Exercise-Induced Secretion of FGF21 and Follistatin Are Blocked by Pancreatic Clamp and Impaired in Type 2 Diabetes

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Context: Hepatokines have emerged as liver-derived hormone-like factors. Plasma fibroblast growth factor (FGF)-21 and follistatin increase with a high glucagon to insulin ratio and exercise, and resting levels are elevated in patients with type 2 diabetes (T2D).

Objective: The objective of the study was to investigate the regulatory roles of glucagon to insulin ratio and T2D on exercise-induced FGF21 and follistatin secretion.

Design /Interventions: Young healthy males performed a 2-hour bicycle exercise bout followed by 5 hours of rest in supine position with and without a pancreatic clamp blocking the increase in the glucagon to insulin ratio. In addition, we evaluated exercise-induced plasma FGF21 and follistatin in patients with T2D compared with healthy controls in response to 1 hour of bicycle exercise followed by a 3-hour recovery period.

Results: In healthy individuals, we observed a 10-fold ($P < .002$) increase in the glucagon to insulin ratio during exercise, which was abolished by the pancreatic clamp. Exercise with the pancreatic clamp completely blunted the exercise-induced increase in FGF21 ($P = .007$), whereas the induction of follistatin was approximately 50% reduced ($P = .04$). Exercise-induced FGF21 secretion was completely absent in patients with T2D, whereas the exercise-induced follistatin increase was impaired.

Conclusions/Interpretation: Exercise-induced increases in plasma FGF21 and follistatin are attenuated by the pancreatic clamp, indicating important roles for glucagon and insulin as upstream regulators. For follistatin, an additional regulatory mechanism must exist. Our data further show that exercise-induced FGF21 and follistatin secretion are impaired in patients with T2D. The magnitude of changes in glucagon and insulin or the sensitivity to these hormones seems central in the regulation of FGF21 and follistatin in humans. **(*J Clin Endocrinol Metab* 101: 2816–2825, 2016)**

Hepatokines emerge as liver-secreted signaling molecules that enable communication between the liver and peripheral tissues (1, 2). The current hypothesis is that the liver responds to humoral and metabolic stimuli by secreting hormone-like proteins to aid maintenance of whole-body homeostasis (1, 2). Fibroblast growth factor

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Abbreviations: BMI, body mass index; CV, coefficient of variation; FFA, free fatty acid; FGF, fibroblast growth factor; NGT, normal glucose tolerance; T2D, type 2 diabetes; VO_2 max, maximal oxygen uptake.

(FGF)-21 is one such hepatokine (3) that is recognized for its favorable metabolic profile as it induces insulin sensitivity (3) and promotes weight loss (4). Follistatin has recently been identified to be liver derived and regulated by exercise in humans (5, 6). It acts as a natural antagonist of TGF-β family members and has multiple functions as promoting muscle growth (7) via inhibition of myostatin (8), stimulating β-cell survival (6, 9), and inhibiting activin signaling (10).

Both follistatin and FGF21 are secreted during energy deprivation such as prolonged fasting (11, 12) and exercise (5, 13–15). In addition, both proteins are secreted upon glucagon administration (16, 17), and this phenomenon seems especially sensitive to changes in the ratio between circulating glucagon and insulin (6, 15). The findings that FGF21 (13, 15) and follistatin (5, 6) increase acutely in response to exercise suggest that they may be involved in mediating the beneficial effects of regular physical activity. On the other hand, circulating levels of FGF21 and follistatin are chronically elevated in metabolic diseases such as type 2 diabetes (T2D) (18, 19) and nonalcoholic steatohepatitis (20, 21), findings that indicate that hepatokine secretion is dysregulated under metabolic pathophysiological conditions. Interestingly, recent evidence suggests that exercise-induced FGF21 secretion is blunted in obese individuals (22), a condition known to predispose to development of T2D. Importantly, exercise-induced hepatokine secretion has not yet been investigated in patients with T2D.

In the present study, we tested the hypothesis that exercise-induced secretion of FGF21 and follistatin is dependent on changes in the glucagon to insulin ratio and that blockade of these changes results in blunted hepatokine secretion. In addition, we evaluated whether exercise-induced secretion of FGF21 and follistatin is affected in patients with T2D compared with healthy control individuals matched for age, body mass index (BMI), and fitness.

Research Design and Methods

Exercise in young healthy subjects

Eight healthy male subjects were recruited to a randomized crossover experimental protocol (for study outline, see Supplemental Figure 1). All subjects were informed orally and in writing about risks and discomfort associated with the experimental protocol. All subjects underwent medical examinations and incremental exercise tests (maximal oxygen uptake [VO_2 max] test) on a bicycle ergometer (Monark Ergomedic 839 E; Monark Ltd) to determine the individual maximum oxygen consumption (VO_2 max) via indirect calorimetry measurements (Quark b^2; CosMed). Of eight subjects included, two subjects were excluded from further analysis because large excursions occurred in plasma glucagon during exercise with a pancreatic clamp (for details, see Supplemental Figure 2). Subject characteristics were age 22.7 ± 0.2 years, BMI 21.7 ± 0.4 kg/m^2, and VO_2 max 52.9 ± 1.9 mL/kg·min (n = 6).

