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Role of exercise-induced hepatokines in metabolic disorders.

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Exercise, Hepatokines and Metabolic Disorders

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Abstract

The health-promoting effects of physical activity to prevent and treat metabolic disorders are numerous. However, the underlying molecular mechanisms are not yet completely deciphered. In recent years, studies have referred to the liver as an endocrine organ, since it releases specific proteins called hepatokines. Some of these hepatokines are involved in whole-body metabolic homeostasis and are theorised to participate in the development of metabolic disease. In this regard, the present review describes the role of FGF21, Fetuin-A, Angiopoietin-like protein 4 and Follistatin in metabolic disease and their production in response to acute exercise. Also, we discuss the potential role of hepatokines in mediating the beneficial effects of regular exercise and the future challenges to the discovery of new exercise-induced hepatokines.

Introduction

The prevalence of metabolic disorders is increasing worldwide and appears as a major public health concern. Management of these pathologies is complicated as practitioners are facing a wide range of new co-morbidities. This is exemplified by the increase of non-alcoholic fatty liver diseases (NAFLD), a spectrum of chronic liver conditions including non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) (22). NAFL is defined as a presence of $\geq$ 5% hepatic steatosis without any sign of hepatocyte injury while NASH is present with $\geq$ 5% hepatic steatosis with inflammation and hepatocyte injury (ballooning) with or without fibrosis (22). For example, it has been underlined that 75 to 100 million people in the United States are suffering from NAFLD (131) and it is the most common liver abnormality in children aged 2 to 19 years (166). While it is well accepted that genetic factors play a significant role, poor quality of life in terms of physical activity and nutrition are also major risk factors in the aetiology of these pathologies (25, 117). In most cases, changing these unhealthy habits is sufficient to significantly improve the physiological profile of patients with obesity, type 2 diabetes (T2D) or NAFLD (2, 95, 161). Also, emerging hypothesis associate NAFLD and the pathogenesis of extrahepatic diseases such as T2D or cardiovascular diseases (CVD). Indeed, elegant reviews report that NAFLD represents an independent risk factor for the incidence of T2D or CVD (1, 113, 151). Moreover, it appears that ectopic fat in the liver represents a stronger risk factor of different stages of prediabetes, insulin resistance or CVD than total or visceral fat mass (37, 74, 153). While hepatic inflammation represents a good candidate (3), the underlying mechanisms linking NAFLD and extrahepatic diseases remain to be deciphered.

The beneficial effects of exercise at the whole-body level are numerous, with particular reference, here, to the adaptive responses occurring in many organs conferring protection

against metabolic diseases, such as obesity and T2D (16). In order to optimise the prescription of physical activity, investigators seek to better understand the underlying mechanisms involved in the beneficial effects of exercise. Recent studies suggest that myokines, released by the skeletal muscle at rest and/or during exercise, might be partially involved (35, 123, 165). Exercise-induced myokines can act locally to regulate skeletal muscle energy metabolism by improving insulin sensitivity, mitochondrial function or inflammation. Myokines also participate in the cross talk during and after exercise between skeletal muscles and other organs (ie: adipose tissues, liver, prancreas). Furthermore, studies show that the liver can also release proteins, referred to here as hepatokines, which may alter whole-body homeostasis at rest and during exercise. Hepatokines can be either beneficial or deleterious in the context of metabolic disease by regulating signalling pathways involved in energy metabolism (109, 149). Interestingly, recent evidence suggests that exercise can modulate the expression of some hepatokines, suggesting that the liver might also participate in tissue cross-talk during physical activity (57, 60, 68).

This review aims to summarise the current literature on hepatokines and their regulation by acute and chronic exercise in the context of metabolic disorders such as obesity, insulin-resistance, T2D, NAFL and NASH.

Hepatokines and metabolic diseases

Liver is a major metabolic organ. It serves as a site of storage and supply of nutrients to ensure metabolic homeostasis. Also, evidence supports that hepatocytes can produce and secrete proteins named as hepatokines (109, 149). Early studies in the area reported that a liver-derived protein, alpha 2-HS Glycoprotein, also known as fetuin-A, can inhibit insulin tyrosine kinase activation and might play a role in the pathogenesis of metabolic disorders (9,

145). However, it is only recently that the progression of NAFLD worldwide (131) has generated great interest in hepatokines. Recent study from Xiong and collaborators compared the liver transcriptome and proteome of control and mice with diet-induced NASH (170). RNA-sequence and proteomic analyses revealed that a total of 156 targets were altered at both mRNA and protein levels. Moreover, the authors reported a profound reprogramming of the hepatic secretome and membrane receptor gene expression during diet-induced NASH. Thus, these results suggest that hepatokine production could remodel metabolic homeostasis. This is exemplified by a number of studies revealing that hepatokines play a pivotal role in metabolism and contribute to the development of obesity, insulin-resistance, T2D, NAFL and NASH (109, 149). So far, about twenty hepatokines have been described to be involved in the regulation of energy and nutrient metabolism by acting directly on the liver or on distal target tissues (Table 1). These proteins regulate glucose and lipid metabolism in the liver, but also in the skeletal muscle or the adipose tissue. Moreover, hepatokines participate in inflammation, beta cell function or mitochondrial function and could participate in the development of CVD. For example, the hepatokine selenoprotein P has been reported to be increased in patients with NAFLD or visceral obesity (27) and to contribute to the development of insulin resistance (110). Selenoprotein P also inhibits vascular endothelial growth factor-stimulated cell proliferation, tubule formation, and migration in human umbilical vein endothelial cells (69). Thus, hepatokines can participate in inter-tissue crosstalk and play an influential role in hepatic and extra hepatic diseases.

