No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC

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ABSTRACT

Myokines may play a role in the health benefits of regular physical activity. Secreted protein acidic rich in cysteine (SPARC) is a pleiotropic myokine that has been shown to be released into the bloodstream by skeletal muscle in response to aerobic exercise. As there is evidence suggesting that SPARC release may be linked to glycogen breakdown and activation of 5' adenosine monophosphate-activated protein kinase (AMPK), we hypothesised that brief supramaximal exercise may also be associated with increased serum SPARC levels. In the present study 10 participants (three women; mean±SD age: 21±3 y, BMI: 22±3 kg·m^{-2}, $\dot{V}O_2$max: 39±6 mL·kg^{-1}·min^{-1}) performed an acute bout of supramaximal cycle exercise (20-s Wingate sprint against 7.5% of body mass, with a 1-min warm-up and a 3-min cool-down consisting of unloaded cycling). Serum SPARC levels were determined pre-exercise as well as 0, 15 and 60 min post-exercise, and corrected for plasma volume change. To determine whether regular exercise affected the acute SPARC response, participants repeated the acute exercise protocol 3 times per week for 4 weeks, and serum SPARC response to supramaximal exercise was reassessed after this period. Acute supramaximal exercise significantly decreased plasma volume (-10%; $p < 0.001$), but was not associated with a significant change in serum SPARC levels at either the pre-training or post-training testing sessions. In conclusion, in contrast to aerobic exercise, a single brief supramaximal cycle sprint is not associated with an increase in serum SPARC levels, suggesting that SPARC release is not related to skeletal muscle glycogen breakdown.

Keywords:

Secreted protein acidic rich in cysteine, osteonectin, basement-membrane protein 40, Wingate sprint

INTRODUCTION

Physical activity is among the most important modifiable risk factors for cardiometabolic health (Garber et al., 2011). However, the mechanisms by which exercise may affect health remain incompletely understood. A growing body of literature supports the notion that skeletal muscle acts as an endocrine organ, secreting proteins into the circulation in order to regulate physiological functioning in other parts of the body such as the liver and adipose tissue (Raschke, Eckardt, Bjorklund Holven, Jensen, & Eckel, 2013; Weigert, Lehmann, Hartwig, & Lehr, 2014), and it has been proposed that these 'myokines' may play a role in the health benefits of regular physical activity. A prominent example is the pleiotropic myokine interleukin 6 (IL-6), which was shown to be released into the circulation by skeletal muscle during aerobic knee extensor exercise in a study by Steensberg *et al.* (2000), and has since been extensively studied by exercise physiologists (Munoz-Canoves, Scheele, Pedersen, & Serrano, 2013; Pal, Febbraio, & Whitham, 2014). IL-6 released during exercise is now generally accepted to play important roles in for example regulating immune function and metabolism (Pal et al., 2014; Pedersen, 2012). However, many more regulatory proteins are secreted by skeletal muscle, and although several of these will fulfil important roles in re-establishing homeostasis following acute exercise and/or bringing about adaptations to chronic exercise training, we are only recently starting to improve our understanding of the extent to which myokines regulate physiological functioning.

With the advent of powerful 'omics' techniques, it has become possible to perform untargeted screenings to identify the full complement of putative novel myokines released in response to acute exercise and/or exercise training in humans: the skeletal muscle 'secretome'. For example, using whole genome gene expression analysis Catoire *et al.* (2014) identified 86 putative myokines that are upregulated in skeletal muscle in response to acute exercise, and 69 putative myokines that are upregulated in response to chronic exercise training. It can be hypothesised that particularly the latter group may include myokines that play a role in the health benefits of regular physical activity. As such, it is important to increase our

understanding of 1) the specific functions of these myokines, 2) the mechanisms by which these myokines are released in response to exercise or training, and 3) the types of exercise that are associated with an increase in the circulating levels of these myokines.

