Circulating leukocyte cell-derived chemotaxin 2 and fibroblast growth factor 21 are negatively associated with cardiorespiratory fitness in healthy volunteers

Running Title: cardiorespiratory fitness and hepatokines

Sundus Malaikah[a,b,c], Scott A. Willis[a,b], Joseph Henson[b,d], Jack A. Sargeant[b,d], Thomas Yates[b,d], Alice E. Thackray[a,b], Fernanda R. Goltz[a,b], Matthew J. Roberts[a,b], Danielle Bernard-Deshong[a], Guruprasad P. Aithal[e,f], David J. Stensel[a,b,g,h], James A. King[a,b]

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

[b]NIHR Leicester Biomedical Research Centre, University Hospitals of Leicester NHS Trust and University of Leicester, UK

[c]Clinical Nutrition Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

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

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

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

[g]Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan

[h]Department of Sport Science and Physical Education, The Chinese University of Hong Kong

Address for correspondence

Dr James King

Senior Lecturer in Exercise Physiology

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

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Competing Interests

The authors declare there are no competing interests.

Abstract

Leukocyte cell-derived chemotaxin-2 (LECT2) and fibroblast growth factor 21 (FGF21) are hepatokines which are regulated by energy balance and mediate insulin sensitivity and glycaemic control. This cross-sectional study examined the independent associations of cardiorespiratory fitness (CRF), moderate-to-vigorous intensity physical activity (MVPA), and sedentary time, with circulating LECT2 and FGF21. Data were combined from two previous experimental studies in healthy volunteers (n=141, male=60%, mean ± SD age=37 ± 19 years, body mass index (BMI)=26.1 ± 6.3 $kg \cdot m^{-2}$). Sedentary time and MVPA were measured via an ActiGraph GT3X+ accelerometer while magnetic resonance imaging quantified liver fat. CRF was assessed using incremental treadmill tests. Generalized-linear models examined the association of CRF, sedentary time and MVPA with LECT2 and FGF21 whilst controlling for key demographic and anthropometric variables. Interaction terms explored the moderating influence of age, sex, BMI, and CRF. In the fully adjusted models, each SD increase in CRF was independently associated with a 24% (95% CI: -37% to -9%, P = 0.003) lower plasma LECT2 concentration and 53% lower FGF21 concentration (95% CI: -73% to -22%, P = 0.004). Each SD increase in MVPA was independently associated with 55% higher FGF21 (95% CI: 12% to 114%, P = 0.006) and this relationship was stronger in those with lower BMI and higher levels of CRF. These findings demonstrate that CRF and wider activity behaviours may independently modulate the circulating concentrations of hepatokines and thereby influence inter-organ cross-talk.

Introduction

The liver plays a central role in system-wide metabolic homeostasis through inter-organ crosstalk with other tissues (Watt et al., 2019). Many 'hepatokines' have been characterised as vehicles of systemic communication, with important roles in regulating energy/substrate metabolism, insulin sensitivity, and glycaemic control (Jensen-Cody & Potthoff, 2021). The regulation of hepatokines has received scientific interest in recent years, with recognition that anthropometric variables, ectopic lipids, and glycaemic control are associated with many hepatokines (Jensen-Cody & Potthoff, 2021). Most prominently, experimental studies have shown that liver fat directly modulates the hepatic proteome (Kirpich et al., 2011; Meex et al., 2015). Emerging evidence indicates that physical activity and cardiorespiratory fitness (CRF) also influence hepatokine metabolism. However, knowledge in this area is rudimentary (Ennequin et al., 2019; Weigert et al., 2019).

Leukocyte cell-derived chemotaxin 2 (LECT2) is a hepatokine known to promote insulin resistance in skeletal muscle and adipose tissue (Jung et al., 2018; Lan et al., 2014). Observational studies have shown that circulating LECT2 is positively associated with body mass index (BMI), circulating lipids, and insulin resistance (Okumura et al., 2013). Moreover, in one analysis, visceral adipose tissue was cited as the strongest predictor of circulating LECT2 concentrations in humans (Tanisawa et al., 2017). Mechanistically, LECT2 is negatively regulated by AMP-activated protein kinase (AMPK), therefore energy balance may also influence LECT2 metabolism (Garcia et al., 2019; Lan et al., 2014). Acute exercise suppressed circulating LECT2 in rodents (Lan et al., 2014); however, this finding has not been replicated in humans (Sargeant et al., 2018; Willis et al., 2019). Additional research is needed to better understand the interaction between physical activity, metabolic status, and LECT2.

A second hepatokine which has received significant attention is fibroblast growth factor 21 (FGF21). Although the FGF21 protein is produced in several tissues, circulating levels are predominantly liver-derived (Markan et al., 2014; van Baak et al., 2020). Preclinical studies have shown that the augmentation of FGF21 action elicits favourable effects on substrate (glucose and lipid) metabolism, insulin sensitivity, ectopic fat, and energy expenditure (Coskun et al., 2008; Xu et al., 2009). In clinical trials, FGF21 analogues have conferred positive metabolic effects in humans (e.g. glycaemic control and lipid metabolism) (Cui et al., 2020). Counter-intuitively, circulating FGF21 levels are positively related to BMI and the metabolic syndrome (Chow et al., 2013), which may be a compensatory response to the metabolic stress of overnutrition (Giralt et al., 2015). Furthermore, FGF21 is acutely regulated by circulating free fatty acids and the glucagon-to-insulin ratio; factors which are sensitive to physical activity (Hansen et al., 2015; Mai et al., 2009). Previous research has identified associations between circulating FGF21, CRF, and habitual physical activity, however, these studies have failed to separate these associations from the confounding influence of liver fat (Cuevas-Ramos et al., 2010, 2012; Matsui et al., 2019; Taniguchi et al., 2014). Given that liver fat is a key determinant of circulating FGF21 levels (Okumura et al., 2013), further studies are warranted to disentangle the relationship between these variables.

Using a sample of precisely phenotyped volunteers, this cross-sectional study examined independent associations between objectively-measured CRF, physical activity and sedentary time with circulating LECT2 and FGF21. A secondary aim was to explore whether sex, age and BMI moderated the associations between exposure and outcome variables.

Methods

Ethical approval

This cross-sectional study pooled data from two experiments which used identical procedures for outcomes included in the present analysis (Goltz et al., 2019; Roberts et al., 2022). Both studies obtained institutional ethical approval and written informed consent from all participants.

Participants

Data were available for 141 individuals (85 men, 56 women) who were white European or South Asian. Participants did not smoke, were weight stable, were not taking medications known to affect study outcomes, and were free of established cardiometabolic disease (e.g., type 2 diabetes and cardiovascular disease). Pre-menopausal women reported not being pregnant and their tests were completed during the follicular phase of the menstrual cycle. Although there was a wide-range, the majority of participants were physically active.

Study procedures

Data collection took place at Loughborough University within laboratory visits that occurred between November 2016 and September 2019. Although the data included in this manuscript were pooled from two separate studies, each study was undertaken in the same laboratory using identical techniques and standard operating procedures. In all cases, participants abstained from alcohol, caffeine, and structured exercise in the 24 h before data collection.

Anthropometry

129 Height and body mass were measured using an integrated stadiometer and scale (Seca Ltd,
130 Hamburg, Germany). Body mass index was calculated as weight (kg) divided by height (m)
131 squared.

132 *Measurement of liver fat*

133 Participants underwent an MRI scan to quantify liver fat. Scans used a dual-echo Dixon fat and
134 water sequence on a 3T MRI scanner (MR750w, GE Healthcare, Chicago, USA). The IDEAL-
135 IQ sequence was used to assess proton density fat fraction (West et al., 2016). After collection,
136 anonymised scans were analysed by AMRA medical using the AMRA^{TM} profiler (AMRA
137 Medical AB, Linköping, Sweden) (Borga et al., 2015).

