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Scott A Willis[1,2], Jack A Sargeant[1,2], Alice E Thackray[1,2], Thomas Yates[2,3], David J Stensel[1,2], Guruprasad P Aithal[4,5], James A King[1,2].

[1]National Centre for Sport and Exercise Medicine, School of Sport, Exercise and Health Sciences, Loughborough University, UK

[2]NIHR Leicester Biomedical Research Centre, UK

[3]Diabetes Research Centre, University of Leicester, UK

[4]Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, UK

[5]NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, UK

Authors' email addresses

Scott A Willis - S.Willis2@lboro.ac.uk

Jack A Sargeant - js928@leicester.ac.uk

Alice E Thackray - A.E.Thackray@lboro.ac.uk

Thomas Yates – ty20@leicester.ac.uk

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

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

James A King - J.A.King@lboro.ac.uk

Address for correspondence

Dr James King

School of Sport, Exercise and Health Sciences

Loughborough University

Leicestershire

United Kingdom

LE11 3TU

Phone: +44(0)1509 228457

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

Abstract

Fibroblast growth factor 21 (FGF21), follistatin and leukocyte cell-derived chemotaxin 2 (LECT2) are novel hepatokines which are modulated by metabolic stresses. This study investigated whether exercise intensity modulates the hepatokine response to acute exercise.

Ten young, healthy men undertook three 8-h experimental trials: moderate-intensity exercise (MOD; 55% $\dot{V}O_2$ peak), high-intensity exercise (HIGH; 75% $\dot{V}O_2$ peak) and control (CON; rest), in a randomised, counterbalanced order. Exercise trials commenced with a treadmill run of varied duration to match gross exercise energy expenditure between trials (MOD vs HIGH; 2475 ± 70 vs 2488 ± 58 kJ). Circulating FGF21, follistatin, LECT2, glucagon, insulin, glucose and non-esterified fatty acids (NEFA) were measured before exercise and at 0, 1, 2, 4 and 7 h post-exercise.

Plasma FGF21 concentrations were increased up to 4 h post-exercise compared to CON ($P \leq 0.022$) with greater increases observed at 1, 2 and 4 h post-exercise during HIGH vs MOD ($P \leq 0.025$). Irrespective of intensity ($P \geq 0.606$), plasma follistatin concentrations were elevated at 4 and 7 h post-exercise ($P \leq 0.053$). Plasma LECT2 concentrations were increased immediately post-exercise ($P \leq 0.046$) but were not significant after correcting for plasma volume shifts. Plasma glucagon (1 h; $P = 0.032$) and NEFA (4 and 7 h; $P \leq 0.029$) responses to exercise were accentuated in HIGH vs MOD.

These findings demonstrate that acute exercise augments circulating FGF21 and follistatin. Exercise-induced changes in FGF21 are intensity-dependent and may support the greater metabolic benefit of high-intensity exercise.

Key words: exercise, physical activity, hepatokines, liver, FGF21, follistatin, LECT2, insulin resistance

Introduction

The liver plays an integral role in the regulation of metabolic homeostasis through inter-organ crosstalk with other metabolically active tissues (Iroz et al. 2015, Meex and Watt 2017). In recent years, several exclusively or predominantly liver-secreted proteins, termed hepatokines, have been identified as molecules possessing the capacity to exert potent metabolic effects, both locally and distally (Meex et al. 2015). These include fibroblast growth factor 21 (FGF21), shown to modulate glucose and lipid metabolism (Kharitonenkov et al. 2005, Potthoff et al. 2009, Xu et al. 2009, BonDurant and Potthoff 2018); follistatin, demonstrated to regulate muscle growth (Amthor et al. 2004, Yaden et al. 2014) and influence pancreatic beta cell function and survival (Zhao et al. 2015, Hansen et al. 2016a); and leukocyte cell-derived chemotaxin 2 (LECT2), which can promote both skeletal muscle and adipose tissue insulin resistance (Lan et al. 2014, Jung et al. 2018).

Chronic positive energy balance (Toye et al. 2007, Kirpich et al. 2011), hepatic steatosis (Meex et al. 2015) and obesity-associated metabolic disease, modulate hepatic protein synthesis and hepatokine secretion (Fu et al. 2012; Meex et al. 2015). Recent research suggests that hepatokines may also be sensitive to acute perturbations in energy and substrate metabolism through coordinating metabolic responses between the liver and extra-hepatic tissues (Xu et al. 2009, Lan et al. 2014, Hansen and Plomgaard 2016). For example, hepatokines are implicated in the coordination of metabolic responses to both fasting and feeding (Lan et al. 2014, Markan et al. 2014). Furthermore, hepatokines may participate in metabolic responses to exercise; potentially contributing to the acute metabolic benefit associated with physical exertion such as improved whole-body substrate metabolism and insulin sensitivity (Kim et al. 2013, Hansen et al. 2015, Weigert et al. 2018).

77 To date, a handful of studies have examined the acute effects of exercise on circulating
78 hepatokines. This research has shown that exercise increases circulating levels of FGF21 and
79 follistatin (Hansen et al. 2011, Kim et al. 2013, Hansen et al. 2015, Hansen et al. 2016a, Hansen
80 et al. 2016b, Sargeant et al. 2018), whilst circulating LECT2 is suppressed after exercise in
81 mice (Lan et al. 2014) but is unaffected in humans (Sargeant et al. 2018). Exercise intensity is
82 a key variable which mediates the metabolic benefit of acute exercise, with protocols of higher
83 intensity typically providing the greatest therapeutic effects (Wahren et al. 1978, Wen et al.
84 2011). Given that FGF21, follistatin and LECT2 responses to exercise are mediated via
85 metabolic factors sensitive to exercise intensity (for example, glucagon, insulin and non-
86 esterified fatty acids) (Hansen et al. 2015, Hansen et al. 2016a), it is possible that exercise
87 intensity may also modulate circulating hepatokines.

88 One previous study explored this hypothesis, demonstrating that circulating FGF21 was higher
89 in healthy men, one hour after 30 minutes of treadmill running at 80% vs 50% of maximum
90 oxygen uptake (Kim et al. 2013). This study was, however, limited by the fact that the exercise
91 protocols were not matched for energy expenditure and no control trial was included.
92 Consequently, further research is required to explore the impact of exercise intensity on
93 circulating hepatokines by matching exercise-related energy expenditure between exercise
94 protocols of different intensities; and to compare responses against a non-exercise control trial.

95 Therefore, the present study investigated the effect of exercise intensity, using two energy-
96 matched exercise trials, on circulating concentrations of FGF21, follistatin and LECT2 in
97 healthy men. Based on previous findings, it was hypothesised that both exercise intensities
98 would lead to transient increases in circulating FGF21 and follistatin, whilst circulating LECT2
99 levels would be reduced following exercise. Furthermore, these responses would be more
100 pronounced after the performance of high- vs moderate-intensity exercise.

Materials and methods

Participants

Following approval from the Institutional Ethical Advisory Committee (ref: R16-P067), 10 healthy men participated in the study. Participant characteristics are presented in Table 1. All participants were non-smokers, metabolically healthy, weight stable for three months prior to participation (< 2 kg body mass change) and were not currently taking any medications. Written informed consent was provided by each participant before data collection commenced.

Insert table 1

Participant pre-assessment

Participants attended an initial pre-assessment session in which they completed a health screen questionnaire, the Physical Activity Readiness Questionnaire (PAR-Q+; Bredin et al. 2013) and food preference questionnaire, before undergoing anthropometric measurements. Height and body mass were measured using a wireless measuring station (Seca 285, Seca Ltd, Germany) and body mass index (BMI) was subsequently calculated. Waist circumference was measured at the smallest circumference between the xiphoid process and the iliac crest, whilst body composition was determined using bio-electrical impedance analysis (BC-418, TANITA, Europe BC, Amsterdam, the Netherlands).

