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Effects of acute exercise and exercise training on plasma GDF15 concentrations and associations with appetite and cardiometabolic health in individuals with overweight or obesity – A secondary analysis of a randomized controlled trial

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ABSTRACT

Growth Differentiation Factor 15 (GDF15) is seemingly involved in appetite control. Acute exercise increases GDF15 concentrations in lean humans, but acute and long-term effects of exercise on GDF15 in individuals with overweight/obesity are unknown. We investigated the effects of acute exercise and exercise training on GDF15 concentrations in individuals with overweight/obesity and associations with appetite and cardiometabolic markers. 90 physically inactive adults (20–45 years) with overweight/obesity were randomized to 6-months habitual lifestyle (CON, n=16), or isocaloric exercise of moderate (MOD, n=37) or vigorous intensity (VIG, n=37), 5 days/week. Testing was performed at baseline, 3, and 6 months. Plasma GDF15 concentrations, other metabolic markers, and subjective appetite were assessed fasted and in response to acute exercise before an *ad libitum* meal. Cardiorespiratory fitness, body composition, insulin sensitivity, and intraabdominal adipose tissue were measured. At baseline, GDF15 increased 18% (95%CI: 4; 34) immediately after acute exercise and 32% (16; 50) 60 min post-exercise. Fasting GDF15 increased 21% (0; 46) in VIG after 3 months (p=0.045), but this attenuated at 6 months (13% (−11; 43), p=0.316) and was unchanged in MOD (11% (−6; 32), p=0.224, across 3 and 6 months). Post-exercise GDF15 did not change in MOD or VIG. GDF15 was not associated with appetite or energy intake. Higher GDF15 was associated with lower cardiorespiratory fitness, central obesity, dyslipidemia, and poorer glycemic control. In conclusion, GDF15 increased in response to acute exercise but was unaffected by exercise training. Higher GDF15 concentrations were associated with a less favorable cardiometabolic profile but not with markers of appetite. This suggests that GDF15 increases in response to acute exercise independent of training state. Whether this has an impact on free-living energy intake and body weight management needs investigation.

1. Introduction

Growth Differentiation Factor 15 (GDF15) is a circulating protein involved in the regulation of inflammatory pathways, apoptosis, cell repair, and growth (Tsai, Husaini, Sainsbury, Brown, & Breit, 2018; Wang et al., 2021). GDF15 has also shown potency to reduce food intake, preference for energy-dense food and body weight in animals, and it is under development as an anti-obesity agent (Tsai et al., 2018;

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Wang et al., 2021). Somewhat contradictory to these effects, observational studies in humans have reported elevated concentrations of GDF15 in individuals with obesity and type 2 diabetes (Vila et al., 2011) and positive associations between GDF15 and diabetes incidence (Bao et al., 2019; Carstensen et al., 2010).

The effects of a single bout of vigorous intensity exercise on appetite are rather well established and include a transient suppression of subjective appetite and of the orexigenic hormone acylated ghrelin and increases in anorexigenic hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), a phenomenon known as *exercise-induced anorexia* (Dorling et al., 2018; King, Burley, & Blundell, 1994; Schubert, Sabapathy, Leveritt, & Desbrow, 2014). In contrast, the effects of regular exercise training on appetite control, including the appetite response to an acute bout of exercise after exercise training, are relatively poorly understood. It has been suggested that habitual exercise improves the sensitivity of the appetite control system (Beaulieu, Hopkins, Blundell, & Finlayson, 2018), but findings on subjective appetite ratings, appetite-regulating hormones, and energy intake in response to exercise training have been inconsistent (Beaulieu et al., 2021; Dorling et al., 2018; Janus et al., 2019; Quist, Blond, et al., 2019).

Plasma GDF15 concentrations have been shown to increase in response to acute vigorous exercise in healthy individuals (Conte et al., 2020; Galliera et al., 2014; Klein et al., 2021; Kleinert et al., 2018; Tchou et al., 2009). Furthermore, exercise training has been associated with increased fasting concentrations of GDF15 in older individuals with obesity, and a greater increase in GDF15 was associated with a greater reduction in fat mass, but no control group was included (Zhang, Fealy, & Kirwan, 2019). These findings indicate that GDF15 is a part of the complex exercise-related control of appetite. However, there is a need for studies investigating the GDF15 response after an acute bout of exercise in sedentary individuals with overweight and obesity and whether this response is changed after habituation to regular exercise. Furthermore, studies investigating whether exercise-induced changes in GDF15 concentrations are involved in appetite control in response to exercise are needed (Klein et al., 2021; Klein, Kleinert, Richter, & Clemmensen, 2022). To our knowledge, no randomized controlled trials have investigated the effects of regular exercise on plasma GDF15 concentrations, nor the interplay between exercise training and exercise intensity, appetite and GDF15 in humans.

We previously reported that both vigorous and moderate-intensity exercise training were associated with reduction in weight and fat mass in individuals with overweight or obesity (Quist et al., 2018). Despite these effects on body energy stores, only vigorous-intensity exercise was associated with changes to appetite-related outcomes; lower subjective appetite and *ad libitum* energy intake after 3 months, and higher fasting and postprandial GLP-1 after 6 months (Quist, Blond, et al., 2019). We did not assess the potential effect of exercise training on GDF15 and whether this was associated with the changes in the anthropometric outcomes.

Therefore, the aim of this analysis of a randomized controlled trial was to investigate acute effects of exercise on plasma GDF15 concentrations and whether 6 months of exercise training of either moderate or vigorous intensity affect plasma GDF15 concentrations in the fasting state and following acute bouts of exercise in previously physically inactive individuals with overweight or obesity. Furthermore, we aimed to investigate whether plasma GDF15 concentrations are associated with *ad libitum* energy intake, subjective appetite, and markers of cardiometabolic health.

2. Material and methods

The present post hoc analysis includes data from the 'Governing Obesity – Active Commuting To Improve health and Wellbeing of Everyday life' (GO-ACTIWE) trial with the overall aim to investigate the health effects of active commuting by bike and leisure-time exercise in adults with overweight and class 1 obesity (Rosenkilde et al., 2017). The study was performed at the Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, from November 2013 to June 2016. Results from the trial are published elsewhere (Blond et al., 2019; Bruhn et al., 2021; Gram et al., 2017; Gram et al., 2018; Kern et al., 2019; Quist, Blond, et al., 2019; Quist, Rosenkilde, et al., 2019; Quist et al., 2018). The study was approved by the Ethical Committee of The Capital Region of Denmark (H-4-2013-108), registered at the Danish Data Protection Agency and at clinicaltrials.gov (NCT01962259 (main trial) & NCT01973686 (energy metabolism substudy)) and adhered to the Declaration of Helsinki. Primary outcomes in GO-ACTIWE were changes in peripheral insulin sensitivity, body fat mass, and endogenous thrombin potential.