Hepatokines and exercise

The beneficial effects of exercise in the context of metabolic disorders are numerous. A recent meta-analysis revealed that exercise, independently of weight loss, improves hepatic steatosis (154). Researches are hence focusing on understanding the molecular mechanism mediating

the health promoting effect of regular exercise. One plausible explanation resides in exercise secreted factors. Firstly, due to its role in locomotion, research has focused on skeletal muscle. The most well-characterized myokine is Interleukin-6 (IL-6) (118, 148). Initial work reported that IL-6 is released and secreted by the contracting skeletal muscle during exercise and stimulates hepatic glucose production to ensure the energy demands of the contracting muscle are adequately met (39). Thus, muscle-derived IL-6 works as an energy sensor to increase release of energy substrates from liver and adipose tissues (124). Secondly, due to its central role in obesity-associated disorders, adipose tissue and adipokines have been investigated. While the effects of a single bout of exercise are modest, exercise training can remodel adipokine expression and secretion. In patients with type 2 diabetes, a recent meta-analysis showed that an aerobic exercise program was associated with a significant change in leptin but did not alter adiponectin levels (63). Finally, studies reported that exercise can trigger the secretion of liver-derived proteins in response to exercise. Using hepatic arterial-to-venous difference, it has been shown that a 1-h single bout of cycling increases HSP72 release from the liver (41). Also, transcriptomic analyses in the liver revealed that exercise induces changes in the mRNA of secreted proteins suggesting that exercise can impact liver secretome (64). It is now clear that a single session of exercise is accompanied by the production of liver-secreted proteins. Hepatokines can also mediate the beneficial effects of chronic exercise or, at least, represent biomarkers of training-induced metabolic improvements (table 1). Interestingly, it has also been reported that selenoprotein-P deficiency increases responsiveness to exercise in mice through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle (111), suggesting that liver-secreted proteins can influence exercise capacity.

Here, we review the current literature on exercise-induced hepatokines implicated in the regulation of metabolism and metabolic diseases. Hepatokines with i) proven release from the

liver using arterial-to-venous difference over the splanchnic bed and increased hepatic mRNA expression (FGF21, Follistatin ANGPTL4) and/or ii) a clear role in the beneficial adaptation to chronic exercise (Fetuin-A) were specifically studied in this review.

Fibroblast growth factor 21 (FGF21)

FGF21 is a 24kDa protein that signals through a cell-surface receptor complex composed of a classic FGF receptor, FGFR1c, and the FGF coreceptor, β-klotho (87, 115). It appears that FGF21 is highly expressed in the liver in both rodent and human (115, 126). Also, while a broad range of tissues are expressing FGF21 (45), it should be specified that, under physiological conditions, FGF21 gene expression is increased in the liver and to a lesser extent the brain (156) and the pancreas (79).

A/ FGF21 in metabolic diseases

Since circulating FGF-21 concentrations increase with obesity (14, 179), T2D (24, 103)and NAFLD (91), FGF21 levels have been reported as a marker of metabolic disorders (122). Also, it is important to notice that, independently of BMI, hepatic triglyceride content is the strongest determinant of hepatic FGF21 production and circulating FGF21 (91, 173). Mechanistically, it appears that high fructose consumption leads to an increase of FGF21 in mice and humans through the activation of ChREBP in the liver (42). Lessons from transgenic mice have contributed considerably to our understanding of the role of FGF21 in energy metabolism regulation. Whole body FGF21 KO mice present with an impairment of glucose metabolism and an excessively abnormal body weight (11). Moreover, in diet-induced obese (DIO) mice, insulin and glucose tolerance is more impaired when mice are conditionally lacking FGF21 in the liver compared with their age- and sex-matched control littermates (101). Also, FGF21 KO mice exhibit severe hepatic insulin resistance when fed with a

ketogenic diet compared with WT controls, when assessed by the gold-standard technique, the hyperinsulinemic–euglycemic clamp. This was associated with an increase in hepatic diacylglycerol content, leading to protein kinase C ε activation, a well-known kinase involved in insulin signalling impairments (19, 136). Moreover, FGF21 KO mice exhibited increased hepatic steatosis and VLDLR protein content through the activation of the eIF2a-ATF4 pathway (178). Conversely, some studies have investigated the potential role for FGF21 as a therapeutic target to prevent and treat metabolic disorders. A first study revealed that 3 to 7 days of subcutaneous administration of FGF-21 to diabetic rodents led to a significant lowering of circulating glucose and triglycerides, as well as a reduction in fasted insulin levels and improved glucose clearance during an oral glucose tolerance test (80). Moreover, treatment for 12 weeks with escalating doses of FGF21 decreased body weight, improved glucose tolerance and reduced concentrations of plasma triglycerides in high fat-fed, obese monkeys (4). Amongst the effects of FGF21 upon the liver, continuous, two week infusion of FGF21 with a miniosmotic pump to diabetic rodents led to a significant decrease in hepatosteatosis (29). It appears that FGF21 treatment abolished *de novo* lipogenesis through the reduction of SREBP-1 and fatty acid synthase in DIO mice (172). Also, *in vivo* hyperinsulinemic-euglycemic clamps in obese, leptin deficient (ob/ob) mice, revealed that 8 days of FGF21 injections improved hepatic insulin sensitivity and decreased hepatic glucose output (13). In a mice model of NASH, Lee and colleagues reported that 3 weeks of injections with the FGF21 analog LY2405319 prevented oxidative stress in the liver, a key component in the development of insulin resistance (30, 82, 89). Finally, clinical trials in patients with T2D revealed that 28 days of treatment with the FGF21 analog LY2405319 decreases low-density lipoprotein cholesterol and triglycerides, increases high-density lipoprotein cholesterol and improves fasting insulin (48). These data provide a scenario whereby metabolic health might be improved via the manipulation of systemic FGF21.