Participants then undertook a submaximal incremental exercise test on a motorised treadmill (Technogym Excite Med, Cesena, Italy), consisting of four, 4-min stages at progressively faster speeds, to determine the relationship between treadmill speed and oxygen consumption ($\dot{V}O_2$)

for each participant. The first stage commenced at a speed between 7-8 $km \cdot h^{-1}$, with each subsequent stage increasing by 1-2 $km \cdot h^{-1}$, according to participant fitness. Expired air was sampled continuously using breath-by-breath indirect calorimetry (Cortex Metalyzer 3B, Leipzig, Germany) to quantify $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$). Heart rate was recorded continuously using short-range telemetry (Polar T31; Polar Electro, Kempele, Finland), whilst rating of perceived exertion (RPE; Borg. 1973) was assessed in the last 30 s of each stage.

After a standardised rest period of 10 min, participants then completed an incremental maximal exercise test to determine peak oxygen uptake ($\dot{V}O_2$ peak). The test commenced at a gradient of 0% and increased by 1% every minute until volitional exhaustion. The maximal exercise test was designed to bring the participants to exhaustion within approximately 8-12 min and the treadmill speed was determined by the individual performance of each participant in the submaximal exercise test. The breath-by-breath data were analysed using a 30 s average (Robergs et al. 2010) to identify the participants' $\dot{V}O_2$ peak.

Experimental design

Participants completed three main experimental trials: a moderate-intensity (MOD) trial, a high-intensity (HIGH) trial and a control (CON) trial. The main trials were carried out in a randomised, counterbalanced order, with at least five days separating each trial. In the 24 h prior to each main trial, participants were asked to standardise their food intake using a weighed food record and to abstain from exercise, caffeine and alcohol. Each main trial lasted 8 h (08:00-16:00) as shown schematically in Figure 1.

Insert figure 1

146

147 ***Experimental protocol***

148 On the day of the main trials, participants arrived at the laboratory at 08:00 following an
149 overnight fast of at least 10 h. During MOD and HIGH trials, participants performed a
150 continuous treadmill run at moderate-intensity (55% $\dot{V}O_2$ peak) and high-intensity (75% $\dot{V}O_2$
151 peak), respectively, resting thereafter. Participants rested throughout the CON trial. Both
152 exercise protocols were designed to elicit a gross energy expenditure of 2510 kJ (600 kcal);
153 meaning the exercise duration differed between MOD and HIGH. The treadmill speed required
154 to elicit the target intensities was predicted via bivariate linear regression using participants'
155 $\dot{V}O_2$ peak and $\dot{V}O_2$-running speed relationship determined in the pre-assessment session. The
156 rate of energy expenditure for each trial was then calculated using equations by Frayn (1983)
157 and the exercise duration required to induce a gross energy expenditure of 2510 kJ was
158 subsequently estimated. Expired air was sampled continuously during exercise to determine
159 the actual gross energy expenditure and percentage substrate oxidation of the exercise period.
160 The speed of the treadmill was adjusted, if necessary, to maintain the target $\dot{V}O_2$. Heart rate
161 was monitored continuously throughout; whilst RPE was recorded every 10 min. Exercise
162 commenced between 08:00 and 09:00 in a staggered fashion so that exercise finished at 09:00.

163 A standardised breakfast (jam sandwich with butter, strawberry breakfast bar, banana and
164 chocolate milkshake) was provided to the participants at 2 h post-exercise (11:00), containing
165 an energy content equal to 35% of the participants' estimated daily energy requirement and a
166 macronutrient content of 69% carbohydrate, 14% protein and 17% fat. Individuals' daily
167 energy requirements were calculated from their estimated basal metabolic rate (BMR), which
168 was multiplied by a physical activity correction factor of 1.4 (Mifflin et al. 1990) to reflect the
169 sedentary nature of prolonged sitting in the laboratory during the main trials. A standardised

lunch (cheese sandwich with butter, salted crisps, mini chocolate roll and apple) was then provided at 6 h post-exercise (15:00), also containing 35% of the participants' estimated daily energy requirement (42% carbohydrate, 13% protein and 45% fat).

Blood sampling

Venepuncture blood samples were taken from an antecubital vein upon arrival (baseline; 08:00) and immediately after exercise (0 h; 09:00). At approximately 0.5 h, a cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein for further venous blood sample collection at 1, 2, 4 and 7 h post-exercise. Following each collection, the cannula was flushed with 10 mL of saline (0.9% w/v sodium chloride, Fannin Ltd, Dublin, Ireland) to maintain patency. To avoid dilution of subsequent samples, residual saline was drawn off immediately prior to each sample collection using a 2 mL syringe. All blood samples were taken with the participant in a semi-supine position to control for any postural changes in plasma volume.

Blood samples were collected into pre-chilled EDTA and lithium heparin monovettes (Sarstedt, Leicester, UK). All monovettes were centrifuged at 1750 *g* for 10 min at 4˚C (Heraeus Labofuge 400R, Thermo Electron, Osterode, Germany) and the resultant plasma supernatant was aliquoted into cryovials. All samples were stored at -80˚C until subsequent (batch) analysis.

Haemoglobin and haematocrit concentrations were determined from each sample using 20 µL micropipettes and 20 µL heparinised microhaematocrit tubes. These measures were taken in duplicate and triplicate, respectively; and were used for subsequent estimation of changes in plasma volume (Dill and Costill 1974).

Biochemical analyses

Commercially available enzyme-linked immunosorbent assays were used to determine plasma concentrations of FGF21(DF2100, R&D Systems, Oxford, UK), follistatin (DFN00, R&D Systems, Oxford UK), LECT2 (5327, MBL International, Massachusetts, USA), insulin (10-113-01, Mercodia AB, Uppsala, Sweden) and glucagon (10-1271-01, Mercodia AB, Uppsala, Sweden). Samples from each participant were analysed on the same assay plate to eliminate inter-assay variation. The mean within-batch coefficients of variation (CVs) for these assays was ≤ 6.0%. Plasma glucose (A11A01668, Horiba Medical, Montpellier, France) and non-esterified fatty acids (NEFA; FA115, Randox Laboratories Ltd., County Antrim, UK) were determined using enzymatic, colorimetric methods using a bench-top analyser (Pentra 400; Horiba Medical, Montpellier, France). The mean within-batch CVs for plasma glucose and NEFA were 0.43% and 1.95%, respectively.

Sample size calculation

Based on published data by our research group examining the effects of moderate-intensity exercise on the total area under the curve (AUC) for FGF21 in normal-weight healthy men (mean difference ± SD: 286 ± 340 $pg \cdot mL^{-1} \cdot 7\ h^{-1}$, giving a standardised difference of 0.84, and with an intra-person correlation of 0.76) (Sargeant et al. 2018), we required nine participants to complete our three-trial cross-over study to detect a main effect of trial for FGF21 total AUC, with 90% power and an alpha error rate of 0.05. To account for participant drop-out or non-compliance, 10 individuals were recruited. Notably, based on similar data from our previous study for follistatin total AUC (mean difference ± SD: 630 ± 942 $pg \cdot mL^{-1} \cdot 7\ h^{-1}$, giving a standardised difference of 0.67, and with an intra-person correlation of 0.88), we also had over 90% power to detect a main effect of trial on follistatin total AUC in the current study.

Statistical analyses

All statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, Illinois). The preprandial (baseline-2 h), postprandial (2-7 h) and total AUC were calculated using the trapezoidal method for all outcomes during each trial. Shapiro-Wilk tests were used to assess the distribution of the data and, when not normally distributed, data were log transformed and reassessed. Differences between exercise characteristics were assessed using paired *t*-tests. One-way repeated-measures analysis of variance (ANOVA) was used to examine differences between trials in baseline concentrations and AUC values for each analyte. Two-way, repeated-measures ANOVA (within-participant factors: trial and time) was used to assess differences in hepatokine and metabolite responses over the duration of the different trials. Where a significant trial and interaction effect was observed, *post-hoc* analyses were performed using one-way ANOVAs and subsequent pairwise comparisons at each time point to locate any differences. A Bonferroni adjustment for multiple comparisons was then applied. If Mauchly's test of sphericity was violated, Greenhouse-Geisser corrected values were used. Correction of FGF21, follistatin, glucose, NEFA, insulin and glucagon data for plasma volume changes did not alter the statistical significance of findings, therefore unadjusted values are presented. The statistical outcomes for LECT2 were altered by plasma volume adjustment, therefore statistical outcomes are presented for both adjusted and unadjusted data. An alpha value of $P < 0.05$ was set to indicate statistical significance. Data are presented as means ± SD or SEM, where indicated.