138 *Measurement of physical activity and sedentary time*

139 Habitual physical activity and sedentary time were assessed over 7-days using a waist-worn
140 ActiGraph GT3X+ accelerometer (ActiGraph, Pensacola, USA). For inclusion, participants
141 were required to submit at least four days of valid wear time (≥ 600 minutes). Data were
142 analysed over 15 second epochs (ActiLife, Actigraph corporation, Florida, USA) and classified
143 as: sedentary time < 25 counts per 15 seconds and MVPA ≥ 488 counts per 15 seconds (Byrom
144 et al., 2016; Freedson et al., 1998). Sixty minutes of continuous zero counts were classified as
145 non-wear time.

146 *Measurement of cardiorespiratory fitness*

147 Cardiorespiratory fitness was assessed as participants' peak oxygen uptake ($\dot{V}O_2$ peak) and was
148 measured directly via indirect calorimetry for 111 individuals. Within these tests, participants
149 completed an incremental protocol on a treadmill until volitional exhaustion, with heart rate
150 (Polar T31; Polar Electro, Kempele, Finland) and perceived exertion (Borg, 1973) measured

throughout. Oxygen uptake was measured continuously during the test via a breath-by-breath analyser (Metalyzer 3B, Cortex, Leipzig, Germany), with $\dot{V}O_2$ peak determined as the highest oxygen consumption value averaged over 20 seconds. In the remaining 30 participants, $\dot{V}O_2$ peak was measured indirectly using the Bruce test (Bruce et al., 1973) given their higher cardio-metabolic risk (central obesity). This indirect measure of $\dot{V}O_2$ peak correlates strongly (r = 0.97) with that measured directly (Bruce et al., 1973; Foster et al., 1984).

Biochemical analysis

Fasted venous blood samples were drawn from an antecubital or forearm vein after participants had rested in a semi-supine position for at least 5 minutes. Samples were collected into pre-chilled EDTA monovettes (Sarstedt, Leicester, United Kingdom) and spun immediately in a refrigerated centrifuge for 10 minutes (4°C, 1500 x *g*) (Labofuge 400R, ThermoScientific, Langenselbold, Germany). The plasma supernatant was collected and stored at -80°C before analysis. Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to measure plasma concentrations of LECT2 (BioVendor, Czech Republic) and FGF21 (R&D Systems, Minneapolis, United States). The coefficient of variation for LECT2 and FGF21 were 4.8% and 5.0%, respectively.

Statistical analyses

A formal sample size calculation was not performed for this exploratory study. Statistical analyses were carried out using SPSS version 26 (SPSS Inc., Chicago, Illinois). To examine the distribution of the data, histograms and Kolmogorov-Smirnov tests were used. Data are presented as the mean ± standard deviation (SD) for normally distributed data, median (interquartile range) for non-normally distributed data, or number (percentage) for categorical groups. Correlations between physical activity variables, CRF, and liver fat with LECT2 and

FGF21 were explored using Pearson's correlation coefficient (*r*) for normally distributed data, or Spearman's correlation coefficient (rho) for non-normally distributed data. LECT2 was normally distributed, whereas FGF21 was not normally distributed. Generalized linear modelling was used to examine the independent associations of CRF, sedentary time, and MVPA with circulating LECT2 and FGF21. All exposure variables were standardised for this analysis. A normal distribution with a log link was used for LECT2 while a gamma distribution with a log link was used for FGF21 (due to the right-skewed distribution of the variable). The consistent use of log links across models and the standardisation (per SD) of exposure variables allowed for the strength of the association to be reported as a fold change per SD for each model, allowing direct comparison between models. In model 1, adjustment was made for demographic variables including study, age (continuous), sex, ethnicity (white European/South Asian), BMI, plus device wear time (continuous) where accelerometer-measured variables (sedentary time and MVPA) were included in the model as exposures. Model 2 was adjusted for the same variables as model 1, as well as for CRF or accelerometer-measured variables including sedentary time and MVPA (all continuous). Model 3 was adjusted for the same variables as model 2 plus liver fat (continuous). Multicollinearity between covariates was assessed using a correlation matrix. Significant associations in model 3 were then explored further by simultaneously adding interactions terms into the models to assess whether these associations were modified by sex, age, and BMI. In addition, because interventions to reduce sedentary behaviour have been shown to be more effective at improving metabolic health in those with lower fitness (McCarthy et al., 2017), we further assessed interactions between CRF and physical activity variables. Only significant interactions are presented. To facilitate interpretation, interactions between continuous variables were also stratified using the median split. Coefficients were back-transformed to generate fold-change. All data for the regression

198 analyses are presented as fold change (95% confidence intervals). Statistical significance was
199 set at $P < 0.05$ for main effects and interactions.

200 **Results**

201 The characteristics of included participants are shown in Table 1. Due to technical issues with
202 the accelerometer, sedentary time and MVPA are presented for $n = 130$. Additionally, liver fat
203 could not be determined in 15 participants due to motion artefacts; therefore, these data are
204 presented for $n = 126$. Moreover, circulating FGF21 data for one participant were removed
205 from the analysis due to being an outlier (z-score = 7); therefore, these values are presented for
206 $n = 140$.

207

208 ***Insert Table 1***

209

210 *Simple correlations between LECT2, FGF21 and key study variables*

211 Figure 1 shows the simple correlations of LECT2 and FGF21 with CRF, sedentary time,
212 MVPA, and liver fat. Pearson's correlation coefficients revealed that plasma LECT2 was
213 negatively associated with CRF ($r = -0.38$, $P < 0.001$) and positively associated with liver fat
214 ($r = 0.35$, $P < 0.001$). Plasma LECT2 was not associated with sedentary time or MVPA ($P >$
215 0.05). Furthermore, Spearman's correlation coefficients revealed that plasma FGF21 was
216 negatively associated with CRF (rho = -0.42, $P < 0.001$) and positively associated with both
217 sedentary time (rho = 0.23, $P = 0.007$) and liver fat (rho = 0.47, $P < 0.001$). Plasma FGF21 was
218 not associated with MVPA ($P > 0.05$).

Independent associations of LECT2 with CRF, sedentary time and MVPA

Adjusted associations of plasma LECT2 with CRF, sedentary time, and MVPA are shown in Table 2 and Figure 2. After adjustment for demographic variables in model 1, only CRF was associated with plasma LECT2 ($P = 0.001$). Each SD increase in CRF was associated with a 26% (95% CI: -40% to -11%) lower plasma LECT2 concentration. After additional adjustment for sedentary time, MVPA and liver fat in model 3, the association remained statistically significant ($P = 0.003$) such that each SD increase in CRF was associated with a 24% (95% CI: -37% to -9%) lower plasma LECT2 concentration. There were no associations between LECT2 and sedentary time, or MVPA. To study the relationship further, interaction analyses with sex, age, and BMI were performed; however, no significant interactions were observed.

Insert Table 2

Independent associations of FGF21 with CRF, sedentary time and MVPA

Associations of plasma FGF21 with CRF, sedentary time, and MVPA are shown in Table 2 and Figure 2. After adjustment for demographic variables (model 1), the association with CRF was statistically significant for plasma FGF21 ($P = 0.007$). Each SD increase in CRF was associated with a 51% (95% CI: -71% to -19%) lower plasma FGF21 concentration. This association remained in the fully adjusted model (model 3) ($P = 0.004$), where each SD increase in CRF was associated with a 53% (95% CI: -73% to -22%) lower plasma FGF21 concentration. Additionally, there was a statistically significant association between plasma

FGF21 and MVPA (P = 0.006), whereby each SD increase in MVPA was associated with a 55% (95% CI: 12% to 114%) higher plasma FGF21 concentration.