2.1. Participants

We recruited physically inactive (structured exercise $\leq$2 h/week and bicycling $\leq$25 km/week), Caucasian women and men aged 20–45 years with overweight/obesity (BMI: 25–35 kg/m^2). The main exclusion criteria were: body fat percentage <32% for women and <25% for men, peak oxygen uptake (VO_2peak) >40 ml O_2/kg/min for women and >45 ml O_2/kg/min for men, diagnosis or family history of type 2 diabetes, smoking, high fasting blood glucose (>6.1 mmol/l), and hypertension ($\geq$140/90 mmHg). Eligible participants received written and oral information and signed informed consent before a screening session.

2.2. Study design and interventions

The study design has been described in detail elsewhere (Rosenkilde et al., 2017). In brief, GO-ACTIWE was a 6-month randomized controlled trial and included identical test periods at baseline and after 3 and 6 months. The original study design included a 1-year intervention, but the duration was shortened to 6 months on February 2nd, 2014, due to an unexpectedly large withdrawal of eligible individuals during inclusion and with no reference to data. After baseline testing, participants were randomly allocated in 1:2:2:2 ratios to non-exercise control (CON, n=18), active commuting by bike (BIKE, n=35), moderate (MOD, n=39), or vigorous intensity leisure-time exercise (VIG, n=38). The present analysis includes only CON, MOD, and VIG since the daily exercise in BIKE was separated into two bouts (commuting to and from work/school) and since exercise intensity was self-selected in BIKE. Diet was *ad libitum* and participants were advised to continue their habitual diet throughout the intervention. VO_2peak was determined during an incremental bicycle test on an electronically braked ergometer bike (Lode Excalibur, Groeningen, Netherlands). During the test, respiratory gaseous exchange was measured (Oxycon Pro, Jaeger, Würzburg, Germany). The test consisted of a 3 × 3 min warm-up at 30, 60, and 90 W for women and 40, 80, and 120 W for men, followed by an increase in workload of 20 W for women and 25 W every min until VO_2peak/exhaustion. VO_2peak was accepted upon a leveling off in VO_2, respiratory exchange ratio >1.15 or age-predicted maximum heart rate (HR) (220-age) as described previously (Rosenkilde et al., 2017).

The exercise was prescribed 5 days/week with exercise energy expenditure (ExEE) at 320 kcal/day for women and 420 kcal/day for men, in line with health recommendations by the World Health Organization and others (Garber et al., 2011; World Health Organization, 2010). MOD and VIG had free access to fitness centers and were instructed to perform different types of aerobic exercise (e.g. walking, running, cycling, or rowing). Target exercise HR was individually prescribed at 50% VO_2peak-reserve in MOD and 70% VO_2peak-reserve in VIG and was adjusted after 6 weeks and 3 months. Participants received and were instructed to wear a HR monitor (RC3 GPS, Polar Electro Oy, Kempele, Finland) during all exercise sessions and to upload exercise data from the HR monitors to an online platform (www.polarpersonaltrainer.com) once per week for project staff to monitor compliance to the prescribed exercise. Every week, exercise compliance was calculated from exercise data recorded during the exercise sessions and the most

recent VO_2peak test. Project staff were in weekly contact with the participants and aimed to keep weekly compliance at 80–120% of the prescribed ExEE. In case of low compliance, participants were instructed to increase ExEE during exercise sessions by up to 25% until the target compliance, i.e., weekly and accumulated ExEE, was achieved. The compliance with the prescribed ExEE was high in both exercise groups but slightly higher in MOD (median (Q1; Q3): 91% (86; 109)) compared with VIG (85% (65; 97)) (Blond et al., 2019).

2.3. Appetite test day

The test day consisted of a standardized meal and an exercise challenge as outlined in Fig. 1 and as described previously (Quist, Blond, et al., 2019). The present analyses include five time points: *'fasting'*, *'pre-exercise'* (180 min), *'exercise'* (255 min), '30 min *recovery'* (285 min), and '60 min *recovery'* (315 min). At 3 and 6-month testing, the last exercise bout was scheduled >36 h before the test day. Participants arrived at 8 a.m. after an overnight fast (≥10 h). While participants rested in a bed, a catheter was inserted into an antecubital vein. After a 15-min rest, fasting blood samples were drawn for assessment of metabolites and hormones, and hunger, satiety, fullness, prospective food consumption, and desire to eat something sweet and fatty were rated using 100-mm visual analog scales (Flint, Raben, Blundell, & Astrup, 2000) (*'fasting'*) (Quist, Blond, et al., 2019). A composite appetite score was calculated based the visual analog scales (Matu et al., 2017; Stubbs et al., 2000): Composite Appetite Score = (hunger (mm) + prospective food consumption (mm) + (100 - fullness (mm) + (100 - satiety (mm) / 4.

2.3.1. Standardized breakfast meal

A standardized breakfast meal containing 460 kcal for women and 600 kcal for men, 64 percent energy (E%) from carbohydrate, 23 E% fat, and 13 E% protein and consisting of bread rolls, cheese, jam, fruit juice, and water was served. During the post-meal period, blood was sampled, and appetite was rated every 30 min for 180 min. A chocolate milk (152 mL, 73 kcal (women) and 200 mL, 96 kcal (men), 61 E% carbohydrate, 8 E% fat, 31 E% protein) was provided for the participants' comfort after the last blood samples and appetite ratings of the meal test (180 min). We did not measure GDF15 during the standardized meal test before the exercise bouts (described below), because of previous observations showing only minor effects of food intake on GDF15 secretion, which suggest that GDF15 does not play a role as a meal-induced satiety factor (Tsai et al., 2015).

2.3.2. Acute exercise bout

An acute exercise bout at 60% VO_2peak was performed on a bicycle ergometer (Monark 928 G3, Monark Exercise AB, Vansbro, Sweden). The ExEE was 320 kcal for women and 420 kcal for men corresponding to the exercise bouts during the intervention. The duration and workload were individually calculated based on the linear HR-VO_2 relationship during submaximal exercise on a bicycle ergometer (Swain & Leutholtz, 1997) during VO_2peak testing on previous test days at baseline, 3, and 6 months (Blond et al., 2019). The exercise bout was initiated at individual time points since it was scheduled to be completed at the same time point (255 min). Immediately after the exercise bout, while participants were still on the bike (255 min, *'exercise')*, as well as at 30 and 60 min during post-exercise recovery (285 min, '30 min *recovery'* and 315 min, '60 min *recovery'*) before the *ad libitum* meal, blood samples were collected and appetite was rated.