Subjects were instructed to refrain from strenuous exercise the days prior to trial days. On both trial days, the subjects reported 7:00 AM at the laboratory fasting from 10:00 PM the day before. For trial 1, a catheter was placed in an antecubital vein for blood sampling. After 30 minutes of supine rest and baseline blood sampling, the subjects exercised 2 hours at 60% of VO_2 max and then rested for 5 hours in supine position. For trial 2, two catheters were placed in antecubital veins: one for blood sampling and one for hormone infusion. After 10 minutes of supine rest and baseline blood sampling, an infusion of somatostatin ((Octreotide; Hospira Nordic) was started at 100 ng/kg·min to block exercise-induced changes in glucagon and insulin. To substitute basal hormone levels, glucagon (GlucaGen; Novo Nordisk) was infused at 0.60 ng/kg·min and insulin (Humulin; Eli Lilly) at 0.05 mU/kg·min. Because somatostatin is effective for up to 6 hours, the infusion was stopped at the end of the exercise bout, whereas glucagon and insulin were infused the entire trial. After 30 minutes of initial rest, the subjects exercised 2 hours at 60% of VO_2 max and then rested for 5 hours in a supine position. Blood glucose was monitored by bedside glucose measurements, and glucose (Fresenius, 100 mg/mL) was infused to keep blood glucose at 5 mmol/L.

During exercise and recovery, blood samples were obtained for hormone analysis. During both trial days, the subjects fasted until the last blood samples were obtained but had free access to water.

Exercise in patients with T2D

Plasma samples from a previously described study were analyzed (23). In brief, patients with T2D (n = 7) and healthy controls (n = 8) were included in the study. The T2D group and the normal glucose tolerance (NGT) group were matched on age, BMI, and fitness (VO_2 max) and were further characterized by an oral glucose tolerance test and glycated hemoglobin to verify the metabolic condition (23). The subjects with T2D paused antidiabetic medication 1 week prior to the experimental day, and all subjects were asked to refrain from strenuous exercise 24 hours prior to the experimental day. In the fasted condition, the subjects completed 60 minutes of bicycle ergometer exercise at 50% of their individual VO_2 max. There was no difference in exercise intensity between the two groups (23). After exercise, the subjects rested in the supine position for 180 minutes. During the experimental day, the subjects fasted until the last blood sample was obtained but had free access to water (see reference 23 for further details including data on plasma insulin and glucagon).

Ethical committee approval

The studies were approved by the Scientific Ethics Committee of the capital region of Denmark (H-1–2012-129, and reference 23) in accordance with the Helsinki Declaration. All subjects provided written informed consent to participate.

Plasma analysis

Blood samples were obtained in tubes containing EDTA for analysis of hormones and aprotinin Becton, Dickinson and Company (BD) for analysis of glucagon. All blood samples were immediately spun at 4°C at 3000 × *g* for 15 minutes. and the plasma fractions were stored at −80°C until analysis. Insulin was ana-

lyzed by electrochemiluminescent immunoassay (Cobas; Roche) at the Department of Clinical Biochemistry, Rigshospitalet. For the second experiment, insulin was analyzed using ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics) (23). Glucagon was analyzed by a RIA (Millipore) and run in duplicates with an intraassay coefficient of variation (CV) of 2%. Follistatin and FGF21 were analyzed by an ELISA (R&D Systems) in duplicates in accordance with the manufacturer's protocol. The intraassay CVs were 2.4% (follistatin) and 5.8% (FGF21); and the interassay CVs were 4.9% (follistatin) and 8.8% (FGF21). Free fatty acids were quantitatively analyzed by a UHPLC 1290 Infinity system (Agilent) coupled to a 6400 Triple Quad mass spectrometry system (Agilent) (23).

Statistical analysis

Data are presented as means ± SEM. Two-way ANOVAs were applied for comparison of repeated measurements between groups. Statistical significance by a two-way ANOVA is marked as follows: # marks the effect of time, £ marks the effect of group, and § marks the effect of time-group interaction. Statistical significance of a one-way ANOVA and the Dunnett's post hoc test is marked as follows: ***, $P < .0001$; **, $P < .01$; and *, $< .05$). $P \leq .05$ was considered statistically significant. All analyses were performed by the use of SAS 9.1 (SAS Institute Inc).

Results

Glucagon to insulin ratio during exercise in healthy subjects

On both trial days, the heart rate increases equally to approximately 140 bpm during exercise and then returns to baseline levels in the recovery period (Figure 1A). During exercise, glucagon increases acutely and peaks at the end of the exercise bout ($P = .004$) and then decreases to baseline levels (Figure 1B). Insulin decreases during the exercise bout ($P = .004$) and returns to baseline levels after cessation of exercise (Figure 1C). Consequently, the glucagon to insulin ratio increases by 10-fold ($P = .002$) and

