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REVIEW

Irisin in metabolic diseases

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Abstract

Introduction Irisin is a myokine/adipokine induced by the exercise in mice and humans, which is proposed to induce "browning" of white adipose tissue, its primary target, thus increasing thermogenesis and energy expenditure. Since its identification, irisin has been linked to favorable effects on metabolic diseases, including obesity, type 2 diabetes mellitus (T2DM), lipid metabolism and cardiovascular disease (CVD), nonalcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), and metabolic bone diseases. Generally, despite the promising profile of irisin in rodents, its effects on human are less recognized.

Review Most, but not all studies show a positive association between irisin and indices of adiposity. In T2DM, NAFLD, and CVD, most observational studies reported lower irisin levels in patients than controls. Regarding metabolic bone diseases, irisin is positively associated with bone mineral density and strength in athletes, and inversely associated with osteoporotic fractures in postmenopausal osteoporosis. In PCOS, data remain largely conflicting. Irisin does not seem to be further reduced when two metabolic diseases, e.g., T2DM and NAFLD, or obesity and NAFLD exist though more data are needed. Furthermore, it seems that diverse confounders may have affected the results of different clinical studies.

Conclusion Irisin remains an appealing molecule from a pathophysiological point of view and an appealing therapeutic target for metabolic diseases, albeit much research is still needed.

Keywords Cardiovascular disease · Diabetes · Irisin · Myokine · Nonalcoholic fatty liver disease · Obesity

Introduction

The skeletal muscle communicates with other tissues, including the adipose tissue, the liver and the bone, through the secretion of hormones, collectively known as myokines, whose pattern of secretion may be altered by the type and the intensity of physical activity [1]. The identification of myokines has led to the hypothesis that some of the beneficial effects of exercise on the metabolic diseases may be related to myokines and their interactions with other systems [1].

Irisin is a novel myokine, first described by Boström et al. [2] and named after the ancient Goddess Iris, who served as a messenger among the Gods in Greek mythology [1]. Initially identified as a myokine, small amounts of irisin are also synthesized and secreted from the liver or adipose tissue [3]. Irisin is induced by the exercise in mice and

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humans. Irisin is proposed to induce the "browning" of white adipose tissue (WAT), which is defined as the occurrence of thermogenic brown adipocytes within the WAT, a process increasing energy expenditure [4]. Irisin induces the expression of a number of pro-myogenic and exercise response genes in myotube; its injection in mice induces significant hypertrophy due to activation of satellite cells and increased protein synthesis, thereby suggesting that irisin functions as a pro-myogenic agent [5].

The molecular sequence leading to irisin secretion is briefly the following: exercise increases the expression of peroxisome proliferator-activated receptor (PPAR)-γ co-activator (PGC)-1α, resulting in the expression of fibronectin type III domain containing (FNDC)5 [2]. FNDC5 is a membrane protein expressed in the brain and skeletal muscle; it is a type of transmembrane protein encoded by the *Fndc5* gene and is the precursor of irisin, i.e., irisin is produced by proteolytic cleavage of FNCD5 at the level of cell membrane [2] by yet unknown proteolytic enzyme(s). Irisin binds to yet unknown receptor(s) of white adipocytes and other cells, including myocytes and hepatocytes [6]. Partly through increasing PPAR-α expression, irisin induces the expression of mitochondrial uncoupling protein (UCP)1 [7], which increases thermogenesis and thus energy expenditure in the skeletal muscle and brown adipose tissue (BAT) [8]. Since this effect seems to improve the metabolic profile, irisin is emerging as a promising therapeutic target for the treatment of metabolic diseases, at least those known to ameliorate with exercise [7].

Important to note, however, that despite the expectations initially generated for irisin, there is justifiable skepticism on the quality (lack of specificity) of commercially available ELISA kits currently used for the measurement of circulating FNDC5 and irisin, and on possible misinterpretation of findings of the relative existing studies [9, 10]. We have previously published on the reliability of assays and have used in our studies the most reliable assay, which is not widely available to all [11, 12].