2.3.3. Ad libitum meal

To determine *ad libitum* energy intake in response to acute exercise, participants were seated in a quiet place on their own and provided a meal consisting of Pasta Bolognese (1440 g, 1912 kcal, 55 E% carbohydrate, 30 E% fat, 15 E% protein, homogenous composition) served with a glass of water (114 ml (women) and 150 ml (men)) 60 min post-exercise. The Pasta Bolognese was served in a large pot and participants were instructed to serve themselves as much and as many times as they liked and to eat until comfortably satiated/full and to drink all the water.

2.4. Body weight, body composition, blood samples, and insulin sensitivity

On another test day, participants arrived in the morning (between 8 and 9 am) after an overnight fast (≥10 h) and blood samples were

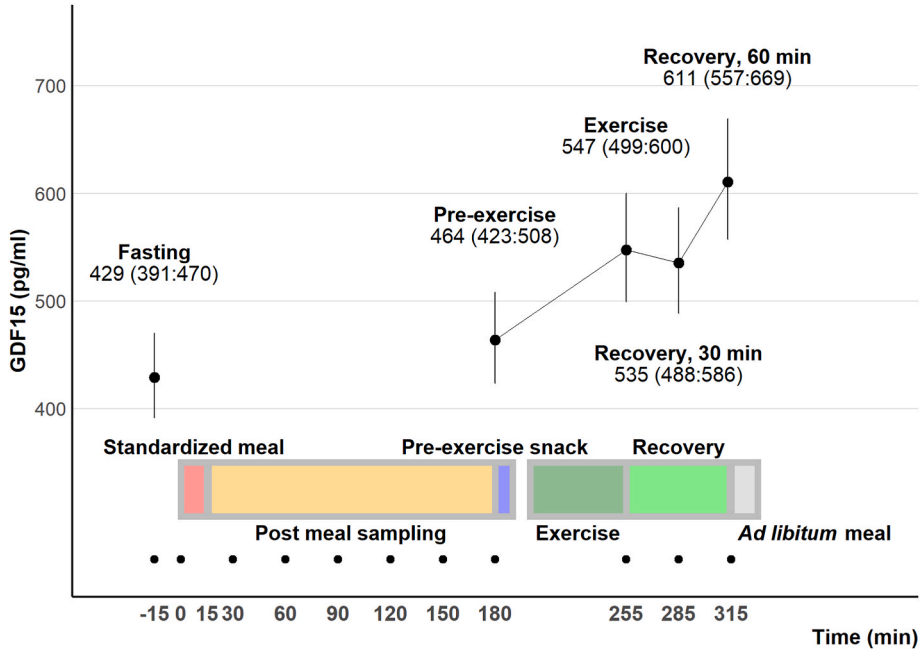

Fig. 1. Acute effect of exercise on plasma GDF15 concentrations. Schematic presentation of the test day and plasma GDF15 concentrations during the test day at the baseline visit in all participants (n=90). • Mark timepoints of blood sampling and appetite rating. Data were log transformed (ln) for analysis and back-transformed for presentation as estimated geometric means and 95%CIs. GDF15, Growth Differentiation Factor 15.

collected after 15 min rest. After measurement of body weight and body composition (Dual-energy X-ray Absorptiometry), peripheral insulin sensitivity was determined during a hyperinsulinemic euglycemic clamp (Blond et al., 2019). Intraabdominal adipose tissue was measured using magnetic resonance on a third test day (Blond et al., 2019).

2.5. Biochemical analyses

GDF15 was measured in blinded human plasma samples using the Quantikine ELISA Human GDF15 Immunoassay (ELISA, R&D Systems, catalog no. DGD150) according to the protocol provided by the manufacturer. Details regarding analysis of plasma glucose, insulin, acylated ghrelin, cholecystokinin, total GLP-1, total PYY, and glucagon have been reported previously (Blond et al., 2019; Quist, Blond, et al., 2019). Plasma samples for each hormone were measured in one batch after completion of the study. Hormones were measured in duplicate and in our hands, the intra-assay coefficients of variation were <10%.

2.6. Statistics

The main analysis was based on the intention to treat principle, but supplementary per protocol analyses including participants who completed the intervention and had a compliance of ≥80% of prescribed ExEE were performed.

GDF15 concentrations were modelled using a baseline corrected repeated measures regression model (Liu, Lu, Mogg, Mallick, & Mehrotra, 2009) specified as: GDF15 = Sex + Age + Sampling condition (factorial) + Sex*Sampling condition (factorial) + Age*Sampling condition (factorial) + Visit (factorial) + Visit (factorial)*Treatment*Sampling condition (factorial). Where "Sampling condition" refers to the condition at the time that GDF15 was sampled, for example *'exercise'* (255 min). In a supplementary analysis, we adjusted for *'pre-exercise'* GDF15 concentrations in the analyses of GDF15 at *'exercise'* and '30 min *recovery'* and '60 min *recovery'*. Results are presented as estimated baseline adjusted mean differences (95%CI) between groups and p-values at 3 months, 6 months and across 3 and 6 months. P-values <0.05 were regarded as statistically significant.

Associations between GDF15 and other outcomes were assessed using a similar regression model, but analyses were performed separately for each sampling condition. GDF15 was the outcome in analyses related to markers of cardiometabolic function and the individual cardiometabolic markers were predictors. GDF15 was the predictor and the measures of appetite were outcomes in the analyses including these. The model used to assess associations in the untrained state: Outcome = Sex + Age + Visit (factorial) + Visit (factorial)*Treatment + Visit (factorial) *Treatment*Predictor. The model used to assess associations between changes: Delta value for outcome = Sex + Age + Baseline value of the outcome + Visit (factorial) + Visit (factorial)*Treatment + Delta value for the predictor. Both delta values for 3 and 6 months were included in the analysis. All variables were standardized to a mean of 0 and a SD of 1 prior to analyses. Results are presented as the standardized regression coefficients and p-values. P-values <0.05 with a false discovery rate <0.1 (Benjamini & Hochberg, 1995) were regarded as statistically significant. False discovery rates were calculated separately for the associations in the untrained state and the associations between changes.

All models were specified using restricted maximum likelihood estimation method, the Kenward-Roger degrees of freedom method, and a repeat (visit) on participant level (unstructured covariance structure). Model fit was evaluated using graphical methods; if relevant, variables were ln-transformed for analyses.

Distribution of raw data was assessed using histograms and QQ-plots. For the residuals of the regression models, distribution of both standard residuals and scaled residuals was assessed using graphical methods to ensure that they were normally distributed and showed no heteroscedasticity.

Statistical analyses were performed using R version 4.0.3 (The R

Table 1
Baseline characteristics.

