum.

Figure 5.10: Histogram and kernel density estimate plot of HbA1c levels frequency with cut-off lines



Histogram: bin n: 21 width: 5, start: 30
Kernel density estimate: bandwidth = 6.2, kernel: Epanechnikov function, n of points evaluated = 50
NICE: ≤ 48 mmol/mol, ADA: ≤ 53 mmol/mol = good control; >75mmol/mol = poor control

Figure 5.11: Histogram and kernel density estimate plot of glucose (fasting) levels frequency with cut-off lines



Histogram: bin n: 20, width: 20, start: 40
Kernel density estimate: bandwidth = 37, kernel: Epanechnikov function, n of points evaluated = 50
ADA: < 70 mg/dl = hypoglycaemia, 130 mg/dl = cut-off for good control vs sub-optimal control (> 130 mg/dl)

The current study was designed to measure adherence to the Mediterranean diet and glycaemic control, but also to assess the association between the two through regression models. There were three OLS regression models applied, with Model 1 a univariate model, while Models 2 and 3 were multivariable models. Model 1 was a simple OLS model with dependent variable the glycaemic control, that is, HbA1c or glucose, and the predictor variable adherence to the Mediterranean diet, as measured by the *MedDietScore* scoring system. Model 2 had the addition of demographic covariates of age, as a continuous variable measured in years, and gender as a categorical variable with two categories; that of female and male. Model 3 was the same as Model 2 with the addition of six additional covariates: that of BMI, as a continuous variable measured in Kg/m^2; C-peptide, as a categorical variable with two categories of detectable ($\geq$ 0.010 ng/ml) and undetectable C-peptide (< 0.010 ng/ml); household income as a categorical variable with two categories, less and higher than 2000 euro per annum; method of insulin injection as a categorical variable, with two categories MDI and CSII; smoking status, with two categories, current smoker and other; and whether they adjusted the insulin dosage to the carbohydrate intake as a categorical variable with two categories, Yes and No. The reasons for the selection of the included covariates are discussed elsewhere. The OLS regression models were examined for any assumption violation using pre-specified methods. Results are presented in one decimal figure in the text, while the complete numbers are presented in the tables.

HbA1c: The models, as related to HbA1c, were initially run with no restrictions, but, due to the violation of a number of assumptions when investigated, it was decided that these should be re-run conditionally by excluding one highly influential observation that appeared to be causing the problem. Indeed, when the models were re-run, all assumptions were nicely met (*Appendix: Chapter 5*). Therefore, I decided to use the models that were conditionally run only on moderate and high adherence to the Mediterranean diet. This was also considered to be legitimate from a clinical perspective (apart from a mathematical one), as the observation excluded, one with a *MedDietScore* equal to ten points, was the only observation within the low adherence category; therefore, the data are not going to be representative of low adherence, with or without the discussed observation. Nevertheless, *post-hoc* MM-estimator regression models (with robust standard errors) were run for Model 1 and Model 2 and an OLS model for Model 3 while

including the discussed observation, with all three models found to be statistically significant ($p < 0.05$; data not shown). However, here only the conditional models are reported, as they are considered to have more statistically efficient estimators (i.e., in simple terms, it can be said that they provide a more accurate estimation of the true association); detailed results for Model 1 are shown in *Table 5.11*, for Model 2 in *Table 5.12* and for Model 3 in *Table 5.13*, while interpretation of the coefficients are provided in *Table 5.10*; graphically, the relationship between adherence to the Mediterranean diet and HbA1c is shown through a scatterplot in *Figure 5.12*.

In all three models, adherence to the Mediterranean diet, as measured by the *MedDietScore*, was a statistically significant predictor of HbA1c (mmol/mol) and well beyond the 5% level of significance and more precisely, the *t* statistic *p*-value of Model 1 was below 0.0005 and of Models 2 and 3 were 0.001 and 0.006, respectively. The coefficient of *MedDietScore* was negative in all models (Model 1, b_1: -0.020, SE(b_1): 0.0046; Model 2, b_1: -0.017, SE(b_1): 0.0051; Model 3, b_1: -0.015, SE(b_1): 0.0054), indicating a negative association, that is, as the *MedDietScore* (or the adherence to the Mediterranean diet) increases by one point, the natural logarithm of HbA1c decreases by about 2 units on average, keeping all other variables constant. *Table 5.10* shows the more clinically meaningful impact of increasing the *MedDietScore* by one point (or the adherence to the Mediterranean diet) had on the reduction of HbA1c (mmol/mol; %Δy) which ranged from 1.9% (95% CIs: 2.8% – 1.0%) on Model 1, to 1.7% (95% CIs: 2.7% – 0.7%) on Model 2, and 1.5% (95% CIs: 2.6% – 0.4%) on Model 3 on average, keeping all other variables constant. Finally, regarding the models, they were all statistically significant ($p < 0.05$; for exact numbers see *Table 5.11*, *Table 5.12* and *Table 5.13*), although no other coefficient than that of Mediterranean diet reached statistical significance ($p > 0.05$; for exact numbers see relevant tables as before). Mediterranean diet seem to explain about 15% of the variation in the HbA1c of the sample (in Model 1) taking into account the sample size; whereas, by adding other variables in Models 2 and 3, the predictive power of the models did not change considerably (15% and 20%, respectively), taking into account all independent variables and that of the sample size (for exact numbers see relevant tables, as before). Adequate degrees of freedom were allowed for the models and appropriate modelling techniques were used.

Glucose: In contrast to the models for HbA1c that were run conditionally to meet the assumptions of the OLS estimator, the models for glucose were run conditionally on

clinical grounds, as it was considered that observations in the hypoglycaemic range (< 70 mg/dl [3.9mmol/l]) were not of clinical interest for the discussed association, that is, the models were restricted to the more relevant part of the non-hypoglycaemic range. The logic behind this was that fasting glucose measurements in the hypoglycaemic range are unlikely to provide the same clinical information as those in the non-hypoglycaemic range; this was pre-specified before the study commenced. The models were run, as predefined, and the assumptions were examined. Two points worth mentioning regarding the assumptions (*Appendix: Chapter 5*) of the OLS models are as follows:

1) the observation that had caused the problems in the initial model of HbA1c, *MedDietScore* = 10, in this case acted as a good leverage point, and because there was no other noticeable influential observation, it was decided to keep it in the models – nevertheless, MM-estimator regression models were also run, *post-hoc*, to compare the results, as good leverage points can sometimes deflate the standard errors (Verardi and Croux, 2009).

2) The distribution of the residuals of Models 1 and 3, according to Shapiro-Wilk tests, were statistically significantly different from a normal distribution – while graphical methods suggested some skewness, this was not suggestive of a major problem, while the skewness-kurtosis test failed to identify any statistically significant deviation from the normal distribution, therefore, for this reason, it was considered that the deviation was likely small. Furthermore, if adequate observations are available, as in the current project, the central limit theorem applies (Mendenhall and Sincich, 2012; Harrell, 2015). For these reasons it was considered that the standard errors (and consequently, any hypothesis testing) would remain reliable and therefore appropriate for this occasion, despite the failure to assume that the residuals follow a normal distribution.

Mediterranean diet as a predictor of glucose levels reached statistical significance in Model 1 ($p = 0.035$), suggesting that, every one point of increase in the adherence to the Mediterranean diet, as measured by the *MedDietScore* scoring system, will cause a decrease in the fasting glucose by about 1.7% (%Δy). In contrast, adherence to the Mediterranean diet as a predictor of glucose levels failed to reach statistical significance after been corrected for co-variates in Models 2 and 3 ($p > 0.05$); detailed results for Model 1 are shown in *Table 5.15*, for Model 2 in *Table 5.16* and for Model 3 in *Table 5.17*, while interpretation of the coefficients is provided in *Table 5.14*; graphically, the

association between adherence to the Mediterranean diet and glucose is shown through a scatterplot, in *Figure 5.13*. Similar *p*-values were obtained when MM-estimator regression models were run, *post-hoc* (Model 1: *p*-value = 0.025; Model 2: *p*-value = 0. 179; Model 3: *p*-value = 0.186). Finally, it is worth mentioning that, in Model 3, age and BMI were statistically significant (*p*-value = 0.017 and < 0.0005, respectively) and the probability that (the variables of) the model does contribute information for predicting glucose only due to random chance alone was small (*F* statistic significance level = 0.12%; for other models and exact numbers see relevant tables as before). Adequate degrees of freedom were allowed for the model and appropriate modelling techniques were used.

Table 5.10: HbA1c – OLS Linear regression

HbA1c ~ adherence to the Mediterranean diet				
Model (*n* = 102[a])	**%Δy[b]**	**95% Confidence Interval[b]**		***p*** **> | *t* |**
Model 1 (df[c] = 100)	-1.9432703	-2.8290512	-1.0494049	**< 0.0005[d]**
Model 2 (df[c] = 98)	-1.6946859	- 2.6851512	-0.69414957	**0.001**
Model 3 (df[c] = 92)	-1.5060243	-2.5556948	-0.44505667	**0.006**

[a]One observation with *MedDietScore* = 10 points were dropped, conditionally, on modelling grounds in all models. [b]Indicates the percentage change in HbA1c for every one point increase in *MedDietScore*, holding all other covariates fixed – whereas the estimated coefficients (b) represent the change in lnHbA1c – the conversion formula is $(e^{\beta i} - 1) \times 100\%$ (Mendenhall and Sincich, 2012), where β_i is the coefficient. [c]Indicates the degrees of freedom that remained in the model; df = *n* – p – 1, where *n* = number of observations and p = number of independent variables. [d]Stata reports the chance of observing a *t*-statistic that large or larger as 0.000, which is Stata's way of indicating a number smaller than 0.0005.
n, number of observations; %Δy, percentage change in the dependent variable; | *t* |, *t* statistic; df, degrees of freedom.

Figure 5.12: Scatterplot and estimated regression line with 95% confidence interval of log-transformed HbA1c and MedDietScore

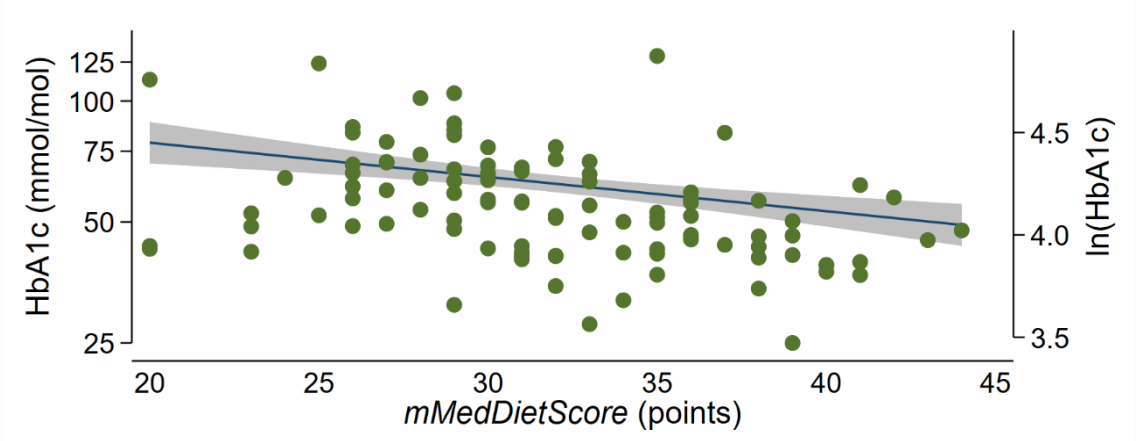


Table 5.11: HbA1c – Model 1

Model 1 (n = 102[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		**$p > \lvert t \rvert$**	**Model specifications**
lnHbA1c [mmol/mol]					$p >$ [b]$\lvert F_{1,100} \rvert <$ **0.00005**[c] R^2 = 0.1555; adj. R^2 = 0.1470
MedDietScore [points]	-0.019624	-0.0286984	-0.0105495	**< 0.0005**[d]	predictor

Table 5.12: HbA1c – Model 2

Model 2 (n = 102[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		**$p > \lvert t \rvert$**	**Model specifications**
ln(HbA1c) [mmol/mol]					$p >$ [b]$\lvert F_{3,98} \rvert =$ **0.0002** R^2 = 0.1796; adj. R^2 = 0.1545
MedDietScore [points]	-0.0170921	-0.0272186	-0.0069657	**0.001**	predictor
gender [female]	0.0561704	-0.0392122	0.151553	0.245	[d]categorical; base: male
ln(age) [years]	-0.0840744	-0.2283255	0.0601766	0.250	

Table 5.13: HbA1c – Model 3

Model 3 (n = 102[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		**$p > \lvert t \rvert$**	**Model specifications**
ln(HbA1c) [mmol/mol]					$p >$ [b]$\lvert F_{9,92} \rvert =$ **0.0004** R^2 = 0.2711; adj. R^2 = 0.1998
MedDietScore [points]	-0.0151748	-0.0258892	-0.0044605	**0.006**	predictor
gender [female]	0.0692392	-0.027805	0.1662834	0.160	[d]categorical; base: male
ln(age) [years]	-0.1426497	-0.2959401	0.0106407	0.068	
ln(BMI) [kg/m^2]	0.2941032	-0.0309702	0.6191766	0.076	
C-peptide [detectable]	-0.0645794	-0.1688208	0.0396619	0.222	[d]categorical; base: undetectable[e]
income [> 20000€]	0.0140219	-0.0868848	0.1149287	0.783	[d]categorical; base: ≤ 20000€
MDI vs CSII [CSII]	-0.146583	-0.3164843	0.0233183	0.090	[d]categorical; base: MDI
smoker [current[f]]	0.0781008	-0.0336801	0.1898818	0.169	[d]categorical; base: other
adjusting insulin:CHO [Yes]	-0.0444882	-0.1769132	0.0879367	0.506	[d]categorical; base: No

[a]One observation with *MedDietScore* = 10 points was dropped, conditionally, on modelling grounds. [b]$\lvert F_{df1, df2} \rvert$: df_1 indicates the degrees of freedom that were spend by the independent variables; df_2 indicates the degrees of freedom that remained in the model; df_1 =p, $df_2 = n - p - 1$, where n = number of observations, p = number of independent variables; df_1 and df_2 are needed for the F statistic calculation and provides information on modelling. [c]Stata reports the chance of observing an F-statistic that large or larger as 0.0000, which is Stata's way of indicating a number smaller than 0.00005. [d]Stata reports the chance of observing a t-statistic that large or larger as 0.000, which is Stata's way of indicating a number smaller than 0.0005. [d]Categorical variable X_p with two categories; if base then $X_i = 0 \rightarrow b_i * X_i = b_i * 0 = 0$; if [non-base] then $X_i = 1 \rightarrow b_i * X_i = b_i * 1 = b_i$, where b_i is the estimated coefficient. [e]Undetectable C-peptide is < 0.010 ng/ml. [f]current smoker was defined as smoking at least one cigarette daily.

n, number of observations; $\lvert t \rvert$, t statistic; $\lvert F_{df1,df2} \rvert$, F statistic with degrees of freedom df_1, df_2; adj R^2, adjusted R^2; MDI, multiple daily injections; CSII, continuous subcutaneous insulin infusion; CHO, carbohydrates.

Table 5.14: Glucose – OLS Linear regression

Glucose ~ adherence to the Mediterranean diet				
Model (*n* = 92[a])	**%Δy[b]**	**95% Confidence Interval[b]**		***p*** **> | *t* |**
Model 1 (df[c] = 90)	-1.7253229	-3.3022547	-0.12267469	**0.035**
Model 2 (df[c] = 88)	-1.1561348	-2.8979113	0.61688495	0.197
Model 3 (df[c] = 82)	-0.79654067	-2.5750453	1.0144308	0.382

[a]Eleven observations with glucose < 70 mg/dl (3.9 mmol/l) were dropped, conditionally, on clinical grounds in all models. [b]Indicates the percentage change in glucose for every one point increase in *MedDietScore*, holding all other covariates fixed – whereas the estimated coefficients (b) represent the change in lnglucose – the conversion formula is $(e^{\beta i} - 1) \times 100\%$ (Mendenhall and Sincich, 2012), where β_i is the coefficient. [c]Indicates the degrees of freedom that remained in the model; df = n – p – 1, where n = number of observations and p = number of independent variables.
n, number of observations; %Δy, percentage change in the dependent variable; | *t* |, *t* statistic; df, degrees of freedom.

Figure 5.13: Scatterplot and estimated regression line with 95% confidence interval of log-transformed glucose and MedDietScore

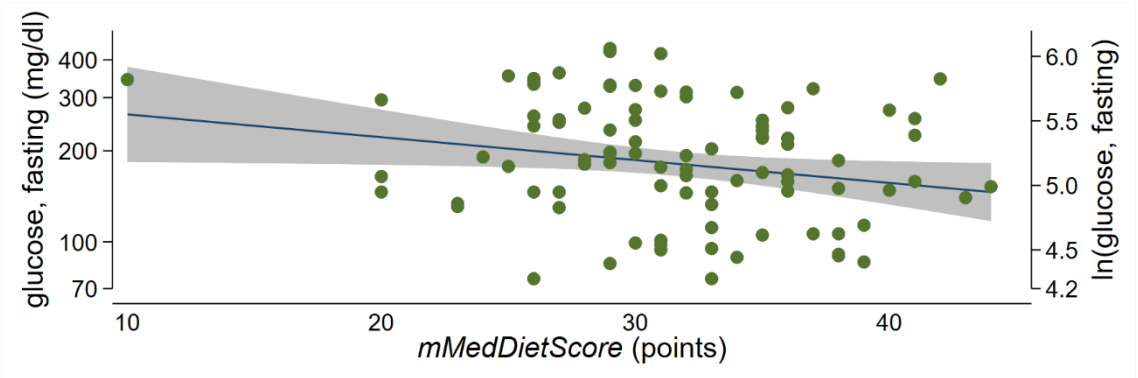


Table 5.15: Glucose – Model 1

Model 1 (*n* = 92[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		***p*** **> | *t* |**	**Model specifications**
ln(glucose) [mg/dl]					*p* > [b]| $F_{1,90}$ | = **0.0353** R^2 = 0.0483; adj. R^2 = 0.0377
MedDietScore [points]	-0.0174038	-0.0335801	-0.0012275	**0.035**	predictor

Table 5.16: Glucose – Model 2

Model 2 (*n* = 92[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		**p > | t |**	**Model specifications**
ln(glucose) [mg/dl]					*p* > [b]| $F_{3,88}$ | = 0.0590 R^2 = 0.0807; adj. R^2 = 0.0494
MedDietScore [points]	-0.0116287	-0.0294073	0.0061499	0.197	predictor
gender [female]	0.0467948	-0.1387065	0.2322961	0.617	[c]categorical; base: male
ln(age) [years]	-0.2253925	-0.5025655	0.0517805	0.110	

Table 5.17: Glucose – Model 3

Model 3 (*n* = 92[a]) **Clinical variables**	**Coefficient**	**95% Confidence Interval**		***p*** **> | *t* |**	**Model specifications**
ln(glucose) [mg/dl]					*p* > [b]| $F_{9,82}$ | = **0.0012** R^2 = 0.2743; adj. R^2 = 0.1946
MedDietScore [points]	-0.0079973	-0.0260878	0.0100932	0.382	predictor
gender [female]	0.0651723	-0.1135521	0.2438967	0.470	[c]categorical; base: male
ln(age) [years]	-0.338033	-0.6134877	-0.0625783	**0.017**	
ln(BMI) [kg/m^2]	1.102507	0.5260757	1.678938	**< 0.0005**[d]	
C-peptide [detectable]	-0.1566576	-0.3442464	0.0309313	0.100	[c]categorical; base: undetectable[e]
income [> 20000€]	-0.0942521	-0.2841568	0.0956526	0.326	[c]categorical; base: ≤ 20000€
MDI vs CSII [CSII]	-0.2200894	-0.539503	0.0993242	0.174	[c]categorical; base: MDI
smoker [current[e]]	-0.1531231	-0.3589643	0.0527181	0.143	[c]categorical; base: other
adjusting insulin:CHO [Yes]	0.160415	-0.0846658	0.4054959	0.197	[c]categorical; base: No

[a]Eleven observations with glucose < 70 mg/dl (3.9 mmol/l) were dropped, conditionally, on clinical grounds. [b]\| $F_{df1, df2}$ \|: df_1 indicates the degrees of freedom that were spend by the independent variables; df_2 indicates the degrees of freedom that remained in the model; df_1 =, df_2 p = *n* – p – 1, where *n*= number of observations, p = number of independent variables; df1 and df2 are needed for the F statistic calculation and provides information on modelling. [c]Categorical variable X_p with two categories; if base then $X_i = 0$ → $b_i * X_i = b_i * 0 = 0$; if [non-base] then $X_i = 1$ → $b_i * X_i = b_i * 1 = b_i$, where b_i is the estimated coefficient. [d]Stata reports the chance of observing a *t*-statistic that large or larger as 0.000, which is Stata's way of indicating a number smaller than 0.0005. [e]Undetectable C-peptide is < 0.010 ng/ml [e]current smoker was defined as smoking at least one cigarette daily.

n, number of observations; | *t* |, *t* statistic; | $F_{df1,df2}$ |, *F* statistic with degrees of freedom df_1, df_2; adj R^2, adjusted R^2; MDI, multiple daily injections; CSII, continuous subcutaneous insulin infusion; CHO, carbohydrates.

HbA1c versus fasting glucose and a potential mechanism (a *post-hoc* analysis)

This section aims to explore the association between the two measures of glycaemic control, namely HbA1c and fasting glucose. Furthermore, I wanted to investigate the hypothesis (or potential mechanism) that, in our data, the effect of the Mediterranean diet on HbA1c was not mediated by the spot fasting glucose measurement, which was based on the results of the glycaemic control and the Mediterranean diet (*Table 5.10* and *Table 5.14*). Therefore, I have computed the following:

i. Pearson product-moment correlation test and a partial correlation of HbA1c (ln) and fasting glucose (ln) adjusted for age (ln) and gender: $r = 0.4239$, $p < 0.00005$,$_p$, $r_p = 0.3789$, $p = 0.0001$.

ii. Simple mediation analysis: the indirect effect of Mediterranean diet on HbA1c (ln) that passes through fasting glucose (ln) is – 0.0026826, $p = 0.180$.

iii. Fasting glucose was added in *Model 3* (*Table 5.13*) of HbA1c (ln): the β of *MedDietScore* is – 0.0142418, $p = 0.005$ and of fasting glucose (ln) is 0.0011189, $p < 0.0005$ (*Model 3*: *MedDietScore* is β = – 0.0151748 , $p = 0.006$; *Table 5.13*).

Based on the above results, we can observe the following:

1. There is a statistically significant correlation between spot fasting glucose and HbA1c independent of other variables (*Figure 5.14*). At the same time, it also indicates that other variables are required to explain the HbA1c in full.
2. The spot measurement of fasting glucose did not significantly mediate the effect of the diet on HbA1c. This is also visible on the two-dimensional contour-line plot (*Figure 5.15*), where there is a visible improvement (reduction) of HbA1c across the *y*-axis (*MedDietScore*) independent of the *x*-axis (fasting glucose).

Figure 5.14: Scatterplot and lowess (locally weighted regression) smoothing lines of log-transformed HbA1c and log-transformed glucose

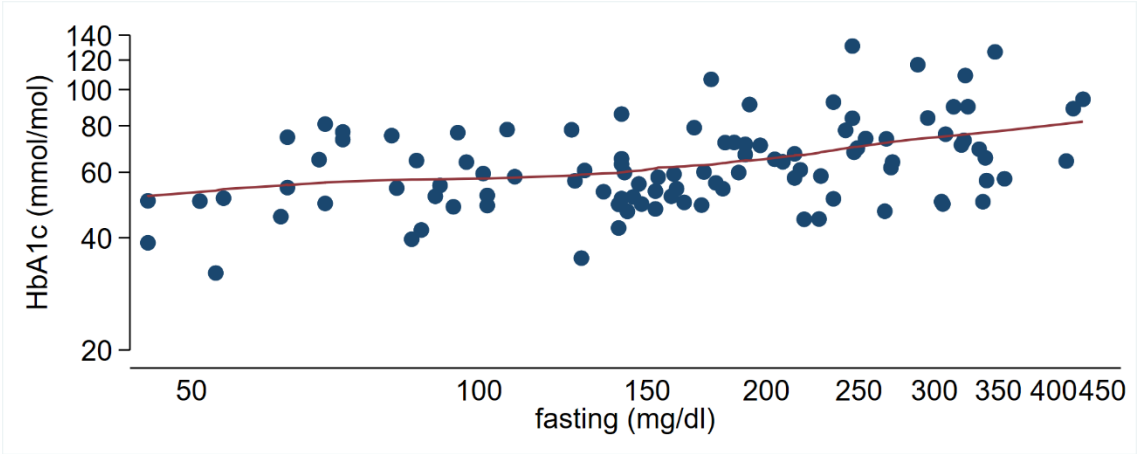
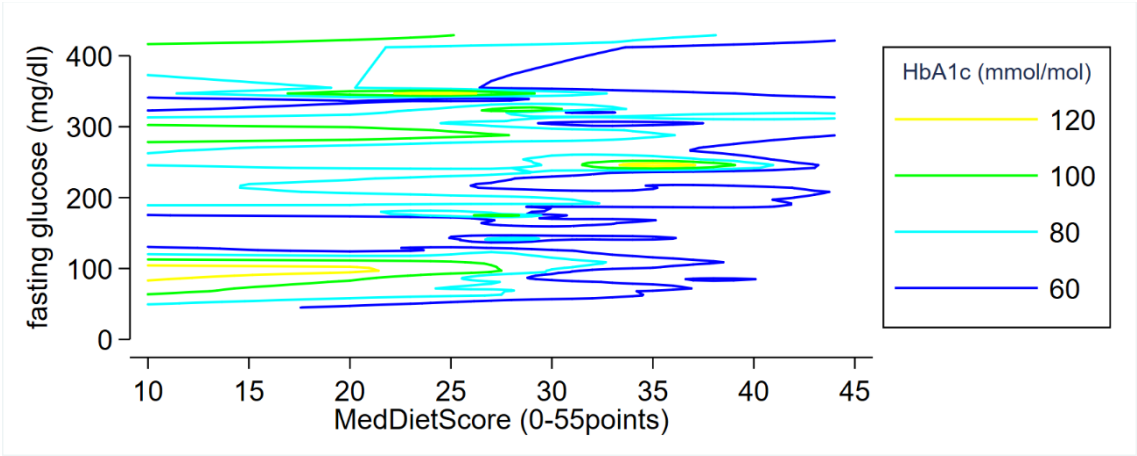


Figure 5.15: Two-dimensional contour-line plot of HbA1c (z-axis or the colour lines), glucose (y-axis) and MedDietScore (x-axis)



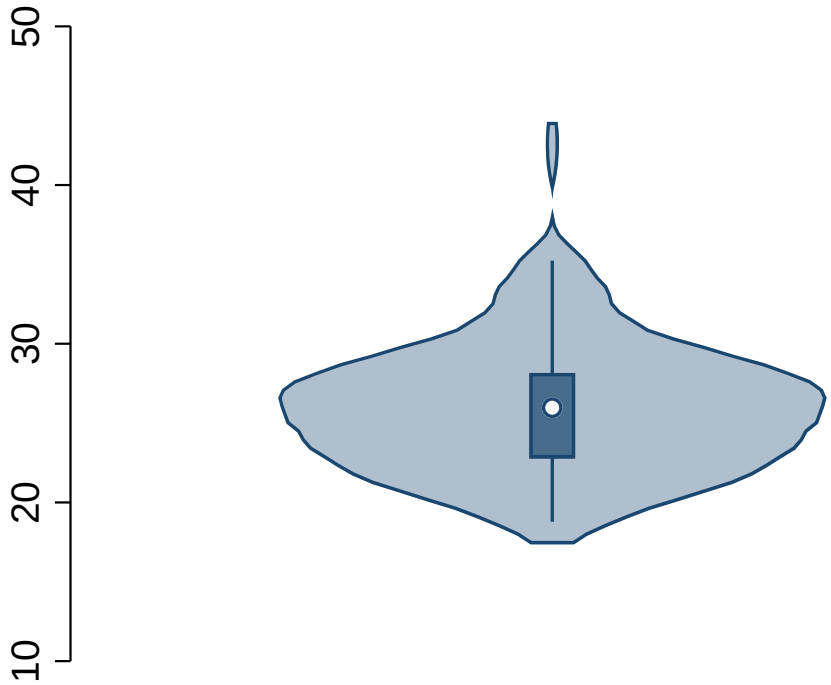
Description of covariates

This section aims to briefly describe the results of the covariates inputted in *Model 3*, other than the demographic characteristics that have been presented previously.

BMI

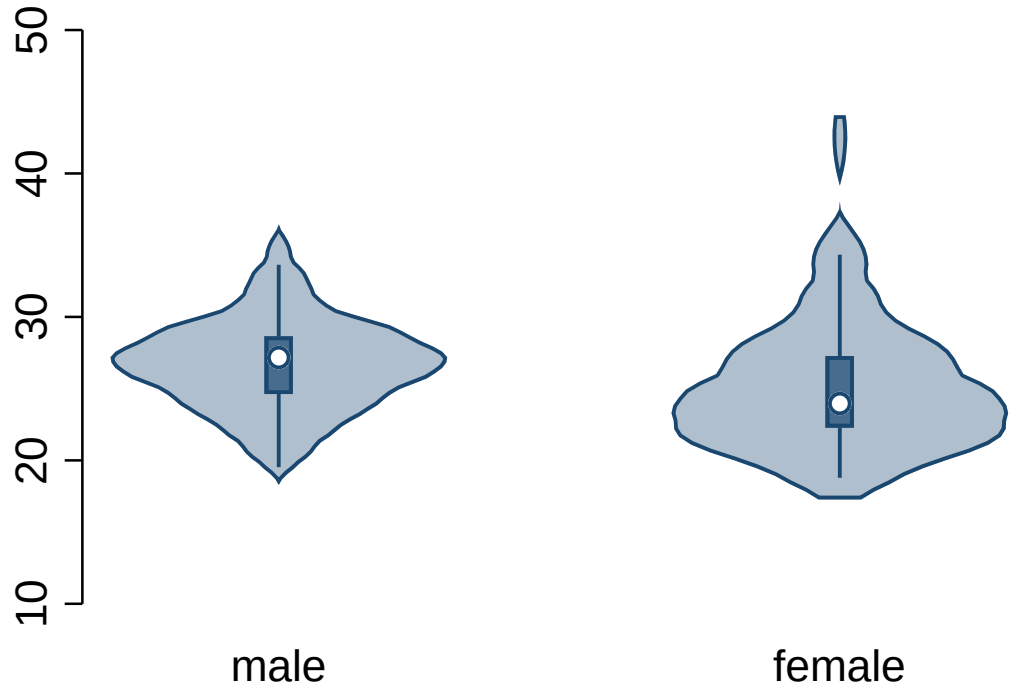
The median BMI of the participants was 26.0 kgm^{-2}, which falls in the overweight category, and the IQR was 22.8 – 28.2 kgm^{-2} (*Figure 5.16*). More precisely, the majority of patients were either overweight or obese (n = 59, 57.3%). The obese patients (n = 12, 11.7%) were mostly *obese class I* (n = 10, 9.7%), while a single participant was *obese class II* (n = 1) and *obese class III* (n = 1). Forty-four participants (42.7%) had a BMI within the healthy range, while there were no underweight participants (n = 0). The median BMI was lower for females (m: 24.0 kgm^{-2} [22.3 – 27.3]) than males (m: 27.2 kgm^{-2} [24.06 – 28.6], p = 0.0077). Furthermore, the violin plots (*Figure 5.17*) show a skewness of the BMI variable for women to the right and the presence of outliers while the one for males seems to have more symmetrical normal distributed shape – this difference in shape is likely to have also affected the p-value. Similarly, in categorical terms, a larger number of male (n = 29, 7, 56.9%, 13.7%, respectively) were within the overweight or obese category (n = 18, 5, 34.6%, 9.6%, respectively, p = 0.025) than the female participants.

Figure 5.16: Violin plot of Body Mass Index



The kernel density smoothing plot characteristics were not specificed; for the default one, see *vioplot* and *kdensity* commands

Figure 5.17: Violin plot of Body Mass Index, by gender

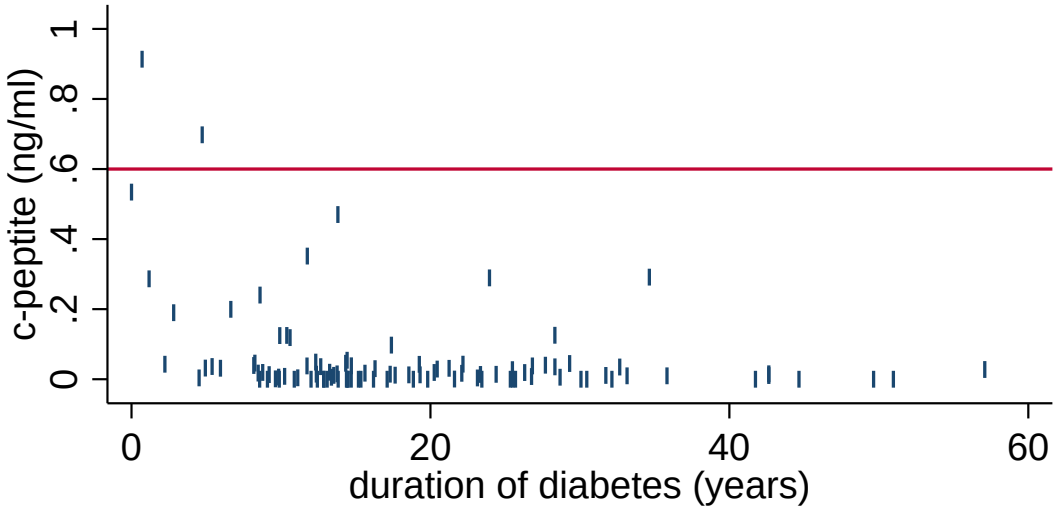


The kernel density smoothing plot characteristics were not specificed; for the default one, see *vioplot* and *kdensity* commands

C-peptide

Thirty-four participants had detectable C-peptide levels (lower limit of detection of C-peptide assay was equal to 0.010 ng/ml) ranging from 0.010 to 0.905 ng/ml. Unsurprisingly, this was related to the diabetes duration (*Figure 5.18*); the median diabetes duration of participants with detectable levels (m = 13 years [8 – 21]) was smaller when compared to participants with undetectable levels of C-peptide (m = 18 years [13 – 27], p = 0.0087 years). The distribution of these variables was similar. Finally, two participants who had a C-peptide measurement above 0.6 ng/ml, were considered as having a diagnosis of Type 1 diabetes (three patients were excluded; *Figure 5.6*). These participants had a diabetes duration of less than a year and four years (*Figure 5.18*).

Figure 5.18: Scatterplot (with spherical random noise) of C-peptide and diabetes duration with y-axis cut-off line



The STATA command *jitter* was applied to allow for spherical random noise in order to avoid points plotted on top of each other; nevertheless, the available data on duration of diabetes was available in years (integer number). Zero values in the scatter plot represent the undetectable levels of C-peptide or C-peptide < 0.010 ng/ml . The cut-off line represents the C-peptide diagnostic value of C-peptide that was considered in the inclusion criteria.

MDI vs CSII

The utilisation of CSII in the study sample was low (n = 9, 8.7%). There was no difference in the use of CSII and MDI by gender (male n = 5, 9.8%; female n = 4, 7.7%, p = 0.741).

Smoking status

More than half of the participants reported that they never smoked (n = 57, 55.3%), while a smaller (but significant) number of participants reported as being current smokers (n = 28, 27.2%), former smokers (n = 12, 11.7%) and occasional smokers (n = 6, 5.8%). Finally, more men were current smokers (n = 15, 29.4%) or ex-smokers (n = 8, 15.7%)

than women (n = 13, 25.0%, n = 4, 7.7%, respectively) while more women reported to have never smoked (n = 32, 61.5% vs n = 25, 49.0%,), although these differences were not statistically significant (p = 0.520).

