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The influence of adiposity and acute exercise on circulating hepatokines in normal weight and overweight/obese men

Jack A Sargeant¹, Guruprasad P Aithal², Toshinari Takamura³, Hirofumi Misu³, Hiroaki Takayama³, Jessica Douglas¹, Mark C Turner¹, David J Stensel¹, Myra A Nimmo⁴, David R Webb⁵, Thomas Yates⁵, James A King¹.

¹School of Sport, Exercise and Health Sciences, Loughborough University, UK

²Nottingham Digestive Diseases Centre, National Institute for Health Research (NIHR) Nottingham Digestive Diseases Biomedical Research Unit, Nottingham University Hospitals NHS Trust and University of Nottingham, UK

³Department of Disease Control & Homeostasis, Kanazawa University Graduate School of Medical Sciences, Japan

⁴College of Life & Environmental Sciences, University of Birmingham, UK

⁵Diabetes Research Centre, University of Leicester, UK

Author's email addresses

Jack A Sargeant – J.A.Sargeant@lboro.ac.uk

Guruprasad P Aithal - Guru.Aithal@nottingham.ac.uk

Toshinari Takamura - ttmedfm@gmail.com

Hirofumi Misu - hmisu@m-kanazawa.jp

Hiroaki Takayama - htakayama@m-kanazawa.jp

Jessica Douglas - jessica.anne.douglas@gmail.com

Mark C Turner - M.C.Turner@lboro.ac.uk

David J Stensel – D.J.Stensel@lboro.ac.uk

Myra A Nimmo – M.A.Nimmo@lboro.ac.uk

26 David R Webb - david.webb@uhl-tr.nhs.uk

27 Thomas Yates – ty20@leicester.ac.uk

28 James A King – J.A.King@lboro.ac.uk

29

30 **Address for correspondence**

31 Dr James King

32 School of Sport, Exercise and Health Sciences

33 Loughborough University

34 Leicestershire

35 United Kingdom

36 LE11 3TU

37 Phone: +44(0)1509 228457

38 Email: J.A.King@lboro.ac.uk

39

Abstract

Hepatokines are liver-secreted proteins with potential to influence glucose regulation and other metabolic parameters. This study investigated differences in adiposity status on five novel hepatokines and characterised their response to acute moderate-intensity exercise in groups of normal weight and overweight/obese men.

Twenty-two men were recruited into normal weight and overweight/obese groups (BMI: 18.5 to 24.9 and 25.0 to 34.9 kg·m⁻²). Each completed two experimental trials, exercise and control. During exercise trials, participants performed 60 min of moderate-intensity treadmill exercise (~60% $\dot{V}O_2$ peak) and then rested for 6 h. Participants rested throughout control trials. Circulating fibroblast growth factor-21 (FGF21), follistatin, leukocyte cell-derived chemotaxin 2 (LECT2), fetuin-A and selenoprotein-P (SeP) were measured throughout.

Fasted (resting) FGF21 and LECT2 were higher in overweight/obese individuals (129% and 55%; $P \leq 0.01$) and correlated with indices of adiposity and insulin resistance; whereas circulating follistatin was lower in overweight/obese individuals throughout trial days (17%, $P < 0.05$). In both groups, circulating concentrations of FGF21 and follistatin were transiently elevated after exercise for up to 6 h ($P \leq 0.02$). Circulating fetuin-A and SeP were no different between groups ($P \geq 0.19$) and, along with LECT2, were unaffected by exercise ($P \geq 0.06$).

These findings show that increased adiposity is associated with a modified hepatokine profile, which may represent a novel mechanism linking excess adiposity to metabolic health. Furthermore, acute perturbations in circulating FGF21 and follistatin after exercise may contribute to the health benefits of an active lifestyle.

Key words: physical activity, insulin resistance, obesity, liver, organokines

Introduction

Recent work characterising the hepatic proteome has identified 168 proteins which can be secreted and potentially exert endocrine-like effects in distal sites (Meex et al. 2015). A number of these ‘hepatokines’ are associated with measures of adiposity (Chen et al. 2011, Xu et al. 2011, Yang et al. 2011, Lan et al. 2014) and have been shown to exert metabolic effects within various central and peripheral tissues (Misu et al. 2010, Camporez et al. 2013, Malin et al. 2013, Lan et al. 2014, Hansen et al. 2016b). Together, this evidence has prompted suggestions that hepatokines represent a potential mechanism linking adiposity and metabolic health and may be novel therapeutic targets to combat obesity-related insulin resistance and associated metabolic disease.

