Original article

MOLECULAR METABOLISM

Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans

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ABSTRACT

Background & aims: Fibroblast growth factor 21 (FGF-21) is a liver-derived metabolic regulator induced by energy deprivation. However, its regulation in humans is incompletely understood. We addressed the origin and regulation of FGF-21 secretion in humans.
Methods: By determination of arterial-to-venous differences over the liver and the leg during exercise, we evaluated the organ-specific secretion of FGF-21 in humans. By four different infusion models manipulating circulating glucagon and insulin, we addressed the interaction of these hormones on FGF-21 secretion in humans.
Results: We demonstrate that the splanchnic circulation secretes FGF-21 at rest and that it is rapidly enhanced during exercise. In contrast, the leg does not contribute to the systemic levels of FGF-21. To unravel the mechanisms underlying the regulation of exercise-induced hepatic release of FGF-21, we manipulated circulating glucagon and insulin. These studies demonstrated that in humans glucagon stimulates splanchnic FGF-21 secretion whereas insulin has an inhibitory effect.
Conclusions: Collectively, our data reveal that 1) in humans, the splanchnic bed contributes to the systemic FGF-21 levels during rest and exercise; 2) under normo-physiological conditions FGF-21 is not released from the leg; 3) a dynamic interaction of glucagon-to-insulin ratio regulates FGF-21 secretion in humans.

Keywords Liver; Hepatic; Hepatokine; Exercise

1. INTRODUCTION

Fibroblast growth factor-21 (FGF-21) is a circulating member of the FGF superfamily, which is primarily expressed in the liver [1]. FGF-21 is regarded as an important endocrine metabolic regulator [2,3] and holds promise as a therapeutic target in metabolic disorders such as type 2 diabetes [4]. However, the regulation of FGF-21 in humans remains incompletely understood.
In humans and particularly in mice, long-term energy deprivation such as fasting has been shown to increase circulating FGF-21 [5,6]. During fasting, several signals have been reported to be involved in mediating an increase in systemic FGF-21. In humans, free fatty acids (FFAs) [7], low protein intake independent of energy restriction [8] and glucagon [9,10] have been linked to increased plasma FGF-21. But whereas glucagon increases circulating FGF-21 [9–11] contrasting findings exist regarding the role of insulin [12,13]. Both animal and *in vitro* studies have demonstrated that FGF-21 is regulated in hepatocytes by peroxisome proliferator-activated receptor (PPAR) α agonism [14–16] and ketone bodies [14]. Collectively, prolonged conditions of energy deprivation induce hepatic FGF-21 secretion and several regulatory signals have been proposed.
During exercise, the body undergoes a state of acute energy deprivation. In order to maintain glucose homoeostasis, circulating levels of glucagon increase whereas insulin levels decrease [17–19]. In addition, free fatty acids (FFAs) increase, and, with prolonged exercise, circulating levels of ketone bodies increase [20,21]. Consequently, energy deprivation by fasting and acute exercise induces a similar pancreatic hormone response and similar metabolite signals [22–25]. Importantly, FGF-21 increases with acute exercise [11,26], suggesting that FGF-21 is regulated within a short time frame.
Many of the effects of FGF-21 signalling are similar to the beneficial effects of physical activity on metabolism, and it is likely that FGF-21 represents a key mediator of the exercise-induced metabolic improvements. FGF-21 signalling leads to metabolically beneficial effects including correction of hyperglycemia, lowering of plasma lipidemia and reduction of hepatic steatosis [2,14,27]. Most of these processes

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Abbreviations: FFA, free fatty acids; FGF-21, fibroblast growth factor-21; PPAR, peroxisome proliferator-activated receptor

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are believed to occur in the adipose tissue and the liver. However, skeletal muscle cells also secrete FGF-21 [28] during mitochondrial stress, and FGF-21 has been suggested to be a so-called "mitokine" [29,30]. Thus, the possibility existed that skeletal muscles could contribute to the circulating levels of FGF-21 during exercise.

The aim of this study was to identify the source of FGF-21 during exercise in humans and identify its regulation. By applying unique invasive techniques, we were able to study net-fluxes over the splanchnic bed as well as skeletal muscle at rest and during exercise, allowing us to determine if FGF-21 is secreted from the liver and/or skeletal muscles in humans. By experimentally mimicking the exercise-induced changes in glucagon/insulin levels in resting subjects, we evaluated hormonal regulatory mechanisms involved in FGF-21 secretion.

2. MATERIALS AND METHODS

2.1. Ethical committee approvals

The studies were approved by the Scientific Ethics Committee of the capital region of Denmark: The exercise study with hepatic vein and brachial artery catheterisation and the hormone infusion study were approved under the same ethical committee number: H-1-2012-129. The ethical committee approval for the exercise study with femoral vein and femoral artery catheterisation has previously been published [31]. All studies were executed in accordance with the Helsinki Declaration. All subjects provided written informed consent to participate.