Insulin adjustment

Through this self-reported question, we aimed to capture the people who take into consideration their carbohydrate intake when estimating their prandial insulin dosage. The limitations of this question have been discussed before. A related question was available in the *medical and diabetes* questionnaire in regard to *diabetes education*, and a further explanatory question was posed to ask participants to specify from whom they received such education. The latter question was initially considered for *Model 3* but then eliminated in favour of the former. This decision was primarily based on the fact that there are no structured education programs in Cyprus. Consequently, it is quite likely that the answers our patients gave to the aforementioned question reflected primarily information received via their regular clinic consultations rather than via structured education programmes. Nevertheless, the majority of the participants answered positively to both questions, although more participants did so for *education* (n = 93, 90.3%) rather than for the *adjustment* question (n = 86, 83.5%, p = 0.010). Furthermore, the question of *education* was also considered *post-hoc* in *Model 3* in place of *insulin adjustment to carbohydrate intake*. The results did not change significantly (data not shown). Finally, far more people reported receiving education from a doctor (n = 76, 73.8%) rather than a dietitian (n = 36, 35.0%, $p < 0.0005$).

Discussion

Adherence to the Mediterranean diet: clinical significance

The participants' adherence to the Mediterranean diet was moderate, with a mean of 58% (or 31.7 points; *Table 5.7*), as measured by the *MedSietScore* scoring system. This provides the first evidence to my knowledge of a significant deviation from the Mediterranean diet in a high-risk group in Cyprus and in Type 1 diabetes patients across the Mediterranean region. At the same time, it was encouraging that only a single observation was below the lower cut-off point – in the low adherence category – indicating that most participants still follow some of the rules of the Mediterranean diet. In fact, the majority of the observations (80%) fell within the moderate adherence category (*Figure 5.9*). These findings were further confirmed by a *post-hoc* computation of the 95% tolerance interval. The lower limit ($\tilde{T}_{0.99}$, = 18.3) of the tolerance intervals

coincides with the first cut-off point (1st cut-off point = 18.3 points). This provides further evidence that the majority of the study population, who are the patients with a diagnosis of Type 1 diabetes residing in Limassol, have an adherence above this lower cut-off point, or fall within the moderate adherence category and above. Furthermore, this tolerance interval and the upper bound limit ($\tilde{T}_{0.99}$ = 45.1; considering that the variable could be assumed to be approximately normally distributed) adds to the evidence that: i. the majority of the study population should lie between the first and second cut-off points (2nd cut-off point = 36.7 points) or in the moderate adherence category, and ii. a smaller portion of the study population has an adherence that is above but nearer to the second cut-off point, or on the lower side of the high adherence category (rather the perfect adherence). Therefore, these findings provide strong evidence that the deviation from the Mediterranean diet is widespread in this population.

These results are also comparable with those of our systematic review (*Chapter 3*). In the systematic review, a widespread moderate adherence was observed in all studies independently of the age of the population included and the scoring system used, similarly to the cross-sectional study. The average total adherence observed in the systematic review of studies using the *MedDietScore* (*Figure 3.2b* and *Figure 3.3*), was slightly lower than the mean adherence (53 vs. 58% or 29 vs. 32 points) of the cross-sectional study. Nevertheless, the cross-sectional study was within the range of reported adherence by the included studies in the systematic review (45 – 64% or 25 – 35 points); moreover, an equal number of studies (four vs. four) reported a higher and lower adherence than the cross-sectional study (*Table 3.2*). This suggests that the adherence by the study population was more likely similar to the general population (the population of the studies of the systematic review) and quite unlikely that it was lower. Furthermore, this is in agreement with studies on patients diagnosed with Type 1 diabetes in North America (USA and Canada) who reported an adherence to the Mediterranean diet similar to the general population (Gingras *et al.*, 2015; Zhong *et al.*, 2016) which was considerably lower than our study. This observation that the adherence to the Mediterranean diet by patients with Type 1 diabetes is in alignment with the background population, allows us to speculate that:

i. Environmental factors, such as the surrounding people and the place of residence (as an indirect measure of other parameters for example access to food), are highly influential on adherence to the Mediterranean diet (and possibly, in general on the

dietary habits) in patients with a diagnosis of Type 1 diabetes. However, which environmental constituents are important and to what degree remains to be seen.

ii. The presence of Type 1 diabetes has no or minimal influence on adherence to the Mediterranean diet (and possibly, in general on the dietary habits). This might have been influenced by the presence of newer insulins (analogues), structured education programmes, and other factors, which confer more flexibility, especially in relation to food habits, and which might allow these patients to follow a similar diet as the rest of the population.

Furthermore, this may partially explain the failure of an RCT study that provided education to patients with Type 1 diabetes (in Canada) on the subject of Mediterranean diet to produce a lasting change (at three months follow-up after the completion of the study) on the adherence, although such change was observed during the trial (Fortin *et al.*, 2018).

These observations have significant public health and policy implications, especially when considering health promotion measures. At the same time, it is important to remember that these patients are of higher risk of micro- and macro-vascular complications than the general population and therefore a better adherence to the Mediterranean diet may potentially confer greater benefits than in the general population.

Mediterranean diet versus gender, age and other demographic characteristics

The age of the participants was linearly correlated with adherence to the Mediterranean diet (*Table 5.6, Figure 5.7*). Furthermore, the difference between males and females was not statistically significant (*Table 5.6, Figure 5.8*). These results are in agreement with the results of our systematic review (*Chapter 3*). This relationship has been poorly explored in patients diagnosed with Type 1 diabetes. In a single study that explored this relationship, the results were not significant for either age or gender (Zhong *et al.*, 2016). Nevertheless, these results (primarily for age) had some methodological flaws, namely, the adherence to the Mediterranean diet score was categorised (in contrast to our study, where it was imputed as a continuous variable), while the high adherence category was significantly smaller (in regard to number of participants) than the rest of the categories. Nevertheless, overall, these results provide further evidence that: i. the adherence of the study sample was comparable to that of the general population; ii. the worrisome trend in the Cypriot general population of lower adherence by younger age groups extends to this

study sample; and iii. men and women deviated equally from the Mediterranean diet in this study sample as in the general population. Finally, other demographic characteristics that were statistically significant in the post-hoc analysis were the co-habitants, the average annual household income (inverse U shape with a top point at 30,000 euro) and the number of children (monotonic relationship); due to their *post-hoc* nature (of demographics other than gender and age) and multiple comparisons, these results need to be interpreted with caution.

Glycaemic control: clinical significance

The current study provided the first epidemiological evidence, to my knowledge, on glycaemic control of patients diagnosed with Type 1 diabetes in Cyprus. These results are far from encouraging, as they indicate suboptimal glycaemic control with the majority of the study sample not meeting the targets. At the same time, the absence of available past data on glycaemic control does not allow for any speculations on how this has changed over time. Finally, the participants included in the present study were more likely to achieve the target HbA1c than patients from other countries (when compared to data available in the literature).

HbA1c: The mean HbA1c was 65 mmol/mol (8.0 %; *Table 5.8*), which is well above the targeted level for optimal glycaemic control set by guidelines (NICE, 2015b; American Diabetes Association, 2019d). This was also true for the majority of the study sample, with less than one in three participants achieving the ADA target and one in eight achieving the stricter NICE criterion for good glycaemic control (*Table 5.8; Figure 5.10*). Even more worrying was that more than one in five had poor glycaemic control – above 75 mmol/mol (9.0 %), which ranged up to 131 mmol/mol (14.1 %). Furthermore, the 95% tolerance interval confirmed the wide range of HbA1c towards the right (or high) side. Even more worrying is that the tolerance intervals predict that more than 3% of the study population is expected to have an HbA1c above the upper limit or the 130.8 mmol. Overall, this evidence of HbA1c suggests that people diagnosed with Type 1 diabetes in Cyprus are far from well controlled.

Glucose: The mean fasting glucose was 185 mg/dl (10.3 mmol/l; *Table 5.9*), which is also well above the target range for optimal glycaemic control set by guidelines (NICE, 2015b; American Diabetes Association, 2019d). This was also true for the majority of the study sample, with only one in five participants achieving the ADA targets for good glycaemic

control (*Table 5.9; Figure 5.11*), while about one in eight were within the hypoglycaemic range. Even more worrying was the range of observations from the dangerous low (hypoglycaemia) to the high (hyperglycaemia) glucose levels. Severe hypoglycaemia and diabetic ketoacidosis due to hyperglycaemia can potentially lead in the short-term to coma and eventually death; fortunately, no severe complications were reported during the study. Furthermore, this may be an indication of not just poorly controlled diabetes, but also an incorrect dosage of long-acting insulin and potentially a significant mismatch between night-time rapid-acting insulin, exercise and food intake (although some may argue that the picture is far more complex than that, for example, tighter control usually results in increased risk of hypoglycaemia). Further, this may potentially reflect the absence of structured education programmes and the provision of appropriate advice by health care professionals on carbohydrate counting in Cyprus. Moreover, the 95% tolerance interval confirmed the wide range of fasting glucose towards both sides; the dangerously low and high blood glucose levels. Overall, these results add further to the results of the HbA1c for poor glycaemic control, while they may also potentially suggest a wide fluctuation of blood glucose levels and inappropriate insulin adjustment.

Glycaemic control: The results for glycaemic control are worrying. Due to the absence of past data on this population we are unable to speculate on trends over time. In *Table 5.18*, it was attempted to compare the data presented during this study against data available on glycaemic control in adults from four countries of interest, namely England and Wales, Scotland, Sweden and the USA. Data were available for HbA1c but not for glucose and were based on national databases and consortiums of (general and specialist) healthcare centres. A larger proportion of participants in the present study achieved the target HbA1c (Category I at *Table 5.18*) than any of the other countries. More precisely, the participants had substantially better glycaemic control than patients in Scotland, which association of the two (categorical) variables was statistically significant, and in England and Wales (there was inadequate data for further comparison). In contrast, although a higher number of participants achieved the target HbA1c, a higher number of participants also had poorly controlled diabetes (Category III at *Table 5.18*) than the patients in Sweden and USA; indeed, with the former there was a statistically significant difference ($p = 0.018$). The following parameters were suggested, which may explain at least partially this discrepancy: i. our data have included a relatively young population (m: 33 years [26 – 43]; *Table 5.6*) and potentially younger than the other studies, also, there was suggestion

of bias towards recruiting younger patients; ii. our population was largely free of complications other than foot and eye disease (data not shown); iii. the data for England and Wales includes all ages and non-adults (especially teenagers) are likely to have worse glycaemic control (Leelarathna *et al.*, 2011) – nevertheless the difference is too large to solely been explained by this parameter; iv. racial differences in HbA1c levels (for same blood glucose levels) exist, although these are usually small (Bergenstal *et al.*, 2017), and the population of the current study was not ethnically diverse (*Table 5.6*); iv. we are unable to account for large fluctuations in blood glucose levels and the presence of hypoglycaemia due to an absence of data on glucose, for example, regular occurrence of hypoglycaemia can push the HbA1c levels lower but not necessary reflect a better glycaemic control. At the same time, similar levels of HbA1c can achieved with low or large fluctuation of blood glucose levels, although the latest is considered detrimental; v. large numbers affect the results of the Pearson's χ^2 test for independence towards a smaller *p*-value, while the Cramér's V, which is unaffected by sample size (Kearney, 2017), was small in all occasions (data not shown); vi. the different recruitment process, which was through the CDA database, contacting the participants, accepting phone invitation and turning up for assessment and laboratory test versus the more universal capture of the large databases (which is also reflected in points *i* and *ii*). Furthermore, we cannot exclude the possibility that the study sample may not be representative of the Cypriot population results as a result of our recruitment method and therefore we may have attracted the more motivated and better controlled individuals; and vii. through categorising the data, although this is clinically useful in the current situation, some information may have been lost, which can affect inferences (Harrell, 2015).

Nevertheless, we are unable to say whether these parameters explain the difference in full, or even partly, and we cannot exclude that such difference is present, especially given the large difference observed. At the same time, such proportions of participants achieving the target levels of HbA1 in not unimaginable and within the range observed in studies before (Mcknight *et al.*, 2015). Finally, some parameters may also potentially have an opposite effect, for example, our data show that the younger population had worse glycaemic control than the older population (*Table 5.6*). Therefore, on balance, the data suggest that our study population was more likely to have a target HbA1c than the populations from the aforementioned studies from the UK, Sweden and the USA. Needless to say, we cannot claim with confidence that the Cypriot population differs

significantly from the aforementioned populations, given the reasons mentioned above, and simply the fact that our study was not designed to answer this question.

Table 5.18: Glycaemic control versus control in patients with Type 1 diabetes in selected countries

Country	HbA1c (mmol/mol)						*p*-value[a] (vs. Cyprus[b])	Comments	Reference
	Category I		Category II		Category III				
	n	%	*n*	%	*n*	%			
	< 58		58 – 75		> 75				
Cyprus	44	42.7	36	35.0	23	22.3		Age: 17 + (*Table 4.7*)	
Scotland	7074	26.6[c]	11379	42.8[c]	8113	30.5[c]	**0.001**	Age: 18 +, > 99% of the population through SCI-Diabetes, 2018	Scottish Diabetes Data Group, 2018
	< 52		52 – 78		> 78				
Cyprus	30	29.1	56	54.4	17	16.5			
Sweden	12977	27.3	30321[d]	63.7	4273	9.0	**0.018**	Age: 18+, > 95% coverage of Type 1 in 2015 through Swedish NDR (Svedbo Engström *et al.*, 2019), 2019	data available online (NDR, 2020)
	≤ 58		> 58						
Cyprus	44	42.7	59	57.3					
England and Wales	[e]	29.9	[e]	70.1			[e]	Age: any age[e], 98.3% of general practices and specialist services in England and Wales through NDA, 2017 – 2018	NHS Digital, 2019
	< 53		53 – 75		≥ 75				
Cyprus	31	30.1	49	47.6	23	22.3	0.082		
USA	1330[f]	21.5	3514[f]	56.9	1337[f]	21.6		Age: 18+, 81 U.S.-based paediatric and adult endocrinology practices in 35 states through T1D Exchange clinic registry, 2016 – 2018	Foster *et al.*, 2019

Note that this table was not intended to be a full list of available data on glycaemic control across countries. [a]The test of choice was the Pearson's χ^2 for independence, which was computed using the immediate STATA command *tabi* by feeding the absolute numbers in each category available, as presented on the table; bold values indicate statistical significance. [b]Based on the data of the cross-sectional study presented in this chapter – data matched for categories and approximately for age when necessary. [c]Calculated based on the people who had a HbA1c record (89.3%) – therefore percentages may differ from those presented in the original document. [d]Calculated from the total number that had available data (47571 out of 48902) minus the other two categories. [e]We were unable to identify raw numbers and data only for adults; therefore, *p*-value was not considered relevant. [f]Calculated from individual data available for 18 – 25, 26 – 50 and ≥ 50 years old.
SCI-Diabetes, Scottish Care Information-Diabetes; Swedish NDR, National Diabetes Register; NDA, National Diabetes Audit.

Glycaemic control versus gender, age and other demographics

The participant age was inversely correlated with HbA1c and fasting glucose (*Table 5.6*, *Figure 5.7*). This association remained significant in the fully adjusted model for fasting glucose but not for HbA1c (*Table 5.13 and Table 5.17*, respectively). At the same time, no difference in glycaemic control was observed between the male and female participants, in the univariate analysis (of location) or in the fully adjusted regression models. A study in the USA using data from the T1D Exchange clinic registry (*Table 5.18*) reported a similar pattern from the age of 18 to 28 years, but, above this age, the HbA1c remained fairly steady (Foster *et al.*, 2019). Another study, which used data from over 300,000 patients diagnosed with Type 1 diabetes from 19 different countries, reported a higher HbA1c for the ages of 15 to 24 when compared to those above the age of 25 years. It also reported small non-significant differences between males and females (Mcknight *et al.*, 2015). Overall, our study observed a significant monotonic and linear relationship between HbA1c and fasting glucose, respectively. These results raise an important question: why might younger people have inferior glycaemic control compared with their older counterparts? Furthermore, this has significant implications in regard to health promotion measures but also for clinical practice. Alternatively, our data suggest that it is unlikely that there is a significant difference between males and females. Finally, other demographic characteristics that were statistically significant in the *post-hoc* analysis were the nationality, marital status, number of children (inverse monotonic relationship), highest educational level (inverse U shape with a top point at University – Bachelor) and the employment time for fasting glucose, while no statistically significant results were observed for HbA1c; due to their *post-hoc* nature (of demographics other than gender and age) and multiple comparisons between these results need to be interpreted with caution.

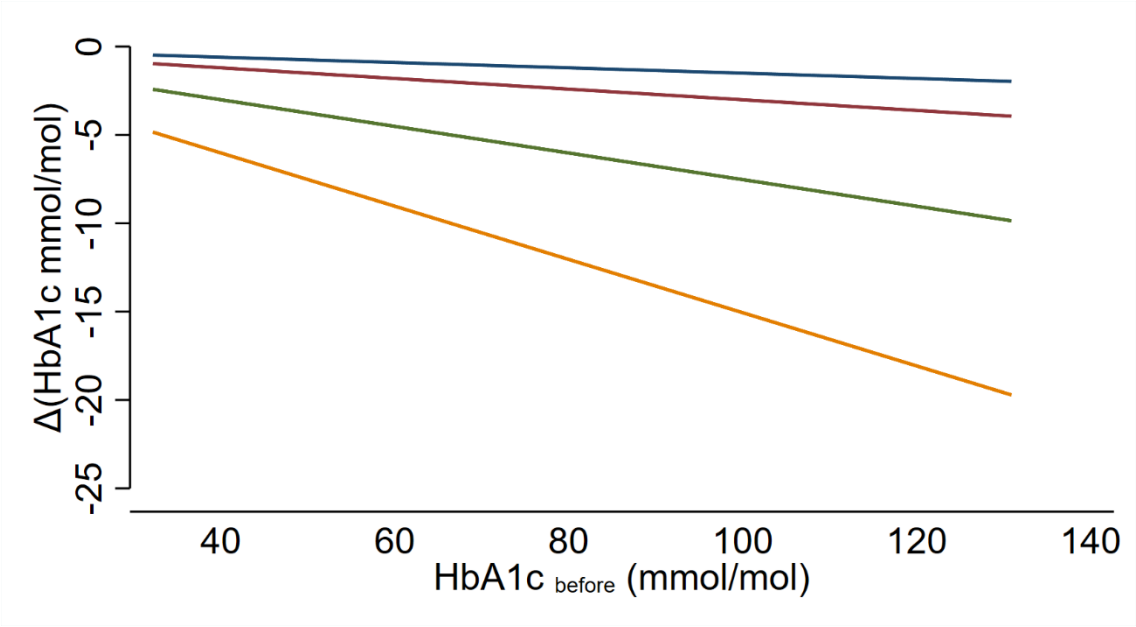
Adherence to the Mediterranean as a predictor of glycaemic control

The Mediterranean diet was a statistically significant predictor of HbA1c (*Table 5.13*) but not of fasting glucose (*Table 5.17*), after adjusting for potential confounders. More precisely, the fully adjusted model (*Table 5.10* and *Table 5.14*) predicted a reduction in HbA1c (mmol/mol) by 1.5% for every additional point in the score of the *MedDietScore* scoring system. This reduction in HbA1c is plotted in *Figure 5.19* and *Figure 5.20*. As can also be observed from these graphs (and as an implication of the models): i. the relationship of the two variables is linear, ii. due to the exponential nature of the

relationship [ln (HbA1c) = f(b *MedDietScore*$_i$) or HbA1c = f(e$^{(b\ MedDietScorei)}$), where f is a function and b_i the coefficient], this change has a multiplicative effect. For example, a 5-point increase in the *MedDietScore* is predicted to cause 5 times the change as 1 point in HbA1c (rather than an additive effect), and iii. this change in HbA1c is predicted to be proportional to the levels of HbA1c, that is, the higher the HbA1c the larger the effect and the opposite. Overall, this effect is clinically significant, especially considering the limited availability of adjuvant medications for this disease (that can be used in conjunction with insulin). Nevertheless, a cause-effect relationship cannot be established due to the cross-sectional nature of the study. Moreover, this relationship cannot be extended to the low adherence category. However, these results are very encouraging. Furthermore, one wonders whether these results have any influence on the favourable glycaemic control observed in the present study over other countries (*Table 5.18*). The relevant studies have been discussed in detail above (*Chapter 1*), including their limitations. In addition, their results are presented in *Table 5.19*.

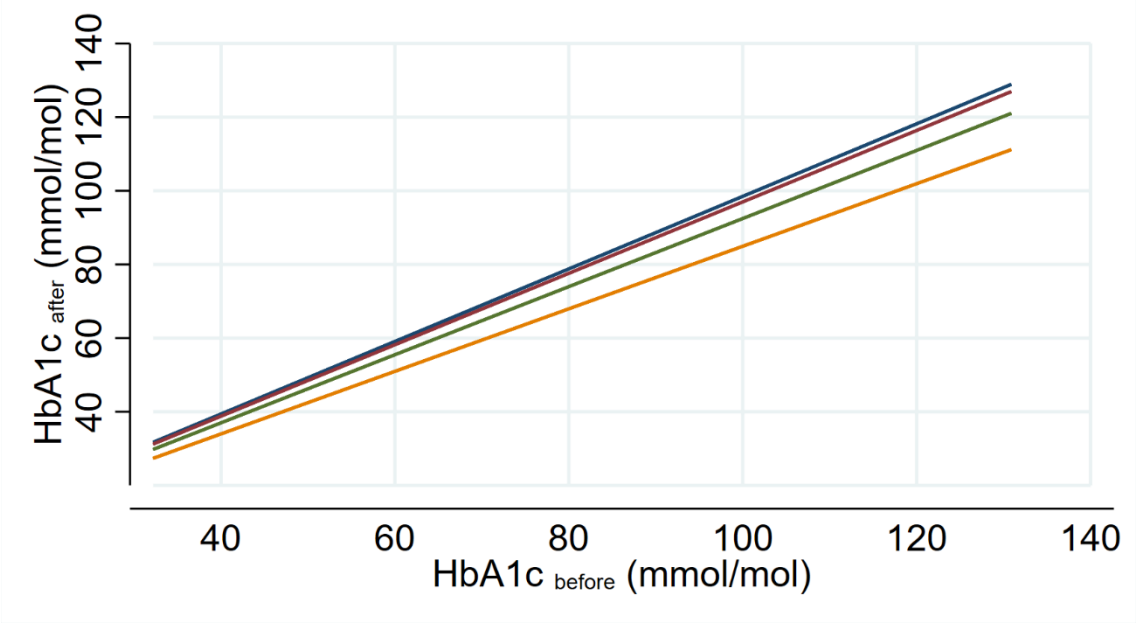
The results of the present study, in combination with the absence of an existing (appropriately designed) interventional study that investigated this outcome, make the need for such an interventional study more urgent. More precisely, this study needs to be adequately powered (number of participants) so as to be able to measure a small but potentially clinically significant effect on glycaemic control. Furthermore, the intervention should be well-designed so it does actually cause an effect on adherence to the Mediterranean diet, while this needs to be measured; correlating the two (i.e., change in Mediterranean diet and glycaemic control) will potentially help infer possible causation by the Mediterranean diet. Finally, this effect should be independent of parameters that are known to cause significant effects on glycaemic control, such as structured education. For example, such study design could be an interventional study with two arms, of which the one arm is structure education alone, and the second arm is structure education plus education on the subject of the Mediterranean diet; this could potentially help clarify whether the Mediterranean diet provides any additional benefits. Finally, if children are to be included, then special care should be sought so that parents are involved, as they are the main providers of food. Nevertheless, given the current evidence, we believe that it is justifiable to provide the patients with information on the Mediterranean diet when considering education and carbohydrate counting and this is potentially a game changer in the field of Type 1 diabetes management.

Figure 5.19: Predicted change in HbA1c (mmol/mol) for four given increases in MedDietScore (points) and a given HbA1c (mmol/mol)



---- 1 point ---- 2 points ---- 5point ---- 10points

Figure 5.20: Predicted HbA1c (mmol/mol) for four given increases in MedDietScore (points) and a given HbA1c (mmol/mol)



---- 1 point ---- 2 points ---- 5point ---- 10points

Table 5.19: Mediterranean diet versus HbA1c, relevant studies

Demographics	Results	Comments
Cross-sectional studies		
(Gingras *et al.*, 2015) Canada, $n = 118$, ≥ 18 years	r = -0.12, p = 0.25, adjusted for age and gender p = 0.29, low vs high adherence	Population with traditionally low adherence to the Mediterranean diet, which was confirmed in the study. The use of partial correlation test explores only the presence of a linear relationship so a different type of relationship could not be excluded. Furthermore, it stills fits a linear model and assumptions hold. No scatter plot of the two variables is available or is mentioned to have been examined. Categorical inference is not providing additional information.
(Zhong *et al.*, 2016) USA, n = 793, ≥ 10 years	β = -0.02, p = 0.02, adjusted for multiple potential confounders p = 0.54, low vs. moderate vs. high adherence	Population with traditionally low adherence to the Mediterranean diet, which was confirmed in the study. Heavily modified *KIDMED* score used (a Mediterranean diet scoring system, see *Chapter 1: Introduction*); This comparison was not a primary outcome but rather a post-hoc analysis; Appropriate statistics used although categorical inference is not providing additional information; The regression model included the following covariates: age, sex, race, diabetes duration, parental education, family income, parental history of diabetes, clinical site, physical activity, sedentary behaviour, smoking, total calories, insulin regimen and daily insulin dose per kg.
Longitudinal studies		
(Zhong *et al.*, 2016) USA, $n_1 = 521$, $n_2 = 501$, ≥ 10 years	β = -0.01, p = 0.07 (lnHbA1c)	Longitudinal at 1 (n_1) and 5 years (n_2); Same as before.
Interventional studies		
(Cadario *et al.*, 2012) Italy, n = 96, children and adolescents	SS in some subgroups only (vs. baseline)	Intervention: structured education training to the Mediterranean diet by a dietitian. The change in adherence to the Mediterranean diet not measured therefore effect or no effect could be attributed to Mediterranean diet.
(Fortin *et al.*, 2018) Canada, $n_1 = 14$, $n_2 = 14$, 18 – 65 years	NS	Intervention: teaching sessions with a registered dietitian, Mediterranean diet (n_1) vs low fat diet (n_2). The study was likely underpowered. Population with traditionally low adherence to the Mediterranean diet, which was confirmed in the study.
(Mouslech *et al.*, 2018) Greece, n = 62, adults	SS	Intervention: Structured diabetes education program and motivated to follow a Mediterranean diet and exercise. Change in the Mediterranean diet was not measured and structured diabetes education is well accepted that improves glycaemic control therefore, it is not possible to infer if the Mediterranean diet has contributed into this improvement in glycaemic control.

A relevant cross-over study of lispro and human insulin (Provenzano et al., 2001) does not report outcomes of interest and therefore is not reported in the table.

SS/NS: statistically significant/ not statistically significant change in glycaemic control (HbA1c).

The Mediterranean diet predicts HbA1c but not spot fasting glucose levels: a potential underlying mechanism of the effect of the Mediterranean diet on glycaemic control

HbA1c and fasting glucose are both indicators of glycaemic control, as has been described previously, whereas the glycaemic control is the glucose levels over a period of time. In more practical terms, HbA1c reflects the average glucose over an approximate period of time equal to the last three months, whereas spot fasting glucose indicates the blood glucose levels at a particular point of time when the blood was drawn under specific conditions, namely fasting. Therefore, the HbA1c reflects the individual glucose measurements over this period and, if categorised, may include the fasting levels (when the patient wakes up before food), pre- and post-meal measurements and other fluctuations in between those. Note that other parameters may interfere in this relationship, making their correlation more complex (Rohlfing *et al.*, 2002), but their effect is expected to be small (or insignificant) for the current study. Based on the above, it should not be much of a surprise that the Mediterranean diet was a significant predictor of HbA1c but not of fasting glucose. Rather, this should be considered as evidence that the potential underlying mechanism of the effect of Mediterranean diet on HbA1c is not indicated through fasting glucose levels, but through the effect on non-fasting levels such as the post-prandial levels. This is not unreasonable, taking into account that food (and consequently, the Mediterranean diet) affects (by definition) the non-fasting (or post-prandial) time frames and, to a lesser extent, the fasting hours (at least directly). Alternatively, the most important parameter that potentially affected the fasting glucose was expected to be the long-acting insulin and, to a lesser extent, parameters such as late exercise and food (although patients were asked to fast), duration of absorption of food last eaten, rapid acting insulin use during the last meal and the presence of hypoglycaemia up to 48 hours beforehand.

Furthermore, a further statistical analysis suggested that the effect of the Mediterranean diet on HbA1c is not mediated by the (spot) fasting glucose measurements. Nevertheless, we recognise the limitations of these results, given the cross-sectional nature of the study (cause-effect relationship could not be assumed) and the spot measurements of glucose (it is widely accepted in Type 1 diabetes that fasting glucose can exhibit large variations from day to day and its usefulness is often disputed). Similarly, in addition to what we have suggested in the previous section, for an interventional study we suggest that this

study should also monitor the blood sugar levels (ideally through an intermittent or a continuous glucose monitoring system) to help elucidate the underlying mechanism. Finally, it worth mentioning that similar results were observed in a meta-analysis of interventions in Type 2 diabetes where the Mediterranean diet did reduce the HbA1c but not the fasting glucose (Carter *et al.*, 2014).

Discussion of Covariates

BMI

The BMI is an index of adiposity, which relates well with health. The median BMI of the participants was 26.0 kgm^{-2}, which falls in the overweight category, and the IQR was 22.8 – 28.2 kgm^{-2} (*Figure 5.16*). These values of obesity (overweight and obese) are considerably higher than for the background population (Eurostat, 2019); although this data should be used with caution as they are likely to be outdated (based on older data). At the same time, the obesity levels (overweight and obese) of the participants were lower than their counterparts in the UK (Scottish Diabetes Data Group, 2018; NHS Digital, 2019), which were comparable to the background population (NHS Digital, 2019). Finally, BMI was a statistically significant predictor of fasting glucose (*Table 5.17*) but not of HbA1c (*Table 5.13*) in the fully adjusted models.

C-peptide

The C-peptide measurement is an indirect measure of the residual endogenous insulin production by the β-cells of the pancreas. Thirty-four participants had detectable C-peptide levels ranging from 0.010 to 0.905 ng/ml. Unsurprisingly, this was related to their diabetes duration (*Figure 5.18*) and the median diabetes duration of participants with detectable levels was smaller when compared to participants with undetectable levels of C-peptide. These results are in agreement with available literature (Oram *et al.*, 2014, 2015; Davis *et al.*, 2015) suggesting that often people with a diagnosis of Type 1 diabetes continue to secrete insulin (especially, when measured using ultrasensitive assays); and that the duration of diabetes is an important predictor of this endogenous insulin secretion.

MDI vs CSII

The utilisation of CSII in the study sample was low. This finding resonates with our clinical experience, although to my knowledge, no official data are available. This rate of CSII is very low when compared to the USA (Foster *et al.*, 2019), but comparable to the Scottish adult population (Scottish Diabetes Data Group, 2018).

Smoking status

Smoking in people with a diagnosis of Type 1 diabetes increases the risk of complications and may be related to worse glycaemic control (Braffett *et al.*, 2019). In the present study, more than half of the participants reported that they never smoked, while a smaller (but significant) number of participants reported as being current smokers, former smokers and occasional smokers. Moreover, more men were current smokers or ex-smokers than women, while more women reported to have never smoked, although these differences were not statistically significant. These numbers are comparable to the Cypriot general population (European Commission, 2017) but higher than their counterparts in the UK (Scottish Diabetes Data Group, 2018; NHS Digital, 2019); who also had a prevalence comparable to the UK background population (NHS Digital, 2019).

Insulin adjustment

The results of this self-reported question and the one on *diabetes education* likely confirm our concern that this question has more likely captured the routine education received via the regular clinic input because it is very unlikely that doctors had the time (and quite often the knowledge and skills) to provide formal education with an emphasis on carbohydrate counting.

Conclusion

The Mediterranean diet was a predictor of glycaemic control in this cohort of patients diagnosed with Type 1 diabetes after adjusting for several potential confounding factors. The fully adjusted model predicted a reduction of 1.5% in HbA1c (mmol/mol) for an increase in the *MedDietScore* by one point. This effect is unlikely to be mediated through the fasting blood glucose levels. Furthermore, in this high-risk population, there was a significant widespread deviation from the Mediterranean diet and sub-optimal glycaemic control. The reduced adherence to the Mediterranean diet and the suboptimal control were more pronounced in the younger participants, but similar between men and women.

Chapter 6: Discussion

Summary

This thesis has investigated the impact of the Mediterranean diet on the glycaemic control of people diagnosed with Type 1 diabetes in Cyprus. More precisely, the degree of adherence to the Mediterranean diet predicted the glycaemic control (HbA1c). Moreover, our data indicated that this effect was mediated through non-fasting (such as the post-prandial) glucose levels. The size of this effect is clinically meaningful and is predicted to be of more benefit to the patients with worse glycaemic control. These results, ideally, should be confirmed in a well-designed interventional study (the desirable characteristics of which has been described previously in *Chapter 5*). Nevertheless, I believe that when these results are considered in combination with the other potential health (such as cardiovascular) benefits of the Mediterranean diet, they do provide adequate evidence to justify the systematic provision of a Mediterranean diet-related advice to people diagnosed with Type 1 diabetes, for example, during structured education and clinical consultations by dietitians, doctors and other health care professionals.

This research has also provided good evidence that the Cypriot population has deviated from the Mediterranean diet and that this deviation extends to people with a diagnosis of Type 1 diabetes. At the same time, our data indicate that not all is lost in regard to the adherence to the Mediterranean diet; instead, it is still followed to a significant degree and, although prompt intervention is needed, it is not yet too late for action to be taken to halt and even reverse this trend. An even stronger argument for the need for urgent implementation of public health measures is the fact that our research revealed that the younger people adhere to a lesser degree to the Mediterranean diet than the older generation, accompanied by a suggestion of the reduction in adherence to the Mediterranean diet over time. This worrisome trend was equally present in men and women and extended to people with a diagnosis of Type 1 diabetes.

We have also presented epidemiological evidence on glycaemic control in patients diagnosed with Type 1 diabetes. Our results were worrisome, as most of the participants did not achieve these targets, while some had dangerously high or low levels of HbA1c or fasting glucose. Furthermore, the worst glycaemic control could be observed in the younger ages, which was similar between male and female participants.

Finally, it is worth acknowledging that significant challenges were encountered during the research process. These included (among others): the absence of appropriate measures, such as questionnaires, validated or appropriate for the Cypriot population; the absence of a central database with anonymised information of people with Type 1 diabetes; and the fragmented nature of the health care system in Cyprus and the limited funding for the study. Nevertheless, through this thesis I have presented the first published systematic review on the subject of adherence to the Mediterranean diet and the first study to provide cumulative results (total adherence stratified by Mediterranean diet score) on this subject. Furthermore, I am the first to explore the glycaemic control of patients with Type 1 diabetes in Cyprus and the first to investigate the effect of a Mediterranean diet on glycaemic control in the adult population of a country in the Mediterranean region.

Strengths and weaknesses

Potential strengths of my results include the following:

a) The recruited sample was derived from a population that by definition had a high adherence to the Mediterranean diet (defined as that of the Cypriot population of the '50s and '60s).
b) The execution of a systematic rather than a narrative review and the presentation of cumulative results (stratified by a Mediterranean diet scoring system).
c) The effect of the Mediterranean diet on glycaemic control was the primary outcome of the study – the study was designed specifically to investigate this outcome.
d) The methodology, including the questionnaires, was extensively tested during the pilot study. Furthermore, a well-studied Mediterranean diet score, the *MedDietScore*, was used.
e) Sound statistical methods were used and, for the primary outcomes, all the assumptions where thoroughly investigated. Furthermore, on some occasions, where it was considered necessary, a second test was also computed to compare the results (for example, MM-robust regression). Moreover, the modelling techniques, such as the model (linear regression) and the covariates were pre-specified.
f) The confirmation of the presence of Type 1 diabetes by means of clinical and biochemical methods and the rigorous random selection process.
g) The use of two measures, namely HbA1c and fasting glucose, for glycaemic control and the inclusion of carbohydrate counting and C-peptide as confounders, despite

their limitations as previously discussed. These parameters, theoretically, may have allowed for more flexibility in food intake accompanied with good glycaemic control.

h) The contributions made to the study by a highly experienced multi-disciplinary team.