The aim of this review is to summarize existing evidence linking irisin with metabolic disorders, including obesity, type 2 diabetes mellitus (T2DM), lipid metabolism and cardiovascular disease (CVD), nonalcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), and metabolic bone diseases.

Irisin in obesity

The WAT is considered the second most important source of irisin after the skeletal muscle [6]. In rodents, FNDC5/irisin is secreted primarily from adipocytes of the subcutaneous adipose tissue (SAT) and in lower amount from adipocytes of the visceral adipose tissue (VAT) [3]. The WAT-derived FNDC5/irisin may represent up to ~30% of its circulating levels [2, 3] and, similarly to skeletal muscle, its secretion is increased after endurance exercise training [3]. In humans though, FNDC5 expression is 100–200 times lower in WAT than in muscle, indicating a minor contribution of WAT in the circulating irisin levels [13–15]. Once released to circulation upon exercise or cold exposure, irisin stimulates UCP1 expression and browning of WAT, resulting in an increase in total body energy expenditure by increased UCP1-mediated thermogenesis [16].

The primary target of irisin is the adipose tissue. Irisin demonstrates differential effects depending on the species (rodents, humans), type of adipocytes (premature or mature adipocytes), and location/type of the adipose tissue (SAT, VAT, BAT). In obese mice, increase of circulating irisin levels with intravenous injection of FNDC5-expressing adenoviral particles or intraperitoneal administration of recombinant human irisin improves glucose metabolism, but has minor effects on body weight [2, 17]. Treatment of stromal vascular fractions from inguinal depots with FNDC5 stimulates browning, indicated by a 7-fold increase in UCP1 [2]. The FNDC5/irisin-treated adipocytes have multilocular lipid droplets, higher density of mitochondria, and increased energy expenditure, which are all signs of a beige/brown phenotype [2]. Additionally, in vitro or in vivo treatment with irisin stimulates basal and isoproterenol-induced lipolysis, increases lipid metabolism, and down-regulates lipid synthesis in mice [18, 19]. Interestingly, recent evidence suggests that irisin is an anorexigenic factor in fish and its actions might be partly mediated by appetite-regulating factors, including cocaine and amphetamine-regulated transcript, orexins, UCP2 and brain agents such as brain-derived neurotropic factor [20]. The role of endogenous irisin on food intake is likely mediated by its actions on other metabolic peptides; unaltered endogenous irisin is required to maintain food intake in zebrafish [21]. In humans, the reduction in irisin concentrations with increased energy intake is consistent with the detrimental metabolic effects of overeating [22].

The browning effects of irisin in humans are less established compared with rodents. In human preadipocytes from SAT, irisin inhibits differentiation to mature adipocytes [23–25]. Interestingly, it does not stimulate browning and rather decreases browning-related genes. In contrast, in mature human adipocytes, irisin stimulates browning, indicated by an increase in UCP1 and PR/SET Domain 16 (PRDM16), probably by activation of the p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) signaling pathway [2, 17, 23–25]. Additionally, irisin stimulates glycolysis demonstrated by an increase in lactate secretion [23]. The irisin-induced browning is observed primarily in human neck adipose depots and secondarily in SAT [26]. Omental or renal

adipose tissue (considered BAT due to high UCP1 expression) are not affected by irisin treatment [25, 26]. This implies that irisin does not further affect BAT.

Based on the in vitro and ex vivo results, several human research studies focused on the potential correlations between circulating irisin levels and obesity. Most, but not all, studies have reported that circulating irisin is positively associated with body mass index (BMI) and weight [6, 13, 27–32]. This association remains positive also in extreme BMI phenotypes. Patients with anorexia nervosa have 15% lower circulating irisin levels compared to normal weight and 30% lower compared to morbidly obese individuals [31, 32]. Moreover, irisin is positively associated with fat mass, waist circumference, waist-to-hip ratio [27, 30, 32, 33] and muscle mass [13, 30]. Notably, obese subjects following an 8-week program of endurance exercise and strength training combined with dietary restriction demonstrated significantly higher irisin levels, which correlated positively with the circulating endothelial progenitor cells [34]. Furthermore, irisin levels are reported to correlate positively with leptin [35] and negatively with adiponectin [36]. A direct interaction between leptin and irisin is thought to be improbable, since leptin administration in humans does not alter circulating irisin [37]. Finally, weight loss reduces irisin levels, which are restored after regaining the lost weight [27, 33].