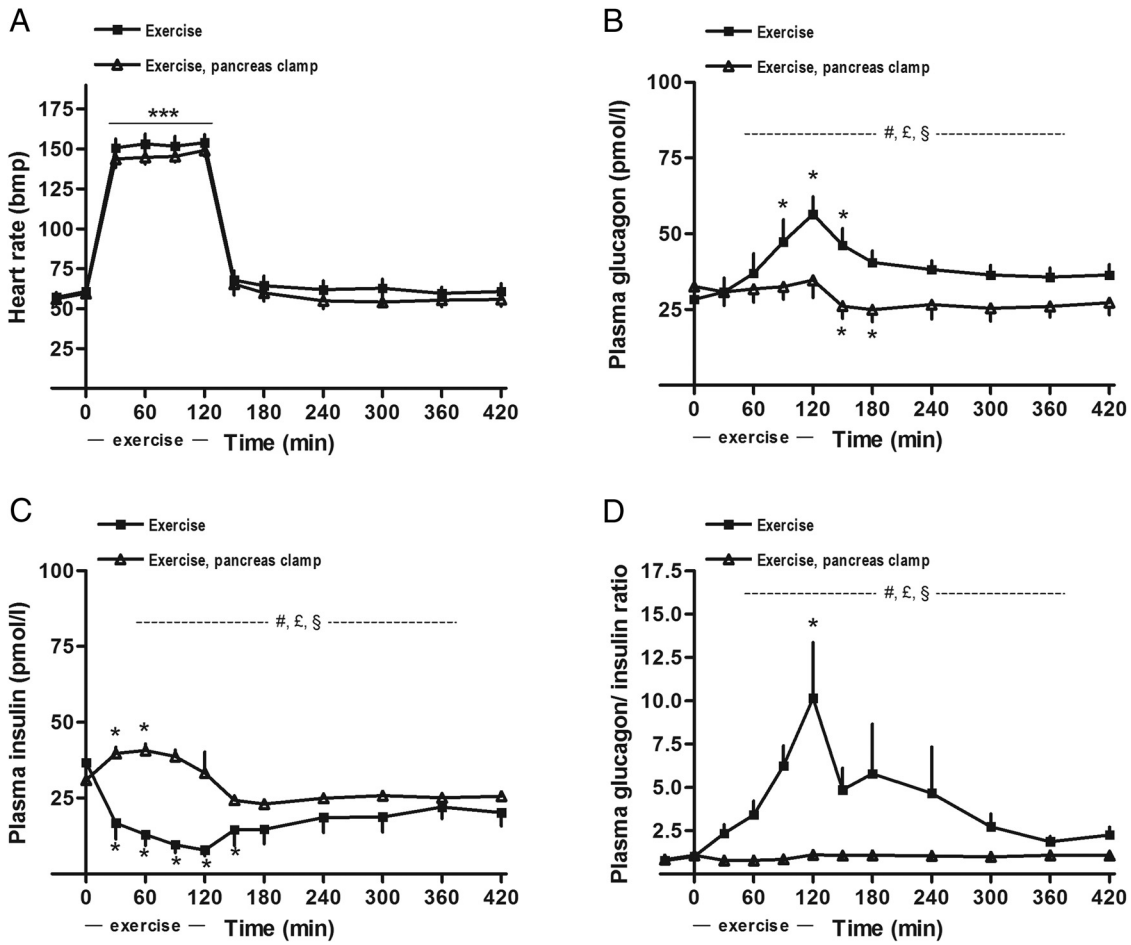

Figure 1. Heart rate, glucagon, insulin, and glucagon to insulin ratio during exercise in healthy young subjects (n = 6). A, In both trials, heart rate increased to approximately 140 bpm during exercise. B, During exercise, glucagon increases to approximately 60 pmol/L and then decreases to baseline levels, whereas it remains at baseline levels during exercise with the pancreatic clamp. C, During exercise, insulin decreases with exercise and then returns toward baseline levels. During exercise with the pancreatic clamp, there is a small initial increase after which it remains at baseline levels. D, During exercise, there is an increase in the glucagon to insulin ratio, whereas it remains unchanged during exercise with the pancreatic clamp. Data are means ± SEM. ■, Exercise; ▲, exercise with the pancreatic clamp. Statistical significance by a two-way ANOVA is marked as follows: #, effect of time; £, effect of group; §, effect of time-group interaction. Statistical significance of a one-way ANOVA and a Dunnett's post hoc test is marked as follows: ***, $P < .0001$; **, $P < .01$; *, $P < .05$.

peaks at the end of the exercise bout (Figure 1D). During exercise with the pancreatic clamp, glucagon was clamped at baseline levels during exercise, although with a minor decrease during recovery ($P < .0001$) (Figure 1B). Comparing glucagon levels of the two trials by a two-way ANOVA revealed a significant effect of time ($P < .0001$), group ($P = .0004$), and time-group interaction ($P = .0009$). During the pancreatic clamp, there is a small initial increase in insulin during the exercise bout ($P < .0001$), and then it decreases to baseline levels (Figure 1C). By a two-way ANOVA (insulin), there is significant effect of time ($P = .003$), group ($P = .0004$), and time-group interaction ($P < .0001$). Importantly, in the pancreatic clamp trial, the glucagon to insulin ratio remains unchanged during the entire trial ($P = .21$) (Figure 1D). By a two-way ANOVA (glucagon to insulin ratio), there is significant effect of time ($P = .008$), group ($P = .001$), and time-group interaction ($P = .008$).

Blood glucose and free fatty acids during exercise

During exercise, blood glucose initially decreases from 5.0 mM at baseline to 4.4 mM and then remains moderately suppressed throughout the trial ($P = .0003$) (Figure 2A). During exercise with the pancreatic clamp, blood glucose was measured bedside every fifth minute, and glucose was continuously infused in an antecubital vein to maintain euglycemia (5.0 mmol/L) (Figure 2A), and a two-way ANOVA revealed a significant effect of time ($P = .004$), group ($P < .0001$), and time-group interaction ($P < .05$). Exogenous glucose administration increased substantially during exercise as seen by the increase in the glucose infusion rate ($P < .0001$) (Figure 2B).

During exercise, free fatty acids (FFAs) increase from 240 μmol/L at baseline to 1270 μmol/L at the end the exercise bout ($P < .0001$). During the recovery period, FFAs remain elevated at approximately 1000 μmol/L (Figure 2C). During exercise with the pancreatic clamp, the FFA response to exercise is blunted and remains at the baseline level throughout the entire trial ($P = .55$) (Figure 2C). There is a significant effect of time, group, and time-group interaction by a two-way ANOVA (all $P < .0001$).

