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Hepatokines - a novel group of exercise factors

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Abstract:	Regular physical activity not only improves the exercise capacity of the skeletal muscle performing the contractions, it is beneficial for the whole body. An extensive search for “exercise factors” mediating these beneficial effects has been going on for decades. Particular skeletal muscle tissue has been investigated as a source of circulating exercise factors, and several myokines have been identified. However, exercise also has an impact on other tissues. The liver is interposed between energy storing and energy utilising tissues and is highly active during exercise, maintaining energy homeostasis. Recently, a novel group of exercise factors termed hepatokines has emerged. These proteins (fibroblast growth factor-21, follistatin, angiopoietin-like protein 4, heat shock protein 72, insulin-like growth factor binding protein 1) are released from the liver and increased in the bloodstream during or in the recovery after an exercise bout. In this narrative review, we evaluate this new group of exercise factors focusing on the regulation and potential function in exercise metabolism and adaptations. These hepatokines may convey some of the beneficial whole-body effects of exercise that could ameliorate metabolic diseases, such as obesity or type 2 diabetes.
Response to Reviewers:	Thank you for your thorough reading and commenting on our manuscript. This has really improved our message and readability. We have prepared a point-to-point answers replying the critique raised. Reviewer #1: OVERALL The present manuscript "Hepatokines - a novel group of exercise factors" provides a very interesting, informative and thorough review on the current literature and view on hepatokines. The two figures support the text well. RESPONSE: Thank you for your positive evaluation and enthusiasm for the livers endocrinology in relation to exercise. SPECIFIC My suggestions are towards minor parts/sentences aiming to make it easier for the

reader. In addition, it is suggested to ensure that the tissue (liver, skeletal muscle) involved and the nature of the measure (plasma, mRNA , protein) are presented throughout the text when referring to previous findings.
RESPONSE: We apologies for the unclear sentence and appreciate the suggestions, which has improved the manuscript. In addition to the specific points raised by the reviewer, we included further revisions in the manuscript to improve the understanding and clarity.

1) It is suggested that more paragraphs are used. Hence, more often use a new paragraph to help the reader follow the focus of the text. Here some examples:
Page 3, line 31-32
RESPONSE: Paragraphs inserted.
Page 6, line 15/16; line 30/31; line 43/44 (the two last might be one paragraph)
RESPONSE: Paragraphs inserted.
Page 7, maybe line 36/37; line 54/55
RESPONSE: Paragraphs inserted.
Page 8, Line 51/52
RESPONSE: Paragraphs inserted.
Page 9, line 9-10
RESPONSE: Paragraphs inserted.
Page 10, line 57/58
RESPONSE: Paragraphs inserted.
Page 11, line 19/20
RESPONSE: Paragraphs inserted.
Page 11, line 56/57
RESPONSE: Paragraphs inserted.
Page 13, line 45
RESPONSE: We changed the order of sentences to improve the clarity of the paragraph.

2) Some sentences are a bit difficult to follow. It is suggested to consider the sentences below:
Page 2: First sentence in abstract: may use "Regular physical activity does not only improve…" and avoid too many "benefit/beneficial".
RESPONSE: thank you, this have been corrected.

Page 2, abstract: consider to revise "…organokines have emerged released from"
Last sentence of abstract: may add "These hepatokines may convey some of the exercise training-induced whole-body…"
RESPONSE: thank you, the sentence has been corrected.

Page 3, line 14-16
RESPONSE: a paragraph has been inserted.
Page 3 line 40-41: "secreted to peripheral organs". Is this a reasonable statement? Should it be: "secreted to the blood with concomitant effects on peripheral organs"?
RESPONSE: Thank you, the sentence has been corrected.
Page 5, line 19-20: "…physical activity must not necessarily…"; may consider: "….because an increased release of proteins is not necessarily reflected in an elevated transcript level, if post-translational regulation or ……."
RESPONSE: Thank you, the sentence has been corrected.
Page 5, line 36-37: "independent from"?? "of" or "in the resting state"?
RESPONSE: Thank you, yes this sentence was unclear and has been corrected.
Page 7. Line 4-7
RESPONSE: a paragraph has been inserted.
Page 7, line 14-18 suggesting: "This is supported by impaired exercise-induced adaptations in FGF21-deficient mice, which failed to improve glucose tolerance and reduce hepatic triglyceride content as observed in wildtype mice on high fat diet".
RESPONSE: Thank you, the sentence adjusted accordingly.
Page 7, line 36-37: does this fit in here? An effect of follistatin is presented, but otherwise regulation of follistatin.
RESPONSE: Thank you, the sentence has been removed.
Page 7, line 49/50: "blunting the glucagon-to-insulin ratio" ? This seems to lack "change of the ratio"?
RESPONSE: Thank you, yes agree the sentence has been corrected.

	Page 8, line8/9: "dysregulated". Is this the intended word/meaning? RESPONSE: Thank you, dysregulated has been removed. Page 8, line 23: Is it clear why TGF-beta is brought up? RESPONSE: Thank you, this has been clarified. Page 8. Line 57; suggesting: "free fatty acids (FFA) increase during fasting (109) acting as ligand for the PPARs. " RESPONSE: Thank you, this has been corrected. Page 9, line 9-18 RESPONSE: Thank you, a paragraph inserted. Page 9, line 26/27: may use: "Based on tissue-specific overexpression models, …" RESPONSE: Thank you, the sentence has been adjusted accordingly. Page 9, line 39-41: "Nevertheless, the acute regulation in plasma could suggest". May adjust to: "Nevertheless, the acute regulation of the plasma ANGPTL4 level may suggest an endocrine.." RESPONSE: Thank you, the sentence has been adjusted accordingly. Page 9, heading: Is it clear from the heading how this section is different from the parts on regulation in the sections above? Should this heading maybe include "intracellular mediated regulation"? RESPONSE: Thank you, the heading has been adjusted accordingly. Page 10: line 10/11: This has been said before. RESPONSE: Thank you, the sentence has been removed. Page 10, line 52-53 "across the liver at rest"? RESPONSE: Thank you, this has been clarified. Page 12: heading - Maybe use: "Exercise-regulated hepatokine candidates" RESPONSE: We removed the heading but clarified in the respective paragraphs that the hepatic origin of these factors has to be clarified. Page 12, line 26-29 RESPONSE: Thank you, a paragraph has been inserted. Page 13, line 14-17: In what tissue was these observations obtained? Maybe adjust to "compared with wildtype mice when mice on HFD were exercise trained". Not clear how ROS come into the picture. This seems to require some supportive explanation. RESPONSE: Thank you, we clarified these points. Page 13, in Concluding remarks: A new protein (fetuin-A) is mentioned and the terms "normoglycemic" and "dysglycemic" are introduced here. Why not in the main text? Moreover, is it relevant to include a reference in "concluding remarks? Could this instead be given in the main text. RESPONSE: Thank you, this has been removed from the conclusion paragraph as circulating fetuin A is not regulated by exercise. 3) There are in some of the paragraphs many VERY short sentences after each other. It is suggested to consider to link or merge some of these sentences to make the text more fluent. Page 6, FGF21 section. Page 7, Follistatin section: RESPONSE: Thank you, excellent point, the short sentences has been changed so the text is less staccato. 4) Table/Figure legends: a) Table 1 legend: please give the time after exercise. "Immediately after exercise"? This is clearly important for acute exercise-induced mRNA responses. RESPONSE: Thank you, this has been clarified in the heading and legend of table1. b) Figure 1 legend, line 40: "exercise induces…" changed to "exercise increases.." "but reduces production of ROS". Has this been shown or is it speculative? Is that clear from this legend? RESPONSE: This has now been clarified in the paragraph about SeP and the legend.

	c) Figure 2 legend, line 37/38. Should there be a "stop" after "factors" and then new sentence? "This leads to increased transcription of…" Line 40-41: suggesting: "Hepatic energy depletion activated FoxO via activation of AMPK and JNK." RESPONSE: Thank you, this has been clarified in the figure legend.

Title:

Hepatokines – a novel group of exercise factors

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Keywords: Hepatokines, liver, exercise, training, energy metabolism, insulin resistance, type 2 diabetes, FGF21, ANGPTL4, Follistatin, Selenoprotein P, HSP72, IGFBP

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Abstract

Regular physical activity has not only benefits for the exercise capacity of the skeletal muscle performing the contractions, but beneficial effects are conveyed to the whole body affecting various tissues and organs. An extensive search for "exercise factors" mediating these beneficial effects has been ongoing for decades. Particularly, the skeletal muscle tissue has been investigated as source of circulating exercise factors and several myokines are identified. However, other tissues are also impacted by exercise. The liver is interpolated between energy storing and energy utilising tissue and is highly active during exercise maintaining energy homeostasis. Recently a novel group of "organokines" has emerged released from the liver termed hepatokines. Several of these proteins (fibroblast growth factor-21, follistatin, angiopoietin like protein 4, heat shock protein 72, insulin-like growth factor binding protein 1) are increased in the bloodstream during or in the recovery after an exercise bout. In this narrative review we evaluate this novel group of exercise factors focusing on the regulation and potential function in exercise metabolism and adaptations. These hepatokines may convey some of the whole-body beneficial effects that could ameliorate metabolic diseases as obesity or type 2 diabetes.

Introduction

Health benefits of exercise are for a long time well-acknowledged and regular physical activity is recommended as therapeutic intervention to prevent and treat chronic disorders including obesity, insulin resistance and type 2 diabetes [95]. Exercise initiates several transcriptional and (post)translational mechanisms in skeletal muscle that increase the capacity and efficiency to utilize and supply fuels with beneficial consequences for whole body energy metabolism [26]. However, several tissues respond to physical activity and exercise performance relies on the orchestration of specific functions tissues bringing the concept of circulating exercise factors into play which mediate the cross-talk. Various tissues produce signalling molecules to regulate energy metabolism and provide the molecular communication between energy storing and energy utilizing tissues. With the discovery of leptin [134] as a signalling molecule secreted by the adipose tissue the term "adipokine" was coined. Transferring the concept of adipokines to the energy utilizing skeletal muscle tissue, interleukin (IL)-6 was the first muscle-derived signalling molecule to be termed a myokine [33], even though that myostatin (growth differentiation factor (GDF)-8) had been classified as a muscle-derived hormone years before [81]. Since then, several endocrine functions have been attributed to the myokines of the working muscle including regulation of body weight, insulin sensitivity, but also tumour suppression and maintenance of cognitive functions [52]. The liver is a central organ in energy metabolism interpolated between energy storage and utilisation and as such maintaining energy homeostasis challenged both during physiological (exercise, fasting, food intake) and pathophysiological (metabolic syndrome, diabetes, obesity, cachexia) conditions. During the last decade hepatokines have emerged as signalling molecules exclusively or predominantly produced in the liver and secreted to peripheral organs [8]. They are preferentially studied in the context of metabolic diseases [108]. Prominent examples are fetuin-A (also known as α-2-HS-glycoprotein) and selenoprotein P (SeP), which are linked to insulin resistance [107,85]. In contrast fibroblast growth factor (FGF)21 has the potency to improve glucose homeostasis and dyslipidemia [62]. In the present narrative review, we summaries and discuss hepatokines in relation to acute exercise as well as physical training and their potential role in energy homeostasis and metabolic adaptations.

The role of the liver in exercise metabolism

The liver plays a pivotal part in exercise metabolism when the energy balance becomes negative and substrates need to be mobilised from the storage or synthesised. Hepatic glucose production is increased via breakdown of glycogen and enhanced de novo synthesis of glucose from lactate, glycerol, and amino acids. The relative contribution of the hepato-splanchnic bed to whole body uptake of free fatty acids (FFA) decreases during exercise, but the absolute uptake increases and FFA are oxidized to provide energy for ATP-consuming biochemical processes [125]. After prolonged exercise, excess FFA are also re-esterified to triglycerides and elevated hepatic triglyceride content can be observed [54,13]. A major regulator for hepatic metabolism during exercise is a change in the glucagon-to-insulin ratio which increases due to both an increase in glucagon and a decrease in insulin. Exercise studies in rodents demonstrate a fall in hepatic energy charge with increased production of AMP which is considered to trigger glycogen mobilisation [117] and to stimulate oxidation of fatty acids via activation of AMPK. The increase in circulating catecholamines in particular during high intensity exercise has been linked to stimulation of hepatic glucose output [39] but hepatic adrenergic blockade in dogs did not reduce glucose delivery from liver [23], thus the contribution of the adrenergic system is not completely clear. Growth hormone (GH) and cortisol are both elevated by exercise and may partially contribute to the adaptation of hepatic metabolism [94]. The increased metabolic activity of the liver during exercise is reflected by a higher hepato-splanchnic oxygen uptake. During moderate exercise hepatic blood flow is unchanged or only slightly reduced [32,31] and hepato-splanchnic oxygen uptake is upregulated more than two-fold [1], presumably due to high levels of oxygen extraction [112]. Of note, exercising with very high intensity can markedly reduce hepato-splanchnic blood flow [96,87], which may compromise the hepatic metabolism [87].

Searching for potential exercise-regulated hepatokines – candidates from mouse microarray data

Upregulation of gene expression is one mechanistic pathway which can lead to increased production and release of adipokines, hepatokines, and myokines. One bout of acute exercise increases the transcript and protein level of several myokines in the contracting muscle [98,19]. Compared with skeletal muscle, even more transcripts in the liver of mice showed pronounced alterations immediately after a non-exhaustive treadmill exercise [51,50]. Filtering these transcripts for genes encoding potentially secreted proteins reveals 55 up-regulated genes after exercise and 29 genes with reduced abundance (Table 1). The list of regulated transcripts is dominated by cytokines, chemokines,

and components of the extracellular matrix. Insulin-like growth factor binding protein (IGFBP1) shows the highest fold change with significant signal intensities in sedentary mice. FGF21 is also strongly increased by acute exercise while almost undetectable in livers of sedentary mice. As described below, the exercise-dependent regulation and secretion of both factors was validated in mice and humans. Further hepatic transcripts regulated by acute exercise in our transcriptomics study, which are already known as liver-derived secreted factors are: follistatin, angiopoietin like 4 protein (ANGPTL4) and inhibin E. Several of the transcripts shown in Table 1 may contribute to changes in the systemic concentration and may be involved in intrahepatic adaptations to exercise, but will not be discussed further due to their wide tissue distribution. Moreover, a transcriptomics approach cannot cover all exercise-regulated hepatokines, since increased release of proteins in response to physical activity must not necessarily be reflected by elevated transcript levels but can be regulated on a (post)translational level or by enhanced secretion of existing protein pools as reported for myokines IL6 and secreted protein acidic and rich in cysteine (SPARC) [3,102]. In the next paragraphs, we will focus on hepatokines with evidence for transcriptional regulation by exercise and hepatic release in to the bloodstream.

Of note, elevated protein abundance in hepatic tissue accompanied by increased plasma concentration is a good hint for an actual release from the liver into the circulation, while best evidence in humans can be achieved as net efflux from the hepato-splanchnic bed analysed as arterial-to-hepatic vein difference. This release from the hepato-splanchnic bed has been reported for FGF21, follistatin, ANGPTL4, and HSP72 [43,47,57,31] and for retinol binding protein 4 and IGFBP1 independent from physical activity [8,16].

Hepatokines in humans:

FGF21

Fibroblast growth factor 21 has emerged as a novel metabolic regulator [63], which is preferentially expressed in the liver [88]. The hepatic origin of circulating FGF21 has elegantly been demonstrated in mice, where knocking-out FGF21 specifically in the liver, results in the absence of circulating FGF21 [80]. In humans, FGF21 is released from the hepato-splanchnic bed both after an over-night fast and during an acute bout of exercise [43]. Thus, evidence supports that FGF21 is a liver-derived factor both in mice and man. In humans, circulating FGF21 is increased after a prolonged fast [35,29],

exercise [65], in type 2 diabetes [20], steatosis [25] non-alcoholic steatohepatitis [25], obesity [25] as well as critical illness [115]. Several mechanisms have been suggested for the regulation. *In vitro* and *in vivo* in mice PPARα activation leads to increased FGF21 expression [56,7]. In humans, treatment with PPAR agonists for 2 weeks only PPARα and β, but not γ agonists increases circulating FGF21 [21]. Increasing FFA for 4 hours in humans also give rise to an increase in plasma FGF21 [79], which is in line with the PPAR mediated FGF21 regulation. The increase of FGF21 in mice and man after PPAR activation or FFA infusion for 4 hours is ~ 1.3-fold [21,79]. The observation that FGF21 increases rapidly after an acute bout of exercise with a higher magnitude (exercise: 2-3-fold versus 1.3-fold with PPAR or FFA activation) suggests that other regulatory mechanisms may exist. The most powerful hormonal signal to induce an increase in the circulating FGF21 is glucagon, where a 2.5-fold increase can be demonstrated [4]. Intriguingly insulin has an inhibitory effect and inducing an increased glucagon-to-insulin ratio in healthy males increases FGF21 with a similar magnitude and kinetic as during exercise [43]. Thus, the glucagon-to-insulin ratio is much more powerful in stimulating hepatic FGF21 secretion than PPAR activation either pharmacologically or by FFA. An increase in the glucagon-to-insulin ratio is a necessary stimulus to increase exercise-induced FGF21, as prevention of an increase in the glucagon-to-insulin ratio during an exercise bout also blunts the exercise-induced increase in FGF21 [44]. An impaired exercise-induced response of FGF21 has been reported both with obesity [105] and type 2 diabetes [44], however Sargeant et al. could not detect an impairment of exercise-induced FGF21 by obesity when compared to a non-obese control group [104]. Slusher et al. studied obesity with insulin resistance, whereas Sargeant et al. studied obesity without insulin resistance. Although conjectural, insulin resistance seems to be the factor impairing the exercise-induced FGF21. This is in line with aforementioned conditions as type 2 diabetes, obesity, prolonged fasting, exercise and critical illness, which all are characterized by reduced or impaired insulin action often in combination with hyperglucagonemia as recently reviewed [46]. The relation of FGF21 to metabolism is not only reflected by its regulation, but indeed in its actions (Figure 1). FGF21 administered to mice [63] and humans [34] has beneficial effects on energy metabolism. Insulin resistant mice treated with FGF21 lost body weight and reduced blood glucose concentrations [63]. Similarly patients with type 2 diabetes treated with a FGF21 analogue experienced a weight loss and reduced fasting glucose levels after 2 weeks of treatment [34]. A recent publication using adenoviral gene transfer to the liver of obese mice demonstrated reduced weight gain, adipose tissue hypertrophy, hepatosteatosis, and inflammation [58]. Moreover, this FGF21 gene therapy prevented insulin resistance associated with aging. The bone loss reported due to FGF21

over-expression [122] was not observed in this study. Liver, adipose tissue, and brain are considered to be the important sites of FGF21 action. In particular rodent studies demonstrate increased fatty acid oxidation and decreased lipogenesis in the liver [56,7] and increased glucose uptake in adipose tissue an effect mainly mediated by brown adipocytes [93]. Human studies support a central regulation of food intake and reduction in sugar consumption by FGF21 [119,106] and increased production of adiponectin [34,113]. As FGF21 holds strong promise as a therapeutic target, exercise-induced FGF21 could be one of the molecular links that mediates the beneficial effects of exercise to the whole-body level. This is supported by impaired exercise adaptations in FGF21-deficient mice. These mice failed to improve glucose tolerance and reduce hepatic triglyceride content compared to wildtype mice under the same high-fat diet [78].

Follistatin

The liver has recently been identified as the source of circulating follistatin [47]. During an acute bout of exercise circulating follistatin is increased 5-7-fold particularly in the hours after the exercise bout [41,60,47,104]. Examining various tissue in mice revealed that the liver exerted a markedly increase in follistatin mRNA expression immediately after an acute exercise bout [41]. In humans follistatin is release from the hepato-splanchnic bed in healthy males at rest and in the recovery after an exercise bout [47]. Collectively these data strongly suggest that the hepato-splanchnic bed (e.g. the liver) is the organ responsible for circulating levels of follistatin. Follistatin has primarily been investigated as a TGF-β modulating protein, due to its inhibition of activins and GDFs. The current perception is that circulating follistatin is a result of spill–over from auto- or paracrine processes. However, follistatin increases with prolonged fast [118], pregnancy [90], type 2 diabetes [42] and critical illness [83,84]. A mutual feature of these conditions is an increase in the glucagon-to-insulin ratio as previously summarized [45]. Examining the regulation of exercise-induced follistatin revealed that the glucagon-to-insulin ratio is important for the increase in follistatin after exercise, but other stimuli must exist as blunting the glucagon-to-insulin ratio during an exercise reduced exercise-induced follistatin by 50% [44]. Thus, the regulation of circulating follistatin is linked to energy metabolism. Exercise-induced follistatin is impaired by insulin resistance [44] but not by obesity per se [104]. Follistatin is a pluripotent molecule and several target tissues has been suggested (Figure 1). Firstly, follistatin acts on the endocrine pancreas modulating both insulin and glucagon secretion, suggesting a feedback regulation [47]. Secondly, follistatin may have a role in regulating muscle hypertrophy

where follistatin acts in concert with myostatin [38], insulin and IGF [10] as well as testosterone [15]. *In vivo* follistatin treatment induces a hypertrophic muscle in ducks [77], mice [36] as well as non-human primates [68]. This has led to testing follistatin gene therapy in a phase 1/2a study including patients with Beckers muscle dystrophy and improvement was observed [82]. Thirdly, a recent study demonstrated that dysregulated chronic activation of FoxO1 drives hepatic expression of follistatin which leads to impaired insulin sensitivity in adipose tissue, potentiation of hepatic glucose production and severe glucose intolerance in mice [114,74]. Even though highly speculative, this insulin unresponsiveness of lipolysis in adipose tissue and unsuppressed hepatic glucose production might be of advantage during endurance exercise and the early recovery phase. To conclude, more studies are needed to understand the physiological role of follistatin as exercise-regulated hepatokine. But follistatin is a pluripotent molecule that could function as communication from the liver to the endocrine pancreas, skeletal muscle or adipose tissue acting as a link between energy metabolism and TGF-β regulated growth and differentiation.