One of the myokines that was identified by Catoire *et al.* (2014) to respond to both acute exercise and exercise training at a gene expression level is secreted protein acidic rich in cysteine (SPARC). SPARC has been reported to exert many potential functions, including for example regulation of GLUT4 expression (Song, Guan, Zhang, Li, & Dong, 2010), insulin secretion (Harries et al., 2013), erythropoiesis (Luo et al., 2012), and suppression of colon tumorigenesis (Aoi et al., 2013). Serum levels of SPARC increase following 30 min of aerobic exercise at an intensity of 70% of $\dot{V}O_2$max, with an augmented response to acute exercise following 4 weeks of aerobic exercise training (Aoi et al., 2013). To our knowledge, no other studies have directly measured serum levels of SPARC following exercise in humans, so it remains unknown whether the release of SPARC is affected by exercise intensity or duration, and whether other types of exercise are also associated with increased serum SPARC levels.

It has been shown that SPARC physically interacts with 5' adenosine monophosphate-activated protein kinase (AMPK), and that AMPK activation increases SPARC expression (Song et al., 2010). AMPK is activated by exercise and is thought to be a critical regulator of energy metabolism and skeletal muscle plasticity (Mounier, Theret, Lantier, Foretz, & Viollet, 2015). AMPK has a glycogen-binding domain, and glycogen-depleting exercise is associated with an increase in free AMPK (Philp, Hargreaves, & Baar, 2012). Thus, glycogen depletion may be a mechanism by which exercise increases SPARC release. Apart from high-volume aerobic exercise, brief supramaximal sprint exercise is a potent means of activating phosphorylase a, the rate-limiting enzyme responsible for breaking down glycogen (Parolin et al., 1999). Brief supramaximal cycle sprint exercise has been demonstrated to deplete skeletal muscle glycogen stores by 20-30% within 15 to 30 seconds (Esbjornsson-Liljedahl, Bodin, & Jansson, 2002; Metcalfe et al., 2015; Parolin et al., 1999), and is associated with activation of AMPK and its downstream targets (Gibala et al., 2009; Metcalfe et al., 2015). We therefore

hypothesised that brief supramaximal exercise would also be associated with an increase in the serum levels of the myokine SPARC, and that similar to aerobic exercise training (Aoi et al., 2013) this effect would be enhanced following regular exercise. Thus, the aim of the present study was to investigate the effects of acute and chronic brief supramaximal exercise on serum SPARC levels.

METHODS

Participants

Eleven apparently healthy volunteers (four women, seven men) were recruited to participate in this study through advertising in the local University student population. One female participant dropped out during the study due to lack of time. Characteristics (mean±SD) of the remaining ten participants are shown in **Table 1**. Exclusion criteria were classification as highly physically active according to the International Physical Activity Questionnaire (IPAQ), contraindications to strenuous exercise according to a standard Physical Activity Readiness Questionnaire (PAR-Q), hypertension (>140/90 mm Hg), resting heart rate >100 bpm, and/or a personal history of metabolic or cardiovascular disease. The study protocol was fully explained to all participants in written and verbal form before they provided written consent. The study was approved by the ethics committee of the Department for Health at the University of Bath (reference number EP 15/16 9).

Experimental Procedures

Participants refrained from performing strenuous exercise and from consuming caffeine and alcohol the day before and the days of testing. $\dot{V}O_2max$ was determined in an incremental cycling test to exhaustion (Excalibur Sport, Lode, Groningen, the Netherlands) as previously described (Metcalfe, Babraj, Fawkner, & Vollaard, 2012). Following a 2-min warm-up at 50 W the intensity was increased by 1 W every 3 s until volitional exhaustion or the inability to maintain a pedal frequency higher than 60 rpm. Oxygen uptake ($\dot{V}O_2$) was determined throughout the test using an online gas analyser (TrueOne 2400, Parvo Medics, Sandy, US) to determine $\dot{V}O_2max$ as the highest value for a 15-breath rolling average. In all tests two or more of the following criteria were met: a plateau in $\dot{V}O_2$ despite increasing intensity, RER>1.15, heart rate within 10 beats of age-predicted maximum, and/or volitional exhaustion (Metcalfe et al., 2012).