B/ Effect of exercise on FGF21

Given the aforementioned possible role of FGF21 in mediating metabolic health, it is of interest to identify ways in which FGF21 secretion can be altered. Exercise alters the expression of FGF21 with initial investigations suggesting that FGF21 is a myokine (71, 72). Indeed, transgenic mice (overexpressing Akt) characterized with increased muscle mass and strength exhibited a significant increase in systemic FGF21 compared with littermate controls. Moreover, in cultured skeletal muscle cells, FGF21 expression and secretion was regulated by Akt transduction supporting the idea that FGF21 is a myokine (71). However, recent studies have questioned this. Hansen et al. analysed the direct production of FGF21 using hepatic and femoral vein and artery catherization (59). They demonstrated, in healthy men, that FGF21 was secreted from the hepatosplanchnic bed but not in the leg during and after a prolonged bout of endurance exercise. In line with these results, investigations involving rodents support the contention that FGF21 is produced by liver. A single bout of endurance exercise significantly increases hepatic *FGF21* mRNA expression, while the results are divergent in skeletal muscle (59, 81, 97, 159). Interestingly, when healthy male subjects were infused with glucagon and somatostatin to mimic exercise (6 ng.kg-1.min-1 and 100 $ng.kg^{-1}.min^{-1}$ respectively) splanchnic FGF21 levels were significantly increased compared to saline infusion (59). Conversely, exercise with a pancreatic clamp (somatostatin, 100 $ng.kg^{-1}.min^{-1}$) completely blunted the exercise-induced increase in plasma FGF21, suggesting a role for pancreatic hormones in the regulation of hepatic FGF21 (60). In line with these results, glucagon receptor knockout mice have a blunted induction of *FGF21* mRNA in the liver in response to exercise (12). Also, resistance exercise, which elicits an increase in plasma insulin, does not induce FGF21 release in the bloodstream (114). Moreover, circulating FGF21 is also under the control of free fatty acids (FFA) levels during exercise in healthy

men (60, 81). Mechanistically, incubation of the FaO cell line with palmitic acid triggered FGF21 transcription through the concomitant action of the activating transcription factor 4 (ATF4) and peroxisome proliferator-activated receptor alpha (PPARα). It could be hypothesized that exercise-induced lipolysis favours FGF21 production by the liver through an ATF4/PPARα pathway. Thus, FGF21 production by the liver during exercise appears to be regulated by a synergetic action of glucagon to insulin ratio and FFA levels. A caveat is that all these experiments were mainly performed in healthy subjects. A recent study revealed that exercise-induced plasma FGF21 elevation was abolished in patients with T2D (60) suggesting that FGF21 production in response to acute exercise is altered in patients with metabolic disruption. While basal FGF21 was higher in T2D patients compared with healthy subjects (60), it appears that hyperinsulinemia or hepatic insulin-resistance would rather impair exercise-induced FGF21 secretion. Indeed, it has been reported that FGF21 secretion is lower in obese patients with hyperinsulinemia compared with healthy subjects (143). Interestingly, there was no difference in basal FGF21 concentrations between both groups, but the clear mechanism affecting FGF21 secretion during exercise in the context of metabolic disease remain to be elucidated.

Other studies have assessed the impact of chronic exercise on circulating levels of FGF21 in the context of metabolic disorders. In humans, the results seem controversial. Some studies support the idea that chronic exercise, combined or not with diet intervention, can significantly decrease circulating FGF21 in obese or elderly people (157, 158, 174) while others did not observe any effect in obese or diabetic patients (5, 15, 84). It is important to note that some methodological issues might explain these discrepancies. Firstly, these studies were performed in heterogeneous populations with respect to metabolic disruption. Also, FGF21 systemic levels are affected by various stimuli such as nutrient intake (98), fasting

status (38) or circadian rhythm (177) that were not specified in these studies. Finally, not all these studies examined changes in systemic levels of insulin or FFA, hepatic fat content, or cardiorespiratory fitness which are seemingly important factors affecting FGF21 levels (60, 157).

In a rodent model of T2D (OLETF), Fletcher et al. have investigated the effect of voluntary wheel running on FGF21 expression (43). The authors observed that active rats had a preserved hepatic mRNA and circulating FGF21 response compared to their sedentary littermates. Additionally, some studies in transgenic mice investigated whether FGF21 is necessary to mediate the effects of chronic exercise on improved energy metabolism. However, voluntary wheel running reduced adiposity, adipose tissue inflammation, hyperinsulinemia, and hepatic fatty acid content and oxidation in both FGF21 KO mice and their control littermates (44, 129). On the contrary, in mice fed with a high fat diet (HFD), voluntary wheel running did not improve hepatic triglyceride content and glucose tolerance but prevented weight and fat mass gain independently of genotype (97). The authors concluded that FGF21 KO mice exhibited an impaired adaptation to exercise training, including reduced AMP-activated protein kinase activity in skeletal muscle. Based on these findings, FGF21 may be necessary for the health-benefits associated with regular exercise under high fat, but not normal, dietary conditions. As the liver is the main source of FGF21 (101), further examinations in liver specific deletion models of FGF21 would help to better understand the cellular adaptations to physical activity.

To sum up, FGF21 plays a pleiotropic role in lipid and glucose metabolism and can improve metabolic-related disorders. It is now well accepted that exercise contributes to the prevention of chronic diseases, but the underlying mechanisms are not well understood. Interestingly, the

metabolic actions of FGF21 share those observed in response to exercise. Thus, the exercise-induced production of FGF21 by the liver might represent one of the cellular mechanisms involved in the metabolic adaptations to exercise. Also, FGF21 interacts with many tissues and its production during exercise might facilitate inter-organ crosstalk.

Fetuin-A

Fetuin-A is a 64 kDa glycoprotein known as an endogenous ligand for Toll-like receptor 4 (TLR4) and encoded by the *AHSG* gene (121). This receptor is expressed in several organs and more specifically in tissues involved in substrate metabolism such as the liver (54), adipose tissue (127) and skeletal muscle (47). Fetuin-A has also been shown to bind the β subunit of the insulin receptor (52).