Angiopoietin-like protein 4

Like follistatin, plasma angiopoietin-like protein 4 (ANGPTL4) increases during exercise and in the recovery phase in healthy subjects [89]. ANGPTL4 was identified in mice as "fasting-induced adipose factor" in both adipose tissue [61] and liver [64]. In humans, ANGPTL4 is highly expressed in liver followed by the pericardium, whereas adipose and muscle tissue expression is low [101]. In mouse liver ANGPTL4 increases acutely at the mRNA level after a bout of exercise [89] and fasting [61]. Interestingly ANGPTL4 is also suggested to be an exercise-induced myokine [18,89]. However, when ANGPTL4 release is measured by arterial-to-venous differences over both a resting and an exercising leg no contribution of ANGPTL4 to the systemic circulation can be detected [57]. In contrast, a release of ANGPTL4 can be detected from the hepato-splanchnic bed during exercise with, no release at rest [57]. Thus, in humans exercise-induced ANGPTL 4 is released in to the circulation via the hepato-splanchnic bed from the liver; however during rest other tissues contribute to the systemic ANGPTL4 level. Induction of ANGPTL4 in adipose tissue during fasting is ascribed to PPARα activation [61]. Other PPARs were also found to have regulatory properties on ANGPTL4 expression [2] which is supported by promoter analysis [130]. This finding was in line with ANGPTL4 being a fasting-induced protein as free fatty acids increases during fasting [109] who acts as a ligand for the PPARs. Fasting and exercise has a common denominator: the increase glucagon-

to-insulin ratio. Indeed, an experimentally induced increase in the glucagon-to-insulin ratio in healthy subjects resting in bed increases systemic levels of ANGPTL4. Furthermore, blunting the increase in the glucagon-to-insulin ratio in healthy males during a bout of bicycle exercise abolishes the exercise-induced ANGPTL4 increase [57]. Taken together these *in vivo* observations in humans demonstrate that the glucagon-to-insulin ratio is pivotal in the regulation of exercise-induced ANGPTL4. The function of ANGPTL4 has been studied *in vivo* and *vitro*, where it acts as an inhibitor of lipoprotein lipase (LPL) [131] (Figure 1). LPL mediates the degradation of triglycerides in lipoproteins into FFA [14] in various tissues and is increased in skeletal muscle tissue by fasting [97]. In line with the observation that ANGPTL4 is an inhibitor of LPL activity, mice lacking ANGPTL4 have a low level of plasma triglyceride [67] whereas mice over-expressing ANGPTL4 have increased levels of circulating triglycerides [131,67]. Human population based studies have revealed that loss-of-function mutations in ANGPTL4 are associated with low levels of triglycerides [101,100]. As ANGPTL4 is an inhibitor of LPL, is seems paradoxical that ANGPTL4 increases in the exact conditions where an increased breakdown of triglyceride is important as it is the case during exercise and fasting. By tissue-specific overexpression models, ANGPTL4 has also been suggested to play a role in lipid partitioning, tissue-specific uptake or release of FFA [132,111], and to improve glucose metabolism [126]. In contrast, a recent study links the genetic inactivation or loss of ANGPTL4 to reduced risk of type 2 diabetes and improvement in glucose homeostasis [40]. An additional function attributed to ANGPTL4 is supporting angiogenesis [6], while recent studies on the regulation of pancreatic α-cells are inconsistent [11,92]. ANGPTL4-induced inhibition of LPL activity is due to direct protein-protein-interaction [111,24], and it is unclear whether an ANGPTL4 receptor exists to mediate other effects related to this protein. Nevertheless, the acute regulation in plasma could suggests an endocrine role, as a liver-derived signal to the peripheral tissues.

Regulation of FGF21, follistatin, and ANGPTL4

An increase in the glucagon-to-insulin ratio regulates FGF21, follistatin and ANGPTL4 plasma concentration *in vivo* in humans [43,47,57]. Stimulation of hepatocytes with glucagon activates the adenylyl cyclase and intracellular cAMP increases [48], while cAMP levels are reduced by insulin via activation of phosphodiesterase (PDE) [48]. Thus, the glucagon-to-insulin ratio sensed by the liver is determining cAMP levels within the hepatocyte. Both glucagon and adrenalin stimulate the adenylyl cyclase, however during exercise glucagon is the determinant of cAMP levels [124]. FoxO

transcription factors appear to play an important role in the enhanced transcription of FGF21, follistatin and ANGPTL4. Exercise does not only induce hepatic cAMP levels, but also activates AMPK and JNK in the liver [17,50] and all these pathways activate FoxO [27]. FoxO1 is an important trigger for hepatic follistatin expression and stimulates ANGPTL4 expression, whereas the inhibitory effect of insulin is mediated by the PI3K/PKB pathway which leads to inactivation of FoxO1 [114]. Exercise also increases lipolysis and the availability of fatty acids which can lead to activation of hepatic PPAR transcription factors. PPARs are established regulators of FGF21 and ANGPTL4 expression, but the contribution of this pathway to exercise-dependent hepatokine production is not fully clarified. Prevention of the increase in the glucagon-to-insulin ratio by somatostatin infusion during exercise also blocked the increase in plasma FFA [44], thus a contribution of elevated FFA to the increase in hepatokine cannot be excluded. Interestingly, elevation of cAMP levels in hepatocytes directly increases ANGPTL4 mRNA levels [57] and the cAMP/PKA pathway can regulate the transcriptional activity of PPARs [71]. Collectively a molecular pathway exists for hepatokine production where secretion is stimulated via cAMP and inhibition occurs via insulin-regulated enzymes PDE and PKB, as summarised in Figure 2. This regulation by the glucagon-to-insulin ratio suggests that systemic levels of these hepatokines are influenced by hepatic insulin resistance and reduction in insulin action can result in less inhibition of hepatokine secretion. Intriguingly, elevated circulating levels of FGF21 [20], ANGPTL4 [116] and follistatin [42] have been demonstrated in patients with type 2 diabetes.

Heat Shock Protein 72

In humans heat shock protein (HSP) 72 increases in the circulation during an acute bout of exercise [121]. Skeletal muscle, liver and brain have been suggested to be the tissues responsible for exercise-induced HSP72 release [103,69]. Using arterial-to-venous differences over the leg and hepato-splanchnic bed during exercise in healthy humans, a release of HSP72 during exercise from the hepato-splanchnic bed could be detected; however no release could be detected over the leg or at rest [31]. In addition, the brain releases HSP72 to the circulation during exercise [69]. Taken together HSP72 is an exercise-induced hepatokine, however contributions from other tissues add to the increase in HSP72 observed with exercise. The regulation of HSP72 during exercise has not been

completely elucidated. As suggested by the name "heat" has been investigated as a possible signal, however heat per se during exercise in humans only influenced exercise-induced HSP72 to a minor degree [123]. In rats stress-induced circulating HSP72 is not affected by hypophysectomy or adrenalectomy, which rules out growth hormone, epinephrine and cortisol as the stimulatory signal [59]. However, phenylephrine increases plasma HSP72 and blocking α1-adrenergic receptor by prazosin blunts stress-induced HSP72 whereas a β-selective antagonist (propranolol) has no effect, which collectively suggests that norepinephrine is a stimulating signal [59]. Interestingly, ingestion of glucose during exercise completely blunts hepato-splanchnic release of HSP72 during exercise, suggesting a role for the glucoregulatory hormones insulin and glucagon [30], however no regulation of liver HSP72 mRNA is observed with 48h fast in rats [91]. Thus, the cross-talk between the adrenergic and glucose-induced hormones needs to be elucidated. The role of HSP72 seems to be multiple and related to whether it is located intra- or extracellular [128]. Overexpression of HSP72 protects against heatstroke [73] which is in line with a muscle-specific overexpression protecting against exercise-induced skeletal muscle damage [76] and preserving muscle function [37] (Figure 1). Interestingly, overexpression of HSP72 in cardiac and skeletal muscle tissue increases exercise performance, oxidative capacity and mitochondrial content and also protects against obesity-induced insulin resistance [22], suggesting a role in energy metabolism. However, the contribution of liver-derived HSP72 to the above-mentioned effects is not validated yet. Since HSP72 also regulates hepatic fatty acid oxidation and maintenance of mitochondrial function in the liver, a paracrine function of exercise-induced HSP72 can be considered as well [5].

IGF-1 and IGFBP1

Both growth hormone (GH) and IGF1 levels increase early during an exercise bout in the circulation [55,9]. During exercise IGF is not released solely from the liver as a contribution from the exercising leg has been demonstrated [12]. Plasma IGFBP1 increases during prolonged exercise in humans [120] and hepatic IGFBP1 mRNA and protein levels are strongly upregulated during acute exercise in mice (Table 1) [50]. The increase is clearly linked to the hepatic energy state and is regulated by hepatic glycogen depletion [70] and thus dependent on the duration of exercise [50]. FoxO proteins are important regulators of IGFBP1 transcription which can be the link between tissue glycogen content and regulation of IGFBP1 production during exercise (Figure 2) [127]. Circulating IGFBPs regulate the bioavailability of IGF1 but the physiological function of elevated plasma levels of

IGFBP1 during prolonged exercise remains elusive. Exercise also induces intrahepatic IGFBP1 protein accumulation in mice [50] raising the possibility that this increase promotes an anti-apoptotic action of IGFBP1 by interfering with the p53 tumour suppressor protein which is also elevated in the liver after acute exercise [75].

Further candidates of exercise-regulated hepatokines:

Inhibin βE

Inhibin βE is one of the hepatokine candidates with a down-regulation of its mRNA after acute exercise (Table 1). It was recently classified as hepatokine which is associated with insulin resistance [110]. Human liver biopsies from insulin-resistant subjects had increased expression levels of inhibin βE mRNA and knock-down of inhibin βE in an obese mouse model shows improved fatty acid oxidation. The influence of acute exercise and regular physical activity on circulating inhibin βE needs to be shown as a role of reduced levels of this hepatokine in the beneficial effects of exercise on glucose tolerance and insulin sensitivity.

GDF 15

GDF15 has recently been identified to increase in the circulation in response to an acute bout exercise [66]. In relation to exercise, GDF15 was initially thought to be a myokine, but no release could be detected from an exercising leg [66]. Interestingly, GDF15 appears as regulated hepatic transcript in our transcriptomic search for liver-derived exercise factors (Table 1). Thus, GDF15 can be a hepatokine. This notion is strengthened by the observation that GDF15 is strongly induced by fasting in mice [133] which support the idea that energy depletion regulates GDF15. In similarity with FGF21, follistatin and ANGPTL4 increased levels of circulating GDF15 are associated with insulin resistance [53]. The receptor for GDF15 has been identified to be the orphan receptor GFRAL which is expressed in the brain and involved in the regulation of food intake [129,28]. Whether GDF15 is released from the liver needs to be addressed and how it is regulated during an acute bout of exercise deciphered.

A role for the hepatokine selenoprotein P in exercise adaptions

The systemic level of hepatokines may alter the response to exercise even if it is not regulated by physical activity. The plasma concentration of the hepatokine selenoprotein P (SeP) has been linked to the training responsiveness in VO_2max [86] in humans and mice. SeP is produced mainly in liver and functions as selenium transport protein supplying selenium to target cells [49]. Hepatic SeP transcripts were not altered by acute exercise or training in mice and plasma SeP concentrations were unchanged after 8 weeks of aerobic exercise training in humans [86]. However, SeP-deficient mice showed enhanced response in PGC1α levels, mitochondrial content, and exercise endurance compared with wildtype mice when training is combined with high fat diet. In middle-aged humans without diabetes and obesity, pre-training SeP plasma levels associate with the improvement in VO_2max. These data provide evidence for a function of liver-derived SeP as regulator of exercise-induced reactive oxygen species (ROS) production in skeletal muscle (Figure 1). Absence or low concentrations of SeP allow higher ROS levels in response to acute exercise which are considered to trigger mitochondrial adaptations [99].

Concluding remarks

During and particular after an exercise bout the liver is secreting potential exercise factors. The exercise-induced hepatokines are regulated by several stimuli where the glucagon-to-insulin seems in particular to be responsible for FGF21, follistatin, and ANGPTL4. These factors are all elevated with insulin resistance, while the response of FGF21 and follistatin to an acute bout of exercise seems to be impaired in subjects with insulin resistance. Obesity per se does not affect the exercise-induced response of these hepatokines. This conjectures that FGF21 and follistatin may discriminate between obesity and insulin resistant phenotype that level of the liver. Another relevant question is whether regular physical activity alters the concentration of circulating hepatokines thereby contributing to the improvement of metabolic disorders. Long-term training leads to a reduction in fetuin-A levels in both, normoglycemic and dysglycemic subjects, paralleled by a reduction in liver fat and improvement in glucose tolerance [72]. After the same training intervention, no change in ANGPTL4 was observed [89], clearly indicating differences in the regulation of these hepatokines. In conclusion, the liver is not merely an organ receiving humoral stimuli, but is also communicating to extrahepatic tissues as the adipose tissue and skeletal muscle. Research is now at the beginning to understand the

physiological role of circulating hepatokines for exercise adaptations and for the beneficial effect of exercise on metabolic homeostasis.

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References

1. Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J (1974) Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. JClinInvest 53:1080-1090
2. Akiyama TE, Lambert G, Nicol CJ, Matsusue K, Peters JM, Brewer HB, Jr., Gonzalez FJ (2004) Peroxisome proliferator-activated receptor beta/delta regulates very low density lipoprotein production and catabolism in mice on a Western diet. Journal of Biological Chemistry 279:20874-20881
3. Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, Sakuma K, Hang LP, Mizushima K, Hirai Y, Koyama R, Wada S, Higashi A, Kokura S, Ichikawa H, Yoshikawa T (2013) A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 62:882-889. doi:10.1136/gutjnl-2011-300776
4. Arafat AM, Kaczmarek P, Skrzypski M, Pruszynska-Oszmalek E, Kolodziejski P, Szczepankiewicz D, Sassek M, Wojciechowicz T, Wiedenmann B, Pfeiffer AF, Nowak KW, Strowski MZ (2013) Glucagon increases circulating fibroblast growth factor 21 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated lipolysis? Diabetologia 56:588-597
5. Archer AE, Rogers RS, Von Schulze AT, Wheatley JL, Morris EM, McCoin CS, Thyfault JP, Geiger PC (2018) Heat Shock Protein 72 Regulates Hepatic Lipid Accumulation. American journal of physiology Regulatory, integrative and comparative physiology. doi:10.1152/ajpregu.00073.2018
6. Babapoor-Farrokhran S, Jee K, Puchner B, Hassan SJ, Xin X, Rodrigues M, Kashiwabuchi F, Ma T, Hu K, Deshpande M, Daoud Y, Solomon S, Wenick A, Lutty GA, Semenza GL, Montaner S, Sodhi A (2015) Angiopoietin-like 4 is a potent angiogenic factor and a novel therapeutic target for patients with proliferative diabetic retinopathy. Proceedings of the National Academy of Sciences of the United States of America 112:E3030-3039. doi:10.1073/pnas.1423765112
7. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E (2007) Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 5:426-437
8. Bahr MJ, Boeker KH, Manns MP, Tietge UJ (2009) Decreased hepatic RBP4 secretion is correlated with reduced hepatic glucose production but is not associated with insulin resistance in patients with liver cirrhosis. ClinEndocrinol(Oxf) 70:60-65
9. Bang P, Brandt J, Degerblad M, Enberg G, Kaijser L, Thoren M, Hall K (1990) Exercise-induced changes in insulin-like growth factors and their low molecular weight binding protein in healthy subjects and patients with growth hormone deficiency. European journal of clinical investigation 20:285-292
10. Barbe C, Kalista S, Loumaye A, Ritvos O, Lause P, Ferracin B, Thissen JP (2015) Role of IGF-I in the Follistatin-induced skeletal muscle hypertrophy. AmJPhysiol EndocrinolMetab:ajpendo
11. Ben-Zvi D, Barrandon O, Hadley S, Blum B, Peterson QP, Melton DA (2015) Angptl4 links alpha-cell proliferation following glucagon receptor inhibition with adipose tissue triglyceride metabolism. ProcNatlAcadSciUSA 112:15498-15503
12. Berg U, Bang P (2004) Exercise and circulating insulin-like growth factor I. Hormone research 62 Suppl 1:50-58. doi:10.1159/000080759
13. Bilet L, Brouwers B, van Ewijk PA, Hesselink MK, Kooi ME, Schrauwen P, Schrauwen-Hinderling VB (2015) Acute exercise does not decrease liver fat in men with overweight or NAFLD. Sci Rep 5:9709. doi:10.1038/srep09709
14. Blanchette-Mackie EJ, Scow RO (1973) Effects of lipoprotein lipase on the structure of chylomicrons. JCell Biol 58:689-708
15. Braga M, Bhasin S, Jasuja R, Pervin S, Singh R (2012) Testosterone inhibits transforming growth factor-beta signaling during myogenic differentiation and proliferation of mouse satellite cells: potential role of follistatin in mediating testosterone action. MolCell Endocrinol 350:39-52

16. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K (1994) Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab 79:872-878. doi:10.1210/jcem.79.3.7521354
17. Camacho RC, Donahue EP, James FD, Berglund ED, Wasserman DH (2006) Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. Am J Physiol Endocrinol Metab 290:E405-408. doi:10.1152/ajpendo.00385.2005
18. Catoire M, Alex S, Paraskevopulos N, Mattijssen F, Evers-van GI, Schaart G, Jeppesen J, Kneppers A, Mensink M, Voshol PJ, Olivecrona G, Tan NS, Hesselink MK, Berbee JF, Rensen PC, Kalkhoven E, Schrauwen P, Kersten S (2014) Fatty acid-inducible ANGPTL4 governs lipid metabolic response to exercise. ProcNatlAcadSciUSA 111:E1043-E1052
19. Catoire M, Mensink M, Kalkhoven E, Schrauwen P, Kersten S (2014) Identification of human exercise-induced myokines using secretome analysis. Physiol Genomics
20. Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H, Boden G (2008) Circulating FGF-21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. ExpClinEndocrinolDiabetes 116:65-68
21. Christodoulides C, Dyson P, Sprecher D, Tsintzas K, Karpe F (2009) Circulating fibroblast growth factor 21 is induced by peroxisome proliferator-activated receptor agonists but not ketosis in man. JClinEndocrinolMetab 94:3594-3601
22. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA (2008) HSP72 protects against obesity-induced insulin resistance. ProcNatlAcadSciUSA 105:1739-1744
23. Coker RH, Krishna MG, Lacy DB, Bracy DP, Wasserman DH (1997) Role of hepatic alpha- and beta-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol 273:E831-838
24. Dijk W, Beigneux AP, Larsson M, Bensadoun A, Young SG, Kersten S (2016) Angiopoietin-like 4 promotes intracellular degradation of lipoprotein lipase in adipocytes. JLipid Res 57:1670-1683
25. Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, Badman MK, Martinez-Chantar ML, Maratos-Flier E (2010) Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456-463
26. Egan B, Zierath JR (2013) Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. Cell Metabolism 17:162-184. doi:10.1016/j.cmet.2012.12.012
27. Eijkelenboom A, Burgering BM (2013) FOXOs: signalling integrators for homeostasis maintenance. Nature reviews Molecular cell biology 14:83-97. doi:10.1038/nrm3507
28. Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, Coskun T, Hamang MJ, Sindelar DK, Ballman KK, Foltz LA, Muppidi A, Alsina-Fernandez J, Barnard GC, Tang JX, Liu X, Mao X, Siegel R, Sloan JH, Mitchell PJ, Zhang BB, Gimeno RE, Shan B, Wu X (2017) The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nature medicine 23:1215-1219. doi:10.1038/nm.4393
29. Fazeli PK, Lun M, Kim SM, Bredella MA, Wright S, Zhang Y, Lee H, Catana C, Klibanski A, Patwari P, Steinhauser ML (2015) FGF21 and the late adaptive response to starvation in humans. J Clin Invest 125:4601-4611. doi:10.1172/JCI83349
30. Febbraio MA, Mesa JL, Chung J, Steensberg A, Keller C, Nielsen HB, Krustrup P, Ott P, Secher NH, Pedersen BK (2004) Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. Cell StressChaperones 9:390-396
31. Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK (2002) Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. The Journal of Physiology 544:957-962. doi:10.1113/jphysiol.2002.025148
32. Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK (2003) Hepatosplanchnic clearance of interleukin-6 in humans during exercise. AmJPhysiol EndocrinolMetab 285:E397-E402