Three days after the max-test, acute responses to a single 20-s 'all-out' cycle sprint were determined in the morning after an overnight fast. Following 15 min of seated rest, a 1.5 mL baseline blood sample was taken through a cannula in a superficial forearm vein. Participants then performed an exercise session on a mechanically-braked cycle ergometer (Ergomedic 874e, Monark, Vansbro, Sweden), involving one min of unloaded pedalling, followed by a 20-s 'all-out' Wingate-type cycle sprint against a resistance of 7.5% of body weight, and a cool-down of 3 min of unloaded pedalling. Further 1.5 mL blood samples were taken directly after and 15 and 60 min after cessation of exercise. Blood samples were analysed for haemoglobin (Hb; HemoCue, Crawley, UK) and haematocrit (Hct; Haematospin 1300; Hawksley & Son Ltd, Lancing, UK) in order to determine acute plasma volume changes in response to exercise using the method of Dill & Costill (Dill & Costill, 1974). Serum was collected after clotting at room temperature for 30 min (0.5 mL clotting activator/serum micro-tubes; Sarstedt, Nümbrecht, Germany), and stored at -80°C for subsequent analysis of SPARC (Human SPARC Quantikine ELISA, RND Systems). In brief, 100 µL of serum was added to 100 µL of assay diluent, and incubated at room temperature for 3 hrs on a microplate shaker. After washing 6 times, 200 µL of cold conjugate was added prior to further incubation on ice for 1 hr. After washing a further 6 times, 200 µL substrate solution was added followed by incubation in the dark at room temperature for 30 min. Finally, 50 µL of stop solution was added and absorbance was read at 450 nm with wavelength correction at 570 nm. Duplicate measures were averaged and serum levels were calculated from a standard curve. Serum levels were corrected for acute plasma volume changes.

In order to determine whether the serum SPARC response to supramaximal exercise is affected by exercise training, participants then started a 4-week training intervention consisting of three weekly supervised cycling sessions identical to the initial acute session. Peak heart rate (Polar RS400, Finland) and peak, mean and end power output (Ergomedic 874e) were recorded during the 3rd and 12th exercise sessions. Rating of perceived exertion on a 15-point Borg scale were taken after the 3rd, 6th, 9th and 12th exercise sessions. Three days following

the final training session, a second $\dot{V}O_2$max test was performed, followed another three days later by one further training session. During the final training session the acute effects of exercise were re-assessed with identical procedures compared to the first training session.

Statistical analysis

All data are presented as mean±SD. Two way repeated measures analysis of variance (trial x time) was used to determine acute exercise-induced changes in plasma volume and levels of SPARC, as well as differences in the response to exercise before and after training. *Post hoc* analysis using Bonferroni correction was performed to determine differences between time points for analyses with a significant main effect of time. Differences between pre- and post-training exercise characteristics and $\dot{V}O_2$max were analysed using paired sample t-tests. Significance was accepted at $p < 0.05$.

RESULTS

Eight participants completed all exercise training sessions, and two participants each missed a single training session. Characteristics of the exercise sessions are shown in **Table 2**. Peak heart rate during the 3rd session was significantly higher (p<0.05) than in the 12th session, but there were no changes in the peak, mean and end power output recorded. Mean RPE scores for the training sessions did not change significantly during the training period (mean±SD RPE: 14.5±1.6, 13.6±1.5, 13.3±1.6 and 13.4±1.8 for the 3rd, 6th, 9th, and 12th sessions respectively). $\dot{V}O_2$max did not significantly change from pre- to post-training (2.62±0.55 L/min and 2.74±0.67 L/min respectively). Plasma volume decreased directly post-exercise (p<0.001) and 15 min post-exercise (p=0.053), but had returned to baseline at 60 min post-exercise (**Figure 1A**). There were no significant differences in the acute decrease in plasma volume between the start and end of training. The effects of single supramaximal cycle sprints on serum SPARC levels are shown in **Figure 1B** (uncorrected for plasma volume change) and **Figure 1C** (corrected). Whereas uncorrected SPARC levels followed a pattern similar to plasma volume change (Figure 1B), SPARC levels corrected for plasma volume change were not significantly different from baseline levels at any time-point during the pre-training acute exercise trial, and this was still the case following four weeks of three weekly sprint sessions.

DISCUSSION

The aim of the present study was to investigate the effects of acute and chronic brief supramaximal exercise on serum SPARC levels. In contrast to 30 min of cycling at 70% of $\dot{V}O_2max$ (Aoi et al., 2013), a brief 'all-out' cycle sprint is not associated with an increase in serum SPARC levels. This suggests that for the release of SPARC from skeletal muscle duration of exercise may be more important than intensity.