To date, much of the research concerning hepatokine function and metabolism has focused on their direct influence on tissue-specific insulin sensitivity and systemic glucose metabolism. The most frequently studied, fibroblast growth factor-21 (FGF21), has been shown to improve glucose metabolism in the liver, skeletal muscle and adipose tissue (Camporez et al. 2013); whilst follistatin may promote pancreatic beta cell survival and suppress circulating glucagon (Hansen et al. 2016b). Other hepatokines may act to promote insulin resistance. For example, within skeletal muscle, leukocyte cell-derived chemotaxin 2 (LECT2) (Lan et al. 2014), selenoprotein-P (SeP) (Misu et al. 2010) and fetuin-A (Malin et al. 2013) have each been shown to directly interfere with distinct aspects of glucose metabolism. Observational evidence in humans has identified associations between these hepatokines and adiposity, insulin resistance, ectopic lipid and the metabolic syndrome (Zhang et al. 2008, Li et al. 2010, Chen et al. 2011, Xu et al. 2011, Yang et al. 2011, Choi et al. 2013, Hansen et al. 2013, Okumura et al. 2013, Lan et al. 2014). However, human experimental research is now required to scrutinise the pathophysiological relevance of these novel proteins *in vivo*.

Current evidence demonstrates that exercise training reduces circulating levels of fetuin-A and FGF21, and responses correlate with improvements in insulin sensitivity and intrahepatic fat (Malin et al. 2013, 2014, Taniguchi et al. 2016). Given that single bouts of exercise transiently enhance insulin sensitivity (Syrow et al. 2017), a handful of studies have also investigated the acute influence of exercise on circulating hepatokines, speculating that modulation of the hepatokine profile may be implicated in the benefits induced. This hypothesis is strengthened by the knowledge that exercise acutely increases circulating non-esterified fatty acids (NEFA) and glucagon, and activates hepatic AMP-activated protein kinase (AMPK) (Camacho et al. 2006, Hansen et al. 2016a). Each of these have been implicated in the regulation of at least one of the hepatokines outlined above (Jung et al. 2013, Lan et al. 2014, Trepanowski et al. 2014, Hansen et al. 2016a). Whilst, these studies remain limited in number, the available evidence shows that moderate- to high-intensity aerobic exercise acutely increases circulating levels of FGF21 and follistatin (Slusher et al. 2015, Hansen et al. 2016a, 2016b), but responses may differ between normal weight and obese individuals, and between individuals with normal and dysregulated glucose metabolism (Slusher et al. 2015, Hansen et al. 2016a). Additional work is required to determine whether the FGF21 and follistatin responses to acute exercise differ between normal weight and overweight/obese individuals who are free of diagnosed metabolic disease, and whether similar responses occur in other relevant hepatokines.

The purpose of this study was two-fold. Firstly, we sought to investigate differences in adiposity status on FGF21, follistatin, LECT2, SeP and fetuin-A in normal weight and overweight/obese men. Secondly, we characterised the effect of an acute bout of moderate-intensity exercise on circulating concentrations of these hepatokines in order to explore their potential role as mediators of exercise-induced improvements in glycaemic control and other

113 metabolic parameters. We hypothesised that overweight/obese individuals would have
114 elevated circulating concentrations of hepatokines at rest and that an acute bout of moderate-
115 intensity exercise would beneficially alter circulating hepatokine profiles; by reducing
116 LECT2, SeP, and fetuin A, whilst increasing FGF21 and follistatin.

Materials and methods

Ethical approval and participant recruitment

After receiving approval from the East Midlands NHS Research Ethics committee (13/EM/0290), 22 non-smoking men were recruited equally into normal weight and overweight/obese groups (BMI: 18.5 to 24.9 and 25.0 to 34.9 kg·m⁻², respectively); providing written, informed consent to participate. This sample size was chosen based on previous studies that documented significant changes in hepatokines (FGF21 and follistatin) in response to acute exercise; as well as differences between participant groups (normal weight vs. obese and normal vs. impaired glucose regulation) (Slusher et al. 2015, Hansen et al. 2016a, 2016b). Participants in the current study were free of diagnosed chronic disease and were not taking medications known to affect glucose or lipid metabolism, or blood pressure. Participants were also ‘inactive’ or ‘moderately active’ according to the International Physical Activity Questionnaire (www.ipaq.ki.se [accessed 03/01/2017]) and were weight stable in the three months prior to enrolment (< 2 kg self-reported weight change).