33. Febbraio MA, Pedersen BK (2005) Contraction,Induced myokine production and release: Is skeletal muscle an endocrine organ.? Exercise and Sport Sciences Reviews 33:114-119. doi:10.1097/00003677-200507000-00003
34. Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333-340
35. Galman C, Lundasen T, Kharitonenkov A, Bina HA, Eriksson M, Hafstrom I, Dahlin M, Amark P, Angelin B, Rudling M (2008) The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. Cell Metab 8:169-174
36. Gangopadhyay SS (2013) Systemic administration of follistatin288 increases muscle mass and reduces fat accumulation in mice. SciRep 3:2441
37. Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA, Lynch GS (2012) Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature 484:394-398
38. Gilson H, Schakman O, Kalista S, Lause P, Tsuchida K, Thissen JP (2009) Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. AmJPhysiol EndocrinolMetab 297:E157-E164
39. Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJ (2016) Liver glycogen metabolism during and after prolonged endurance-type exercise. Am J Physiol Endocrinol Metab 311:E543-553. doi:10.1152/ajpendo.00232.2016
40. Gusarova V, O'Dushlaine C, Teslovich TM, Benotti PN, Mirshahi T, Gottesman O, Van Hout CV, Murray MF, Mahajan A, Nielsen JB, Fritsche L, Wulff AB, Gudbjartsson DF, Sjogren M, Emdin CA, Scott RA, Lee WJ, Small A, Kwee LC, Dwivedi OP, Prasad RB, Bruse S, Lopez AE, Penn J, Marcketta A, Leader JB, Still CD, Kirchner HL, Mirshahi UL, Wardeh AH, Hartle CM, Habegger L, Fetterolf SN, Tusie-Luna T, Morris AP, Holm H, Steinthorsdottir V, Sulem P, Thorsteinsdottir U, Rotter JI, Chuang LM, Damrauer S, Birtwell D, Brummett CM, Khera AV, Natarajan P, Orho-Melander M, Flannick J, Lotta LA, Willer CJ, Holmen OL, Ritchie MD, Ledbetter DH, Murphy AJ, Borecki IB, Reid JG, Overton JD, Hansson O, Groop L, Shah SH, Kraus WE, Rader DJ, Chen YI, Hveem K, Wareham NJ, Kathiresan S, Melander O, Stefansson K, Nordestgaard BG, Tybjaerg-Hansen A, Abecasis GR, Altshuler D, Florez JC, Boehnke M, McCarthy MI, Yancopoulos GD, Carey DJ, Shuldiner AR, Baras A, Dewey FE, Gromada J (2018) Genetic inactivation of ANGPTL4 improves glucose homeostasis and is associated with reduced risk of diabetes. Nat Commun 9:2252. doi:10.1038/s41467-018-04611-z
41. Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, Pedersen BK, Plomgaard P (2010) Exercise induces a marked increase in plasma follistatin: Evidence that follistatin is a contraction-induced hepatokine. Endocrinology 152:164-171
42. Hansen J, Rinnov A, Krogh-Madsen R, Fischer CP, Andreasen AS, Berg RM, Moller K, Pedersen BK, Plomgaard P (2013) Plasma follistatin is elevated in patients with type 2 diabetes: relationship to hyperglycemia, hyperinsulinemia, and systemic low-grade inflammation. Diabetes Metab ResRev 29:463-472
43. Hansen JS, Clemmesen JO, Secher NH, Hoene M, Drescher A, Weigert C, Pedersen BK, Plomgaard P (2015) Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans. MolMetab 4:551-560
44. Hansen JS, Pedersen BK, Xu G, Lehmann R, Weigert C, Plomgaard P (2016) Exercise-induced secretion of FGF21 and follistatin are blocked by pancreatic clamp and impaired in type 2 diabetes. JClinEndocrinolMetab 101:2816-2825
45. Hansen JS, Plomgaard P (2016) Circulating follistatin in relation to energy metabolism. MolCell Endocrinol 433:87-93
46. Hansen JS, Plomgaard P (2016) Fibroblast growth factor 21: new insights from human studies. Cardiovascular Endocrinology 5:112-116. doi:10.1097/xce.0000000000000084

47. Hansen JS, Rutti S, Arous C, Clemmesen JO, Secher NH, Drescher A, Gonelle-Gisport C, Halban PA, Pedersen BK, Weigert C, Bouzakri K, Plomgaard P (2015) Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. JClinEndocrinolMetab 101:550-560
48. Heyworth CM, Wallace AV, Houslay MD (1983) Insulin and glucagon regulate the activation of two distinct membrane-bound cyclic AMP phosphodiesterases in hepatocytes. BiochemJ 214:99-110
49. Hill KE, Wu S, Motley AK, Stevenson TD, Winfrey VP, Capecchi MR, Atkins JF, Burk RF (2012) Production of selenoprotein P (Sepp1) by hepatocytes is central to selenium homeostasis. J Biol Chem 287:40414-40424. doi:10.1074/jbc.M112.421404
50. Hoene M, Franken H, Fritsche L, Lehmann R, Pohl AK, Haring HU, Zell A, Schleicher ED, Weigert C (2010) Activation of the mitogen-activated protein kinase (MAPK) signalling pathway in the liver of mice is related to plasma glucose levels after acute exercise. Diabetologia 53:1131-1141. doi:10.1007/s00125-010-1666-3
51. Hoene M, Weigert C (2010) The stress response of the liver to physical exercise. ExercImmunolRev 16:163-183
52. Hoffmann C, Weigert C (2017) Skeletal Muscle as an Endocrine Organ: The Role of Myokines in Exercise Adaptations. Cold Spring Harbor perspectives in medicine 7. doi:10.1101/cshperspect.a029793
53. Hong JH, Chung HK, Park HY, Joung KH, Lee JH, Jung JG, Kim KS, Kim HJ, Ku BJ, Shong M (2014) GDF15 Is a Novel Biomarker for Impaired Fasting Glucose. Diabetes & metabolism journal 38:472-479. doi:10.4093/dmj.2014.38.6.472
54. Hu C, Hoene M, Zhao X, Haring HU, Schleicher E, Lehmann R, Han X, Xu G, Weigert C (2010) Lipidomics analysis reveals efficient storage of hepatic triacylglycerides enriched in unsaturated fatty acids after one bout of exercise in mice. PLoSONE 5:e13318
55. Hunter WM, Fonseka CC, Passmore R (1965) Growth hormone: important role in muscular exercise in adults. Science 150:1051-1053
56. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA (2007) Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 5:415-425. doi:10.1016/j.cmet.2007.05.003
57. Ingerslev B, Hansen JS, Hoffmann C, Clemmesen JO, Secher NH, Scheler M, Hrabe de Angelis M, Haring HU, Pedersen BK, Weigert C, Plomgaard P (2017) Angiopoietin-like protein 4 is an exercise-induced hepatokine in humans, regulated by glucagon and cAMP. Mol Metab 6:1286-1295. doi:10.1016/j.molmet.2017.06.018
58. Jimenez V, Jambrina C, Casana E, Sacristan V, Munoz S, Darriba S, Rodo J, Mallol C, Garcia M, Leon X, Marco S, Ribera A, Elias I, Casellas A, Grass I, Elias G, Ferre T, Motas S, Franckhauser S, Mulero F, Navarro M, Haurigot V, Ruberte J, Bosch F (2018) FGF21 gene therapy as treatment for obesity and insulin resistance. EMBO molecular medicine. doi:10.15252/emmm.201708791
59. Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Fleshner M (2005) Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. JApplPhysiol (1985) 99:1789-1795
60. Kerschan-Schindl K, Thalmann MM, Weiss E, Tsironi M, Foger-Samwald U, Meinhart J, Skenderi K, Pietschmann P (2015) Changes in serum levels of myokines and wnt-antagonists after an ultramarathon race. PLoSONE 10:e0132478
61. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. Journal of Biological Chemistry 275:28488-28493
62. Kharitonenkov A, DiMarchi R (2015) FGF21 Revolutions: Recent Advances Illuminating FGF21 Biology and Medicinal Properties. Trends in endocrinology and metabolism: TEM 26:608-617. doi:10.1016/j.tem.2015.09.007
63. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB (2005) FGF-21 as a novel metabolic regulator. JClinInvest 115:1627-1635

64. Kim I, Kim HG, Kim H, Kim HH, Park SK, Uhm CS, Lee ZH, Koh GY (2000) Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. BiochemJ 346 Pt 3:603-610
65. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS (2013) Acute exercise induces FGF21 expression in mice and in healthy humans. PLoSONE 8:e63517
66. Kleinert M, Clemmensen C, Sjoberg KA, Carl CS, Jeppesen JF, Wojtaszewski JFP, Kiens B, Richter EA (2018) Exercise increases circulating GDF15 in humans. Mol Metab 9:187-191. doi:10.1016/j.molmet.2017.12.016
67. Koster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC, Yang DD, Heuer JG, Jaskunas SR, Eacho P (2005) Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. Endocrinology 146:4943-4950
68. Kota J, Handy CR, Haidet AM, Montgomery CL, Eagle A, Rodino-Klapac LR, Tucker D, Shilling CJ, Therlfall WR, Walker CM, Weisbrode SE, Janssen PM, Clark KR, Sahenk Z, Mendell JR, Kaspar BK (2009) Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. SciTranslMed 1:6ra15
69. Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L (2004) Exercise induces the release of heat shock protein 72 from the human brain in vivo. Cell StressChaperones 9:276-280
70. Lavoie JM, Fillion Y, Couturier K, Corriveau P (2002) Exercise Effects on Muscle Insulin Signaling and Action - Selected Contribution: Evidence that the decrease in liver glycogen is associated with the exercise-induced increase in IGFBP-1. Journal of Applied Physiology 93:798-804. doi:10.1152/japplphysiol.00125.2002
71. Lazennec G, Canaple L, Saugy D, Wahli W (2000) Activation of peroxisome proliferator-activated receptors (PPARs) by their ligands and protein kinase A activators. MolEndocrinol 14:1962-1975
72. Lee S, Norheim F, Gulseth HL, Langleite TM, Kolnes KJ, Tangen DS, Stadheim HK, Gilfillan GD, Holen T, Birkeland KI, Jensen J, Drevon CA (2017) Interaction between plasma fetuin-A and free fatty acids predicts changes in insulin sensitivity in response to long-term exercise. Physiol Rep 5. doi:10.14814/phy2.13183
73. Lee WC, Wen HC, Chang CP, Chen MY, Lin MT (2006) Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heatstroke. JApplPhysiol (1985) 100:2073-2082
74. Leong I (2018) Follistatin inactivation improves glucose tolerance. Nature reviews Endocrinology. doi:10.1038/s41574-018-0052-y
75. Leu JIJ, George DL (2007) Hepatic IGFBP1 is a prosurvival factor that binds to BAK, protects the liver from apoptosis, and antagonizes the proapoptotic actions of p53 at mitochondria. Genes & Development 21:3095-3109. doi:10.1101/gad/1567107
76. Liu CC, Lin CH, Lin CY, Lee CC, Lin MT, Wen HC (2013) Transgenic overexpression of heat shock protein 72 in mouse muscle protects against exhaustive exercise-induced skeletal muscle damage. JFormosMedAssoc 112:24-30
77. Liu HH, Wang JW, Yu HY, Zhang RP, Chen X, Jin HB, Dai F, Li L, Xu F (2012) Injection of duck recombinant follistatin fusion protein into duck muscle tissues stimulates satellite cell proliferation and muscle fiber hypertrophy. ApplMicrobiolBiotechnol
78. Loyd C, Magrisso IJ, Haas M, Balusu S, Krishna R, Itoh N, Sandoval DA, Perez-Tilve D, Obici S, Habegger KM (2016) Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. Journal of applied physiology (Bethesda, Md : 1985) 121:687-698. doi:10.1152/japplphysiol.00456.2016
79. Mai K, Andres J, Biedasek K, Weicht J, Bobbert T, Sabath M, Meinus S, Reinecke F, Mohlig M, Weickert MO, Clemenz M, Pfeiffer AF, Kintscher U, Spuler S, Spranger J (2009) Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. Diabetes 58:1532-1538

80. Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ (2014) Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 63:4057-4063
81. McPherron AC, Lawler AM, Lee SJ (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83-90
82. Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, Alfano LN, Berry K, Meadows E, Lewis S, Braun L, Shontz K, Rouhana M, Clark KR, Rosales XQ, Al-Zaidy S, Govoni A, Rodino-Klapac LR, Hogan MJ, Kaspar BK (2015) A phase 1/2a follistatin gene therapy trial for becker muscular dystrophy. MolTher 23:192-201
83. Michel U, Ebert S, Phillips D, Nau R (2003) Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia. EurJEndocrinol 148:559-564
84. Michel U, Shintani Y, Nau R (1998) Serum follistatin concentrations are increased in patients with septicaemia. ClinEndocrinol(Oxf) 48:413-417
85. Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K, Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Yamashita T, Honda M, Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim HJ, Lee IK, Minokoshi Y, Saito Y, Takahashi K, Yamada Y, Takakura N, Kaneko S (2010) A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab 12:483-495. doi:10.1016/j.cmet.2010.09.015
86. Misu H, Takayama H, Saito Y, Mita Y, Kikuchi A, Ishii KA, Chikamoto K, Kanamori T, Tajima N, Lan F, Takeshita Y, Honda M, Tanaka M, Kato S, Matsuyama N, Yoshioka Y, Iwayama K, Tokuyama K, Akazawa N, Maeda S, Takekoshi K, Matsugo S, Noguchi N, Kaneko S, Takamura T (2017) Deficiency of the hepatokine selenoprotein P increases responsiveness to exercise in mice through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle. Nature medicine 23:508-516. doi:10.1038/nm.4295
87. Nielsen HB, Clemmesen JO, Skak C, Ott P, Secher NH (2002) Attenuated hepatosplanchnic uptake of lactate during intense exercise in humans. Journal of applied physiology (Bethesda, Md : 1985) 92:1677-1683. doi:10.1152/japplphysiol.00028.2001
88. Nishimura T, Nakatake Y, Konishi M, Itoh N (2000) Identification of a novel FGF, FGF-21, preferentially expressed in the liver. BiochimBiophysActa 1492:203-206
89. Norheim F, Hjorth M, Langleite TM, Lee S, Holen T, Bindesboll C, Stadheim HK, Gulseth HL, Birkeland KI, Kielland A, Jensen J, Dalen KT, Drevon CA (2014) Regulation of angiopoietin-like protein 4 production during and after exercise. Physiol Rep 2. doi:10.14814/phy2.12109
90. O'Connor AE, McFarlane JR, Hayward S, Yohkaichiya T, Groome NP, de Kretser DM (1999) Serum activin A and follistatin concentrations during human pregnancy: a cross-sectional and longitudinal study. HumReprod 14:827-832
91. Oarada M, Tsuzuki T, Nikawa T, Kohno S, Hirasaka K, Gonoi T (2012) Refeeding with a high-protein diet after a 48 h fast causes acute hepatocellular injury in mice. BrJNutr 107:1435-1444
92. Okamoto H, Cavino K, Na E, Krumm E, Kim S, Stevis PE, Harp J, Murphy AJ, Yancopoulos GD, Gromada J (2017) Angptl4 does not control hyperglucagonemia or alpha-cell hyperplasia following glucagon receptor inhibition. Proceedings of the National Academy of Sciences of the United States of America 114:2747-2752. doi:10.1073/pnas.1620989114
93. Owen BM, Ding X, Morgan DA, Coate KC, Bookout AL, Rahmouni K, Kliewer SA, Mangelsdorf DJ (2014) FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. Cell Metab 20:670-677. doi:10.1016/j.cmet.2014.07.012
94. Peake JM, Tan SJ, Markworth JF, Broadbent JA, Skinner TL, Cameron-Smith D (2014) Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. Am J Physiol Endocrinol Metab 307:E539-552. doi:10.1152/ajpendo.00276.2014
95. Pedersen BK, Saltin B (2015) Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scandinavian Journal of Medicine & Science in Sports 25:1-72. doi:10.1111/sms.12581

96. Perko MJ, Nielsen HB, Skak C, Clemmesen JO, Schroeder TV, Secher NH (1998) Mesenteric, coeliac and splanchnic blood flow in humans during exercise. JPhysiol 513 (Pt 3):907-913
97. Pilegaard H, Saltin B, Neufer PD (2003) Effect of short-term fasting and refeeding on transcriptional regulation of metabolic genes in human skeletal muscle. Diabetes 52:657-662
98. Pourteymour S, Eckardt K, Holen T, Langleite T, Lee S, Jensen J, Birkeland KI, Drevon CA, Hjorth M (2017) Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines. Mol Metab 6:352-365. doi:10.1016/j.molmet.2017.01.007
99. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America 106:8665-8670. doi:10.1073/pnas.0903485106
100. Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, Cohen JC (2007) Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. NatGenet 39:513-516
101. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, Cohen JC (2009) Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. JClinInvest 119:70-79
102. Rosendal L, Sogaard K, Kjaer M, Sjogaard G, Langberg H, Kristiansen J (2005) Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. Journal of Applied Physiology 98:477-481. doi:10.1152/japplphysiol.00130.2004
103. Salo DC, Donovan CM, Davies KJ (1991) HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. Free RadicBiolMed 11:239-246
104. Sargeant JA, Aithal GP, Takamura T, Misu H, Takayama H, Douglas JA, Turner MC, Stensel DJ, Nimmo MA, Webb DR, Yates T, King JA (2018) The influence of adiposity and acute exercise on circulating hepatokines in normal-weight and overweight/obese men. Appl Physiol Nutr Metab 43:482-490. doi:10.1139/apnm-2017-0639
105. Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj M, Huang CJ (2015) Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese individuals. NutrMetab CardiovascDis 25:839-845
106. Soberg S, Sandholt CH, Jespersen NZ, Toft U, Madsen AL, von Holstein-Rathlou S, Grevengoed TJ, Christensen KB, Bredie WLP, Potthoff MJ, Solomon TPJ, Scheele C, Linneberg A, Jorgensen T, Pedersen O, Hansen T, Gillum MP, Grarup N (2017) FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans. Cell Metab 25:1045-1053.e1046. doi:10.1016/j.cmet.2017.04.009
107. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB (2008) Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 57:2762-2767. doi:10.2337/db08-0538
108. Stefan N, Haring HU (2013) The role of hepatokines in metabolism. NatRevEndocrinol 9:144-152
109. Streja DA, Marliss EB, Steiner G (1977) The effects of prolonged fasting on plasma triglyceride kinetics in man. Metabolism 26:505-516
110. Sugiyama M, Kikuchi A, Misu H, Igawa H, Ashihara M, Kushima Y, Honda K, Suzuki Y, Kawabe Y, Kaneko S, Takamura T (2018) Inhibin beta E (INHBE) is a possible insulin resistance-associated hepatokine identified by comprehensive gene expression analysis in human liver biopsy samples. Plos One 13. doi:10.1371/journal.pone.0194798
111. Sukonina V, Lookene A, Olivecrona T, Olivecrona G (2006) Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. ProcNatlAcadSciUSA 103:17450-17455
112. Takala J (1996) Determinants of splanchnic blood flow. British journal of anaesthesia 77:50-58. doi:10.1093/bja/77.1.50