Altogether, irisin levels increase in obesity. This increase may only partially be explained by the higher fat mass and presumably the higher concentration of adipose tissue-derived irisin. It may represent a counterbalancing mechanism to increase energy expenditure and improve insulin sensitivity, though it remains to be elucidated. It may also indicate resistance to the action of irisin, similar to insulin and leptin resistance observed in obesity and T2DM [1]. Whether irisin treatment can induce browning, increase energy expenditure, reduce weight or have other positive metabolic effects in humans could be answered at the setting of prospective interventional studies.

Irisin in diabetes and gestational diabetes

Insulin resistance (IR) and diabetes mellitus

Irisin has potential multiple favorable effects on glucose homeostasis and insulin sensitivity by promoting energy expenditure, glucose uptake, and glycogenolysis and reducing gluconeogenesis, adipogenesis, and lipid accumulation [6, 38, 39]. Interventional animal studies have shown that irisin improves [2, 17] blood glucose levels by reducing IR. Additionally, it may promote pancreatic beta-cell survival and improve glucose-induced insulin secretion in lipotoxic conditions [40].

Several studies have examined the association between irisin and blood glucose levels, beta-cell function and IR in non-diabetic subjects. Fasting blood glucose has been generally positively associated with irisin levels [13, 29, 41]. In a cross-sectional study including 254 subjects with normal glucose regulation, irisin was positively correlated with beta-cell function according to the homeostasis model assessment (HOMA)-β, even after adjusting for anthropometric and metabolic confounders [42]. However, acute changes in glucose or insulin levels, as during an OGTT, do not affect irisin levels [29]. A weak but significant positive association of circulating irisin with IR in non-diabetic subjects was shown in a recent meta-analysis of 17 studies comprising of 1912 individuals [43]. Ethnic variability might affect the association, since it was found significant in people of Asiatic and American origin, but not in Europeans. This provides a plausible explanation to the contradictory findings of negative irisin association with both blood glucose and IR in a Saudi Arabian children population, in which very young age might also be a confounder [44]. Accordingly, insulin sensitivity indices show an inverse association with irisin [43, 45], with the exemption of a European study, in which a positive association was reported [15]. Interestingly, in apparently healthy individuals, higher irisin levels independently predicted the onset of diabetes in a longitudinal 2.6-year study in a Korean population [46].

In the diabetic state, irisin levels seem to dissociate between the two diabetes types. In type 1 diabetes mellitus (T1DM), irisin has been consistently reported to be higher than in controls [47, 48]. On the contrary, the majority of studies [41, 49–53], including two meta-analyses [25, 54], have shown lower irisin levels in T2DM patients than controls. The more recent meta-analysis, which included 15 studies with 1289 T2DM patients and 834 controls, showed lower irisin in T2DM [54]. Ethnicity, again, emerged as a determinant, since the association between T2DM and irisin was more pronounced in Asian than European populations. This might explain the contradictory findings of slightly higher irisin levels in Caucasian [55] and Saudi Arabian [56] T2DM patients, in two studies not included in the previous meta-analysis. Similarly, lower irisin levels have been reported in populations with prediabetes [57, 58] and in drug naïve T2DM patients [57, 59].

Irisin levels have also been independently associated to diabetic microvascular complications [51, 60–62] and macrovascular risk factors [63, 64]. In 100 newly diagnosed, non-obese, drug-naïve T2DM patients, serum irisin levels were negatively correlated with urinary albumin excretion [62]. Furthermore, T2DM patients and macroalbuminuria have significantly lower irisin levels than T2DM patients with normoalbuminuria/microalbuminuria and healthy controls [51, 52]. A more pronounced reduction