Participant pre-assessment

During a pre-assessment visit, participants were screened to determine eligibility and suitability for exercise testing. Participants underwent full medical evaluation, including assessment of cardiovascular disease risk, fasted capillary blood glucose and completion of the Physical Activity Readiness Questionnaire (www.csep.ca/en/publications [accessed 03/01/2017]). Participants were excluded if blood pressure was greater than 160/100 mmHg, whilst resting and exercise electrocardiograms were performed to confirm the absence of established cardiac arrhythmias. Body fat was assessed using bioelectrical impedance analysis (BC-418, TANITA Europe BV, Amsterdam, The Netherlands) under standardised conditions and waist circumference was measured at the level of the umbilicus.

142

143 On a separate occasion, participants completed a 16-min, progressive sub-maximal exercise
144 test on a motorised treadmill (Excite Med, Technogym, Italy), which was subsequently
145 followed by a ramped peak oxygen uptake test. Expired air was collected continuously
146 throughout both tests for quantification of oxygen consumption ($\dot{V}O_2$) and carbon dioxide
147 production ($\dot{V}CO_2$) using breath-by-breath indirect calorimetry (Metalyser 3B, Cortex,
148 Germany). Peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) was calculated and bivariate linear regression was
149 used to estimate the treadmill speed necessary to elicit 60% of $\dot{V}O_{2\text{ peak}}$ during exercise trials.

150

151 In the 48 h before main trials, participants refrained from strenuous physical activity, alcohol
152 and caffeine, and standardised their dietary intake using weighed records. On the evening
153 before main trials, participants were provided with a standardised meal (3138 kJ; 71%
154 carbohydrate, 18% fat, 11% protein) to consume before 21:00 after which only water was
155 permitted until the start of the trials.

156

157 *Experimental trials*

158 Participants completed two experimental trials, control and exercise, in a counterbalanced
159 order and separated by approximately one week. On the morning of each trial, participants
160 arrived at the laboratory at 08:30, at which point an intravenous cannula (21 G; Venflon,
161 Becton Dickinson, Sweden) was inserted into an antecubital vein. Trials were then initiated
162 with a venous blood sample taken at ~09:00 (0 h) and additional samples were collected at 1,
163 1.5, 2.75, 4 and 7 h. During exercise trials, participants completed a 60-min bout of
164 moderate-intensity treadmill exercise (60% of $\dot{V}O_{2\text{ peak}}$) between 0 and 1 h, and then rested
165 in the laboratory for the remainder of the trial (1-7 h). Heart rate (RS100, Polar, United
166 Kingdom) and rate of perceived exertion (RPE) (Borg 1970) were recorded every 15 min

during exercise, and expired air was collected throughout for ongoing measurement of $\dot{V}O_2$ and $\dot{V}CO_2$. If necessary, treadmill speed was adjusted to maintain the desired exercise intensity. Participants rested for the entirety of control trials and samples of expired air were collected between 0 and 1 h to quantify resting energy expenditure; allowing the calculation of net energy expenditure elicited by exercise. Participants were provided with a standardised breakfast (2690 kJ; 72% carbohydrate, 18% fat, 10% protein) and lunch (3138 kJ; 43% carbohydrate, 25% fat, 32% protein) at 1.5 and 4 h, respectively.

Biochemical analyses

Venous blood samples were collected into ice-cooled monovettes pre-treated with anticoagulant (Sarstedt, Leicester, UK). Monovettes were spun immediately in a refrigerated centrifuge (4 °C) for 10 min at 2383 x g and the plasma supernatant was removed and aliquoted for storage at -80 °C. At each time point, commercially available enzyme-linked immunosorbent assays were used to measure plasma concentrations of FGF21, follistatin, fetuin-A (R & D Systems, Oxford, UK), LECT2 (MBL International, Massachusetts, USA), insulin and glucagon (Mercodia, Uppsala, Sweden), whilst plasma concentrations of full-length SeP were measured using a sol particle homogeneous immunoassay as previously reported (Saito et al. 2001, Tanaka et al. 2016). The mean within-batch co-efficient of variation (CV) for these assays was $\leq 5.5\%$. Circulating concentrations of NEFA, glucose, triacylglycerol (TAG), total cholesterol, liver aminotransferases (AST, ALT) and gamma-glutamyl transpeptidase (GGT) were analysed by enzymatic colorimetric methods using a benchtop analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France; within batch CV $\leq 4.2\%$). All were measured at 0 h only with the exception of plasma NEFA, which was measured at each sample time point. Insulin resistance was assessed by HOMA-IR and Adipo-IR as previously described (Matthews et al. 1985, Gastaldelli et al. 2007).