Interaction analyses showed that the relationship between plasma FGF21 and MVPA was moderated by BMI (P = 0.052) (Table 3). Specifically, each SD increase in MVPA (per 30 minute) was associated with an 86% (95% CI: -48% to 576%) higher plasma FGF21 concentration in those with a lower BMI (< 26.1 $kg \cdot m^{-2}$), whereas no relationship was evident in those with a higher BMI (≥ 26.1 $kg \cdot m^{-2}$) (Table 3). Additionally, CRF also moderated the relationship between plasma FGF21 and MVPA (P = 0.088), such that each SD increase in MVPA (per 30 minute) was associated with a 104% (95% CI: 35% to 202%) higher plasma FGF21 concentration in those with a higher CRF (≥ 40.1 $mL \cdot kg^{-1} \cdot min^{-1}$) (Table 3).

Insert Table 3

Discussion

This study examined the associations of circulating LECT2 and FGF21 with CRF, sedentary time, and MVPA. The primary findings are that circulating concentrations of LECT2 and FGF21 are inversely associated with CRF. Importantly, these relationships were apparent after controlling for liver fat and other anthropometric, demographic, and accelerometer-measured variables, each of which are key mediators of circulating hepatokine concentrations. Whilst observational in nature, these data suggest that an individual's CRF may be an additional mediator of hepatokine metabolism.

LECT2 is a hepatokine that has several detrimental effects on metabolic homeostasis (Slowik & Apte, 2017). Specifically, LECT2 has been shown to promote skeletal muscle and adipose tissue insulin resistance in preclinical models (Jung et al., 2018; Lan et al., 2014) and has most recently been implicated in the development of hepatic inflammation (Takata et al., 2021). In humans, circulating LECT2 concentrations are elevated in individuals with obesity (Okumura et al., 2013), type 2 diabetes (Zhang et al., 2018) and NAFLD (Yoo et al., 2017), and are positively associated with adiposity, dyslipidaemia, and markers of insulin resistance (Lan et al., 2014; Okumura et al., 2013; Zhang et al., 2018). Liver fat is thought to be a key mediator of LECT2 production given that LECT2 mRNA is almost exclusively expressed in the liver and circulating concentrations are positively associated with the presence of NAFLD (Yamagoe et al., 1998; Yoo et al., 2017). Our data support and extend these findings as we observed novel positive associations between circulating LECT2 and MRI-derived liver fat in both simple correlations and fully adjusted generalised linear models (data not shown in results tables).

In the present study, we report for the first time that circulating concentrations of LECT2 are inversely and independently associated with CRF. Only one previous study has examined the relationship between circulating LECT2 and CRF, and the authors reported no statistically significant associations after adjusting for age and visceral adipose tissue (Tanisawa et al., 2017). We opted to adjust our models for liver fat given the importance of liver fat in the regulation of LECT2. Furthermore, discrepancies between the studies may be related to the more homogenous population of middle-aged and elderly men in the study by Tanisawa et al. (2017), and cultural/lifestyle differences between their Japanese population and our European population. Importantly, our findings were independent of liver fat, suggesting that CRF may be an additional mediator, and raises the possibility that improving CRF through exercise

training could potentially reduce circulating LECT2 concentrations. Previous research has demonstrated that an acute, exhaustive bout of exercise in mice can reduce hepatic LECT2 expression and secretion via increasing phosphorylation of hepatic AMPK (Lan et al., 2014); however, human studies are yet to replicate these findings (Sargeant et al., 2018; Willis et al., 2019). Experimental studies are required to determine whether improvements in CRF reduce circulating LECT2 concentrations in humans.

No previous studies have explored the associations of circulating LECT2 with objectively measured physical activity and sedentary time. In our fully adjusted model, we found no statistically significant associations between circulating LECT2 and sedentary time, or MVPA. Whilst it may be expected that MVPA would be negatively associated with circulating LECT2 given our observed association with CRF, it is important to note that CRF is determined by both genetic factors and habitual physical activity, in which vigorous intensity is a key determinant (Bouchard et al., 2015). Notably, our objective measurement of MVPA does not enable us to differentiate between these two intensities; thus, it is possible that the inclusion of moderate intensity physical activity dampened our ability to detect differences. Therefore, further research is warranted to specifically examine the associations of circulating LECT2 with more purposeful physical activity of vigorous intensity.

FGF21 is another hepatokine that has gained extensive attention due to its favorable effects on glucose and lipid metabolism (BonDurant & Potthoff, 2018). Synthetic FGF21 analogues have shown promise as novel medicinal therapies for metabolic disease (Cui et al., 2020). Administration of recombinant FGF21 reduces body weight, liver fat content and circulating glucose and lipid concentrations, and improves insulin sensitivity in mice with obesity and type 2 diabetes (Berglund et al., 2009; Coskun et al., 2008; Kharitonenkov et al., 2005). FGF21 is considered a marker of physiological stress since its production may be induced by several

309 acute metabolic stress signals such as fasting (Nygaard et al., 2018), (Sargeant, et al., 2018;
310 Willis et al., 2019), and overfeeding (Lundsgaard et al., 2017; Willis et al., 2020). Notably,
311 however, circulating FGF21 concentrations are chronically elevated in obesity, the metabolic
312 syndrome (Zhang et al., 2008), type 2 diabetes (Chavez et al., 2009), and NAFLD (Dushay et
313 al., 2010), potentially as a compensatory mechanism to alleviate the obesity-related metabolic
314 dysfunction. Similar to LECT2, liver fat may also be an important determinant of circulating
315 FGF21 concentrations (Okumura et al., 2013). Our data corroborate this notion as circulating
316 FGF21 was positively associated with liver fat in our sample of volunteers.

317 Furthermore, we found circulating FGF21 to be negatively associated with CRF independent
318 of anthropometric, demographic, physical activity variables, and liver fat. This finding is in
319 agreement with two previous studies reporting inverse associations between circulating FGF21
320 and $\dot{V}O_2$ peak in middle-aged and elderly men and women (Matsui et al., 2019; Taniguchi et
321 al., 2014). These data are also consistent with experimental research by Taniguchi et al. (2016)
322 who showed that five weeks of exercise training reduces circulating FGF21 concentrations
323 alongside improvements in CRF and reductions in liver fat content (Taniguchi et al., 2016). In
324 a subsequent regression analysis, the authors concluded that the liver fat reduction may be
325 mediating the exercise-induced decrease in circulating FGF21. Importantly, our regression
326 analysis demonstrated that the negative association between FGF21 and CRF was independent
327 of liver fat. Henceforth, this raises the possibility that interventions aimed at improving CRF
328 may be able to reduce FGF21 independent of changes in liver fat. Due to the observational
329 nature of the present study, future studies are needed to confirm this in experimental trials.

330 Additionally, we found that circulating FGF21 concentrations were independently positively
331 associated with greater objectively measured MVPA. Given our inverse association between
332 circulating FGF21 and CRF, the positive association observed with MVPA may appear

unexpected. However, our data are consistent with the work of others (Cuevas-Ramos et al., 2010, 2012) who have previously observed positive associations between circulating FGF21 and MVPA when measured using self-report questionnaires. However, the study by Cuevas-Ramos et al. (2012) demonstrated that two weeks of daily supervised physical activity was sufficient to increase circulating FGF21 concentrations. Consequently, our observed positive association with MVPA could represent a more transient acute response to recent physical activity, whereas CRF is a global marker of longer-term trends in higher intensity physical activity and healthy lifestyle practices. In contrast to our findings, Matsui et al. (2020) recently reported an inverse association between circulating FGF21 concentrations and objectively measured MVPA after adjustment for potential confounders (Matsui et al., 2020). It must be noted, however, that this association was only evident in their older cohort (mean age = 70 years), whilst the participants in the present study ranged from 18 to 59 years; thus, older age may be an important factor mediating this relationship.