113. Talukdar S, Zhou Y, Li D, Rossulek M, Dong J, Somayaji V, Weng Y, Clark R, Lanba A, Owen BM, Brenner MB, Trimmer JK, Gropp KE, Chabot JR, Erion DM, Rolph TP, Goodwin B, Calle RA (2016) A Long-Acting FGF21 Molecule, PF-05231023, Decreases Body Weight and Improves Lipid Profile in Non-human Primates and Type 2 Diabetic Subjects. Cell Metab 23:427-440. doi:10.1016/j.cmet.2016.02.001
114. Tao R, Wang C, Stohr O, Qiu W, Hu Y, Miao J, Dong XC, Leng S, Stefater M, Stylopoulos N, Lin L, Copps KD, White MF (2018) Inactivating hepatic follistatin alleviates hyperglycemia. Nature medicine 24:1058-1069. doi:10.1038/s41591-018-0048-0
115. Thiessen SE, Vanhorebeek I, Derese I, Gunst J, Van den Berghe G (2015) FGF21 response to critical illness: effect of blood glucose control and relation with cellular stress and survival. JClinEndocrinolMetab 100:E1319-E1327
116. Tjeerdema N, Georgiadi A, Jonker JT, van Glabbeek M, Alizadeh Dehnavi R, Tamsma JT, Smit JW, Kersten S, Rensen PC (2014) Inflammation increases plasma angiopoietin-like protein 4 in patients with the metabolic syndrome and type 2 diabetes. BMJ Open Diabetes Res Care 2:e000034. doi:10.1136/bmjdrc-2014-000034
117. Trefts E, Williams AS, Wasserman DH (2015) Exercise and the Regulation of Hepatic Metabolism. Progress in molecular biology and translational science 135:203-225. doi:10.1016/bs.pmbts.2015.07.010
118. Vamvini MT, Aronis KN, Chamberland JP, Mantzoros CS (2011) Energy deprivation alters in a leptin- and cortisol-independent manner circulating levels of activin A and follistatin but not myostatin in healthy males. JClinEndocrinolMetab 96:3416-3423
119. von Holstein-Rathlou S, BonDurant LD, Peltekian L, Naber MC, Yin TC, Claflin KE, Urizar AI, Madsen AN, Ratner C, Holst B, Karstoft K, Vandenbeuch A, Anderson CB, Cassell MD, Thompson AP, Solomon TP, Rahmouni K, Kinnamon SC, Pieper AA, Gillum MP, Potthoff MJ (2016) FGF21 Mediates Endocrine Control of Simple Sugar Intake and Sweet Taste Preference by the Liver. Cell Metab 23:335-343. doi:10.1016/j.cmet.2015.12.003
120. Wallace JD, Cuneo RC, Baxter R, Orskov H, Keay N, Pentecost C, Dall R, Rosen T, Jorgensen JO, Cittadini A, Longobardi S, Sacca L, Christiansen JS, Bengtsson BA, Sonksen PH (1999) Responses of the growth hormone (GH) and insulin-like growth factor axis to exercise, GH administration, and GH withdrawal in trained adult males: a potential test for GH abuse in sport. J Clin Endocrinol Metab 84:3591-3601. doi:10.1210/jcem.84.10.6037
121. Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA (2001) Exercise increases serum Hsp72 in humans. Cell StressChaperones 6:386-393
122. Wang X, Wei W, Krzeszinski JY, Wang Y, Wan Y (2015) A Liver-Bone Endocrine Relay by IGFBP1 Promotes Osteoclastogenesis and Mediates FGF21-Induced Bone Resorption. Cell Metab 22:811-824. doi:10.1016/j.cmet.2015.09.010
123. Whitham M, Laing SJ, Jackson A, Maassen N, Walsh NP (2007) Effect of exercise with and without a thermal clamp on the plasma heat shock protein 72 response. JApplPhysiol (1985) 103:1251-1256
124. Winder WW (1988) Role of cyclic AMP in regulation of hepatic glucose production during exercise. MedSciSports Exerc 20:551-559
125. Wolfe RR, Klein S, Carraro F, Weber JM (1990) Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. Am J Physiol 258:E382-389. doi:10.1152/ajpendo.1990.258.2.E382
126. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, Lam KS (2005) Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. ProcNatlAcadSciUSA 102:6086-6091
127. Yalley A, Schill D, Hatta M, Johnson N, Cirillo LA (2016) Loss of Interdependent Binding by the FoxO1 and FoxA1/A2 Forkhead Transcription Factors Culminates in Perturbation of Active Chromatin Marks and Binding of Transcriptional Regulators at Insulin-sensitive Genes. J Biol Chem 291:8848-8861. doi:10.1074/jbc.M115.677583

128. Yamada P, Amorim F, Moseley P, Schneider S (2008) Heat shock protein 72 response to exercise in humans. Sports Med 38:715-733
129. Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, Wu X, Huang T, Hultman K, Paulsen SJ, Wang J, Bugge A, Frantzen JB, Norgaard P, Jeppesen JF, Yang Z, Secher A, Chen H, Li X, John LM, Shan B, He Z, Gao X, Su J, Hansen KT, Yang W, Jorgensen SB (2017) GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nature medicine 23:1158-1166. doi:10.1038/nm.4394
130. Yoshida K, Ono M, Koishi R, Furukawa H (2004) Characterization of the 5' regulatory region of the mouse angiopoietin-like protein 4. VetResCommun 28:299-305
131. Yoshida K, Shimizugawa T, Ono M, Furukawa H (2002) Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. JLipid Res 43:1770-1772
132. Yu X, Burgess SC, Ge H, Wong KK, Nassem RH, Garry DJ, Sherry AD, Malloy CR, Berger JP, Li C (2005) Inhibition of cardiac lipoprotein utilization by transgenic overexpression of Angptl4 in the heart. ProcNatlAcadSciUSA 102:1767-1772
133. Zhang M, Sun W, Qian J, Tang Y (2018) Fasting exacerbates hepatic growth differentiation factor 15 to promote fatty acid beta-oxidation and ketogenesis via activating XBP1 signaling in liver. Redox biology 16:87-96. doi:10.1016/j.redox.2018.01.013
134. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432

Table 1. Transcripts of potential hepatokines regulated after 60 min non-exhaustive treadmill exercise in mice

Gene Symbol	Gene Description	FC	sed	run	SL	ECM	Accession
Igfbp1	insulin-like growth factor binding protein 1	32,3	46,3	1496	yes		P47876
Fgf21	fibroblast growth factor 21	26,5	bd	26,5	yes		Q9JJN1
Serpine2	serine peptidase inhibitor, clade E, member 2	18,3	bd	18,3	yes	yes	Q07235
Esm1	endothelial cell-specific molecule 1	12,1	2,2	26,6	yes		Q9QYY7
Impg2	interphotoreceptor matrix proteoglycan 2	11,0	bd	11		yes	Q80XH2
Ccl5	chemokine (C-C motif) ligand 5	9,1	1,3	11,8	yes		P30882
Chgb	chromogranin B	8,8	1,3	11,5	yes		P16014
Gpc3	glypican 3	8,8	2,3	20,2	yes	yes	Q8CFZ4
Matn2	matrilin 2	7,0	bd	7	yes	yes	O08746
Ang2	angiogenin	6,6	3,4	22,4	yes		Q64438
Ephb6	Eph receptor B6	6,4	2,2	14	yes		O08644
Il1b	interleukin 1 beta	6,0	10,2	61,7	yes		P10749
Adm2	adrenomedullin 2	5,8	1,8	10,4	yes		Q7TNK8
Serpine1	serine peptidase inhibitor, clade E, member 1	5,4	2,2	11,8	yes	yes	P22777
Sbsn	suprabasin	4,7	2,3	10,9	yes	yes	Q8CIT9
Podn	podocan	4,7	4	18,9	yes	yes	Q7TQ62

Col4a4	collagen, type IV, alpha 4	4,5	4	18,1	yes	yes	Q9QZR9
Acrbp	proacrosin binding protein	4,5	bd	4,5	yes		Q3V140
Cxadr	coxsackie virus and adenovirus receptor	4,5	bd	4,5	yes		P97792
Tfpi	tissue factor pathway inhibitor	4,4	bd	4,4	yes		O54819
Pcolce2	procollagen C-endopeptidase enhancer 2	4,3	2,9	12,4	yes		Q8R4W6
Edn1	endothelin 1	3,9	2,7	10,5	yes		P22387
Pcsk5	proprotein convertase subtilisin/kexin type 5	3,5	3,6	12,5	yes		Q04592
Efna1	ephrin A1	3,4	304	1033	yes		P52793
Igfbp3	insulin-like growth factor binding protein 3	3,4	6	20,3	yes		P47878
Xcl1	chemokine (C motif) ligand 1	3,4	3,7	12,4	yes		P47993
Angptl4	angiopoietin-like 4	3,2	1056	3334	yes	yes	Q9Z1P8
Lrch3	leucine-rich repeats and CH domain 3	3,1	bd	3,1	yes		Q8BVU0
Gdf15	growth differentiation factor 15	3,1	38,3	118,6	yes		Q9Z0J7
Scube2	signal peptide, CUB domain, EGF-like 2	3,0	3,6	10,7	yes		D3YVM9
Hist2h4	histone cluster 2, H4	3,0	3,6	10,7		yes	P62806
Cxcl13	chemokine (C-X-C motif) ligand 13	3,0	7,8	23,1	yes		O55038
Col11a2	collagen, type XI, alpha 2	2,9	6,2	18	yes	yes	Q64739
Ran	RAN, member RAS oncogene family	2,9	bd	2,9		yes	P62827
Ccl6	chemokine (C-C motif) ligand 6	2,9	6,5	18,7	yes		P27784
Gdf5	growth differentiation factor 5	2,8	5,3	14,9	yes		P43027
Plaur	plasminogen activator, urokinase receptor	2,8	4,5	12,4	yes		P35456
Fst	follistatin	2,7	32,3	88,2	yes		P47931
Cfl1	cofilin 1, non-muscle	2,7	bd	2,7		yes	P18760
Smpdl3b	sphingomyelin phosphodiesterase, acid-like	2,6	7,9	20,9	yes		P58242
Ctgf	connective tissue growth factor	2,5	63	160,5	yes	yes	P29268
Fbn1	fibrillin 1	2,5	5,8	14,6	yes	yes	Q61554
Cd14	CD14 antigen	2,5	6,9	17,2	yes		P10810
Fbln5	fibulin 5	2,5	1,9	4,7	yes	yes	Q9WVH9
Serpina7	serine (or cysteine) peptidase inhibitor	2,5	81	199,4	yes		P61939
Gsn	gelsolin	2,4	4,7	11,1	yes		P13020
Lepr	leptin receptor	2,4	4	9,4	yes		P48356
Tomm20	translocase of outer mitochondrial membrane 20 homolog (yeast)	2,3	3,8	8,9		yes	Q9DCC8
Fgfr2	fibroblast growth factor receptor 2	2,2	7,7	17,2		yes	P21803
Loxl3	lysyl oxidase-like 3	2,2	2,2	4,8	yes		Q9Z175
Mep1b	meprin 1 beta	2,2	9,2	19,8	yes		Q61847
Thbs2	thrombospondin 2	2,1	10,3	21,9		yes	Q03350
Lifr	leukemia inhibitory factor receptor	2,1	16	33,2	yes		P42703
Insl5	insulin-like 5	2,0	5,2	10,4	yes		Q9WUG6
S100a8	S100 calcium binding protein A8	2,0	2,7	5,4	yes		P27005
Itgb4	integrin beta 4	0,5	12,8	6,4		yes	A2A863
Prok2	prokineticin 2	0,5	10,4	5,1	yes		Q9QXU7
Camp	cathelicidin antimicrobial peptide	0,5	17,8	8,5	yes		P51437
Il1a	interleukin 1 alpha	0,5	2,1	Bd	yes		P01582
Cort	cortistatin	0,5	10,1	4,6	yes		P56469
Cxcl1	chemokine (C-X-C motif) ligand 1	0,4	165,9	73,1	yes		P12850
Bmp5	bone morphogenetic protein 5	0,4	2,3	Bd	yes		P49003
Rpn1	ribophorin I	0,4	14,2	6,1		yes	Q91YQ5
Retn	resistin	0,4	20,7	8,8	yes		Q99P87
Tub	tubby candidate gene	0,4	11,3	4,7	yes		P50586
Nodal	nodal	0,4	13,6	5,5	yes		P43021
Trh	thyrotropin releasing hormone	0,4	10,3	4,1	yes		Q62361
Casp14	caspase 14	0,4	10,9	4		yes	O89094
Anxa1	annexin A1	0,3	14,7	5,1	yes		P10107
Mmp15	matrix metallopeptidase 15	0,3	25,2	8,6		yes	O54732
Col1a2	collagen, type I, alpha 2	0,3	3	Bd	yes	yes	Q01149
Fgl2	fibrinogen-like protein 2	0,3	23,7	7,7	yes		P12804
Fgf22	fibroblast growth factor 22	0,3	9,3	3	yes		Q9ESS2

Ndfip1	Nedd4 family interacting protein 1	0,3	3,1	Bd	yes		Q8R0W6
Icam4	intercellular adhesion molecule 4	0,3	14,6	4,6	yes		Q9ERM2
Angptl2	angiopoietin-like 2	0,3	3,9	Bd	yes		Q9R045
Pla2g10	phospholipase A2, group X	0,2	16,3	3,7	yes		Q9QXX3
Ucn	urocortin	0,2	4,5	Bd	yes		P81615
Inhbe	inhibin beta E	0,2	109,5	18,2	yes		O08717
Cxcl14	chemokine (C-X-C motif) ligand 14	0,2	6,1	Bd	yes		Q9WUQ5
Cgref1	cell growth regulator with EF hand domain 1	0,1	7,6	Bd	yes		Q8R1U2
Col12a1	collagen, type XII, alpha 1	0,1	11,6	1,2	yes	yes	Q60847
Cfh	complement component factor h	0,1	11,1	Bd	yes		P06909
Ccl1	chemokine (C-C motif) ligand 1	0,1	18,4	1,2	yes		P10146

Transcripts with 2-fold regulation and secreted protein annotation (SL-0243, Uniprot) or annotation as extracellular matrix protein (ECM; GO:0031012) are shown. Mice ran 60 min at 14 m/min and 14º inclination on a treadmill. Sedentary mice remained in their cages. Sed, run; Signal intensity of transcripts on Illumina mouseRef-8 v1.0 Expression bread Chip array (see (Illumina, San Diego, CA, USA) and respective Swissprot accession number. Experimental conditions are described in reference [50].

Figure 1.

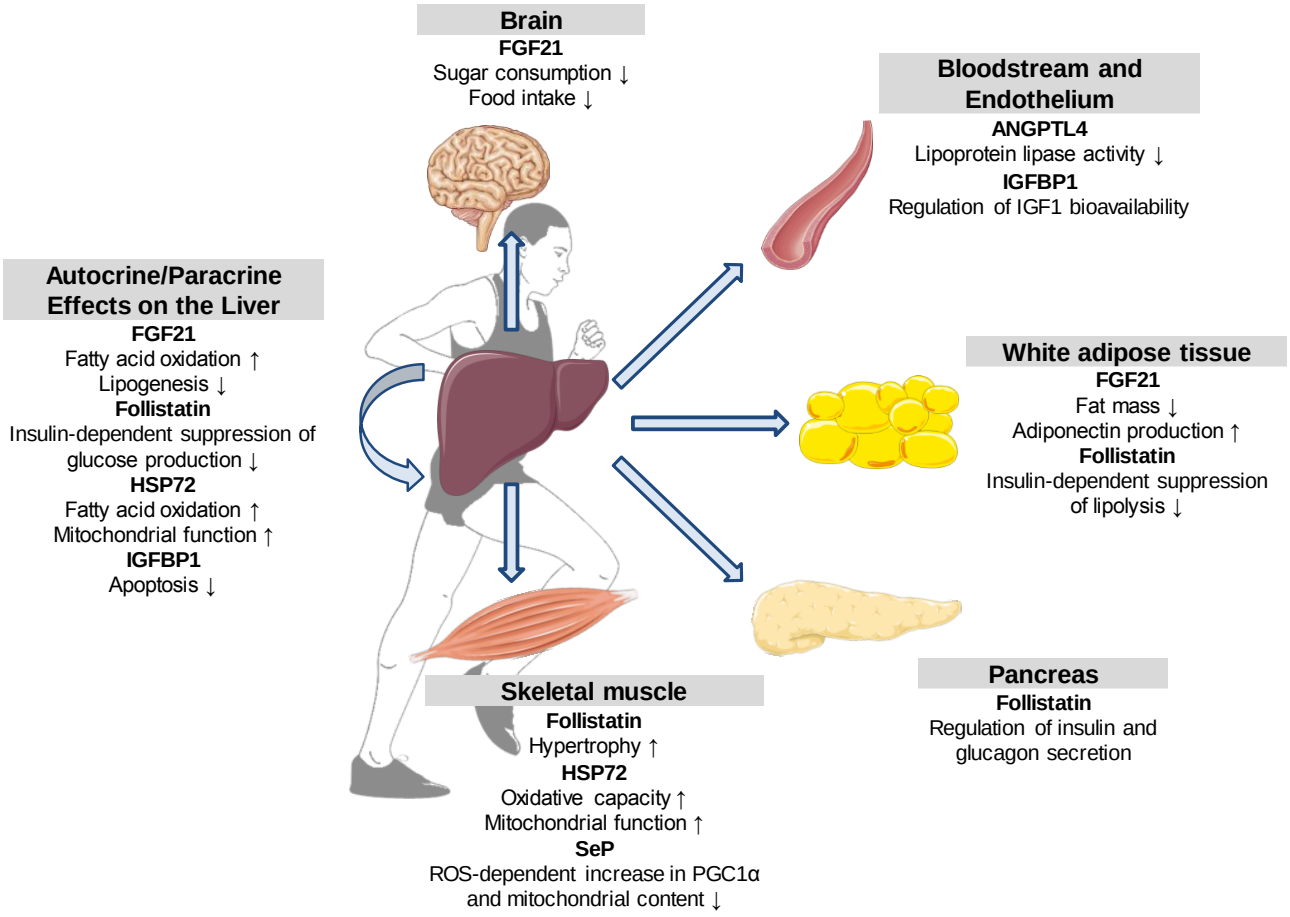

Figure 1. Potential effects of exercise-regulated hepatokines.

Hepatokines can act in an autocrine/paracrine manner on the liver and as endocrine proteins on brain, endothelial lipoprotein lipase, adipose tissue, pancreas and skeletal muscle. In most cases, exercise induces the systemic concentration of hepatokines. Selenoprotein P (SeP) is not regulated by exercise, but reduces production of reactive oxygen species (ROS) during exercise. This figure was created using illustrations provided by Servier medical art.

Figure 2

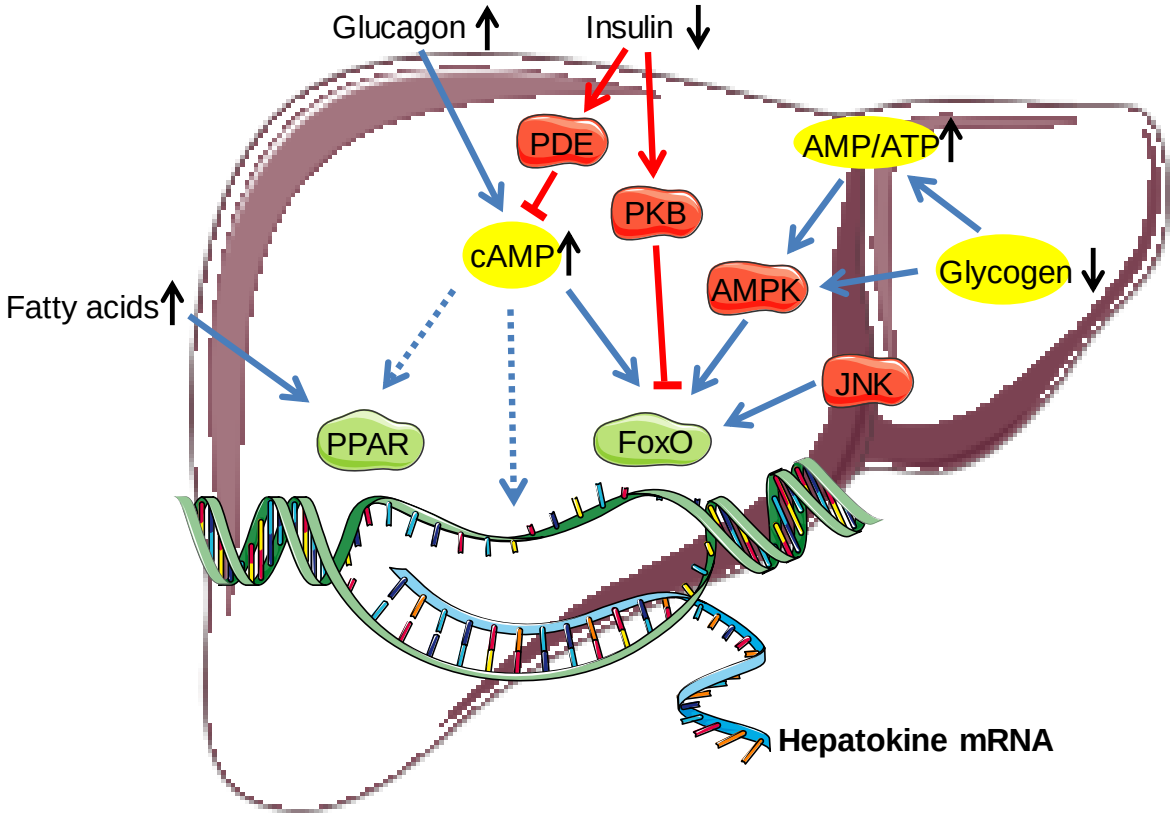

Figure 2. Regulation of hepatokines during exercise.