A/ Fetuin A in metabolic disease

Much like FGF21, Fetuin-A has been proposed as a biomarker for metabolic diseases (122). For example, in a large cohort of 3170 community-living elderly individuals, a ten year follow up revealed that higher plasma Fetuin-A was associated with an increased incidence of T2D (70). More generally, it has been reported that circulating levels of Fetuin-A are positively correlated with impaired glucose tolerance, insulin resistance, T2D and liver fibrosis (119, 120, 152, 175), while an association with hepatic fat accumulation remains unclear (7). Significantly, several works assert that Fetuin-A might play a pivotal role in the pathogenesis of metabolic disorders. Firstly, data from transgenic mice demonstrate that Fetuin-A participates in the onset of metabolic dysfunction (106, 107). Indeed, Fetuin-A KO mice were protected from the deleterious effects of high fat diet with improved glucose clearance rate. This was associated with a higher insulin-stimulated phosphorylation of insulin receptor and the downstream signalling molecules MAPK and Akt in both liver and skeletal

muscle (107). Secondly, it has been reported that a single injection of fetuin-A inhibits insulin-stimulated insulin receptor autophosphorylation and IRS-1 phosphorylation in the liver and skeletal muscle of rats suggesting that fetuin-A may participate in the development of insulin resistance. Finally, *in vivo* and *in vitro* models of insulin resistance reinforce the idea that Fetuin-A is upregulated and released in the context of metabolic disruption. *In vivo*, it was observed that the expression of *Fetuin-A* mRNA in liver was increased by a high fat diet in rats (96). Also, F-box and WD repeat domain-containing 7 (FBXW7), an E3 ubiquitin protein ligase involved in Fetuin-A ubiquitination and degradation, is markedly downregulated in the liver of obese patients (181). *In vitro*, Takata and colleagues reported that glucose infusion increased Fetuin-A protein expression and *AHSG* transcription through the activation of ERK1/2 signalling in HepG2 cells (155). Also, palmitate incubation increased Fetuin-A protein expression and secretion through activation of NF-κB in HepG2 cells and rat hepatocytes (33). When secreted, Fetuin-A represents an endogenous ligand for TLR4 through which FFA induces insulin resistance, macrophage infiltration and inflammation in adipocytes (121, 150). It is important to note that TLR4 KO mice have been shown to be protected from insulin resistance induced by lipid infusion or by HFD (139). Similarly, TLR4 activation in adipocytes resulted in insulin resistance (144). An effect of Fetuin-A has also been shown in the pancreas. While Fetuin-A promotes lipotoxicity of β-cells through a TLR4-signaling pathway (138), it also impairs glucose-induced insulin secretion in a TLR-4-independent manner (49). Finally, Fetuin-A may also promote insulin resistance by direct binding to the β subunit of insulin receptors, leading to decreased tyrosine kinase activity of the receptor (52, 105). Together, these results support that Fetuin-A affects insulin secretion and resistance, adipose tissue inflammation and thus may participate in the pathogenesis of metabolic disorders.

B/ Effect of exercise on Fetuin-A

There are only two published articles that have investigated the effect of a single bout of exercise on Fetuin-A. The results show that a 60min session of cycling/treadmill exercise (60% of VO_{2max}) does not modify circulating levels of Fetuin-A in both healthy and obese subjects (137). In obese individuals, serum phosphofetuin-A (Ser312) levels were immediately increased after a single bout of exercise (60-70% VO_{2max} expending 500 kcals) which decreased to baseline in 24 hours (104). Interestingly, glucose and insulin during OGTT were significantly decreased 24 hours after the session of exercise suggesting that exercise-induced lowering of Fetuin-A might participate in this acute health-benefit of exercise.

All other studies assessed the effect of chronic exercise on Fetuin-A. In rodents, Sakr et al. (2014) have shown that 16 weeks of swimming exercise in male Sprague Dawley rats suffering from metabolic syndrome significantly decreases Fetuin-A serum levels and improves HOMA-IR index (135). Similar findings were reported in human by Malin et al (2013) who studied the effect of 7 days of endurance training (60min at 85%HR_{max}) on plasma Fetuin-A concentrations in obese patients with NAFLD (99). The authors observed a significant decrease in circulating Fetuin-A which was positively correlated with a reduced insulin resistance index and improved glucose tolerance. Later, the same team revealed that 12 weeks of endurance training induced a significant decrease of plasma Fetuin-A, which correlated with a decrease in hepatic, but not skeletal muscle or adipose insulin resistance (100). Interestingly, the effect of exercise on the decrease of Fetuin-A levels was not associated with a change in hepatic triglyceride content (99). One plausible explanation would be that exercise-induced changes in fetuin-A may relate to changes in blood lipids rather than liver fat content. Indeed, Lee and colleagues reported that the decrease in plasma fetuin-A and FFA interacted to improve glucose infusion rate in sedentary and overweight disglycemic

men in response to 12 weeks of concurrent training (90). Importantly, the decrease in plasma concentration of fetuin-A predicted changes in gene expression related to inflammatory TLR-signalling in macrophages in adipose tissue.

It is now well established that chronic exercise is beneficial for diseases associated with low-grade inflammation such as obesity, T2D, NAFLD or NASH (18, 75, 76). Altogether, we could hypothesize that exercise-induced lowering of fetuin-A through the downregulation of TLR4 pathway is one mechanism that participates in this anti-inflammatory process. Also, these studies suggest that regular exercise improves whole body and liver insulin sensitivity in patients with metabolic disease by decreasing circulating Fetuin-A levels. Thus, if exercise regulates Fetuin A expression, this might thus be one mechanism by which physical activity can influence the development of metabolic disease. One major limitation is that we are lacking studies examining the effect of a single bout of exercise on hepato-splanchnic production of fetuin-A. Also, it would be interesting to investigate the cellular modifications of the Fetuin-A signalling pathway in the liver in response to exercise.