Plasma volume was calculated in each whole blood sample using established equations (Dill and Costill 1974). Circulating protein concentrations were corrected, as previously described (Sherk et al. 2013), when plasma volume deviated significantly from baseline.

Statistical analyses

Unless otherwise stated, data are presented as mean $\pm$ SD or SEM for participant characteristics and outcome variables, respectively. Statistical analyses were performed using commercially available software (SPSS version 23.0, SPSS Inc., United States). Where appropriate, data were log transformed to meet assumptions for parametric statistical testing. Two-tailed, independent samples *t*-tests were used to compare differences in participant characteristics, fasted plasma protein and metabolite concentrations, and characteristics of the exercise performed between normal weight and overweight/obese groups. When parametric assumptions were not met before or after log transformation, non-parametric Wilcoxon matched-pairs signed rank test was used. Relationships between fasted hepatokine concentrations and other participant characteristics were assessed using bivariate Pearson's and Spearman's correlation analyses as appropriate. Three-way, mixed-design analysis of variance (ANOVA), consisting of two within-participant factors (trial and sample time) and one between-participant factor (group), was used to assess hepatokine responses to exercise. After inspection of main effects, the three-way interaction between trial, time and group was used to assess whether hepatokine responses during and after exercise, when compared to the control trial, differed between normal weight and overweight/obese groups. When this was not significant, the two-way interaction between trial and time was used to investigate the hepatokine response to exercise in the two groups combined. Statistically significant two-way interactions were investigated further with two-tailed paired samples *t*-tests to identify the times at which circulating concentrations differed between control and exercise trials. Due to

217 the sample size in this study, no correction for multiple comparisons was applied. To help
218 clarity in graphical presentation, total area under the concentration-time curve (AUC) was
219 also calculated for each experimental trial and these data were analysed statistically using
220 two-way mixed design ANOVA. Probability (*P*-) values ≤ 0.05 were considered statistically
221 significant.

Results

Participant characteristics

Descriptive characteristics of the normal weight and overweight/obese groups can be found in Table 1. By design, the overweight/obese group had higher BMI, body mass, body fat percentage and waist circumference, but age was similar between groups. There was no difference in absolute cardiorespiratory fitness between groups but relative fitness was greater in the normal weight individuals due to their lower body mass. Fasted plasma glucose, insulin and HOMA-IR were similar between groups, but fasted plasma lipids were greater in the overweight/obese individuals, whilst Adipo-IR tended to be higher. There were no significant differences in AST, ALT or GGT between groups (data not shown).

Insert table 1 here

Fasted plasma hepatokine concentrations and associations with metabolic variables

The overweight/obese individuals had greater fasted plasma concentrations of LECT2 and FGF21, but fasted concentrations of follistatin, fetuin-A and SeP were similar between groups (Table 1). Fasted circulating LECT2 and FGF21 were positively correlated with each other ($\rho^2 = 36.9\%$, $P = 0.03$), body mass, BMI, WC, body fat, NEFA, TAG, Adipo-IR and glucagon ($r^2 \geq 19.4\%$, $P \leq 0.02$ or $\rho^2 \geq 17.6\%$, $P \leq 0.05$), and negatively with relative $\dot{V}O_2$ peak ($r^2 \geq 27.0\%$, $P \leq 0.01$). FGF21 was also marginally positively correlated with fasted plasma glucose ($r^2 = 18.1\%$, $P = 0.048$), whilst LECT2 was strongly positively correlated with HOMA-IR ($\rho^2 = 43.2\%$, $P = 0.001$). Significant negative correlations were found between fasted concentrations of follistatin and the AST:ALT ratio ($r^2 = 18.5\%$, $P = 0.05$), fetuin-A and age ($\rho^2 = 25\%$, $P = 0.02$), and between fasted concentrations of SeP and ALT

($r^2 = 19.4\%$, $P = 0.04$). Further details of all significant correlations can be found in supplementary material.

Exercise characteristics

Participants in the normal weight group exercised at a greater treadmill speed due to their higher relative cardiorespiratory fitness. However, the relative intensity of the exercise performed was similar between groups (Table 2). Consequently, given the higher energy cost of exercise in the overweight/obese group as a result of their higher body mass, the net energy expenditure during exercise trials was similar between groups ($P = 0.98$). During the exercise trials, there was a significant reduction in plasma volume immediately post-exercise irrespective of group (0 vs 1 h: 58.5 ± 0.7 vs. $53.7 \pm 0.7\%$; $P < 0.01$), which returned to baseline by 1.5 h.