Characteristic	All, n=90	CON, n=16	MOD, n=37	VIG, n=37
Age (years)	36 (28, 40)	37 (30, 40)	32 (26, 38)	38 (31, 40)
Sex				
Men	43 (48%)	9 (56%)	17 (46%)	17 (46%)
Women	47 (52%)	7 (44%)	20 (54%)	20 (54%)
Body mass (kg) (women)	81.7 (75.6, 89.4)	84.2 (80.8, 91.0)	85.9 (74.0, 90.7)	78.6 (75.2, 83.4)
Body mass (kg) (men)	98.9 (91.8, 104.6)	98.9 (91.5, 105.3)	95.1 (91.1, 103.2)	100.6 (94.2, 111.6)
BMI (kg/m^2)	29.6 (2.3)	30.1 (2.3)	29.2 (2.1)	29.8 (2.5)
Fat mass (kg) (women)	36.8 (6.1)	38.8 (5.1)	38.0 (7.7)	35.0 (4.1)
Fat mass (kg) (men)	32.9 (6.9)	34.0 (6.3)	30.4 (4.5)	34.8 (8.6)
Fat free mass (kg) (women)	45.2 (4.3)	46.8 (3.5)	44.9 (4.9)	44.9 (3.8)
Fat free mass (kg) (men)	66.5 (6.5)	65.2 (8.7)	65.7 (6.2)	68.0 (5.6)
Total body fat percentage (women)	44.0 (42.3, 47.5)	44.0 (43.0, 46.0)	45.2 (42.7, 49.5)	43.9 (42.0, 45.8)
Total body fat percentage (men)	32.7 (29.4, 35.0)	33.6 (32.7, 34.4)	32.3 (29.0, 33.4)	33.8 (28.3, 37.4)
Waist circumference (cm) (women)	87.6 (7.1)	91.7 (3.1)	88.1 (9.2)	85.7 (4.9)
Waist circumference (cm) (men)	101.0 (7.6)	101.9 (8.3)	98.0 (7.4)	103.5 (6.7)
Cardiorespiratory fitness (ml/kg/min) (women)	25.0 (23.2, 29.8)	23.5 (22.1, 24.5)	25.3 (24.1, 29.0)	26.2 (23.5, 31.4)
Cardiorespiratory fitness (ml/kg/min) (men)	31.4 (29.6, 36.0)	33.5 (31.1, 37.8)	33.2 (30.5, 35.8)	30.5 (29.2, 33.7)
Ad libitum energy intake (kcal)	635 (516, 836)	764 (561, 839)	608 (547, 846)	651 (427, 777)
Total cholesterol (mM)	4.7 (0.8)	4.5 (0.6)	4.6 (0.7)	4.8 (1.0)
LDL cholesterol (mM)	2.9 (2.3, 3.4)	2.6 (2.5, 3.3)	2.9 (2.5, 3.2)	3.0 (2.3, 3.5)
HDL cholesterol (mM) (women)	1.5 (0.3)	1.3 (0.4)	1.5 (0.3)	1.5 (0.3)
HDL cholesterol (mM) (men)	1.0 (0.2)	1.2 (0.1)	1.0 (0.2)	1.0 (0.2)
Plasma triglycerides (mM) (women)	0.92 (0.72, 1.13)	0.93 (0.85, 0.99)	0.92 (0.74, 1.40)	0.91 (0.68, 1.10)
Plasma triglycerides (mM) (men)	1.21 (0.87, 2.04)	0.93 (0.80, 1.17)	1.39 (1.00, 2.12)	1.42 (1.07, 1.96)
Fasting plasma glucose (mM))	5.4 (0.4)	5.5 (0.5)	5.3 (0.3)	5.4 (0.4)
Fasting plasma insulin (pmol/l)	71 (53, 90)	70 (47, 99)	77 (55, 95)	70 (54, 86)
Fasting plasma GDF15 (pg/ml)	408 (327, 555)	500 (347, 618)	457 (326, 608)	378 (320, 528)
Pre-exercise GDF15 (pg/ml)	460 (372, 606)	460 (407, 735)	443 (350, 610)	494 (378, 575)
Exercise plasma GDF15 (pg/ml)	533 (413, 692)	640 (392, 962)	500 (409, 639)	540 (425, 683)
Recovery plasma GDF15 (pg/ml), 30 min	494 (403, 650)	569 (407, 911)	492 (374, 621)	484 (442, 640)
Recovery plasma GDF15 (pg/ml), 60 min	547 (437, 793)	635 (398, 1,218)	530 (416, 736)	624 (458, 790)

Data are presented as median (Q1, Q3), n (%) or mean (SD). BMI, body mass index; CON, control group; GDF15, growth differentiation factor 15; HDL, high density lipoprotein; LDL, low density lipoprotein; MOD, moderate intensity exercise group; VIG, vigorous intensity exercise group.

Foundation for Statistical Computing, www.R-project.org) and SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

Baseline characteristics are presented in Table 1 and Supplementary Table 1.

3.1. Acute effect of exercise on plasma GDF15 concentrations

In the untrained state at baseline, including participants from all three groups, GDF15 concentrations increased by 18% (4; 34) during exercise, a level which was maintained at *'30 min recovery'* but was further increased to 32% (16; 50) at *'60 min recovery'* when compared with *'pre-exercise'* concentrations (Fig. 1 and Supplementary Table 2).

3.2. Effects of exercise training on plasma GDF15 concentrations

Although fasting GDF15 concentrations increased by 21% (0; 46) in VIG compared with CON at 3 months (p=0.045), there was no difference between the two groups at 6 months, and consequently we found no statistically significant difference when averaging the effects across the visits at 3 and 6 months (17% (−2; 40), p=0.084) (Fig. 2 and Table 2). Furthermore, there were no differences between MOD and VIG (Fig. 2 and Table 2).

Plasma GDF15 concentrations measured immediately after acute exercise (*'exercise'*) did not change in response to exercise training in MOD or VIG and there were no differences between MOD and VIG (Fig. 2 and Table 2).

There were no differences in plasma GDF15 concentrations at *'30 min recovery'* in MOD or VIG compared with CON when assessing the changes across the measurements at 3 and 6 months. However, we did observe that plasma GDF15 concentrations at *'30 min recovery'* were 27% (0; 61) higher in VIG compared with CON at 6 months (p=0.047), but this difference was mainly driven by a 19% (−33; −3) reduction in GDF15 within CON from baseline to 6 months (Fig. 2 and Table 2). Furthermore, the effect disappeared after adjustment for *'pre-exercise'* plasma GDF15 concentrations (Supplementary Table 3). Similarly, there was no overall effect of exercise training on plasma GDF15 concentrations at *'60 min recovery'* in MOD or VIG. GDF15 at *'60 min recovery'* was 29% (3; 62) higher in MOD compared with CON at 6 months (p=0.025), which was mainly attributable to a 20% (−33; −3) reduction within CON from baseline to 6 months. However, the difference between groups disappeared after adjustment for *'pre-exercise'* plasma GDF15 concentrations (Supplementary Table 3). There were no differences in plasma GDF15 concentrations during recovery between MOD and VIG (Fig. 2 and Table 2).