Results

Exercise characteristics

The characteristics for the MOD and HIGH exercise protocols are presented in Table 2. Treadmill speed, oxygen uptake, and relative exercise intensity were greater during HIGH compared to MOD (all $P \leq 0.001$). However, the exercise duration was shorter during HIGH than MOD ($P < 0.001$); resulting in similar gross energy expenditures between the two exercise protocols ($P = 0.459$). Respiratory exchange ratio (RER) and percentage carbohydrate oxidation were significantly higher, and percentage fat oxidation significantly lower, in HIGH compared with MOD (all $P \leq 0.001$). Heart rate and RPE were also greater in HIGH in comparison to MOD (both $P \leq 0.001$).

Insert table 2

Hepatokine responses to exercise

Baseline plasma concentrations of FGF21 were similar between trials ($P = 0.235$). Two-way ANOVA showed a significant main effect of trial and trial by time interaction for plasma FGF21 concentrations (both $P < 0.001$; Figure 2A). *Post-hoc* analyses revealed significantly higher plasma FGF21 concentrations during MOD compared to CON at 0, 1 and 2 h; and during HIGH compared to CON at 1, 2 and 4 h (all $P \leq 0.017$). Additionally, plasma FGF21 concentrations were significantly higher during HIGH vs MOD at 1, 2 and 4 h (all $P \leq 0.025$). Table 3 details the preprandial (baseline-2 h), postprandial (2-7 h) and total FGF21 AUC.

255 Compared with control, the FGF21 AUC was higher preprandially in MOD and HIGH ($P \leq$
256 0.009) yet remained elevated postprandially only in HIGH ($P = 0.001$).

257 Boxplot analysis of total trial AUC values for plasma follistatin identified one participant as an
258 outlier (Field 2009). This was due to the participant exhibiting mean plasma follistatin
259 concentrations during HIGH which were 13 times greater than the remaining group SD on the
260 same trial. Consequently, all data for this participant were removed from follistatin analyses
261 and thus results are presented for $n = 9$. No differences were observed in fasting plasma
262 follistatin concentrations between trials at baseline ($P = 0.138$). Two-way ANOVA showed a
263 significant main effect of trial, time and trial by time interaction for plasma follistatin
264 concentrations (all $P \leq 0.002$; Figure 2B). *Post-hoc* analyses revealed that when compared to
265 CON, plasma follistatin concentrations tended to be higher at 4 h ($P = 0.053$) and were
266 significantly higher at 7 h during MOD ($P = 0.007$). Furthermore, plasma follistatin
267 concentrations were significantly higher at 4 and 7 h during HIGH compared to CON (both P
268 ≤ 0.003). Examination of AUC data showed that circulating follistatin concentrations were
269 greater in HIGH vs CON postprandially ($P = 0.003$; Table 3).

270 Baseline plasma concentrations of LECT2 did not differ between trials ($P = 0.108$). Two-way
271 ANOVA showed a significant main effect of time and trial by time interaction for plasma
272 LECT2 concentrations (both $P \leq 0.004$; Figure 2C). *Post-hoc* analyses identified elevated
273 plasma LECT2 concentrations immediately post-exercise during MOD and HIGH trials
274 compared to CON (both $P \leq 0.046$), with a similar response between exercise trials; however,
275 following adjustment for plasma volume changes across the trials, statistical significance did
276 not remain (both $P \geq 0.243$). No differences in AUC values were observed between trials (Table
277 3).

278

279 ***Insert figure 2***

280

281 ***Insert table 3***

282

283 ***Hormone and metabolite responses to exercise***

284 Plasma hormone and metabolite responses at baseline and after exercise are shown in Figure 3.
285 Baseline plasma concentrations of glucose, NEFA and glucagon were not statistically different
286 between trials (all $P \geq 0.124$). However, plasma insulin was significantly higher and the
287 glucagon to insulin ratio was significantly lower at baseline in MOD compared to CON ($P \leq$
288 0.01). In response to exercise, two-way ANOVA showed a significant main effect of time and
289 trial by time interaction for glucose and NEFA (all $P \leq 0.001$), whilst a significant main effect
290 of trial was also observed for NEFA ($P < 0.001$). *Post-hoc* analyses revealed significantly
291 higher plasma NEFA concentrations during MOD compared to CON at 0 and 2 h; and during
292 HIGH compared to CON at 0, 2, 4 and 7 h (all $P \leq 0.044$). Additionally, plasma NEFA
293 concentrations were significantly higher during HIGH in comparison to MOD at 4 and 7 h
294 (both $P \leq 0.029$). No significant differences in plasma glucose were found between trials at any
295 time point ($P \geq 0.084$).

296 There was a significant main effect of time and trial by time interaction for glucagon, insulin
297 and glucagon to insulin ratio (all $P \leq 0.002$), whilst a significant main effect of trial was also
298 observed for glucagon ($P < 0.001$). *Post-hoc* analyses revealed that when compared to CON,
299 plasma glucagon concentrations were significantly higher at 0, 1 and 2 h during MOD and
300 HIGH (all $P \leq 0.045$). Furthermore, plasma glucagon concentrations were significantly higher
301 at 1 h (P = 0.032) and tended to be higher at 2 h ($P = 0.061$) during HIGH compared to MOD.

Subsequently, a significantly greater glucagon to insulin ratio was observed at 1 h during MOD, and at 1 and 2 h during HIGH, when compared to CON (all $P \leq 0.006$). However, no differences were observed between the two exercise trials ($P \geq 0.656$). No significant differences in plasma insulin were found between trials at any time point post-exercise ($P \geq 0.378$).

Insert figure 3

Discussion

The present study explored the acute effect of exercise intensity on circulating concentrations of FGF21, follistatin and LECT2 in a population of healthy men. Our novel observations are that high-intensity exercise elicited a greater post-exercise increase in circulating FGF21 when compared to moderate-intensity exercise. Furthermore, circulating follistatin was elevated after exercise independently of exercise intensity; whilst LECT2 appeared not to respond to acute bouts of exercise.

FGF21 is regarded as a metabolically beneficial hepatokine which is upregulated by obesity and obesity-related diseases (Chavez et al. 2009, Dushay et al. 2010), potentially as a compensatory mechanism to preserve metabolic homeostasis (Li et al. 2018). FGF21 has also been shown to be acutely responsive to metabolic stresses such as fasting, protein restriction and exercise (Galman et al. 2008, Kim et al. 2013, Laeger et al. 2014). The findings from the present study demonstrate that the FGF21 response to acute exercise is intensity dependent, with a greater response evident after an isoenergetic bout of high- vs moderate-intensity exercise. This finding is supported by previous observations suggesting that greater post-exercise increases in circulating FGF21 may be present with increasing exercise intensity (Kim et al. 2013). It must be noted, however, this previous study failed to isolate the effect of exercise intensity from the confounding influence of exercise-related energy expenditure (Kim et al. 2013). Our findings also demonstrate that the exercise-induced increase in circulating FGF21 persisted for up to four hours post-exercise which is consistent with previous reports from our laboratory (Sargeant et al. 2018) and elsewhere (Kondo et al. 2011).

Although it is known that exercise enhances the expression and secretion of FGF21 from the liver (Kim et al. 2013, Hansen et al. 2015), the mechanisms underpinning the exercise intensity-dependent increase in circulating FGF21 cannot be delineated from the present study. Our

results cannot distinguish whether the identified difference in systemic FGF21 was due to altered protein expression, secretion or clearance. Our data may, however, indicate an influential role of glucagon. Specifically, whilst no intensity-dependent differences were apparent in circulating NEFA, insulin or the glucagon to insulin ratio (all of which have been shown to modulate hepatic FGF21 secretion; Inagaki et al. 2007, Arafat et al. 2013, Hansen et al. 2015, Hansen et al. 2016b), circulating glucagon was two-fold higher in response to high- vs moderate-intensity exercise. It is therefore possible that the greater FGF21 response to high-intensity exercise was mediated by a relatively larger glucagon response. This possibility is supported by preclinical data which has demonstrated an independent role of glucagon as a stimulus of hepatic FGF21 expression and secretion (Arafat et al. 2013, Habegger et al. 2013, Cyphert et al. 2014, Alonge et al. 2017, Kim et al. 2018); however, the descriptive nature of the present study prevents us from unpicking this further.