Interestingly, although represented as statistical tendencies, our interaction analyses showed that the relationship between circulating FGF21 and MVPA may be modified by both BMI and CRF. Specifically, the positive association between circulating FGF21 and MVPA was stronger in those with lower BMI, and higher levels of CRF. This finding is in agreement with Slusher et al. (2015) who observed that the circulating FGF21 response to an acute bout of exercise was blunted in individuals with overweight or obesity, potentially due to the greater FGF21 resistance in these individuals (Slusher et al., 2015). Therefore, when split based on median CRF and BMI, the fitter and leaner individuals in our study cohort may possess a greater FGF21 sensitivity and are thus more responsive to regular bouts of physical activity. This supports the idea that chronic exercise training may act as an FGF21 sensitizer (Fletcher et al., 2012), potentially through increasing CRF and reducing body weight, which in turn could

increase the responsiveness of FGF21 to regular physical activity. Appropriately designed rodent and human studies are required to test this hypothesis in an experimental setting.

A crucial strength of the present study is our robust measurement of physical activity variables and sedentary time using accelerometers, and the use of MRI to quantify liver fat percentage. Furthermore, our sample is a diverse group of community volunteers spanning a wide range of demographic and physical variables. Some limitations of this study must also be recognized, however. The cross-sectional nature of the present study means that causality cannot be inferred. Notably, CRF is a global marker of overall health status that reflects genetic, environmental, and behavioural factors. Therefore, the associations reported here, could be confounded by unmeasured determinants of CRF. Additionally, the study participants were free from chronic disease; thus, future studies are needed to test our identified associations in clinical populations such as type 2 diabetes and NAFLD. Finally, whilst this study examined whether activity behaviours were independently associated with hepatokines, future studies should determine the interactive effects of sedentary time and physical activity (Julian et al., 2022).

In conclusion, the present study found that in a sample of community volunteers, CRF is negatively associated with both circulating LECT2 and FGF21 concentrations. Furthermore, circulating FGF21 is positively associated with MVPA, and this relationship may be stronger in those with a lower BMI and higher CRF. These findings suggest that independent of key demographics, sedentary time, physical activity, and liver fat, CRF is an important determinant of circulating concentrations of LECT2 and FGF21. Additional studies are now required to determine if reported association are in causal nature by undertaking interventions aimed at increasing CRF through chronic structured exercise training in both community volunteers and clinical populations.

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Authors Contribution

SM, JAK and SAW developed the initial idea for this secondary data analysis, which was further refined with DJS and SM. MJR, FRG, AET and DB collected the primary data which this secondary analysis is based on. SM, SAW and JAK led the analysis of this paper with support from JH, DHB and all other authors. All authors approved the final version of this manuscript and hold accountability for all aspects of the work.

Data Availability Statement

The datasets analysed during the current study are available from the corresponding author on reasonable request.

References

Berglund, E. D., Li, C. Y., Bina, H. A., Lynes, S. E., Michael, M. D., Shanafelt, A. B.,

403 Kharitonenkov, A., & Wasserman, D. H. (2009). Fibroblast growth factor 21 controls
404 glycemia via regulation of hepatic glucose flux and insulin sensitivity. *Endocrinology*,
405 *150*(9), 4084–4093. https://doi.org/10.1210/en.2009-0221

406 BonDurant, L. D., & Potthoff, M. J. (2018). Fibroblast Growth Factor 21: A Versatile
407 Regulator of Metabolic Homeostasis. *Annual Review of Nutrition*, *38*(1), 173–196.
408 https://doi.org/10.1146/annurev-nutr-071816-064800

409 Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. *Medicine and*
410 *Science in Sports*, *5*(2), 90–93.

411 Borga, M., Thomas, E. L., Romu, T., Rosander, J., Fitzpatrick, J., Dahlqvist Leinhard, O., &
412 Bell, J. D. (2015). Validation of a fast method for quantification of intra-abdominal and
413 subcutaneous adipose tissue for large-scale human studies. *NMR in Biomedicine*, *28*(12),
414 1747–1753. https://doi.org/10.1002/nbm.3432

415 Bouchard, C., Blair, S. N., & Katzmarzyk, P. T. (2015). Less sitting, more physical activity,
416 or higher fitness? *Mayo Clinic Proceedings*, *90*(11), 1533–1540.
417 https://doi.org/10.1016/j.mayocp.2015.08.005

418 Bruce, R. A., Kusumi, F., & Hosmer, D. (1973). Maximal oxygen intake and nomographic
419 assessment of functional aerobic impairment in cardiovascular disease. *American Heart*
420 *Journal*, *85*(4), 546–562. https://doi.org/10.1016/0002-8703(73)90502-4

421 Byrom, B., Stratton, G., McCarthy, M., & Muehlhausen, W. (2016). Objective measurement
422 of sedentary behaviour using accelerometers. *International Journal of Obesity*, *40*(11),
423 1809–1812. https://doi.org/10.1038/ijo.2016.136

424 Chavez, A. O., Molina-Carrion, M., Abdul-Ghani, M. A., Folli, F., DeFronzo, R. A., &

425 Tripathy, D. (2009). Circulating fibroblast growth factor-21 is elevated in impaired
426 glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin
427 resistance. *Diabetes Care*, *32*(8), 1542–1546. https://doi.org/10.2337/dc09-0684

428 Chow, W., Xu, A., Woo, Y. C., Tso, A. W. K., Cheung, S. C. W., Fong, C. H. Y., Tse, H. F.,
429 Chau, M. T., Cheung, B. M. Y., & Lam, K. S. L. (2013). Serum fibroblast growth
430 factor-21 levels are associated with carotid atherosclerosis independent of established
431 cardiovascular risk factors. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *33*(10),
432 2454–2459. https://doi.org/10.1161/ATVBAHA.113.301599

433 Coskun, T., Bina, H. A., Schneider, M. A., Dunbar, J. D., Hu, C. C., Chen, Y., Moller, D. E.,
434 & Kharitonenkov, A. (2008). Fibroblast growth factor 21 corrects obesity in mice.
435 *Endocrinology*, *149*(12), 6018–6027. https://doi.org/10.1210/en.2008-0816

436 Cuevas-Ramos, D., Almeda-Valdes, P., Gómez-Pérez, F. J., Elena Meza-Arana, C., Cruz-
437 Bautista, I., Arellano-Campos, O., Navarrete-López, M., & Aguilar-Salinas, C. A.
438 (2010). Daily physical activity, fasting glucose, uric acid, and body mass index are
439 independent factors associated with serum fibroblast growth factor 21 levels. *European*
440 *Journal of Endocrinology*, *163*(3), 469–477. https://doi.org/10.1530/EJE-10-0454

441 Cuevas-Ramos, D., Almeda-Valdes, P., Meza-Arana, C. E., Brito-Córdova, G., Gómez-
442 Pérez, F. J., Mehta, R., Oseguera-Moguel, J., & Aguilar-Salinas, C. A. (2012). Exercise
443 increases serum fibroblast growth factor 21 (FGF21) levels. *PLoS ONE*, *7*(5), 1–8.
444 https://doi.org/10.1371/journal.pone.0038022

445 Cui, A., Li, J., Ji, S., Ma, F., Wang, G., Xue, Y., Liu, Z., Gao, J., Han, J., Tai, P., Wang, T.,
446 Chen, J., Ma, X., & Li, Y. (2020). The Effects of B1344, a Novel Fibroblast Growth