Nevertheless, my results had some potential limitations, including the following:

a) The systematic review was restricted to the population of Cyprus and Greece and thus the results do not necessarily reflect populations other than those described here. Furthermore, the number of studies conducted in Cyprus was limited.
b) A cause-effect relationship between a Mediterranean diet and glycaemic control cannot be established due to the cross-sectional nature of the study. Furthermore, this relationship cannot be extended to the low adherence category.
c) The absence of validated questionnaires in the Cypriot population that could have been used in the current project.
d) The absence of formal structured diabetes education programmes in Cyprus so that our results may not necessarily extend to populations that receive such training.
e) The absence of a national database in Cyprus of people diagnosed with Type 1 diabetes. Therefore, our results (such as those relating to glycaemic control) may not necessarily be representative of this population in Cyprus.

Critical review of the study design for the intended aims of the study

This section was a reflection on the study design on meeting the aims of the cross–sectional study and the implications of it on the results. Initially the study design was discussed from the perspective of the primary outcomes and then selected aspects of the study design were considered.

Outcomes

Epidemiological evidence (Mediterranean diet and glycaemic control): The present study provided epidemiological evidence on adherence to Mediterranean diet and glycaemic control for people with Type 1 diabetes in Cyprus and this is in agreement with the aims and objectives of the study. These results are expected to be significant from a clinical and public health perspective because they are the *first evidence* available on these important epidemiological parameters for this particular population (in Cyprus). Nevertheless, and as previously mentioned (including the *limitations* section in the current chapter), we cannot say definitely if these results are representative of the target

population i.e., patients with Type 1 diabetes residing in Limassol and by extension in Cyprus. This is primarily due to the limitations of the CDA database, as discussed elsewhere. Having said that, we tried hard to reduce selection bias and there is good chance that the CDA database included a significant portion of the target population as all people with Type 1 diabetes in Cyprus are required to register with the CDA in order to obtain medications and devices free of charge (Skordis *et al.*, 2012). Nevertheless, these results are in full agreement with our clinical experience and available research data (as discussed below), and substantially reinforce the argument that public health and other measures are required in this high-risk population; some of these potential measures are discussed in the next section of this chapter (*Suggestions for public health measures (and clinical)*).

Association (of adherence to Mediterranean diet and glycaemic control): The present study provided evidence on the significant association between adherence to Mediterranean diet and glycaemic control for people with Type 1 diabetes in Cyprus, which is a Mediterranean country, and this is in agreement with the aims and objectives of the study. The study was adequately powered to investigate this outcome and was the first to adjust for confounders that are known to improve the glycaemic control, such as education. However, and as described before, including in the *limitations* section of the current chapter, a cause-effect effect could not be inferred and our results may not apply to non-Mediterranean populations as the number of participants with low adherence was limited. Nevertheless, our results are encouraging and they reinforce the current clinical practice, i.e. to provide advice on the Mediterranean diet to people with Type 1 diabetes for complications prevention and potentially for glycaemic control. Recognizing the limitations of the current study, we have discussed further research suggestion to cover these gaps, in *Chapter 5* and in the next section of the current chapter (*Further academic and research suggestions*).

Selected aspects of the study design

Reflection on sample size analysis: The aimed sample size was decided based on PSS analysis and other calculations, and was defined as 100 participants, who had sufficient data to be included in the analysis of the primary outcomes. These calculations were very useful as they provided us with the invaluable information: i. the aimed sample (i.e., 100 participants) was adequately powered to detect the defined smallest clinically significant effect (of the Mediterranean diet on glycaemic control) ii. allowed us to forecast that the

CDA database could provide us with this number of participants iii. and eventually, allowed us to define the aimed sample size based on theoretical and practical parameters, including the data collected through the pilot study. In contrast, for example, if all patients were conducted and based on our data (33.3%) this would have potentially resulted in additional 12 participants being recruited without any significant research benefit raising ethical issues while increasing the financial and practical burden of the study. Finally, the pilot study provided us with good estimates of the parameters used in the calculations and the participation rate (29.2% and 30%) was similar, helping us to better design the main study. At the same time, the epidemiological outcomes were not considered in these calculations since the potentially large sample studied (in comparison to the target population) would have allowed us to adequate answer the epidemiological questions, as discussed elsewhere.

Reflection on the recruitment process (random selection process): The method of recruitment, namely the random selection process, was chosen on research, clinical and ethical grounds. For example, it helped to avoid i. the CDA secretarial staff potentially cherry-picking participants based on familiarity with patients i.e., selecting people to benefit from the free blood and urine tests ii. recruiting by order of registration numbers in the CDA database, which potentially may reflect the diabetes duration of the patients i.e., patients most often are registered when they are still hospitalized and on the first days of diagnosis of the diabetes. Furthermore, there was no statistically significant difference between those we attempted (n = 36) and did not attempt (n = 353) to recruit in respect to age and gender. Nevertheless, due to the fact that a significant proportion of the population was conducted (92.5%; which was of no surprise as this was forecast by the sample size calculations) and the flaws of the database used, the benefit of this method on the results was potentially reduced. More precisely, this may have introduced bias in the generalizability of the epidemiological outcomes. However, our clinical experience and currently available evidence, for example, the systematic review and other studies on the background population, and studies with participants with Type 1 diabetes in other countries, support the hypothesis that these results reflect the target population. Nevertheless, this study provided the useful clinical and public health information that this large proportion of the target population studied, consistently deviates from optimal glycaemic control and adherence to Mediterranean diet; these parameters are good predictors of complications and cardiovascular health in this high-risk group and hence

the results will have a negative impact on the CVS health of this Mediterranean population. At the same time, this potential bias does not reduce the other benefits mentioned before i.e. the 36 patients not invited to participate was based on random chance (and the patients conducted before them). Finally, the recruitment method is reported in a clear and detail manner to allow the readers of this project to make an informed decision on the relevance of our results on their clinical practice and to replicate our methods if and when needed.

Reflection on diabetes parameters: A rigorous process was applied to identify patients with Type 1 diabetes based on clinical and biochemical criteria (described in detail before and improved following the pilot study), therefore we can confidently say that the appropriate sample with the correct diagnosis was included in this study. Other aspects of diabetes not considered in the current project and are potential clinically significant parameters, such as glycaemic variability, are briefly discussed in the current chapter and in *Chapter 5*.

Summary: Overall, the design of the main study was successful in achieving the aims of the study (while ethical considerations were taken on board), namely, to produce the first and currently only available epidemiological evidence on Mediterranean diet and glycaemic control in people with Type 1 diabetes in Cyprus; and the first to explore the association between them, whilst also adjusting for potentially significant confounders.

Suggestions for future research and public health measures

Further academic and research suggestions

This thesis has provided some important insights that can be utilised to guide us and other researchers into future research work. In this section, I provide some suggestions for this research, which are as follows:

1. Through the study on Type 1 diabetes (*Chapter 5*), I have collected a large amount of data. These data extended beyond the aims (primary outcomes) of the current thesis and therefore were not presented here. Nevertheless, it is of paramount importance that these data are analysed in the near future as they could provide us with valuable information. For example, they could provide information on the epidemiological aspects (such as the lifestyle) of the people living with Type 1 diabetes but also

explore relationships (in variables) of interest (such suggestions are presented in *Appendix: Chapter 6*). Furthermore, we might attempt to input on the models of predicting the glycaemic control (*Chapter 5*) a *Mediterranean lifestyle* score (*Chapter 1*) in place of the Mediterranean diet score (*MedDietScore*); it will be of interest to see whether the new variable remains statistically significant but also its effect on the model (such as its predicting power).

2. The absence of validated questionnaires in the Cypriot population has led us to construct them from scratch or adapt existing questionnaires. This process was meticulous and thus very time-consuming and is partly described in the pilot study (*Chapter 3*). Although it is wide-spread and acceptable to use adapted questionnaires (especially given the similarity between the populations in which the questionnaires have been validated), I feel that it will be a great benefit that these questionnaires are formally validated in Cyprus (and published accordingly). Through such a process, the degree of certainty for the results will improve, while it will be helpful and time-saving for other researchers in Cyprus who potentially could use these questionnaires.

3. There is an urgent need for well-designed interventional studies that investigate the effect of the Mediterranean diet on glycaemic control. Some of the characteristics of such a study have been described previously (*Chapter 5*) and include the need for the results to be adequately controlled for variables that are well-accepted for improving diabetes control, such as carbohydrate counting and appropriate insulin adjustment. Furthermore, more studies are required to investigate the mechanism of this effect on diabetes control and to confirm or disprove our suggested mechanism (*Chapter 5*). It will also be of interest to see whether our results apply beyond the Mediterranean region and in populations with traditionally low adherence to the Mediterranean diet.

4. A well-designed systematic review(s) (and possible meta-analysis) is required to summarise the available evidence on different approaches to improving adherence to the Mediterranean diet and, to my knowledge, no such study exists. These different approaches may have different efficacy; while this efficacy in turn may apply only in certain circumstances and populations. Furthermore, these approaches need to be extended beyond the group education sessions to population-based approaches and technology-smart solutions. The results would provide policymakers and others with

effective and population-tailored tools for improving adherence to the Mediterranean diet.

5. My analysis has suggested that people diagnosed with Type 1 diabetes follow similar patterns to those of the general population, including in terms of their diet. Therefore, it is likely, but this needs further investigation, that more active involvement of those in the wider social circles (the people living with, such as family members and spouses) of people diagnosed with Type 1 diabetes in their care (such as in structured education programmes) will be of additional benefit.

Suggestions for public health measures (and clinical)

This development and implementation of public health measures is potentially the most difficult element to achieve, as it requires more than just the willingness of the scientific community. It also requires the will of policymakers, such as the government, the ministry of health, the politicians in general, along with input from journalists, the industry and other interested parties and bearing in mind the potential conflicting priorities and interests of these various groups. Here I suggest a brief list of such measures that may be of benefit:

1. The implementation of measures that will improve adherence to the Mediterranean diet in the Cypriot population (and beyond). This is of paramount importance, taking into account that the published literature indicates that such interventions have significant health benefits, including in populations that traditionally do not follow the Mediterranean diet. Additional emphasis should be placed on the younger generation, who are more vulnerable to adopting a more Westernised diet. Furthermore, these measures have the potential of providing additional benefits in selected groups, such as people diagnosed with diabetes. On the contrary, if no such measures are implemented, we can foresee a deterioration in the health of the people caused by a trend of further deviation from the Mediterranean diet over time, as reflected by our data, especially in the younger age group. Nevertheless, we are not oblivious to the fact that such a major change cannot be achieved without significant structural changes in society, for example, in socioeconomic and financial aspects, advertising, food affordability and availability, to name just a few.

2. The provision of information on the Mediterranean diet and the implementation of evidence-based approaches to improve adherence through diabetes-structured

education programmes and regular clinical consultations of people diagnosed with Type 1 diabetes (and in general in diabetes) would be greatly beneficial. Based on my results (*Chapter 5*), such a measure has the potential to improve glycaemic control significantly. Nevertheless, this also has the potential to improve other parameters in this high-risk group, for example, CVD disease, which is also reflected in the available guidelines (Dyson *et al.*, 2018; American Diabetes Association, 2020). This will also require the appropriate training of doctors, dietitians, nurses and other health care professionals involved in the care of people with a diagnosis of Type 1 diabetes, on the matter of the Mediterranean diet.

3. I strongly support the development of a national database in Cyprus, which should try to include all people with a diagnosis of Type 1 diabetes. This database could also include some relevant epidemiological information, such as HbA1c, presence of complications, screening, etc. This database can be modelled on the structure of available databases in other countries (such as those in Scotland and Sweden), while the characteristics of the health care system are taken into account. This will allow for a more regular monitoring of diabetes-related information and trends, such as glycaemic control and vascular events, and could potentially allow for better monitoring and management of these patients.

4. The implementation of formal structured education in Cyprus, which could be based on the model of DAFNE in the UK. I strongly believe that such a step will have a significant positive effect on the glycaemic control (HbA1c, fluctuations and rates of hypoglycaemia) of people diagnosed with Type 1 diabetes in Cyprus, while improving their quality of life (probably through more flexibility). Furthermore, through such a programme we expect the younger ages to benefit the most, and who, according to our data have the worst glycaemic control (*Chapter 5*). Nevertheless, I hope that the results of this study on glycaemic control (*Chapter 5*) should be adequate evidence to alert the relevant authorities in Cyprus for the urgent need of formulating and implementing a national strategy to improve glycaemic control in this population.

Conclusion

This thesis showed that an improvement in glycaemic control is viable through a new approach, the Mediterranean diet. This approach is invariably side-effect free and

potentially has other benefits, such as a reduction in CVD risk. Nevertheless, this needs to be translated into everyday practice, while more research is needed to further verify and clarify aspects of our results.

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Appendices

Chapter 3

Equations for calculating the mean score and standard deviation from sub-groups

When a study only reported the mean score of each sex group ($\bar{X}_{female}$ and $\bar{X}_{male}$), the total mean score ($\bar{X}_{total}$) was estimated as follows:

$$\bar{X}_f \cdot n_f = \sum_{i=1}^{n_f} x_{f_i} \qquad \bar{X}_m \cdot n_m = \sum_{i=1}^{n_m} x_{m_i}$$

$$\bar{X}_{total} = \frac{\sum_{i=1}^{n_f} x_{f_i} + \sum_{i=1}^{n_m} x_{m_i}}{n_{total}}$$

Where *n* represents the number of participants in each group.

Similarly, for the estimation of the total mean score's standard deviation (s_{total}), the following procedure was used:

$$(n_f - 1)s_f^2 + n_f \cdot \bar{X}_f^2 = \sum_{i=1}^{n_f} x_{f_i}^2 \qquad (n_m - 1)s_m^2 + n_m \cdot \bar{X}_m^2 = \sum_{i=1}^{n_m} x_{m_i}^2$$

$$s_{total} = \sqrt{\frac{(\sum_{i=1}^{n_f} x_{f_i}^2 + \sum_{i=1}^{n_m} x_{m_i}^2) - n_{total} \cdot \bar{X}_{total}^2}{n_{total} - 1}}$$

Where:

$\bar{X}_f$ represents the mean score for females

$\bar{X}_m$ represents the mean score for males

$\bar{X}_{total}$ represents the total mean score

n_f is the number of female participants

n_m is the number of male participants

n_{total} is the total number of participants

x_{f_i} is the Mediterranean score for the i^{th} female individual

x_{m_i} is the Mediterranean score for the i^{th} male individual

s_{total} is total mean-score's standard deviation

s_f^2 is the mean-score's variance for the female participants

s_m^2 is the mean-score's variance for the male participants

Journal publication

- Alexis Kyriacou, Josie M.M. Evans, Nicholas Economides, Angelos Kyriacou; Mediterranean diet adherence in Cyprus and Greece: A systematic review; *European Journal of Public Health*; June 2015; Vol. 25, No. 6, 1012–1018 | DOI: 10.1093/eurpub/ckv124 (see *First page of the Journal Publication*)

Conference related presentation

- Invited presentation: Mediterranean diet in Cyprus & Greece: cause for concern? *3rd Public Health Day: Research in Cyprus 2017*
  Cyprus University of Technology (ΤΕΠΑΚ), Limassol, Cyprus – 21st Sep 2017

Popular press related publications

- The place and the role of Mediterranean diet in the 21st century [In Greek] *Politis newspaper* – 17 January 2016 (see *Politis newspaper: The place and the role of Mediterranean diet in the 21st century [In Greek]*)
- Cypriots have abandoned the Mediterranean diet [In Greek] *Philelefθeros newspaper* – 19 November 2015

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Adherence to the Mediterranean diet by the Greek and Cypriot population: a systematic review

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Background: The traditional Mediterranean diet is defined as the dietary pattern in the countries of the Mediterranean basin between the 1950s and 1960s, and it is now widely accepted that has a beneficial effect on health. A debate exists from empirical and research data if the traditional Mediterranean diet remains the main dietary pattern of the region or if it has changed overtime. **Aims:** This systematic review addresses whether the people of Cyprus and Greece still follow the traditional Mediterranean diet or whether the diet has become more 'Westernised'. **Methods:** The MEDLINE database was searched using relevant free terms and independently reviewed by two authors. In addition, all reference lists of identified studies were hand-searched to identify additional, relevant studies. **Results:** The review resulted in 18 research papers that met the inclusion and exclusion criteria and represented 15 independent studies. The main outcome was consistent between studies and indicated moderate adherence of the Greek, and (probably) of the Cypriot, population to the Mediterranean diet. The majority of studies found no statistically significant differences by gender. There was an observed inter-study lower adherence to the Mediterranean diet by the younger population. Few studies addressed intra-study variations by age. **Conclusions:** This review shows that adherence to the Mediterranean diet is moderate in Greece (and probably also in Cyprus).This suggests a continuing transition from dietary patterns in the 50s–60s towards a more Westernized diet.

Introduction

Interest in the Mediterranean diet increased after the widely cited Seven Countries study[1] suggested a cardio-protective role of the Mediterranean dietary pattern. According to Trichopoulou et al.[2] 'The Mediterranean diet could be considered as the dietary pattern found in the olive oil-growing area of the Mediterranean region in the late 1950s and early 1960s, before the invasion of the fast food culture in the area'. The traditional Mediterranean diet is based mainly on plant sources such as vegetables, fruits, whole grains, legumes and seeds. Fish and poultry are consumed weekly and dairy products daily, in low to moderate amounts, with an emphasis on fish. The primary source of fat intake is from olive oil and meals are accompanied by moderate amounts of wine.[2]

The Mediterranean diet is associated with reduction in risk of a number of diseases and conditions. It has been found to reduce all-cause mortality,[3] the incidence and mortality from cardiovascular disease and cancer[2,3] and the incidence of neurodegenerative diseases such as Alzheimer and Parkinson's disease[3] and of Type 2 diabetes.[4]

Despite these health benefits, there is debate as to whether adherence to the Mediterranean diet in modern Greek and Cypriot society remains the same or whether people have moved towards a more 'Westernised/Americanised' diet. Ecological studies using the food balance sheets of the United Nations' Food and Agriculture Organization have shown a significant drop in the Mediterranean dietary pattern among the Mediterranean countries over the last decades, with Greece experiencing the biggest drop and Cyprus also having a considerable negative change.[5–7] We aimed to examine the evidence for the current/recent adherence of the Greek and Cypriot population to the Mediterranean diet, after the significant changes that have appeared over the last decades, including food availability.

Methods

This study aims to systematically review the evidence on adherence of the Greek and Cypriot population to the Mediterranean diet.

Search strategy

A MEDLINE search was conducted, up to 15 July 2013, using the free terms 'adheren* or prevelan*'(topic) and 'Mediterranean diet*'(topic) and 'gre* or cypr*'(topic). The search was conducted independently by two researchers (A.K. and N.E.), and any disagreement was settled after discussion between the two. The papers were reviewed progressively in stages on the basis of the title, abstract and finally full text. One of the authors (A.K.) hand-searched references of all the identified papers (figure 1). The search was not limited to language, date or any other limitations.

To be included in the review, the study had to fulfil the following criteria:

- The study was conducted in a Cypriot or Greek population or both nationalities who were permanently residing in Cyprus or Greece or both.
- Adherence to the Mediterranean diet was measured as a primary or secondary outcome of the total study population and/or of the total population of each gender of the study and/or of different age groups.
- A Mediterranean diet score was used.
- A direct method of data collection such as questionnaires, interviews, self-reported individual methods and surveys was used.
- The data were not collected after an intervention; i.e. the data must have originated from an observational cross-sectional or longitudinal study or at baseline of an intervention study (before the intervention commenced).

Studies which were not original (e.g. reviews and meta-analysis) or that used an indirect method of measurement of data collection

Politis newspaper: The place and the role of Mediterranean diet in the 21st century [In Greek]

Η θέση και ο ρόλος της μεσογειακής δίαιτας στον 21ο αιώνα

Μεσογειακή δίαιτα ονομάζουμε την παραδοσιακή διατροφή των λαών της Μεσογείου, κυρίως μεταξύ της περιόδου του 1950 και 1960. Είναι μια δίαιτα πλούσια σε φρούτα, λαχανικά, λιγότερο επεξεργασμένα δημητριακά (π.χ. ολικής αλέσεως ψωμί, μακαρόνια και καστανό ρύζι), πατάτες, όσπρια και ξηρούς καρπούς. Το γάλα και τα γαλακτοκομικά προϊόντα αποτελούν μέρος της καθημερινότητας, αλλά σε μικρές προς μέτριες ποσότητες.

Τα πουλερικά και το ψάρι καταναλώνονται επί εβδομαδιαίας βάσης, με έμφαση στα ψάρια. Το ελαιόλαδο είναι το κύριο συνοδευτικό λάδι του φαγητού και γενικότερα η κυριότερη πηγή λιπαρών στη διατροφή. Παράλληλα η Μεσογειακή διατροφή είναι χαμηλή σε κόκκινο κρέας, φαγητά πλούσια σε ζάχαρη, επεξεργασμένα φαγητά και απουσιάζουν παντελώς τα έτοιμα φαγητά "fast food". Τέλος, τα γεύματα συνοδεύονται από 1-2 μέτρια ποτήρια κόκκινου κρασιού. Τέτοιες διατροφικές συνήθειες συνοδεύονται και με ένα παραδοσιακό τρόπο ζωής που χαρακτηρίζεται από αυξημένα επίπεδα άσκησης και δραστηριότητας (π.χ. στο σπίτι και στα χωράφια).

> Είναι μια δίαιτα πλούσια σε φρούτα, λαχανικά, λιγότερο επεξεργασμένα δημητριακά (π.χ. ολικής αλέσεως ψωμί, μακαρόνια και καστανό ρύζι), πατάτες, όσπρια και ξηρούς καρπούς.

Επιδημιολογικές και άλλες επιστημονικές έρευνες απέδειξαν, πέραν κάθε αμφιβολίας, ότι η τήρηση της μεσογειακής δίαιτας συνεπάγεται

σημαντικά οφέλη για την ανθρώπινη υγεία. Στα θετικά αποτελέσματα της μεσογειακής δίαιτας περιλαμβάνονται η μείωση των κρουσμάτων εμφάνισης τύπου 2 διαβήτη, υπέρτασης, υπερλιπιδαιμίας, καρδιοπαθειών, αλλά και κάποιων τύπων καρκίνου. Κοινός παρονομαστής για αρκετές από αυτές τις ασθένειες είναι η παχυσαρκία, στην οποία η μεσογειακή δίαιτα, και γενικότερα ο παραδοσιακός μεσογειακός τρόπος ζωής, φαίνεται να έχει μια θετική επίδραση. Επίσης υπάρχουν ενδείξεις πως μειώνει τον κίνδυνο εμφάνισης νευροεκφυλιστικών παθήσεων, όπως οι νόσοι Αλτζχάιμερ και Πάρκινσον, και παράλληλα αυξάνει το προσδόκιμο ζωής. Τα θετικά ευρήματα είναι τέτοιων διαστάσεων ώστε να προωθείται η μεσογειακή δίαιτα σε διάφορους πληθυσμούς ανά την υφήλιο, συμπεριλαμβανομένων και λαών που παραδοσιακά έχουν πολύ διαφορετικές διατροφικές συνήθειες από τους μεσογειακούς λαούς. Υπάρχει μια αβεβαιότητα κατά πόσον η τήρηση της μεσογειακής διατροφής έχει μειωθεί ή διατηρείται στα ίδια επίπεδα στη σύγχρονη εποχή. Αυτή την ερώτηση προσπαθήσαμε να απαντήσουμε με μια έρευνα στο πλαίσιο της οποίας συγκεντρώσαμε συστηματικά όλες τις μελέτες που υπάρχουν στο θέμα της συχνότητας τήρησης της μεσογειακής δίαιτας. Με αυτόν τον τρόπο φέραμε τα δεδομένα των διάφορων ερευνών μαζί με σκοπό να δημιουργήσουμε μια μεγάλη βάση δεδομένων (Systematic review & Meta-analysis) για να μας επιτρέψει να ερευνήσουμε μεγάλο αριθμό ασθενών και για μεγάλη χρονική περίοδο. Η έρευνα διεξήχθη και σε συνεργασία με το νοσοκομείο του Σάλφορτ (Μάντσεστερ, Αγγλία) και το Πανεπιστήμιο του Στέρλινγκ (Σκωτία). Στην ανάλυσή μας συμπεριλήφθηκαν 18 έρευνες με 35.964 συμμετέχοντες από Κύπρο και Ελλάδα.

Τα αποτελέσματα της έρευνάς μας έδειξαν μια μέτρια προσκόλληση στη μεσογειακή δίαιτα από το 2000 μέχρι σήμερα, ενώ συνολικά παρατηρήσαμε μια σημαντική πτώση στην τήρηση της μεσογειακής δίαιτας της τάξης του 50% σε σύγκριση με τις δεκαετίες του 1950 και 1960. Το φαινόμενο αυτό ήταν εντονότερο στις πιο νεαρές ομάδες πληθυσμού. Επιπλέον, δεν παρατηρήσαμε ιδιαίτερες διαφορές μεταξύ ανδρών και γυναικών. Τα αποτελέσματα της έρευνάς μας συμφωνούν με μια προηγούμενη έρευνα που έδειξε πτώση στην προσκόλληση στη μεσογειακή δίαιτα στην Ελλάδα από την 1η στη 10η θέση και Κύπρο από την 20ή στην 27η θέση από το 1961-65 μέχρι το 2000-03. Ιδιαίτερο ενδιαφέρον παρουσιάζει μια άλλη έρευνα στο πλαίσιο της οποίας έγινε σύγκριση των διατροφικών συνηθειών φοιτητών από Ελλάδα και Ολλανδία, και έδειξε πως οι Ολλανδοί φοιτητές ήταν πιο πιθανό να ακολουθούν τη μεσογειακή διατροφή απ' ό,τι οι Έλληνες!

Η πιο πιθανή ερμηνεία των ευρημάτων της έρευνας αυτής είναι ότι με την πάροδο του χρόνου και με μια υιοθέτηση ξενόφερτων, δυτικών τρόπων ζωής, η μεσογειακή δίαιτα έχει κάπως περιοριστεί. Αυτό τυγχάνει να έχει αρνητικό αντίκτυπο στην υγεία, με φαινόμενα όπως αύξηση των ποσοστών εμφάνισης διαβήτη, παχυσαρκίας και καρδιοπαθειών, αλλά και στην οικονομία π.χ. άμεσο κόστος λόγω ιατρικής περίθαλψης και έμμεσο κόστος λόγω χαμένων ωρών εργασίας και πρόωρης θνησιμότητας. Συνεπώς, απαιτείται μια συντονισμένη προσπάθεια προώθησης της μεσογειακής διατροφής από τους αρμόδιους φορείς και τα οργανωμένα σύνολα της κοινωνίας. Κατά την προσωπική μας γνώμη, η εκπαίδευση των νέων στα σχολεία ούτως ώστε να μαθαίνουν πώς να μαγειρεύουν παραδοσιακά γεύματα, η καθολική πληροφόρηση του πληθυσμού για τα πλεονεκτήματα μιας ισορροπημένης και υγιούς διατροφής και τρόπου ζωής (συμπεριλαμβανομένων της μεσογειακής δίαιτας, της άσκησης και της διακοπής του καπνίσματος) και πιθανόν η αύξηση των τελών σε κακής ποιότητας φαγητά (π.χ. σακχαρώδη ροφήματα και τροφές υψηλές σε λιπαρά) θα αποτελούσαν βήματα προς τη σωστή κατεύθυνση.

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Chapter 4

Algorithm

The algorithm was split into four parts, namely *standardisation of servings (of individual foods)*, *total number of servings of (individual) MedDietScore components*, *scoring of servings* and *total MedDietScore score*. As previously mentioned, we tried to keep the algorithms as simple as possible, as more complicated ones (for example, by taking seasonality into consideration) may have more accurately reflected the true food intake but would need to be validated in a new study, before been applied.

Standardisation of servings (of individual foods): Initially, the reported number of servings – that one serving is equal to one pre-defined portion of the respective food – are all converted into number of servings per month; this allows us, to produce standardised data that can be further processed. The algorithm, for the i^{th} participant, is as follows:

if n_{di} servings reported, then $n_{mi} = 30 \, . \, n_{di}$
if n_{wi} servings reported, then $n_{mi} = 4 \, . \, n_{wi}$
if n_{mi} servings reported, then $n_{mi} = 1 \, . \, n_{mi}$
if n_i servings in rarely/never† column reported or if $n_{mi} < 1$, then $n_{mi} = 0 \, . \, n_{(m)i}$

where: n_{di} = number of servings (of the pre-defined portion of a food) reported to be consumed daily, for example, 5 servings of (*1 medium slice – 30 g*) white bread are consumed per day or in practise the participant adds the number 5 at the row of the white bread and the column of daily; n_{wi} = number of servings reported to be consumed weekly; n_{mi} = number of servings reported to be consumed per month; †for the purpose of the olive oil, the rarely and never are distinct entities and are scored separately, for more see following sections.

Total number of servings of (individual) *MedDietScore* components: The difficulties of calculating portion sizes have been described previously, as also the reason the algorithm was kept as simple as possible (unweighted addition – although in practise can be said that the algorithm is weighted by the pre-defined portion sizes); and why some food groups are constructed from several foods while others are measured directly. For each food group, and for the i^{th} participant, the calculations were as follow:

Non-refined cereals $servings_i$ = no of (*bread, wheat, wholemeal*: 1 medium slice, 30g portion) $servings_i$ + no of (*rusk, wholemeal*: 1 medium, 45g portion) $servings_i$ + no of

(*breakfast cereals, wholegrain*: ¾ of a mug, 30g portion) $servings_i$ + no of (*pasta, all types, wholemeal*: 1 mug, 250ml, 150g portion) $servings_i$ + no of (*rice, all types, brown, wholegrain*: 1 mug, 250ml, 150g portion) $servings_i$

Potatoes $servings_i$ = no of (*chips*: 10 pieces, 50g portion) $servings_i$ + no of (*roasted potatoes*: 1 medium, 80g portion) $servings_i$ + no of (*boiled potatoes*: 1 medium, 80g portion) $servings_i$ + no of (*mashed potato*: 1 glass, 200ml, 200g portion) $servings_i$

Fruits $servings_i$ = no of (*fruits, any*: 1 handful, 150g portion) $servings_i$

Vegetables $servings_i$ = no of (*cucumber*: 1 medium, 80g portion) $servings_i$ + no of (*tomato*: 1 medium, 80g portion) $servings_i$ + no of (*onion*: ½ medium, 150g portion) $servings_i$ + no of (*briam, mixed vegetable dish*: 1 cup, 250ml, 180g portion) $servings_i$ + no of (*garlic*: 1 glove, 3g portion) $servings_i$ + no of (*mushrooms*: 1 medium, 20g portion) $servings_i$ + no of (*aubergines*: 1 medium, 150g portion) $servings_i$ + no of (*beetroots*: 1 small, 40g portion) $servings_i$ + no of (*artichokes*: 1 artichoke, 80g portion) $servings_i$ + no of (*courgette*: 1 small, 120g portion) $servings_i$ + no of (*peppers*: 1 medium, 130g portion) $servings_i$ + no of (*asparagus*: 2 cooked asparagus, 40g portion) $servings_i$ + no of (*spinach*: 1 cup, 250ml, 40g portion) $servings_i$ + no of (*carrots*: 1 medium, 80g portion) $servings_i$ + no of (*parsley*: 2 springs, 6g portion) $servings_i$ + no of (*spearmint*: 2 springs, 6g portion) $servings_i$ + no of (*village salad, vegetables only*: 1 cup, 250ml, 80g portion) $servings_i$ + no of (*cauliflower*: 1 cup, 250ml, 120g portion) $servings_i$ + no of (*Broccoli*: 1 cup, 250ml, 120g portion) $servings_i$ + no of (*Greek salad*: 1 cup, 250ml, 100g portion) $servings_i$ + no of (*Russian salad*: 1 cup, 250ml, 150g portion) $servings_i$ + no of (*greens, wild, cooked*: 1 cup, 250ml, 80g portion) $servings_i$ + no of (*peas*: 5 table spoons, 100g portion) $servings_i$ + no of (*ladies' fingers*: 10 pieces, 80g portion) $servings_i$ + no of (*green beans*: 10 pieces, 30g portion) $servings_i$

Legumes $servings_i$ = no of (*white kidney beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*black eye beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*broad beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*lentils*: 5 table spoons, 100g portion) $servings_i$

Fish $servings_i$ = no of (*fish, any*: 1 palm, 100g portion) $servings_i$

Red meat and products $servings_i$ = no of (*bacon*: 1 slice, 10g portion) $servings_i$ + no of (*ham*: 1 slice, 10g portion) $servings_i$ + no of (*mortadella*: 1 slice, 20g portion)

servings$_i$ + no of (*parizaki*, cooked salami: 1 slice, 20g portion) servings$_i$ + no of (*salami*: 1 slice, 10g portion) servings$_i$ + no of (*sausages*, 1 piece, 80g portion) servings$_i$ + no of (*veal and beef meat, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of *(pork meat, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of (*lamb meat, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of (*goat meat, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of (*liver*: 1 palm, 100g portion) servings$_i$ + no of (*offal, other than liver*: 1 palm, 100g portion) servings$_i$ + no of (*souvlaki, pork, meat only*: 1 skewer portion) servings$_i$ + no of (*souvlaki, pork, with pitta bread*: 1 Cyprus pitta bread portion) servings$_i$ + no of (*kebab, pork*: 1 Cyprus pitta bread portion) servings$_i$ + no of (*Kebab, beef*: Cyprus pitta portion) servings$_i$ + no of (*keftedes, meatballs*: 4 medium, 100g portion) servings$_i$ + no of (*soutsoukakia, meatballs in tomato sauce*: 3 pieces, 100g portion) servings$_i$ + no of (*burger, various ways of cooking*: 1 palm, 100g portion) servings$_i$

Poultry servings$_i$ = no of (*turkey*, cold cut, 1 slice, 30g portion) servings$_i$ + no of (*eggs*: 1 medium, 60g portion) servings$_i$ + no of (*game*: 1 palm, 100g portion) servings$_i$ + no of (*chicken, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of (*turkey, various ways of cooking*: 1 palm, 100g portion) servings$_i$ + no of (*souvlaki, chicken, meat only*: 1 skewer portion) servings$_i$ + no of (*souvlaki, chicken, with pitta bread*: 1 Cyprus pitta bread portion) servings$_i$ + no of (*kebab, chicken*: 1 Cyprus pitta bread portion) servings$_i$ + no of (*stifado, rabbit*: 1 palm, 100g portion) servings$_i$

Full fat dairy products servings$_i$ = no of (*milk, full-fat*: 1 mug, 250ml portion) servings$_i$ + no of (*milk, condensed, sweetened*: 1 mug, 250ml portion) servings$_i$ + no of (*milk, chocolate flavoured*: 1 mug, 250ml portion) servings$_i$ + no of (*yoghurt, full fat*: small pot, 125g portion) servings$_i$ + no of (*yoghurt with fruits*: small pot, 125g portion) servings$_i$ + no of (*feta cheese*: matchbox size, 30g portion) servings$_i$ + no of (*kasseri cheese*: matchbox size, 30g portion) servings$_i$ + no of (*anari cheese, fresh*: matchbox size, 30g portion) servings$_i$ + no of (*anari cheese, dry*: matchbox size, 30g portion) servings$_i$ + no of (*graviera cheese*: matchbox size, 30g portion) servings$_i$ + no of (*kefalotyri cheese*: matchbox size, 30g portion) servings$_i$ + no of (*halloumi cheese*, matchbox size, 30g portion) servings$_i$ + no of (*cheese, other*: matchbox size, 30g portion) servings$_i$ + no of (*tzatziki, yogurt and garlic*: 1 table spoon, 40g portion) servings$_i$

Use of olive oil in cooking $servings_i$ = max (never, rarely, no of [*Olive* oil: 1 table spoon, 10g portion]) $servings_i^{\dagger}$

Alcoholic beverages $servings_i^{\dagger\dagger}$ = no of (*wine*: 1 regular wine glass, 100ml portion) $servings_i$ + no of (*beer*: 1 pint, 250ml portion) $servings_i$ + no of (*ouzo, with water and ice*: 1 glass, 200ml portion) $servings_i$ + no of (*brandy*: 1 pub measure, 30ml portion) $servings_i$ + no of (*whiskey*: 1 pub measure, 30ml portion) $servings_i$ + no of (*liquor*: 1 pub measure, 30ml portion) $servings_i$ + no of (*zivania*: 1 pub measure, 30ml portion) $servings_i$ + no of (*rum*: 1 pub measure, 30ml portion) $servings_i$ + no of (*alcoholic beverages, other*: 1 pub measure, 30ml portion) $servings_i$

where: no = number; 1 serving = the pre-defined food portion (in the FFQ) in household measures or grams; no of servings = the number of serving per month reported by the participants; †max = maximum of the function; never < rarely < 1 portion per month, where, they can be broadly defined as never = 0, rarely → 0; for the actual methods on olive oil see algorithm below; ††The portion size of alcoholic drinks (in the FFQ) was chosen to approximately match 12g of alcohol, which was the portion size required by the *MedDietScore* scoring system and at the same time provided meaningful portions for the participants (in Cyprus).