It has been suggested that exercise-related augmentation of FGF21 may contribute to the beneficial metabolic effects of exercise (Hansen et al. 2016b, Weigert et al. 2018). Notably, in Wistar rats, Loyd et al (2016) recently demonstrated that FGF21 is essential for the beneficial effects of exercise training on hepatic steatosis and glycaemic control to be realised during a period of high-fat feeding. In the present study, the physiological relevance of the greater, yet transient augmentation of circulating FGF21 in response to high- vs moderate-intensity exercise, is not clear. It is possible that the enhanced response may contribute to the greater metabolic benefits associated with high-intensity exercise, including greater improvements in insulin sensitivity and glycaemic control (Hayashi et al. 2005, Rynders et al. 2014); or it may reflect a participatory role of the protein in the metabolic response to exercise and recovery. Carefully designed experiments utilising animal models are required to shed more light on this question.

Previous research has identified follistatin as another hepatokine which is transiently increased in the circulation in response to a single bout of exercise (Hansen et al. 2011, Hansen et al. 2016a, Hansen et al. 2016b, Sargeant et al. 2018). In the present study, both exercise intensities led to a similar increase in circulating follistatin concentrations. Previous studies have shown that the circulating glucagon to insulin ratio is a potent stimulus of hepatic follistatin release in response to exercise (Hansen et al. 2016a, Hansen et al. 2016b). The comparable follistatin response between exercise intensities may therefore be due to equivalent changes in the circulating glucagon to insulin ratio. The importance of these gluco-regulatory hormones may help explain recent findings showing that circulating follistatin was elevated after both high-intensity interval exercise and resistance exercise; but not low-to-moderate intensity continuous exercise (Perakakis et al. 2018). Although not measured in this previous study, it is likely that only the high-intensity exercise modalities would have triggered an increase in circulating glucagon and/or a decrease in circulating insulin; as opposed to the low-to-moderate intensity exercise (Wolfe et al. 1986, Lira et al. 2012).

Whilst the physiological relevance of the follistatin response to exercise is not fully understood, follistatin has been identified to play a beneficial role in several metabolic actions which may be relevant to exercise. This includes promoting skeletal muscle growth and preventing degradation through its actions as a natural antagonist of myostatin (Amthor et al. 2004, Yaden et al. 2014). Moreover, chronic overexpression of follistatin promotes pancreatic β-cell survival (Zhao et al. 2015, Hansen et al. 2016a), whilst acute administration of follistatin may reduce glucagon secretion from human islets in a potential negative feedback loop (Hansen et al. 2016a). Therefore, an exercise-induced increase in circulating follistatin may contribute in some capacity to the positive metabolic effects of exercise; however, the exact role needs to be delineated further.

When interpreting the follistatin results in the present study, one important methodological consideration warranting recognition was the decision to feed participants after exercise. In previous studies, follistatin responses to exercise have been examined with study participants remaining fasted throughout experimental trials (Hansen et al. 2011; Hansen et al. 2016b). Notably, the magnitude of increase in circulating follistatin after exercise in these previous studies was considerably greater than observed in the present study. It is therefore possible that food consumption may have blunted the follistatin response after exercise in the present study. In particular, evidence suggests that insulin may inhibit follistatin production (Tao et al. 2018); including the response to exercise (Hansen et al. 2016b). Raised postprandial insulin may have blunted our ability to detect an effect of exercise intensity on circulating follistatin within our post-exercise observation period. An additional experiment is needed to test this hypothesis.

LECT2 is a novel hepatokine whose circulating levels are elevated in obesity, type 2 diabetes and non-alcoholic fatty liver disease (Okumura et al. 2013, Zhang et al. 2018). *In vitro* studies have shown that LECT2 triggers inflammation in vascular endothelium (Hwang et al. 2015) and directly inhibits insulin signalling in C2C12 myocytes (Lan et al. 2014) and 3T3-L1 adipocytes (Jung et al. 2018). It has therefore been suggested that LECT2 may provide a mechanistic link between NAFLD, T2DM and cardiovascular disease (Meex and Watt 2017).

In our previous research, 60 min of moderate-intensity treadmill exercise had no impact on circulating LECT2 concentrations for up to 6 h post-exercise in healthy lean and overweight men (Sargeant et al. 2018). Based on previous rodent data showing a negative regulatory influence of hepatic AMPK-phosphorylation on LECT2 expression (Lan et al. 2014), we hypothesised in the present study that high-intensity exercise would suppress circulating LECT2. In contrast, we observed a significant increase in circulating LECT2 concentrations immediately after both moderate- and high-intensity exercise; with no differences apparent in

404 the hours thereafter. It must be noted, however, that statistical significance was lost at the 0 h
405 time point following adjustment for plasma volume changes, despite similar effect sizes (ES)
406 remaining (Cohen's *d* before vs after plasma volume adjustment: ES = 0.41 vs ES = 0.49 and
407 ES = 0.42 vs ES = 0.45 for MOD and HIGH compared to CON, respectively). Therefore,
408 exercise-induced haemoconcentration may have contributed, in part, to the observed increase
409 in plasma LECT2 immediately after exercise.

410 Lan et al (2014) previously showed that hepatic LECT2 expression and circulating
411 concentrations were suppressed immediately after 3 h of treadmill running in C57BL/6J mice.
412 This contrasting finding with our work may be related to species differences but may also be
413 due to the nature of the exercise utilised and/or the study participants. Specifically, given the
414 negative regulatory influence of hepatic AMPK, longer duration exercise with a greater
415 metabolic challenge may be needed to suppress LECT2 in humans. It is also possible that this
416 response may only occur in individuals with obesity in which circulating LECT2 is elevated;
417 however additional studies in populations with obesity are needed to test these hypotheses.

418 A key strength of our study is the precision with which exercise-related energy expenditure
419 was matched. Conversely, a limitation of the present study is that the observed hepatokine
420 responses occurred in a population of young, healthy men. Circulating levels of hepatokines
421 are modulated by adiposity and associated metabolic disease and, therefore, we cannot be
422 certain that our findings translate to different populations. The therapeutic benefits of exercise
423 are greater in clinical populations and therefore additional studies should investigate the
424 influence of exercise of varied intensities and durations in individuals with obesity and
425 associated metabolic disease.

426 In conclusion, this study demonstrates that circulating concentrations of FGF21 and follistatin
427 are transiently increased in response to acute bouts of aerobic exercise, whereas circulating

LECT2 concentrations may not be sensitive to acute exercise stimuli. Furthermore, exercise increases circulating FGF21 in an intensity-dependent manner, whilst the exercise-induced increase in circulating follistatin may be independent of exercise intensity. These findings support the notion that FGF21 and follistatin may contribute to the favourable metabolic effects of exercise and a dose-dependent increase in FGF21 secretion may be partly responsible for the additional benefits of high-intensity exercise. Additionally, these data extend our understanding of the acute regulation of hepatokines in humans and may help to inform future therapeutic interventions targeted at manipulating hepatokine concentrations.

Conflict of interest statement

The authors declare no conflicts of interest.

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443 **References**

444 Alonge, K.M., Meares, G.P., and Hillgartner, F.B. 2017. Glucagon and insulin cooperatively
445 stimulate fibroblast growth factor 21 gene transcription by increasing the expression of
446 activating transcription factor 4. J. Biol. Chem. **292**(13): 5239-5252.
447 doi:10.1074/jbc.M116.762922.