Plasma FGF21 and follistatin during exercise in healthy young subjects

During exercise, plasma FGF21 remains at baseline levels the first hour and then increases from approximately 75 ng/L at 60 minutes to its peak at approximately 215 ng/L at 180 minutes ($P < .0001$) (Figure 3A). Two hours after its peak, plasma FGF21 is back to baseline levels (Figure 3A). Relative to baseline levels, plasma FGF21 is elevated by more than 8-fold at its peak ($P < .0001$) (Figure 3B). In contrast, during exercise with the pancreatic clamp,

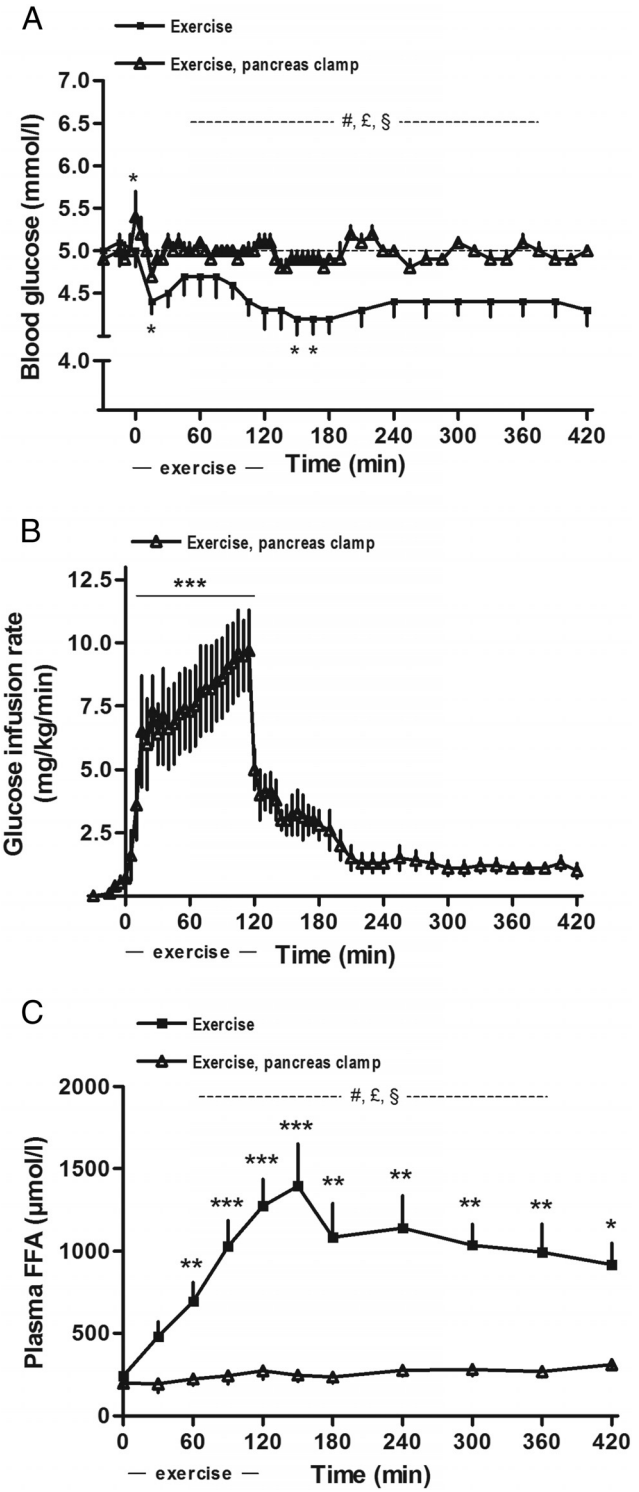

Figure 2. Blood glucose, glucose infusion rate, and FFAs in healthy young subjects (n = 6). A, During exercise, blood glucose initially decreases and then remains moderately suppressed, whereas it remains at 5 mM during exercise with the pancreatic clamp. B, During exercise with the pancreatic clamp, there is a significant increase in the glucose infusion rate during exercise. C, During exercise, the plasma FFAs increase to 1270 μmol/L and remains moderately elevated, whereas there is no change in plasma FFAs during exercise with the pancreatic clamp. Data are means ± SEM. ■, Exercise; ▲, exercise with pancreatic clamp. Statistical significance by a two-way ANOVA is marked as follows: #, effect of time; £, effect of group; §, effect of time-group interaction. Statistical significance of a one-way ANOVA and a Dunnett's post hoc test is marked as follows: ***, $P < .0001$; **, $P < .01$; *, $P < .05$.