Factor 21 Analog, on Nonalcoholic Steatohepatitis in Nonhuman Primates. *Diabetes*, *69*(8), 1611–1623. https://doi.org/10.2337/DB20-0209

Dushay, J., Chui, P. C., Gopalakrishnan, G. S., Varela-Rey, M., Crawley, M., Fisher, F. M., Badman, M. K., Martinez-Chantar, M. L., & Maratos-Flier, E. (2010). Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology*, *139*(2), 456–463. https://doi.org/10.1053/j.gastro.2010.04.054

Ennequin, G., Sirvent, P., & Whitham, M. (2019). Role of exercise-induced hepatokines in metabolic disorders. *American Journal of Physiology - Endocrinology and Metabolism*, *317*(1), E11–E24. https://doi.org/10.1152/ajpendo.00433.2018

Fletcher, J. A., Meers, G. M., Harold Laughlin, M., Ibdah, J. A., Thyfault, J. P., & Rector, R. S. (2012). Modulating fibroblast growth factor 21 in hyperphagic OLETF rats with daily exercise and caloric restriction. *Applied Physiology, Nutrition and Metabolism*, *37*(6), 1054–1062. https://doi.org/10.1139/H2012-091

Foster, C., Jackson, A. S., Pollock, M. L., Taylor, M. M., Hare, J., Sennett, S. M., Rod, J. L., Sarwar, M., & Schmidt, D. H. (1984). Generalized equations for predicting functional capacity from treadmill performance. *American Heart Journal*, *107*(6), 1229–1234. https://doi.org/10.1016/0002-8703(84)90282-5

Freedson, P. S., Melanson, E., & Sirard, J. (1998). Calibration of the Computer Science and Applications, Inc. accelerometer. *Medicine & Science in Sports & Exercise*, *30*(5). https://journals.lww.com/acsm-msse/Fulltext/1998/05000/Calibration_of_the_Computer_Science_and.21.aspx

Gaich, G., Chien, J. Y., Fu, H., Glass, L. C., Deeg, M. A., Holland, W. L., Kharitonenkov,

A., Bumol, T., Schilske, H. K., & Moller, D. E. (2013). The effects of LY2405319, an FGF21 Analog, in obese human subjects with type 2 diabetes. *Cell Metabolism*, *18*(3), 333–340. https://doi.org/10.1016/j.cmet.2013.08.005

Garcia, D., Hellberg, K., Chaix, A., Wallace, M., Herzig, S., Badur, M. G., Lin, T., Shokhirev, M. N., Pinto, A. F. M., Ross, D. S., Saghatelian, A., Panda, S., Dow, L. E., Metallo, C. M., & Shaw, R. J. (2019). Genetic Liver-Specific AMPK Activation Protects against Diet-Induced Obesity and NAFLD. *Cell Reports*, *26*(1), 192-208.e6. https://doi.org/10.1016/j.celrep.2018.12.036

Giralt, M., Gavaldà-Navarro, A., & Villarroya, F. (2015). Fibroblast growth factor-21, energy balance and obesity. *Molecular and Cellular Endocrinology*, *418*, 66–73. https://doi.org/10.1016/j.mce.2015.09.018

Goltz, F. R., Thackray, A. E., Varela-Mato, V., King, J. A., Dorling, J. L., Dowejko, M., Mastana, S., Thompson, J., Atkinson, G., & Stensel, D. J. (2019). Exploration of associations between the FTO rs9939609 genotype, fasting and postprandial appetite-related hormones and perceived appetite in healthy men and women. *Appetite*, *142*(March), 104368. https://doi.org/10.1016/j.appet.2019.104368

Hansen, J. S., Clemmesen, J. O., Secher, N. H., Hoene, M., Drescher, A., Weigert, C., Pedersen, B. K., & Plomgaard, P. (2015). Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans. *Molecular Metabolism*, *4*(8), 551–560. https://doi.org/10.1016/j.molmet.2015.06.001

Jensen-Cody, S. O., & Potthoff, M. J. (2021). Hepatokines and metabolism: Deciphering communication from the liver. *Molecular Metabolism*, *44*(101138), 1–17.

https://doi.org/10.1016/j.molmet.2020.101138

Julian, V., Bergsten, P., Ennequin, G., Forslund, A., Ahlstrom, H., Ciba, I., Dahlbom, M., Furthner, D., Gomahr, J., Kullberg, J., Maruszczak, K., Morwald, K., Olsson, R., Pixner, T., Schneider, A., Pereira, B., Ring-Dimitriou, S., Thivel, D., & Weghuber, D. (2022). Association between alanine aminotransferase as surrogate of fatty liver disease and physical activity and sedentary time in adolescents with obesity. *European Journal of Pediatrics*, *181*(8), 3119–3129. https://doi.org/10.1007/S00431-022-04539-Z

Jung, T. W., Chung, Y. H., Kim, H. C., Abd El-Aty, A. M., & Jeong, J. H. (2018). LECT2 promotes inflammation and insulin resistance in adipocytes via P38 pathways. *Journal of Molecular Endocrinology*, *61*(1), 37–45. https://doi.org/10.1530/JME-17-0267

Kharitonenkov, A., Shiyanova, T. L., Koester, A., Ford, A. M., Micanovic, R., Galbreath, E. J., Sandusky, G. E., Hammond, L. J., Moyers, J. S., Owens, R. A., Gromada, J., Brozinick, J. T., Hawkins, E. D., Wroblewski, V. J., Li, D. S., Mehrbod, F., Jaskunas, S. R., & Shanafelt, A. B. (2005). FGF-21 as a novel metabolic regulator. *Journal of Clinical Investigation*, *115*(6), 1627–1635. https://doi.org/10.1172/JCI23606

Kirpich, I. A., Gobejishvili, L. N., Homme, M. B., Waigel, S., Cave, M., Arteel, G., Barve, S. S., McClain, C. J., & Deaciuc, I. V. (2011). Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease. *Journal of Nutritional Biochemistry*, *22*(1), 38–45. https://doi.org/10.1016/j.jnutbio.2009.11.009

Lan, F., Misu, H., Chikamoto, K., Takayama, H., Kikuchi, A., Mohri, K., Takata, N., Hayashi, H., Matsuzawa-Nagata, N., Takeshita, Y., Noda, H., Matsumoto, Y., Ota, T.,

513 Nagano, T., Nakagen, M., Miyamoto, K. I., Takatsuki, K., Seo, T., Iwayama, K., …
514 Takamura, T. (2014). LECT2 functions as a hepatokine that links obesity to skeletal
515 muscle insulin resistance. *Diabetes*, *63*(5), 1649–1664. https://doi.org/10.2337/db13-
516 0728

517 Lundsgaard, A. M., Fritzen, A. M., Sjøberg, K. A., Myrmel, L. S., Madsen, L.,
518 Wojtaszewski, J. F. P., Richter, E. A., & Kiens, B. (2017). Circulating FGF21 in humans
519 is potently induced by short term overfeeding of carbohydrates. *Molecular Metabolism*,
520 *6*(1), 22–29. https://doi.org/10.1016/j.molmet.2016.11.001

521 Mai, K., Andres, J., Biedasek, K., Weicht, J., Bobbert, T., Sabath, M., Meinus, S., Reinecke,
522 F., Möhlig, M., Weickert, M. O., Clemenz, M., Pfeiffer, A. F. H., Kintscher, U., Spuler,
523 S., & Spranger, J. (2009). Free fatty acids link metabolism and regulation of the insulin-
524 sensitizing fibroblast growth factor-21. *Diabetes*, *58*(7), 1532–1538.
525 https://doi.org/10.2337/db08-1775