448 Amthor, H., Nicholas, G., McKinnell, I., Kemp, C.F., Sharma, M., Kambadur, R., et al. 2004.
449 Follistatin complexes myostatin and antagonises myostatin-mediated inhibition of
450 myogenesis. Dev. Biol. **270**(1): 19-30. doi:10.1016/j.ydbio.2004.01.046.

451 Arafat, A.M., Kaczmarek, P., Skrzypski, M., Pruszyńska-Oszmalek, E., Kołodziejski, P.,
452 Szczepankiewicz, D., et al. 2013. Glucagon increases circulating fibroblast growth factor 21
453 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated
454 lipolysis? Diabetologia. **56**(3): 588-597. doi:10.1007/s00125-012-2803-y.

455 BonDurant, L.D., and Potthoff, M.J. 2018. Fibroblast growth factor 21: A versatile regulator
456 of metabolic homeostasis. Annu. Rev. Nutr. **38**: 173-196. doi:10.1146/annurev-nutr-071816-
457 064800.

458 Borg, G.A. 1973. Perceived exertion: a note on "history" and methods. Med. Sci. Sports.
459 **5**(2): 90-93.

460 Bredin, S.S., Gledhill, N., Jamnik, V.K., and Warburton, D.E. 2013. PAR-Q+ and ePARmed-
461 X+: new risk stratification and physical activity clearance strategy for physicians and patients
462 alike. Can. Fam. Physician. **59**(3): 273-277.

463 Chavez, A.O., Molina-Carrion, M., Abdul-Ghani, M.A., Folli, F., Defronzo, R.A., and
464 Tripathy, D. 2009. Circulating fibroblast growth factor-21 is elevated in impaired glucose

tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care **32**(8): 1542-1546. doi:10.2337/dc09-0684.

Cyphert, H.A., Alonge, K.M., Ippagunta, S.M., and Hillgartner, F.B. 2014. Glucagon stimulates hepatic FGF21 secretion through a PKA- and EPAC-dependent posttranscriptional mechanism. PLoS One. **9**(4): e994996. doi: 10.1371/journal.pone.0094996.

Dill, D.B., and Costill, D.L. 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J. Appl. Physiol. **37**(2): 247-248. doi:10.1152/jappl.1974.37.2.247.

Dushay, J., Chui, PC., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al. 2010. Increased fibroblast growth factor 21 in obesity and non-alcoholic fatty liver disease. Gastroenterology. **139**(2): 456-463. doi:10.1053/j.gastro.2010.04.054.

Field, A. 2009. Discovering Statistics using SPSS, edn 3. London, UK: Sage.

Frayn, K.N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. **55**(2): 628-634. doi:10.1152/jappl.1983.55.2.628.

Fu, S., Fan, J., Blanco, J., Giminez-Cassina, A., Danial, N.N., Watkins, S.M., et al. 2012. Polysome profiling in liver identifies dynamic regulation of endoplasmic reticulum translatome by obesity and fasting. PLoS Genet. **8**(8): e1002902. doi:10.1371/journal.pgen.1002902.

Gälman, C., Lundåsen, T., Kharitonenkov, A., Bina, H.A., Eriksson, M., Hafström, I., et al. 2008. The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. Cell Metab **8**(2): 169-174. doi:10.1016/j.cmet.2008.06.014.

487 Habegger, K.M., Stemmer, K., Cheng, C., Müller, T.D., Heppner, K.M., Ottaway, N., et al.
488 2013. Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes **62**(5): 1453-
489 1463. doi:10.2337/db12-1116.

490 Hansen, J.S., and Plomgaard, P. 2016. Circulating follistatin in relation to energy metabolism.
491 Mol. Cell. Endocrinol. **433**: 87-93. doi:10.1016/j.mce.2016.06.002.

492 Hansen, J.S., Brandt, C., Nielsen, A.R., Hojman, P., Whitham, M., Febbraio, M.A., et al.
493 2011. Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a
494 contraction-induced hepatokine. Endocrinology. **152**(1): 164-171. doi: 10.1210/en.2010-
495 0868.

496 Hansen, J.S., Clemmesen, J.O., Secher, N.H., Hoene, M., Drescher, A., Weigert, C., et al.
497 2015. Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans.
498 Mol. Metab. **4**(8): 551-560. doi:10.1016/j.molmet.2015.06.001.

499 Hansen, J.S., Rutti, S., Arous, C., Clemmesen, J.O., Secher, N.H., Drescher, A., et al. 2016a.
500 Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. J. Clin.
501 Endocrinol. Metab. **101**(2): 550-560. doi: 10.1210/jc.2015-3668.

502 Hansen, J.S., Pedersen, B.K., Xu, G., Lehmann, R., Weigert, C., and Plomgaard, P. 2016b.
503 Exercise-induced secretion of FGF21 and follistatin are blocked by pancreatic clamp and
504 impaired in type 2 diabetes. J. Clin. Endocrinol. Metab. **101**(7): 2816-2825.
505 doi:10.1210/jc.2016-1681.

506 Hayashi, Y., Nagasaka, S., Takahashi, N., Kusaka, I., Ishibashi, S., Numao, S., et al. 2005. A
507 single bout of exercise at higher intensity enhances glucose effectiveness in sedentary men. J.
508 Clin. Endocrinol. Metab. **90**(7): 4035-4040. doi:10.1210/jc.2004-2092.

509 Hwang, H.J., Jung, T.W., Hong, H.C., Seo, J.A., Kim, S.G., Kim, N.H., et al. 2015. LECT2
510 induces atherosclerotic inflammatory reaction via CD209 receptor-mediated JNK
511 phosphorylation in human endothelial cells. Metabolism **64**(9): 1175-1182.
512 doi:10.1016/j.metabol.2015.06.001.

513 Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., et al. 2007.
514 Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast
515 growth factor 21. Cell. Metab. **5**(6): 415-425. doi:10.1016/j.cmet.2007.05.003.

516 Iroz, A., Couty, J.P., and Postic, C. 2015. Hepatokines: unlocking the multi-organ network in
517 metabolic diseases. Diabetologia. **58**(8): 1699-1703. doi:10.1007/s00125-015-3634-4.

518 Jung, T.W., Chung, Y.H., Kim, H.C., Abd El-Aty, A.M., and Jeong, J.H. 2018. LECT2
519 promotes inflammation and insulin resistance in adipocytes via P38 pathways. J. Mol.
520 Endocrinol. **61**(1): 37-45. doi:10.1530/JME-17-0267.

521 Kharitonenkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Galbreath, E.J.,
522 et al. 2005. FGF-21 as a novel metabolic regulator. J. Clin. Invest. **115**(6): 1627-1635. doi:
523 10.1172/JCI23606.

524 Kim, K.H., Kim, S.H., Min, Y.K., Yang, H.M., Lee, J.B., and Lee, M.S. 2013. Acute exercise
525 induces FGF21 expression in mice and in healthy humans. PLoS One. **8**(5): e63517.
526 doi:10.1371/journal.pone.0063517.

527 Kim, T., Holleman, C.L., Nason, S., Arble, D.M., Ottaway, N., Chabenne, J., et al. 2018.
528 Hepatic glucagon-receptor signalling enhances insulin-stimulated glucose disposal in rodents.
529 Diabetes. **67**(11): 2157-2166. doi:10.2337/db18-0068.

Kirpich, I.A., Gobejishvili, L.N., Bon Homme, M., Waigel, S., Cave, M., Arteel, G., et al. 2011. Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced non-alcoholic fatty liver disease. J. Nutr. Biochem. **22**(1): 38-45. doi:10.1016/j.jnutbio.2009.11.009.

Kondo, H., Hojo, Y., Saito, T., Ikemoto, T., Katsuki, T., Shimada, K., et al. 2011. Relationship between fibroblast growth factor 21 and extent of left ventricular remodelling after acute myocardial infarction. J. Clinic. Experiment. Cardiol. **2**(6) 1000139. doi:10.4172/2155-9880.1000139.

Laeger, T., Henagan, T.M., Albarado, D.C., Redman, L.M., Bray, G.A., Noland, R.C., et al. 2014. FGF21 is an endocrine signal of protein restriction. J. Clin. Invest. **124**(9): 3913-3922. doi:10.1172/JCI74915.