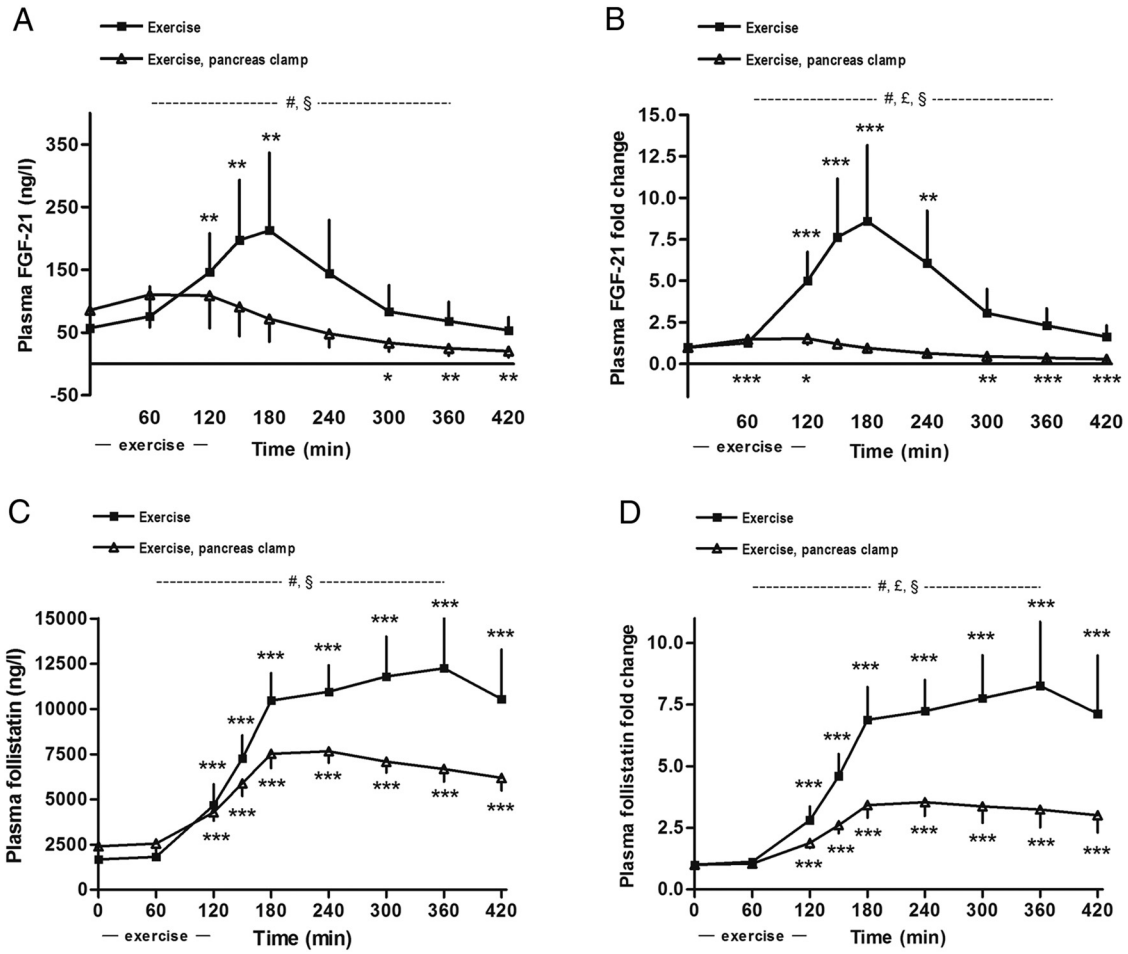

Figure 3. Circulating FGF21 and follistatin in response to exercise in healthy young subjects (n = 6). A, Circulating FGF21 increases by the end of the exercise bout and peaks 1 hour into recovery after which it return to baseline levels. During exercise with the pancreatic clamp, circulating FGF21 does not increase. B, Relative to baseline levels, plasma FGF21 is elevated by greater than 8-fold with exercise, whereas it decreases during exercise with the pancreatic clamp. C, Circulating follistatin increases late in the exercise bout and reaches a plateau 4 hours into recovery. During exercise with the pancreatic clamp, circulating follistatin increases late in the exercise bout and peaks 2 hours into recovery. D, Circulating follistatin increases greater than 8-fold relative to baseline levels with exercise, whereas there is only a 3.5-fold increase during exercise with pancreatic clamp. Data are means ± SEM. ■, Exercise; ▲, exercise with the pancreatic clamp. Statistical significance by a two-way ANOVA is marked as follows: #, effect of time; £, effect of group; §, effect of time-group interaction. Statistical significance of a one-way ANOVA and a Dunnett's post hoc test is marked as follows: ***, $P < .0001$; **, $P < .01$; *, $P < .05$.

plasma FGF21 decreases and is significantly decreased in the last part of the trial ($P = .003$) (Figure 3A). Accordingly, when expressed as a relative change from baseline, plasma FGF21 is significantly decreased in the last part of the trial ($P < .0001$) (Figure 3B). When analyzed by a two-way ANOVA (Figure 3A), there is a significant effect of time ($P < .0001$) and time-group interaction ($P = .007$) and borderline significant effect of group ($P = .057$).

During exercise, plasma follistatin increases in the last part of the exercise bout and is significantly increased at 120 minutes ($P < .0001$). Follistatin then increases further from approximately 4500 ng/L at 120 minutes to a plateau of 10 000–12 000 ng/L from 180 to 420 minutes (all $P < .0001$) (Figure 3C). During exercise with the pancreatic clamp, plasma follistatin also increases ($P < .0001$), showing a similar kinetics, but it does not reach the same level as during normal exercise. It increases from approximately 4500 ng/L at 120 minutes to approximately 7500 ng/L at 240 minutes (Figure 3C). When analyzed by a two-way ANOVA (Figure 3C), there is a significant effect of time ($P < .0001$) and time-group interaction ($P = .04$) and a borderline significant effect of group ($P = .08$).

The increase in follistatin during exercise corresponds to a more than 8-fold increase relative to baseline levels ($P = .004$), whereas there is only a 3.5-fold increase during exercise with the pancreatic clamp ($P < .0001$) (Figure 3D). When analyzed by a two-way ANOVA (Figure 3D), there is a significant effect of time, group, and time-group interaction ($P < .0001$, $P = .0002$, and $P = .02$, respectively).