in irisin levels is seen in stage 5 chronic kidney disease [60], although the reduction in muscle mass and/or the significant reduction in renal function may affect irisin levels. Thus, it seems that there is a decrease in serum irisin level in T2DM and even more substantial reduction in diabetic nephropathy patients [65]. Besides, T2DM patients with proliferative diabetic retinopathy had significantly lower serum and vitreous irisin levels than controls and patients without retinopathy [51]. In nonproliferative diabetic retinopathy, an interaction between interleukin (IL)-17A, a proinflammatory cytokine implicated in the pathogenesis of diabetic retinopathy, and irisin levels were suggested [61]. Irisin levels are also associated with indices of endothelial function, as a positive association of irisin concentration with arterial flow-mediated dilatation has been reported [62, 63]. Moreover, in Chinese patients with T2DM, irisin levels negatively correlated with advanced glycation end-products, which are established contributors to the pathogenesis of diabetic complications [53].

Irisin might be considered for the future management of T2DM. Currently, there are some experimental studies toward this direction. First, metformin was shown to increase FDNC5 mRNA/protein expression and irisin levels in diabetic mice, in an adenosine monophosphate (AMP)-activated protein kinase (AMPK) independent manner [66]. This finding implies a novel molecular mechanism by which metformin exerts its positive metabolic effects. Next, it was demonstrated that irisin administration in diabetic [39, 67] and insulin-resistant [68, 69] mice models were followed by reduced gluconeogenesis, increased glycogenesis and improvement in muscle IR, which is mediated thought the AMPK pathway [68]. Furthermore, irisin was shown to improve endothelial function in diabetic mice models [70]. Likewise, irisin can promote beta-cell survival by restraining apoptosis induced by either high glucose [71] or high saturated fatty acids [40]. Recent data indicate that β-arrestin-2 plays a crucial role in irisin-induced glucose metabolism in T2DM by regulating the p38 MAPK signaling, thereby possibly representing a novel therapeutic target of diabetes treatment; β-arrestin-2 improves glucose utilization in diabetes by increasing the glucose uptake and insulin sensitivity, as shown in mice overexpressing β-arrestin-2 [72].

Gestational diabetes mellitus (GDM)

Gestation is a physiologic condition of IR. In normal pregnancies, irisin levels have been positively correlated with fasting insulin and HOMA-IR [73, 74]. A number of studies have examined second trimester maternal circulating irisin levels in GDM in comparison to control pregnancies. The results were conflicting, showing higher [75], lower [54, 76–81], or comparable [73, 74, 82] irisin levels in GDM vs. healthy pregnancies, respectively. However, the majority of studies and a meta-analysis [80] support that irisin levels are lower in pregnancies complicated with diabetes. Furthermore, in the only study that reported higher irisin levels in GDM, this result was only apparent after model adjustment for BMI, lipids, and glucose [75].

Interestingly, one prospective study, in which irisin levels were measured both early (6–11 weeks of gestation) and later in the second trimester, showed lower early irisin levels in women who subsequently developed GDM; nevertheless, this difference was attenuated in the second trimester [73]. This finding suggests that maternal serum irisin might serve as a prognostic factor for GDM. Finally, the presence of GDM did not seem to affect cord blood irisin levels [75, 79, 83].

Irisin in NAFLD

FNDC5 is expressed in the liver, albeit in lower amounts than the skeletal muscle or adipose tissue [13]. Irisin expression is detected immunohistochemically in hepatocytes, Kupffer cells, and sinusoidal endothelial cells [84, 85]. A study with ^{125}I-labeled irisin and single-photon emission computed tomography (SPECT/CT) showed high radioactivity in the mouse liver, implying the liver as a target for irisin [86]. Moreover, irisin was high in the gallbladder too, indicating that the hepatobiliary system possibly plays a role in irisin clearance [86]. Irisin was located inside mouse hepatic cells (AML12) and primary hepatocytes after irisin treatment [87], a finding implying hepatocyte as a target of irisin, but also a potential intrahepatic irisin receptor, warranting further research. More interestingly, irisin expression increased in parallel with the activation of hepatic stellate cells and irisin treatment increased fibrogenic markers in activated hepatic stellate cells [88]. Hepatic irisin production can be induced, at least partly, by the constitutive androstane receptor, a nuclear receptor capable to directly induce FNDC5 expression in the liver, thus increasing circulating irisin levels in mice [38]. Irisin was shown to be involved in hepatic glucose and lipid metabolism in cell lines and animal models. In human hepatocellular carcinoma cells (HepG2), mouse and human primary hepatocytes and in diabetic mice, irisin reduced hepatic gluconeogenesis and increased glycogenesis, thereby improving glucose homeostasis [38, 39, 68, 89]. These effects were achieved via phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) [39] and AMPK [38, 68, 89] pathways. As to lipid metabolism, it was initially shown that irisin treatment prevented the palmitic acid-induced lipid accumulation in hepatocytes by inhibiting two main regulators of lipogenesis (liver X receptor-α and sterol regulatory element-binding protein [SREBP]1c) in mouse