526 Markan, K. R., Naber, M. C., Ameka, M. K., Anderegg, M. D., Mangelsdorf, D. J., Kliewer,
527 S. A., Mohammadi, M., & Potthoff, M. J. (2014). Circulating FGF21 is liver derived and
528 enhances glucose uptake during refeeding and overfeeding. *Diabetes*, *63*(12), 4057–
529 4063. https://doi.org/10.2337/db14-0595

530 Matsui, M., Kosaki, K., Akazawa, N., Tanahashi, K., Kuro-o, M., & Maeda, S. (2019).
531 Association between circulating fibroblast growth factor 21, aerobic fitness, and aortic
532 blood pressure in middle-aged and older women. *The Journal of Physical Fitness and*
533 *Sports Medicine*, *8*(5), 195–201. https://doi.org/10.7600/jpfsm.8.195

534 Matsui, M., Kosaki, K., Tanahashi, K., Akazawa, N., Osuka, Y., Tanaka, K., Kuro-o, M., &

Maeda, S. (2020). Relationship between physical activity and circulating fibroblast growth factor 21 in middle-aged and older adults. *Experimental Gerontology*, *141*. https://doi.org/10.1016/j.exger.2020.111081

McCarthy, M., Edwardson, C. L., Davies, M. J., Henson, J., Bodicoat, D. H., Khunti, K., Dunstan, D. W., King, J. A., & Yates, T. (2017). Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks. *Medicine and Science in Sports and Exercise*, *49*(11), 2216–2222. https://doi.org/10.1249/MSS.0000000000001338

Meex, R. C., Hoy, A. J., Morris, A., Brown, R. D., Lo, J. C. Y., Burke, M., Goode, R. J. A., Kingwell, B. A., Kraakman, M. J., Febbraio, M. A., Greve, J. W., Rensen, S. S., Molloy, M. P., Lancaster, G. I., Bruce, C. R., & Watt, M. J. (2015). Fetuin B Is a Secreted Hepatocyte Factor Linking Steatosis to Impaired Glucose Metabolism. *Cell Metabolism*, *22*(6), 1078–1089. https://doi.org/10.1016/J.CMET.2015.09.023

Nygaard, E. B., Ørskov, C., Almdal, T., Vestergaard, H., & Andersen, B. (2018). Fasting decreases plasma FGF21 in obese subjects and the expression of FGF21 receptors in adipose tissue in both lean and obese subjects. *Journal of Endocrinology*, *239*(1), 73–80. https://doi.org/10.1530/JOE-18-0002

Okumura, A., Unoki-Kubota, H., Matsushita, Y., Shiga, T., Moriyoshi, Y., Yamagoe, S., & Kaburagi, Y. (2013). Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver. *BioScience Trends*, *7*(6), 276–283. https://doi.org/10.5582/bst.2013.v7.6.276

Roberts, M. J., Thackray, A. E., Wadley, A. J., Alotaibi, T. F., Hunter, D. J., Thompson, J., Fujihara, K., Miyashita, M., Mastana, S., Bishop, N. C., O'Donnell, E., Davies, M. J.,

King, J. A., Yates, T., Webb, D., & Stensel, D. J. (2022). Effect of acute walking on endothelial function and postprandial lipaemia in South Asians and white Europeans. *Medicine & Science in Sports & Exercise*, *(In Press)*.

Sargeant, J. A., Aithal, G. P., Takamura, T., Misu, H., Takayama, H., Douglas, J. A., Turner, M. C., Stensel, D. J., Nimmo, M. A., Webb, D. R., Yates, T., King, J. A., Misu, H., & Yates, T. (2018). The influence of adiposity and acute exercise on circulating hepatokines in normal-weight and overweight/obese men. *Appl. Physiol. Nutr. Metab.*, *43*(5), 482–490. https://doi.org/10.1139/apnm-2017-0639

Slowik, V., & Apte, U. (2017). Leukocyte Cell-Derived Chemotaxin-2: It's Role in Pathophysiology and Future in Clinical Medicine. *Clinical and Translational Science*, *10*(4), 249–259. https://doi.org/10.1111/cts.12469

Slusher, A. L., Whitehurst, M., Zoeller, R. F., Mock, J. T., Maharaj, M., & Huang, C. J. (2015). Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese individuals. *Nutrition, Metabolism and Cardiovascular Diseases*, *25*(9), 839–845. https://doi.org/10.1016/j.numecd.2015.06.002

Takata, N., Ishii, K. aki, Takayama, H., Nagashimada, M., Kamoshita, K., Tanaka, T., Kikuchi, A., Takeshita, Y., Matsumoto, Y., Ota, T., Yamamoto, Y., Yamagoe, S., Seki, A., Sakai, Y., Kaneko, S., & Takamura, T. (2021). LECT2 as a hepatokine links liver steatosis to inflammation via activating tissue macrophages in NASH. *Scientific Reports*, *11*(1), 1–10. https://doi.org/10.1038/s41598-020-80689-0

Taniguchi, H., Tanisawa, K., Sun, X., Cao, Z. B., Oshima, S., Ise, R., Sakamoto, S., & Higuchi, M. (2014). Cardiorespiratory fitness and visceral fat are key determinants of

serum fibroblast growth factor 21 concentration in Japanese men. *Journal of Clinical Endocrinology and Metabolism*, *99*(10), E1877–E1884. https://doi.org/10.1210/jc.2014-1877

Taniguchi, H., Tanisawa, K., Sun, X., Kubo, T., & Higuchi, M. (2016). Endurance exercise reduces hepatic fat content and serum fibroblast growth factor 21 levels in elderly men. *Journal of Clinical Endocrinology and Metabolism*, *101*(1), 191–198. https://doi.org/10.1210/jc.2015-3308

Tanisawa, K., Taniguchi, H., Sun, X., Ito, T., Kawamori, R., Sakamoto, S., & Higuchi, M. (2017). Visceral fat area is a strong predictor of leukocyte cell-derived chemotaxin 2, a potential biomarker of dyslipidemia. *PLoS ONE*, *12*(3), 1–14. https://doi.org/10.1371/journal.pone.0173310

van Baak, M. A., Vink, R. G., Roumans, N. J. T., Cheng, C. C., Adams, A. C., & Mariman, E. C. M. (2020). Adipose tissue contribution to plasma fibroblast growth factor 21 and fibroblast activation protein in obesity. *International Journal of Obesity*, *44*(2), 544–547. https://doi.org/10.1038/s41366-019-0433-x

Watt, M. J., Miotto, P. M., De Nardo, W., & Montgomery, M. K. (2019). The Liver as an Endocrine Organ—Linking NAFLD and Insulin Resistance. *Endocrine Reviews*, *40*(5), 1367–1393. https://doi.org/10.1210/er.2019-00034

Weigert, C., Hoene, M., & Plomgaard, P. (2019). Hepatokines—a novel group of exercise factors. In *Pflugers Archiv European Journal of Physiology* (Vol. 471, Issue 3, pp. 383–396). Springer Verlag. https://doi.org/10.1007/s00424-018-2216-y

West, J., Leinhard, O. D., Romu, T., Collins, R., Garratt, S., Bell, J. D., Borga, M., &

Thomas, L. (2016). Feasibility of MR-based body composition analysis In large scale population studies. *PLoS ONE*, *11*(9). https://doi.org/10.1371/journal.pone.0163332

Willis, S. A., Sargeant, J. A., Thackray, A. E., Yates, T., Stensel, D. J., Aithal, G. P., & King, J. A. (2019). Effect of exercise intensity on circulating hepatokine concentrations in healthy men. *Applied Physiology, Nutrition and Metabolism*, *44*(10), 1065–1072. https://doi.org/10.1139/apnm-2018-0818