Lan, F., Misu, H., Chikamoto, K., Takayama, H., Kikuchi, A., Mohri, K., et al. 2014. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. Diabetes. **63**(5): 1649-1664. doi:10.2337/db13-0728.

Loyd, C., Magrisso, I.J., Haas, M., Balusu, S., Krishna, R., Itoh, N., et al. 2016. Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. J. Appl. Physiol. **121**(3): 687-698. doi:10.1152/japplphysiol.00456.2016.

Li, H., Wu, G., Fang, Q., Zhang, M., Hui, X., Sheng, B., et al. 2018. Fibroblast growth factor 21 increases insulin sensitivity through specific expansion of subcutaneous fat. Nat. Commun. **9**(1): 272. doi:10.1038/s41467-017-02677-9.

Lira, F.S., Carnevali Jr, L.C., Zanchi, N.E., Santos, R.V.T., Lavoie, J.M., and Seelaender, M. 2012. Exercise intensity modulation of hepatic lipid metabolism. J. Nutr. Metab. 809576. doi:10.1155/2012/809576.

553 Markan, K.R., Naber, M.C., Ameka, M.K., Anderegg, M.D., Mangelsdorf, D.J., Kliewer,
554 S.A., et al. 2014. Circulating FGF21 is liver derived and enhances glucose uptake during
555 refeeding and overfeeding. Diabetes. **63**(12): 4057-4063. doi:10.2337/db14-0595.

556 Meex, R.C.R., and Watt, M.J. 2017. Hepatokines: linking non-alcoholic fatty liver disease
557 and insulin resistance. Nat. Rev. Endocrinol. **13**(9): 509-520. doi:10.1038/nrendo.2017.56.

558 Meex, R.C.R., Hoy, A.J., Morris, A., Brown, R.D., Lo, J.C., Burke, M., et al. 2015. Fetuin B
559 is a secreted hepatocyte factor linking steatosis to impaired glucose metabolism. Cell. Metab.
560 **22**(6): 1078-1089. doi:10.1016/j.cmet.2015.09.023.

561 Mifflin, M.D., St Jeor, S.T., Hill, L.A., Scott, B.J., Daugherty, S.A., and Koh, Y.O. 1990. A
562 new predictive equation for resting energy expenditure in healthy individuals. Am. J. Clin.
563 Nutr. **51**(2): 241-247. doi:10.1093/ajcn/51.2.241.

564 Okumura, S., Unoki-Kubota, H., Matsushita, Y., Shiga, T., Moriyoshi, Y., Yamagoe, S. et al.
565 2013. Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and
566 fatty liver. Biosci. Trends. **7**(6): 276-283.

567 Perakakis, N., Mougios, V., Fatouros, I., Siopi, A., Draganidis, D., Peradze, N., et al. 2018.
568 Physiology of activins/follistatins: associations with metabolic and anthropometric variables
569 and response to exercise. J. Clin. Endocrinol. Metab. **103**(10): 3890-3899.
570 doi:10.1210/jc.2018-01056.

571 Potthoff, M.J., Inagaki, T., Satapati, S., Ding, X., He, T., Goetz, R., et al. 2009. FGF21
572 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the
573 adaptive starvation response. Proc. Natl. Acad. Sci. U. S. A. **106**(26): 10853-10858.
574 doi:10.1073/pnas.0904187106.

575 Robergs, R.A., Dwyer, D., and Astorino, T. 2010. Recommendations for improved data
576 processing from expired gas analysis indirect calorimetry. Sports. Med. **40**(2): 95-111.
577 doi:10.2165/11319670-000000000-00000.

578 Rynders, C.A., Weltman, J.Y., Jiang, B., Breton, M., Patrie, J., Barrett, E.J., et al. 2014.
579 Effects of exercise intensity on postprandial improvement in glucose disposal and insulin
580 sensitivity in prediabetic adults. J. Clin. Endocrinol. Metab. **99**(1): 220-228.
581 doi:10.1210/jc.2013-2687.

582 Sargeant, J.A., Aithal, G.P., Takamura, T., Misu, H., Takayama, H., Douglas, J.A., et al.
583 2018. The influence of adiposity and acute exercise on circulating hepatokines in normal-
584 weight and overweight/obese men. Appl. Physiol. Nutr. Metab. **43**(5): 482-490.
585 doi:10.1139/apnm-2017-0639.

586 Tao, R., Wang, C., Stöhr, O., Qiu, W., Hu, Y., Miao, J., et al. 2018. Inactivating hepatic
587 follistatin alleviates hyperglycaemia. Nat. Med. **24**(7): 1058-1069. doi:10.1038/s41591-018-
588 0048-0.

589 Toye, A.A., Dumas, M.E., Blancher, C., Rothwell, A.R., Fearnside, J.F., Wilder, S.P., et al.
590 2007. Subtle metabolic and liver gene transcriptional changes underlie diet-induced fatty liver
591 susceptibility in insulin-resistant mice. Diabetologia. **50**(9): 1867-1879. doi:10.1007/s00125-
592 007-0738-5.

593 Wahren, J., Felig, P., and Hagenfeldt, L. 1978. Physical exercise and fuel homeostasis in
594 diabetes mellitus. Diabetologia. **14**(4): 213-222.

595 Weigert, C., Hoene, M., and Plomgaard, P. 2018. Hepatokines - a novel group of exercise
596 factors. Pflügers. Arch. doi:10.1007/s00424-018-2216-y. [Epub ahead of print].

Wen, C.P., Wai, J.P., Tsai, M.K., Yang, Y.C., Cheng, T.Y., Lee, M.C., et al. 2011. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet. **378**(9798): 1244-1253. doi:10.1016/S0140-6736(11)60749-6.

Wolfe, R.R., Nadel E.R., Shaw, J.H., Stephenson, L.A., and Wolfe, M.H. 1986. Role of changes in insulin and glucagon in glucose homeostasis in exercise. J. Clin. Invest. **77**(3): 900-907. doi:10.1172/JCI112388.

Xu, J., Stanislaus, S., Chinookoswong, N., Lau, Y.Y., Hager, T., Patel, J., et al. 2009. Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models--association with liver and adipose tissue effects. Am. J. Physiol. Endocrinol. Metab. **297**(5): E1105-E1114. doi:10.1152/ajpendo.00348.2009.

Yaden, B.C., Croy, J.E., Wang, Y., Wilson, J.M., Datta-Mannan, A., Shetler, P., et al. 2014. Follistatin: a novel therapeutic for the improvement of muscle regeneration. J. Pharmacol. Exp. Ther. **349**(2): 355-371. doi:10.1124/jpet.113.211169.

Zhang, Z., Zeng, H., Lin, J., Hu, Y., Yang, R., Sun, J., et al. 2018. Circulating LECT2 levels in newly diagnosed type 2 diabetes mellitus and their association with metabolic parameters: An observational study. Medicine. **97**(15): e0354. doi:10.1097/MD.0000000000010354.

Zhao, C., Qiao, C., Tang, R.H., Jiang, J., Li, J., Martin, C.B., et al. 2015. Overcoming insulin insufficiency by forced follistatin expression in β-cells of db/db mice. Mol. Ther. **23**(5): 866-874. doi:10.1038/mt.2015.29.

Tables

Table 1. Participant characteristics (n = 10).

Characteristic	Mean ± SD
Age (years)	26 ± 2
Height (m)	1.77 ± 0.07
Body mass (kg)	80.2 ± 6.2
BMI ($kg \cdot m^{-2}$)	25.6 ± 1.7
Waist circumference (cm)	83.7 ± 4.1
Body fat (%)	15.4 ± 4.4
$\dot{V}O_2$ peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	49.8 ± 5.3

Note: BMI, body mass index; $\dot{V}O_2$ peak, peak oxygen uptake.

Table 2. Exercise characteristics (n = 10).