Insert table 2 here

Circulating hepatokine responses to exercise

Plasma FGF21 concentrations were higher in the overweight/obese group, irrespective of trial or time (Figure 1a; $P = 0.003$), but there was no interaction between trial, time and group ($P = 0.19$). With groups combined, there was a significant two-way interaction between trial and time for circulating FGF21 (Figure 2a; $P < 0.001$), and post-hoc analyses revealed that circulating concentrations were significantly higher at 1, 1.5 and 4 h in the exercise trial, compared with control (all $P \leq 0.005$). Accordingly, the total AUC for FGF21 was significantly greater in the overweight/obese compared with the normal weight individuals (Figure 1b; $P = 0.003$), and in the exercise versus the control trials (Figure 2b; $P = 0.003$). However, there was no interaction between group and trial ($P = 0.65$).

Circulating follistatin was lower in the overweight/obese versus the normal weight group (Figure 1c; $P = 0.05$), but the interaction between trial, time and group was not significant ($P = 0.94$). In the whole study population, there was a significant two-way interaction between trial and time for plasma follistatin (Figure 2c; $P = 0.001$). Post-hoc analyses identified significantly higher concentrations at 2.75, 4 and 7 h in the exercise trial ($P \leq 0.02$). Similarly, the total AUC for follistatin was significantly lower in the overweight/obese group (Figure 1d; $P = 0.05$) and greater in the exercise trials (Figure 2d; $P < 0.01$), but there was no interaction between group and trial ($P = 0.41$).

Circulating LECT2 was higher in the overweight/obese group versus the normal weight group, (Figure 1e; $P = 0.009$), but there was no interaction between trial, time and group ($P = 0.38$). For plasma concentrations of LECT2, the two-way interaction between trial and time in the whole study population was also not significant (Figure 2e; $P = 0.06$). The total AUC analyses for LECT2 mirrored these results with a significantly greater AUC in the overweight/obese group (Figure 1f; $P < 0.01$), but no significant difference between the control and exercise trials (Figure 2f; $P = 0.07$) and no interaction between group and trial ($P = 0.45$).

Circulating concentrations of fetuin-A and SeP were similar between groups (Figures 1g and 1i; $P \geq 0.20$) and there were no interactions between trial, time and group ($P \geq 0.07$). Furthermore, with groups combined, there were no interactions between trial and time (Figures 2g and 2i; $P \geq 0.11$). In accordance, the total AUC for fetuin-A (Figures 1h and 2h) and SeP (Figures 1j and 2j) were similar between groups and between trials, and there were no significant interactions (all $P \geq 0.17$).

Insert figure 1

Insert figure 2

Circulating responses of NEFA, glucagon and insulin to exercise

Despite differences in the fasted state, circulating concentrations of NEFA were similar between groups throughout experimental trials ($P = 0.09$), and there was no significant interaction between trial, time and group ($P = 0.13$). However, with groups combined there was a significant two-way interaction between trial and time (Figure 3a; $P < 0.001$) and post-hoc analyses revealed significantly higher concentrations of NEFA in the exercise trial at 1, 1.5, 2.75 and 7 h ($P \leq 0.04$).

Circulating concentrations of glucagon, insulin and glucagon to insulin ratio were also similar between groups ($P \geq 0.27$), and the three-way interactions between trial, time and group were not significant for any of these outcomes ($P \geq 0.16$). However, in the whole study population, there were significant two-way interactions between trial and time for all of glucagon, insulin and the glucagon to insulin ratio (Figure 3b-d; $P \leq 0.03$). Post-hoc tests revealed the glucagon to insulin ratio was significantly greater at 1 and 1.5 h in the exercise trial when compared to the control trial ($P \leq 0.02$). This was primarily driven by significantly higher concentrations of glucagon ($P \leq 0.01$) and occurred despite significantly higher concentrations of insulin at 1.5 h in the exercise trial ($P = 0.02$). Glucagon remained elevated in the exercise trial at 2.75 h ($P = 0.03$) but the consequential increase in the glucagon to insulin ratio was not statistically significant ($P = 0.08$).