Scoring of servings: The details of *MedDietScore* scoring system have been discussed before, including the edits on the original score – the final version is shown in *Table 4.2*. Here, the actual algorithm is presented, for the i^{th} participant, and is sub-dived in three steps. The first two are needed to standardize the servings consumed with the scoring system, whereas the third step is the actual scoring of the serving using the *adapted MedDietScore* scoring system.

Step 1: The servings are converted to weekly

$s_{mi\,/\,4} = s_{wi}$ for all (11) food groups as calculated previously

where: s_{mi} = servings per month; s_{wi} = serving per week

Step 2: Weekly serving are converted to integer number as the *MedDietScore scoring system* has non-integer gaps between numbers

$s_{wi} \rightarrow S_{wi}$

where s_{wi} = servings per week with α decimal places (as per calculation above); S_{wi} = servings per month as an integer number

Step 3

$f_s : S_{wi} \rightarrow$ as per *MedDieScore*, then

where f_s is a function, based on the *MedDietScore* scoring system, with S_{wi} (the input) that is the servings for the particular component or food group; in practise, $f_s(S_{wi})$ is the score, measured in points (range 0 – 5), for each of the component or food group – shown below:

Non-refined cereals score: $f_c(S_w)$

if $S_{wi} = 0$, then $f_c(S_w) = 0$, if $1 \leq S_{wi} \leq 6$ then $f_c(S_w) = 1$, if $7 \leq S_{wi} \leq 12$ then $f_c(S_w) = 2$, if $13 \leq S_{wi} \leq 18$ then $f_c(S_w) = 3$, if $19 \leq S_{wi} \leq 31$ then $f_c(S_w) = 4$, if $S_{wi} > 32$ then $f_c(S_w) = 5$

Potatoes score: $f_p(S_w)$

if $S_{wi} = 0$, then $f_p(S_w) = 0$, if $1 \leq S_{wi} \leq 4$ then $f_p(S_w) = 1$, if $5 \leq S_{wi} \leq 8$ then $f_p(S_w) = 2$, if $9 \leq S_{wi} \leq 12$ then $f_p(S_w) = 3$, if $13 \leq S_{wi} \leq 18$ then $f_p(S_w) = 4$, if $S_{wi} > 18$ then $f_p(S_w) = 5$

Fruits score: $f_f(S_w)$

if $S_{wi} = 0$, then $f_f(S_w) = 0$, if $1 \leq S_{wi} \leq 4$ then $f_f(S_w) = 1$, if $5 \leq S_{wi} \leq 8$ then $f_f(S_w) = 2$, if $9 \leq S_{wi} \leq 15$ then $f_f(S_w) = 3$, if $16 \leq S_{wi} \leq 21$ then $f_f(S_w) = 4$, if $S_{wi} > 22$ then $f_f(S_w) = 5$

Vegetables score: $f_v(S_w)$

if $S_{wi} = 0$, then $f_v(S_w) = 0$, if $1 \leq S_{wi} \leq 6$ then $f_v(S_w) = 1$, if $7 \leq S_{wi} \leq 12$ then $f_v(S_w) = 2$, if $13 \leq S_{wi} \leq 20$ then $f_v(S_w) = 3$, if $21 \leq S_{wi} \leq 32$ then $f_v(S_w) = 4$, if $S_{wi} > 33$ then $f_v(S_w) = 5$

Legumes score: $f_l(S_w)$

if $S_{wi} = 0$, then $f_l(S_w) = 0$, if $0 < S_{wi} \leq 1$ then $f_l(S_w) = 1$, if $1 \leq S_{wi} \leq 2$ then $f_l(S_w) = 2$, if $3 \leq S_{wi} \leq 4$ then $f_l(S_w) = 3$, if $5 \leq S_{wi} \leq 6$ then $f_l(S_w) = 4$, if $S_{wi} > 6$ then $f_l(S_w) = 5$

Fish score: $f_h(S_w)$

if $S_{wi} = 0$, then $f_h(S_w) = 0$, if $0 < S_{wi} \leq 1$ then $f_h(S_w) = 1$, if $1 \leq S_{wi} \leq 2$ then $f_h(S_w) = 2$, if $3 \leq S_{wi} \leq 4$ then $f_h(S_w) = 3$, if $5 \leq S_{wi} \leq 6$ then $f_h(S_w) = 4$, if $S_{wi} > 6$ then $f_h(S_w) = 5$

Red meat and products score: $f_r(S_w)$

if $S_{wi} > 10$, then $f_r(S_w) = 0$, if $8 \leq S_{wi} \leq 10$ then $f_r(S_w) = 1$, if $6 \leq S_{wi} \leq 7$ then $f_r(S_w) = 2$, if $4 \leq S_{wi} \leq 5$ then $f_r(S_w) = 3$, if $2 \leq S_{wi} \leq 3$ then $f_r(S_w) = 4$, if $S_{wi} \leq 1$ then $f_r(S_w) = 5$

Poultry score: $f_y(S_w)$

if $S_{wi} > 10$, then $f_y(S_w) = 0$, if $9 \leq S_{wi} \leq 10$ then $f_y(S_w) = 1$, if $7 \leq S_{wi} \leq 8$ then $f_y(S_w) = 2$, if $5 \leq S_{wi} \leq 6$ then $f_y(S_w) = 3$, if $S_{wi} = 4$ then $f_y(S_w) = 4$, if $S_{wi} \leq 3$ then $f_y(S_w) = 5$

Full fat dairy products score: $f_d(S_w)$

if $S_{wi} > 30$, then $f_d(S_w) = 0$, if $29 \leq S_{wi} \leq 30$ then $f_d(S_w) = 1$, if $21 \leq S_{wi} \leq 28$ then $f_d(S_w) = 2$, if $16 \leq S_{wi} \leq 20$ then $f_d(S_w) = 3$, if $11 \leq S_{wi} \leq 15$ then $f_d(S_w) = 4$, if $S_{wi} \leq 10$ then $f_d(S_w) = 5$

Olive oil score: $f_o(S_w)$

To solve the problem of never and rarely as both couldn't be = 0, and → 0 can't be used in an algorithm – extra columns were created, named for the current purpose as *12a* and *12b*, and were as follows:

If never then *12a* = 1, otherwise *12a* = 0
If rarely then *12b* = 1, otherwise *12b* = 0

Therefore, the algorithm was as follows:

if $S_{wi} = 0$ and *12a* > 0, then $f_o(S_w) = 0$, if $S_{wi} = 0$ and *12b* > 0, then $f_o(S_w) = 1$, if $0 < S_{wi} \leq 1$ then $f_o(S_w) = 2$, if $1 \leq S_{wi} \leq 3$ then $f_d(S_w) = 3$, if $4 \leq S_{wi} \leq 6$ then $f_d(S_w) = 4$, if $S_{wi} \geq 7$ then $f_d(S_w) = 5$

Alcoholic beverages score: $f_a(S_w)$

Note that 1 serving equals approximately 12g of alcohol or 100ml of a regular wine; furthermore, note the polytonic nature of the scoring function for alcoholic beverages.

if $S_{wi} > 7$, then $f_a(S_w) = 0$, if $6 \leq S_{wi} \leq 7$ then $f_a(S_w) = 1$, if $5 \leq S_{wi} < 6$ then $f_a(S_w) = 2$, if $4 \leq S_{wi} < 5$ then $f_a(S_w) = 3$, if $3 \leq S_{wi} < 4$ then $f_a(S_w) = 4$, if $0 < S_{wi} < 3$ then $f_a(S_w) = 5$, if $S_{wi} = 0$ then $f_d(S_w) = 0$

Total MedDietScore score: The total *MedDietScore* score algorithm was as follows, for the i^{th} participant:

Total *MedDietScore* score (points; range 0 – 55) = $f_{ci} + f_{pi} + f_{fi} + f_{vi} + f_{li} + f_{hi} + f_{ri} + f_{yi} + f_{di} + f_{oi} + f_{ai}$

where: f_{ci} = non refined cereals score, f_{pi} = potatoes score, f_{fi} = fruits score, f_{vi} = vegetables score, f_{li} = legumes score, f_{hi} = fish score, f_{ri} = red meat and products score, f_{yi} = poultry score, f_{di} = full dairy products, f_{oi} = olive oil score, f_{ai} = alcoholic beverages score.

Results of data collected

Anthropometric and clinical examination data

The measurements collected through the anthropometry and the clinical examination are presented in *Table 7.1*.

Table 7.1: Anthropometry and clinical examination measurements

Measurements (units)	Total ***n***	Variables	***n***	%	Mean	sd	Min	Max
Weight (Kg)	9				79.6	24.7	57.0	121.2
Height (m)	9				1.70	0.09	1.57	1.87
BMI (Kg/m^2)	9	Total			27.0	5.5	20.9	36.6
	4	Male			31.7	4.7	25.2	36.6
		Normal[1]	0	0				
		Overweight[2]	1	25.0				
		Obese[3]	3	75.0				
	5	Female			23.3	2.2	20.9	25.3
		Normal[1]	3	60.0				
		Overweight[2]	2	40.0				
		Obese[3]	0	0				
Waist circumference (cm)	9	Total			95.3	16.8	78	122
	4	Male			109	16.3	86	122
		Normal[4]	1	25.0				
		Increased risk[5]	3[6]	75.0				
	5	Female			84.4	5.3	78	91
		Normal[7]	1	20.0				
		Increased risk[8]	4[9]	80.0				
Blood pressure Systolic (mmHg)	9	Total			13.1	1.8	10	15
	4	Male			13.6	1.3	12	15
	5	Female			12.6	2.1	10	15
		Normal[10]	5	55.6				
		High[11]	4	44.4				
Blood pressure diastolic (mmHg)	9	Total			8.1	1.3	6	10
	4	Male			8.5	1.3	7	10
	5	Female			7.8	1.3	6	9.4
		Normal[12]	3	33.3				
		High[13]	6	66.7				
Lipohypetrophy or lipoatrophy[14]	9	Absent	9	100				
		Present	0	0				
Current injection sites	9	Arms only	1	11.1				
		Abdomen only	3	33.3				
		Legs only	1	11.1				
		Arms & abdomen	2	22.2				
		Arms & abdomen & legs	2	22.2				

[1]Normal BMI = 18.5 – 24.9 Kg/m^2. [2]Overweight BMI = 25.0 – 29.9 Kg/m^2. [3]obese BMI ≥ 30.0 Kg/m^2. [4]waist circumference < 94 cm. [5]Waist circumference ≥ 94 cm. [6]All patients were ≥ 102, that is, considered as having substantially increased risk. [7]Waist circumference < 80cm. [8]Waist circumference ≥ 80 cm. [9]One patient was ≥ 88, that is, considered as having substantially increased risk. [10]Blood pressure < 140 mmHg. [11] Blood pressure ≥ 140 mmHg. [12]Blood pressure < 80 mmHg. [13]Blood pressure ≥ 80 mmHg. [14]At injection sites.

Food and beverages consumption

This section (*Table 7.2*) presents the food and beverages intake reported by the participants of the foods available in the FFQ, and the frequency in which they used various cooking methods. Furthermore, the results of the question on food cost are also presented.

Table 7.2: Foods and beverages consumption

Food category	Food name	Intake reported (***n***)[1]	Consumption, excluding zero (servings per month)[2]			
			Mean	sd	Min	Max
Cereals and cereal products	Bread, wheat, white	5	9.8	5.7	1	16
	Bread, wheat, brown	4	33.5	30.9	2	60
	Bread, wheat, wholemeal	3	24.7	31.0	2	60
	Bread, rye	0	-	-	-	-
	Bread, corn	0	-	-	-	-
	Rusk, white	1	4	-	-	-
	Rusk, wholemeal	1	2	-	-	-
	Breakfast cereals, regular	3	8.3	3.5	5	12
	Breakfast cereals, wholegrain	3	21.3	15.0	4	30
	Breakfast cereals, with fruits	0	-	-	-	-
	Breakfast cereals, chocolate	0	-	-	-	-
	Pasta, all types, white	7	5.6	4.0	1	12
	Pasta, all types, wholemeal	5	6.8	5.2	4	16
	Rice, all types, white	3	4.3	3.2	2	8
	Rice, all types, wholegrain	1	2	-	-	-
	Pourgouri	3	3.3	1.2	2	4
	Orzo	4	2	0.8	1	3
	Spanakorizo	1	1	-	-	-
	Gemista	1	1	-	-	-
	Koupepia	3	2.0	1.7	1	4
	Makaronia tou fournou	2	1.5	0.7	1	2
	Moussaka	2	1.5	0.7	1	2
	Rice pudding	0	-	-	-	-
	Pizza	6	3.8	2.6	1	8

Food category	Food name	Intake reported (***n***)[1]	Consumption, excluding zero (servings per month)[2]			
			Mean	sd	Min	Max
Salads and vegetables	Cucumber	7	36.0	17.7	12	60
	Tomato	8	32.1	19.7	5	60
	Onion	5	26.6	22.1	5	60
	Briam	2	6.0	2.8	4	8
	Garlic	3	9.0	13.0	1	24
	Mushrooms	6	7.8	5.7	1	16
	Aubergine	2	3.0	1.4	2	4
	Beetroot	3	5.3	5.8	2	12
	Artichoke	2	2.0	0	2	2
	Courgette	3	2.7	1.2	2	4
	Peppers	3	14.7	14.2	2	30
	Asparagus	1	4	-	-	-
	Spinach	3	3.0	1.7	1	4
	Carrots	7	14.4	20.8	2	60
	Parsley	1	8	-	-	-
	Spearmint	3	9.7	5.7	5	16
	Village salad	5	17.6	12.1	4	30
	Cauliflower	4	7.8	4.9	3	12
	Broccoli	3	8.0	4.0	4	12
	Skordalia	1	6	-	-	-
	Greek salad	3	15.3	12.7	8	30
	Russian salad	0	-	-	-	-
	Swiss chard	3	2.0	1.7	1	4
Fruits	Fruits, any	8	40.5	30.8	8	90
	Olives	6	19	22.9	2	60
	Lemon, on food	6	38.3	31.5	4	90
	Orange	6	10.0	11.2	1	30
	Mandarin orange (seasonal)	2	3.5	3.5	1	6
	Apple	7	26.7	24.2	5	60
	Pear (seasonal)	3	5.0	3.0	2	8
	Watermelon (seasonal)	4	45.0	50.7	10	120
	Melon (seasonal)	3	22.0	9.2	12	30
	Peach (seasonal)	4	25.3	26.0	2	60
	Grapes (seasonal)	4	42.0	53.2	2	120
	Cherries (seasonal)	3	11.7	16.0	1	30
	Strawberries (seasonal)	5	9.8	11.6	2	30
	Banana	7	17.1	10.9	4	30
	Figs (seasonal)	4	12.3	12.0	4	30
	Pomegranate (seasonal)	4	15.3	11.2	3	30
	Prickly pear (seasonal)	4	11.3	12.7	3	30
	Avocado	5	12.6	11.4	1	30
	Tinned fruit	1	30	-	-	-
	Tomato ketchup	5	10.6	11.7	1	30

Food category	Food name	Intake reported (*n*)[1]	Consumption, excluding zero (servings per month)[2]			
			Mean	sd	Min	Max
Dried fruits and nuts	Dried fruits	4	3.0	1.8	1	5
	Nuts, salted	6	8.5	11.2	2	30
	Nuts, unsalted	3	6.0	5.3	2	12
Legumes and ladies' fingers	White beans	6	6.3	3.7	2	12
	Black-eyed beans	7	6.0	3.5	2	12
	Broad beans	4	4.3	2.9	1	8
	Lentils	7	5.9	3.7	1	12
	Peas	4	4.0	2.8	2	8
	Ladies' fingers	3	3.3	4.0	1	8
	Green beans	4	4.8	2.8	2	8
Fish	Fish, any	7	7.9	4.2	3	16
	Prawns	2	2.5	0.7	2	3
	Calamari	4	2.5	1.3	1	4
	Tuna	4	4.3	2.6	2	8
	Octopus	1	2	-	-	-
	Taramasalata	0	-	-	-	-
	Oily fish	5	4.0	2.5	2	8
Meat, eggs and products	Bacon	3	3.7	3.8	1	8
	Ham	3	6.7	2.3	4	8
	Mortadella	0	-	-	-	-
	Parizaki, lunch meats	2	14.0	14.1	4	24
	Turkey, lunch meats	4	53.5	84.6	4	180
	Salami	0	12.0	4.0	8	16
	Sausages	3	4.3	3.5	1	8
	Eggs	6	10.0	11.2	2	30
	Veal and beef meat, any cooking method	2	2.0	0	2	2
	Pork meat, any cooking method	4	3.0	2.0	8	12
	Lamb meat, any cooking method	3	2.3	1.5	1	4
	Goat meat, any cooking method	1	8	-	-	-
	Game, any cooking method	0	-	-	-	-
	Liver, any cooking method	0	-	-	-	-
	Offal (other than liver), any cooking method	0	-	-	-	-
	Chicken, any cooking method	6	12.7	9.3	2	30
	Turkey, any cooking method	1	3	-	-	-
Potatoes	Chips	7	9.0	8.1	2	24
	Roasted potatoes	7	4.1	3.4	1	9
	Boiled potatoes	3	2.7	2.1	1	5
	Mashed potato	4	3.5	3.3	1	8

Food category	Food name	Intake reported (***n***)[1]	Consumption, excluding zero (servings per month)[2]			
			Mean	sd	Min	Max
Meat, eggs and products	Souvlaki, chicken, meat only	5	3.4	2.8	1	8
	Souvlaki, pork, meat only	3	2.0	1.0	1	3
	Souvlaki, chicken, with pitta bread	3	2.0	1.0	1	3
	Souvlaki, pork, with pitta bread	2	4.5	4.9	1	8
	Gyros, pork	2	5.5	3.5	3	8
	Gyros, beef	1	4	-	-	-
	Gyros, chicken	1	2	-	-	-
	Keftedes	5	2.8	1.6	1	4
	Soutzoukakia	0	-	-	-	-
	Burger, meat only, any cooking method	4	3.8	3.1	1	8
	Rabbit (stifado)	3	1.7	0.6	1	2
	Mayonnaise	3	16.7	11.7	8	30
Milk and dairy products	Milk, goat	0	-	-	-	-
	Milk, full-fat	0	-	-	-	-
	Milk, semi-skimmed	1	60	-	-	-
	Milk, skimmed	3	60.7	59.0	2	120
	Milk, evaporated, full-fat	1	4	-	-	-
	Milk, evaporated, light	0	-	-	-	-
	Milk, condensed, sweetened	0	-	-	-	-
	Milk, chocolate	0	-	-	-	-
	Yoghurt, full-fat	0	-	-	-	-
	Yoghurt with fruits	3	7.7	4.5	3	12
	Yoghurt, skimmed	4	25.5	25.7	4	60
	Feta cheese	6	8.7	10.6	2	30
	Kasseri cheese	2	4.5	4.9	1	8
	Anari cheese, fresh	5	8.0	12.4	1	30
	Anari cheese, dry	4	10.3	13.5	1	30
	Graviera cheese	1	1	-	-	-
	Kefalotyri cheese	3	13.0	15.1	1	30
	Halloumi cheese	7	18.6	14.5	2	40
	Cheese, other	6	17.8	11.6	1	32
	Tzatziki	3	3.0	2.6	1	6
Fats and oils	Olive oil	8	65.3	30.7	12	90
	Margarine spread	1	8	-	-	-
	Butter	4	27.5	23.7	8	60
	Crisps	4	7.3	4.6	1	12
Pastries	Cheese pie	4	3.5	1.7	1	5
	Spinach pie	0	-	-	-	-
	Meat pie	0	-	-	-	-
	Chicken pie	1	1	-	-	-
Fast foods and ready meals	Fast food	5	7.4	7.1	3	20
	Ready meals	2	8.0	5.7	4	12

Food category	Food name	Intake reported (*n*)[1]	Consumption, excluding zero (servings per month)[2]			
			Mean	sd	Min	Max
Soups	Trahanas soup	6	4.3	5.8	1	16
	Avgolemono	3	6.7	8.1	1	16
	Vegetable soup	4	9.3	7.9	1	16
	Meat soup (other than chicken)	1	1	-	-	-
	Chicken soup	0	-	-	-	-
	Fish soup	1	2	-	-	-
	Lentil soup	1	2	-	-	-
	Chickpea soup	0	-	-	-	-
	Bean soup	0	-	-	-	-
	Louvana	2	5.0	4.2	2	8
	Giouvarlakia soup	1	1	-	-	-
Sugar and sugary foods	Sugar	0	-	-	-	-
	Honey	2	2.5	2.1	1	4
	Marmalade	0	-	-	-	-
	Cake	1	8	-	-	-
	Biscuits, sweet	3	2.3	0.6	2	3
	Biscuits, salty	1	3	-	-	-
	Cyprus spoon sweets	1	2	-	-	-
	Baklava and kataifi desserts	1	1	-	-	-
	Kourabiedes	1	4	-	-	-
	Galaktoboureko	1	1	-	-	-
	Melomakarona	0	-	-	-	-
	Halva	1	8	-	-	-
	Candies	0	-	-	-	-
	Chocolate	6	3.3	3.4	1	10
	Ice cream	4	5.5	4.4	2	12

Beverage category	Beverage name	Intake reported (*n*)[1]	Consumption, excluding zero (servings per month)[2]			
			mean	sd	Min	Max
Non-alcoholic beverages	Coffee, Cypriot	10	16.5	15.6	2	30
	Coffee, instant	5	90.0	47.4	30	150
	Coffee, decaffeinated	0	-	-	-	-
	Coffee, other	4	49.0	32.9	16	90
	Tea, instant	3	13.3	4.6	8	16
	Tea, herbal infusion	1	8	-	-	-
	Fruit juice, fresh	2	7.0	7.1	2	12
	Fruit juice, packaged	2	16.0	19.8	2	30
	Squash	1	30	-	-	-
	Vegetable juice	0	-	-	-	-
	Cola-type soft drinks	2	4.5	4.9	1	8
	Other type soft drinks with sugar	0	-	-	-	-
	Soft drinks, diet (diet, zero, max, light)	6	24.2	34.0	3	90
	Energy drinks	2	2.5	2.1	1	4

Beverage category	Beverage name	Intake reported (***n***)[1]	Consumption, excluding zero (servings per month)[2]			
			mean	sd	Min	Max
Alcoholic drinks	Wine	7	15.1	20.3	4	60
	Beer	4	31.0	21.8	8	60
	Ouzo	0	-	-	-	-
	Brandy	0	-	-	-	-
	Whiskey	1	5	-	-	-
	Liquor	0	-	-	-	-
	Zivania	2	13.0	4.2	10	16
	Rum	0	-	-	-	-
	Alcoholic drink, other	0	-	-	-	-

Cooking method	Cooking method reported (***n***)[1]	Frequency, excluding zero (times per month)[2]			
		mean	sd	Min	Max
Frying	4	9.3	10.2	1	24
Boiling	6	13.4	9.3	1	30
Roasting	7	19.7	9.9	8	30
Grilling	7	17.1	12.6	2	30
Yiachni	4	2.5	1.3	1	4
Stir-frying	4	3.5	3.3	1	8
Deep-frying	4	3.8	5.5	1	12

Food cost	Answers
The financial cost of food for people with Type 1 diabetes is higher than for people without Type 1 diabetes.	Yes
	No
	I don't know

[1]Defined as consumption of at least one portion per month or frequency of use of a particular cooking method at least once a month, otherwise the intake or frequency was zero. [2]When applicable; descriptive statistics only for the participants that reported consuming a food or a drink or using a cooking method at least once a month.

Bioelectrical impedance analysis data

The results of the body composition analysis measurements collected via the BIA method are presented in *Table 7.3*. There was no control for parameters, such as hydration status, the menstrual cycle, food and drink consumption, and exercise, for practical reasons.

Table 7.3: Body Composition analysis through the BIA method

BIA	kg				%				Within normal range?[3] ***n***[%]		
	Mean	sd	Min	Max	Mean	sd	Min	Max	Yes	No	
Healthy body composition[2]									4[44.4]	5[55.6]	
Male									1[25.0]	3[75.0]	
female									3[60.0]	2[40.0]	
									Yes	No, ↑	No, ↓
Lean mass[4]	54.1	12.3	39.6	75.0	69.3	6.5	59.5	78.6	4[44.4]	1[11.1]	4[44.4]
Male	65.5	8.2	55.2	75.0	66.1	7.7	59.5	76.8	1[25.0]	0	3[75.0]
Female	44.9	4.1	39.6	51.0	72.0	4.5	67.2	78.6	3[60.0]	1[20.0]	1[20.0]
Fat mass	25.6	13.0	12.2	46.2	30.7	6.5	21.4	40.5	4[44.4]	4[44.4]	1[11.1]
Male	35.5	13.9	16.7	46.2	34.0	7.7	23.2	40.5	1[25.0]	3[75.0]	0
Female	17.6	3.9	12.2	22.0	28.0	4.5	21.4	32.8	3[60.0]	1[20.0]	1[20.0]
Water	39.6[5]	9.0	29.0	54.9	50.8	4.7	43.6	57.5	5[55.6]	1[11.1]	3[33.3]
Male	48.0[5]	6.0	40.4	54.9	48.4	5.6	43.6	56.2	1[25.0]	0	3[75.0]
Female	32.9[5]	3.0	29.0	37.3	52.7	3.3	49.2	57.5	4[80.0]	1[20.0]	0
RMR[6]	1571	280	1197	2078							
Male[6]	1834	170	1685	2078							
Female[6]	1361	102	1197	1445							

[1]Measured using a hand-to-food BIA device, described at *Methods*. [2]Defined as within normal range if the body fat and the lean mass were within normal range. [3]Compared to the normal range provided by the BIA device for each participant; note that a general description of the normal range for different ages, male vs. females, etc. used by the BIA device is unknown thus the subject is further discussed in *Chpater 4: Discussion and key recommendations* section. [4]The term 'lean mass' is used here to described the body weight other than fat mass and includes the muscle mass, bones, fluids and body organs; in practice, the body fat mass and the lean mass should constitute 100% or the total weight (kg) of the body of a participant. [5]Fluids are measured in litres. [6]RMR is measured in kcal. RMR, resting metabolic rate.

Medical and Diabetes characteristics

The results of the *medical and diabetes* questionnaire are presented in *Table 7.4*.

Table 7.4: Medical- and diabetes-related characteristics

Table 7.4a: Diabetes diagnosis

Characteristics	Total ***n***	Variables	***n***	%	Mean	sd	Min	Max
Who made the diagnosis of diabetes?	9	GP	3	33.3				
		Internist	4	44.4				
		Endocrinologist	0	-				
		Other	2	22.2				
How was the diagnosis made?	9	Symptoms and fasting glucose	3	33.3				
		Symptoms and HbA1c	1	11.1				
		DKA	2	22.2				
		Incidentally on laboratory testing	1	11.1				
		Could not recall	2	22.2				
Duration of diabetes (years)	9				16.9	8.9	5	33
Family members who had diabetes [a]	9	Present	2	22.2				
	2	If present, that relative was a sibling	1	50.0				

[a]Any combination of the answers *mum*, *dad*, *grandchild(ren)* and *grandparent(s)* was possible; one patient did not specify the family member with diabetes
GP, General Practitioner; HbA1c, glycosylated haemoglobin; DKA, Diabetic Ketoacidosis

Table 7.4b: Insulin

Characteristics	Total ***n***	Variables	***n***	%	Mean	sd	Min	Max
MDI vs CSII	9	MDI	9	100				
		CSII	0	0				
MDI therapy (insulins combination)	8	Rapid acting and long acting insulin	6	75.0				
		Rapid acting and pre-mixed insulin[b]	1	12.5				
		Rapid acting analogue only[b]	1	12.5				
Total daily insulin	8	Dosage (units)			52.0	30.2	20[c]	114
		Frequency (times)			4.0	1.2	2	6
Total daily slow acting insulin	6	Dosage (units)			29	12.7	12	44
		Frequency (times)			1.3	0.5	1	2
Total daily rapid acting insulin	8	Dosage (units)			27.8	24.3	5[c]	70
		Frequency (times)			2.9	0.6	2	4

[b]The management of type 1 diabetes with a rapid acting insulin alone is not indicated and is unlikely to provide good glycaemic control, while the use of rapid acting insulin with a mixed insulin is also an unusual way to manage Type 1 diabetes. [c]The 20 units of total insulin is a small dosage; taken into account that this participant reported administrating only 5 units of total daily rapid acting insulin may reflect a limited total daily carbohydrate intake of about 50g.
MDI, Multiple Daily Insulin Injections; CSII, Continuous Subcutaneous Insulin Infusion

Table 7.4c: Diabetes care, lifestyle and diabetes education

Characteristics	Total ***n***	Variables	***n***	%	Mean	sd	Min	Max
Adjusting insulin to carbohydrate intake	8	Yes	7	87.5				
		No	1	12.5				
Ever received education on how to adjust insulin	8	Yes	7	87.5				
		No	1	12.5				
Who was/were the provider(s) of the education[d]	7	Doctor only	4	57.1				
		Doctor and dietitian	3	42.9				
Main provider of care[e]	8	None	2	25.0				
		Public sector (hospital)	4	50.0				
		Private sector	2	25.0				
Ever had a dietetic or a diet-related input	8	No	3	37.5				
		Yes	5	62.5				
Who was the provider of the diet input[e]	5	Doctor	2	40.0				
		Dietitian	3	60.0				
Reason for the diet input[f]	5	Weight loss	2	40.0				
		Diabetes	3	60.0				
Ever had a podiatric or a foot-related input	8	No	5	62.5				
		Yes	3	37.5				
Who was the provider of the foot input[g]	3	Podiatrist	3	100				
Reason for the foot input[h]	3	Routine screening	3	100				
Input from a podiatrist over the last 12 months	8	Yes	1	12.5				
		No	7	87.5				
Input from an ophthalmologist over the last 12 months	8	Yes	4	50.0				
		No	4	50.0				
Smoking habits[i] (no. of cigarette packets smoked weekly)	8	Current	2	25.0	6.0	1.4	5	7
		Non-smoker	2	25.0				
		Ex-smoker	1	12.5				
		Occasional	3	37.5				

[d]The questionnaire-software allowed the selection of maximum two answers from the three available answers, namely *doctor*, *dietitian* and *other*. [e]Other available answer was *other*. [f]Other available answers were *renal disease* and *other*. [g]Other available answers were *doctor* and *other*. [h]Other available answers were *ulcer treatment* and *other*. [i]The term *smoker* was defined as the smoking of at least one cigarette per day, *ex-smoker* as having stopped smoking for at least one year, and *occasional smoker* as smoking less than 7 cigarettes per week.

Table 7.4d: Potential diabetes complications

Characteristics	Total *n*	Variables	n	%	Years since diagnosis or treatment
Macrovascular complications					
Total	8	Present	0	-	-
MI		Present	0	-	-
IHD		Present	0	-	-
CCF		Present	0	-	-
CVA		Present	0	-	-
PVD		Present	0	-	-
Microvascular complications					
Total	8	Present	0	-	-
Renal disease		Present	0	-	-
On dialysis		Present	0	-	not asked
Ever had a urine test for proteinuria?		Yes	3	37.5	n/a
		No	5	62.5	n/a
Eye disease		Present	0	-	-
Ever received treatment for eye disease[j]		Yes	0	-	not asked
Foot disease		Present	0	-	-
Ever received treatment for foot disease[k]		Yes	0	-	-
Sexual health – males					
Ever had the testosterone levels checked?	4	Yes	1	25.0	n/a
		No	3	75.0	n/a
Low testosterone levels	1	Present	0	-	n/a
Erectile dysfunction	4	Present	0	-	n/a
Loss of libido		Present	0	-	n/a
Loss of early morning erections		Present	0	-	n/a
Ever received treatment for erectile dysfunction	1	Yes	0	-	n/a
Sexual health – females					
Loss of libido	4	Present	1	25.0	n/a
		Absent	3	75.0	n/a

[j]If a participant had answered yes then (s)he could had further selected the type and/or reason for the treatment; available answers were *laser treatment*, *cataract*, *glaucoma*, *observation*, *glaucoma surgery* and *other*. [k]If a participant had answered yes then (s)he could had further selected the type and/or reason for the treatment; available answers were *foot ulcer*, *amputation*, *osteomyelitis* and *other*

MI, myocardial infraction; IHD, ischaemic heart disease; CCF, congestive cardiac failure; CVA, cerebrovascular accident; PVD, peripheral vascular disease.

Table 7.4e: Medical history, medications and supplements

Characteristics	Total ***n***	Variables	***n***	%
Medical history				
Hypertension[l]	**8**	Present	3	37.5
		Absent	5	62.5
Hyperlipidaemia[l]		Present	3	37.5
		Absent	5	62.5
Lung disease, for example, COPD and asthma[l]		Present	0	-
		Absent	8	100
Autoimmune disease, for example, thyroid disease and RA[l]		Present	1	12.5
		Absent	7	87.5
Other disease(s)[m]		Present	2	25.0
		Absent	6	75.0
PCOS[m]	2	Present	1	12.5
Hashimoto's thyroiditis and epilepsy[m]		Present	1	12.5
Medications[m]				
Any medications	8	Yes	4	50.0
		No	4	50.0
Telmisartan	4	Yes	2	25.0
Metformin		Yes	1	12.5
Simvastatin		Yes	1	12.5
Levothyroxine		Yes	1	12.5
Levetiracetam		Yes	1	12.5
Supplements[m]				
Any supplements	8	Yes	0	-
		No	8	100

[l]The only available answers were the *Yes* or the *No* which indicated the presence or the absence of the disease(s). [m]These are based on the manual entries recorded by the participants on their clinical history, and of the medications and the supplements that they were taking at the time of completion.

COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis; PCOS, polycystic ovary syndrome.

Poster presentation and abstract publication

Title: Mediterranean diet and glycaemic control in a Mediterranean population with type 1 diabetes: a pilot study

Poster Presentation:

Conference: 19th European Congress of Endocrinology (ECE) 2017, Lisbon, Portugal

Date: 20 - 23 May 2017

Authors: Alexis Kyriacou, Josie M.M. Evans, Angelos Kyriacou

Abstract Publication**:**

Journal: Endocrine Abstracts

Date: May 2017

Volume: 49 EP481 | **DOI**: 10.1530/endoabs.49.EP481

Authors: Alexis Kyriacou, Josie M.M. Evans, Angelos Kyriacou

Conference related presentation

- Invited presentation: Mediterranean diet and Diabetes
  Annual Symposium of Cyprus Endocrinology Association 2018
  Limassol, Cyprus (14th April 2018)

Abstract

Endocrine Abstracts (2017) **49** EP481 | DOI: 10.1530/endoabs.49.EP481

Mediterranean diet and glycaemic control in a Mediterranean population with type 1 diabetes: a pilot study

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Background: The Mediterranean diet (MD) is the traditional diet of the people living in the Mediterranean basin and has been linked with positive health outcomes e.g. reduced incidence of cardiovascular and neoplastic disease. No study has investigated the relationship between the MD and glycaemic control in a Mediterranean population with type 1 diabetes mellitus (T1DM). Furthermore, it is unknown how well controlled are such patients and whether they follow the MD.