Overall, results from the supplementary per protocol analysis confirmed those from the intention to treat analysis (Supplementary Table 4). For summary of raw data see Supplementary Table 5.

3.3. Associations between plasma GDF15 concentrations and appetite and energy intake

Across all participants at the baseline visit, neither fasting nor post-exercise plasma GDF15 concentrations were associated with *ad libitum* energy intake, subjective appetite (composite appetite score), or desire to eat something sweet or fatty and there were no associations between changes (Fig. 3).

3.4. Associations between plasma GDF15 concentrations and markers of cardiometabolic health

At baseline, in the physically inactive state, plasma GDF15 concentrations were associated with lower cardiorespiratory fitness, central obesity, dyslipidemia, poorer glycemic control during a meal test, and higher age (all $p<0.05$ and FDR<0.1) (Fig. 4). There were no associations between changes in GDF15 and changes in other outcomes (Fig. 4).

4. Discussion

To our knowledge, this is the first randomized controlled trial in which the effects of acute and regular exercise on plasma GDF15 concentrations in individuals with overweight and obesity have been investigated. We found that plasma GDF15 concentrations increased in response to an acute bout of exercise and that it was further increased after 1 h of recovery. We also report that an exercise training intervention consisting of exercise of either moderate or vigorous intensity for 6 months did not affect plasma GDF15 concentrations – neither during fasting nor in relation to a bout of moderate-intensity exercise. Plasma GDF15 concentrations were associated with a poorer cardiometabolic profile in the physically inactive state, but this did not translate into associations between changes in GDF15 and changes in other cardiometabolic markers in response to exercise training. Interestingly, plasma GDF15 concentrations were not associated with energy intake, subjective appetite, or the desire to eat sweet or fatty foods.

Our finding that plasma concentrations of GDF15 increased during and following an acute bout of exercise in people with overweight or obesity is in line with previous observations in lean men (Klein et al., 2021; Kleinert et al., 2018). We observed that the fasting level of GDF15 was higher after 3 months of vigorous-intensity exercise, but this was not evident after 6 months of vigorous exercise training, indicating no lasting effect. In the non-controlled trial by Zhang et al., fasting GDF15 increased in response to 12 weeks of vigorous intensity exercise training in previously physically inactive older adults with obesity (Zhang et al., 2019). Also, fasting serum GDF15 increased in response to 35 days of intensive resistance and aerobic exercise training in elite rugby players, but no control group was included (Galliera et al., 2014). In contrast, in a recent non-controlled study by Cai et al., fasting GDF15 concentrations did not change in response to 3 weeks of exercise training of moderate to vigorous intensity exercise in individuals with overweight or obesity (Cai et al., 2021). If indeed fasting GDF15 increases in response to shorter-term regular exercise training, it is somewhat contrary to observational data indicating higher fasting GDF15 in physically inactive compared with physically active individuals (Conte et al., 2020). However, the higher plasma GDF15 concentrations among physically inactive individuals could be explained by a compensatory increase in response to a more unfavorable metabolic profile compared with physically active individuals. Whether there is a transient effect of vigorous-intensity exercise training on the fasting concentrations of GDF15 needs to be addressed by additional studies designed for this purpose.

There were no effects of moderate or vigorous intensity exercise training on the GDF15 response to acute exercise. Our data suggest that exercise training does not modify the effect of acute exercise on GDF15 in individuals with overweight or obesity. However, our findings that plasma GDF15 concentrations increase in response to acute exercise independent of training status might be an important observation, since it suggests that people who exercise regularly are exposed to increased GDF15 concentrations in the post-exercise period in response to each exercise session. Whether this has any physiological relevance, e.g., an impact on free-living energy intake and/or contributing to the benefits of exercise on body weight management needs investigation in future studies.

In the present study, cross-sectionally or over time, GDF15 was not associated with *ad libitum* energy intake, subjective appetite, or desire to eat something sweet or fatty. We observed no associations between GDF15 and the appetite-related hormones measured, which corresponds well with infusion studies in humans showing only a minor increase in GDF15 in response to cholecystokinin infusion and no effect of GLP-1 or PYY infusion on plasma GDF15 concentrations (Tsai et al., 2015). However, in mice, pharmacological administration of GDF15 reduces