Willis, S. A., Sargeant, J. A., Yates, T., Takamura, T., Takayama, H., Gupta, V., Brittain, E., Crawford, J., Parry, S. A., Thackray, A. E., Varela-Mato, V., Stensel, D. J., Woods, R. M., Hulston, C. J., Aithal, G. P., & King, J. A. (2020). Acute hyperenergetic, high-Fat feeding increases circulating FGF21, LECT2, and fetuin - A in healthy men. *Journal of Nutrition*, *150*(5), 1076–1085. https://doi.org/10.1093/jn/nxz333

Xu, J., Lloyd, D. J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., Vonderfecht, S., Hecht, R., Li, Y. S., Lindberg, R. A., Chen, J. L., Jung, D. Y., Zhang, Z., Ko, H. J., Kim, J. K., & Véniant, M. M. (2009). Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes*, *58*(1), 250–259. https://doi.org/10.2337/db08-0392

Yamagoe, S., Mizuno, S., & Suzuki, K. (1998). Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. *Biochimica et Biophysica Acta - Gene Structure and Expression*, *1396*(1), 105–113. https://doi.org/10.1016/S0167-4781(97)00181-4

Yoo, H. J., Hwang, S. Y., Choi, J. H., Lee, H. J., Chung, H. S., Seo, J. A., Kim, S. G., Kim, N. H., Baik, S. H., Choi, D. S., & Choi, K. M. (2017). Association of leukocyte cell-

derived chemotaxin 2 (LECT2) with NAFLD, metabolic syndrome, and atherosclerosis. *PLoS ONE*, *12*(4), 1–11. https://doi.org/10.1371/journal.pone.0174717

Zhang, X., Yeung, D. C. Y., Karpisek, M., Stejskal, D., Zhou, Z., Liu, F., Wong, R. L. C., Chow, W., Tso, A. W. K., & Lam, K. S. L. (2008). Serum FGF21 Levels Are Increased in Obesity and Are Independently Associated With the Metabolic Syndrome in Humans. *Diabetes*, *57*(May), 1246–1253. https://doi.org/10.2337/db07-1476.Additional

Zhang, Z., Zeng, H., Lin, J., Hu, Y., Yang, R., Sun, J., Chen, R., & Chen, H. (2018). Circulating LECT2 levels in newly diagnosed type 2 diabetes mellitus and their association with metabolic parameters. *Medicine (United States)*, *97*(15). https://doi.org/10.1097/MD.0000000000010354

643 **Table 1. Participant characteristics.**

Demographic variables	Combined (*n* = 141)		Female (*n* = 56)		Male (*n* = 85)	
Ethnicity (white European)	119	[84]	51	[91]	68	[80]
Age (years)	37.0	(19.0)	34.5	(14.7)	38.0	(17.0)
Height (cm)	172.8	± 8.9	165.2	± 6.1	177.8	± 6.6
Body mass (kg)	80.9	± 19.7	66.5	± 11.1	90.3	± 18.3
Anthropometric variables						
BMI ($kg \cdot m^{-2}$)	26.1	(6.3)	24.1	(4.9)	27.4	(6.4)
MRI-derived variables						
Liver fat (%) [a]	1.8	(2.1)	1.3	(0.9)	2.3	(5.8)
Cardiorespiratory fitness, sedentary time, and physical activity						
CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	40.8	± 9.8	38.9	± 6.0	42.1	± 11.5
Sedentary time ($mins \cdot d^{-1}$) [a]	580	± 95	557	± 82	595	± 100
MVPA ($mins \cdot d^{-1}$) [a]	50	(41)	46	(41)	50	(41)
Device wear time ($mins \cdot d^{-1}$) [a]	925	(73)	917	(63)	926	(83)
Hepatokines						
LECT2 ($ng \cdot mL^{-1}$)	25	± 6	25	± 5	25	± 7
FGF21 ($pg \cdot mL^{-1}$) [a]	116	(162)	88	(107)	145	(211)

644 Data are presented as mean ± SD, median (interquartile range) or number [column percentage]. BMI,
645 body mass index; CRF, cardiorespiratory fitness; MRI, magnetic resonance imaging; MVPA, moderate-
646 vigorous intensity physical activity; LECT2, leukocyte cell–derived chemotaxin 2; FGF21, fibroblast
647 growth factor 21. [a]*Please note n = 130 for physical activity data, n = 126 for liver fat data and n = 140*
648 *for FGF21 data*

649 **Table 2. Associations of cardiorespiratory fitness and objectively measured sedentary time and physical activity with circulating**
650 **hepatokines**

	CRF **(mL·kg^{-1}·min^{-1})**[bc]		**Sedentary time (per 30 mins)**[ac]		**MVPA (per 30 mins)**[ab]	
	Fold-change (95% CI)	***P*-value**	**Fold-change (95% CI)**	***P*-value**	**Fold-change (95% CI)**	***P*-value**
Model 1						
LECT2 (ng.mL^{-1})	**0.74 (0.60 to 0.89)**	**0.001**	1.10 (0.95 to 1.23)	0.233	1.00 (0.89 to 1.12)	0.971
FGF21 (pg.mL^{-1})	**0.49 (0.29 to 0.81)**	**0.007**	1.20 (0.85 to 1.70)	0.306	1.20 (0.91 to 1.62)	0.202
Model 2						
LECT2 (ngm.L^{-1})	**0.72 (0.59 to 0.87)**	**0.001**	1.10 (0.95 to 1.26)	0.178	1.07 (0.95 to 1.20)	0.234
FGF21 (pgm.L^{-1})	**0.42 (0.25 to 0.71)**	**0.001**	1.35 (0.93 to 1.91)	0.109	**1.12 (1.12 to 2.09)**	**0.009**
Model 3						
LECT2 (ngm.L^{-1})	**0.76 (0.63 to 0.91)**	**0.003**	1.07 (0.93 to 1.23)	0.276	1.10 (0.98 to 1.23)	0.130
FGF21 (pgm.L^{-1})	**0.47 (0.27 to 0.78)**	**0.004**	1.29 (0.91 to 1.78)	0.150	**1.55 (1.12 to 2.14)**	**0.006**

651 Data were back-transformed to show fold-change (95% CI). Model 1 adjusted for study, sex, ethnicity, age, BMI, and device wear time. Model 2
652 adjusted for all of the previous plus [a]*CRF,* [b]*sedentary time, or* [c]*MVPA*. Model 3 adjusted for all of the previous covariates plus liver fat. CRF,
653 cardiorespiratory fitness; MVPA, moderate-vigorous intensity physical activity; LECT2, leukocyte cell–derived chemotaxin 2; FGF21, fibroblast
654 growth factor 21.

Table 3. Statistically significant interaction analyses with body mass index, cardiorespiratory fitness and objectively measured physical activity.

Outcome	Variable	n	*P*-value for interaction	Category 1 Fold-change (95% CI)	Category 2 Fold-change (95% CI)
BMI				**< 26.1 kg·m^{-2}**	**≥ 26.1 kg·m^{-2}**
FGF21 (pg·mL^{-1})	MVPA (per 30 mins)[ab]	129	0.052	*1.86 (0.52 to 6.76)*	0.83 (0.46 to 1.51)
Cardiorespiratory fitness				**< *40.1* mL·kg^{-1}·min^{-1}**	**≥ *40.1* mL·kg^{-1}·min^{-1}**
FGF21 (pg·mL^{-1})	MVPA (per 30 mins)[ab]	129	0.088	1.07 (0.91 to 2.82)	2.04 (1.35 to 3.02)

Models adjusted for study, sex, ethnicity, age, device wear time, BMI, interaction term and all previous plus [a]*CRF and* [b]*sedentary time. Data are presented as P-values* for the interaction term and as fold-changes (95% confidence intervals) for categorical variables and variables stratified using the median split. BMI, body mass index; CRF, cardiorespiratory fitness; FGF21, fibroblast growth factor 21; LECT2, leukocyte cell–derived chemotaxin 2; MVPA, moderate-vigorous intensity physical activity.