Glucagon to insulin ratio and FFA in T2D

Patients with T2D and matched healthy controls had comparable plasma glucagon concentrations, whereas the T2D group had higher insulin concentrations during the

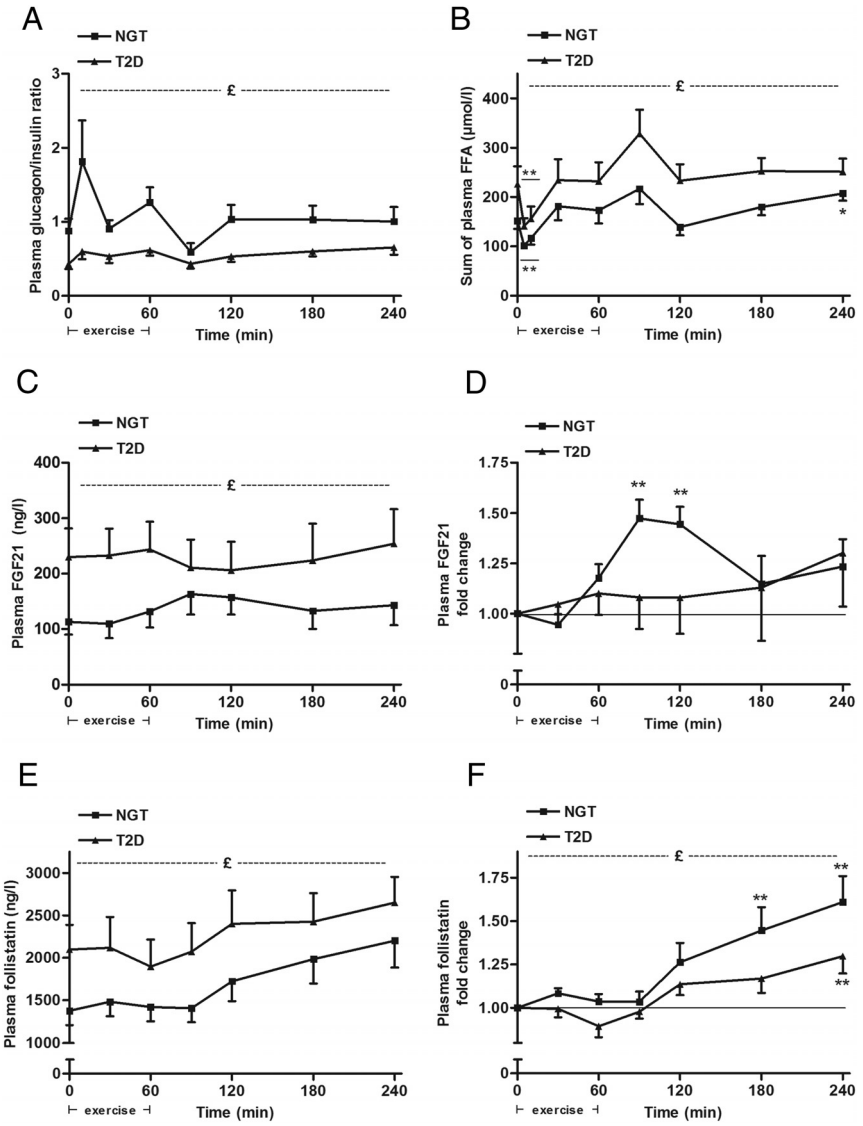

Figure 4. Glucagon to insulin ratio, FFAs, FGF21, and follistatin in patients with T2D compared with healthy control subjects. A, Glucagon to insulin ratio is constantly higher in healthy controls compared with patients with T2D (due to hyperinsulinemia in the T2D group). In the healthy controls, there is a borderline significant effect of time, whereas there is no change in the T2D group. B, Plasma FFAs are higher in the T2D group than in the NGT group. Shown is the sum of all detected plasma FFAs as reported elsewhere (23). In both groups, there is a significant effect of time ($P < .0001$). C, When analyzed in absolute concentrations, no change is observed in FGF21 in either group in response to exercise. D, When analyzed as fold change, FGF21 is increased by approximately 50% in the NGT group in response to exercise, whereas it does not increase in the T2D group. E, Follistatin tends to increase in response to exercise in the NGT group ($P = .07$), whereas it does not increase in the T2D group (0.73) when analyzed as absolute concentrations. F, Follistatin increases by 60% in the NGT group and by 30% in the T2D group in response to exercise when analyzed as fold change. Data are means ± SEM. ■, NGT group; ▲, T2D group. Statistical significance by a two-way ANOVA is marked as follows: £, effect of group. Statistical significance of a one-way ANOVA and a Dunnett's post hoc test is marked as follows: **, $P < .01$; *, $P < .05$.

60-minute exercise bout (23). Consequently, the glucagon to insulin ratio is higher in the NGT group than in the T2D group (two way ANOVA, effect of group, $P < .0001$) (Figure 4A). In the healthy controls, the glucagon to insulin ratio increases initially with exercise with a borderline significant effect of time (one way ANOVA, $P = .056$), whereas there is no change in the T2D group (one way ANOVA, $P = .47$). The sum of all detected plasma FFAs (individually reported in reference 23) is higher in the T2D group compared with the NGT group (two way ANOVA, effect of group, $P = .049$) (Figure 4B). In both groups, FFAs initially decrease and then increase during the last part of the exercise bout and remain moderately elevated during the recovery period (one way ANOVA, both $P < .0001$).

Exercise-induced FGF21 secretion in T2D

Circulating FGF21 is constantly higher in the patients with T2D compared with healthy controls (two way ANOVA, effect of group, $P = .0004$) (Figure 4C). In healthy controls, exercise has no effect on circulating FGF21 when analyzed in absolute concentrations (one way ANOVA, $P = .80$) (Figure 4C) due to the wide range of individual levels. However, relative to baseline levels, there is a 1.5-fold increase in response to exercise (one way ANOVA, $P = .0008$) with maximum increases at 30 minutes and 60 minutes after the exercise bout (Figure 4D). In contrast, the patients with T2D do not respond to exercise: there is no effect of exercise on plasma FGF21 neither when analyzed as absolute concentrations (one way ANOVA, $P = 1.0$) nor fold changes from baseline (one way ANOVA, $P = .92$) (Figure 4, C and D). When the fold changes of FGF21 between the T2D and NGT group are analyzed by a two-way ANOVA, there is a borderline significant effect of group ($P = .056$).