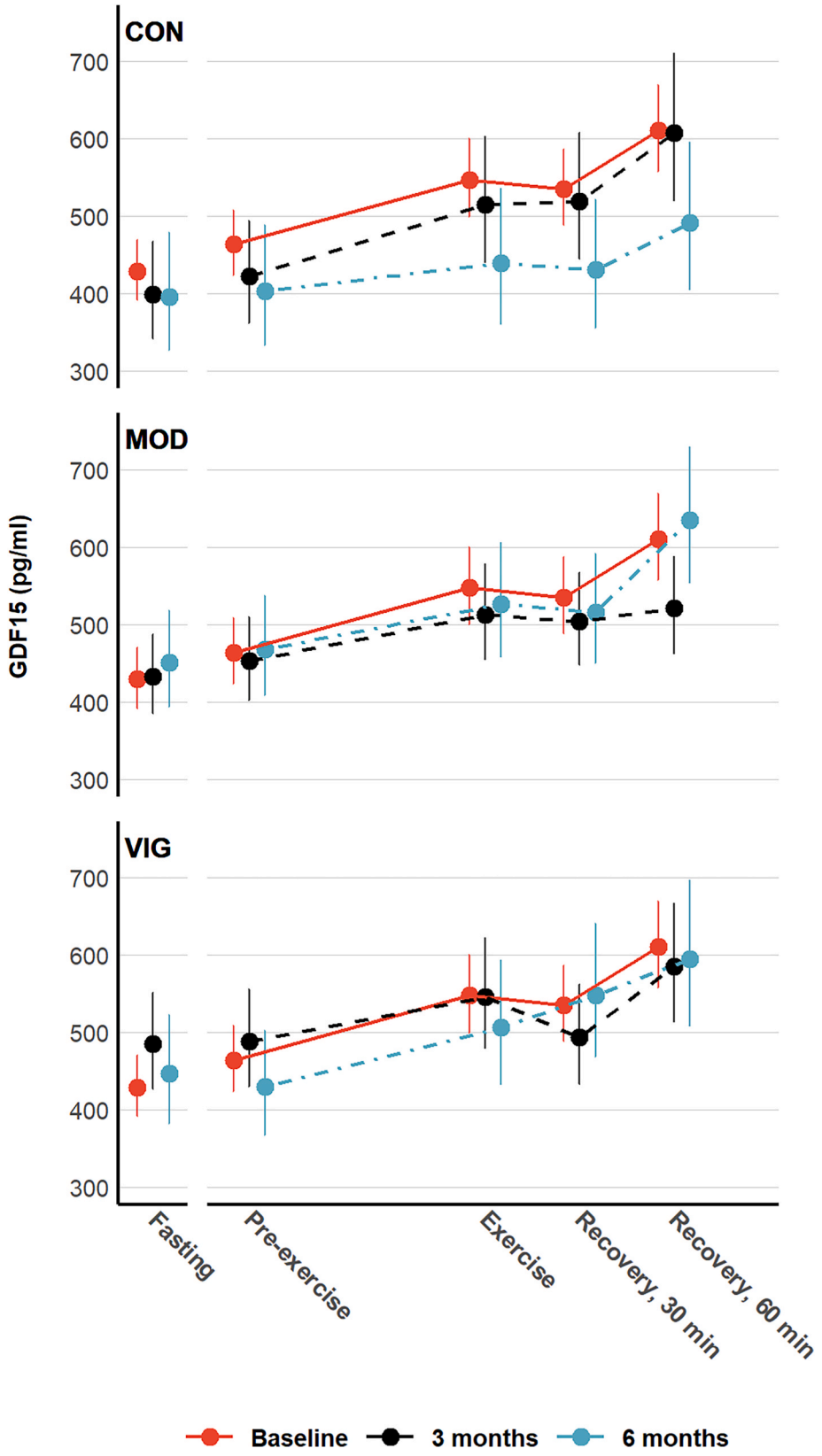

Fig. 2. Effects of exercise training on plasma GDF15 concentrations. Plasma GDF15 during the test days at baseline (visit 1) and after 3 (visit 2) and 6 months (visit 3) in CON, MOD, and VIG. Data were log transformed (ln) for analysis and back-transformed for presentation as estimated geometric means and 95%CIs. CON, control group, GDF15, growth differentiation factor, MOD, moderate intensity exercise group; VIG, vigorous intensity exercise group.

Table 2
Estimated GDF15 concentrations and differences between groups.

Condition	Group	Visit	GDF15 (pg/ml)	Within group changes (%)	Difference from CON (%)	P-value	Difference from MOD (%)	P-value
Fasting	CON	3 months	399 (342: 467)	−7 (−20: 8)				
	CON	6 months	396 (327: 479)	−8 (−24: 11)				
	MOD	Across visits			11 (−6: 32)	0.224		
	MOD	3 months	432 (384: 486)	−1 (−11: 11)	8 (−10: 30)	0.392		
	MOD	6 months	451 (393: 517)	−5 (−17: 9)	14 (−9: 43)	0.250		
	VIG	Across visits			17 (−2: 40)	0.084	5 (−9: 22)	0.494
	VIG	3 months	485 (426: 551)	13 (0: 28)	21 (0: 46)	0.045	12 (−4: 31)	0.157
	VIG	6 months	446 (382: 522)	4 (−11: 21)	13 (−11: 43)	0.316	−1 (−19: 20)	0.916
Pre-exercise	CON	3 months	423 (362: 494)	−9 (−22: 6)				
	CON	6 months	403 (333: 488)	−13 (−28: 5)				
	MOD	Across visits			11 (−6: 32)	0.212		
	MOD	3 months	452 (402: 509)	2 (−8: 15)	7 (−11: 28)	0.464		
	MOD	6 months	468 (408: 536)	−1 (13: 13)	16 (−7: 45)	0.194		
	VIG	Across visits			11 (−7: 33)	0.252	0 (−14: 16)	0.959
	VIG	3 months	489 (430: 555)	5 (−7: 19)	16 (−4: 40)	0.132	8 (−8: 27)	0.339
	VIG	6 months	430 (367: 502)	−7 (−20: 8)	7 (−16: 35)	0.599	−8 (−24: 12)	0.393
Exercise	CON	3 months	515 (441: 603)	−6 (−19: 10)				
	CON	6 months	439 (361: 535)	−20 (−34: 3)				
	MOD	Across visits			9 (−8: 30)	0.319		
	MOD	3 months	512 (454: 577)	7 (−5: 20)	−1 (−17: 19)	0.943		
	MOD	6 months	527 (458: 605)	4 (−9: 19)	20 (−5: 51)	0.122		
	VIG	Across visits			10 (−8: 32)	0.277	1 (−13: 18)	0.871
	VIG	3 months	546 (480: 621)	0 (−12: 13)	6 (−12: 28)	0.551	7 (−9: 25)	0.434
	VIG	6 months	507 (433: 593)	−7 (−21: 8)	15 (−9: 47)	0.247	−4 (−21: 17)	0.697
Recovery, 30 min	CON	3 months	520 (445: 608)	−3 (−17: 13)				
	CON	6 months	431 (356: 522)	−19 (−33: 3)				
	MOD	Across visits			8 (−9: 28)	0.393		
	MOD	3 months	504 (448: 567)	6 (−5: 19)	−3 (−19: 16)	0.734		
	MOD	6 months	515 (449: 591)	4 (−9: 18)	20 (−4: 49)	0.117		
	VIG	Across visits			10 (−8: 31)	0.303	2 (−12: 18)	0.794
	VIG	3 months	493 (433: 562)	−8 (−19: 5)	−5 (−21: 15)	0.589	−2 (−17: 15)	0.797
	VIG	6 months	548 (468: 640)	2 (−12: 19)	27 (0: 61)	0.047	6 (−13: 29)	0.542
Recovery, 60 min	CON	3 months	608 (520: 710)	0 (−15: 16)				
	CON	6 months	491 (406: 595)	−20 (−33: 3)				
	MOD	Across visits			5 (−11: 25)	0.557		
	MOD	3 months	520 (462: 586)	17 (−5: 32)	−14 (−29: 3)	0.095		
	MOD	6 months	635 (553: 728)	−4 (−16: 10)	29 (3: 62)	0.025		
	VIG	Across visits			8 (−10: 29)	0.398	3 (−12: 19)	0.731
	VIG	3 months	585 (514: 666)	−4 (−15: 9)	−4 (−20: 16)	0.694	12 (−4: 32)	0.153
	VIG	6 months	595 (509: 696)	−3 (−16: 13)	21 (−4: 53)	0.111	−6 (−23: 14)	0.513

Presented as baseline adjusted estimates (95% CI). "Across visits" indicate overall effect across visits at 3 months and 6 months. GDF15 data were ln-transformed for analysis and back-transformed for presentation. Results are from the intention to treat analysis. CON, control group; GDF15, growth differentiation factor 15; MOD, moderate intensity exercise group; VIG, vigorous intensity exercise group.

food intake (Klein et al., 2021; Xiong et al., 2017) and preference for energy-dense food (Xiong et al., 2017).