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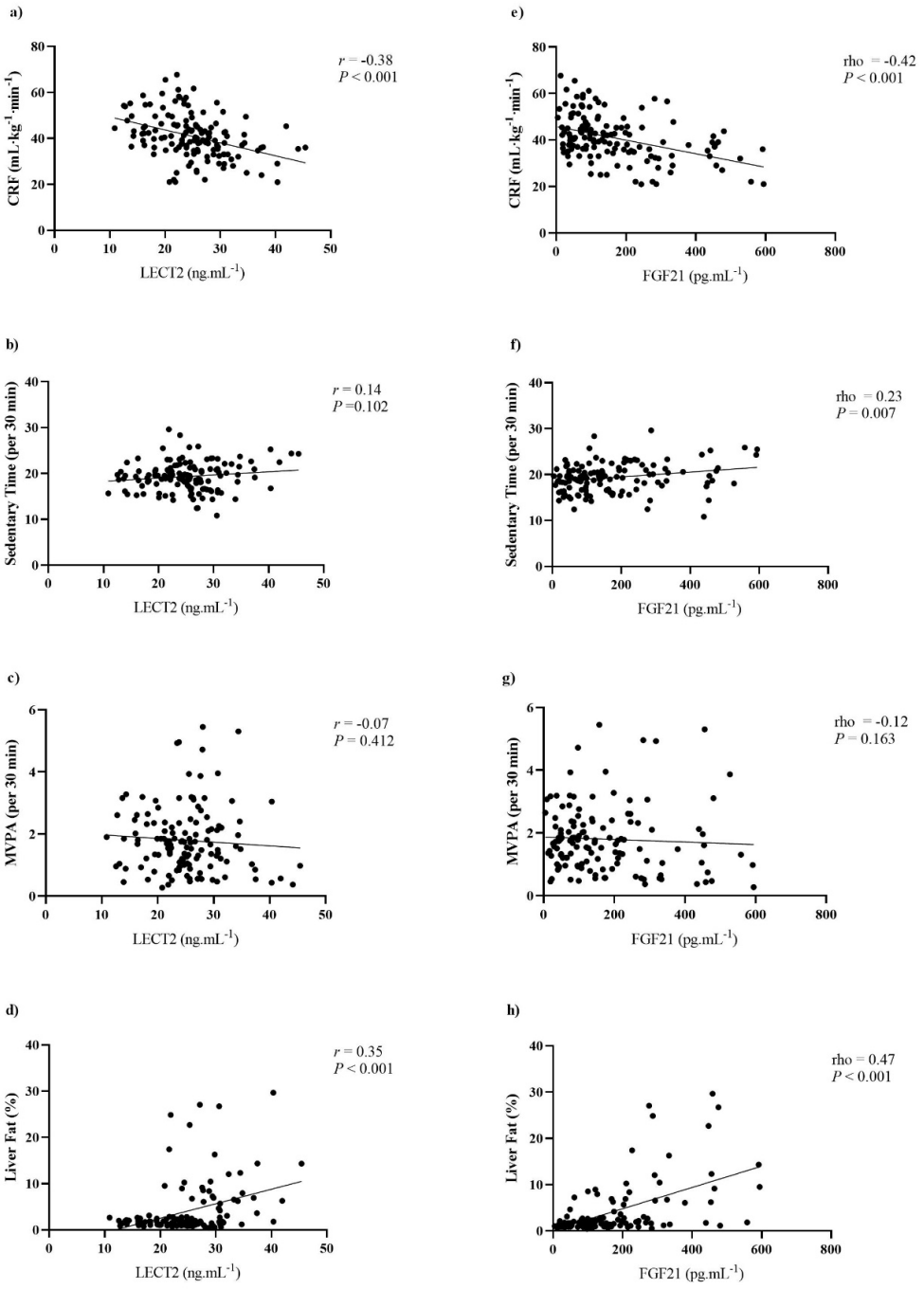

662

663 **Figure 1.** Correlations of plasma LECT2 and FGF21 with CRF (a, e), sedentary time (b, f),
664 MVPA (c, g), and liver fat (d, h). CRF, cardiorespiratory fitness; FGF21, fibroblast growth
665 factor 21; LECT2, leukocyte cell–derived chemotaxin 2; MVPA, moderate-vigorous intensity
666 physical activity.

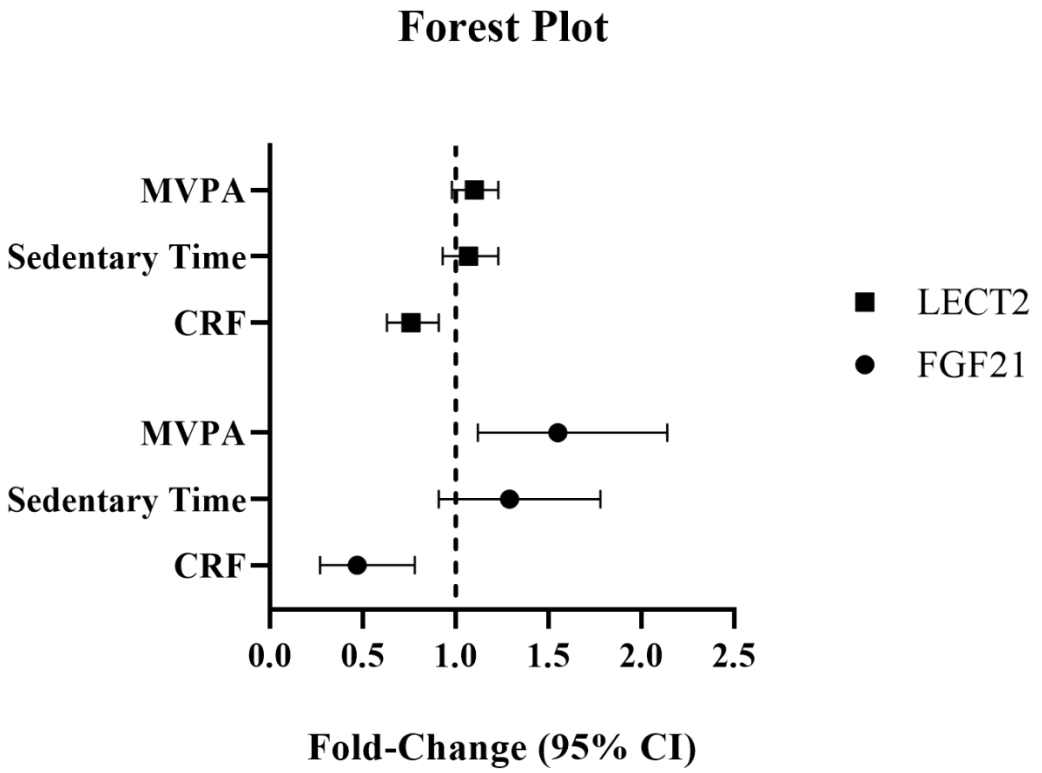

Figure 2. Forest plot showing the associations of cardiorespiratory fitness, sedentary time and objectively measured moderate-vigorous physical activity with plasma LECT2 and FGF21. Values represent fold-change and 95% CI for each SD change in CRF and physical activity metrics (model 3). CRF, cardiorespiratory fitness; FGF21, fibroblast growth factor 21; LECT2, leukocyte cell–derived chemotaxin 2; MVPA, moderate-vigorous intensity physical activity.