hepatic cells (AML12) and primary hepatocytes [87]. Subsequently, irisin treatment was shown to reduce cholesterol content in mouse primary hepatocytes and in high fat diet obese mice [90], or triglyceride content in human HepG2 cells [89], human primary hepatocytes and *ob/ob* mice [38], via activating AMPK and subsequent inhibition of SREBP2 and other lipogenic enzymes [38, 89, 90]. Of importance, injection of adenovirus carrying human FNDC5 cDNA increased serum irisin, suppressed hepatic gluconeogenic and lipogenic enzymes, thus improving IR and decreasing hepatic triglyceride (but not cholesterol) levels in *ob/ob* mice [38], a leptin-deficient model exerting severe steatosis [91]. These results were confirmed in irisin transgenic mice, showing 35-fold higher irisin levels than wild-type mice [91].

Irisin may also play a role in the oxidative stress and hepatic cell survival, which are closely related to the pathogenesis of NAFLD [92]. Reactive oxygen species induced by hydrogen peroxide in mouse hepatic cells were slightly reduced after treatment with irisin [93]. Formaldehyde inhalation damaged the liver (as well as the lung) together with decreasing irisin levels, which were restored after carnosine supplementation in Sprague-Dawley rats [94]. Furthermore, irisin treatment attenuated palmitic acid-induced oxidative stress in mouse hepatic cells and primary hepatocytes [87]. Some studies showed that irisin promotes cell survival in HepG2 cells in an AMPK-dependent manner [89]. More recent data indicate that augmented irisin levels might have protective roles in liver malignant cells via partial activation of the PI3K/Akt pathway, which may facilitate hepatocellular carcinoma (HCC) and decrease the sensitivity to chemotherapy [95]. Of note, NAFLD is an important risk factor for HCC development [92]. Therefore, further research is needed on the association between irisin, oxidative stress, and cell survival.

Data on irisin in human NAFLD remain controversial. In the first histologically confirmed study in NAFLD adults, we observed similar circulating irisin levels among patients with simple steatosis, nonalcoholic steatohepatitis (NASH) and obese controls, whereas higher irisin in lean controls [96]. Notably, irisin was independently associated with portal inflammation in NAFLD patients; irisin also tended to be higher, albeit statistically nonsignificantly, by increasing the severity of steatosis, lobular inflammation, and fibrosis [96]. In a post hoc analysis of the same study, we also observed that circulating irisin and homocysteine were independently and inversely associated in NAFLD patients [97]. In a more recent study with histological confirmation, both circulating irisin levels and hepatic irisin expression were higher in patients with higher steatosis grade and fibrosis stage, and in NASH patients [98]. Interestingly, the rs3480 (A>G) variant of FNDC5, but not the rs726344 variant, was protective against hepatic fibrosis (F2–F4), albeit not the severity of steatosis [98]. However, irisin levels were not associated with either FNDC5 polymorphisms. Lower irisin levels in NAFLD (assessed by transient elastography) patients than lean controls were confirmed in a more recent study [99]. Irisin levels were similar between NAFLD patients with and without T2DM [99]. On the contrary, higher irisin levels in NAFLD (assessed by ultrasonography) adults than controls of lower BMI were observed in another study [100]. Of interest, within the NAFLD group, higher irisin levels were observed in patients with mild steatosis than those with moderate-to-severe steatosis [100]. In another study, irisin levels were independently inversely associated with hepatic triglyceride content (assessed by ^{1}H magnetic resonance spectroscopy) in obese Chinese adults [101]. In a children cohort, I148M polymorphism of patatin-like phospholipase domain-containing protein (*PNPLA*)*3* gene was independently positively associated with plasma irisin [102]. Specifically, irisin gradually increased from individuals with the I148I variant (CC homozygous) to I148M variant (GC heterozygous) and then to M148M (GG homozygous) variant [102]. This study provided the first evidence for an association between circulating irisin and PNPLA3 gene I148M polymorphism. It is highlighted that *PNPLA3* is a strong genetic factor for NAFLD and its severity; it is the most validated gene polymorphism strongly associated with the full spectrum of NAFLD, being simple steatosis, NASH, NASH-related cirrhosis, and HCC [12]. Although this study refers to children without established NAFLD [102] and the fact that *PNPLA3* gene is also expressed in extra-hepatic tissues (e.g., the kidneys, brain), the results of this study warrant further research in NAFLD populations. There is no secure explanation for any discrepancies among existing clinical data on circulating irisin in human NAFLD; however, differences might be partly attributed to population (ethnicity, age, gender, BMI, sample size) and methodological differences (mainly the method used for the diagnosis of NAFLD), as well as potential differences between ELISA kits, as we have previously summarized [12].