The increase in the glucagon-to-insulin ratio during exercise leads to elevated cAMP levels via stimulation of the adenyl cyclase and decreased activation of phosphodiesterase (PDE), which can activate via Forkhead box (Fox)O, peroxisome proliferator-activated receptors (PPAR) and other factors the transcription of fibroblast growth factor (FGF)21, follistatin, angiopoietin protein like 4 (ANGPTL4) and insulin like growth factor binding protein (IGFBP). Hepatic energy depletion via 5' AMP-activated protein kinase (AMPK) and activation of c-Jun N-terminal kinase (JNK) also activates FoxO. Insulin inactivates FoxO via activation of protein kinase (PKB) and reduces cAMP levels. Fatty acid signalling via PPARs can contribute to the transcriptional regulation of hepatokines. This figure was created using illustrations provided by Servier medical art.

Hepatokines – a novel group of exercise factors

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Abstract

Regular physical activity not only improves the exercise capacity of the skeletal muscle performing the contractions, it is beneficial for the whole body. An extensive search for "exercise factors" mediating these beneficial effects has been going on for decades. Particular skeletal muscle tissue has been investigated as a source of circulating exercise factors, and several myokines have been identified. However, exercise also has an impact on other tissues. The liver is interposed between energy storing and energy utilising tissues and is highly active during exercise, maintaining energy homeostasis. Recently, a novel group of exercise factors - termed hepatokines - has emerged. These proteins (fibroblast growth factor-21, follistatin, angiopoietin-like protein 4, heat shock protein 72, insulin-like growth factor binding protein 1) are released from the liver and increased in the bloodstream during or in the recovery after an exercise bout. In this narrative review, we evaluate this new group of exercise factors focusing on the regulation and potential function in exercise metabolism and adaptations. These hepatokines may convey some of the beneficial whole-body effects of exercise that could ameliorate metabolic diseases, such as obesity or type 2 diabetes.

Introduction

Health benefits from exercise have long been acknowledged, and regular physical activity is recommended as therapeutic intervention to prevent and treat a range of chronic disorders, including obesity, insulin resistance and type 2 diabetes [94]. Exercise initiates several transcriptional and (post)translational mechanisms in skeletal muscle, which increases the capacity and efficiency for fuel uptake and utilization, thus improving whole-body energy metabolism [26]. However, several tissues respond to physical activity, for which reason the orchestration of tissue-specific functions is crucial, bringing the concept of circulating exercise factors into play which could mediate the cross-talk. To regulate energy metabolism, various tissues produce signalling molecules to communicate between energy storing and energy utilizing tissues. With the discovery of leptin [133] as a signalling molecule secreted by the adipose tissue, the term "adipokine" was coined. Transferring the concept of adipokines to the energy utilizing skeletal muscle tissue, interleukin (IL)-6 was the first muscle-derived signalling molecule to be termed a myokine [33], even though myostatin (growth differentiation factor (GDF)-8) had been classified as a muscle-derived hormone years before [80]. Since then, several endocrine functions have been attributed to the myokines secreted by the working muscle including regulation of body weight and insulin sensitivity, as well as tumour suppression and maintenance of cognitive functions [52].

The liver is a central organ in energy metabolism, interposed between energy storage and utilisation and as such maintaining energy homeostasis challenged both during physiological (exercise, fasting, food intake) and pathophysiological (metabolic syndrome, diabetes, obesity, cachexia) conditions. In analogy to adipo- and myokines, signalling molecules exclusively or predominantly produced by the liver and secreted into the bloodstream that impact extrahepatic tissues/organs [8] have been termed "hepatokines". During the last decade, hepatokines have principally been studied in the context of metabolic diseases [107]. Prominent examples are fetuin-A (also known as α-2-Heremans-Schmid-glycoprotein) and selenoprotein P (SeP), which are linked to insulin resistance [106,84]. In contrast, fibroblast growth factor (FGF)21 has the potency to improve glucose homeostasis and dyslipidemia [62]. In the present narrative review, we summarize and discuss hepatokines in relation to acute exercise and physical training as well as their potential role in energy homeostasis and metabolic adaptations.

The role of the liver in exercise metabolism

The liver plays a pivotal role in exercise metabolism when the energy balance becomes negative and substrates need to be mobilised from storage or synthesised. Hepatic glucose production is increased via breakdown of glycogen as well as enhanced *de novo* synthesis of glucose from lactate, glycerol and amino acids. The relative contribution of the hepato-splanchnic bed to the whole-body uptake of free fatty acids (FFA) decreases during exercise, but the absolute uptake increases, and FFA are oxidized to provide energy for ATP-consuming biochemical processes [124]. After prolonged exercise, excess FFA are also re-esterified to triglycerides, and elevated hepatic triglyceride content can be observed [54,13].

A major regulator of hepatic metabolism during exercise is a change in the glucagon-to-insulin ratio, which increases due to both an increase in glucagon and a decrease in insulin in the circulation. Exercise studies in rodents demonstrate a fall in hepatic energy charge with increased production of AMP, which is considered to trigger glycogen mobilisation [116] and to stimulate oxidation of fatty acids via activation of AMPK. The increase in circulating catecholamines during high intensity exercise has been suggested to stimulate hepatic glucose output [39], but hepatic adrenergic blockade in dogs did not reduce glucose delivery from the liver [23], and thus the contribution of the adrenergic system is not completely clear. Growth hormone (GH) and cortisol are both elevated by exercise and may partially contribute to the adaptation of hepatic metabolism [93]. The increased metabolic activity of the liver during exercise is reflected by a higher hepato-splanchnic oxygen uptake. During moderate exercise, hepatic blood flow is unchanged or only slightly reduced [32,31] while, hepato-splanchnic oxygen uptake is upregulated more than two-fold [1], presumably due to high levels of oxygen extraction [111]. Of note, exercising with very high intensity can markedly reduce hepato-splanchnic blood flow [95,86], which may compromise hepatic metabolism [86].

Searching for potential exercise-regulated hepatokines – candidates from mouse microarray data

Upregulation of gene expression is one mechanistic pathway which can lead to increased production and release of adipokines, hepatokines and myokines. One bout of acute exercise increases the transcript and protein levels of several myokines in the contracting muscle [97,19]. Compared with

skeletal muscle, even more transcripts in the liver of mice showed pronounced alterations immediately after a non-exhaustive treadmill exercise [51,50]. Filtering these transcripts for genes encoding potentially secreted proteins revealed 55 upregulated genes after exercise and 29 transcripts with reduced abundance (Table 1). The list of regulated transcripts is dominated by cytokines, chemokines, and components of the extracellular matrix. Insulin-like growth factor binding protein (IGFBP1) showed the highest change with significant signal intensities already in sedentary mice. FGF21 was also strongly increased by acute exercise while being almost undetectable in livers of sedentary mice. The exercise-dependent regulation and secretion of both factors has been validated in mice and humans as described below in further detail. Additional hepatic transcripts regulated by acute exercise in our transcriptomics study, which are already known as liver-derived secreted factors are: follistatin, angiopoietin-like 4 protein (ANGPTL4) and inhibin E.

Several of the transcripts listed in Table 1 may contribute to changes in the systemic concentration and may be involved in intrahepatic adaptations to exercise, but these factors will not be discussed further due to their wide tissue distribution. Moreover, a transcriptomics approach cannot cover all exercise-regulated hepatokines because an increased release of proteins is not necessarily reflected in an elevated transcript level. For instance, post-translational regulation or an enhanced secretion of existing protein pools, which is not reflected on the transcriptional level have been reported for two myokines, IL6 and secreted protein acidic and rich in cysteine (SPARC) [3,101].

In the following paragraphs, we will focus on hepatokines with evidence for transcriptional regulation by exercise and hepatic release into the bloodstream. Of note, elevated protein abundance in hepatic tissue accompanied by increased plasma concentration is a good hint for an actual release from the liver into the circulation, but the best evidence in humans is a net efflux from the hepato-splanchnic bed, which can be analysed as arterial-to-hepatic vein difference. This release from the hepato-splanchnic bed has been reported for FGF21, follistatin, ANGPTL4 and heat shock protein (HSP) 72 in relation to an acute exercise bout [43,47,57,31] and in the resting condition for IGFBP1 [16].

Hepatokines in humans:

FGF21

FGF21 has emerged as a novel metabolic regulator [63], which is preferentially expressed in the liver [87]. The hepatic origin of circulating FGF21 has elegantly been demonstrated in mice, where liver-specific knock-out of FGF21 results in the absence of circulating FGF21 [79]. In humans, FGF21 is released from the hepato-splanchnic bed both after an over-night fast and during an acute bout of exercise [43], supporting that FGF21 is a liver-derived factor both in mouse and man. In humans, circulating FGF21 is increased after a prolonged fast [35,29], exercise [65], in type 2 diabetes [20], steatosis [25], non-alcoholic steatohepatitis [25], obesity [25] and during critical illness [114]. Several mechanisms have been suggested for the regulation of FGF21, where the predominant one both *in vitro* and *in vivo* in mice is PPARα activation, which leads to increased FGF21 expression [56,7]. In humans, 2 weeks' treatment with PPARα and β, but not γ agonists increases circulating FGF21 [21], which is in line with the fact that elevation of FFA - the endogenous agonist of PPAR α and β - for 4 hours by infusion gives rise to an increase in plasma FGF21 [78]. The kinetics and the low magnitude of the FGF21 response to either PPAR activation or FFA infusion for 4 hours [21,78] are not consistent with the observation that FGF21 increases rapidly after an acute bout of exercise with a higher magnitude (exercise: 2-3-fold versus 1.3-fold with PPAR or FFA activation), indicating that other regulatory mechanisms may exist.

The most powerful hormonal signal to induce an increase in circulating FGF21 is glucagon, where a 2.5-fold increase can be demonstrated [4]. Intriguingly, insulin has an inhibitory effect, and inducing an increased glucagon-to-insulin ratio in healthy males increases FGF21 with a similar magnitude and kinetic as during exercise [43]. Thus, the glucagon-to-insulin ratio is much more powerful in stimulating hepatic FGF21 secretion than activating PPAR, either pharmacologically or by FFA. During physical exercise, an increase in the glucagon-to-insulin ratio is crucial, as prevention of an increase in the glucagon-to-insulin ratio during an exercise bout blunts the exercise-induced increase in FGF21 [44]. An impaired exercise-induced FGF21 response has been reported both in obesity associated with insulin resistance [104] and in type 2 diabetes [44]. However, obesity per se does not impair exercise-induced FGF21 [103]. Although conjectural, insulin resistance seems to be related to an impaired the exercise-induced FGF21 as recently reviewed [46].

The relation of FGF21 to metabolism is not only reflected by its regulation, but indeed by its actions (Figure 1). FGF21 administered to mice [63] and humans [34] has beneficial effects on energy metabolism. Insulin resistant mice treated with FGF21 reduce body weight and blood glucose concentrations [63], and similarly, patients with type 2 diabetes treated with an FGF21 analogue

experience weight loss and reduced fasting glucose levels after 2 weeks of treatment [34]. Adenoviral gene transfer to the liver of obese mice causes a reduction in weight gain, adipose tissue hypertrophy, hepatosteatosis and inflammation [58]. Moreover, this FGF21 gene therapy prevent insulin resistance associated with aging, while a FGF21 induced bone loss as potential adverse effect of FGF21 over-expression [121] was not observed. Liver, adipose tissue and brain are considered the most important sites of FGF21 action. Rodent studies in particular demonstrate that FGF21 mediates increased fatty acid oxidation and decreased lipogenesis in the liver [56,7] as well as increased glucose uptake in adipose tissue - an effect mainly accounted for by brown adipocytes [92]. Human studies support a central regulation of food intake and reduction in sugar consumption by FGF21 [118,105] and increased production of adiponectin [34,112]. As FGF21 holds strong promise as a therapeutic target, exercise-induced FGF21 could be one of the molecular links that mediates the beneficial effects of exercise to the whole-body level. This is supported by the impaired exercise adaptations in FGF21-deficient mice, which fail to improve glucose tolerance and to reduce hepatic triglyceride content compared to wildtype mice under the same high-fat diet [77].

Follistatin

The liver has recently been identified as the source of circulating follistatin [47]. Particularly during the first hours after an exercise bout circulating follistatin is increased 5-7-fold [41,60,47,103]. Examination of various tissue in mice revealed that the liver exhibits a marked increase in follistatin mRNA expression immediately after an acute exercise bout [41], which is supported by the observation in humans that follistatin is released from the hepato-splanchnic bed in the recovery state after an exercise bout [47]. Collectively, these data strongly suggest that the liver is the organ mainly accounting for circulating follistatin.

The initial perception was that circulating follistatin is a result of spill–over from auto- or paracrine processes, yet, follistatin increases with prolonged fast [117], pregnancy [89], type 2 diabetes [42] and critical illness [82,83]. A mutual feature of these conditions is an increase in the glucagon-to-insulin ratio as previously summarized [45]. Examining the regulation of exercise-induced follistatin revealed that the glucagon-to-insulin ratio is important for the increase in follistatin after exercise [44]. Thus, the regulation of circulating follistatin is clearly linked to energy metabolism. Additional stimuli must exist, as blunting the increase in the glucagon-to-insulin ratio

during an exercise bout only reduces the exercise-induced rise in follistatin levels by 50%. Moreover, exercise-induced follistatin is impaired by insulin resistance [44] but not by obesity per se [103].

Follistatin has primarily been investigated as a TGF-β modulating protein due to its inhibition of activins and GDFs, but follistatin is a pluripotent molecule and several target tissues have been suggested (Figure 1). Firstly, follistatin acts on the endocrine pancreas modulating both insulin and glucagon secretion, which suggests a feedback regulation [47]. Secondly, follistatin may have a role in regulating muscle hypertrophy where follistatin acts in concert with myostatin [38], insulin and IGF [10] as well as testosterone [15]. *In vivo* follistatin treatment induces muscle hypertrophy in mice [36] and non-human primates [68], and follistatin gene therapy has been tested in a phase 1/2a study in patients with Becker's muscle dystrophy, where an improvement in muscle function was observed [81]. Thirdly, a recent study demonstrated that ~~dysregulated~~ chronic activation of FoxO1 drives hepatic expression of follistatin, which leads to impaired insulin sensitivity in adipose tissue, potentiation of hepatic glucose production and severe glucose intolerance in mice [113,73]. Even though highly speculative, a lack of insulin suppression of adipose tissue lipolysis and hepatic glucose production might be of advantage during endurance exercise and the early recovery phase. More studies are needed to understand the physiological role of follistatin as exercise-regulated hepatokine. However, follistatin is a pluripotent molecule and could function as a means of communication from the liver to the endocrine pancreas, skeletal muscle or adipose tissue, acting as a link between energy metabolism and tissue growth and differentiation.

ANGPTL4

Like follistatin, plasma ANGPTL4 increases during exercise and in the recovery phase in healthy subjects [88]. ANGPTL4 was identified in mice as "fasting-induced adipose factor" in both adipose tissue [61] and liver [64]. In humans, ANGPTL4 is highly expressed in the liver followed by the pericardium, whereas adipose and skeletal muscle tissue expression is low [100]. Skeletal muscle has been suggested as a source of elevated plasma levels of ANGPTL4 in response to exercise [18,88]. However, when ANGPTL4 release is measured by arterial-to-venous differences over both a resting and an exercising leg, no contribution of ANGPTL4 to the systemic circulation can be detected [57]. In contrast, a release of ANGPTL4 can be detected from the hepato-splanchnic bed during exercise with no release at rest [57]. Thus, in humans, exercise-induced ANGPTL4 is released in to the

circulation via the hepato-splanchnic bed from the liver while other tissues contribute to the systemic ANGPTL4 level in the resting state.

Induction of ANGPTL4 in adipose tissue during fasting is ascribed to PPARα activation [61], but other PPARs were also found to have regulatory effects on ANGPTL4 expression [2], which has been supported by promoter analysis [129]. These findings are in line with ANGPTL4 being a fasting-induced protein as FFA, which are the ligands of PPARs increase during fasting [108]. A common denominator of fasting and exercise is the increase in the glucagon-to-insulin ratio. Indeed, an experimentally induced increase in the glucagon-to-insulin ratio in healthy subjects resting in bed increases systemic levels of ANGPTL4, and blunting the increase in the glucagon-to-insulin ratio in healthy males during a bout of bicycle exercise abolishes the exercise-induced ANGPTL4 increase [57]. Taken together, these *in vivo* observations in humans demonstrate that the glucagon-to-insulin ratio is pivotal for the induction of ANGPTL4 during physical exercise.

The function of ANGPTL4 has been studied *in vivo* and *in vitro*, where it acts as an inhibitor of lipoprotein lipase (LPL) [130] (Figure 1). LPL mediates the degradation of lipoprotein triglycerides into FFA in various tissues [14] and is increased in skeletal muscle tissue by fasting [96]. In line with the observation that ANGPTL4 is an inhibitor of LPL activity, circulating triglycerides levels are decreased in mice lacking ANGPTL4 [67] and increased in mice over-expressing ANGPTL4 [130,67]. Population-based studies in humans have revealed that loss-of-function mutations in ANGPTL4 are associated with low levels of triglycerides [100,99]. As ANGPTL4 is an inhibitor of LPL, it seems paradoxical that ANGPTL4 increases under the exact conditions where an increased breakdown of triglycerides is important - as it is the case during exercise and fasting. Based on tissue-specific overexpression models, ANGPTL4 has also been suggested to play a role in lipid partitioning and tissue-specific uptake or release of FFA [131,110], and to improve glucose metabolism [125]. In contrast, a recent study linked the genetic inactivation or loss of ANGPTL4 to a reduced risk of type 2 diabetes and improvement in glucose homeostasis [40]. An additional function attributed to ANGPTL4 is supporting angiogenesis [6], while recent studies on the regulation of pancreatic α-cells are inconsistent [11,91]. ANGPTL4-induced inhibition of LPL activity is due to direct protein-protein interaction [110,24], and it is unclear whether an ANGPTL4 receptor exists to mediate other effects attributed to this protein. Nevertheless, the acute regulation of plasma ANGPTL4 levels may suggest an endocrine role as a liver-derived signal to the peripheral tissues.

A pathway jointly regulating the hepatic FGF21, follistatin and ANGPTL4 response to exercise

A rise in the glucagon-to-insulin ratio increases the plasma concentration of FGF21, follistatin and ANGPTL4 *in vivo* in humans [43,47,57]. Stimulation of hepatocytes with glucagon activates the adenylyl cyclase and intracellular cAMP increases [48], while cAMP levels are reduced by insulin via activation of phosphodiesterase (PDE) [48]. Thus, the glucagon-to-insulin ratio sensed by the liver determine cAMP levels within the hepatocyte. Both glucagon and adrenalin can stimulate the adenylyl cyclase during exercise, however, glucagon seems to be the determinant of cAMP levels during exercise [123]. FoxO transcription factors appear to play an important role in the enhanced hepatic transcription of FGF21, follistatin and ANGPTL4. Exercise does not only induce hepatic cAMP levels, but also activates AMPK and JNK in the liver [17,50] and all these pathways activate FoxO [27]. The inhibitory effect of insulin on the transcription of these hepatokines may be mediated by the PI3K/PKB pathway, which leads to inactivation of FoxO1 [113]. ~~Exercise also increases lipolysis and the availability of fatty acids which can lead to activation of hepatic PPAR transcription factors.~~

In addition, PPARs are established regulators of FGF21 and ANGPTL4 expression, but the contribution of this pathway to exercise-dependent hepatokine production has not been fully clarified. Prevention of the increase in the glucagon-to-insulin ratio by somatostatin infusion during exercise also blocked the increase in plasma FFA [44], and thus, a contribution of elevated FFA to the increase in hepatokines cannot be excluded. Interestingly, elevation of cAMP levels in hepatocytes directly increases ANGPTL4 mRNA levels [57], and the cAMP/PKA pathway can regulate the transcriptional activity of PPARs [71]. Collectively, a molecular pathway exists for hepatokine production where secretion is stimulated via cAMP, and inhibition occurs via the insulin-regulated enzymes PDE and PKB, as summarized in Figure 2. This regulation by the glucagon-to-insulin ratio suggests that systemic levels of these hepatokines are influenced by hepatic insulin resistance, and reduction in insulin action can result in less inhibition of hepatokine secretion. As discussed above, elevated circulating levels of FGF21 [20], ANGPTL4 [115] and follistatin [42] have been demonstrated in patients with type 2 diabetes.