Discussion

This study investigated the impact of adiposity and acute exercise on five candidate hepatokines which have been identified as novel circulating proteins linking the liver and peripheral metabolism. Our findings suggest that circulating levels of LECT2, FGF21 and follistatin are modulated by adiposity and are associated with various anthropometric measurements and biomarkers of metabolic health. Additionally, our findings show that circulating levels of FGF21 and follistatin are transiently elevated after a single bout of moderate-intensity exercise, and these responses are preserved in overweight/obese individuals. These responses may help mediate the favourable metabolic impact of exercise, but further research is needed to assess causality.

Previous reports have shown increased LECT2 in obese individuals, with or without nonalcoholic fatty liver disease (NAFLD), in two large Japanese cohorts (Okumura et al. 2013, Lan et al. 2014). The current study is the first, however, to show that LECT2 is elevated in European men that are overweight/obese and correlates with BMI in a population of normal weight, overweight and obese individuals. In mice, hepatic expression and circulating concentrations of LECT2 are negatively regulated by hepatic AMPK (Lan et al. 2014). Furthermore, eight weeks of high-fat overfeeding increased circulating concentrations of LECT2, alongside increases in body mass (Lan et al. 2014). As such, circulating LECT2 may be increased in overweight and obese individuals due to chronic reduction of hepatic AMPK activity resulting from sustained energy surplus. In agreement with previous studies (Okumura et al. 2013, Lan et al. 2014), we show significant associations between LECT2 and fasted plasma insulin and HOMA-IR, whilst we also report, for the first time, significant correlations between fasted concentrations of LECT2, NEFA and Adipo-IR. LECT2 has been shown to inhibit insulin signalling in C2C12 myotubes via activation of Jun NH₂-terminal

kinase (JNK) (Lan et al. 2014) but its effects on other peripheral tissues, including hepatic and adipose tissues, warrant further investigation.

Previous reports have shown that fasted concentrations of FGF21 are elevated in obese individuals with normal or dysregulated glucose metabolism (Zhang et al. 2008, Chen et al. 2011). Our findings are in agreement with these studies and show that FGF21 is also increased in individuals that are overweight/obese. Hepatic expression and secretion of FGF21 is increased during periods of starvation via the activation of peroxisome proliferator-activated receptor alpha (PPAR- α) by circulating NEFA (Badman et al. 2007). Plasma concentrations of NEFA are also increased with obesity (Boden 2008), offering a potential mechanism to explain the increased FGF21 concentrations seen in the present study. In support of this, we report elevated fasted concentrations of NEFA in the overweight/obese group and a strong positive correlation between circulating concentrations of FGF21 and NEFA. Alternatively, a state of 'FGF21 resistance' may also result in elevated concentrations of FGF21 group (Potthoff et al. 2012) but it was beyond the scope of this study to investigate this hypothesis.

Despite no statistically significant difference in the fasted state, we showed lower concentrations of follistatin in the overweight/obese group throughout trial days. Our findings are consistent with previous data which identified lower follistatin levels in obese individuals with T2DM (Ueland et al. 2012), yet contrast those of Hansen *et al.* (2013) who identified higher follistatin in T2DM patients. The reasons for these discrepancies are not clear at this time and further work is therefore needed to more fully understand the metabolism of follistatin in health and disease.

We report no differences between groups in concentrations of fetuin-A or SeP either in the fasted state or throughout trial days. This may suggest that the development of metabolic complications, and not adiposity *per se*, may be required to disrupt fetuin-A and SeP metabolism. Previous research has found no independent effect of obesity on fetuin-A concentrations (Obuchi et al. 2014), whilst studies reporting differences in SeP have recruited individuals with NAFLD or dysregulated glucose metabolism (Yang et al. 2011, Choi et al. 2013).

In the current study we demonstrate that circulating concentrations of FGF21 are increased immediately after an acute 60-min bout of moderate-intensity aerobic exercise, peaking 30 min after the cessation of exercise, and remaining elevated for up to 3 h. A similar, albeit delayed, increase in circulating follistatin also occurred. These findings support previous studies showing that FGF21 and follistatin are increased with acute aerobic exercise (Slusher et al. 2015, Hansen et al. 2016a, 2016b). FGF21 and follistatin production are positively regulated by the glucagon to insulin ratio (Hansen et al. 2016a, 2016b), whilst FGF21 may also be increased via activation of PPAR- α by circulating NEFA (Kim et al. 2013, Hansen et al. 2016a). The systemic glucagon to insulin ratio and circulating NEFA were both elevated in response to exercise in the present study. FGF21 improves glucose metabolism in skeletal muscle, adipose tissue and the liver, whilst follistatin may promote pancreatic beta cell survival, reduce circulating glucagon and preserve skeletal muscle mass (Camporez et al. 2013, Hansen et al. 2016b). Transient increases in the circulating levels of these hepatokines may represent potential mechanisms which contribute to the short-term improvements in glycaemic control after acute exercise (SyLOW et al. 2017).