Exercise-induced follistatin secretion in T2D

At baseline, the plasma follistatin is elevated in the T2D group compared with controls and remain higher during the entire trial (two way ANOVA, effect of group, $P = .0002$) (Figure 4E). In both patients with T2D and healthy controls, plasma follistatin is unchanged during the exer-

cise bout and then increases in the recovery period. In the NGT group, there is a borderline significant effect of time by a one-way ANOVA ($P = .07$) (Figure 4E). When expressed as fold change from baseline, a 1.6-fold increase at the end of recovery is revealed (one way ANOVA, $P < .0001$) (Figure 4F). When expressed as absolute concentrations, there is no effect of exercise on plasma follistatin in the T2D group (one way ANOVA, $P = .73$) (Figure 4E). However, when expressed as fold change from baseline, there is a 1.3-fold increase at the end of the recovery period (one way ANOVA, $P = .0007$) (Figure 4F). When the fold changes of follistatin between the T2D and NGT group are analyzed by a two-way ANOVA, there is a significant effect of group ($P = .002$) (Figure 4F).

Discussion

The present study demonstrates the following: 1) exercise-induced secretion of FGF21 is blocked by a pancreatic clamp, demonstrating an important role for glucagon and insulin as upstream regulators, 2) the pancreatic clamp does not completely block exercise-induced secretion of follistatin, indicating an additional regulatory mechanism, and 3) exercise-induced FGF21 and follistatin secretion are impaired in patients with T2D.

Plasma levels of follistatin and FGF21 increased markedly in response to exercise, and their secretion is impaired by the pancreatic clamp during exercise. We recently demonstrated that the exercise-induced increases in circulating FGF21 and follistatin are largely determined by hepatosplanchnic production (6, 15) and that FGF21 and follistatin are regulated by the glucagon to insulin ratio at rest (6, 15). One limitation is that the exercise-induced increases of FGF21 and follistatin in the present data are systemic measurements, and that is why we cannot rule out that the impaired response of FGF21 and follistatin is due to an increased clearance/degradation with the pancreatic clamp rather than an impaired release; however, this seems unlikely in light of the identification of the hepatosplanchnic release of both FGF21 and follistatin during exercise. In particular, for FGF21, changes in glucagon to insulin ratio appear to be an important upstream regulatory mechanism during exercise because exercise-induced FGF21 secretion is completely blunted by the pancreatic clamp. Of note as insulin and glucagon are released from the endocrine pancreas, they pass by the liver before entering the systemic circulation; thus, the liver is exposed to a high concentration, which is reflected in the higher concentration in the hepatic vein. Furthermore, the liver degrades/clears insulin and glucagon (24), which further adds to the difference between hepatic exposure and systemic concentration. Hence, our data support the findings by Berglund et al (25), who demonstrated that exercise-induced hepatic FGF21 gene expression is lost in gcgr$^{-/-}$ mice, indicating that glucagon-mediated intracellular signaling is necessary for this increase in FGF21. During the pancreatic clamp procedure, there is a small increase in the insulin level during the exercise bout (in contrast to the exercise induced decrease), which may have decreased the FGF21 response further.

Importantly, FFAs are also significant regulators of FGF21 via peroxisomal proliferator-activated receptor-α activation (26). In the present study, the exercise-induced FFA response is completely blunted by the pancreatic clamp. This is likely a consequence of the lack of decreasing insulin levels and to a less extent the lack of increasing glucagon levels. In addition, GH-driven lipolysis is likely also absent. Hence, the relative contribution of the glucagon to insulin ratio and FFAs on exercise-induced FGF21 secretion cannot by distinguished from these data. Notably, a synergistic effect of glucagon and lipid signaling on hepatic FGF21 mRNA has been reported (27, 28). Thus, the increased hepatic FGF21 gene expression and secretion are likely a result of combined glucagon and FFA stimulation both during exercise and prolonged fasting (Figure 5).

Interestingly, changes in the glucagon to insulin ratio seem to represent only approximately 50% of the regulatory stimulus for exercise-induced follistatin secretion. When follistatin increases in response to exercise (with and without pancreatic clamp), the two curves follow a similar kinetic pattern, but the secretion is induced to a different magnitude in the two experiments. This indicates that the early phase of the follistatin response is driven by additional mechanisms, whereas the late phase seems to be driven by the glucagon to insulin ratio, ie, changes in the glucagon to insulin ratio lead to a sustained follistatin secretion. Thus, for follistatin, a regulatory mechanism independent of changes in the glucagon to insulin ratio must exist. Because the FFA response is blunted by the pancreatic clamp, the additional regulatory mechanism is also independent of hepatic FFA signaling (Figure 5). This is in line with the observation that follistatin is not regulated by a lipid infusion in humans (6). The follistatin gene promoter contains several response elements responsive to cAMP (29), and intracellular cAMP induces its secretion in hepatocytes (6). Epinephrine, which increases with exercise, acts via cAMP, making it a plausible candidate as an inducer of follistatin secretion. Although conflicting results are reported (5), evidence exist that hepatic follistatin gene expression is induced by phenylephrine (17), which could potentially explain the induction of follistatin during exercise despite the pancreatic clamp.