HSP72

In humans, HSP72 increases in the circulation during an acute bout of exercise [120]. Skeletal muscle, liver and brain have been suggested to be the tissues responsible for exercise-induced HSP72 release [102,69]. Measurement of arterial-to-venous differences in healthy humans during exercise demonstrates a release of HSP72 from the hepato-splanchnic bed, while no release is detected over the exercising leg or at rest [31]. In addition, the brain releases HSP72 to the circulation during exercise [69]. Taken together, HSP72 is an exercise-induced hepatokine, however contributions from other tissues add to the increase in HSP72 observed with exercise.

The regulation of HSP72 during exercise has not been completely elucidated. Different from what its name suggests heat per se only influences HSP72 induction to a minor degree during exercise in humans [122]. In rats, stress-induced circulating HSP72 is not affected by hypophysectomy or adrenalectomy, which rules out growth hormone, epinephrine and cortisol as major stimulatory signals [59]. However, phenylephrine increases plasma HSP72, and blocking of α1-adrenergic receptor by prazosin blunts stress-induced HSP72, whereas a β-selective antagonist (propranolol) has no effect, which collectively suggests that norepinephrine is a stimulating signal [59]. Interestingly, ingestion of glucose during exercise completely blunts hepato-splanchnic release of HSP72, suggesting a role for the glucoregulatory hormones insulin and glucagon [30]. However, no regulation of liver HSP72 mRNA is observed with a 48h fast in rats [90]. Thus, the cross-talk between the adrenergic and glucose-induced hormones still needs to be elucidated.

The roles of HSP72 seem to be diverse and related to whether it is located intra- or extracellularly [127]. Overexpression of HSP72 protects against heatstroke [72], which is in line with a muscle-specific overexpression protecting against exercise-induced skeletal muscle damage [75] and preserving muscle function [37] (Figure 1). Interestingly, overexpression of HSP72 in cardiac and skeletal muscle tissue increases exercise performance, oxidative capacity and mitochondrial content and also protects against obesity-induced insulin resistance [22], suggesting a role in energy metabolism. However, the contribution of liver-derived HSP72 to the above-mentioned effects has not been validated yet. Since HSP72 also regulates hepatic fatty acid oxidation and maintenance of mitochondrial function in the liver, a paracrine function of exercise-induced HSP72 can be considered as well [5].

IGF-1 and IGFBP1

Both GH and IGF1 levels increase at an early stage during an exercise bout in the circulation [55,9]. During exercise IGF is not released solely from the liver, as a contribution from the exercising leg has also been demonstrated [12]. Plasma IGFBP1 increases during prolonged exercise in humans [119], and hepatic IGFBP1 mRNA and protein levels are strongly upregulated during acute exercise in mice (Table 1) [50]. The increase is clearly linked to the hepatic energy state and is regulated by hepatic glycogen depletion [70] and thus dependent on the duration of exercise [50]. FoxO proteins are important regulators of IGFBP1 transcription, which may be the link between tissue glycogen content and regulation of IGFBP1 production during exercise (Figure 2) [126].

Circulating IGFBPs regulate the bioavailability of IGF1, but the physiological function of elevated plasma levels of IGFBP1 during prolonged exercise remains elusive. Exercise also induces intrahepatic IGFBP1 protein accumulation in mice [50], raising the possibility that this increase promotes an anti-apoptotic effect by interfering with the p53 tumour suppressor protein, which is also elevated in the liver after acute exercise [74].

GDF15

GDF15 is a hepatokine candidate based on its upregulation in the liver of mice during an acute bout of exercise, as shown in Table 1. This notion is strengthened by the observation that GDF15 is strongly induced by fasting in mice [132], which supports the idea that energy depletion regulates hepatic GDF15 mRNA levels. GDF15 protein has recently been shown to increase in the circulation in response to an acute bout of exercise [66]. In relation to exercise, GDF15 was initially thought to be a myokine, but no release could be detected from an exercising leg [66]. In similarity with FGF21, follistatin and ANGPTL4, increased levels of circulating GDF15 are associated with insulin resistance [53]. The receptor for GDF15 has been identified to be the orphan receptor GFRAL, which is expressed in the brain and is involved in the regulation of food intake [128,28]. Whether GDF15 is released from the liver and how it is regulated during an acute bout of exercise still needs to be addressed.

Inhibin βE

Inhibin βE is one of the hepatokine candidates with a down-regulation of its mRNA after acute exercise (Table 1). It was recently classified as hepatokine, which is associated with insulin resistance [109]. Human liver biopsies from insulin-resistant subjects have increased expression levels of inhibin βE mRNA, and knock-down of inhibin βE in an obese mouse model improves fatty acid oxidation. The influence of acute exercise and regular physical activity on circulating inhibin βE needs to be investigated. Reduced levels of circulating inhibin βE may be involved in mediating the beneficial effects of exercise on glucose tolerance and insulin sensitivity.

A role for the hepatokine selenoprotein P in exercise adaption

The systemic level of a hepatokine may alter the response to exercise even if it is not regulated by physical activity. The plasma concentration of the hepatokine selenoprotein P (SeP) has been linked to the training responsiveness in VO_2max [85] in humans and mice. SeP is produced mainly in the liver and functions as a transport protein supplying selenium to extrahepatic tissues [49]. Hepatic SeP transcript levels are not altered by acute exercise or training in mice, and plasma SeP concentrations are unchanged after 8 weeks of aerobic exercise training in humans [85]. However, SeP-deficient mice on a high fat diet have enhanced responses in muscle PGC1α mRNA levels, mitochondrial content and exercise endurance after training when compared to wildtype mice [85]. Further data provide evidence for a function of liver-derived SeP as regulator of exercise-induced reactive oxygen species (ROS) production in skeletal muscle [85]. Absence or low concentrations of SeP allows higher levels of ROS, which are considered to trigger mitochondrial adaptations [98] in response to exercise (Figure 1). SeP may also modulate the effects of exercise in humans, since pre-training SeP plasma levels are inversely associated with the improvement in VO2 max in middle-aged humans without diabetes and obesity [85].

Conclusion and outlook

During and particularly after an exercise bout the liver secretes potential exercise factors. The exercise-induced hepatokines are regulated by several stimuli where the glucagon-to-insulin ratio seems to be most relevant for FGF21, follistatin and ANGPTL4. These factors are all elevated with insulin resistance, while the response of FGF21 and follistatin to an acute bout of exercise appears to

be impaired in subjects with insulin resistance. Obesity per se does not affect the exercise-induced response of these hepatokines. This conjectures that FGF21 and follistatin may discriminate between distinct effects of steatosis and insulin resistance of the liver. Another relevant question is whether regular physical activity alters the concentration of circulating hepatokines associated with insulin resistance thereby contributing to the improvement of metabolic disorders.

In conclusion, the liver is not merely an organ receiving humoral stimuli, it is also communicating to extrahepatic tissues such as the adipose tissue and skeletal muscle. Research is now beginning to unravel the physiological role of circulating hepatokines in the adaptation to physical exercise and in the beneficial effect of exercise on whole-body metabolic homeostasis.

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References

1. Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J (1974) Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. JClinInvest 53:1080-1090
2. Akiyama TE, Lambert G, Nicol CJ, Matsusue K, Peters JM, Brewer HB, Jr., Gonzalez FJ (2004) Peroxisome proliferator-activated receptor beta/delta regulates very low density lipoprotein production and catabolism in mice on a Western diet. Journal of Biological Chemistry 279:20874-20881
3. Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, Sakuma K, Hang LP, Mizushima K, Hirai Y, Koyama R, Wada S, Higashi A, Kokura S, Ichikawa H, Yoshikawa T (2013) A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 62:882-889. doi:10.1136/gutjnl-2011-300776
4. Arafat AM, Kaczmarek P, Skrzypski M, Pruszynska-Oszmalek E, Kolodziejski P, Szczepankiewicz D, Sassek M, Wojciechowicz T, Wiedenmann B, Pfeiffer AF, Nowak KW, Strowski MZ (2013) Glucagon increases circulating fibroblast growth factor 21 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated lipolysis? Diabetologia 56:588-597
5. Archer AE, Rogers RS, Von Schulze AT, Wheatley JL, Morris EM, McCoin CS, Thyfault JP, Geiger PC (2018) Heat Shock Protein 72 Regulates Hepatic Lipid Accumulation. American journal of physiology Regulatory, integrative and comparative physiology. doi:10.1152/ajpregu.00073.2018
6. Babapoor-Farrokhran S, Jee K, Puchner B, Hassan SJ, Xin X, Rodrigues M, Kashiwabuchi F, Ma T, Hu K, Deshpande M, Daoud Y, Solomon S, Wenick A, Lutty GA, Semenza GL, Montaner S, Sodhi A (2015) Angiopoietin-like 4 is a potent angiogenic factor and a novel therapeutic target for patients with proliferative diabetic retinopathy. Proceedings of the National Academy of Sciences of the United States of America 112:E3030-3039. doi:10.1073/pnas.1423765112
7. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E (2007) Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 5:426-437
8. Bahr MJ, Boeker KH, Manns MP, Tietge UJ (2009) Decreased hepatic RBP4 secretion is correlated with reduced hepatic glucose production but is not associated with insulin resistance in patients with liver cirrhosis. ClinEndocrinol(Oxf) 70:60-65
9. Bang P, Brandt J, Degerblad M, Enberg G, Kaijser L, Thoren M, Hall K (1990) Exercise-induced changes in insulin-like growth factors and their low molecular weight binding protein in healthy subjects and patients with growth hormone deficiency. European journal of clinical investigation 20:285-292
10. Barbe C, Kalista S, Loumaye A, Ritvos O, Lause P, Ferracin B, Thissen JP (2015) Role of IGF-I in the Follistatin-induced skeletal muscle hypertrophy. AmJPhysiol EndocrinolMetab:ajpendo
11. Ben-Zvi D, Barrandon O, Hadley S, Blum B, Peterson QP, Melton DA (2015) Angptl4 links alpha-cell proliferation following glucagon receptor inhibition with adipose tissue triglyceride metabolism. ProcNatlAcadSciUSA 112:15498-15503
12. Berg U, Bang P (2004) Exercise and circulating insulin-like growth factor I. Hormone research 62 Suppl 1:50-58. doi:10.1159/000080759
13. Bilet L, Brouwers B, van Ewijk PA, Hesselink MK, Kooi ME, Schrauwen P, Schrauwen-Hinderling VB (2015) Acute exercise does not decrease liver fat in men with overweight or NAFLD. Sci Rep 5:9709. doi:10.1038/srep09709
14. Blanchette-Mackie EJ, Scow RO (1973) Effects of lipoprotein lipase on the structure of chylomicrons. JCell Biol 58:689-708
15. Braga M, Bhasin S, Jasuja R, Pervin S, Singh R (2012) Testosterone inhibits transforming growth factor-beta signaling during myogenic differentiation and proliferation of mouse satellite cells: potential role of follistatin in mediating testosterone action. MolCell Endocrinol 350:39-52

16. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K (1994) Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab 79:872-878. doi:10.1210/jcem.79.3.7521354
17. Camacho RC, Donahue EP, James FD, Berglund ED, Wasserman DH (2006) Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. Am J Physiol Endocrinol Metab 290:E405-408. doi:10.1152/ajpendo.00385.2005
18. Catoire M, Alex S, Paraskevopulos N, Mattijssen F, Evers-van GI, Schaart G, Jeppesen J, Kneppers A, Mensink M, Voshol PJ, Olivecrona G, Tan NS, Hesselink MK, Berbee JF, Rensen PC, Kalkhoven E, Schrauwen P, Kersten S (2014) Fatty acid-inducible ANGPTL4 governs lipid metabolic response to exercise. ProcNatlAcadSciUSA 111:E1043-E1052
19. Catoire M, Mensink M, Kalkhoven E, Schrauwen P, Kersten S (2014) Identification of human exercise-induced myokines using secretome analysis. Physiol Genomics
20. Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H, Boden G (2008) Circulating FGF-21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. ExpClinEndocrinolDiabetes 116:65-68
21. Christodoulides C, Dyson P, Sprecher D, Tsintzas K, Karpe F (2009) Circulating fibroblast growth factor 21 is induced by peroxisome proliferator-activated receptor agonists but not ketosis in man. JClinEndocrinolMetab 94:3594-3601
22. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA (2008) HSP72 protects against obesity-induced insulin resistance. ProcNatlAcadSciUSA 105:1739-1744
23. Coker RH, Krishna MG, Lacy DB, Bracy DP, Wasserman DH (1997) Role of hepatic alpha- and beta-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol 273:E831-838
24. Dijk W, Beigneux AP, Larsson M, Bensadoun A, Young SG, Kersten S (2016) Angiopoietin-like 4 promotes intracellular degradation of lipoprotein lipase in adipocytes. JLipid Res 57:1670-1683
25. Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, Badman MK, Martinez-Chantar ML, Maratos-Flier E (2010) Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456-463
26. Egan B, Zierath JR (2013) Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. Cell Metabolism 17:162-184. doi:10.1016/j.cmet.2012.12.012
27. Eijkelenboom A, Burgering BM (2013) FOXOs: signalling integrators for homeostasis maintenance. Nature reviews Molecular cell biology 14:83-97. doi:10.1038/nrm3507
28. Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, Coskun T, Hamang MJ, Sindelar DK, Ballman KK, Foltz LA, Muppidi A, Alsina-Fernandez J, Barnard GC, Tang JX, Liu X, Mao X, Siegel R, Sloan JH, Mitchell PJ, Zhang BB, Gimeno RE, Shan B, Wu X (2017) The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nature medicine 23:1215-1219. doi:10.1038/nm.4393
29. Fazeli PK, Lun M, Kim SM, Bredella MA, Wright S, Zhang Y, Lee H, Catana C, Klibanski A, Patwari P, Steinhauser ML (2015) FGF21 and the late adaptive response to starvation in humans. J Clin Invest 125:4601-4611. doi:10.1172/JCI83349
30. Febbraio MA, Mesa JL, Chung J, Steensberg A, Keller C, Nielsen HB, Krustrup P, Ott P, Secher NH, Pedersen BK (2004) Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. Cell StressChaperones 9:390-396
31. Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK (2002) Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. The Journal of Physiology 544:957-962. doi:10.1113/jphysiol.2002.025148
32. Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK (2003) Hepatosplanchnic clearance of interleukin-6 in humans during exercise. AmJPhysiol EndocrinolMetab 285:E397-E402

33. Febbraio MA, Pedersen BK (2005) Contraction,Induced myokine production and release: Is skeletal muscle an endocrine organ.? Exercise and Sport Sciences Reviews 33:114-119. doi:10.1097/00003677-200507000-00003
34. Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333-340
35. Galman C, Lundasen T, Kharitonenkov A, Bina HA, Eriksson M, Hafstrom I, Dahlin M, Amark P, Angelin B, Rudling M (2008) The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. Cell Metab 8:169-174
36. Gangopadhyay SS (2013) Systemic administration of follistatin288 increases muscle mass and reduces fat accumulation in mice. SciRep 3:2441
37. Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA, Lynch GS (2012) Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature 484:394-398
38. Gilson H, Schakman O, Kalista S, Lause P, Tsuchida K, Thissen JP (2009) Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. AmJPhysiol EndocrinolMetab 297:E157-E164
39. Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJ (2016) Liver glycogen metabolism during and after prolonged endurance-type exercise. Am J Physiol Endocrinol Metab 311:E543-553. doi:10.1152/ajpendo.00232.2016
40. Gusarova V, O'Dushlaine C, Teslovich TM, Benotti PN, Mirshahi T, Gottesman O, Van Hout CV, Murray MF, Mahajan A, Nielsen JB, Fritsche L, Wulff AB, Gudbjartsson DF, Sjogren M, Emdin CA, Scott RA, Lee WJ, Small A, Kwee LC, Dwivedi OP, Prasad RB, Bruse S, Lopez AE, Penn J, Marcketta A, Leader JB, Still CD, Kirchner HL, Mirshahi UL, Wardeh AH, Hartle CM, Habegger L, Fetterolf SN, Tusie-Luna T, Morris AP, Holm H, Steinthorsdottir V, Sulem P, Thorsteinsdottir U, Rotter JI, Chuang LM, Damrauer S, Birtwell D, Brummett CM, Khera AV, Natarajan P, Orho-Melander M, Flannick J, Lotta LA, Willer CJ, Holmen OL, Ritchie MD, Ledbetter DH, Murphy AJ, Borecki IB, Reid JG, Overton JD, Hansson O, Groop L, Shah SH, Kraus WE, Rader DJ, Chen YI, Hveem K, Wareham NJ, Kathiresan S, Melander O, Stefansson K, Nordestgaard BG, Tybjaerg-Hansen A, Abecasis GR, Altshuler D, Florez JC, Boehnke M, McCarthy MI, Yancopoulos GD, Carey DJ, Shuldiner AR, Baras A, Dewey FE, Gromada J (2018) Genetic inactivation of ANGPTL4 improves glucose homeostasis and is associated with reduced risk of diabetes. Nat Commun 9:2252. doi:10.1038/s41467-018-04611-z
41. Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, Pedersen BK, Plomgaard P (2010) Exercise induces a marked increase in plasma follistatin: Evidence that follistatin is a contraction-induced hepatokine. Endocrinology 152:164-171
42. Hansen J, Rinnov A, Krogh-Madsen R, Fischer CP, Andreasen AS, Berg RM, Moller K, Pedersen BK, Plomgaard P (2013) Plasma follistatin is elevated in patients with type 2 diabetes: relationship to hyperglycemia, hyperinsulinemia, and systemic low-grade inflammation. Diabetes Metab ResRev 29:463-472
43. Hansen JS, Clemmesen JO, Secher NH, Hoene M, Drescher A, Weigert C, Pedersen BK, Plomgaard P (2015) Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans. MolMetab 4:551-560
44. Hansen JS, Pedersen BK, Xu G, Lehmann R, Weigert C, Plomgaard P (2016) Exercise-induced secretion of FGF21 and follistatin are blocked by pancreatic clamp and impaired in type 2 diabetes. JClinEndocrinolMetab 101:2816-2825
45. Hansen JS, Plomgaard P (2016) Circulating follistatin in relation to energy metabolism. MolCell Endocrinol 433:87-93
46. Hansen JS, Plomgaard P (2016) Fibroblast growth factor 21: new insights from human studies. Cardiovascular Endocrinology 5:112-116. doi:10.1097/xce.0000000000000084

47. Hansen JS, Rutti S, Arous C, Clemmesen JO, Secher NH, Drescher A, Gonelle-Gisport C, Halban PA, Pedersen BK, Weigert C, Bouzakri K, Plomgaard P (2015) Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. JClinEndocrinolMetab 101:550-560
48. Heyworth CM, Wallace AV, Houslay MD (1983) Insulin and glucagon regulate the activation of two distinct membrane-bound cyclic AMP phosphodiesterases in hepatocytes. BiochemJ 214:99-110
49. Hill KE, Wu S, Motley AK, Stevenson TD, Winfrey VP, Capecchi MR, Atkins JF, Burk RF (2012) Production of selenoprotein P (Sepp1) by hepatocytes is central to selenium homeostasis. J Biol Chem 287:40414-40424. doi:10.1074/jbc.M112.421404
50. Hoene M, Franken H, Fritsche L, Lehmann R, Pohl AK, Haring HU, Zell A, Schleicher ED, Weigert C (2010) Activation of the mitogen-activated protein kinase (MAPK) signalling pathway in the liver of mice is related to plasma glucose levels after acute exercise. Diabetologia 53:1131-1141. doi:10.1007/s00125-010-1666-3
51. Hoene M, Weigert C (2010) The stress response of the liver to physical exercise. ExercImmunolRev 16:163-183
52. Hoffmann C, Weigert C (2017) Skeletal Muscle as an Endocrine Organ: The Role of Myokines in Exercise Adaptations. Cold Spring Harbor perspectives in medicine 7. doi:10.1101/cshperspect.a029793
53. Hong JH, Chung HK, Park HY, Joung KH, Lee JH, Jung JG, Kim KS, Kim HJ, Ku BJ, Shong M (2014) GDF15 Is a Novel Biomarker for Impaired Fasting Glucose. Diabetes & metabolism journal 38:472-479. doi:10.4093/dmj.2014.38.6.472
54. Hu C, Hoene M, Zhao X, Haring HU, Schleicher E, Lehmann R, Han X, Xu G, Weigert C (2010) Lipidomics analysis reveals efficient storage of hepatic triacylglycerides enriched in unsaturated fatty acids after one bout of exercise in mice. PLoSONE 5:e13318
55. Hunter WM, Fonseka CC, Passmore R (1965) Growth hormone: important role in muscular exercise in adults. Science 150:1051-1053
56. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA (2007) Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 5:415-425. doi:10.1016/j.cmet.2007.05.003
57. Ingerslev B, Hansen JS, Hoffmann C, Clemmesen JO, Secher NH, Scheler M, Hrabe de Angelis M, Haring HU, Pedersen BK, Weigert C, Plomgaard P (2017) Angiopoietin-like protein 4 is an exercise-induced hepatokine in humans, regulated by glucagon and cAMP. Mol Metab 6:1286-1295. doi:10.1016/j.molmet.2017.06.018
58. Jimenez V, Jambrina C, Casana E, Sacristan V, Munoz S, Darriba S, Rodo J, Mallol C, Garcia M, Leon X, Marco S, Ribera A, Elias I, Casellas A, Grass I, Elias G, Ferre T, Motas S, Franckhauser S, Mulero F, Navarro M, Haurigot V, Ruberte J, Bosch F (2018) FGF21 gene therapy as treatment for obesity and insulin resistance. EMBO molecular medicine. doi:10.15252/emmm.201708791
59. Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Fleshner M (2005) Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. JApplPhysiol (1985) 99:1789-1795
60. Kerschan-Schindl K, Thalmann MM, Weiss E, Tsironi M, Foger-Samwald U, Meinhart J, Skenderi K, Pietschmann P (2015) Changes in serum levels of myokines and wnt-antagonists after an ultramarathon race. PLoSONE 10:e0132478
61. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. Journal of Biological Chemistry 275:28488-28493
62. Kharitonenkov A, DiMarchi R (2015) FGF21 Revolutions: Recent Advances Illuminating FGF21 Biology and Medicinal Properties. Trends in endocrinology and metabolism: TEM 26:608-617. doi:10.1016/j.tem.2015.09.007
63. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB (2005) FGF-21 as a novel metabolic regulator. JClinInvest 115:1627-1635