Supramaximal exercise causes a substantial disturbance of homeostasis within a very brief amount of time. In the present study this was evidenced by a significant 10% acute decrease in plasma volume, and a mean peak exercise heart rate of ~90% of maximal heart rate. The decrease in plasma volume is likely caused by fluid shifts secondary to the hyperosmotic state caused by rapid glycogen depletion (Metcalfe et al., 2015). Glycogen depletion following 'all-out' Wingate-type sprints has previously been determined to be between 20-30% (Esbjornsson-Liljedahl et al., 2002; Metcalfe et al., 2015; Parolin et al., 1999). Thus the lack of an increase in serum SPARC levels suggests that exercise-induced SPARC release is not dependent on glycogen depletion. Unlike IL-6 release, which occurs both in response to aerobic exercise (Steensberg et al., 2000) and brief supramaximal exercise (Abedelmalek et al., 2013), SPARC release is not mediated by single bouts of supramaximal exercise. As repeated supramaximal sprints (sprint interval training; SIT) have been shown to be associated with many of the adaptations that are also seen following aerobic exercise training (Babraj et al., 2009; Metcalfe et al., 2012; Metcalfe, Tardif, Thompson, & Vollaard, 2016), further studies are needed to determine whether performing multiple sprints may result in SPARC release from skeletal muscle.

There were a number of limitations to our study. Firstly, we did not confirm pre-study physical activity levels, or maintenance of pre-study physical activity levels during the study, using an objective measure such as accelerometer data. Secondly, the sample size was too small to formally compare potential sex differences in the SPARC response to exercise. Thirdly, we did not take muscle biopsies in the present study, and therefore we were unable to take direct

measures of changes in SPARC mRNA expression, muscle glycogen levels, AMPK activation, etc. Further studies are needed to confirm that skeletal muscle SPARC expression is unaffected by supramaximal exercise and/or glycogen depletion. However, the lack of an increase in serum SPARC levels following both acute supramaximal exercise and 4 weeks of regularly performing supramaximal exercise suggests that any potential increase in SPARC gene expression is not associated with increased SPARC protein release into the bloodstream with supramaximal exercise. As SPARC levels uncorrected for plasma volume change significantly increased directly post-exercise and followed the same pattern as plasma volume changes (**Figure 1B**) we are confident that we would have been able to demonstrate a change in serum SPARC levels if it had occurred.

In conclusion, in the first study to examine the effects of supramaximal exercise on circulating levels of the pleiotropic myokine SPARC, we demonstrate that serum SPARC levels are not affected by acute or chronic Wingate-type sprints. This suggests that muscle glycogen breakdown is not a mechanism for exercise-induced skeletal muscle SPARC release.

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Table 1: Participant characteristics

	Men (n=7)	Women (n=3)	All (n=10)
Age (y)	21±3	21±3	21±3
BMI (kg·m^{-2})	21.8±3.5	20.6±0.8	21.5±2.9
$\dot{V}O_2$max (mL·kg^{-1}·min^{-1})	40.0±6.4	37.8±4.9	39.3±5.7

Values shown are mean±SD.

Table 1: Training characteristics

	3rd session	12th session
Peak HR (bpm)	182±7	172±12*
Peak power output (W)	699±154	642±135
Mean power output (W)	501±132	512±106
End power output (W)	354±124	383±95

*Values shown are mean±SD. Differences between the 3rd and 12th session: * $p<0.05$.*

Figure 1: Effect of supramaximal cycle sprint exercise on **A**) plasma volume, **B**) uncorrected serum SPARC levels, and **C**) serum levels of SPARC corrected for changes in plasma volume. For plasma volume and uncorrected serum SPARC levels there was a significant main effect of time ($p<0.001$), but no effects of trial or trail x time interaction effects. Post hoc analysis indicated similar patterns for both parameters; a: $p<0.001$ compared to pre-exercise; b: $p<0.01$ compared to post 15 min; c: $p<0.001$ compared to post 60 min; d: $p<0.05$ compared to post 60 min; e: $p<0.01$ compared to pre-exercise; f: $p<0.01$ compared to post 60 min.