Methods: Patients known with T1DM were randomly conducted through the registry of the Cyprus Diabetes Association. Ethics: Received from the University of Stirling and the Cyprus National Bioethics Committee.

Results: Twenty patients were conducted; eight patients fulfilled the inclusion criteria and completed antropometrics and the questionnaires; six had biochemistry. Age was 34.6±10.7 years. All patients were classified as having moderate adherence to the MD using the MedDietScore scoring system (28.9±5.2; max score 55). Lowest score was seen for potatoes and non-refined cereals (1.4±0.7 and 1.4±0.9; max score 5) and the highest for use of vegetables and olive oil in cooking (4.1±1.5 and 4.8±0.7; max score 5). All six patients with biochemical testing had undetectable levels of fasting blood c-peptide. HbA1c was 63.5±5.8 mmol/mol and fasting glucose levels 227.7±64.8 mg/dl; none had optimal diabetes control i.e. HbA1c≤53 mmol/mol. MD score was unrelated to HbA1c (ρ=−0.65; P=0.16) or fasting glucose (ρ=−0.66; P=0.15). Mean BMI was 27.0±5.5; 67% were overweight or obese; BMI was marginally related to HbA1c (ρ=0.8117; P=0.0499).

Discussion: Our results show a no association between the MD and glycaemic control although a type 2 statistical error is plausible. Adherence to the MD diet was moderate in-line with our previous research that showed a moderate and reducing adherence in the general population in Cyprus. Furthermore, our results are worrying regarding glycaemic control. A larger study is underway to further investigate these relationships.

Poster

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Salford Royal NHS
NHS Foundation Trust

Mediterranean diet and glycaemic control in a Mediterranean population with Type 1 diabetes: a pilot study

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Introduction

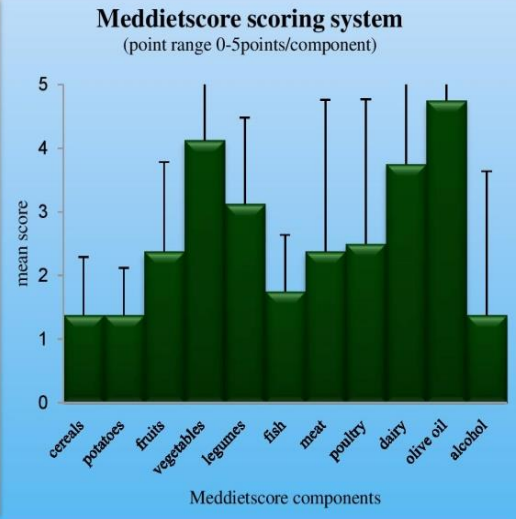
- Mediterranean diet is defined as the dietary pattern found in the olive oil-growing area of the Mediterranean region in the late 1950s and early 1960s, before the invasion of the fast food culture in the area[1]
- There is good evidence which shows a reduction of HbA1c and fasting blood glucose with better adherence to the Mediterranean diet in patients with Type 2 diabetes mellitus[2]
- Limited evidence exists regarding the relationship of the Mediterranean diet and glycaemic control in patients with Type 1 diabetes and no such studies have been conducted in the Mediterranean population[3]
- Dietary habits in the Mediterranean region are changing towards a more Westernized diet but it is not known if such is the trend for patients with Type 1 diabetes[3]

Methods

- The pilot part of the study aimed to include a small number of adult patients with Type 1 diabetes residing in Limassol, Cyprus
- Mediterranean diet was measured using an adapted Mediterranean diet score ('Meddiet score') after extrapolating data from a food frequency questionnaire
- Statistical analysis was performed using STATA 11.2 (StataCorp LP, College station, TX, USA); Any association was investigated using the Spearman's rank correlation coefficient and gender differences using Mann-Whitney test. Demographics are reported as mean±standard deviation
- The study has received ethics approval by the School of Health Sciences Research Ethics Committee of the University of Stirling & the Cyprus National Bioethics Committee

Results

- Eight patients completed diet related and anthropometric measurements of which 6 also had a blood & urine test
- Mean age was 34.6 ± 10.7 years & 50% were female
- All patients had a moderate adherence to the Mediterranean diet (n=8; mean Meddiet score = 28.9±5.2points; range: 0-50points). Females had a non-statistical significant better adherence to the Mediterranean diet (p=0.38; ♀:30.3±5.7; ♂:27.5±4.9)
- Olive oil was the better adhered while non-refined cereals, potatoes and alcohol the least (*see 'Meddietscore scoring system' 'barchart'*)
- All patients had a suboptimal glycaemic control i.e. HbA1c ≤ 53mmol/mol (n=6; mean HbA1c= 63.5±5.8mmol/mol; fasting blood glucose = 227.7±64.8mg/dl)
- BMI was 27.0±5.5kg/m^2 (n=8); 6 patients were overweight or obese
- C-peptide levels were undetectable i.e. <0.10ng/ml (n=8)
- Mediterranean diet score was unrelated to HbA1c (rho=-0.65; p=0.16) or fasting glucose (rho=-0.66; p=0.15)
- BMI was marginally related to HbA1c (rho=0.8117; p=0.0499)



Conclusions & Discussion

- This is the first study to explore the adherence to the Mediterranean diet in a Mediterranean population with Type 1 diabetes & the first to study the epidemiological characteristics of people with Type 1 diabetes in Cyprus.
- In this small population sample (required for a pilot study) of patients with Type 1 diabetes
  - Adherence to the Mediterranean diet was moderate as shown in reviews of the Mediterranean general population[3]
  - Sub-optimal glycaemic control & obesity were worryingly prevalent
  - Glycaemic control was not associated with Mediterranean diet and marginally associated with BMI
- Following this pilot study, a similar but larger study is under way to confirm or disprove the above results

[1]Trichopoulou, A., Lagiou, P., Kuper, H. & Trichopoulos, D. Cancer and Mediterranean dietary traditions. Cancer Epidemiology Biomarkers and Prevention 9, 869–873 (2000). [2]Ajala, O., English, P. & Pinkney, J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *Am. J. Clin. Nutr.* 97, 505–516 (2013). [3]Kyriacou, A., Evans, J. M. M., Economides, N. & Kyriacou, A. Adherence to the Mediterranean diet by the Greek and Cypriot population: a systematic review. *Eur. J. Public Health* 25(6), 1012-1018 (2015).

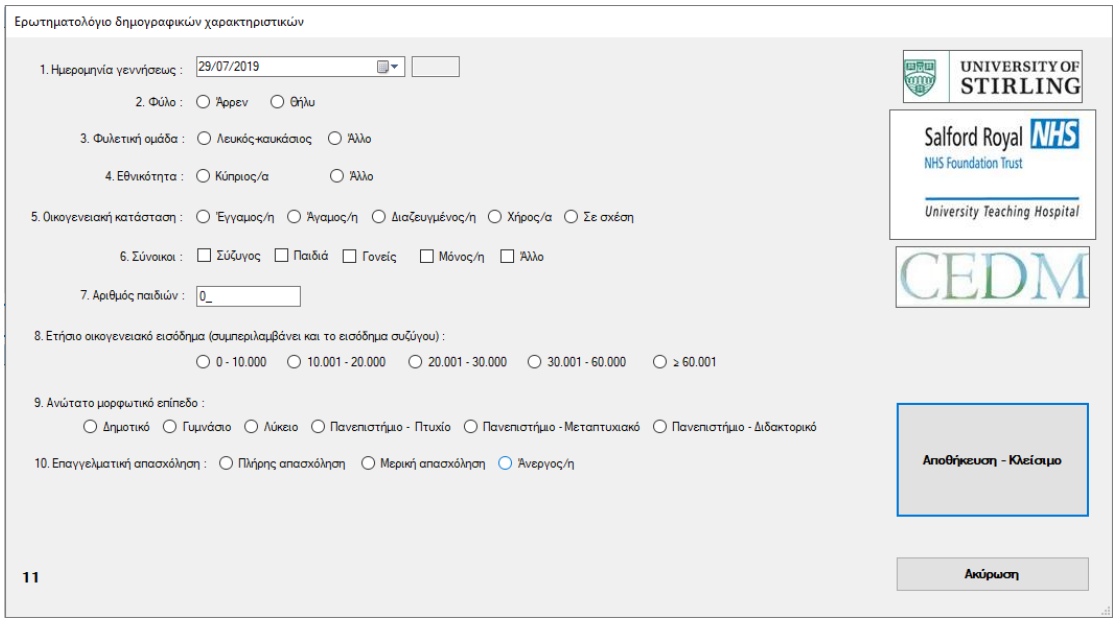
Chapter 5

Questionnaires

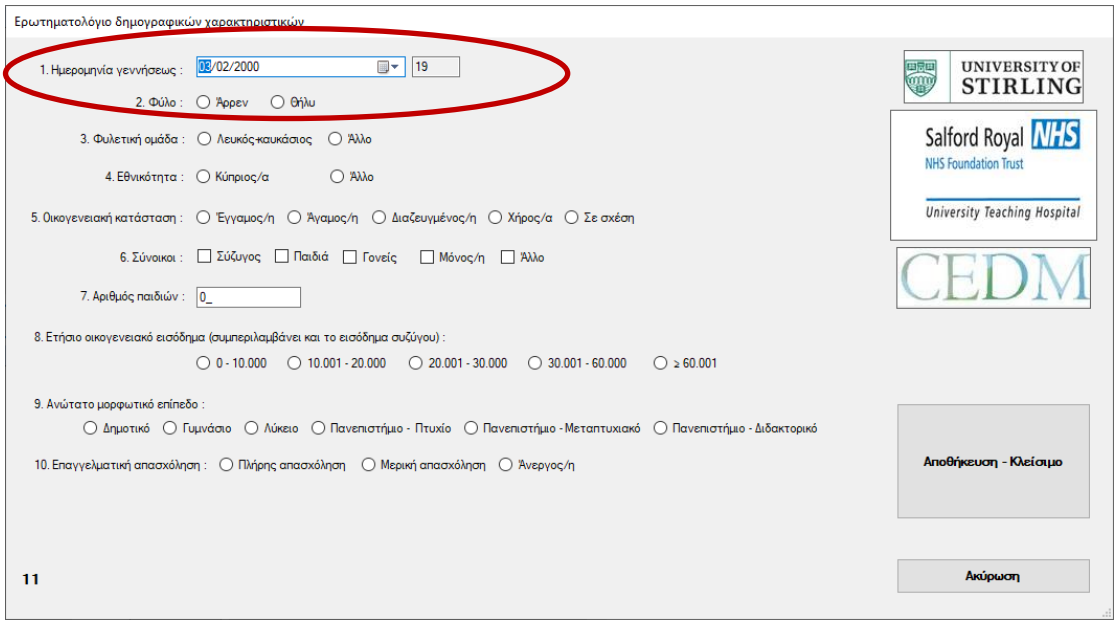
Demographic characteristics questionnaire

Electronic format (in Greek language)

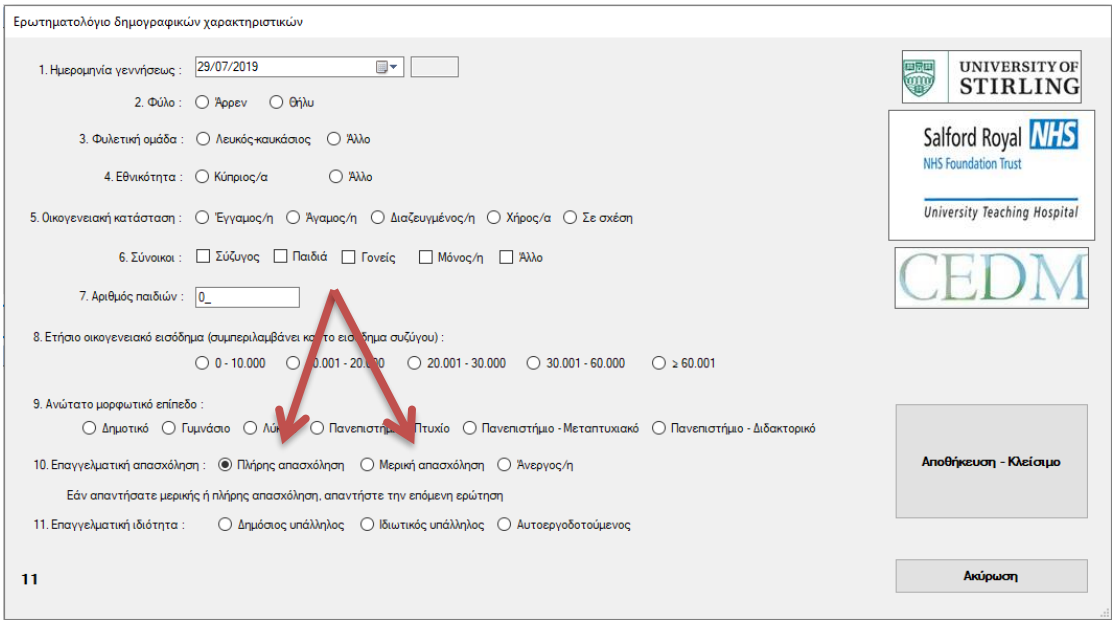
Page 1 of 1: As was administrated



Page 1 of 1: The date of birth ‘03/02/2020’ was entered at question 1



Page 1 of 1: When the *full-time* or the *part-time* answer (1st or 2nd box, respectively) was selected at question 10



Description and translation in English

The demographic characteristics questionnaire was a short, one-page questionnaire and is pictorially presented at *Page 1 of 1: As was administrated*. The translation of the questionnaire was as follows:

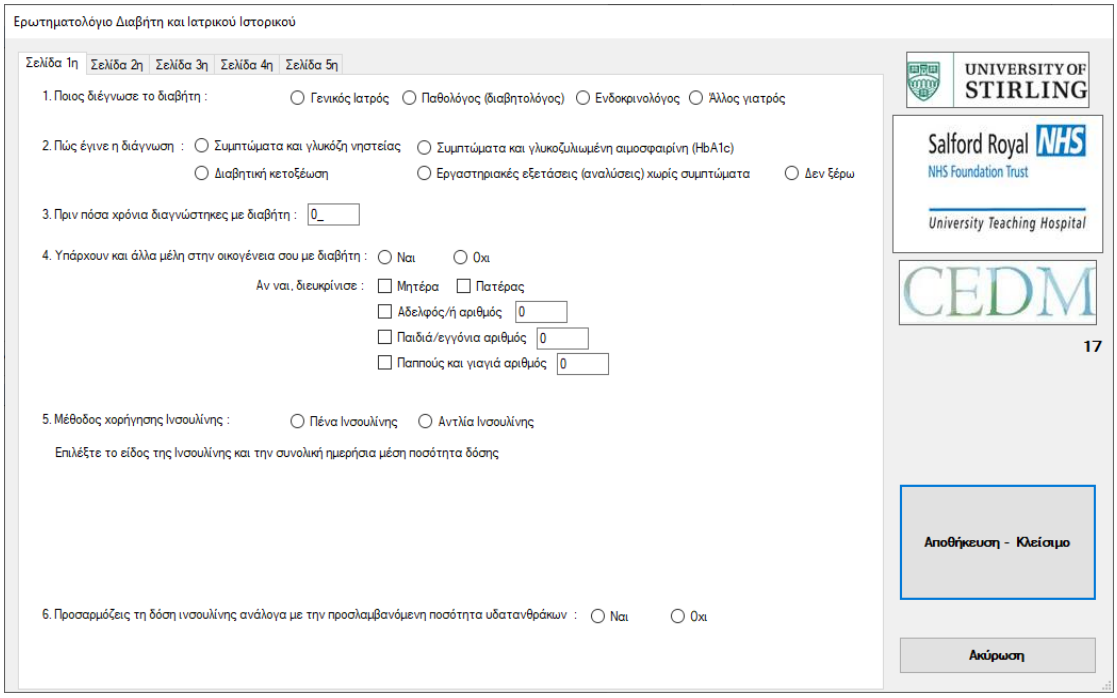
1. Date of Birth: *DD/MM/YYYY* | *XX*
2. Gender: *Male* | *Female*
3. Ethnic group: *White-Caucasian* | *Other*
4. Nationality: *Cypriot* | *Other*
5. Marital status: *Married* | *Single* | *Divorced* | *Widowed* | *In a relationship*
6. Co-habitants: *Spouse* | *Children* | *Parents* | *Alone* | *Other*
7. Number of children: *0*
8. Average annual household income (includes the income of spouse): *0 – 10.000* | *10.001 – 20.000* | *20.001 – 30.000* | *30.001 – 60.000* | *≥ 60.001*
9. Highest educational level: *Primary school* | *Gymnasium* | *Lyceum* | *University - Bachelor* | *University - Master* | *University - Doctorate*
10. Employment: *Full-time* | *Part-time* | *Unemployed*
11. Employer: *Public sector employee* | *Private sector employee* | *Self-employed*

Notes: At question 1, the *DD/MM/YYYY* was the Day/Month/Year, which was to be added by the participant either manually or through a drop-down calendar. The *XX* was the age in years, which was a computerized calculation, as shown in picture *Page 1 of 1: The date of birth '03/02/2020' was entered at question 1*. The default answer at questions 7, was *0* and could be changed accordingly. The answers at question 9, corresponded to primary school (6 – 12 years) for *dimotiko*, to lower secondary school (12 – 15 years) for *gymnasium* and to upper secondary school (15 – 18 years) for *lyceum*. The ◯ indicated that only one answer (box) could be selected. Therefore, if a second box was ticked, then the selection (answer) changed to the latest one. In contrast, the☐ indicated that one or more answers (boxes) could be selected. Therefore, if more than one box was ticked, all boxes remained selected unless they were unselected by the participant. Finally, the question 11 was available only if the answer selected at question 10 was *full-time* or *part-time*, as shown in picture *Page 1 of 1: When the full-time or the part-time answer (1st or 2nd circle, respectively) was selected at question 10*.

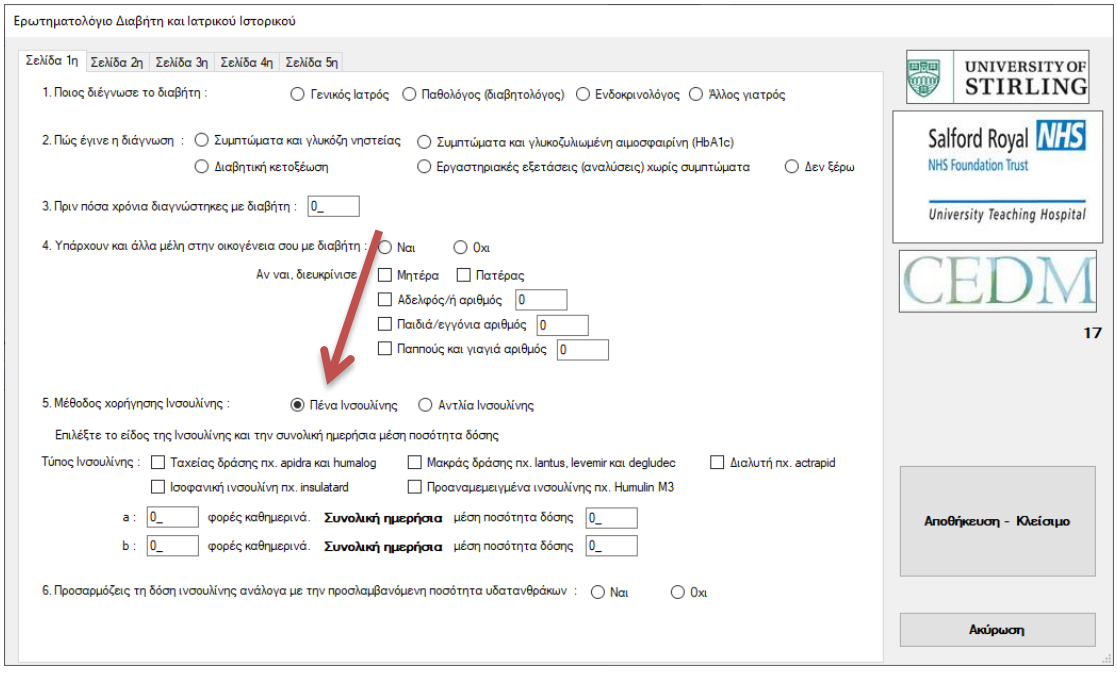
Medical and diabetes questionnaire

Electronic format (in Greek language)

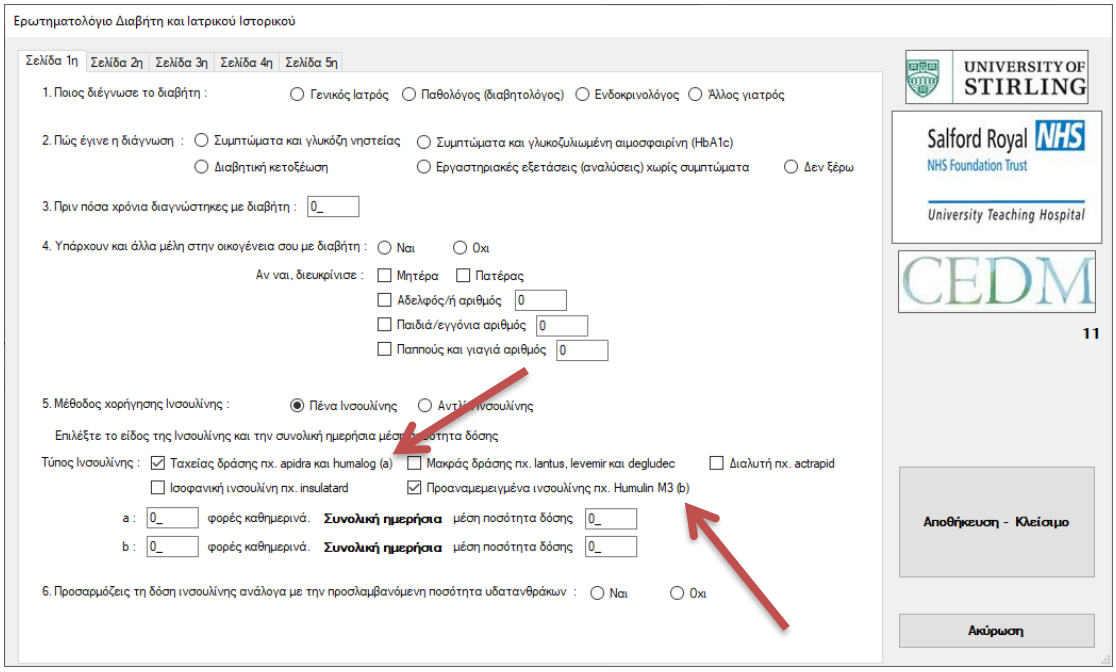
Page 1 of 5: As was administrated



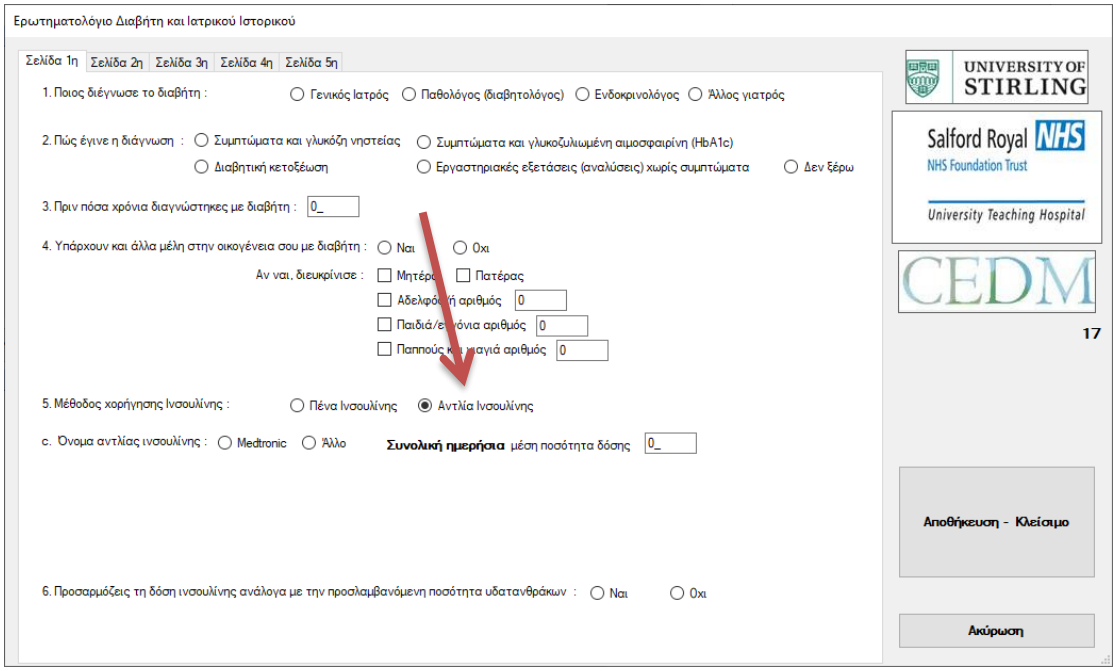
Page 1 of 5: When the MDI option was selected at question 5



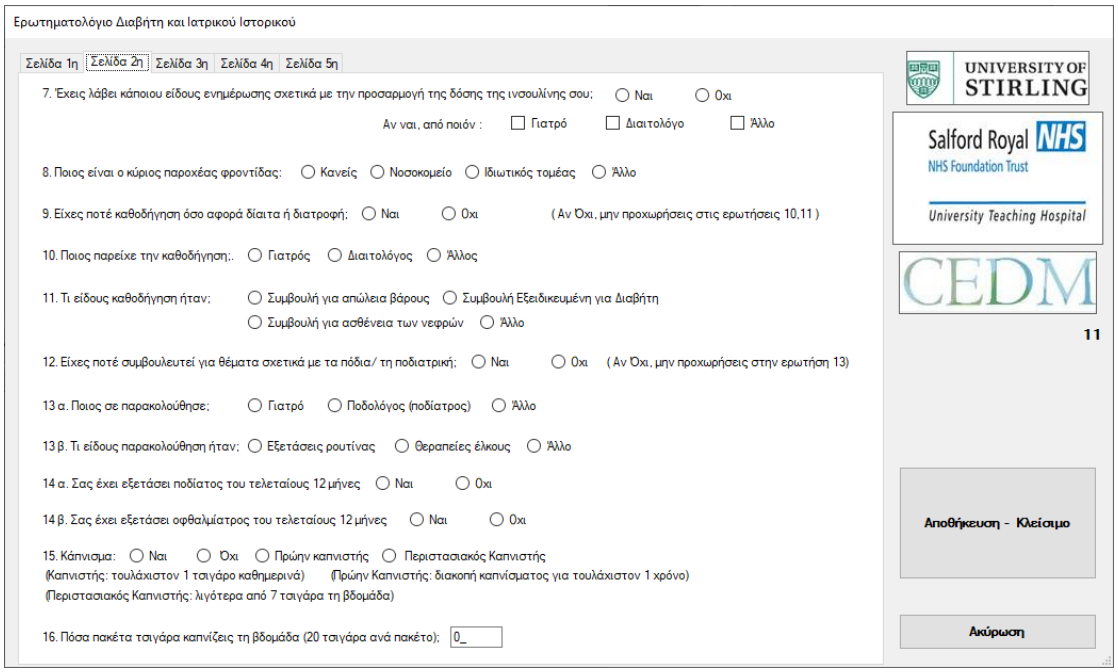
Page 1 of 5: At question 5, when the insulins used, were selected, then they were marked as (a) and (b)



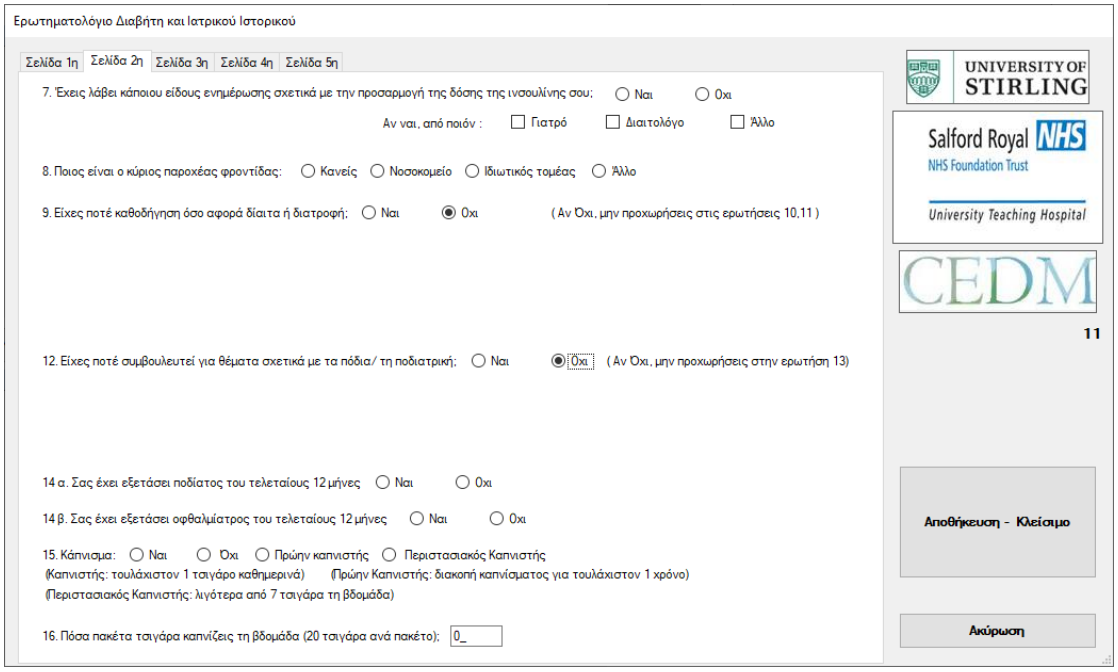
Page 1 of 5: When the CSII option was selected at question 5



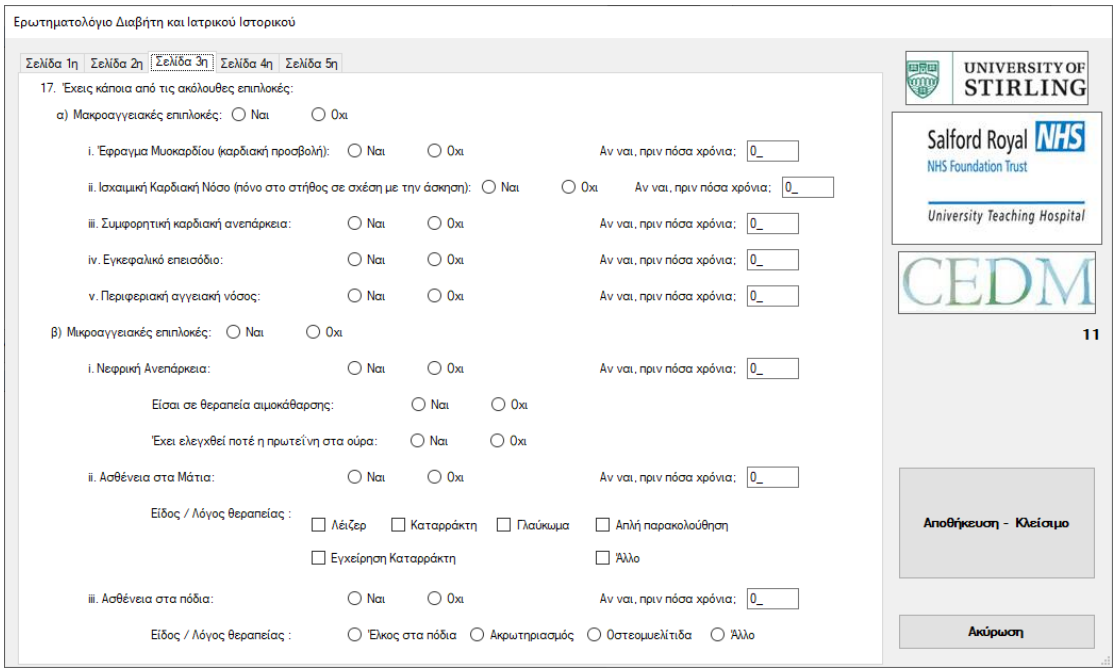
Page 2 of 5: As was administrated



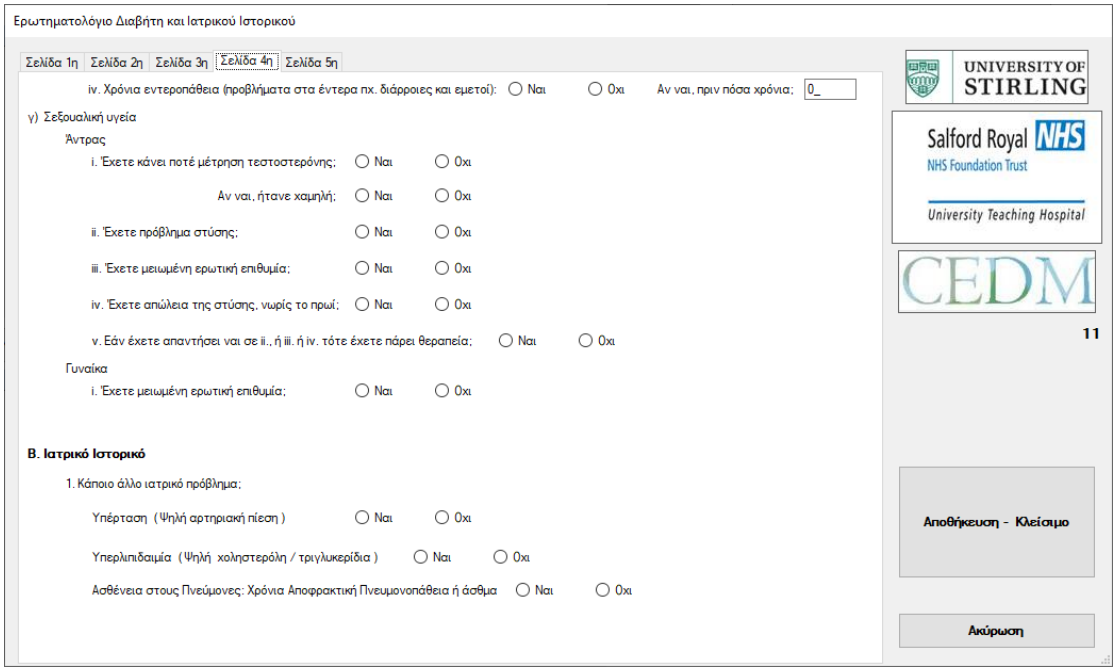
Page 2 of 5: When the option *No* was selected in question 9 and 10



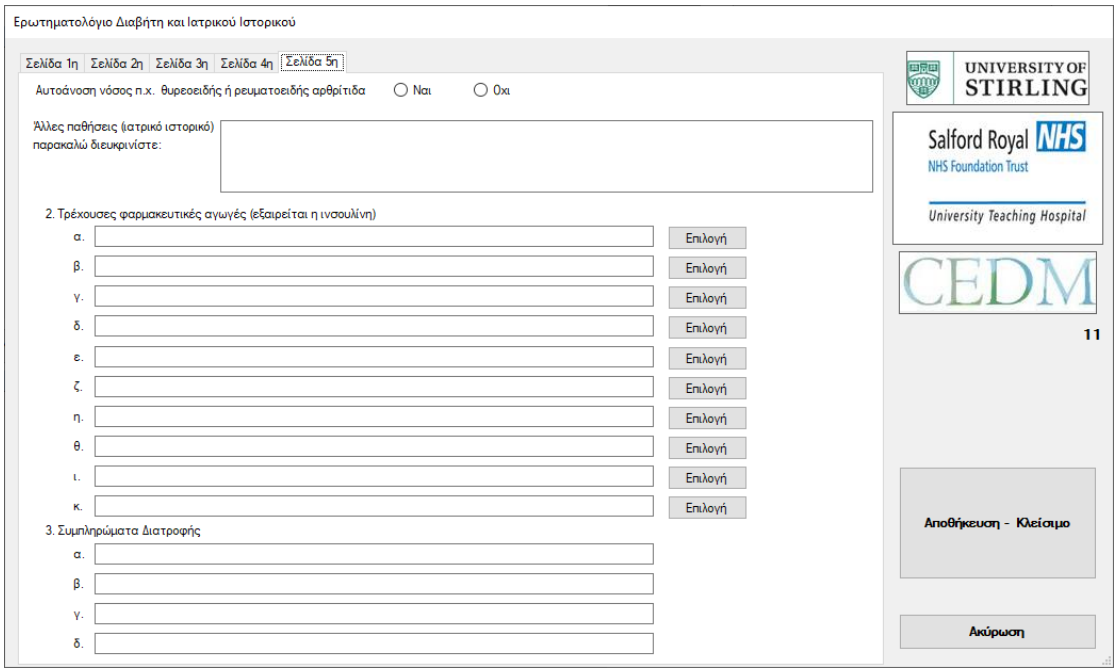
Page 3 of 5: As was administrated



Page 4 of 5: As was administrated



Page 5 of 5: As was administrated



Page 5 of 5: A sample of the medication list, which was available when the browse option [Επιλογή] was used at the *current medications* question