Angiopoietin-like protein 4 (ANGPTL4)

Angiopoietin like protein 4 (ANGPTL4) a 45–65 kDa glycosylated and secreted protein which belongs to the angiopoietin-like gene family. *ANGPTL4* mRNA is expressed in liver but also in adipose tissue and to a lesser extent in skeletal muscle (78).

A/ ANGPTL4 in metabolic disease

Little is known about the determinants of plasma ANGPTL4 and its clinical relevancy in metabolic disorders. So far, a clear, positive relationship with plasma FFA has been shown (78, 83, 132). Furthermore, obese subjects generally have higher levels of plasma ANGPTL4

while an association with OGTT- and hyperinsulinemic-euglycemic clamp–derived indexes of insulin sensitivity are not clear (146). It is well established that ANGPTL4 participates in the regulation of lipid metabolism via the stimulation of lipolysis in adipocytes in a fasting state (53) and the inhibition of lipoprotein lipase (LPL) activity (88). LPL is an enzyme responsible for the hydrolysis of the triglyceride (TG) core of circulating TG-rich lipoproteins resulting in FFA which can be either stored or oxidized. Thus, overexpression of ANGPTL4 in mice resulted in a dramatic increase in circulating triglycerides and cholesterol, associated with a decrease in LPL activity, compared with wild-type littermates (85). Concerning glucose metabolism, the role of ANGPTL4 is unclear. Overexpression of Angptl4 by adenovirus improved glucose tolerance in mice compared with control, and reduced hepatic glucose production in rat hepatocytes (171). On the contrary, hyperinsulinemic-euglycemic clamp analyses revealed that whole-body transgenic overexpression of ANGPTL4 causes impaired glucose utilisation and insulin resistance, and higher insulin-mediated suppression of glucose production in the liver (94). Recently, Janssen and colleagues investigated the effect of whole-body deletion of ANGPTL4 on glucose homeostasis and metabolic function using a diet-induced obesity model (73). The authors observed that ANGPTL4$^{-/-}$ mice exhibited elevated fat mass, visceral fat mass and inflammation but, interestingly, improved glucose tolerance compared with wild type controls. Specific adipose tissue deletion of ANGPTL4 also resulted in improved glucose metabolism, associated with decreased ectopic lipid deposition in the liver and skeletal muscle (8). Overall, ANGPTL4 seems to display a dichotomous effect on lipid and glucose metabolism.

B/ Effect of exercise on ANGPTL4

Kersten and colleagues (2009) first reported that endurance cycling exercise (50% VO_{2max} for 2 h) increased ANGPTL4 circulating levels in fasted but not fed young healthy males (78).

Lately, micro-array analysis of *vastus lateralis* samples following a single bout of one-legged cycle exercise (60min at 50% of maximum workload (Wmax)) revealed a significant increase in *ANGPLT4* mRNA, interestingly in both legs with a more pronounced elevation in the non-exercising limb (21). To better understand this difference and the role of ANGPTL4 in exercise-induced metabolic adaptations, Catoire and colleagues repeated this one leg exercise protocol to bring to light the regulatory mechanism (20). The authors revealed that induction of ANGPTL4 in non-exercising muscle is mediated by elevated plasma free fatty acids via PPARδ, presumably leading to prevent fat overload and provide fatty acids to the active skeletal muscle. However, it is unclear whether the increase in circulating ANGPTL4 levels is triggered by an increased mRNA and production of ANGPTL4 from skeletal muscle or whether other tissues contribute as well. Recently, an elegant investigation from Ingerslev and colleagues depicted the mechanism of ANGPTL4 production in response to exercise (68). By assessing arterial-to-venous differences over the leg and the hepato-splanchnic bed, the authors revealed that the increase in plasma ANGPTL4 in exercising humans is liver-derived with no contribution of the exercising muscles. Moreover, when exercise was performed under pancreatic clamp to inhibit the increase in glucagon-to-insulin ratio and FFA, ANGPTL4 production was blunted. This suggests that glucagon-to-insulin ratio and FFA plays a pivotal role in ANGPTL4 production. *In vitro*, hormonal infusions revealed that the glucagon-to-insulin ratio through the activation of the cAMP-PKA pathway triggered *ANGPTL4* mRNA production in hepatocytes (68). Together, these data suggest that ANGPTL4 is an exercise-induced hepatokine and that the skeletal muscle is not involved in the increase of the plasma concentration. Notwithstanding, ANGPTL4 production by the skeletal muscle during exercise may have an autocrine function (20, 146). Furthermore, while a single bout of endurance exercise (60min at 14m.min−1 and 14° inclination) increased mRNA expression of *ANGPLT4* in liver of mice (65), it is unclear whether hepatocytes are

responsible for the increase of the serum protein level in response to exercise in this rodent model. Also, Norheim and colleagues observed a significant increase in serum concentration of ANGPTL4 in response to 60min of cycling (70% of VO2max) which was even more pronounced in dysglycemic subjects compared to controls (116).

Regarding chronic exercise, little is known about the impact of endurance training on circulating ANGPTL4. In healthy humans, Catoire et al. (2014) observed that 2 weeks (a session of 45 min, 3-min intervals at 70% and 35% Wmax alternated with a session of 120 min at 50% Wmax) or 12 weeks of endurance training (three times per week for 47.5 ± 2.5 min at 40% VO_{2max}) did not alter circulating ANGPTL4 (20). In obese patients, it has been shown that 6 months of endurance training (3 times per week for 60-75 min at 70% of heart rate (HR) reserve) resulted in a significant weight loss and an increase of serum ANGPTL4 (31).