	MOD	HIGH	P-value
Treadmill speed ($km \cdot h^{-1}$)	8.2 ± 1.4	11.6 ± 1.6	**<0.001**
Oxygen uptake ($L \cdot min^{-1}$)	2.17 ± 0.26	2.98 ± 0.37	**<0.001**
Exercise intensity (%$\dot{V}O_2$ peak)	54.6 ± 1.7	74.7 ± 1.8	**<0.001**
Exercise duration (min)	57 ± 8	42 ± 6	**<0.001**
Gross energy expenditure (kJ)	2475 ± 70	2488 ± 58	0.459
Respiratory exchange ratio	0.90 ± 0.03	0.99 ± 0.03	**<0.001**
Carbohydrate oxidation (%)	67 ± 10	94 ± 8	**<0.001**
Fat oxidation (%)	33 ± 10	6 ± 8	**<0.001**
Heart rate ($beats \cdot min^{-1}$)	142 ± 10	168 ± 9	**<0.001**
Rating of perceived exertion	11 ± 2	16 ± 1	**<0.001**

Note: Data are presented as means ± SD. $\dot{V}O_2$ peak, peak oxygen uptake.

621 **Table 3.** Area under the hepatokine concentration vs time curve during CON, MOD and
622 HIGH trials for the preprandial (BL-2 h), postprandial (2-7 h) and total trial (8 h) periods.

	CON	MOD	HIGH
FGF21 (n = 10)			
Preprandial AUC ($pg \cdot mL^{-1}$ BL-2 h)	144 ± 124	230 ± 156[a]	334 ± 249[a§]
Postprandial AUC ($pg \cdot mL^{-1}$ 2-7 h)	264 ± 209	323 ± 233	433 ± 271[a]
Total AUC ($pg \cdot mL^{-1}$ 8 h)	407 ± 330	553 ± 377[a]	768 ± 491[a§]
Follistatin (n = 9)			
Preprandial AUC ($pg \cdot mL^{-1}$ BL-2 h)	3848 ± 515	4518 ± 1148	4566 ± 962[#]
Postprandial AUC ($pg \cdot mL^{-1}$ 2-7 h)	6615 ± 823	8504 ± 2118[#]	9275 ± 1406[a]
Total AUC ($pg \cdot mL^{-1}$ 8 h)	10463 ± 1162	13022 ± 3175[#]	13841 ± 2110[a]
LECT2 (n = 10)			
Preprandial AUC ($ng \cdot mL^{-1}$ BL-2 h)	157 ± 59	165 ± 64	166 ± 62[#]
Postprandial AUC ($ng \cdot mL^{-1}$ 2-7 h)	253 ± 98	267 ± 102	268 ± 99
Total AUC ($ng \cdot mL^{-1}$ 8 h)	410 ± 156	432 ± 166	434 ± 160

623 **Note:** Data are presented as means ± SD. FGF21, fibroblast growth factor 21; AUC, area
624 under curve; BL, baseline; LECT2, leukocyte cell-derived chemotaxin 2.

625 [a]Significantly different from CON ($P < 0.05$).

626 [#]Tended to differ from CON ($P < 0.07$).

627 [§]Tended to differ from MOD ($P < 0.07$).

628 **Figure captions**

629 **Figure 1.** Schematic illustration of the main trial protocol. Duration of exercise in MOD and
630 HIGH = 57 ± 8 and 42 ± 6 min, respectively. Participants were provided with 35% of
631 estimated daily energy requirements at both 2 h and 6 h.

632

633 **Figure 2.** Plasma concentrations of fibroblast growth factor 21 (FGF21) (A), follistatin (B)
634 and leukocyte cell-derived chemotaxin 2 (LECT2) (C) during the control (open circles),
635 moderate-intensity exercise (black circles) and high-intensity exercise (grey circles) trials.
636 Data are presented as means ± SEM, n = 10 for FGF21 and LECT2 data, and n = 9 for
637 follistatin data. Black rectangle indicates moderate-intensity exercise, grey rectangle indicates
638 high-intensity exercise and hatched rectangles indicate test meals. BL, baseline. [a]MOD
639 significantly different from CON ($P < 0.05$); [b]HIGH significantly different from CON ($P <$
640 0.05); [c]HIGH significantly different from MOD ($P < 0.05$).

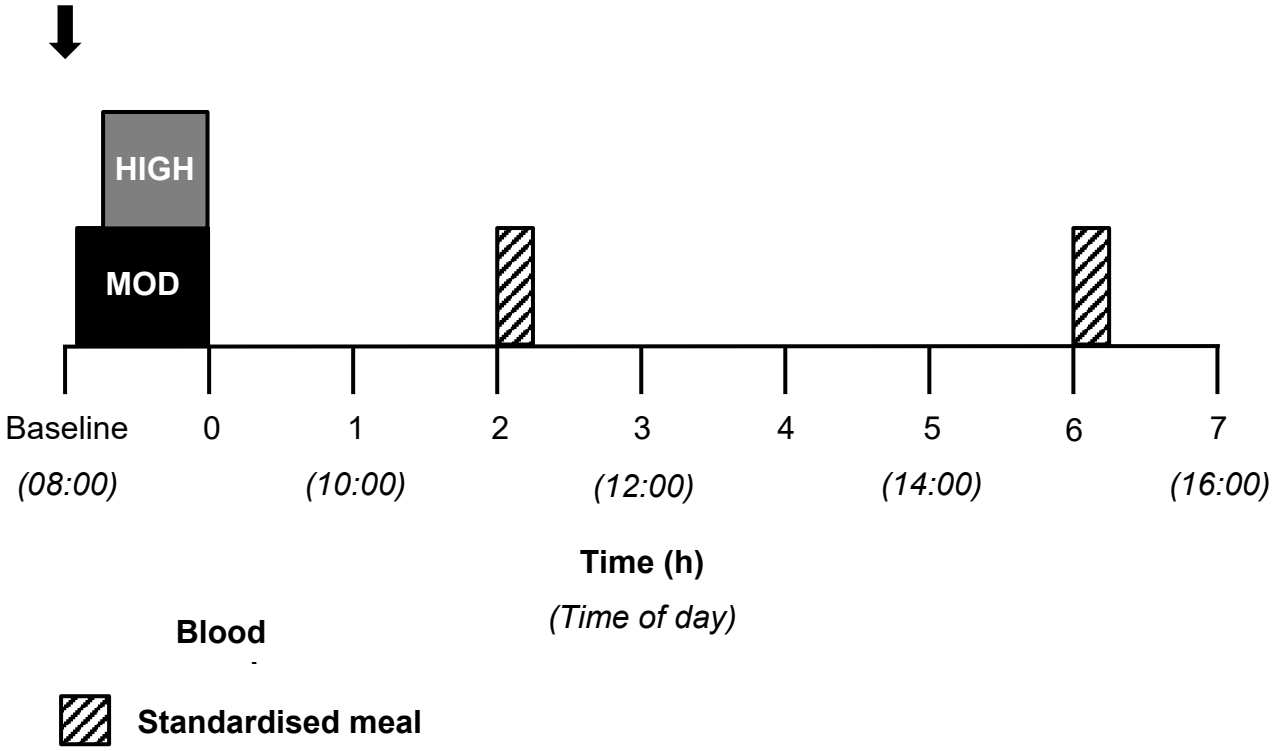
641

642 **Figure 3.** Plasma concentrations of glucose (A), non-esterified fatty acids (NEFA) (B),
643 glucagon (C), insulin (D) and the glucagon to insulin ratio (E) during the control (open
644 circles), moderate-intensity exercise (black circles) and high-intensity exercise (grey circles)
645 trials. Data are presented as means ± SEM. Black rectangle indicates moderate-intensity
646 exercise, grey rectangle indicates high-intensity exercise and hatched rectangles indicate test
647 meals. BL, baseline. [a]MOD significantly different from CON ($P < 0.05$); [b]HIGH significantly
648 different from CON ($P < 0.05$); [c]HIGH significantly different from MOD ($P < 0.05$).