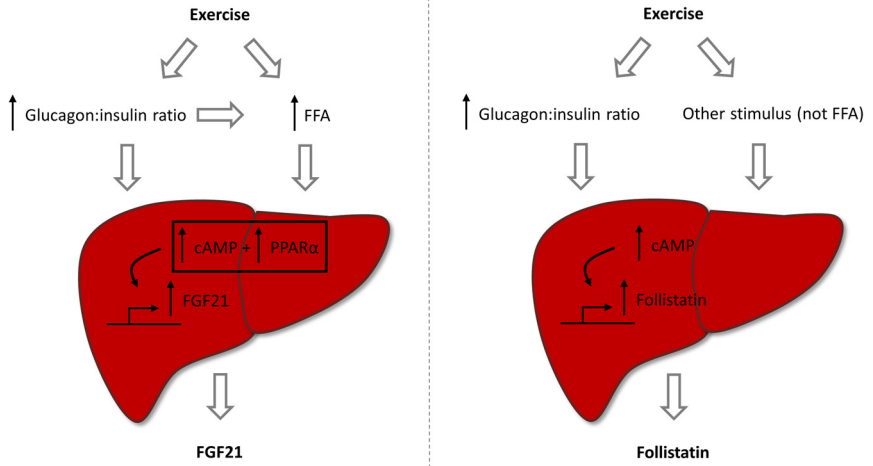

Figure 5. Illustration of the hypothesis of exercise-induced regulation of FGF21 and follistatin. An acute bout of exercise increases circulating glucagon, whereas circulating insulin decreases it. This results in a marked increase in the glucagon to insulin ratio. In addition, acute exercise increases circulating FFAs, partly due to the increased glucagon to insulin ratio. The combination of the increased glucagon to insulin ratio and the increased FFAs leads to the induction of hepatic FGF21 secretion. Hepatic follistatin is also induced by the increased glucagon to insulin ratio, whereas an additional regulatory mechanism also must exist. PPAR, peroxisomal proliferator-activated receptor.

We also observe that healthy individuals experience elevated levels of both FGF21 (50%) and follistatin (60%) after exercise, whereas follistatin increases by only 30% and the FGF21 response is completely absent in patients with T2D. These data are in line with the finding that exercise-induced FGF21 secretion is blunted in obese individuals without T2D but with an increase in insulin resistance (22). Hence, a dysregulated exercise-induced FGF21 response could be an early event in metabolic diseases, and our data suggest that the impairment of exercise-induced FGF21 secretion persists in manifest metabolic disease as T2D. These data indicate that in patients with metabolic diseases, the liver may not respond adequately to the exercise-induced signals that promote hepatokine secretion, which could be due to the presence of insulin resistance or other features as hepatic fat accumulation. In the present study, there was no difference in glucagon levels, whereas the T2D group had elevated insulin levels (23). This results in a suppressed glucagon to insulin ratio in the T2D group, which might attenuate the hepatokine response. Although hepatic insulin resistance must be suspected in the patients with T2D, hyperinsulinemia may also contribute to the attenuation of hepatokine secretion.

Exercise induced only a minor systemic glucagon response (23); however, due to a marked hepatic clearance of glucagon (30), the systemic glucagon concentration reflects only part of the hepatic/portal glucagon concentration. Because the glucagon response did not differ between the groups (23), these data open the door for the speculation that impaired exercise-induced hepatokine may be caused by reduced hepatic glucagon sensitivity. In T2D, reduced hepatic glucagon-stimulated adenylyl cyclase activity has been reported (31), indicative of reduced hepatic glucagon sensitivity, whereas others find no evidence of a reduced glucagon sensitivity in T2D (32). Based on this inconsistency, it is difficult to estimate to what extent reduced hepatic glucagon sensitivity in T2D affects exercise-induced hepatokine secretion. Thus, this calls for a direct evaluation of glucagon-induced hepatokine secretion in insulin-resistant states. In addition, the chronic elevation of FGF21 and follistatin in T2D may itself hinder an exercise-induced response. Therefore, it could be speculated that exercise training, weight loss, a gastric bypass procedure, or other metabolic interventions would lead to a lowering of chronic FGF21 and follistatin levels due to increased insulin sensitivity, which in turn could lead to the normalization of the exercise-induced responses of FGF21 and follistatin.

FGF21 and follistatin may represent beneficial metabolic factors mediating some of metabolic improvements observed with regular physical exercise because FGF21 increases insulin sensitivity (4) and energy expenditure (33) and induces weight loss (4), whereas follistatin regulates glucagon secretion and promotes β-cell survival (6, 9). FGF21 (15) and follistatin (6) are acutely induced by changes in the glucagon to insulin ratio, which is in line with the finding by Berglund et al (25), who demonstrated that glucagon receptor signaling is required for exercise-induced reversal of hepatic steatosis in mice. Evidence exist that glucagon receptor content (34) and sensitivity (35) are increased by exercise training. In light of this, an important question arises: can dysregulated hepatokine secretion be reversed by regular physical exercise, eg, repeated bouts of exercise may assist normalization of exercise-induced hepatokine secretion via increased (short term) glucagon action.

In conclusion, we demonstrate that exercise-induced FGF21 and follistatin secretions are attenuated by a pancreatic clamp, suggestive of glucagon to insulin ratio as an important upstream regulatory mechanism for exercise-induced hepatokine secretion. Moreover, an additional

regulatory mechanism must exist for follistatin secretion. We further demonstrate that exercise-induced hepatokine secretion, especially FGF21, is impaired in patients with T2D compared with healthy individuals. Because hepatokine actions may be important mechanisms promoting metabolic health, it remains to be investigated whether an impairment of exercise-induced hepatokine secretion can be corrected by exercise training regimens.

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