Physical activity triggers short- and long-term adaptations to supply the energetic demands of the body. Lipid metabolism is one of the key components and multiple mechanisms underpin the adaptive responses to acute and chronic exercise. We describe here work suggesting that ANGPTL4 regulates LPL activity and thus, plays a pivotal role in lipid metabolism. As ANGPTL4 is an exercise-induced hepatokine, this mechanism could participate in the adaptation of lipid metabolism to physical activity. However, it is now necessary to decipher whether production of ANGPTL4 during exercise participates in the health-benefits of physical activity to prevent and treat metabolic disease. Finally, as ANGPTL4 appears to play an important role in skeletal muscle lipid metabolism (20), this hepatokine might participate in exercise-induced inter-organ crosstalk between the liver and the skeletal muscle.

Follistatin (Fst)

Fst is a glycosylated plasma protein, which is a member of the TGFβ superfamily. Fst was first described for its role in reproduction (86), but is also implicated in the regulation of the skeletal muscle mass (134). Recently, it was reported that Fst is highly expressed in the liver but also in skeletal muscle and white and brown adipose tissues (17, 57). There are two Fst isoforms: Fst 288 and Fst 315 (140).

A/ Fst in metabolic disease

It is thought that Fst levels are increased in patients with T2D, NAFLD and NASH compared with control subjects and that they correlate positively with HbA1c, fasting blood glucose, and impaired glucose tolerance (58, 60, 176). Also, Polyzos and colleagues assessed the circulating levels of Fst in lean and obese subjects and patients with NAFLD or NASH (128). There was no difference between subjects but Fst levels were associated with NASH within NAFLD patients. The authors suggested that Fst may underlie the progression from NAFLD to NASH (128). Finally, recent findings reported that bariatric surgery significantly decreased Fst and this correlates with improved Hba1c in obese patients with diabetes (160). In line with these observations, *in vivo* and *in vitro* investigations support the idea that Fst plays a pivotal role in glucose metabolism. It has been shown that Fst participates in systemic metabolic dysregulation by hepatic FoxO1 activity (160). Also, during HFD, overexpression of Fst315 by adenovirus in mice impaired the glycaemic response to OGTT compared with control mice (160). Interestingly, *Fst315*-KO mice exhibit steatosis while hepatic insulin signalling, as assessed by phospho-Akt in response to insulin injection, was improved (163). Recently, an elegant study from Tao and colleagues suggested that Fst targets hepatic glucose production (160). In a mouse model of insulin resistance (LDKO), silencing the hepatic Fst allele restored glucose tolerance and insulin levels compared with control LDKO. Also,

458 hyperinsulinemic-euglycemic clamps revealed an improvement in insulin sensitivity, through
459 an increase in Akt signalling in white adipose tissue and a decrease in hepatic glucose
460 production. Thus, it appears that Fst is a hepatokine which participates in the development of
461 metabolic disorders. However, further clinical studies are needed to clearly establish the role
462 of Fst in metabolic disorders. Also, it is important to note that Fst seems to have opposite
463 functions depending on the tissues. For example, overexpression of Fst in pancreatic β-cells
464 improved fasting blood glucose in db/db mice (180) suggesting a complex role of Fst in
465 metabolism.
466
467 B/ Effect of exercise on Fst
468 Fst was studied in the area of exercise because of its role in regulating skeletal muscle
469 hypertrophy by antagonizing myostatin (32, 34, 51). Recent findings reported that Fst is
470 released in the bloodstream in response to an acute bout of exercise. A first study performed
471 by Hansen and colleagues revealed that 3h of cycling at 50% of VO_{2max} increased circulating
472 levels of Fst but not *Fst* mRNA content in the vastus lateralis of healthy subjects (57). When
473 the authors assessed the response of 1h of swimming in mice in several tissues, they observed
474 a marked increase of mRNA content and protein level of Fst in the liver but not in skeletal
475 muscle. This prompted the authors to determine the source of Fst during exercise in humans
476 using liver vein catheterization (61). A significant increase in Fst in both hepatic vein and
477 artery in response to 2h of cycling at 60% of VO_{2max} was observed. More importantly,
478 arterial-to-venous differences was negative during the exercise session demonstrating a
479 constant hepatic secretion of Fst from the splanchnic bed. This secretion can partly be
480 explained by an increase in glucagon to insulin ratio during exercise (60). Indeed, combined
481 somatostatin-glucagon infusion increased plasma Fst while its secretion in response to
482 exercise during a pancreatic clamp was partially blunted in humans (60, 61). This hypothesis

was reinforced by *in vitro* investigations that revealed that glucagon increases, and insulin inhibits Fst production through the secondary messenger cAMP in hepatocytes (61). It is important to note that Fst secretion during exercise is impaired in patients with T2D (60) but not in obese subjects (137). Together, these studies suggest that an acute bout of exercise leads to Fst liver secretion.

While the acute regulation of Fst by exercise is partially characterised, the relationship between chronic exercise and Fst has not been extensively studied. It has been reported that resistance training is associated with an increase in circulating Fst in elderly overweight women (66). Also, high-intensity interval training (HIIT) increase Fst levels in sedentary but not life-long active elderly subjects (36). Regarding hepatic Fst, one study observed that 4 weeks of swimming training decreased similarly mRNA content of *Fst* in both lean and obese rats when compared with controls (141).

Regular physical activity is well known to promote glucose control and insulin sensitivity. We summarize here that Fst may participate in the regulation of these processes and in the development of metabolic disorders. Evidence suggests that Fst is an exercise-induced hepatokine, but little is known about its long-term adaptation to regular exercise. However, due to its biological properties mentioned above, it could be speculated that Fst participates in the cellular adaptation to exercise and to metabolic disease prevention. Also, Fst is involved in skeletal muscle mass hypertrophy and in β-cell function, and could mediate exercise-induced inter-organ crosstalk.

Methodological limitations and future directions.