Klein et al. showed that in healthy individuals prolonged endurance exercise increased plasma GDF15 concentrations to concentrations observed in disease states that are associated with anorexia and weight loss (Klein et al., 2021). One could speculate that the increase in GDF15 concentrations in response to the exercise bout in the present study was too small to elicit a measurable anorexigenic effect; however, animal studies have suggested that even small changes in plasma GDF15 concentrations can lower food intake (Breen et al., 2020; Coll et al., 2020; Lu et al., 2022).

Despite the relatively narrow BMI range and none of the participants having diabetes, we observed that higher plasma GDF15 concentrations are associated with a cardiometabolic profile characterized by lower cardiorespiratory fitness, central obesity, dyslipidemia, and poorer glycemic control during a meal, supporting an association between GDF15 and poorer metabolic status. On the other hand, our findings do not support that GDF15 is associated with peripheral insulin sensitivity. The association with cardiorespiratory fitness is in agreement with previous cross-sectional observations of higher fasting GDF15 concentrations in physically inactive compared with physically active individuals independent of BMI (Conte et al., 2020). Our observations correspond well with previous observational studies reporting higher fasting GDF15 in individuals with obesity and type 2 diabetes (Vila et al., 2011), and associations with glucose intolerance, HOMA-IR (Hong et al., 2014; Yalcin et al., 2016), and diabetes incidence (Bao et al., 2019; Carstensen et al., 2010). It has been suggested that these findings may be driven by a compensatory increase in GDF15 in response to poor metabolic function (Carstensen et al., 2010; Zhang et al., 2019).

In the present trial, we observed significant and meaningful improvements in several markers of cardiometabolic health, including cardiorespiratory fitness, waist circumference, and intraabdominal adipose tissue (Blond et al., 2019; Quist et al., 2018). Hence, exercise training improved some of the markers associated with GDF15 at baseline, without GDF15 being affected. This indicates that these markers do not affect the concentrations of GDF15, though the reverse could still be true. It would therefore be interesting to examine whether changes in GDF15 can affect these markers. Our observations are in contrast to the study by Zhang et al., in which the increase in fasting GDF15 after 3 months of exercise training was associated with greater loss of total and abdominal fat in individuals with obesity (Zhang et al., 2019). Furthermore, our results are also contrary to the observations by Cai et al. that increases in GDF15 were associated with reductions in waist circumference and fasting plasma glucose but not body weight during 3 weeks of exercise training in individuals with overweight/-obesity (Cai et al., 2021). However, Cai et al. did not observe any effects of exercise training on fasting serum GDF15 and the short intervention duration may have been insufficient to induce relevant mean changes in the outcomes of interest on a group level (Cai et al., 2021). Lastly, our findings confirm the well-known association between GDF15 and age

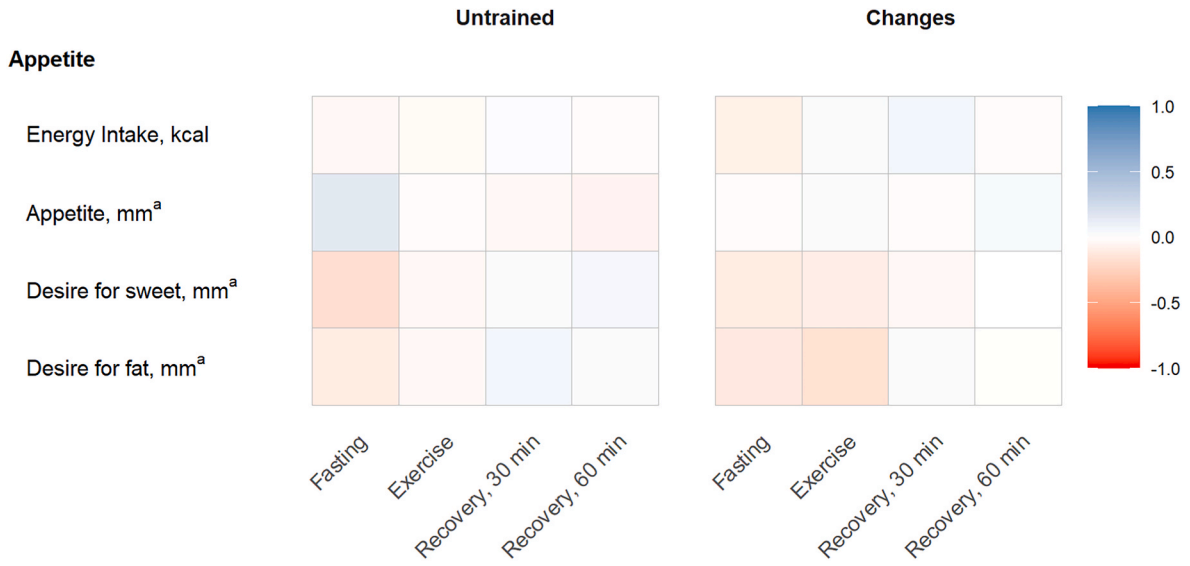

Fig. 3. Cross-sectional associations between GDF15 concentrations and markers of appetite at baseline in all three groups combined and associations between changes in GDF15 concentrations and changes in markers of appetite. Associations between changes were assessed across the visits at 3 and 6 months. Results are presented as standardized estimates (95%CIs) with markers of appetite as the outcome, i.e., SD change per 1 SD change in GDF15. x indicates $p<0.05$ plus FDR <0.1 and (x) indicates $p<0.05$, but FDR ≥ 0.1. GDF15 data were ln-transformed for analysis. [a]The given marker was sampled at the same time as GDF15. Appetite, Composite Appetite Score; GDF15, growth differentiation factor 15.

(Conte et al., 2019, 2020), regardless of the rather narrow age range of the participants in the study (20–45 years).

Our observation that the GDF15 response to an acute bout of exercise is maintained following 6 months of increased habitual exercise, suggests that the increased appetite in response to increased exercise often seen after some time, is not due to an attenuated post-exercise response in GDF15. However, we only measured GDF15 and energy intake 60 min after the exercise bout, which may limit the practical implications of our findings.

Strengths of the present study include the randomized controlled design, the relatively large study sample, and the repeated assessment of GDF15 and other appetite-regulating hormones and subjective appetite, as well as *ad libitum* food intake in response to an acute bout of exercise, before, during (3 months) and after a period of habitual exercise training (6 months). Furthermore, the study included repeated assessments of cardiometabolic health including peripheral insulin sensitivity measured by the gold standard hyperinsulinemic euglycemic clamp and intraabdominal adipose tissue by magnetic resonance imaging. The high compliance to the prescribed exercise is an additional strength of the study.

