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

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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

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

Measurement of liver fat

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

Measurement of physical activity and sedentary time

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

Measurement of cardiorespiratory fitness

Cardiorespiratory fitness was assessed as participants' peak oxygen uptake ($\dot{V}O_2$ peak) and was measured directly via indirect calorimetry for 111 individuals. Within these tests, participants completed an incremental protocol on a treadmill until volitional exhaustion, with heart rate (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.

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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)

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

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

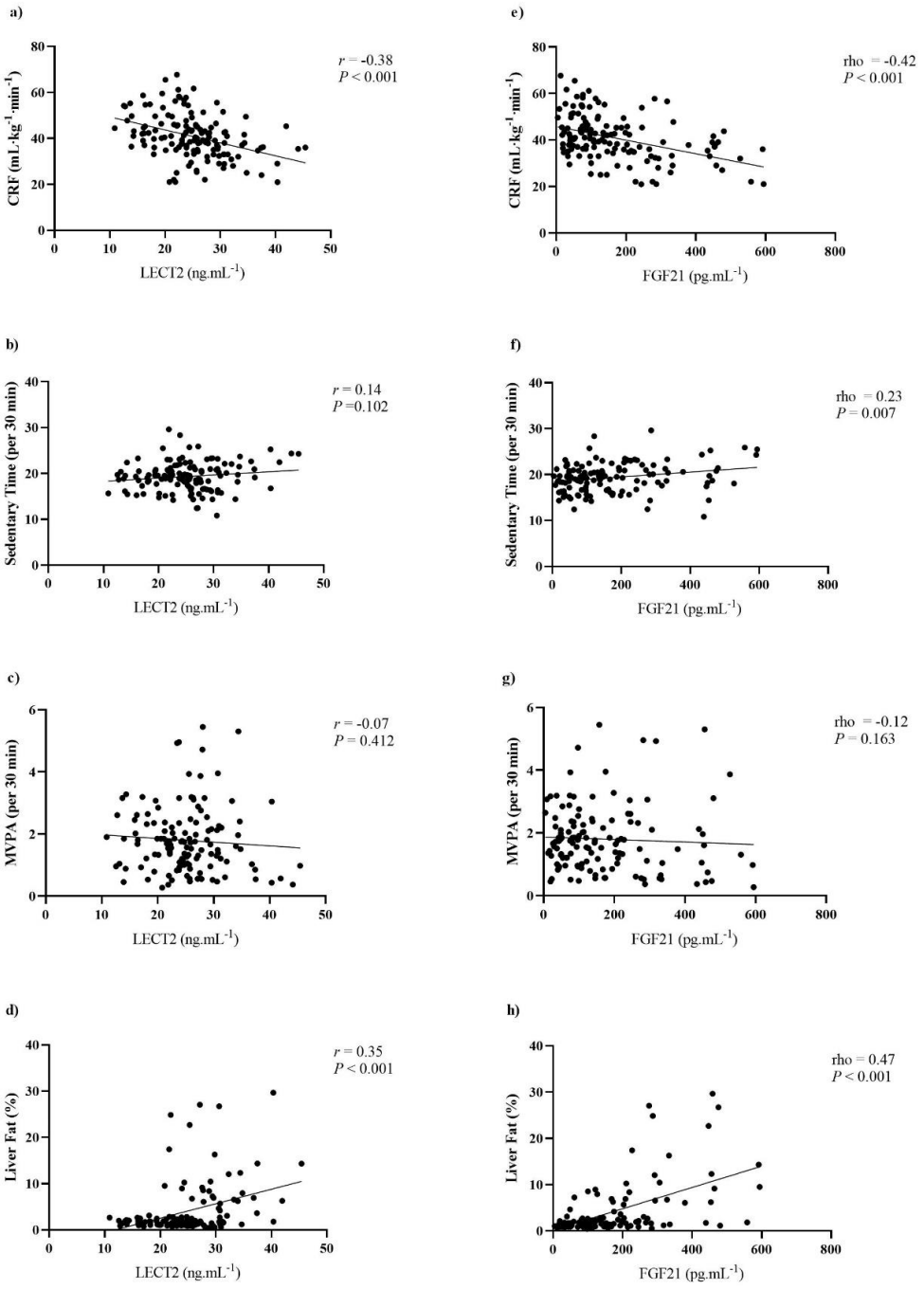
Data were back-transformed to show fold-change (95% CI). Model 1 adjusted for study, sex, ethnicity, age, BMI, and device wear time. Model 2 adjusted for all of the previous plus [a]*CRF*, [b]*sedentary time, or* [c]*MVPA*. Model 3 adjusted for all of the previous covariates plus liver fat. CRF, cardiorespiratory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

661



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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.

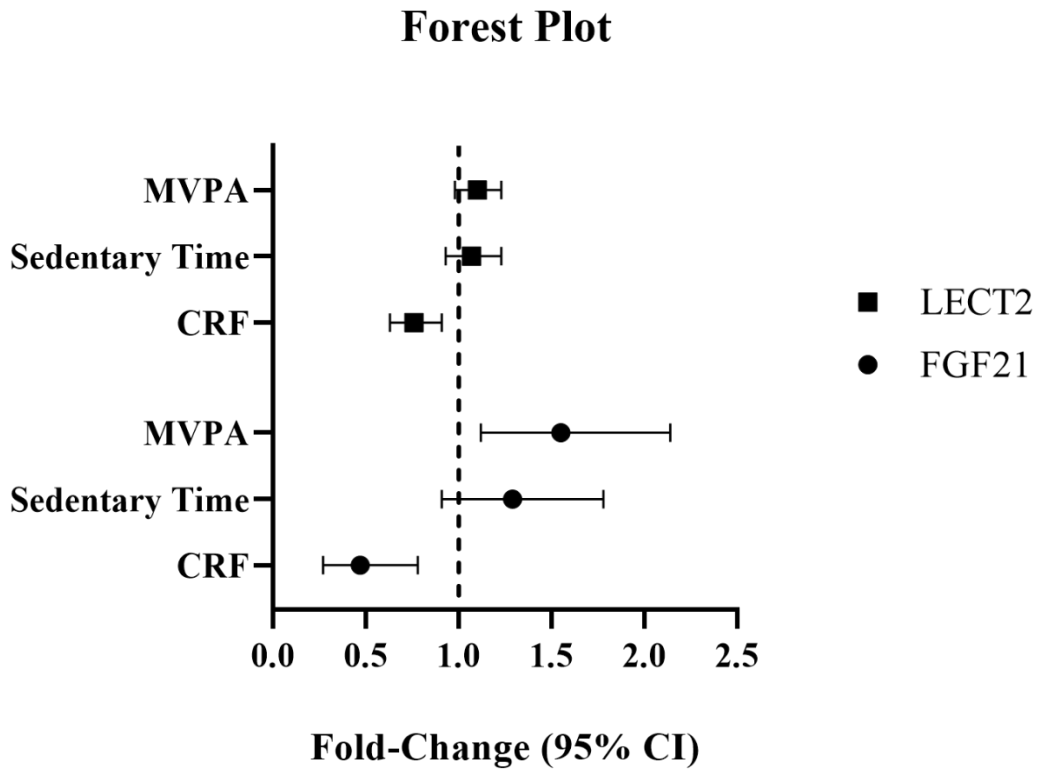

Figure 2. Forest plot showing the associations of cardiorespiratory fitness, sedentary time and objectively measured moderate-vigorous physical activity with plasma LECT2 and FGF21. Values represent fold-change and 95% CI for each SD change in CRF and physical activity metrics (model 3). CRF, cardiorespiratory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