Description and translation in English

The *medical and diabetes* questionnaire was a detailed, five pages questionnaire. The translation of the questionnaire was as follows:

[Page 1, for pictorial see *Page 1 of 5: As was administrated*]

1. Who made the diagnosis of diabetes: *Family doctor* | *Internist* | *Endocrinologist* | *Other*
2. How was the diagnosis made: *Symptoms and fasting glucose* | *Symptoms and glycosylated haemoglobin (HbA1c)* | *Diabetic ketoacidosis* | *Biochemically while asymptomatic* | *I don't remember*
3. How many years ago was the diagnosis made: *0*
4. Do you have any other family members with diabetes: *Yes* | *No*
   If yes, specify: *Mother* | *Father*
   Sibling(s), number *0*
   Children and grandchildren, number *0*
   Grandparents, number *0*
5. Method of insulin delivery: *Insulin Pen* | *Insulin pump*

[*Insulin Pen*]

Select the type of insulin and the daily insulin dosage

Type of insulin: *Rapid acting e.g. apidra and humalog* | *Long acting e.g. lantus, levemir and degludec* | *Soluble e.g. actrapid* | *Isophane insulin e.g. insulatard* | *Pre-mixed insulin e.g. humulin M3*

a: *0* times per day. **Total daily** average dosage *0*
b: *0* times per day. **Total daily** average dosage *0*

[*Insulin Pump*]

Type of insulin device: *medtronic* | *other*. **Total daily** average dosage *0*

6. Do you adjust the insulin dose depending on your carbohydrate intake: *Yes* | *No*

[Page 2, for pictorial see *Page 2 of 5: As was administrated*]

7. Have you received instructions on how to adjust your insulin dose: *Yes* | *No*
   If yes, then by who: *Doctor* | *Dietitian* | *Other*
8. Who is your main provider of care: *None* | *Public sector* | *Private sector* | *Other*

9. Have you ever received dietetic or diet-related input: *Yes* | *No*
   (If No, then you don't need to answer to the questions 10 and 11)
10. Who was the provider of the diet input: *Doctor* | *Dietitian* | *Other*
11. What type of diet input was it: *Advise for weight loss* | *Specialist advise for diabetes* | *Advise for renal disease* | *Other*
12. Have you ever received podiatric or foot-related input: *Yes* | *No*
    (If No, then you don't need to answer to the question 13)
13. a. Who was the provider of the foot input: *Doctor* | *Podiatrist* | *Other*
    b. What type of foot input was it: *Routine screening* | *Ulcer treatment* | *Other*
14. a. Have you been examined by a podiatrist over the last 12 months: *Yes* | *No*
    b. Have you been examined by an ophthalmologist over the last 12 months: *Yes* | *No*
15. Smoking: *Yes* | *No* | *Ex-smoker* | *Occasional smoker*
    (Smoker: at least one cigarette per day; Ex-smoker: stopped smoking for at least one year; Occasional smoker: less than 7 cigarettes per week)
16. How many packets of cigarettes do you smoke per week (20 cigarettes are one packet): *0*

[Page 3, for pictorial see *Page 3 of 5: As was administrated*]

17. Do you have any of the following complications:

    a. Macrovascular complications: *Yes* | *No*
       i. Myocardial infarction (heart attack): *Yes* | *No*. if yes, how many years ago: *0*
       ii. Ischemic heart disease (chest pain during exercise): *Yes* | *No*. if yes, how many years ago: *0*
       iii. Congestive cardiac failure: *Yes* | *No*. if yes, how many years ago: *0*
       iv. Cerebrovascular accident: *Yes* | *No*. if yes, how many years ago: *0*
       v. Peripheral vascular disease: *Yes* | *No*. if yes, how many years ago: *0*

    b. Microvascular complications: *Yes* | *No*
       i. Renal disease: *Yes* | *No*. if yes, how many years ago: *0*
          Are you on dialysis: *Yes* | *No*
          Have you ever had a urine test to check for the presence of protein: *Yes* | *No*
       ii. Eyes disease: *Yes* | *No*. if yes, how many years ago: *0*

Type or reason for treatment: *LASER* | *Cataract* | *Glaucoma* | *Regular follow up for monitoring* | *Cataract surgery* |*Other*

iii. Foot disease: *Yes* | *No*. if yes, how many years ago: *0*

Type or reason for treatment: *Food ulcer* | *Amputation* | *Osteomyelitis* | *Other*

[Page 4, for pictorial see *Page 4 of 5: As was administrated*]

iv. Chronic enteropathy (gastrointestinal problems e.g. diarrhoea and vomiting): *Yes* | *No*. if yes, how many years ago: *0*

c. Sexual health

Males

i. Have you ever had your testosterone levels checked: *Yes* | *No*

If yes, then was it low: *Yes* | *No*

ii. Do you have erectile dysfunction: *Yes* | *No*

iii. Do you have reduced libido: *Yes* | *No*

iv. Do you have a loss of early morning erections: *Yes* | *No*

If you have answered yes to ii. or iii. or iv., then have you received treatment: *Yes* | *No*

Females

i. Do you have reduced libido: *Yes* | *No*

B. Medical history

1. Do you have other medical problems:

Hypertension (high blood pressure): *Yes* | *No*

Hyperlipidaemia (high cholesterol or triglycerides): *Yes* | *No*

Lung disease: Chronic Obstructive Pulmonary Disease or Asthma: *Yes* | *No*

[Page 5, for pictorial see *Page 5 of 5: As was administrated*]

Autoimmune disease e.g. thyroid or rheumatoid arthritis: *Yes* | *No*

Other conditions (medical history), please specify:

… [Free Text] …

2. Current medications (other than insulin)

a. ... *[Free Text]* ... Browse
b. ... *[Free Text]* ... Browse
c. ... *[Free Text]* ... Browse
d. ... *[Free Text]* ... Browse
e. ... *[Free Text]* ... Browse
f. ... *[Free Text]* ... Browse

3. Nutritional supplements
    a. ... *[Free Text]* ...
    b. ... *[Free Text]* ...
    c. ... *[Free Text]* ...
    d. ... *[Free Text]* ...

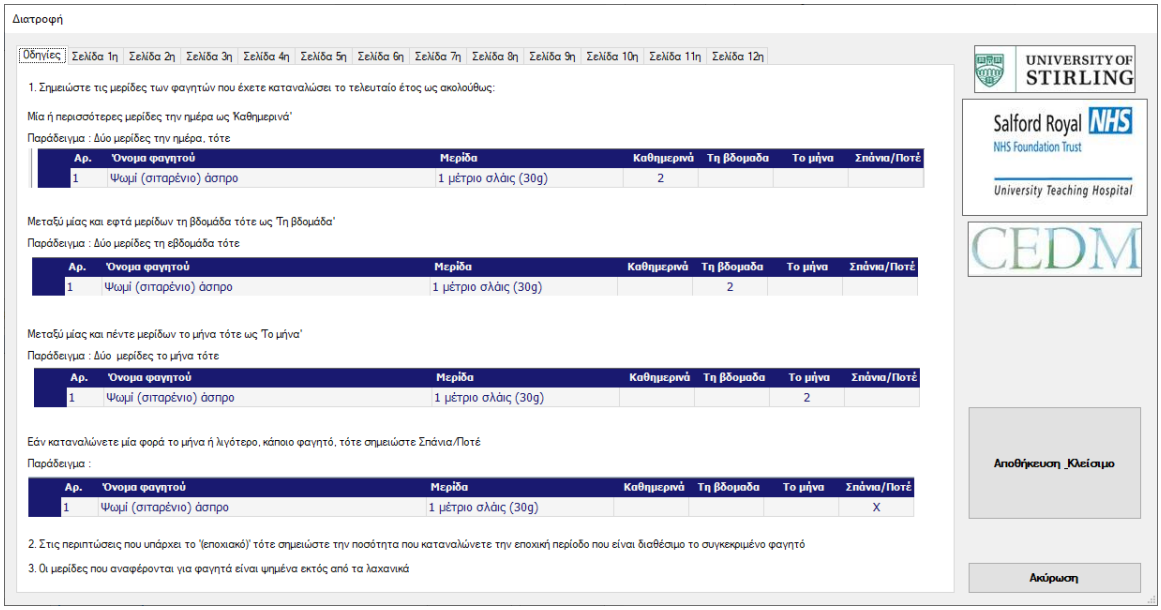
Notes: Note that, the current translation is a rough translation of the *medical and diabetes* questionnaire and reflects an effort to accommodate a precise translation in English. In question 1, the *family doctor* and *internist* are rough translations of the corresponding Greek terms. This is primarily due to the fact that Cyprus suffers from poor legislation and oversight of specialties in that unspecialised doctors, general practitioners, internists and sometimes other specialties are grouped together and the general public cannot in most cases distinguish between such specialists. The default answer(s) at questions 3, 4, 5, 16, and17 was/were *0* and could be changed accordingly. The set of potential answers was different for the *inulin pen* and *insulin pump* option as shown in the relevant pictures, the *Page 1 of 5: When the MDI option was selected at question 5* and the *Page 1 of 5: When the CSII option was selected at question 5*. Furthermore, the insulin selections were marked with (a) and (b) to allow for their distinction when answering the following questions in regard to their dosage, as shown in picture *Page 1 of 5: At question 5, when the insulins used, were selected, then they were marked as (a) and (b)*. In question 8, the answer *νοσοκομείο* (*hospital)* has been translated as *public sector* since the use of the word hospital is most often used as a reference to the government run hospitals. The questions 10 and 11, and 13 a. and 13 b. were available only if the answer selected at questions 9 and 12 was *yes*, respectively, as shown in picture *Page 2 of 5: When the option No was selected in question 9 and 10*. The *... [Free Text] ...* indicates that the answers are entered manually by the participants. In the case of *current medications,* the medications could also be browsed through the latest formulary by the pharmaceutical services of the Republic of Cyprus and which allowed searching for both commercial

names and the active ingredient(s), as shown in picture *Page 5 of 5: A sample of the medication list, which was available when the browse option [Επιλογή] was used at the current medications question.* The ◯ indicated that only one answer (box) could be selected. Therefore, if a second box was ticked, then the selection (answer) changed to the latest one. In contrast, the ☐ indicated that one or more answers (boxes) could be selected. Therefore, if more than one box was ticked, all boxes remained selected unless they were unselected by the participant.

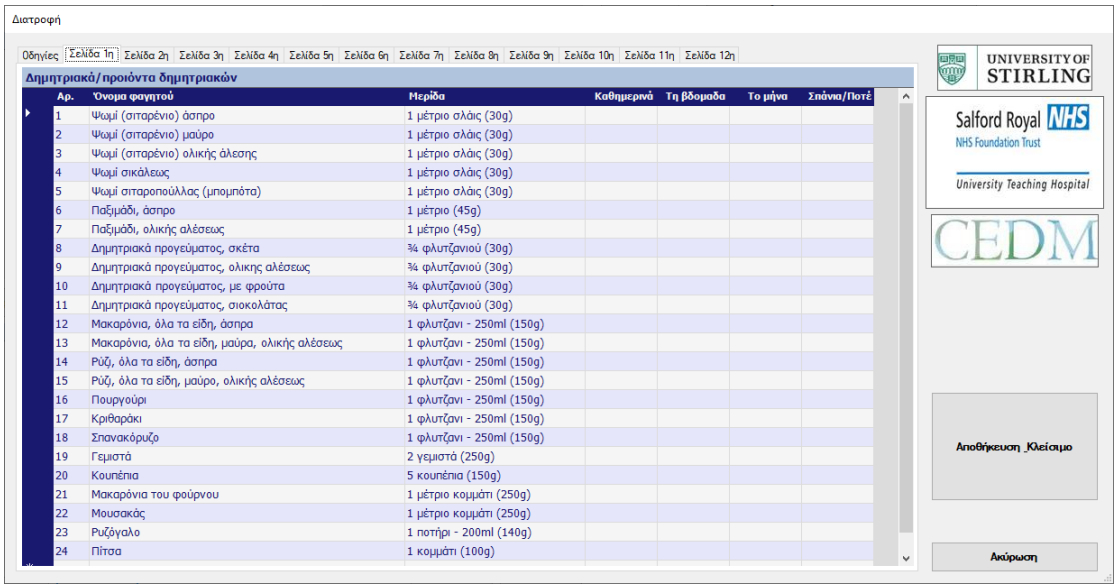
Food frequency questionnaire

Electronic format (in Greek language)

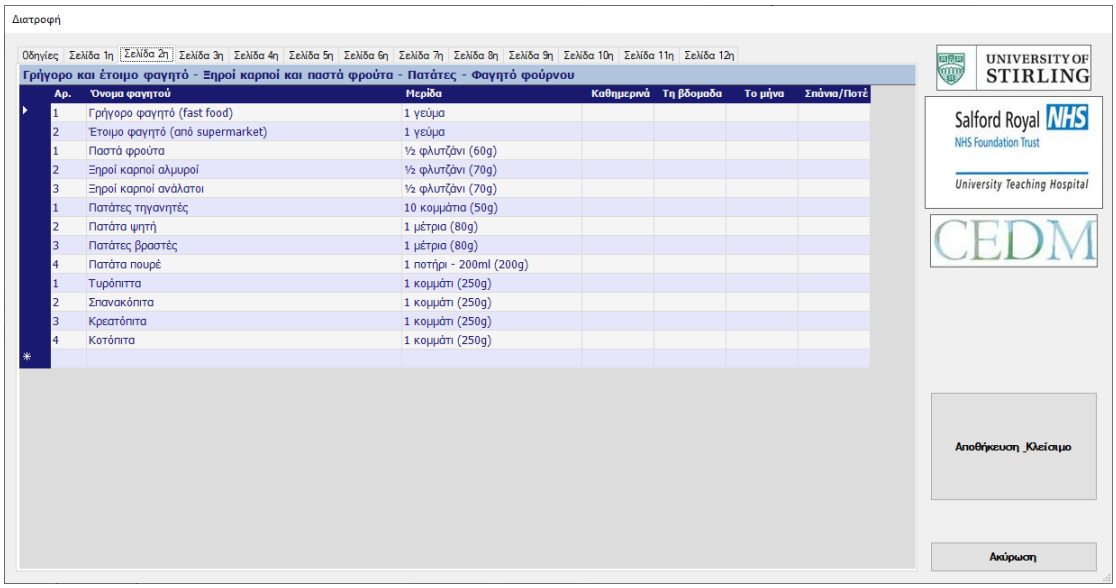
Page 0 of 12: Instructions



Page 1 of 12: Cereals and cereal products

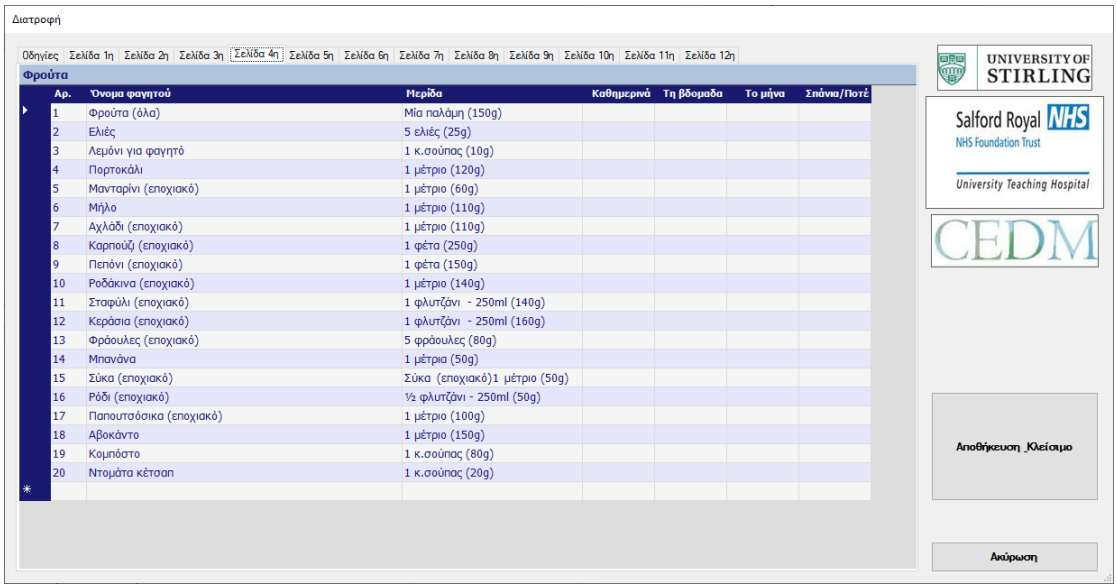


Page 2 of 12: Fast foods and ready meals, nuts and dried fruits, potatoes and pastries

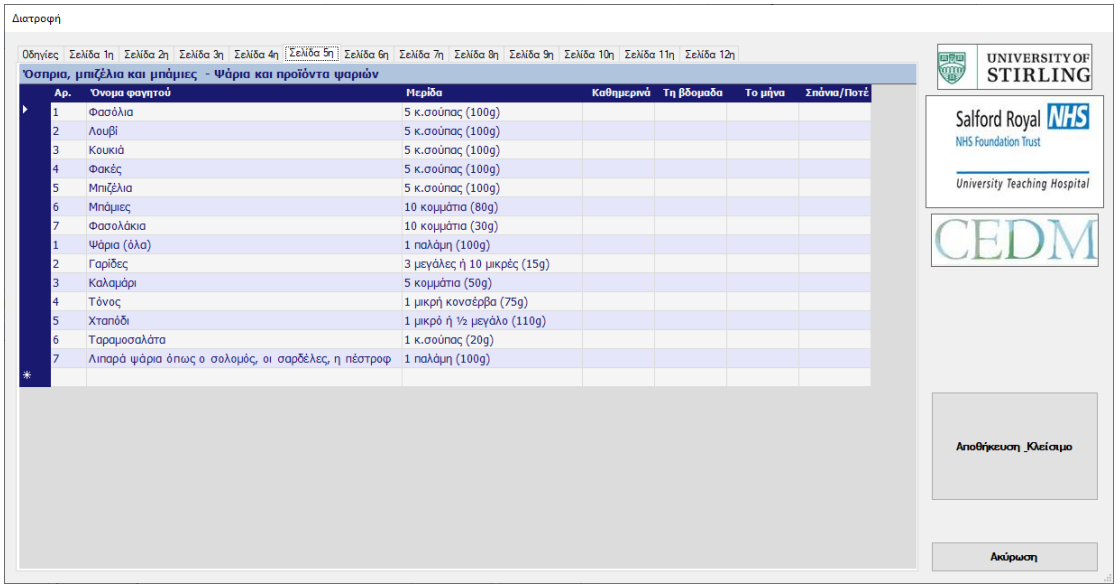


Page 3 of 12: Salads and vegetables

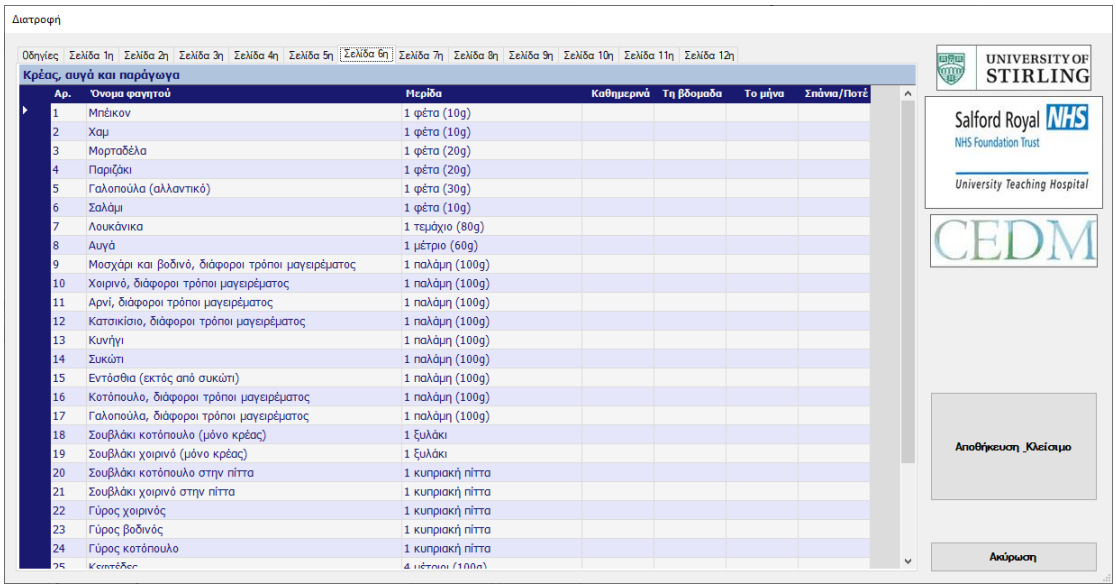
Page 4 of 12: Fruits



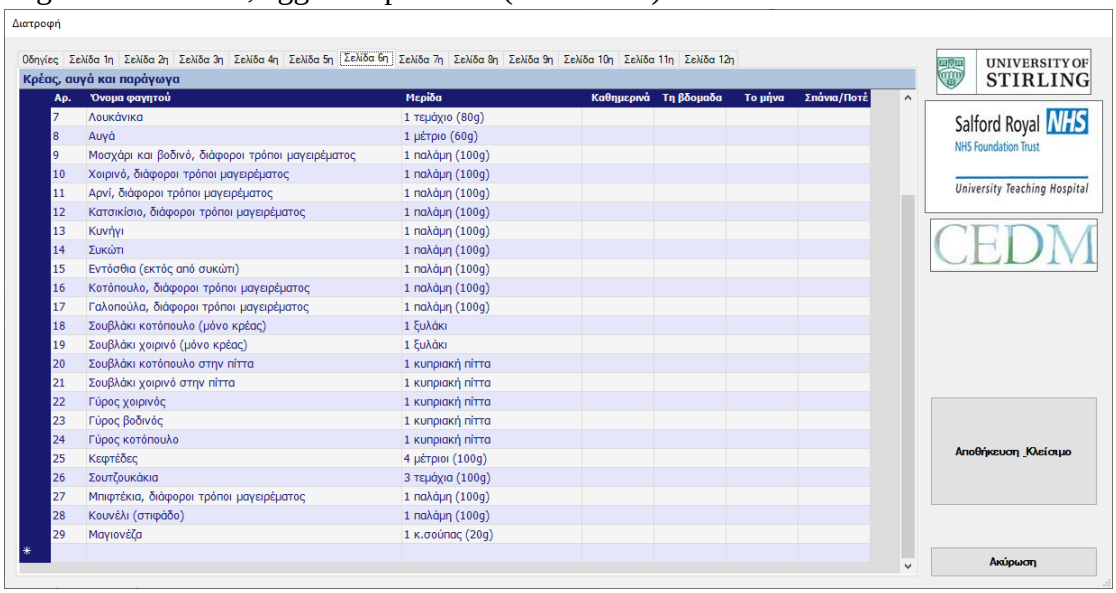
Page 5 of 12: Legumes, peas and ladies' fingers, and fish and fish products



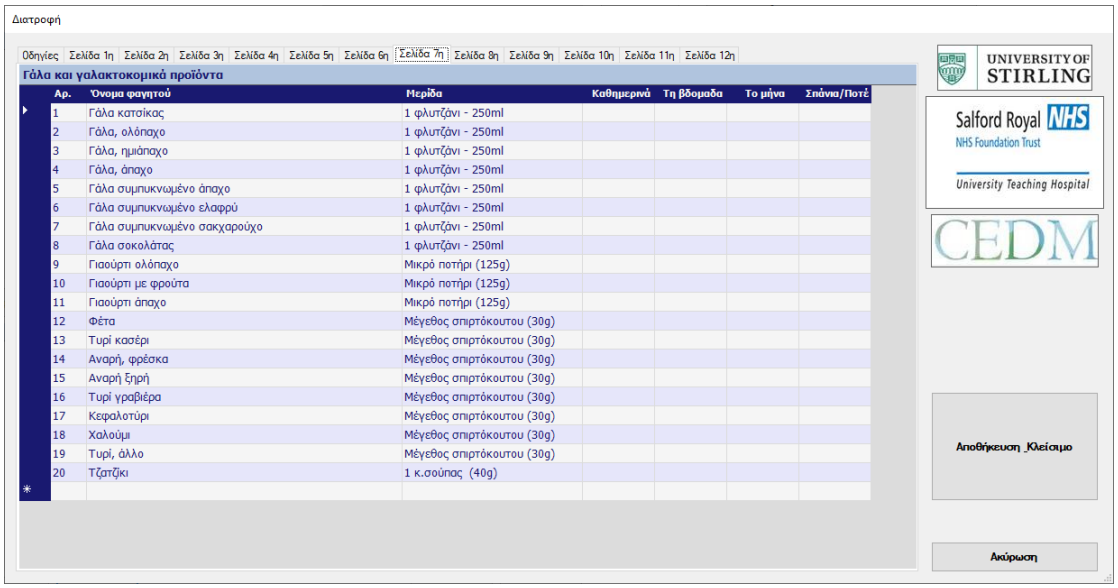
Page 6 of 12: Meat, eggs and products (Upper half)



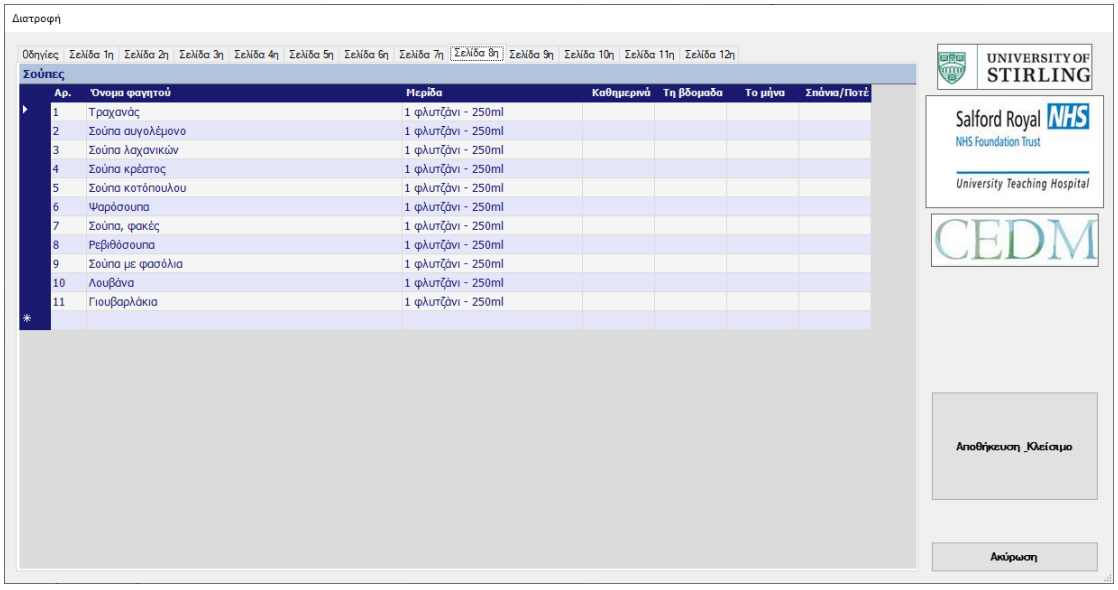
Page 6 of 12: Meat, eggs and products (Lower end)



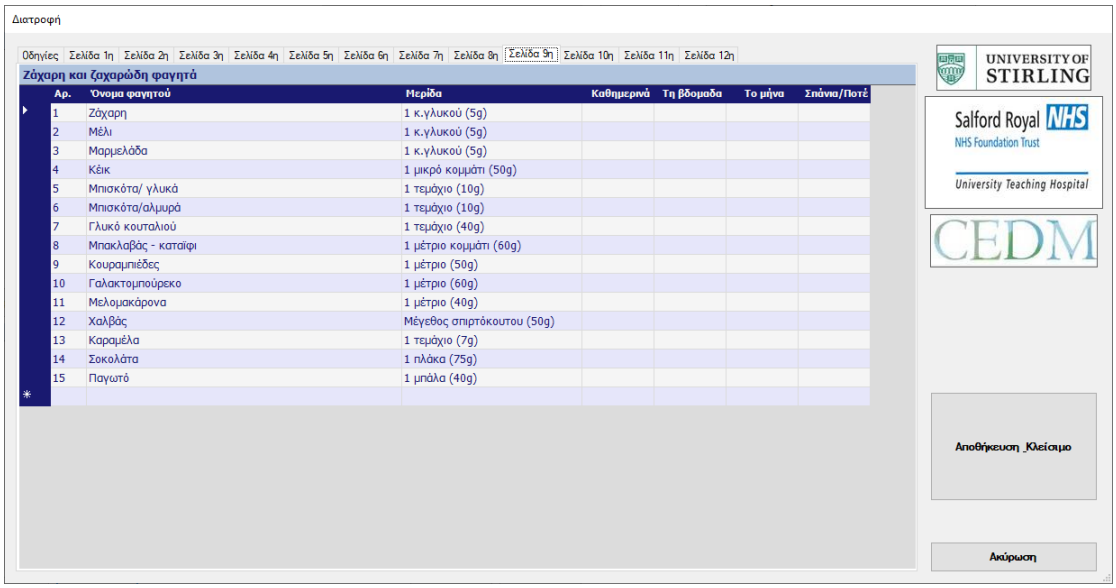
Page 7 of 12: Milk and dairy products



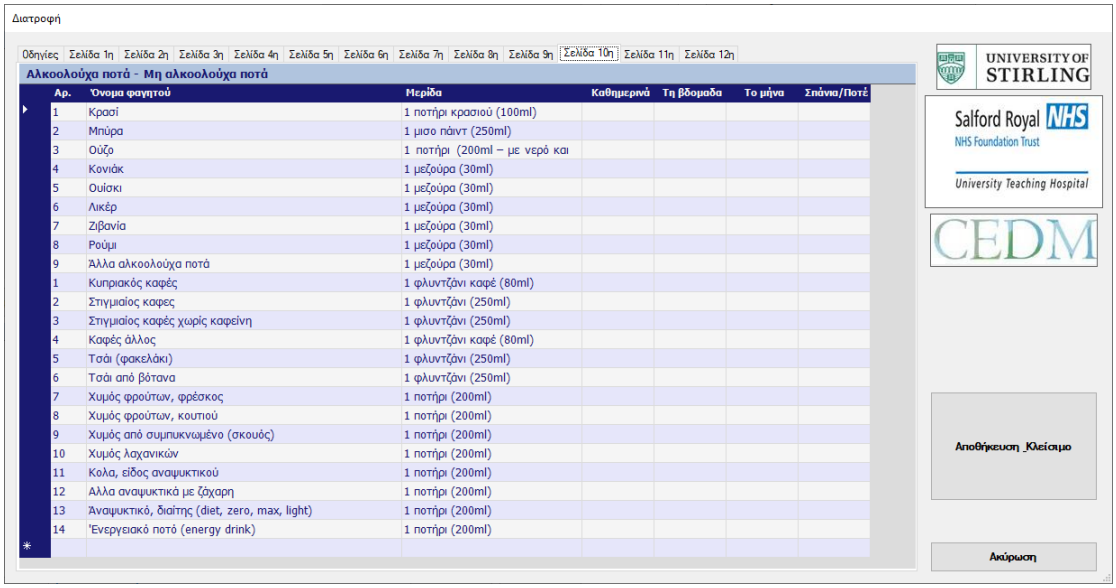
Page 8 of 12: Soups



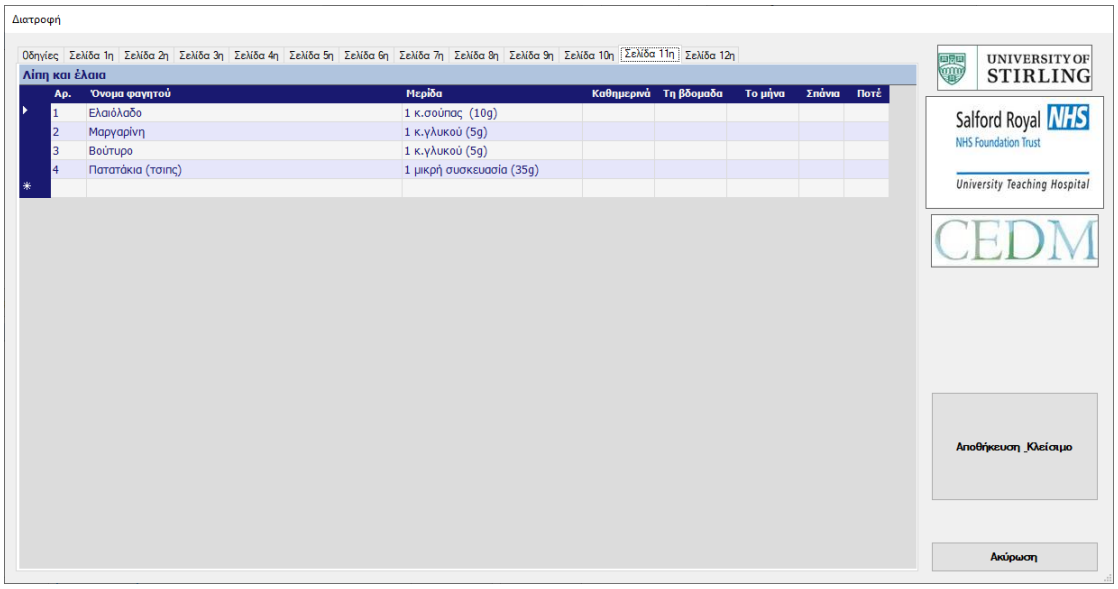
Page 9 of 12: Sugar and sugary foods



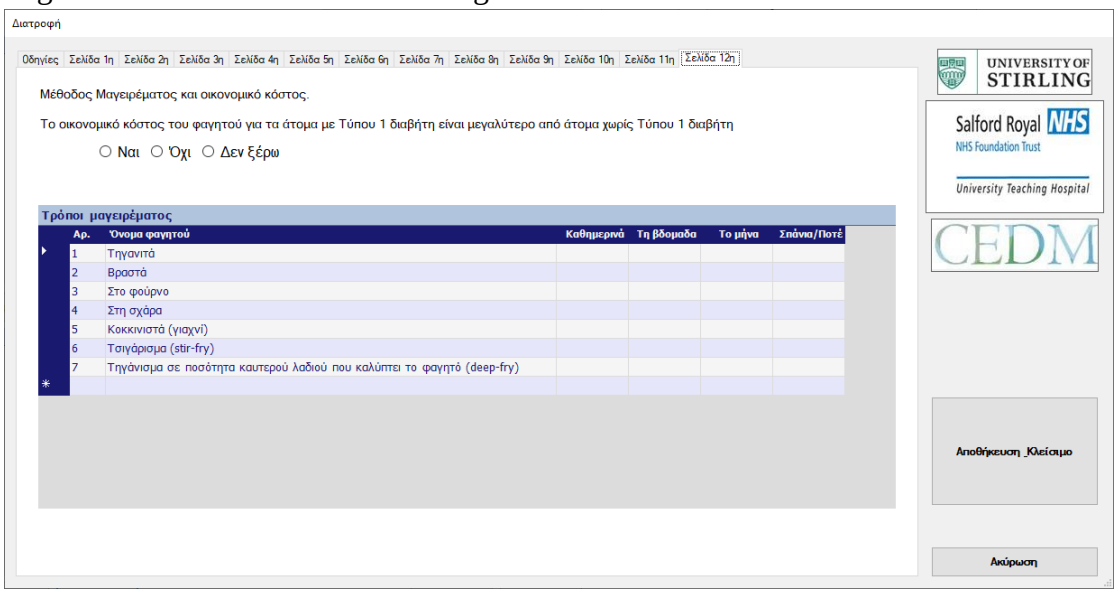
Page 10 of 12: Alcoholic and non-alcoholic beverages



Page 11 of 12: Fats and oils



Page 12 of 12: Food cost and cooking methods



Description and translation in English

The *FFQ* covered a wide range of foods and drinks, which are commonly found in Cyprus; in addition, it explored various cooking methods and included a question regarding the food cost. The translation of the questionnaire was as follows:

Instructions (Page 0)

1. Record the portions of the foods, you have consumed over the last year, as follows:

If one or more portions per day, then as 'Daily'
For example: Two portions per day, then

No.	Food name	Portion	Daily	Weekly	Monthly	Rarely/Never
1	Bread (wheat) white	1 medium slice (30g)	2			

Between one and seven portions per week, then as 'Weekly'
For example: Two portions per week, then

No.	Food name	Portion	Daily	Weekly	Monthly	Rarely/Never
1	Bread (wheat) white	1 medium slice (30g)		2		

Between one and five portions per month, then as 'Monthly'
For example: Two portions per month, then

No.	Food name	Portion	Daily	Weekly	Monthly	Rarely/Never
1	Bread (wheat) white	1 medium slice (30g)			2	

If you consume less than one portion per month, then mark the ‘Rarely/Never’
For example:

No.	Food name	Portion	Daily	Weekly	Monthly	Rarely/Never
1	Bread (wheat) white	1 medium slice (30g)				X

2. If a food has the note '(seasonal)', then report the food portion that you consume the seasonal period that the food is available

3. The portions sizes reported correspond to cooked foods with the exception of vegetables

No	Food name	Portion
	Cereal and cereal products	
1	Bread (wheat) white	1 medium slice (30g)
2	Bread (wheat) brown	1 medium slice (30g)
3	Bread (wheat) wholemeal	1 medium slice (30g)
4	Bread, rye	1 medium slice (30g)
5	Bread, corn	1 medium slice (30g)
6	Rusk, white	1 medium (45g)
7	Rusk, wholemeal	1 medium (45g)
8	Breakfast cereals, regular	¾ of a mug (30g)
9	Breakfast cereals, wholegrain	¾ of a mug (30g)
10	Breakfast cereals, with fruits	¾ of a mug (30g)
11	Breakfast cereals, chocolate	¾ of a mug (30g)
12	Pasta, all types, white	1 mug – 250ml (150g)
13	Pasta, all types, wholemeal	1 mug – 250ml (150g)
14	Rice, all types, white	1 mug – 250ml (150g)
15	Rice, all types, wholegrain	1 mug – 250ml (150g)
16	Pourgouri[a]	1 mug – 250ml (150g)
17	Orzo	1 mug – 250ml (150g)
18	Spanakorizo[b]	1 mug – 250ml (150g)
19	Gemista[c]	2 gemista (250g)
20	Koupepia[d]	5 koupepia (150g)
21	Makariona tou fournou[r]	1 medium piece (250g)
22	Moussaka[f]	1 medium piece (250g)
23	Rice pudding	1 glass - 200ml (140g)
24	Pizza	1 piece (100g)

[a]a bulgur wheat-based dish; [b]rice with spinach; [c]peppers, tomatoes and courgettes stuffed with a mixture of rice and mincemeat; [d]vine leaves stuffed with a mixture of rice and mincemeat; [e]oven-baked pasta; [f]a Greek, aubergine-based dish with mincemeat and cream.