In the current study the responses of circulating FGF21 and follistatin to exercise were similar in both normal weight and overweight/obese participants. It has been previously shown that the FGF21 and follistatin responses to acute exercise are blunted in individuals with T2DM, and this may be the result of differences in the exercise-induced changes in circulating NEFA and the glucagon to insulin ratio (Hansen et al. 2016a). Furthermore, the response of FGF21 to 30 min of exercise at 75% $\dot{V}O_2$ peak has been shown to be reduced in obese individuals compared with healthy, normal weight controls (Slusher et al. 2015). Notably, although the participants in the study by Slusher and colleagues (Slusher et al. 2015) were reportedly healthy, the obese group had a mean HOMA-IR of 4.36, which approaches the 5.13 threshold previously used to distinguish insulin resistant individuals (Wildman et al. 2008). The participants in the current study were free from chronic disease and fasted plasma glucose, insulin and HOMA-IR suggested they were not insulin resistant. It could, therefore, be speculated that exercise-induced increases in circulating FGF21 and follistatin are maintained in overweight/obese individuals with preserved glycaemic control but not once a degree of insulin resistance has developed. Notably, the exercise-induced changes in NEFA and the glucagon to insulin ratio were no different between groups in the current study. This speculative hypothesis, however, should be tested further.

The current study is the first to investigate the acute effects of aerobic exercise on fetuin-A, LECT2 and SeP, but these hepatokines were unaffected by exercise. Fetuin-A, LECT2 and SeP are all negatively regulated by hepatic AMPK (Jung et al. 2013, Lan et al. 2014, Trepanowski et al. 2014) which is activated by exercise in an intensity-dependent manner (Camacho et al. 2006). It may be that higher intensity exercise is required to acutely modulate fetuin-A, LECT2 or SeP (Trepanowski et al. 2014), or that repeated bouts of exercise are required to elicit benefits; as shown previously for fetuin-A (Malin et al. 2013, 2014).

421

422 This study is not without limitation. Most prominently, this trial was conducted using a
423 relatively small sample of normal weight and overweight/obese men. Given the lack of prior
424 evidence we were unable to determine *a priori* whether our sample size was sufficient for all
425 of our outcomes. The novel data presented in this manuscript may, however, be utilised to
426 inform power calculations for future studies, particularly those investigating the effects of
427 acute exercise on LECT2, SeP and fetuin-A. Our results also cannot be generalised to
428 individuals with chronic metabolic disease or women. Notably, we did not directly measure
429 intrahepatic fat or insulin sensitivity in the current study. These are important considerations
430 because the development of metabolic disease may influence hepatokine metabolism.
431 Furthermore, the heightened propensity for fatty liver development in men, and the potential
432 metabolic influence of sex hormones, underscores the necessity for additional research to be
433 undertaken in women.

434

435 In conclusion, this study has identified higher circulating concentrations of FGF21 and
436 LECT2, and lower follistatin, in overweight/obese men when compared to normal weight
437 individuals. Moreover, circulating FGF21 and follistatin are acutely increased after moderate-
438 intensity aerobic exercise, and this beneficial shift in hepatokine profile is similar in both
439 groups. These data provide new information regarding the effect of adiposity on the
440 metabolism of several novel hepatokines and supports evidence for a potential role of FGF21
441 and follistatin in the metabolic response to exercise. Additional work is needed to better
442 understand the interaction between these novel proteins, obesity and chronic disease; as well
443 as to better define their interaction with exercise and other metabolic perturbations.

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450

451 **Disclosures**

452 The authors declare no conflicts of interest.

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574 Table 1 – Participant Characteristics