2.2. Exercise study with hepatic vein and brachial artery catheterisation

In ten healthy males, catheters were placed in an antecubital vein, the right hepatic vein and the brachial artery of the non-dominant arm. The subjects exercised on an adjusted cycle ergometer in semi-supine position at 60% of VO_2 max for 2 h and then rested for 4 h in the same position. Estimation of hepatic blood flow was performed by the indocyanine green (ICG) technique [32]. For further details on the experimental procedures, see Supplementary Materials and Methods.

2.3. Exercise study with femoral vein and femoral artery catheterisation

This study has previously been described [31]. Nine healthy males performed 2 h of one-legged knee extensor exercise at 50% of maximum workload with catheters inserted into the right and left femoral vein and the femoral artery of the resting leg. Femoral arterial blood flow was assessed by Doppler ultrasound (CFM-800, Wingmed A/S, Horten, Norway) [33]. None of the data presented here have been published previously. None of these subjects were included in the other studies presented here. For further details on the experimental procedures, see Supplementary Materials and Methods.

2.4. Animal experiments

Treadmill experiments have been described [34]. In brief, 12-week-old male C57Bl/6J mice ran after 5 min warm-up for 60 min at 14 m/min and 14° uphill slope. Immediately after the run, the mice were anesthetized by intraperitoneal injection of ketamine (150 μg/g body weight) and xylazine (10 μg/g body weight) and killed by decapitation. Tissues were immediately removed and frozen in liquid nitrogen. For further details on the experimental procedures, see Supplementary Materials and Methods. Animal data are presented as a Supplementary Figure (A4).

2.5. Hormone infusions

Ten healthy males went through four experimental protocols separated by at least 2 weeks (test days 1–4). Test day 1: glucagon (GlucaGen, Novo Nordisk Scandinavia, Copenhagen, Denmark) was infused for 1 h at 6 ng/kg/min. Test day 2: to identify the isolated effect of glucagon, an infusion of somatostatin (Octreotide, Hospira Nordic AB, Stockholm, Sweden) at 100 ng/kg/min was started 10 min prior to the glucagon infusion and was infused for additional 2 h. Glucagon was infused for 1 h at 6 ng/kg/min. Test day 3: somatostatin was infused at 100 ng/kg/min for 130 min. Test day 4: saline was infused for 1 h with same rate as the glucagon infusion rate. For further details on the experimental procedures, see Supplementary Materials and Methods. Of the included subjects, 3 subjects in the somatostatin infusion trial also participated in the exercise study with hepatic vein catheterisation and brachial artery catheterisation.

2.6. Statistics

Data are presented as means ± SEM. For analyses of hormone and blood glucose kinetics one-way ANOVAs with Dunnett's post hoc tests were applied. Where relevant, Student's t-tests were applied * significant by one-way ANOVA. # significant by one-way ANOVA and Dunnett's post hoc test. † significant by Student's t-test. $p \leq 0.05$ was considered statistically significant. Analyses were performed by SAS 9.1, SAS Institute Inc., Cary, NC, USA and linear regression analyses by GraphPad Prism 4, GraphPad Software Inc, La Jolla, CA, USA.

3. RESULTS

3.1. Splanchnic FGF-21 secretion and acute regulation during exercise in humans

Here, we quantified the hepatic FGF-21 production in healthy humans and evaluated the kinetics of the exercise-induced FGF-21 increase. First, we evaluated hepatic plasma flow (Supplementary Figure A1) and glucose and lactate flux over the splanchnic bed during exercise, which confirmed enhanced glucose production and lactate uptake by the liver (Supplementary Figure A2).

At rest we observed an arterial-hepatic vein (a-hv) difference of −30.2 ± 6.6 ng/l in plasma FGF-21 ($p = 0.001$) (Figure 1A). When taking hepatic blood flow into consideration (Supplementary Figure A1), this equals a hepatic FGF-21 production of 30.5 ± 7.4 ng/min ($p = 0.002$) at rest (Figure 1B). These data demonstrate a constant hepatic FGF-21 release in humans at rest after an overnight fast. During exercise, plasma FGF-21 increases in the hepatic vein from ~200 ng/l at rest to a peak at ~670 ng/l 30 min after exercise ($p < 0.0001$) (Figure 1C), an ~5-fold increase ($p < 0.0001$) (individual data presented in Supplementary Figure A3). After exercise, plasma FGF-21 rapidly decreases and returns to baseline after 120 min. Thus, plasma FGF-21 is acutely regulated by exercise, and these data indicate a short plasma half-life. The a-hv difference for FGF-21 is negative at all time points (significant by Student's t-test) (Figure 1D), demonstrating a constant secretion of FGF-21 to the splanchnic circulation. During exercise, the a-hv difference of FGF-21 increases and thus net hepatic production peaks at 108 ng/min 30 min after the end of exercise ($p = 0.03$ and $p = 0.04$, resp.) (Figure 1E). Collectively, exercise increases the hepatic FGF-21 secretion ~4-fold ($p = 0.01$).

In mice, gene expression analysis confirmed the previous finding [26] that FGF-21 mRNA levels are up-regulated 3-fold in the liver immediately after exercise ($p = 0.02$) (Supplementary Figure A4), while they remained low in fat and soleus and tibialis muscles of sedentary as well as exercised mice.