649

650

Fig 1


HIGH
MOD
Baseline
0
1
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Time (h)
(Time of day)
Blood
Standardised meal

677 Fig 2

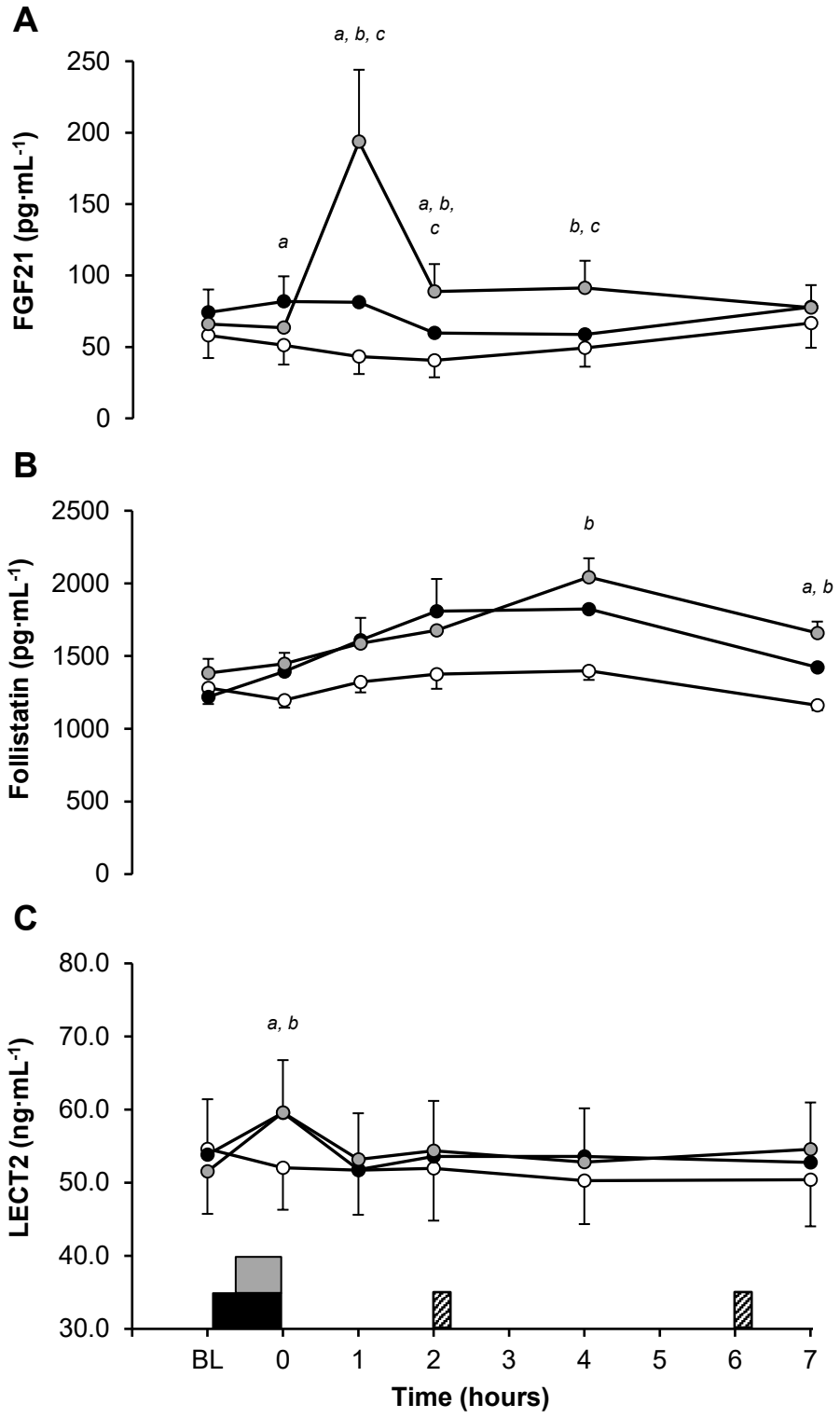

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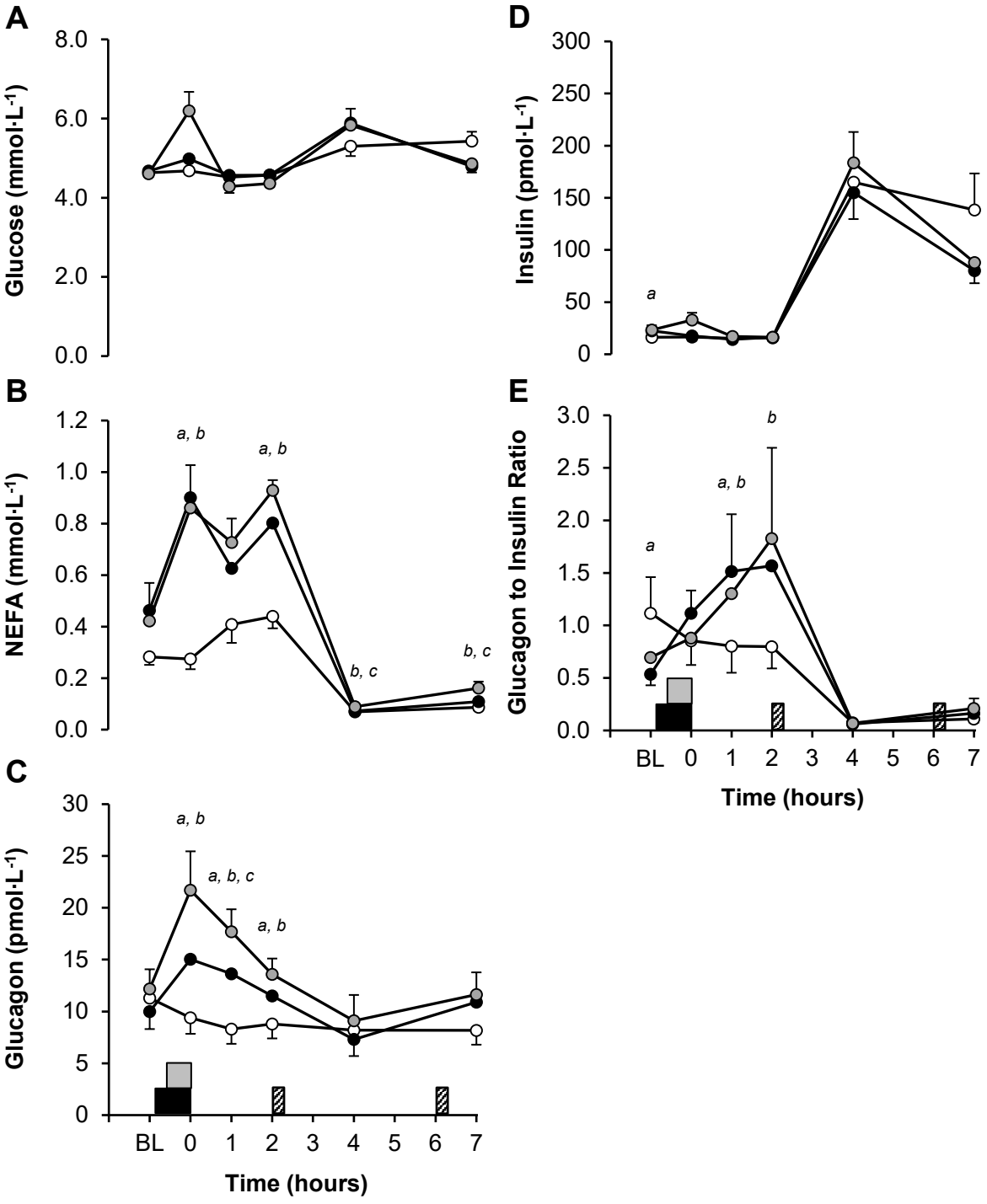
Fig 3


A
Glucose (mmol·L-1)
8.0
6.0
4.0
2.0
0.0
D
Insulin (pmol·L-1)
300
250
200
150
100
50
0
a
B
NEFA (mmol·L-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a, b
a, b
b, c
b, c
E
Glucagon to Insulin Ratio
3.0
2.5
2.0
1.5
1.0
0.5
0.0
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a, b
b
BL 0 1 2 3 4 5 6 7
Time (hours)
C
Glucagon (pmol·L-1)
30
25
20
15
10
5
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a, b
a, b, c
a, b
BL 0 1 2 3 4 5 6 7
Time (hours)