	Normal weight	Overweight/obese
	(n=11)	(n=11)
<i>Anthropometry</i>		
BMI (kg•m ⁻²) ^a	23.4 (1.6)	29.2 (4.5) [‡]
Age (years)	36 ± 15	45 ± 14
Body weight (kg)	69.8 ± 1.5	92.3 ± 3.4 [‡]
Body fat (%)	16.9 ± 3.6	26.4 ± 4.0 [‡]
Waist circumference (cm)	81.6 ± 5.3	96.0 ± 7.8 [‡]
<i>Cardiorespiratory Fitness</i>		
Absolute $\dot{V}O_2$ peak (L•min ⁻¹)	3.46 ± 0.74	3.21 ± 1.21
Relative $\dot{V}O_2$ peak (mL•kg BW ⁻¹ •min ⁻¹)	50.1 ± 11.9	38.5 ± 9.7 [†]
<i>Circulating Metabolic Risk Factors</i>		
Total cholesterol (mmol•L ⁻¹)	4.12 ± 0.73	4.91 ± 0.89*
TAG (mmol•L ⁻¹)	1.04 ± 0.15	1.82 ± 0.24 [†]
NEFA (mmol•L ⁻¹)	0.39 ± 0.18	0.58 ± 0.14 [†]
FPG (mmol•L ⁻¹)	4.9 ± 0.2	5.0 ± 0.3
FPI (pmol•L ⁻¹)	30.0 ± 12.2	37.1 ± 19.3
<i>Insulin Sensitivity</i>		
HOMA-IR	0.95 ± 0.39	1.21 ± 0.67
Adipo-IR	12.57 ± 8.99	21.98 ± 13.01
<i>Hepatokines</i>		
FGF21 (pg•mL ⁻¹)	83 ± 55	190 ± 74 [‡]
Follistatin (pg•mL ⁻¹)	795 ± 257	670 ± 154
LECT2 (ng•mL ⁻¹)	31 ± 10	48 ± 17 [†]
Fetuin-A (µg•mL ⁻¹)	541 ± 137	497 ± 99
SeP (µg•mL ⁻¹)	3.01 ± 0.39	2.81 ± 0.30

575 BMI: Body mass index; $\dot{V}O_2$ peak: Peak oxygen uptake; TAG: Fasted triacylglycerol; NEFA:
576 Fasted non-esterified fatty acids; FPG: Fasted plasma glucose; FPI: Fasted plasma insulin;
577 HOMA-IR: Homeostatic model assessment of insulin resistance; Adipo-IR: Adipose tissue
578 insulin resistance index; FGF21: Fibroblast growth factor 21; LECT2: Leukocyte cell-derived
579 chemotaxin 2; SeP: Selenoprotein P. ^a Heterogeneous variance between groups, non-
580 parametric analyses performed and data presented as median (interquartile range). All other
581 data presented as mean $\pm$ SD. Symbols indicate statistically significant differences between
582 groups (* < 0.05; † $\leq$ 0.01; ‡ < 0.001).

583 **Table 2 – Exercise Characteristics**

	Normal weight	Overweight/obese
	(n=11)	(n=11)
Treadmill speed (km•h ⁻¹)	7.6 ± 1.0	6.8 ± 1.0*
$\dot{V}O_2$ elicited (% $\dot{V}O_2$ peak)	59.3 ± 2.8	57.9 ± 2.3
Net energy expenditure (kJ)	2211 ± 507	2217 ± 509
Heart rate (beats•min ⁻¹)	141 ± 29	139 ± 19
Rate of perceived exertion (6-20)	13 ± 1	12 ± 1

584 $\dot{V}O_2$: oxygen uptake; $\dot{V}O_2$ peak: peak oxygen uptake. Data presented as mean ± SD. * indicates
585 a statistically significant difference between groups ($P < 0.05$).

Figure captions

Figure 1 – Hepatokine responses in normal weight and overweight/obese groups.

Circulating plasma concentrations FGF21 (a-b), follistatin (c-d), LECT2 (e-f), fetuin-A (g-h) and SeP (i-j) during control and exercise trials in both normal weight and overweight/obese groups. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean $\pm$ SEM. AUC represents the total area under the concentration-time curve for the given experimental day. *P*-values denote significant main effect of group irrespective of time or trial.

Figure 2 – Hepatokine exercise responses with groups combined to a single population.

Circulating plasma concentrations FGF21 (a-b), follistatin (c-d), LECT2 (e-f), fetuin-A (g-h) and SeP (i-j) during control and exercise trials in the whole study population combined. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean $\pm$ SEM. AUC represents the total area under the concentration-time curve for the given experimental day. * indicates significant difference from control trial at the same time point (all $P \leq 0.02$). *P*-values on AUC plots denote significant difference between control and exercise trials.

Figure 3 – Responses in NEFA, glucagon and insulin with groups combined to a single

population. Circulating plasma concentrations (a) NEFA (b) glucagon (c) insulin and (d) the glucagon to insulin ratio during control and exercise trials in the whole study population combined. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean $\pm$ SEM. * indicates significant difference from control trial at the same time point (all $P \leq 0.04$).

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