In this review, we aimed to summarize the current literature regarding some proposed hepatokines involved in metabolic functions that are secreted in response to an acute session

of exercise and their regulation in response to training. While results are promising to better understand the cellular and molecular adaptations to exercise, several challenges need to be overcome. From a methodological point of view, key points need to be addressed before considering a protein as an exercise-induced hepatokine that participates in the health-promoting benefits of exercise. Firstly, clearly demonstrating a protein is secreted by the liver is technically challenging and we have mentioned how researchers have used arterial-venous difference analyses to overcome this (59, 61, 68). Secondly, determining the key function of a protein released from the liver is difficult. However, the generation of hepatocyte-specific gene knockout mouse models is a useful approach. For example, employing the Cre/Lox system, Markan and colleagues generated a model of mice lacking FGF21 specifically in the liver (101) which could be used to assess if FGF21 is necessary for the beneficial metabolic adaptions to exercise. Similarly, liver specific adenoviral overexpression of FGF21 (93) could help clarify the role of FGF21 in training.

Another challenge is to discover new exercise-responsive hepatokines that are released from the liver to influence whole-body glucose or lipid homeostasis. To do so, deep proteomic analyses associated with mass spectrometry may allow identification of new hepatokine candidates. Recently, Meex and colleagues suggested that purified hepatocytes can secrete more than 500 proteins with 114 differentially expressed under steatotic conditions (108). A similar approach could be envisaged to test the effect of exercise upon the liver secretome. When identified, specific attention should be paid on the cellular mechanisms involved in hepatokine expression, secretion and action. For instance, there are two proposed mechanisms that trigger hepatokine release in response to exercise: glucagon to insulin ratio and FFA levels (**Fig. 1**). The hormonal changes during exercise generally occur to ensure cardiovascular adjustments, energy substrate disposal and/or hydration (55). Thus, it could be hypothesized that hepatokine secretion acts as a conduit for the adaptation to exercise.

Regarding their actions, we reported here that hepatokines are secreted in to the bloodstream in response to a single bout of endurance exercise. As hepatokines can interact with other tissues, we can speculate that exercise-induced secreted protein from the liver participates in inter-tissue crosstalk.

It is well accepted that whole body homeostasis is influenced differently by exercise depending on its modality and the conditions in which it is performed. There exists a broad range of resistance or aerobic exercises such as classical moderate intensity continuous training (MICT), as well as the more recently proposed HIIT programs or sprint interval training (SIT) (77). Interestingly, it appears that short period HIIT training is well tolerated by patients and has a pronounced impact on glycemic control in patients with T2D (46). Thus, further studies are warranted to determine the optimal modalities of exercise that trigger hepatokine secretion to help the clinician to prescribe physical activity. In addition, nutritional status (fed vs fasted) or strategies (*ie* post exercise carbohydrate consumption) should be investigated in the context of hepatokine secretion. For example, exercise-induced plasma ANGPTL4 increases were blunted in the fed compared with the fasted state (78).

Finally, the aforementioned studies regarding exercise and hepatokines were performed in a broad range of subjects with respect to sex, age and metabolic disruptions. Thus, clinical studies are warranted in large cohort of patients with a long term follow up to decipher the contribution of hepatokines in metabolic adaptations to physical activity and, ultimately, improve the management of obesity, insulin-resistance, T2D, NAFLD and NASH through adapted training programs.

Review criteria

Searches for original articles or abstracts published between 1990 and December 2018 focusing on hepatokines in metabolic diseases and in exercise were performed in MEDLINE

and PubMed. The search terms used were “liver”, “exercise”, “physical activity”, “hepatokine”, “fetuin-A”, “follistatin”, “fibroblast growth factor 21”, “angiopoietin-like protein 4”, “obesity”, “inflammation”, “type 2 diabetes”, “nonalcoholic fatty liver disease” and “insulin resistance”. All articles identified were in the English-language. We apologise in advance to any researchers whose relevant work may have been missed using this criteria.

Declaration of interest statement

Pascal Sirvent is employed by Valbiotis S.A.S.

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1124 **Table 1. Metabolic roles of hepatokines and their systemic regulation by metabolic diseases and exercise.**

Hepatokines	Metabolic roles	Biomarkers in metabolic diseases	Acute exercise		Chronic exercise (metabolic diseases)	Refs
			Healthy	Metabolic diseases		
α1-microglobulin	Promotes adipose tissue inflammation	-	-	-	-	(56)
Activin βE	Stimulates energy expenditure and increases insulin sensitivity through brown and beige adipocyte activation	-	-	-	-	(62)
ANGPTL4	Major role in lipid metabolism, systemic levels of lipids and liver steatosis.	?	↗	↗	↗	(68, 116, 164, 171)
DPP4	Promotes adipose tissue inflammation and insulin resistance	↗ (if inflammation)	-	-	↘	(50, 130)
EDA	Promotes insulin resistance	↗ (mouse only)	-	-	-	(10)
Fam3C	Improves insulin resistance and fatty liver, suppressing hepatic gluconeogenesis	-	-	-	-	(26)
Fetuin A	Promotes adipose tissue inflammation, lipotoxicity of β-cells and insulin resistance	↗	-	↔		(137, 162)
Fetuin B	Impairs glucose tolerance	↗	-	-	-	(108, 125)
FGF21	Improves glucose tolerance, insulin sensitivity, steatosis, lipids profile and β-cell function. Promote adipose tissue browning	↗	↗	↔	?	(59, 60, 143, 147)
Follistatin	Promotes glucose metabolism disruption by enhancing hepatic glucose production	↗	↗	↔	-	(57, 58, 60, 160)
Hepassocin/ HFREP1	Promotes insulin resistance and hepatic lipid accumulation	↗	-	-	-	(167, 168)
HSP72	Promotes insulin sensitivity and mitochodrial function, reduces hepatic lipid accumulation and inflammation	↘	↗	-	-	(6, 28, 40, 102)