In summary, in experimental models, irisin, acting mainly via the AMPK pathway, improves hepatic glucose homeostasis and steatosis, whereas more data are required on its effect on hepatic oxidative stress and apoptosis. On the other hand, data from clinical studies are conflicting, largely implicated by the fact that all studies are to date cross-sectional. Cohort studies in histologically confirmed NAFLD are needed to clarify the prognostic role of irisin in NAFLD and its related HCC. As a next step, a phase I randomized controlled trial is needed to evaluate the effect of recombinant irisin in NASH and fibrosis. Nonetheless, results of recombinant irisin on hepatic fibrosis, the main prognostic endpoint for advanced NASH, may be limited

similar to the limited effects of metformin, which acts mainly via the same pathway (AMPK) [103].

Irisin in lipid metabolism and CVD

Lipid metabolism

As mentioned above, besides myocytes, FNDC5 is also expressed and irisin is also secreted by the adipocytes [15]. Experimental data suggest that FNDC5 expression and/or irisin administration inhibit lipid synthesis and stimulate lipolysis and intracellular lipid metabolism by regulating the expression of genes, such as *Pnpla2* (which encodes adipose triglyceride lipase), hormone-sensitive lipase, and of proteins such as fatty acid-binding protein 4 [18, 19, 23]. Adipocytes treated with irisin are smaller and accumulate fewer lipids than control adipocytes [19], while ex vivo irisin and FNDC5 treatment reduce the differentiation of human preadipocytes leading to decreased fat mass [18]. In obese mice, FNDC5 overexpression and irisin perfusion reduced hyperlipidemia and hyperinsulinemia [19], while subcutaneous irisin infusion induced a reduction in plasma cholesterol through inhibition of hepatic cholesterol synthesis via AMPK-SREBP2 pathway [90].

However, in human studies data are conflicting. A positive correlation between irisin levels and an unfavorable lipid profile has been reported in most studies, e.g., circulating irisin was positively associated with fasting triglycerides in sedentary subjects [104] and patients with metabolic syndrome [105], with total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and fasting fatty acids in Chinese patients [106] and with TC, LDL-C and triglycerides in Korean adolescents [107], while negatively associated with high-density lipoprotein cholesterol in patients at high cardiometabolic risk [105, 108]. On the other hand, circulating irisin has been associated with a favorable lipid profile in a Central European general population [109], while in other studies no correlation of irisin with lipid parameters has been observed in healthy young individuals [110] in normal weight individuals with increased body fat (>30%) [111] and in men with mild hypercholesterolemia [112]. The reason for these discrepancies in different studies is unclear. Differences in studied populations or different ELISA kits used for irisin measurements could be responsible. Another explanation could be that, although FNDC5 expression in adipose tissue and circulating irisin is proportionally lower in obese or diabetic subjects compared to lean controls [14, 15], the increased fat mass in these individuals, who traditionally have an unfavorable lipid profile, could result in an overall irisin release that is equal or even higher than in lean controls. Statin (simvastatin) treatment has been reported to increase FNDC5 mRNA expression and irisin secretion both in vitro and in vivo [112].