No	Food name	Portion
	Fast foods and ready meals	
1	Fast food	1 meal
2	Ready meals (from supermarket)	1 meal
	Nuts and dried fruits	
1	Dried fruits	½ cup (60g)
2	Nuts, salted	½ cup (70g)
3	Nuts, unsalted	½ cup (70g)
	Potatoes	
1	Chips	10 pieces (50g)
2	Roasted potatoes	1 medium (80g)
3	Boiled potatoes	1 medium (80g)
4	Mashed potato	1 glass – 200ml (200g)
	Pastries	
1	Cheese pie	1 piece (250g)
2	Spinach pie	1 piece (250g)
3	Meat pie	1 piece (250g)
4	Chicken pie	1 piece (250g)

No	Food name	Portion
	Salads and vegetables	
1	Cucumber	1 medium (80g)
2	Tomato	1 medium (80g)
3	Onion	½ medium (150g)
4	Briam (aubergines, courgettes)[a]	1 mug – 250ml (180g)
5	Garlic	1 clove (3g)
6	Mushrooms	1 medium (20g)
7	Aubergine	1 medium (150g)
8	Beetroot	1 small (40g)
9	Artichoke	1 artichoke (80g)
10	Courgette	1small (120g)
11	Peppers	1 medium (130g)
12	Asparagus	2 pieces, cooked (40g)
13	Spinach	1 cup – 250ml (40g)
14	Carrots	1 medium (80g)
15	Parsley	2 sprigs (6g)
16	Spearmint	2 sprigs (6g)
17	Village salad[b]	1 mug – 250ml (100g)
18	Cauliflower	1 mug – 250ml (120g)
19	Broccoli	1 mug – 250ml (120g)
20	Skordalia[c]	1 table spoon (15g)
21	Greek salad[d]	1 mug – 250ml (100g)
22	Russian salad[e]	1 mug – 250ml (150g)
23	Swiss chard	1 mug – 250ml, cooked (80g)

[a]a Greek dish of roasted mixed vegetables; [b]vegetables only; [c]a garlic-based dish, mostly used as a dip; [d]with feta cheese; [e]also known as Olivier salad.

No	Food name	Portion
	Fruits	
1	Fruits (any)	A handful (150g)
2	Olives	5 olives (25g)
3	Lemon (on food)	1 table spoon (10g)
4	Orange	1 medium (120g)
5	Mandarin orange (seasonal)	1 medium (60g)
6	Apple	1 medium (110g)
7	Pear (seasonal)	1 medium (110g)
8	Watermelon (seasonal)	1 slice (250g)
9	Melon (seasonal)	1 slice (150g)
10	Peach (seasonal)	1 medium (140g)
11	Grapes (seasonal)	1 mug – 250ml (140g)
12	Cherries (seasonal)	1 mug – 250ml (160g)
13	Strawberries (seasonal)	5 strawberries (80g)
14	Banana	1 medium (50g)
15	Figs (seasonal)	1 medium (50g)
16	Pomegranate (seasonal)	½ mug – 250ml (50g)
17	Prickly pear (seasonal)	1 medium (100g)
18	Avocado	1 medium (150g)
19	Tinned fruit	1 table spoons (80g)
20	Tomato ketchup	1 table spoons (20g)

No	Food name	Portion
	Legumes and ladies’ fingers	
1	White beans	5 table spoons (100g)
2	Black-eyed beans	5 table spoons (100g)
3	Broad beans	5 table spoons (100g)
4	Lentils	5 table spoons (100g)
5	Peas	5 table spoons (100g)
6	Ladies' fingers	10 pieces (80g)
7	Green beans	10 pieces (30g)
	Fish and fish products	
1	Fish (any)	1 palm (100g)
2	Prawns	3 large or 10 small (15g)
3	Calamari	5 pieces (50g)
4	Tuna	1 small tin (75g)
5	Octopus	1 small ή ½ large (110g)
6	Taramasalata[a]	1 table spoon (20g)
7	Oily fish	1 palm (100g)

[a]a fish roe-based dish.

No	Food name	Portion
	Meat, eggs and products	
1	Bacon	1 slice (10g)
2	Ham	1 slice (10g)
3	Mortadella	1 slice (20g)
4	Parizaki, lunch meats	1 slice (20g)
5	Turkey, lunch meats	1 slice (30g)
6	Salami	1 slice (10g)
7	Sausages	1 piece (80g)
8	Eggs	1 medium (60g)
9	Veal and beef meat, various cooking methods	1 palm (100g)
10	Pork meat, various cooking methods	1 palm (100g)
11	Lamb meat, various cooking methods	1 palm (100g)
12	Goat meat, various cooking methods	1 palm (100g)
13	Game	1 palm (100g)
14	Liver	1 palm (100g)
15	Offal (other than liver)	1 palm (100g)
16	Chicken, various cooking methods	1 palm (100g)
17	Turkey, various cooking methods	1 palm (100g)
18	Souvlaki, chicken (meat only)[a]	1 skewer
19	Souvlaki, pork (meat only)[a]	1 skewer
20	Souvlaki, chicken, with pitta bread[a]	1 Cyprus pitta
21	Souvlaki, pork, with pitta bread[a]	1 Cyprus pitta
22	Gyros, pork [a]	1 Cyprus pitta
23	Gyros, beef [a]	1 Cyprus pitta
24	Gyros, chicken[a]	1 Cyprus pitta
25	Keftedes[b]	4 medium (100g)
26	Soutzoukakia[c]	3 pieces (100g)
27	Burger, various cooking methods	1 palm (100g)
28	Rabbit (stifado[d])	1 palm (100g)
29	Mayonnaise	1 table spoons (20g)

[a]various types of kebab, similar to the shish and the donner kebab, respectively; [b]fried meatballs; [c]a Greek dish of meatballs in tomato sauce; [d]rabbit stew.

No	Food name	Portion
	Milk and dairy products	
1	Milk, goat	1 mug – 250ml
2	Milk, full-fat	1 mug – 250ml
3	Milk, semi-skimmed	1 mug – 250ml
4	Milk, skimmed	1 mug – 250ml
5	Milk, evaporated, full-fat	1 mug – 250ml
6	Milk, evaporated, light	1 mug – 250ml
7	Milk, condensed, sweetened	1 mug – 250ml
8	Milk, chocolate	1 mug – 250ml
9	Yoghurt, full-fat	Small pot (125g)
10	Yoghurt with fruits	Small pot (125g)
11	Yoghurt, skimmed	Small pot (125g)
12	Feta cheese	Matchbox size (30g)
13	Kasseri cheese	Matchbox size (30g)
14	Anari cheese, fresh	Matchbox size (30g)
15	Anari cheese, dry	Matchbox size (30g)
16	Graviera cheese	Matchbox size (30g)
17	Kefalotyri cheese	Matchbox size (30g)
18	Halloumi cheese	Matchbox size (30g)
19	Cheese, other	Matchbox size (30g)
20	Tzatziki[a]	1 table spoon (40g)

[a]a yoghurt-based dip.

No	Food name	Portion
	Soups	
1	Trahanas soup[a]	1 mug – 250ml
2	Avgolemono[b]	1 mug – 250ml
3	Vegetable soup	1 mug – 250ml
4	Meat soup	1 mug – 250ml
5	Chicken soup	1 mug – 250ml
6	Fish soup	1 mug – 250ml
7	Lentil soup	1 mug – 250ml
8	Chickpea soup	1 mug – 250ml
9	Bean soup	1 mug – 250ml
10	Louvana[c]	1 mug – 250ml
11	Giouvarlakia soup[d]	1 mug – 250ml

[a]a grain and yoghurt-based soup; [b]a rice, eggs and lemon-based soup; [c]a yellow split pea-based soup [d]a soup with meatballs.

No	Food name	Portion
	Sugar and sugary foods	
1	Sugar	1 tea spoon (5g)
2	Honey	1 tea spoon (5g)
3	Marmalade	1 tea spoon (5g)
4	Cake	1 small piece (50g)
5	Biscuits, sweet	1 piece (10g)
6	Biscuits, salty	1 piece (10g)
7	Cyprus spoon sweets[a]	1 piece (40g)
8	Baklava and kataifi desserts	1 medium piece (60g)
9	Kourabiedes[b]	1 medium (50g)
10	Galaktoboureko[c]	1 medium (60g)
11	Melomakarona[d]	1 medium (40g)
12	Halva[e]	Matchbox size (50g)
13	Candies	1 piece (7g)
14	Chocolate	1 bar (75g)
15	Ice cream	1 scoop (40g)

[a]a traditional sweet fruit, vegetable or peel preserve; [b]almond-based sweet biscuits; [c]a custard-based sweet; [d]honey-based sweet biscuits; [e]tahini (sesame)-based dessert.

No	Food name	Portion
	Alcoholic drinks	
1	Wine	1 regular wine glass (100 ml)
2	Beer	1 pint (250 ml)
3	Ouzo	1 glass (200 ml – with water and ice)
4	Brandy	1 pub measure (30 ml)
5	Whiskey	1 pub measure (30 ml)
6	Liquor	1 pub measure (30 ml)
7	Zivania[a]	1 pub measure (30 ml)
8	Rum	1 pub measure (30 ml)
9	Alcoholic drinks, other	1 pub measure (30 ml)
	Non-alcoholic beverages	
1	Coffee, Cypriot[b]	1 coffee cup (80ml)
2	Coffee, instant	1 mug (250ml)
3	Coffee, decaffeinated	1 mug (250ml)
4	Coffee, other	1 coffee cup (80ml)
5	Tea (instant)	1 mug (250ml)
6	Tea, herbal infusion	1 mug (250ml)
7	Fruit juice, fresh	1 glass (200ml)
8	Fruit juice, packaged	1 glass (200ml)
9	Juice made from squash	1 glass (200ml)
10	Vegetable juice	1 glass (200ml)
11	Cola-type soft drinks	1 glass (200ml)
12	Other type soft drinks with sugar	1 glass (200ml)
13	Soft drinks, diet (diet, zero, max, light)	1 glass (200ml)
14	Energy drinks	1 glass (200ml)

[a]a traditional, grape pomace-based spirit; [b]a traditional unfiltered coffee

No	Food name	Portion
	Fats and oils	
1	Olive oil	1 table spoon (10g)
2	Margarine spread	1 tea spoon (5g)
3	Butter	1 tea spoon (5g)
4	Crisps	1 small packet (35g)

Cooking methods and financial cost

The financial cost of food for people with Type 1 diabetes is higher than for people without Type 1 diabetes

Yes | *No* | *I don't know*

No	Food name	
	Cooking methods	
1	Frying	
2	Boiling	
3	Roasting	
4	Grilling	
5	Yiachni[a]	
6	Stir-frying	
7	Deep-frying	

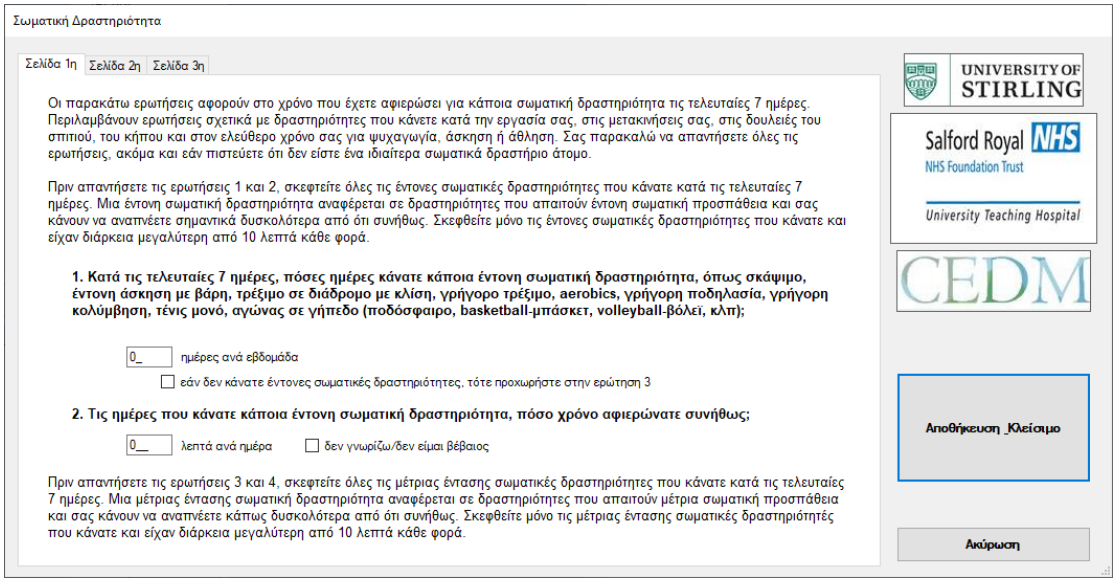
[a]Stewed with tomatoes and onions.

Notes: Note that, in the FFQ we did not make a distinction between the words portion and serving since the participants were unlikely to recognise the difference and furthermore, this could have caused them some confusion. The *seasonal* term was used since some foods, especially some locally produced fruits and vegetables, are mostly consumed seasonally. Also note that, although some further explanations for local and traditional foods were provided (subscriptions), these notes were not intended to be the food recipes of the foods described or to provide a full list of the ingredients contained in these foods. The categories of *rarely* and *never* are distinct for the *fats and oils* food category that is the olive oil, margarine spread, butter and crisps, as shown in *Page 11 of 12: Oils and products*. This was necessary due to the distinction of the two categories for the olive oil in the *MedDietScore* scoring system.

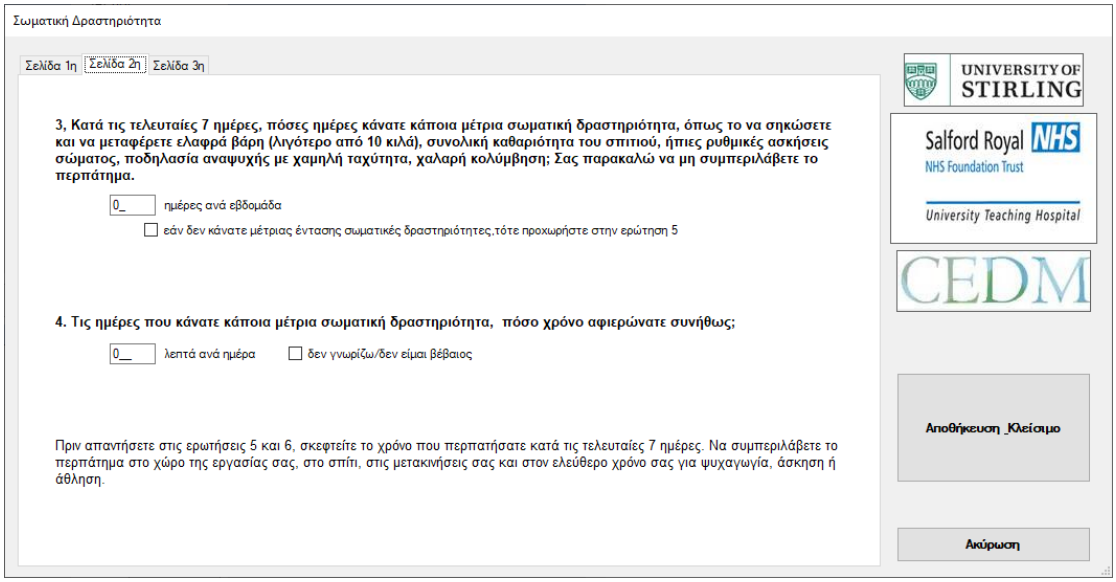
Physical Activity Questionnaire

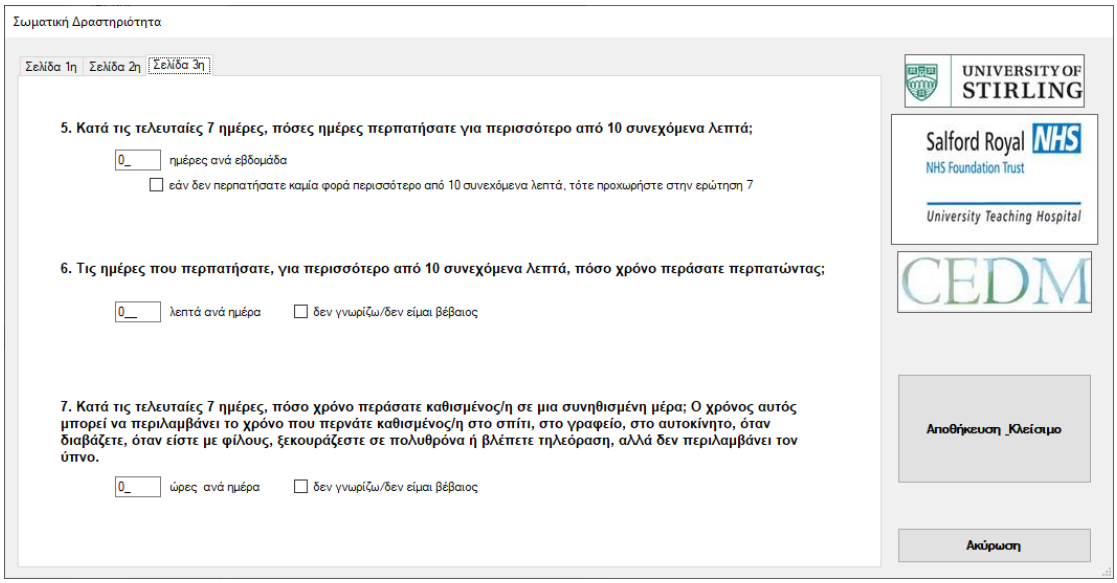
Electronic format (in Greek language)

Page 1 of 3



Page 2 of 3




Σωματική Δραστηριότητα
Σελίδα 1η
Σελίδα 2η
Σελίδα 3η
5. Κατά τις τελευταίες 7 ημέρες, πόσες ημέρες περπατήσατε για περισσότερο από 10 συνεχόμενα λεπτά;
0_
ημέρες ανά εβδομάδα
εάν δεν περπατήσατε καμία φορά περισσότερο από 10 συνεχόμενα λεπτά, τότε προχωρήστε στην ερώτηση 7
6. Τις ημέρες που περπατήσατε, για περισσότερο από 10 συνεχόμενα λεπτά, πόσο χρόνο περάσατε περπατώντας;
0__
λεπτά ανά ημέρα
δεν γνωρίζω/δεν είμαι βέβαιος
7. Κατά τις τελευταίες 7 ημέρες, πόσο χρόνο περάσατε καθισμένος/η σε μια συνηθισμένη μέρα; Ο χρόνος αυτός μπορεί να περιλαμβάνει το χρόνο που περνάτε καθισμένος/η στο σπίτι, στο γραφείο, στο αυτοκίνητο, όταν διαβάζετε, όταν είστε με φίλους, ξεκουράζεστε σε πολυθρόνα ή βλέπετε τηλεόραση, αλλά δεν περιλαμβάνει τον ύπνο.
0_
ώρες ανά ημέρα
δεν γνωρίζω/δεν είμαι βέβαιος
UNIVERSITY OF STIRLING
Salford Royal NHS
NHS Foundation Trust
University Teaching Hospital
CEDM
Αποθήκευση _Κλείσιμο
Ακύρωση

Description and translation in English

The current questionnaire on physical activity is based on the available official translation in the Greek language (Papathanasiou *et al.*, 2009) of the widely-used, short-version IPAQ questionnaire (Craig *et al.*, 2003). The translation presented here is based on the original English version of the IPAQ (Craig *et al.*, 2003) with minor changes, mainly in the presentation structure, in order to match the electronic version of our questionnaire; this was as follows:

[Page 1]

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. **During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?**

   0 days per week

   ☐ no vigorous physical activities → skip to question 3

2. **How much time did you usually spend doing vigorous physical activities on one of those days?**

   0 minutes per day ☐ don't know/not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

[Page 2]

3. **During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.**

   0 days per week

   ☐ no moderate physical activities → skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

   0 minutes per day ☐ don't know/not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

[Page 3]

5. **During the last 7 days, on how many days did you walk for at least 10 minutes at a time?**

   0 days per week

   ☐ no walking → skip to question 7

6. **How much time did you usually spend walking on one of those days?**

   0 minutes per day ☐ don't know/not sure

7. **The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television. During the last 7 days, how much time did you spend sitting on a week day?**

   0 hours per day ☐ don't know/not sure

Notes: The default answer was *0* and could be changed accordingly.

Algorithm

The algorithm was split into four parts, namely *standardisation of servings (of individual foods), total number of servings of (individual) MedDietScore components, scoring of servings* and *total MedDietScore score*. This is an updated version of the algorithm presented previously (*Appendix: Chapter 4*)

Standardisation of servings (of individual foods): Initially, the reported number of servings – that one serving is equal to one pre-defined portion of the respective food – are all converted into number of servings per month; this allows us, to produce standardised data that can be further processed. The algorithm, for an *i* participant, is as follows:

if n_{di} servings reported, then $n_{mi} = 28 \, . \, n_{di}$
if n_{wi} servings reported, then $n_{mi} = 4 \, . \, n_{wi}$
if n_{mi} servings reported, then $n_{mi} = 1 \, . \, n_{mi}$
if n_i servings in rarely/never† column reported or if $n_{mi} < 1$, then $n_{mi} = 0 \, . \, n_{(m)i}$

where: n_{di} = number of servings (of the pre-defined portion of a food) reported to be consumed daily; n_{wi} = number of servings reported to be consumed weekly; n_{mi} = number of servings reported to be consumed per month; †for the purpose of the olive oil, the rarely and never are distinct entities and are scored separately, for more see following sections.

Total number of servings of (individual) MedDietScore components: This section has been updated to reflect the unpublished protocol provided by the authors of the *MedDietScore* scoring system. Note that the algorithm is a weighted summation of individual foods (in contrast to unweighted, previously) and that some foods have been added or removed. For each food group and for the *i* participant, the calculations were as follow:

Non-refined cereals $servings_i$ = no of (*bread, wheat, wholemeal*: 1 medium slice, 30g portion) $servings_i$ + no of (*rusk, wholemeal*: 1 medium, 45g portion) $servings_i$ + no of (*breakfast cereals, wholegrain*: ¾ of a mug, 30g portion) $servings_i$ + [no of (*pasta, all types, wholemeal*: 1 mug, 250ml, 150g portion) $servings_i$ * 2] + [no of (*rice, all types, brown, wholegrain*: 1 mug, 250ml, 150g portion) $servings_i$ * 2]

Potatoes $servings_i$ = no of (*chips*: 10 pieces, 50g portion) $servings_i$ + no of (*roasted potatoes*: 1 medium, 80g portion) $servings_i$ + no of (*boiled potatoes*: 1 medium, 80g portion) $servings_i$ + no of (*mashed potato*: 1 glass, 200ml, 200g portion) $servings_i$

Fruits $servings_i$ = no of (*fruits, any*: 1 handful, 150g portion) $servings_i$ + [no of (*fruit juice, fresh*: 1 glass, 200ml) $servings_i$ * 2]

Vegetables $servings_i$ = no of (*cucumber*: 1 medium, 80g portion) $servings_i$ + no of (*tomato*: 1 medium, 80g portion) $servings_i$ + no of (*onion*: ½ medium, 150g portion) $servings_i$ + [no of (*briam, mixed vegetable dish*: 1 cup, 250ml, 180g portion) $servings_i$ * 2] + [no of (*garlic*: 1 glove, 3g portion) $servings_i$ / 30] + [no of (*aubergines*: 1 medium, 150g portion) $servings_i$ * 2] + [no of (*beetroots*: 1 small, 40g portion) $servings_i$ / 2] + no of (*artichokes*: 1 artichoke, 80g portion) $servings_i$ + no of (*courgette*: 1 small, 120g portion) $servings_i$ + no of (*peppers*: 1 medium, 130g portion) $servings_i$ + [no of (*asparagus*: 2 cooked asparagus, 40g portion) $servings_i$ / 2] + no of (*spinach*: 1 cup, 250ml, 40g portion) $servings_i$ + no of (*carrots*: 1 medium, 80g portion) $servings_i$ + [no of (*parsley*: 2 springs, 6g portion) $servings_i$ / 15] + [no of (*spearmint*: 2 springs, 6g portion) $servings_i$ / 15] + no of (*village salad, vegetables only*: 1 cup, 250ml, 80g portion) $servings_i$ + no of (*cauliflower*: 1 cup, 250ml, 120g portion) $servings_i$ + no of (*Broccoli*: 1 cup, 250ml, 120g portion) $servings_i$ + no of (*Greek salad*: 1 cup, 250ml, 100g portion) $servings_i$ + no of (*Russian salad*: 1 cup, 250ml, 150g portion) $servings_i$ + no of (*greens, wild, cooked*: 1 cup, 250ml, 80g portion) $servings_i$ + no of (*peas*: 5 table spoons, 100g portion) $servings_i$ + no of (*ladies' fingers*: 10 pieces, 80g portion) $servings_i$ + [no of (*green beans*: 10 pieces, 30g portion) $servings_i$ / 3]

Legumes $servings_i$ = no of (*white kidney beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*black eye beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*broad beans*: 5 table spoons, 100g portion) $servings_i$ + no of (*lentils*: 5 table spoons, 100g portion) $servings_i$

Fish $servings_i$ = no of (*fish, any*: 1 palm, 100g portion) $servings_i$ + [no of (*fish soup*: 1 mug, 250ml) $servings_i$ / 3]

Red meat and products $servings_i$ = [no of (*bacon*: 1 slice, 10g portion) $servings_i$ / 10] + [no of (*ham*: 1 slice, 10g portion) $servings_i$ / 10] + [no of (*mortadella*: 1 slice, 20g portion) $servings_i$ /5] + [no of (*parizaki*, cooked salami: 1 slice, 20g portion) $servings_i$ / 5] + [no of (*salami*: 1 slice, 10g portion) $servings_i$ / 10] + no of (*sausages*, 1 piece, 80g portion) $servings_i$ + no of (*veal and beef meat, various ways of cooking*: 1 palm, 100g portion) $servings_i$ + no of *(pork meat, various ways of cooking*: 1 palm, 100g portion) $servings_i$ + no of (*lamb meat, various ways of cooking*: 1 palm, 100g portion) $servings_i$ + no of (*goat*

meat, various ways of cooking: 1 palm, 100g portion) $servings_i$ + no of (*liver*: 1 palm, 100g portion) $servings_i$ + no of (*offal, other than liver*: 1 palm, 100g portion) $servings_i$ + no of (*souvlaki, pork, meat only*: 1 skewer portion) $servings_i$ + [no of (*souvlaki, pork, with pitta bread*: 1 Cyprus pitta bread portion) $servings_i$ * 2] + [no of (*kebab, pork*: 1 Cyprus pitta bread portion) $servings_i$ * 2] + [no of (*Kebab, beef*: Cyprus pitta portion) $servings_i$ * 2] + no of (*keftedes, meatballs*: 4 medium, 100g portion) $servings_i$ + no of (*soutsoukakia, meatballs in tomato sauce*: 3 pieces, 100g portion) $servings_i$ + no of (*burger, various ways of cooking*: 1 palm, 100g portion) $servings_i$

Poultry $servings_i$ = [no of (*turkey,* cold cut, 1 slice, 30g portion) $servings_i$ / 3] + no of (*game*: 1 palm, 100g portion) $servings_i$ + no of (*chicken, various ways of cooking*: 1 palm, 100g portion) $servings_i$ + no of (*turkey, various ways of cooking*: 1 palm, 100g portion) $servings_i$ + no of (*souvlaki, chicken, meat only*: 1 skewer portion) $servings_i$ + [no of (*souvlaki, chicken, with pitta bread*: 1 Cyprus pitta bread portion) $servings_i$ * 2] + [of (*kebab, chicken*: 1 Cyprus pitta bread portion) $servings_i$ * 2] + no of (*stifado, rabbit*: 1 palm, 100g portion) $servings_i$

Full fat dairy products $servings_i$ = no of (*milk, full-fat*: 1 mug, 250ml portion) $servings_i$ + no of (*milk, condensed, sweetened*: 1 mug, 250ml portion) $servings_i$ + no of (*milk, chocolate flavoured*: 1 mug, 250ml portion) $servings_i$ + [no of (*yoghurt, full fat*: small pot, 125g portion) $servings_i$ * 2/3] + [no of (*yoghurt with fruits*: small pot, 125g portion) $servings_i$ * 2/3] + no of (*feta cheese*: matchbox size, 30g portion) $servings_i$ + no of (*kasseri cheese*: matchbox size, 30g portion) $servings_i$ + no of (*anari cheese, fresh*: matchbox size, 30g portion) $servings_i$ + no of (*anari cheese, dry*: matchbox size, 30g portion) $servings_i$ + no of (*graviera cheese*: matchbox size, 30g portion) $servings_i$ + no of (*kefalotyri cheese*: matchbox size, 30g portion) $servings_i$ + no of (*halloumi cheese*, matchbox size, 30g portion) $servings_i$ + no of (*cheese, other*: matchbox size, 30g portion) $servings_i$ + [no of (*tzatziki, yogurt and garlic*: 1 table spoon, 40g portion) $servings_i$ /4]

Use of olive oil in cooking $servings_i$ = max (never, rarely, no of [*Olive* oil: 1 table spoon, 10g portion) $servings_i^{\dagger}$

Alcoholic beverages $servings_i^{\dagger\dagger}$ = no of (*wine*: 1 regular wine glass, 100ml portion) $servings_i$ + no of (*beer*: 1 pint, 250ml portion) $servings_i$ + no of (*ouzo, with water and ice*: 1 glass, 200ml portion) $servings_i$ + no of (*brandy*: 1 pub measure, 30ml portion) $servings_i$ + no of (*whiskey*: 1 pub measure, 30ml portion) $servings_i$ + no of (*liquor*: 1 pub measure,

30ml portion) servings$_i$ + no of (*zivania*: 1 pub measure, 30ml portion) servings$_i$ + no of (*rum*: 1 pub measure, 30ml portion) servings$_i$ + no of (*alcoholic beverages, other*: 1 pub measure, 30ml portion) servings$_i$

where: no = number; 1 serving = the pre-defined food portion (in the FFQ) in household measures or grams; no of servings = the number of serving per month reported by the participants; †max = maximum of the function; never < rarely < 1 portion per month, where, they can be broadly defined as never = 0, rarely → 0; for the actual methods on olive oil see algorithms below; ††The portion size of alcoholic drinks (in the FFQ) was chosen to approximately match 12g of alcohol, which was the portion size required by the *MedDietScore* scoring system and at the same time provided meaningful portions for the participants (in Cyprus).

Scoring of servings: The details of *MedDietScore* scoring system have been discussed before, including the edits on the original score – the final version is shown in *Table 5.4*. Here, the actual algorithm is presented, for an *i* participant, and is sub-dived in two steps. The first step is needed to standardize the servings consumed with the scoring system, whereas the second step is the actual scoring of the serving using the *adapted MedDietScore* scoring system.