LECT2	Promotes insulin resistance, hepatic lipid accumulation and inflammation	↗	↔	↔		(67, 137)
MSP	Promotes hepatic inflammation but conversely inhibit hepatic lipid accumulation and regulates hepatic gluconeogenesis	-	-	-	-	(23)
RBP 4	Promotes hepatic lipid accumulation and adipose tissue inflammation	↗	↔	↗	?	(92, 112, 169)
Selenoprotein P	Impair insulin signalling and secretion, and dysregulate glucose metabolism. *Deficiency of selenoprotein P increases exercise responsiveness through upregulation of reactive oxygen species in muscle.*	↗	↔	↔	↔	(111, 137)
SHBG	Prevent obesity and fatty liver suppresses inflammation and lipid accumulation in adipose tissue	↘	-	-	↗	(133, 142)
Tsukushi	Decrease adipose tissue thermogenesis	↗ (mouse only)	-	-	-	(170)

↗ Systemic increase; ↘ Systemic decrease; ↔ No change; - No data; ? Conflicting results; ANGPTL4, angiopoietin-like protein 4; DPP4, Dipeptidyl peptidase 4; EDA, ectodysplasin A; Fam3C, Family with sequence similarity 3C; FGF21, Fibroblast growth factor 21; HFREP1, Hepatocyte-derived fibrinogen-related protein 1; HSP72, Heat shock protein 72; LECT2, Leukocyte cell-derived chemotaxin-2; MSP, Macrophage stimulating protein; RBP4, Retinol-binding protein 4; SHBG, Sex Hormone Binding Globulin.

Figure legend

Fig. 1 Exercise-induced hepatokines production and release. Prolonged endurance exercise promotes an increase in glucagon-to-insulin ratio and FFA. This results in the activation of two distinct pathways ATF4/cAMP/PPARα and cAMP that trigger FGF21, Fst and ANGPTL4 production and release. This secretion is altered by metabolic diseases and nutritional state (fasting *vs* fed). Other mechanisms might participate in the production of exercise-induced hepatokine such as FGF21, Fst, ANGPTL4 and Fetuin-A.

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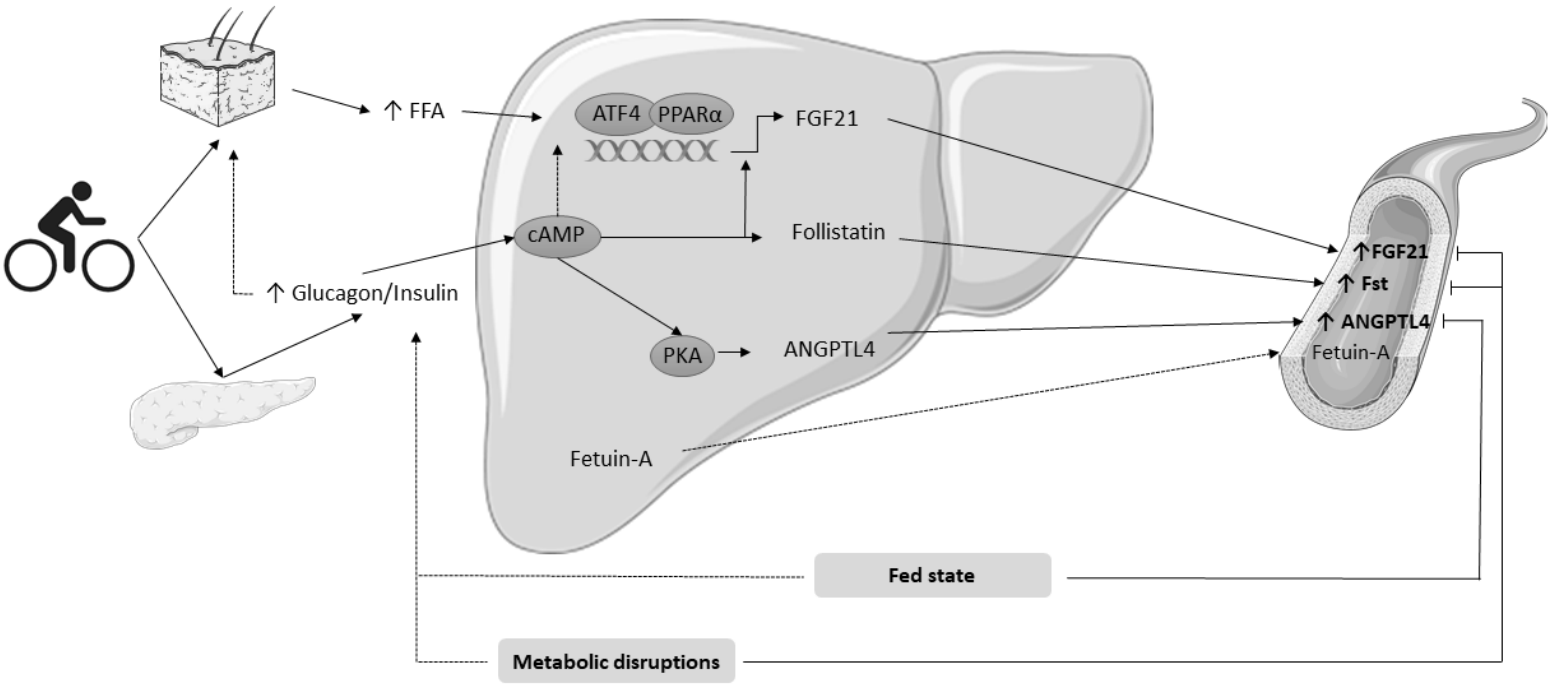
↑ FFA
ATF4
PPARα
FGF21
cAMP
Follistatin
↑ Glucagon/Insulin
PKA
ANGPTL4
Fetuin-A
↑FGF21
↑ Fst
↑ ANGPTL4
Fetuin-A
Fed state
Metabolic disruptions