Cardiovascular disease

Myocardium, being one of the largest muscles in the human body, secretes irisin and its contribution to circulating irisin levels is substantial, at least in animals [84]. Ischemic conditions of the heart, i.e., coronary artery disease (CAD) and its severe outcome, myocardial infarction (MI), modify irisin secretion [113]. This effect could be direct, through stress or damage of the cardiomyocytes, or indirect, through the unfavorable lipid profile that accompanies such conditions, as described above [104, 108]. In respect to the direct effect of CAD/MI on irisin secretion, it was initially thought that increased release from the damaged cardiomyocytes would result in higher irisin levels following MI. However, in rats, irisin tissue expression and circulating levels were lower in isoproterenol-induced MI and negatively correlated with established markers of cardiac damage, such as troponin and creatine phosphokinase-myocardial band isoenzyme (CK-MB) [114]. Similar findings have been reported in humans: not only MI [113, 115], but also CAD patients [113, 116] had lower irisin levels than controls. In addition, T2DM patients with macrovascular disease had lower irisin levels than patients without macrovascular disease and healthy controls [64], while lower irisin levels have been associated with endothelial dysfunction [117]. These findings imply that irisin is not passively released as a result of cardiomyocyte injury, as does CK-MB, but is rather energetically secreted reflecting the sufficiency of blood supply, and thus the functional potential of the cardiac muscle [113]. In the latter study [113], it has been hypothesized that, in state of reduced blood and oxygen supply, myocardium may release less irisin to restrain the metabolic demands of the heart in an attempt to compensate for the lower energy availability. This hypothesis is supported by studies suggesting regulation of FNDC5 expression by the oxidative stress [2, 112] and a significant role of irisin in energy homeostasis and cardiomyoblast growth and function [118].

Recombinant irisin treatment has shown positive cardiovascular effects in several animal studies. Specifically, irisin exerted anti-apoptotic effects on ischemic cardiomyocytes, through protection of mitochondrial function [119], and protected apolipoprotein E-deficient mice from atherosclerosis through endothelial cell proliferation [120] and/or suppression of oxidized LDL-induced cell inflammation and apoptosis [121], while inhibited vascular smooth muscle cells proliferation [122]. Similar results were reported in obese [123] and streptozotocin-induced diabetic apolipoprotein E-Null mice, through activation of the AMPK-endothelial nitric oxide synthase pathway [124].

Furthermore, irisin induced dose-dependent relaxation in mice mesenteric arteries with or without endothelium [125] and increased diastolic volume, heart rate and cardiac output in zebrafish, while knockdown of irisin showed opposing effects on cardiovascular function [21]. Regarding diabetic cardiomyopathy, recent data indicate that recombinant irisin low-dose treatment attenuates cardiac fibrosis and left ventricular function in diabetic mice, while high-dose irisin fails to diminish ventricular function impairment and augmented collagen deposition. The possible mechanism underlying the effect of low dosage involves irisin-mediated inhibition of high glucose-induced endothelial-to-mesenchymal transition; in contrast, irisin high dosage increases high glucose-induced matrix metalloproteinase expression by inducing MAPK (p38 and ERK) signaling and cardiac fibroblast proliferation and migration, thereby exerting a dose-dependent bidirectional effect on diabetic cardiomyopathy [126]. Regarding cardio-cerebrovascular disease, irisin diminishes ischemia-induced neuronal injury through the Akt and ERK1/2 signaling pathways activation and contributes to the physical exercise neuroprotective effect against cerebral ischemia, signifying that irisin might be a factor linking metabolism and cardio-cerebrovascular disease [127]. Likewise, irisin diminishes oxygen-glucose deprivation-induced neuronal injury in part through inhibiting reactive oxygen species-NOD-like receptor pyrin 3 inflammatory signaling pathway, suggesting a relative mechanism for irisin-induced therapeutic effect in ischemic stroke [128].