64. Kim I, Kim HG, Kim H, Kim HH, Park SK, Uhm CS, Lee ZH, Koh GY (2000) Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. BiochemJ 346 Pt 3:603-610
65. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS (2013) Acute exercise induces FGF21 expression in mice and in healthy humans. PLoSONE 8:e63517
66. Kleinert M, Clemmensen C, Sjoberg KA, Carl CS, Jeppesen JF, Wojtaszewski JFP, Kiens B, Richter EA (2018) Exercise increases circulating GDF15 in humans. Mol Metab 9:187-191. doi:10.1016/j.molmet.2017.12.016
67. Koster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC, Yang DD, Heuer JG, Jaskunas SR, Eacho P (2005) Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. Endocrinology 146:4943-4950
68. Kota J, Handy CR, Haidet AM, Montgomery CL, Eagle A, Rodino-Klapac LR, Tucker D, Shilling CJ, Therlfall WR, Walker CM, Weisbrode SE, Janssen PM, Clark KR, Sahenk Z, Mendell JR, Kaspar BK (2009) Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. SciTranslMed 1:6ra15
69. Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L (2004) Exercise induces the release of heat shock protein 72 from the human brain in vivo. Cell StressChaperones 9:276-280
70. Lavoie JM, Fillion Y, Couturier K, Corriveau P (2002) Exercise Effects on Muscle Insulin Signaling and Action - Selected Contribution: Evidence that the decrease in liver glycogen is associated with the exercise-induced increase in IGFBP-1. Journal of Applied Physiology 93:798-804. doi:10.1152/japplphysiol.00125.2002
71. Lazennec G, Canaple L, Saugy D, Wahli W (2000) Activation of peroxisome proliferator-activated receptors (PPARs) by their ligands and protein kinase A activators. MolEndocrinol 14:1962-1975
72. Lee WC, Wen HC, Chang CP, Chen MY, Lin MT (2006) Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heatstroke. JApplPhysiol (1985) 100:2073-2082
73. Leong I (2018) Follistatin inactivation improves glucose tolerance. Nature reviews Endocrinology. doi:10.1038/s41574-018-0052-y
74. Leu JIJ, George DL (2007) Hepatic IGFBP1 is a prosurvival factor that binds to BAK, protects the liver from apoptosis, and antagonizes the proapoptotic actions of p53 at mitochondria. Genes & Development 21:3095-3109. doi:10.1101/gad/1567107
75. Liu CC, Lin CH, Lin CY, Lee CC, Lin MT, Wen HC (2013) Transgenic overexpression of heat shock protein 72 in mouse muscle protects against exhaustive exercise-induced skeletal muscle damage. JFormosMedAssoc 112:24-30
76. Liu HH, Wang JW, Yu HY, Zhang RP, Chen X, Jin HB, Dai F, Li L, Xu F (2012) Injection of duck recombinant follistatin fusion protein into duck muscle tissues stimulates satellite cell proliferation and muscle fiber hypertrophy. ApplMicrobiolBiotechnol
77. Loyd C, Magrisso IJ, Haas M, Balusu S, Krishna R, Itoh N, Sandoval DA, Perez-Tilve D, Obici S, Habegger KM (2016) Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. Journal of applied physiology (Bethesda, Md : 1985) 121:687-698. doi:10.1152/japplphysiol.00456.2016
78. Mai K, Andres J, Biedasek K, Weicht J, Bobbert T, Sabath M, Meinus S, Reinecke F, Mohlig M, Weickert MO, Clemenz M, Pfeiffer AF, Kintscher U, Spuler S, Spranger J (2009) Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. Diabetes 58:1532-1538
79. Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ (2014) Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 63:4057-4063

80. McPherron AC, Lawler AM, Lee SJ (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83-90
81. Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, Alfano LN, Berry K, Meadows E, Lewis S, Braun L, Shontz K, Rouhana M, Clark KR, Rosales XQ, Al-Zaidy S, Govoni A, Rodino-Klapac LR, Hogan MJ, Kaspar BK (2015) A phase 1/2a follistatin gene therapy trial for becker muscular dystrophy. MolTher 23:192-201
82. Michel U, Ebert S, Phillips D, Nau R (2003) Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia. EurJEndocrinol 148:559-564
83. Michel U, Shintani Y, Nau R (1998) Serum follistatin concentrations are increased in patients with septicaemia. ClinEndocrinol(Oxf) 48:413-417
84. Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K, Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Yamashita T, Honda M, Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim HJ, Lee IK, Minokoshi Y, Saito Y, Takahashi K, Yamada Y, Takakura N, Kaneko S (2010) A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab 12:483-495. doi:10.1016/j.cmet.2010.09.015
85. Misu H, Takayama H, Saito Y, Mita Y, Kikuchi A, Ishii KA, Chikamoto K, Kanamori T, Tajima N, Lan F, Takeshita Y, Honda M, Tanaka M, Kato S, Matsuyama N, Yoshioka Y, Iwayama K, Tokuyama K, Akazawa N, Maeda S, Takekoshi K, Matsugo S, Noguchi N, Kaneko S, Takamura T (2017) Deficiency of the hepatokine selenoprotein P increases responsiveness to exercise in mice through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle. Nature medicine 23:508-516. doi:10.1038/nm.4295
86. Nielsen HB, Clemmesen JO, Skak C, Ott P, Secher NH (2002) Attenuated hepatosplanchnic uptake of lactate during intense exercise in humans. Journal of applied physiology (Bethesda, Md : 1985) 92:1677-1683. doi:10.1152/japplphysiol.00028.2001
87. Nishimura T, Nakatake Y, Konishi M, Itoh N (2000) Identification of a novel FGF, FGF-21, preferentially expressed in the liver. BiochimBiophysActa 1492:203-206
88. Norheim F, Hjorth M, Langleite TM, Lee S, Holen T, Bindesboll C, Stadheim HK, Gulseth HL, Birkeland KI, Kielland A, Jensen J, Dalen KT, Drevon CA (2014) Regulation of angiopoietin-like protein 4 production during and after exercise. Physiol Rep 2. doi:10.14814/phy2.12109
89. O'Connor AE, McFarlane JR, Hayward S, Yohkaichiya T, Groome NP, de Kretser DM (1999) Serum activin A and follistatin concentrations during human pregnancy: a cross-sectional and longitudinal study. HumReprod 14:827-832
90. Oarada M, Tsuzuki T, Nikawa T, Kohno S, Hirasaka K, Gonoi T (2012) Refeeding with a high-protein diet after a 48 h fast causes acute hepatocellular injury in mice. BrJNutr 107:1435-1444
91. Okamoto H, Cavino K, Na E, Krumm E, Kim S, Stevis PE, Harp J, Murphy AJ, Yancopoulos GD, Gromada J (2017) Angptl4 does not control hyperglucagonemia or alpha-cell hyperplasia following glucagon receptor inhibition. Proceedings of the National Academy of Sciences of the United States of America 114:2747-2752. doi:10.1073/pnas.1620989114
92. Owen BM, Ding X, Morgan DA, Coate KC, Bookout AL, Rahmouni K, Kliewer SA, Mangelsdorf DJ (2014) FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. Cell Metab 20:670-677. doi:10.1016/j.cmet.2014.07.012
93. Peake JM, Tan SJ, Markworth JF, Broadbent JA, Skinner TL, Cameron-Smith D (2014) Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. Am J Physiol Endocrinol Metab 307:E539-552. doi:10.1152/ajpendo.00276.2014
94. Pedersen BK, Saltin B (2015) Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scandinavian Journal of Medicine & Science in Sports 25:1-72. doi:10.1111/sms.12581
95. Perko MJ, Nielsen HB, Skak C, Clemmesen JO, Schroeder TV, Secher NH (1998) Mesenteric, coeliac and splanchnic blood flow in humans during exercise. JPhysiol 513 (Pt 3):907-913

96. Pilegaard H, Saltin B, Neufer PD (2003) Effect of short-term fasting and refeeding on transcriptional regulation of metabolic genes in human skeletal muscle. Diabetes 52:657-662
97. Pourteymour S, Eckardt K, Holen T, Langleite T, Lee S, Jensen J, Birkeland KI, Drevon CA, Hjorth M (2017) Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines. Mol Metab 6:352-365. doi:10.1016/j.molmet.2017.01.007
98. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America 106:8665-8670. doi:10.1073/pnas.0903485106
99. Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, Cohen JC (2007) Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. NatGenet 39:513-516
100. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, Cohen JC (2009) Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. JClinInvest 119:70-79
101. Rosendal L, Sogaard K, Kjaer M, Sjogaard G, Langberg H, Kristiansen J (2005) Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. Journal of Applied Physiology 98:477-481. doi:10.1152/japplphysiol.00130.2004
102. Salo DC, Donovan CM, Davies KJ (1991) HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. Free RadicBiolMed 11:239-246
103. Sargeant JA, Aithal GP, Takamura T, Misu H, Takayama H, Douglas JA, Turner MC, Stensel DJ, Nimmo MA, Webb DR, Yates T, King JA (2018) The influence of adiposity and acute exercise on circulating hepatokines in normal-weight and overweight/obese men. Appl Physiol Nutr Metab 43:482-490. doi:10.1139/apnm-2017-0639
104. Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj M, Huang CJ (2015) Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese individuals. NutrMetab CardiovascDis 25:839-845
105. Soberg S, Sandholt CH, Jespersen NZ, Toft U, Madsen AL, von Holstein-Rathlou S, Grevengoed TJ, Christensen KB, Bredie WLP, Potthoff MJ, Solomon TPJ, Scheele C, Linneberg A, Jorgensen T, Pedersen O, Hansen T, Gillum MP, Grarup N (2017) FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans. Cell Metab 25:1045-1053.e1046. doi:10.1016/j.cmet.2017.04.009
106. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB (2008) Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 57:2762-2767. doi:10.2337/db08-0538
107. Stefan N, Haring HU (2013) The role of hepatokines in metabolism. NatRevEndocrinol 9:144-152
108. Streja DA, Marliss EB, Steiner G (1977) The effects of prolonged fasting on plasma triglyceride kinetics in man. Metabolism 26:505-516
109. Sugiyama M, Kikuchi A, Misu H, Igawa H, Ashihara M, Kushima Y, Honda K, Suzuki Y, Kawabe Y, Kaneko S, Takamura T (2018) Inhibin beta E (INHBE) is a possible insulin resistance-associated hepatokine identified by comprehensive gene expression analysis in human liver biopsy samples. Plos One 13. doi:10.1371/journal.pone.0194798
110. Sukonina V, Lookene A, Olivecrona T, Olivecrona G (2006) Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. ProcNatlAcadSciUSA 103:17450-17455
111. Takala J (1996) Determinants of splanchnic blood flow. British journal of anaesthesia 77:50-58. doi:10.1093/bja/77.1.50
112. Talukdar S, Zhou Y, Li D, Rossulek M, Dong J, Somayaji V, Weng Y, Clark R, Lanba A, Owen BM, Brenner MB, Trimmer JK, Gropp KE, Chabot JR, Erion DM, Rolph TP, Goodwin B, Calle RA (2016) A Long-Acting FGF21 Molecule, PF-05231023, Decreases Body Weight and Improves Lipid Profile in Non-

human Primates and Type 2 Diabetic Subjects. Cell Metab 23:427-440. doi:10.1016/j.cmet.2016.02.001
113. Tao R, Wang C, Stohr O, Qiu W, Hu Y, Miao J, Dong XC, Leng S, Stefater M, Stylopoulos N, Lin L, Copps KD, White MF (2018) Inactivating hepatic follistatin alleviates hyperglycemia. Nature medicine 24:1058-1069. doi:10.1038/s41591-018-0048-0
114. Thiessen SE, Vanhorebeek I, Derese I, Gunst J, Van den Berghe G (2015) FGF21 response to critical illness: effect of blood glucose control and relation with cellular stress and survival. JClinEndocrinolMetab 100:E1319-E1327
115. Tjeerdema N, Georgiadi A, Jonker JT, van Glabbeek M, Alizadeh Dehnavi R, Tamsma JT, Smit JW, Kersten S, Rensen PC (2014) Inflammation increases plasma angiopoietin-like protein 4 in patients with the metabolic syndrome and type 2 diabetes. BMJ Open Diabetes Res Care 2:e000034. doi:10.1136/bmjdrc-2014-000034
116. Trefts E, Williams AS, Wasserman DH (2015) Exercise and the Regulation of Hepatic Metabolism. Progress in molecular biology and translational science 135:203-225. doi:10.1016/bs.pmbts.2015.07.010
117. Vamvini MT, Aronis KN, Chamberland JP, Mantzoros CS (2011) Energy deprivation alters in a leptin- and cortisol-independent manner circulating levels of activin A and follistatin but not myostatin in healthy males. JClinEndocrinolMetab 96:3416-3423
118. von Holstein-Rathlou S, BonDurant LD, Peltekian L, Naber MC, Yin TC, Claflin KE, Urizar AI, Madsen AN, Ratner C, Holst B, Karstoft K, Vandenbeuch A, Anderson CB, Cassell MD, Thompson AP, Solomon TP, Rahmouni K, Kinnamon SC, Pieper AA, Gillum MP, Potthoff MJ (2016) FGF21 Mediates Endocrine Control of Simple Sugar Intake and Sweet Taste Preference by the Liver. Cell Metab 23:335-343. doi:10.1016/j.cmet.2015.12.003
119. Wallace JD, Cuneo RC, Baxter R, Orskov H, Keay N, Pentecost C, Dall R, Rosen T, Jorgensen JO, Cittadini A, Longobardi S, Sacca L, Christiansen JS, Bengtsson BA, Sonksen PH (1999) Responses of the growth hormone (GH) and insulin-like growth factor axis to exercise, GH administration, and GH withdrawal in trained adult males: a potential test for GH abuse in sport. J Clin Endocrinol Metab 84:3591-3601. doi:10.1210/jcem.84.10.6037
120. Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA (2001) Exercise increases serum Hsp72 in humans. Cell StressChaperones 6:386-393
121. Wang X, Wei W, Krzeszinski JY, Wang Y, Wan Y (2015) A Liver-Bone Endocrine Relay by IGFBP1 Promotes Osteoclastogenesis and Mediates FGF21-Induced Bone Resorption. Cell Metab 22:811-824. doi:10.1016/j.cmet.2015.09.010
122. Whitham M, Laing SJ, Jackson A, Maassen N, Walsh NP (2007) Effect of exercise with and without a thermal clamp on the plasma heat shock protein 72 response. JApplPhysiol (1985) 103:1251-1256
123. Winder WW (1988) Role of cyclic AMP in regulation of hepatic glucose production during exercise. MedSciSports Exerc 20:551-559
124. Wolfe RR, Klein S, Carraro F, Weber JM (1990) Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. Am J Physiol 258:E382-389. doi:10.1152/ajpendo.1990.258.2.E382
125. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, Lam KS (2005) Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. ProcNatlAcadSciUSA 102:6086-6091
126. Yalley A, Schill D, Hatta M, Johnson N, Cirillo LA (2016) Loss of Interdependent Binding by the FoxO1 and FoxA1/A2 Forkhead Transcription Factors Culminates in Perturbation of Active Chromatin Marks and Binding of Transcriptional Regulators at Insulin-sensitive Genes. J Biol Chem 291:8848-8861. doi:10.1074/jbc.M115.677583
127. Yamada P, Amorim F, Moseley P, Schneider S (2008) Heat shock protein 72 response to exercise in humans. Sports Med 38:715-733

128. Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, Wu X, Huang T, Hultman K, Paulsen SJ, Wang J, Bugge A, Frantzen JB, Norgaard P, Jeppesen JF, Yang Z, Secher A, Chen H, Li X, John LM, Shan B, He Z, Gao X, Su J, Hansen KT, Yang W, Jorgensen SB (2017) GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nature medicine 23:1158-1166. doi:10.1038/nm.4394
129. Yoshida K, Ono M, Koishi R, Furukawa H (2004) Characterization of the 5' regulatory region of the mouse angiopoietin-like protein 4. VetResCommun 28:299-305
130. Yoshida K, Shimizugawa T, Ono M, Furukawa H (2002) Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. JLipid Res 43:1770-1772
131. Yu X, Burgess SC, Ge H, Wong KK, Nassem RH, Garry DJ, Sherry AD, Malloy CR, Berger JP, Li C (2005) Inhibition of cardiac lipoprotein utilization by transgenic overexpression of Angptl4 in the heart. ProcNatlAcadSciUSA 102:1767-1772
132. Zhang M, Sun W, Qian J, Tang Y (2018) Fasting exacerbates hepatic growth differentiation factor 15 to promote fatty acid beta-oxidation and ketogenesis via activating XBP1 signaling in liver. Redox biology 16:87-96. doi:10.1016/j.redox.2018.01.013
133. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432

Table 1. Transcripts of potential secreted hepatokines differentially regulated immediately after 60 min non-exhaustive treadmill exercise in mice

Gene Symbol	Gene Description	FC	sed	Run	SL	ECM	Accession
Igfbp1	insulin-like growth factor binding protein 1	32.3	46.3	1496	yes		P47876
Fgf21	fibroblast growth factor 21	26.5	bd	26.5	yes		Q9JJN1
Serpine2	serine peptidase inhibitor, clade E, member 2	18.3	bd	18.3	yes	yes	Q07235
Esm1	endothelial cell-specific molecule 1	12.1	2.2	26.6	yes		Q9QYY7
Impg2	interphotoreceptor matrix proteoglycan 2	11.0	bd	11		yes	Q80XH2
Ccl5	chemokine (C-C motif) ligand 5	9.1	1.3	11.8	yes		P30882
Chgb	chromogranin B	8.8	1.3	11.5	yes		P16014
Gpc3	glypican 3	8.8	2.3	20.2	yes	yes	Q8CFZ4
Matn2	matrilin 2	7.0	bd	7	yes	yes	O08746
Ang2	angiogenin	6.6	3.4	22.4	yes		Q64438
Ephb6	Eph receptor B6	6.4	2.2	14	yes		O08644
Il1b	interleukin 1 beta	6.0	10.2	61.7	yes		P10749
Adm2	adrenomedullin 2	5.8	1.8	10.4	yes		Q7TNK8
Serpine1	serine peptidase inhibitor, clade E, member 1	5.4	2.2	11.8	yes	yes	P22777
Sbsn	suprabasin	4.7	2.3	10.9	yes	yes	Q8CIT9
Podn	podocan	4.7	4	18.9	yes	yes	Q7TQ62
Col4a4	collagen, type IV, alpha 4	4.5	4	18.1	yes	yes	Q9QZR9
Acrbp	proacrosin binding protein	4.5	bd	4.5	yes		Q3V140