Step 1: The servings are converted to weekly or daily

$s_{mi\,/\,4} = S_{wi}$ for all food groups (as calculated previously) other than alcoholic beverages
$s_{mi\,/\,28} = S_{di}$ for alcoholic beverages (as calculated previously) only

where: s_{mi} = servings per month; S_{wi} = servings per week; S_{di} = servings per day

Step 2

$f_s : S_{wi}$ or $f_s : S_{di}$ → as per *MedDieScore*, then

where f_s is a function, based on the *MedDietScore* scoring system, with S_{wi} or S_{di} (the input) that is the servings for the particular component or food group; in practise, $f_s(S_{wi})$ or $f_s(S_{di})$ is the score, measured in points (range 0 – 5), for each of the component or food group – shown below:

Non-refined cereals score: $f_c(S_w)$

if $S_{wi} < 1$, then $f_c(S_w) = 0$, if $1 \leq S_{wi} < 7$ then $f_c(S_w) = 1$, if $7 \leq S_{wi} < 13$ then $f_c(S_w) = 2$, if $13 \leq S_{wi} < 19$ then $f_c(S_w) = 3$, if $19 \leq S_{wi} \leq 32$ then $f_c(S_w) = 4$, if $S_{wi} > 32$ then $f_c(S_w) = 5$

Ptatoes score: $f_p(S_w)$

if $S_{wi} < 1$, then $f_p(S_w) = 0$, if $1 \leq S_{wi} < 5$ then $f_p(S_w) = 1$, if $5 \leq S_{wi} < 9$ then $f_p(S_w) = 2$, if $9 \leq S_{wi} < 13$ then $f_p(S_w) = 3$, if $13 \leq S_{wi} \leq 18$ then $f_p(S_w) = 4$, if $S_{wi} > 18$ then $f_p(S_w) = 5$

Fruits score: $f_f(S_w)$

if $S_{wi} < 1$, then $f_f(S_w) = 0$, if $1 \leq S_{wi} < 5$ then $f_f(S_w) = 1$, if $5 \leq S_{wi} < 9$ then $f_f(S_w) = 2$, if $9 \leq S_{wi} < 16$ then $f_f(S_w) = 3$, if $16 \leq S_{wi} \leq 22$ then $f_f(S_w) = 4$, if $S_{wi} > 22$ then $f_f(S_w) = 5$

Vegetables score: $f_v(S_w)$

if $S_{wi} < 1$, then $f_v(S_w) = 0$, if $1 \leq S_{wi} < 7$ then $f_v(S_w) = 1$, if $7 \leq S_{wi} < 13$ then $f_v(S_w) = 2$, if $13 \leq S_{wi} < 21$ then $f_v(S_w) = 3$, if $21 \leq S_{wi} \leq 33$ then $f_v(S_w) = 4$, if $S_{wi} > 33$ then $f_v(S_w) = 5$

Legumes score: $f_l(S_w)$

if $S_{wi} = 0$, then $f_l(S_w) = 0$, if $0 < S_{wi} < 1$ then $f_l(S_w) = 1$, if $1 \leq S_{wi} < 3$ then $f_l(S_w) = 2$, if $3 \leq S_{wi} < 5$ then $f_l(S_w) = 3$, if $5 \leq S_{wi} \leq 6$ then $f_l(S_w) = 4$, if $S_{wi} > 6$ then $f_l(S_w) = 5$

Fish score: $f_h(S_w)$

if $S_{wi} = 0$, then $f_h(S_w) = 0$, if $0 < S_{wi} < 1$ then $f_h(S_w) = 1$, if $1 \leq S_{wi} < 3$ then $f_h(S_w) = 2$, if $3 \leq S_{wi} < 5$ then $f_h(S_w) = 3$, if $5 \leq S_{wi} \leq 6$ then $f_h(S_w) = 4$, if $S_{wi} > 6$ then $f_h(S_w) = 5$

Red meat and products score: $f_r(S_w)$

if $S_{wi} > 10$, then $f_r(S_w) = 0$, if $8 \leq S_{wi} \leq 10$ then $f_r(S_w) = 1$, if $6 \leq S_{wi} < 8$ then $f_r(S_w) = 2$, if $4 \leq S_{wi} < 6$ then $f_r(S_w) = 3$, if $2 \leq S_{wi} < 4$ then $f_r(S_w) = 4$, if $S_{wi} < 2$ then $f_r(S_w) = 5$

Poultry score: $f_y(S_w)$

if $S_{wi} > 10$, then $f_y(S_w) = 0$, if $9 \leq S_{wi} \leq 10$ then $f_y(S_w) = 1$, if $7 \leq S_{wi} < 9$ then $f_y(S_w) = 2$, if $5 \leq S_{wi} < 7$ then $f_y(S_w) = 3$, if $4 \leq S_{wi} < 5$ then $f_y(S_w) = 4$, if $S_{wi} < 4$ then $f_y(S_w) = 5$

Full fat dairy products score: $f_d(S_w)$

if $S_{wi} > 30$, then $f_d(S_w) = 0$, if $29 \leq S_{wi} \leq 30$ then $f_d(S_w) = 1$, if $21 \leq S_{wi} < 29$ then $f_d(S_w) = 2$, if $16 \leq S_{wi} < 21$ then $f_d(S_w) = 3$, if $11 \leq S_{wi} < 16$ then $f_d(S_w) = 4$, if $S_{wi} < 11$ then $f_d(S_w) = 5$

Olive oil score: $f_o(S_w)$

To solve the problem of never and rarely as both couldn't be = 0, and → 0 can't be used in an algorithm – extra columns were created, named for the current purpose as *12a* and *12b,* and were as follows:

If never then *12a* = 1, otherwise *12a* = 0
If rarely then *12b* = 1, otherwise *12b* = 0

Therefore, the algorithm was as follows:

if $S_{wi} = 0$ and *12a* > 0, then $f_o(S_w) = 0$, if $S_{wi} = 0$ and *12b* > 0, then $f_o(S_w) = 1$, if $0 < S_{wi} < 1$ then $f_o(S_w) = 2$, if $1 \leq S_{wi} \leq 3$ then $f_d(S_w) = 3$, if $3 < S_{wi} < 7$ then $f_d(S_w) = 4$, if $S_{wi} \geq 7$ then $f_d(S_w) = 5$

Alcoholic beverages score: $f_a(S_d)$

Note that 1 serving equals approximately 12g of alcohol or 100ml of a regular wine; furthermore, note the polytonic nature of the scoring function for alcoholic beverages and the use of S_{di}.

if $S_{di} > 7$, then $f_a(S_d) = 0$, if $6 \leq S_{di} \leq 7$ then $f_a(S_d) = 1$, if $5 \leq S_{di} < 6$ then $f_a(S_d) = 2$, if $4 \leq S_{di} < 5$ then $f_a(S_d) = 3$, if $3 \leq S_{di} < 4$ then $f_a(S_d) = 4$, if $0 < S_{di} < 3$ then $f_a(S_d) = 5$, if $S_{di} = 0$ then $f_d(S_d) = 0$

Total MedDietScore score: The total *MedDietScore* score algorithm was as follows, for an *i* participant:

Total *MedDietScore* score (points; range 0 – 55) = $f_{ci} + f_{pi} + f_{fi} + f_{vi} + f_{li} + f_{hi} + f_{ri} + f_{yi} + f_{di} + f_{oi} + f_{ai}$

where: f_{ci} = non refined cereals score, f_{pi} = potatoes score, f_{fi} = fruits score, f_{vi} = vegetables score, f_{li} = legumes score, f_{hi} = fish score, f_{ri} = red meat and products score, f_{yi} = poultry score, f_{di} = full dairy products, f_{oi} = olive oil score, f_{ai} = alcoholic beverages score.

Statistics methodology – Distribution, outliers and transformations (extended)

Distribution and normality

The distribution of variables and residuals (e) was examined using the following methods:

Moments (skewness and kurtosis) and other descriptive statistics

Moments of skewness and kurtosis: The sample skewness calculation – as defined by the third moment – indicated a positive or a right skew if it was above zero and a negative or a left skew if it was below zero (Daniel and Cross, 2013). More precisely, the sample skewness calculation was defined as indicating an approximately symmetric distribution (i.e., no skew) if the third moment was less than | 0.5 |, a moderately skewed distribution if it was between | 0.5 | and | 1.0 |, and highly skewed if it was above | 1.0 | (Bulmer, 1979). In the current project, the sample excess kurtosis – as defined by the fourth moment – was used, which is equal to the moment of kurtosis minus three. Excess kurtosis calculation indicated a leptokurtic distribution if it was above zero, a platykurtic distribution if it was below zero and a mesokurtic distribution if it was approaching zero (Daniel and Cross, 2013). Note that the moments of skewness and kurtosis have significant limitations and their usefulness has been long debated (Cox, 2010), hence they were used with caution in this project.

Trimmed means: A less formal method to examine whether a sample distribution is symmetrical, is to compare the value of mean to that of trimmed means (Rosner, 2016). The trimmed means that were used in the current study were the 50% trimmed mean, which (as a limiting case) is essentially the median (after leaving one or two values behind required to determine the median), and the 10% trimmed mean, which is based on a less radically trimmed method and thus it retains some of the characteristics of the original (i.e., untrimmed) sample distribution (Hamilton, 1991; Rosner, 2016; Cox, 2013). In a perfectly symmetrical distribution, the mean, the 10% and the 50% trimmed mean should meet (i.e., have similar values). At the same time, a higher mean value indicated a positive skew and a lower mean value indicated a negative skew. The advantage of the trimmed means was that they are more resistant to outliers that otherwise could substantially inflate the calculation of moments of skewness and kurtosis (Hamilton, 1991; StataCorp LLC, 2019a).

Shapiro-Wilk W test for normality

The Shapiro-Wilk W test for normality was favoured in the current project over other related tests, such as the Kolmogorov-Smirnov test. The reason was that the Shapiro-Wilk W test seems to outperform (i.e., provides more power) other comparable tests, especially when the sample size is around 100 participants or less (Razali and Wah, 2011; Ghasemi and Zahediasl, 2012; Kaltenbach, 2012; StataCorp LLC, 2019a); and it is the recommended test for non-aggregated data or ungrouped data (StataCorp LLC, 2019a). The H_0 of the Shapiro-Wilk W was that the examined sample derives from a normal distribution (Ghasemi and Zahediasl, 2012; StataCorp LLC, 2019a).

Histogram

The results of the Shapiro-Wilk W test (or any other comparable test) for normality, should be confirmed by a graphical method and therefore I also used the histogram (Miller and Brown, 1997; Rosner, 2016; StataCorp LLC, 2019a). The histogram was exported with the addition of an appropriately scaled normal density, which had the same mean and standard deviation as the sample data, in order to aid with the visual evaluation of the assumption of normality.

Outliers

Outliers, especially severe outliers, have a profound impact on distributions and normality (Rosner, 2016) and are discussed separately.

Skewness and kurtosis test for normality

If the normal distribution of a sample could not be assumed or was in doubt, the skewness and kurtosis test for normality was used to determine the source of the problem. Note that the skewness and kurtosis test is not advocated to be used as the primary test for testing for normality. Nevertheless, the distribution could be assumed to approximately normal (H_o) if the *p*-value < 0.05, while the assumption of normality could not be rejected if the *p*-value ≥ 0.05 (StataCorp LLC, 2019a).

Q-Q and P-P diagnostic plots (for residuals only)

In the cases of ANOVA or regression, the e rather than the actual variables were inspected for the assumption of normality. An e_i is the distance between the fitted and the predicted value and corresponds to the more theoretical value of error term (ε_i) (Miller and Brown, 1997; Daniel and Cross, 2013; Rosner, 2016). The distribution of the residuals was

examined through the methods described in the current section but also through the Q-Q and P-P diagnostic plots, as is recommended by several sources (Miller and Brown, 1997; Bewick, Cheek and Ball, 2003, 2004; StataCorp LLC, 2019a). The diagnostic plot used was the Quantile-normal (Q-Q) plot (i.e., the quantiles of the variable were plotted against the quantiles of the normal distribution), which was accompanied by the normal-probability (P-P) plot (i.e., a standardized normal probability plot), although not always. The reason for the use of the Quantile-normal plot was that it emphasises the tails of the distribution, that is, the part of the distribution that the other tests and methods are more likely to neglect; in contrast, the P-P plots place the focus on the centre of the distribution (Miller and Brown, 1997; StataCorp LLC, 2019a). Furthermore, although the histogram was also employed as a visual method, it has the disadvantage that it requires the imputation of an arbitrary bin width and origin and the data are aggregated into bins, therefore it is more difficult to identify individual points that do not feed the data (Cox, 2005). The normal plots were used only for the e_i and not for the rest of the variables; this is due to the fact that the statistical tests employed in the current project, other than the regression and the ANOVA such as, the *t*-test, tend to be slightly more resistant to small deviation from normality (Whitley and Ball, 2002).

Treatment

In the case of variables or residuals that could not be assumed to have a normal distribution, some sort of action was required. The methods of data curation were similar to those described in the *outliers* section. If a variable was transformed, then the resulting variable's distribution was re-examined, as is described in the section below.

Outliers

Univariate: All variables were inspected for the presence of outliers. The data were visually examined for outliers using a boxplot graph; and, thereafter, the number and type of outliers were identified using the STATA community-contributed *iqr* command. Outliers were defined as the values that fall below or above the lower and upper bound, respectively. The lower and upper bound were defined as follows (Hamilton, 1991; Rosner, 2016):

Lower bound = Q_1 - 1.5 * IQR

Upper bound = Q_3 + 1.5 * IQR

where the IQR = $Q_3 - Q_1$, the $Q_3 = 75^{th}$ percentile and the $Q_1 = 25^{th}$ percentile.

Once the outliers were identified, they were further classified into mild outliers (inner fences) or severe outliers (outer fences). The inner and outer fence outliers were defined as follows (Hamilton, 1991; Rosner, 2016):

Mild outlier = $Q_1 - 3 * IQR \leq x < Q_1 - 1.5 * IQR$ (lower bound) or

$Q_3 + 1.5 * IQR < x \leq Q_3 + 3 * IQR$ (upper bound)

Severe outlier = $x < Q_1 - 3 * IQR$ (lower bound) or

$x > Q_3 + 3 * IQR$ (upper bound)

where the $IQR = Q_3 - Q_1$, the $Q_3 = 75^{th}$ percentile and the $Q_1 = 25^{th}$ percentile.

Bivariate (for Pearson product-moment correlation only): In addition to the univariate absence of outliers, the bivariate absence of outliers – and leverage points – was also required for the Pearson product-moment correlation as they can potentially have a profound effect on the estimates. The presence of extreme observation was explored through a scatterplot of the two variables that were compared, in combination with the community-contributed *BACON* command (Billor, Hadi and Velleman, 2000; Weber, 2010). Once the *BACON* command was run, thereafter the observations were plotted on a scatterplot as 0s and 1s, which indicated the *BACON* proposed regular observations and potential extreme values, respectively. Default settings were used for the *bacon* command including the *p(0.15)* i.e. the 0.85 percentile of the χ^2 distribution to be used as a threshold to separate potential outliers (Weber, 2010).

Residuals (for OLS regression only): This is discussed elsewhere (*Ordinary least-squares (OLS) linear regression*).

Treatment: Initially, the identified outliers were examined for possible typographical errors during data cleaning/curation or for a potential incorrect insertion by the participant. The erroneous data were to be corrected – if the correct value was available – otherwise, the observation was to be excluded and clearly labelled as '# observation deleted due to incorrect value insertion'. If no profound typographical error was identified (that was the case in all occasions in the current project), a number of options were considered, depending on the type and the number of outliers present. Mild outliers are not uncommon in data samples and thus action was considered only if at least two mild outliers were present and, at the same time, if it was deemed beneficial for action to be taken. The presence of any severe outlier should be sufficient evidence to reject normality at a 5% significance level as they lie far out enough to have a substantial effect on means,

standard deviations, and other classical statistics and thus action was always considered to be necessary. The following methods were considered (Rosner, 2016):

- To transform the variable, for example, by using the natural logarithm (ln) of the variable.
- To perform the data analysis (or the statistical inference test) with and without the outlier(s) and compare the results.
- To use a non-parametric method or a different estimator that was less sensitive to outliers.

If any of the above methods was used, the method and the results are clearly stated.

Transformations

Variable transformations were considered for the Pearson's moment correlation and the linear regression models, but not for the other parametric tests used in the main study, for which the non-parametric alternative tests were preferred. This was for no other reason than because of my own preference and familiarity with the interpretability of results. For the variables that were transformed, the new variable $f(x)$ or its residuals were re-checked for normality and the presence of outliers, heteroscedasticity and linearity as required. The most often used function – preferred mainly due to its convenient characteristics – was the natural logarithm (ln), but other transformations were also considered. The resulting $f(x)$ was considered only if the interpretation of it made some logical sense, otherwise other methods were preferred, such as non-parametric related tests.

OLS Regression assumptions

Linearity: Essentially, the OLS regression model attempts to fit a straight line between the dependent variable Y and the predictors x_0, x_1…, x_n, or, more accurately, the OLS regression model is assumed to be the linear in the regression parameters β_0, β_1, …, β_x so that $Y = \beta_0 + \beta_1 x_1$, …, $\beta_x x_n$ (Chatterjee and Hadi, 2012; Gareth *et al.*, 2014). The assumption of linearity for simple regression i.e. one predictive variable, was examined through a scatterplot of Y against X, which was plotted against the regression line and the local linear smooth (lowess) plot (which further assisted in detecting non-linearity). In the case of the multiple regression, the augmented component-plus-residual plot (acprplot; or augmented partial residual plot) was plotted against the regression line and the lowess plot. The acprplot plot attempts to project multidimensional data back to the two-dimensional world for each of the original regressors, i.e., it draws the relationship

of Y versus X_i but takes into account the effect of the other predictors in the model (StataCorp LLC, 2019a). If the acprplot plot was suggestive of non-linearity, this was followed by a generating a scatterplot matrix for further examination. It can be argued that the assumption of linearity in essence is an assumption of correct functional form, thus a transformation tends to solve the problem most of the time. Other more complex potential treatments, such as non-linear regressions (Gareth *et al.*, 2014), were not required, based on the data analysis; hence ,they were not considered in the current study.

Homoscedasticity: Homoscedasticity is one of the main assumptions of the OLS regression; an OLS regression model suffers from heteroscedasticity if the $V(Y|X)$ [variance of Y given X or in practice, the variance of the residuals, $V(e_i|X)$ for all i = 1, 2, … n] is not constant (Gareth *et al.*, 2014; Wooldridge, 2015). The variance of the residuals was initially examined graphically, through the residual-versus-fitted plot (rvfplot) that was plotted against a line at $y = 0$ (which further assisted in detecting heteroscedasticity). This was accompanied by the more robust methods of White's test and Breusch-Pagan test, which tested the H_0: $V(e_i|X) = \sigma^2$, where σ is a constant; i.e., the residuals are homoscedastic. Note that these tests used are very sensitive to the assumption of normality (StataCorp LLC, 2019a), therefore, the assumption of normality and the presence of outliers were investigated before performing these tests. For the same reason, the appropriate correction of the functional form by means of transformations or by treating the outliers can often help meet the assumption of homoscedasticity. If these methods failed to help meet the assumption of homoscedasticity, the robust standard errors were used. This is a model that is less sensitive to the assumption of homoscedasticity but usually has the consequence of increasing the CI (Wooldridge, 2015); for this reason, and although considered a good treatment, it was applied only if the other treatments of heteroscedasticity (mentioned) were not possible or if they had failed to correct the problem.

Autocorrelation: Another important assumption of the linear regression model is that the error terms, $\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_n$, are uncorrelated, so that, for example, the fact that e_i is positive provides little or no information about the sign of e_{i+1}. Note that, although the correlation of the error terms is usually consider as a time-series assumption, it can also occur outside of time-series data, such as cross-sectional studies (Gareth *et al.*, 2014). The assumption of correlated error terms was initially investigated graphically, through a scatter plot of the residuals versus the time variable. Because of the absence of a time variable *per se* in

this main study, the participation number was used instead, as an indicative time variable; in reality, the participation number indicated the order and hence the time frame within which the participants attended their initial appointment for the data collection. Similarly to the linearity assumption, the regression plot should be $E(e|X_t) = 0$ [the expected value of residuals given *X*-time variable should have a mean function equal to 0] or, simply, the residual plot should look like a null plot (Weisberg, 2005). Furthermore, the assumption of no correlated error terms was examined using the Breusch–Godfrey and the Durbin's alternative test for serial correlation (the more robust estimator of Durbin's alternative test was used if heteroscedasticity was present). The data were examined only for first order serial correlation using the default settings (StataCorp LLC, 2019d, 2019a). The Breusch–Godfrey and Durbin's alternative test have the H_0: $\rho_1 = 0,\ldots, \rho_p = 0$ with the alternative hypothesis being that, at least one of the pairwise correlations is non-zero (StataCorp LLC, 2019a). Correlation in the error terms suggests that there is additional information in the data that has not been exploited in the particular model and, hence, the correction of the functional form or the addition of an omitted variable most often resolves the problem (Chatterjee and Hadi, 2012). The more complex remedy of generalised difference equation was not required, based on the data analysis; hence, it was not considered in the current study.

Normality of residuals: The OLS regression model assumes that the residuals have a normal distribution. Therefore, the distribution of the residuals was assessed for normality. Furthermore, the residuals were predicted using the command *double* so as to decrease the mean of the residuals closer to zero and, more precisely, to 10^{-14} (StataCorp LLC, 2019a). If the residuals could not be assumed as normally distributed, then the functional form of the appropriate variables was transformed.

Multicollinearity: The OLS regression requires that there is no multicollinearity in the model. Multicollinearity refers to the situation when two or more predictive variables are highly correlated (Gareth *et al.*, 2014). The presence of collinearity was investigated through Pearson's moment correlation coefficient (*r*) and the more robust methods of variation inflation factor (VIF), which runs auxiliary regressions for each of the predicted variable against the rest of the independent variables. The centered VIF was defined as indicating significant multicollinearity if any VIF value is greater than 10, while for *r*, this was defined as any value above 0.90. The problem of multicollinearity was to be solved by either removing one of the collinear variables or combining the collinear

variables in a meaningful way. Other more complex possible treatments, such as ridge regression and weighted least squares (Chatterjee and Hadi, 2012; Gareth *et al.*, 2014; StataCorp LLC, 2019a), were not required, based on the data analysis; hence, they were not considered.

Exogeneity and model specification: Endogeneity is a strong but usually underestimated assumption of regression that can be loosely defined as a correlation between the explanatory variables (X_1, X_2,… X_n) and the error term in a regression (Constantinides, Harris and Stulz, 2013) due to an omitted cause (Antonakis *et al.*, 2010). There are many reasons why the *X* variable might be endogenous, including that of omitted variables, simultaneity, that is, when the *y* and one or more of the *X*'s are determined in equilibrium, and measurement error, for example, in cases where proxies are used for unobservable or difficult to quantify variables (Antonakis *et al.*, 2010; Constantinides, Harris and Stulz, 2013). The models were checked for omitted variables through the Ramsey's test for omitted variables, which essentially, it looks for a pattern in the residuals (StataCorp LLC, 2019a). Furthermore, the models were tested using the more general link test for model specification. The link test for model specification is based on the idea that, if a regression or regression-like equation is properly specified, you should be able to find no additional independent variables that are significant except by chance. In practice, both tests create new variable that are refitted in the regression and, if these new variables are then significant, the model has some kind of misspecification (StataCorp LLC, 2019a). If the general remedy methods applied for other assumptions, for example, transformations, correcting functional form or trying to control for more factors in a multiple regression analysis fails to treat endogeneity, then the method of single-equation instrumental-variables regression with a two-stage least squares estimator (Antonakis *et al.*, 2010) was to be considered; nevertheless, this more complex method was not required, based on the data analysis; hence, it was not considered.

Furthermore, good modelling rules were applied, such as allowing a minimum of 10 to 15 observations for each predictor or cofactor added to a model (Babyak, 2004) or, in the case of the primary outcomes, preferring the superior method (Babyak, 2004) of pre-specifying the models. This allowed me to preserve adequate degrees of freedom (including phantom ones) and therefore for the model to produce trusted results that can be generalised (Babyak, 2004).

Unusual and influential data: The absence of unusual and influential data, strictly speaking, is not an assumption of OLS linear regression. Nevertheless, it is considered to be of paramount importance that data are investigated for the presence of unusual and influential observations, as they can have a significant effect on the estimates of the regression model, while they may be the cause and/or the remedy of not meeting OLS assumptions (Harrell, 2015). These kinds of data points can be classified into outliers, high leverage points and influential observations. An outlier is an observation with large residual e_i, that is, a large unusual difference between the observed y_i and the predicted value $\hat{y}_i$. In contrast, high leverage points are outliers in the *x*-space, that is, a large distance between the high leverage observation and the rest of the observations; leverage points can be located close to the regression line (good leverage points) or far from the regression line (good leverage points). Influential points are those observations that have a large effect on the estimates, so that, if that point was to be deleted, this will have a dramatic effect on the predicted line ŷ; influential points tend to be either outliers or/and observations with high leverage (Chatterjee and Hadi, 2012; Gareth *et al.*, 2014; StataCorp LLC, 2019a). To detect such points, the leverage-versus-squared-residual plot (L-R plot) was examined in combination with DFITS and DFBETA tests. The L-R plot is a graph of leverage against the normalized residuals squared accompanied by a *y*-line equal to the average leverage and an *x*-line equal to the average normalized residuals squared – observations further away from the lines point towards high leverage points and outliers, respectively (Gareth *et al.*, 2014; StataCorp LLC, 2019a). This was accompanied by the more general statistic DFITS, that is, the change in the predicted $X\beta$ (where $X\beta = \beta_0 + \beta_1 X_1 + \ldots + \beta_k X_k$) when the observation is dropped (Harrell, 2015). Essentially, both the L-R plot and the DFITS test try to combine the points of high leverage and outliers into one graph and one statistic respectively (StataCorp LLC, 2019a). Results that deserved further examination were the $|\text{DFITS}_i| > 2\sqrt{\frac{k}{n}}$,where *k* = number of independent variables and *n* = number of observations in the regression model (StataCorp LLC, 2019a), although other sources report different cut-off points (Harrell, 2015). Furthermore, this was followed by the more specific tests of DFBETAS, that is, the change in the vector of regression coefficient estimated upon deletion of each observation in turn, scaled by their standard error (Harrell, 2015; StataCorp LLC, 2019a). When DFBETAs variables are calculated (one DFBETA variable is produced for each

predictor in the original model) they are plotted together (i.e., in the same graph) against an *X*-variable (usually the dependent variable of the original regression model is used – but mostly for convenience rather for any significance) against the *y*-line = $\frac{2}{\sqrt{n}}$, where *n* is the number of observations. Points above or below (i.e., $|DFBETA_i| > \frac{2}{\sqrt{n}}$) this line indicate observations that are worth further investigation, but special attention is given to observations that have changed the estimation by more than 1 SE (i.e., $|DFBETA_i| > 1$) (StataCorp LLC, 2019a). Finally, an observation that was deemed unusual when using the described measures was initially checked for its accuracy (Harrell, 2015). If no data entry error could be identified, the option was either to remove the observation – under the strict condition that the analysis is made conditional on observations being unlike the influential one (Harrell, 2015) – or, in the more likely case that the assumption could not be made, then the option of the MM-regression estimator was preferred, that is, an estimator more resistant than the OLS estimator to extreme values (Chatterjee and Hadi, 2012).

STATA statistical software

Table 7.5 STATA community–contributed packages

Command name (alphabetically)	Title	Author(s)
bacon	BACON algorithm to identify multivariate outliers	Sylvain Weber
dftol	Distribution-free tolerance intervals	Ignacio López de Ullibarri
dunntest	Dunn's test of multiple comparisons using rank sums outliers	Alexis Dinno
iqr	Interquartile range, etc	Lawrence C. Hamilton
mmregress	MM-robust regression	V. Verardi and C. Croux
tolerance	Generate tolerance intervals from input data	Peter A. Lachenbruch
vioplot	Violin plots	Austin Nichols

Regression Assumptions of Primary Outcomes

Table 7.6 Assumption of OLS regression for primary outcomes: brief description

I. HbA1c ~ adherence to the Mediterranean diet + covariates

Model	Pre-specified conditions (n in the model)	Can we assume the following assumptions?							Conclusion & Comments
		Linearity	Homoscedasticity	Autocorrelation	Normality of residuals	Multicollinearity	Exogeneity & Model specification	Unusual & influential data	
Model 1	Nil (n = 103)	No Comment: deviates due to presence of 'MedDietScore = 10' observation, a leverage point; for the rest can assume linearity	No Comment: the statistical tests suggest the presence of heteroscedasticity; rvfplot indicates that the problem is more likely due to the presence of an extreme value, the 'MedDietScore = 10' observation rather that the model is truly heteroscedastic	Yes	Yes	n/a	No Comment: problem with model specification and suggestion of omitted variable	No Comment: the 'MedDietScore = 10' is a mild outlier, a high leverage point and a highly influential point	Model estimates are highly unreliable Comment: likely due to the presence of an extreme value → prefer excluding conditionally; otherwise MM-estimator regression
Model 2	Nil (n = 103)	No Comment: see Model 1	No Comment: see Model 1	Yes	Yes	Yes	Yes	No Comment: the 'MedDietScore = 10' is a high leverage and highly influential point	Model estimates are highly unreliable Comment: see Model 1
Model 3	Nil (n = 103)	No Comment: see Model 1	Yes	Yes	Yes	Yes	Yes	Yes Comment: 'MedDietScore = 10' considerably less influential	Model estimates are unreliable Comment: conditional model; otherwise OLS model

II. HbA1c ~ adherence to the Mediterranean diet + covariates

Model	Pre-specified conditions (n)	Can we assume the following assumptions?							Conclusion & Comments
		Linearity	Homoscedasticity	Autocorrelation	Normality of residuals	Multicollinearity	Exogeneity & Model specification	Unusual & influential data	
Model 1	Conditionally – on moderate & high adherence – exclusion of one observation 'MedDietScore = 10points' with low adherence (n = 102)	Yes	Yes	Yes	Yes	n/a	Yes	Yes	Model estimates are reliable
Model 2	See model 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Model estimates are reliable
Model 3	See model 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Model estimates are reliable

Notes: The models described at *I. HbA1c ~ adherence to the Mediterranean diet + covariates* were dropped and rather the models described at *II. HbA1c ~ adherence to the Mediterranean diet + covariates* are presented, conditionally - on modelling grounds, as described; Furthermore, additional post-hoc MM-estimator/OLS regression models, as appropriate, were run without excluding the troubling observation (n = 103); The results are presented in the *Results* sections as also the description of the modelling techniques used for the current analysis, see *Results: Primary Outcomes: Adherence to the Mediterranean diet and glycaemic control*. The regressions methods, statistical tests and graphical techniques used to examine the assumptions are described in the Appendix – results presented here, are based on those methods, see *Appendix: Chapter 6: OLS Regression* assumptions. No results, either statistical tests or graphical results, are presented mainly due to space constrains but also because they were considered beyond the scope of this thesis – nevertheless, these have been saved as either *STATA Do-files*, that contain all the *STATA commands* used to run the regression models and investigate the assumptions, or as *STATA log-file* that contain a more raw format of data results and are available on request.

III. Glucose ~ adherence to the Mediterranean diet + covariates

Model	Pre-specified conditions (n)	Can we assume the following assumptions?							Conclusion & Comments
		Linearity	Homoscedasticity	Autocorrelation	Normality of residuals	Multicollinearity	Exogeneity & Model specification	Unusual & influential data	
Model 1	Conditionally on non-hypoglycaemia – exclusion of eleven observations with glucose < 70mg/dl; based on clinical grounds (n = 92)	Yes	Yes	Yes	No Comment: Shapiro-Wilk test is significant but sktest could not identify the problem – histogram, and other graphics show some negative skewness but not gross problem → run model if this the only problem	n/a	Yes	Yes Comment: although L-R plot points 'MedDietScore = 10', the DFITS & DFBETA suggest that is not an influential point → can stay in the model	Model estimates can be considered reliable
Model 2	See model 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes Comment: see model 1	Model estimates are reliable
Model 3	See model 1	Yes	Yes	Yes	No Comment: see Model 1	Yes	Yes	Yes Comment: see model 1	Model estimates can be considered reliable

Notes: The models described at *III. Glucose ~ adherence to the Mediterranean diet + covariates* were run, conditionally – but in contrast to the modelling grounds of HbA1c, the restriction of model were pre-specified and was based solely on clinical grounds; No additional models were run post-hoc that included the observation in the hypoglycaemic range that were initially excluded – to avoid providing void results by spending phantom degrees of freedom or in simple words testing until we find a statistical significant result – nevertheless robust-to-outliers models were run post-hoc as good leverage points can have an effect on estimated standard errors and consequently on the t statistic (Verardi and Croux, 2009).

Chapter 6

Potential secondary outcomes not considered in the current thesis

Epidemiological outcomes:

- Medical, diabetes and other epidemiological characteristics collected using the *medical and diabetes questionnaire*.
- The presence (or prevalence) of lifestyle risk or protective factors – individually and in combination – including: dietary habits such as healthy eating as defined by available international or local guidelines (no such guidelines exist, to our knowledge, designed explicitly for Cyprus; collected using the FFQ), physical activity and sedentary lifestyle (calculated using the IPAQ), smoking (collected through the *Medical and diabetes questionnaire*) and obesity, quantified through the BMI, the waist circumference indicating the central obesity and the high levels of body fat, estimated through BIA.
- The presence (or prevalence) of comorbidities such as hyperlipidaemia and hypertension, either as self-reported (collected through the *Medical and diabetes questionnaire*) or identified by the clinical examination, and the blood and the urine tests.
- The presence (or prevalence) of complications such eye disease, foot, renal and CVD complications, gastroparesis and sexual dysfunction, which were identified through the *medical and diabetes questionnaire*, the foot screening, and the blood and the urine tests.
- The presence (or prevalence) of hypertrophy that is an insulin-use related complication, identified through the clinical examination.
- The adherence to a Mediterranean lifestyle; by encompassing factors that have been measured on the current study, and based (measured) on the Mediterranean lifestyle scoring systems (*Chapter 1*) available in the literature.
- The energy, and nutrient (of macronutrients and micronutrients of interest) intake such as carbohydrates, protein, fat, dietary fibre, (total) sugars, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), ethanol, sodium (Na), iodine (I) and vitamin C, estimated using the data available in the FFQ by using algorithms.

- The intake of selected foods (and drinks) and food groups of interest such as fruits, vegetables, oily fish, processed and fast foods, and alcohol, as reported or calculated using the foods from the FFQ.

Association between selected outcomes:

- Mediterranean lifestyle (encompassing factors that have been measured on the current study, and based on the Mediterranean lifestyle scoring systems available in the literature) *vs* glycaemic control (HbA1c and glucose).
- Energy, macro and micro-nutrient intake of interest *vs* recommended energy and nutrient intake as defined by available international or local guidelines (no such guidelines exist, to our knowledge, designed explicitly for Cyprus).
- Energy, macro and micro-nutrient intake of interest *vs* glycaemic control (HbA1c and glucose).
- Foods and food groups intake such as fruits, and soft drinks (with sucrose or artificial sweeteners; number of servings reported) vs. glycaemic control (HbA1c and glucose).
- Iodine intake in women of child-bearing age (UIC – a biochemical indicator of recent dietary iodine intake) *vs* recommended intake in the specific population group.
- Vitamin C (number of servings reported) vs. foot problems such as foot ulcers.
- Gluten estimated intake (number of servings reported) *vs* glycaemic control (HbA1c and glucose) and thyroid functions (fT4 and TSH).
- Adherence to the Mediterranean diet (*MedDeitScore*) *vs* comorbidities such as obesity – through (quantified by) BMI or through waist circumference indicating central obesity or high levels of body fat, estimated through BIA, hyperlipidaemia and hypertension, either as self-reported or as measured at the clinical examination, and by the blood and urine tests .
- Adherence to the Mediterranean diet (*MedDeitScore*) *vs* sexual dysfunction (self-reported).
- Adherence to the Mediterranean diet (*MedDeitScore*) *vs* foot screening results such as the monofilament response score (indicative of sensory neuropathy) and the doppler ultrasound assessment (indicative of peripheral vascular disease).
- Adherence to the Mediterranean diet (*MedDietScore*) *vs* inflammatory effect (quantified using) such as CRP and hs-CRP.
- Prevalence of obesity *vs* thyroid function (fT4 and TSH).

- Use of HMG-CoA reductase inhibitors (statins; self-reported) *vs* glycaemic control (HbA1c and glucose).
- Cholesterol medication potency used (self-reported) *vs* cholesterol medication potency that should be used (decided from reported medical history).
- Subjective (self-reported) data on comorbidities, such as hyperlipidaemia and hypertension, and complications, such as renal and foot disease vs the objective (measured) data obtained on comorbidities and complications.
- Insulin delivery method (MDI or CSII; self-reported) *vs* glycaemic control (HbA1c and glucose).
- C-peptide (blood test) *vs* glycaemic control (HbA1c and glucose).
- Hypertrophy (Yes or No) *vs* glycaemic control (HbA1c and glucose) and insulin dosage (self-reported).

Predictions (forecast): The current study has gathered a large of amount data that can be used to forecast important parameters, such as the glycaemic control (primarily of HbA1c; within the limitations of the study); in combination with more modern and sophisticated statistical techniques current available, such as least absolute shrinkage and selection operator (lasso), ridge and elastic net regression (or older methods such principal component analysis, PCA), that can be used (and are considered relative reliable methods) for variable selection and prediction purposes.

Validation of questionnaires: The validation of a questionnaire (validity and reproducibility) assures that any future usage provides reliable and accurate information; for example, the validation of the FFQ for estimating iodine intake, through the UIC (urine) test.