The study also has limitations. It is a post hoc explorative analysis of a randomized controlled trial and the multiple statistical tests are associated with a risk of false-positive findings, especially so for the 45 statistical tests related to GDF15 concentrations, as these were not adjusted for multiple testing. In the trial, we experienced a greater than expected drop-out in the vigorous-intensity exercise group, which resulted in missing data and lower power and generalizability of the findings.

In conclusion, acute exercise increases plasma GDF15 concentrations in individuals with overweight or obesity, and this is unaffected by 6 months of regular exercise training and is not associated with appetite. This implies that plasma GDF15 concentrations increase in response to each exercise bout independently of training state. Whether this has an impact on free-living energy intake and thus body weight management in response to exercise training needs to be elucidated in future studies. Plasma GDF15 concentrations were, however, associated with markers of cardiometabolic risk and low cardiorespiratory fitness, but there were no associations between changes during the exercise interventions, suggesting that GDF15 is not involved in the benefits of exercise training on cardiometabolic health.

Author contributions

Conceptualization: JSQ, ABK, CC, KF, BS, and MBB; designed trial: JSQ, MBB, ASG, AS, BS, MR; conducted trial: JSQ, MBB, ASG, AS, BS, and MR; performed biochemical analysis of GDF15: ABK; analyzed data: MBB and JSQ; interpreted results: all authors; drafted manuscript: JSQ; contributed to the writing of the manuscript: MBB, ABK, CC; critically reviewed the manuscript and read and approved the final manuscript: all authors.

Funding and declaration of interests

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Ethical statement

The study was approved by the Ethical Committee of The Capital Region of Denmark (H-4-2013-108), registered at the Danish Data Protection Agency and at clinicaltrials.gov (NCT01962259 (main trial) & NCT01973686 (energy metabolism substudy)) and adhered to the Declaration of Helsinki.

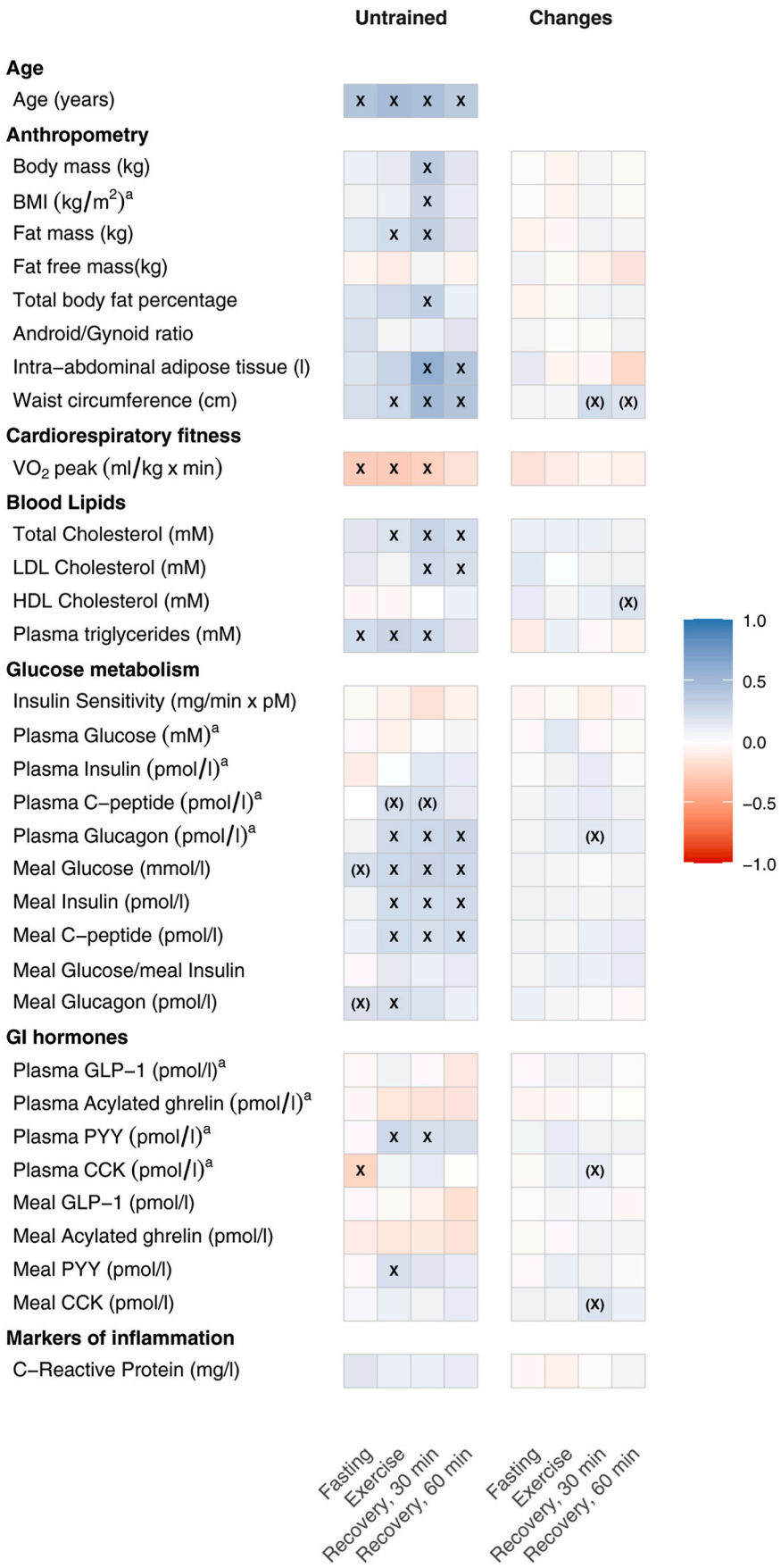

Fig. 4. Cross-sectional associations between GDF15 and cardiometabolic markers at baseline in all three groups combined and associations between changes in GDF15 and changes in cardiometabolic markers. Associations between changes were assessed across the visits at 3 and 6 months. Results are presented as standardized estimates (95%CIs) with GDF15 as the outcome i.e., SD change in GDF15 per 1 SD change in the predictor. X indicates $p<0.05$ plus FDR <0.1 and (x) indicates $p<0.05$, but FDR ≥ 0.1. [a]The given marker was sampled at the same time as GDF15. BMI, body mass index; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; GDF15, growth differentiation factor 15; HDL, high density lipoprotein; LDL, low density lipoprotein; PYY, peptide YY; VO_2peak, peak oxygen consumption.

Declaration of competing interest

Jonas Salling Quist and Kristine Færch have received funding from Novo Nordisk A/S for another project. Kristine Færch holds shares in Novo Nordisk A/S.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2022.106423.

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