Cxadr	coxsackie virus and adenovirus receptor	4.5	bd	4.5	yes		P97792
Tfpi	tissue factor pathway inhibitor	4.4	bd	4.4	yes		O54819
Pcolce2	procollagen C-endopeptidase enhancer 2	4.3	2.9	12.4	yes		Q8R4W6
Edn1	endothelin 1	3.9	2.7	10.5	yes		P22387
Pcsk5	proprotein convertase subtilisin/kexin type 5	3.5	3.6	12.5	yes		Q04592
Efna1	ephrin A1	3.4	304	1033	yes		P52793
Igfbp3	insulin-like growth factor binding protein 3	3.4	6	20.3	yes		P47878
Xcl1	chemokine (C motif) ligand 1	3.4	3.7	12.4	yes		P47993
Angptl4	angiopoietin-like 4	3.2	1056	3334	yes	yes	Q9Z1P8
Lrch3	leucine-rich repeats and CH domain 3	3.1	bd	3.1	yes		Q8BVU0
Gdf15	growth differentiation factor 15	3.1	38.3	118.6	yes		Q9Z0J7
Scube2	signal peptide, CUB domain, EGF-like 2	3.0	3.6	10.7	yes		D3YVM9
Hist2h4	histone cluster 2, H4	3.0	3.6	10.7		yes	P62806
Cxcl13	chemokine (C-X-C motif) ligand 13	3.0	7.8	23.1	yes		O55038
Col11a2	collagen, type XI, alpha 2	2.9	6.2	18	yes	yes	Q64739
Ran	RAN, member RAS oncogene family	2.9	bd	2.9		yes	P62827
Ccl6	chemokine (C-C motif) ligand 6	2.9	6.5	18.7	yes		P27784
Gdf5	growth differentiation factor 5	2.8	5.3	14.9	yes		P43027
Plaur	plasminogen activator, urokinase receptor	2.8	4.5	12.4	yes		P35456
Fst	Follistatin	2.7	32.3	88.2	yes		P47931
Cfl1	cofilin 1, non-muscle	2.7	bd	2.7		yes	P18760
Smpdl3b	sphingomyelin phosphodiesterase, acid-like	2.6	7.9	20.9	yes		P58242
Ctgf	connective tissue growth factor	2.5	63	160.5	yes	yes	P29268
Fbn1	fibrillin 1	2.5	5.8	14.6	yes	yes	Q61554
Cd14	CD14 antigen	2.5	6.9	17.2	yes		P10810
Fbln5	fibulin 5	2.5	1.9	4.7	yes	yes	Q9WVH9
Serpina7	serine (or cysteine) peptidase inhibitor	2.5	81	199.4	yes		P61939
Gsn	Gelsolin	2.4	4.7	11.1	yes		P13020
Lepr	leptin receptor	2.4	4	9.4	yes		P48356
Tomm20	translocase of outer mitochondrial membrane 20 homolog (yeast)	2.3	3.8	8.9		yes	Q9DCC8
Fgfr2	fibroblast growth factor receptor 2	2.2	7.7	17.2		yes	P21803
Loxl3	lysyl oxidase-like 3	2.2	2.2	4.8	yes		Q9Z175
Mep1b	meprin 1 beta	2.2	9.2	19.8	yes		Q61847
Thbs2	thrombospondin 2	2.1	10.3	21.9		yes	Q03350
Lifr	leukemia inhibitory factor receptor	2.1	16	33.2	yes		P42703
Insl5	insulin-like 5	2.0	5.2	10.4	yes		Q9WUG6
S100a8	S100 calcium binding protein A8	2.0	2.7	5.4	yes		P27005
Itgb4	integrin beta 4	0.5	12.8	6.4		yes	A2A863
Prok2	prokineticin 2	0.5	10.4	5.1	yes		Q9QXU7
Camp	cathelicidin antimicrobial peptide	0.5	17.8	8.5	yes		P51437
Il1a	interleukin 1 alpha	0.5	2.1	Bd	yes		P01582
Cort	Cortistatin	0.5	10.1	4.6	yes		P56469
Cxcl1	chemokine (C-X-C motif) ligand 1	0.4	165.9	73.1	yes		P12850
Bmp5	bone morphogenetic protein 5	0.4	2.3	Bd	yes		P49003
Rpn1	ribophorin I	0.4	14.2	6.1		yes	Q91YQ5
Retn	Resistin	0.4	20.7	8.8	yes		Q99P87
Tub	tubby candidate gene	0.4	11.3	4.7	yes		P50586
Nodal	Nodal	0.4	13.6	5.5	yes		P43021
Trh	thyrotropin releasing hormone	0.4	10.3	4.1	yes		Q62361
Casp14	caspase 14	0.4	10.9	4		yes	O89094
Anxa1	annexin A1	0.3	14.7	5.1	yes		P10107
Mmp15	matrix metallopeptidase 15	0.3	25.2	8.6		yes	O54732
Col1a2	collagen, type I, alpha 2	0.3	3	Bd	yes	yes	Q01149
Fgl2	fibrinogen-like protein 2	0.3	23.7	7.7	yes		P12804
Fgf22	fibroblast growth factor 22	0.3	9.3	3	yes		Q9ESS2
Ndfip1	Nedd4 family interacting protein 1	0.3	3.1	Bd	yes		Q8R0W6
Icam4	intercellular adhesion molecule 4	0.3	14.6	4.6	yes		Q9ERM2

Angptl2	angiopoietin-like 2	0.3	3.9	Bd	yes		Q9R045
Pla2g10	phospholipase A2, group X	0.2	16.3	3.7	yes		Q9QXX3
Ucn	Urocortin	0.2	4.5	Bd	yes		P81615
Inhbe	inhibin beta E	0.2	109.5	18.2	yes		O08717
Cxcl14	chemokine (C-X-C motif) ligand 14	0.2	6.1	Bd	yes		Q9WUQ5
Cgref1	cell growth regulator with EF hand domain 1	0.1	7.6	Bd	yes		Q8R1U2
Col12a1	collagen, type XII, alpha 1	0.1	11.6	1.2	yes	yes	Q60847
Cfh	complement component factor h	0.1	11.1	Bd	yes		P06909
Ccl1	chemokine (C-C motif) ligand 1	0.1	18.4	1.2	yes		P10146

Transcripts with 2-fold regulation and secreted protein annotation (SL-0243, Uniprot) or annotation as extracellular matrix protein (ECM; GO:0031012) are shown. Mice ran 60 min at 14 m/min and 14° uphill inclination on an electric treadmill and livers obtained immediately after the run. Sedentary mice remained in their cages. Sed, run; Signal intensity of transcripts on Illumina mouseRef-8 v1.0 Expression bread Chip array (see (Illumina, San Diego, CA, USA) and respective Swissprot accession number. Experimental conditions are described in reference [50].

Figure 1.

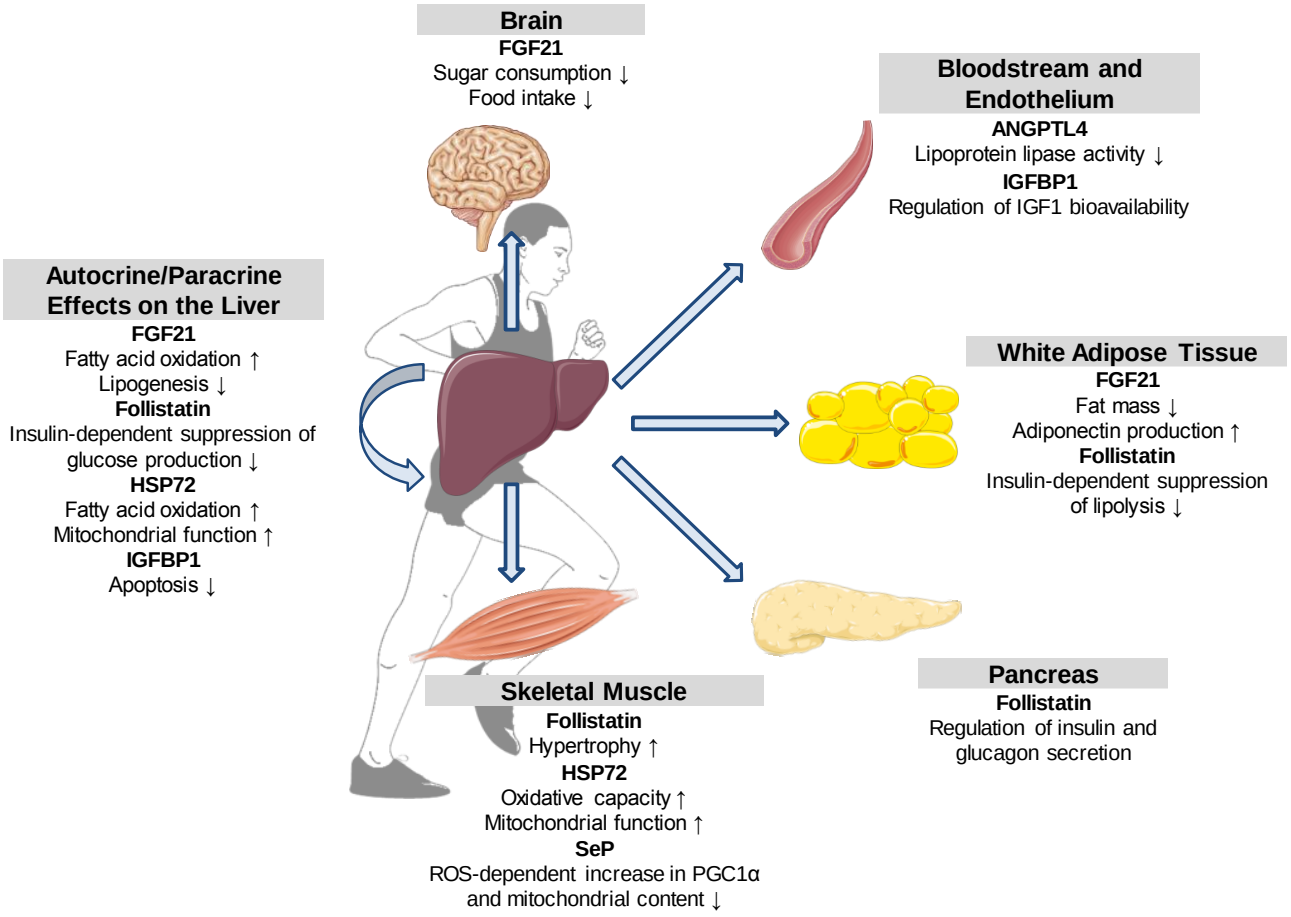

Figure 1. Potential effects of exercise-regulated hepatokines.

Hepatokines can act in an autocrine/paracrine manner on the liver and as endocrine factors on brain, endothelium, adipose tissue, pancreas and skeletal muscle. In most cases, an acute bout of exercise increases the systemic concentration of hepatokines. Selenoprotein P (SeP) is not regulated by exercise but reduces production of reactive oxygen species (ROS) in the exercising skeletal muscle in an endocrine manner. This figure was created using illustrations provided by Servier medical art.

Figure 2

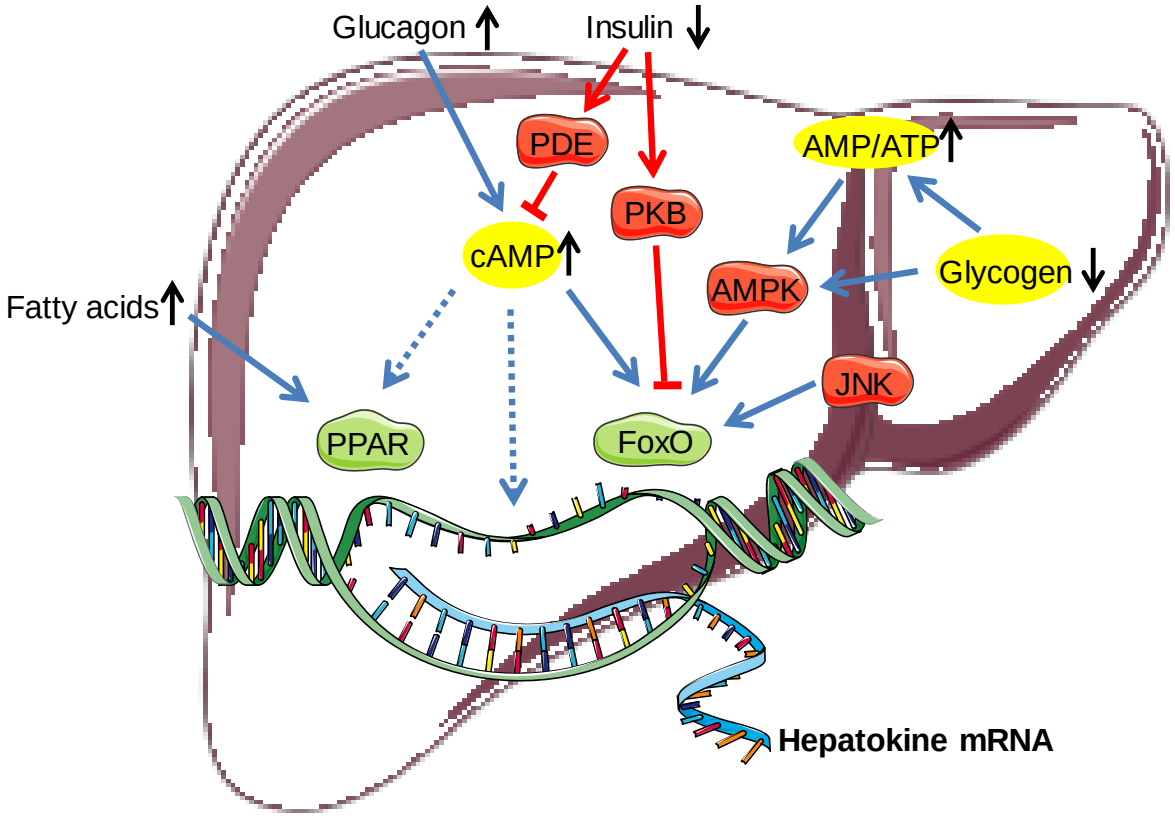

Figure 2. Regulation of hepatokines during exercise.

The increase in the glucagon-to-insulin ratio during exercise leads to elevated cAMP levels via stimulation of the adenyl cyclase and inactivation of phosphodiesterase (PDE). This can activate Forkhead box (Fox)O, peroxisome proliferator-activated receptors (PPAR) and other factors. This increases the transcription of fibroblast growth factor (FGF)21, follistatin, angiopoietin protein-like 4 (ANGPTL4) and insulin like growth factor binding protein (IGFBP)1. Hepatic energy depletion activates FoxO via 5' AMP-activated protein kinase (AMPK) and activation of c-Jun N-terminal kinase (JNK). Insulin inactivates FoxO via activation of protein kinase (PKB) and reduces cAMP levels. Fatty acid signalling via PPARs can contribute to the transcriptional regulation of hepatokines. This figure was created using illustrations provided by Servier medical art.

Thank you for your thorough reading and commenting on our manuscript. This has really improved our message and readability. We have prepared a point-to-point answers replying the critique raised.

Reviewer #1: OVERALL
The present manuscript "Hepatokines - a novel group of exercise factors" provides a very interesting, informative and thorough review on the current literature and view on hepatokines. The two figures support the text well.
RESPONSE: Thank you for your positive evaluation and enthusiasm for the livers endocrinology in relation to exercise.

SPECIFIC
My suggestions are towards minor parts/sentences aiming to make it easier for the reader. In addition, it is suggested to ensure that the tissue (liver, skeletal muscle) involved and the nature of the measure (plasma, mRNA , protein) are presented throughout the text when referring to previous findings.

RESPONSE: We apologies for the unclear sentence and appreciate the suggestions, which has improved the manuscript. In addition to the specific points raised by the reviewer, we included further revisions in the manuscript to improve the understanding and clarity.

1) It is suggested that more paragraphs are used. Hence, more often use a new paragraph to help the reader follow the focus of the text. Here some examples:
Page 3, line 31-32
RESPONSE: Paragraphs inserted.
Page 6, line 15/16; line 30/31; line 43/44 (the two last might be one paragraph)
RESPONSE: Paragraphs inserted.
Page 7, maybe line 36/37; line 54/55
RESPONSE: Paragraphs inserted.
Page 8, Line 51/52
RESPONSE: Paragraphs inserted.
Page 9, line 9-10
RESPONSE: Paragraphs inserted.
Page 10, line 57/58
RESPONSE: Paragraphs inserted.
Page 11, line 19/20
RESPONSE: Paragraphs inserted.
Page 11, line 56/57
RESPONSE: Paragraphs inserted.
Page 13, line 45
RESPONSE: We changed the order of sentences to improve the clarity of the paragraph.

2) Some sentences are a bit difficult to follow. It is suggested to consider the sentences below:
Page 2: First sentence in abstract: may use "Regular physical activity does not only improve…" and avoid too many "benefit/beneficial".

RESPONSE: thank you, this have been corrected.

Page 2, abstract: consider to revise "…organokines have emerged released from"
Last sentence of abstract: may add "These hepatokines may convey some of the exercise training-induced whole-body…"

RESPONSE: thank you, the sentence has been corrected.

Page 3, line 14-16

RESPONSE: a paragraph has been inserted.

Page 3 line 40-41: "secreted to peripheral organs". Is this a reasonable statement? Should it be: "secreted to the blood with concomitant effects on peripheral organs"?

RESPONSE: Thank you, the sentence has been corrected.

Page 5, line 19-20: "…physical activity must not necessarily…"; may consider: "….because an increased release of proteins is not necessarily reflected in an elevated transcript level, if post-translational regulation or ……."

RESPONSE: Thank you, the sentence has been corrected.

Page 5, line 36-37: "independent from"?? "of" or "in the resting state"?

RESPONSE: Thank you, yes this sentence was unclear and has been corrected.

Page 7. Line 4-7

RESPONSE: a paragraph has been inserted.

Page 7, line 14-18 suggesting: "This is supported by impaired exercise-induced adaptations in FGF21-deficient mice, which failed to improve glucose tolerance and reduce hepatic triglyceride content as observed in wildtype mice on high fat diet".

RESPONSE: Thank you, the sentence adjusted accordingly.

Page 7, line 36-37: does this fit in here? An effect of follistatin is presented, but otherwise regulation of follistatin.

RESPONSE: Thank you, the sentence has been removed.

Page 7, line 49/50: "blunting the glucagon-to-insulin ratio" ? This seems to lack "change of the ratio"?

RESPONSE: Thank you, yes agree the sentence has been corrected.

Page 8, line8/9: "dysregulated". Is this the intended word/meaning?

RESPONSE: Thank you, dysregulated has been removed.

Page 8, line 23: Is it clear why TGF-beta is brought up?

RESPONSE: Thank you, this has been clarified.

Page 8. Line 57; suggesting: "free fatty acids (FFA) increase during fasting (109) acting as ligand for the PPARs. "

RESPONSE: Thank you, this has been corrected.

Page 9, line 9-18

RESPONSE: Thank you, a paragraph inserted.

Page 9, line 26/27: may use: "Based on tissue-specific overexpression models, …"

RESPONSE: Thank you, the sentence has been adjusted accordingly.

Page 9, line 39-41: "Nevertheless, the acute regulation in plasma could suggest". May adjust to: "Nevertheless, the acute regulation of the plasma ANGPTL4 level may suggest an endocrine.."

RESPONSE: Thank you, the sentence has been adjusted accordingly.

Page 9, heading: Is it clear from the heading how this section is different from the parts on regulation in the sections above? Should this heading maybe include "intracellular mediated regulation"?

RESPONSE: Thank you, the heading has been adjusted accordingly.

Page 10: line 10/11: This has been said before.

RESPONSE: Thank you, the sentence has been removed.

Page 10, line 52-53 "across the liver at rest"?

RESPONSE: Thank you, this has been clarified.

Page 12: heading - Maybe use: "Exercise-regulated hepatokine candidates"

RESPONSE: We removed the heading but clarified in the respective paragraphs that the hepatic origin of these factors has to be clarified.

Page 12, line 26-29

RESPONSE: Thank you, a paragraph has been inserted.

Page 13, line 14-17: In what tissue was these observations obtained? Maybe adjust to "compared with wildtype mice when mice on HFD were exercise trained". Not clear how ROS come into the picture. This seems to require some supportive explanation.

RESPONSE: Thank you, we clarified these points.

Page 13, in Concluding remarks: A new protein (fetuin-A) is mentioned and the terms "normoglycemic" and "dysglycemic" are introduced here. Why not in the main text? Moreover, is it relevant to include a reference in "concluding remarks? Could this instead be given in the main text.

RESPONSE: Thank you, this has been removed from the conclusion paragraph as circulating fetuin A is not regulated by exercise.

3) There are in some of the paragraphs many VERY short sentences after each other. It is suggested to consider to link or merge some of these sentences to make the text more fluent.
Page 6, FGF21 section.
Page 7, Follistatin section:
RESPONSE: Thank you, excellent point, the short sentences has been changed so the text is less staccato.

4) Table/Figure legends:

a) Table 1 legend: please give the time after exercise. "Immediately after exercise"?
This is clearly important for acute exercise-induced mRNA responses.

RESPONSE: Thank you, this has been clarified in the heading and legend of table1.

b) Figure 1 legend, line 40: "exercise induces…" changed to "exercise increases.."
"but reduces production of ROS". Has this been shown or is it speculative? Is that clear from this legend?

RESPONSE: This has now been clarified in the paragraph about SeP and the legend.

c) Figure 2 legend, line 37/38. Should there be a "stop" after "factors" and then new sentence? "This leads to increased transcription of…"
Line 40-41: suggesting: "Hepatic energy depletion activated FoxO via activation of AMPK and JNK."

RESPONSE: Thank you, this has been clarified in the figure legend.