Recombinant irisin treatment has not been tested in human studies so far. In conclusion, irisin levels may represent a biomarker for CVD, including MI, as well as a promising treatment option for such conditions.

Irisin in PCOS

PCOS has been closely linked with obesity and other metabolic manifestations of IR syndrome, including NAFLD [129]. Data on circulating irisin levels between PCOS women and controls are to date derived from case-control studies and are inconclusive. Most studies have reported higher irisin levels in PCOS women than controls [130–133], whereas some other studies reported similar [134, 135] or lower [136] circulating irisin levels in PCOS women than controls. Irisin was higher in overweight/obese PCOS women than normal weight ones in some [131, 134, 137], but not all [135] studies having reported on this association. It should be highlighted that PCOS women had higher BMI than controls in all but two studies [133, 136], which implicates the interpretation of the results regarding irisin, since adiposity is a given confounder. In neither study irisin was higher in PCOS than controls [133, 136]. A recent meta-analysis suggested that irisin was higher in PCOS women, possibly independently of IR [138].

Some studies reported higher irisin levels in PCOS women with high-free androgen index (FAI) than those with low FAI, even after adjustment for potential confounders [137]. However, no correlation was observed between irisin and circulating androgens in another study [134]. Notably, a 6-month metformin treatment decreased circulating irisin in the PCOS women together with IR [131]. Furthermore, insulin infusion during euglycemic hyperinsulinemic clamp decreased irisin levels to a similar degree with the controls [131, 133]. These limited results may imply a possible role of irisin against IR or irisin resistance in PCOS women.

Differences in the populations and/or ELISA kits for irisin may partly explain the existing controversy. Further studies in cautiously selected populations and matched controls are needed.

Irisin in metabolic bone disease

Physical activity is a rather potent stimulus for bone formation, exerting beneficial effects both on bone mineral density (BMD) and fracture risk, although through yet unclear molecular mechanisms [139–141]. The association of exercise and skeletal health is further supported by the opposite condition of decreased physical activity and the invariable bone loss and profound increase in fracture risk in several conditions [142–144]. Since the discovery of irisin, as a myokine produced in response to exercise by the skeletal muscles, it has been suggested that this molecule might be the missing link for the cross-talk between muscles and bones [139, 145].

The hypothesis that bone tissue could be a source of FNDC5 and/or irisin upon exercise has been recently tested in 5-week-old mice: FNDC5 protein expression and irisin increased over 6-fold in bone tissue and in less extent in articular cartilage after exercise [16]. In addition, the osteogenic potential of irisin has been validated through the assessment of its effect on undifferentiated bone marrow stromal cells [146]. In this study, irisin increased osteoblast differentiation through the Wingless (Wnt)/β-catenin pathway [146], while more recently, in a study using both an anti-FNDC5 antibody and an irisin-ELISA Kit, irisin was reported to activate the p38 MAPK and ERK, thereby inducing the proliferation, differentiation, alkaline phosphatase activity, and mineralization of cultured osteoblasts [147]. In addition, the inhibition of p38 MAPK or pERK abolished the proliferative and upregulatory effects of irisin, confirming its direct effect on osteoblasts through these pathways [147]. Moreover, irisin has been reported to inhibit in vitro the receptor activator of nuclear factor-kappa

B ligand (RANKL)/nuclear factor-activated T-cells (NFAT) c1 and consequently to suppress the formation of osteoclasts [148].

In the first pre-clinical in vivo study evaluating the effect of irisin on bone, recombinant irisin (100 mcg/kg/week) was administered in young healthy male mice for 4 weeks; bone formation was induced, notable reductions in osteoclast numbers were reported, cortical bone mass and strength was increased, although no effect on trabecular bone was found [139]. In addition, the expression of the most abundant bone matrix protein, namely osteopontin, was found higher, while the expression of the well-known inhibitor of Wnt/β-catenin pathway, sclerostin, was found significantly lower within the tibiae of mice treated with irisin [139]. As mechanical stimulation exerts the same effects on these bone molecules, it was speculated that irisin could be the mediator of loading-induced changes in bone [149]. Finally, it was recently reported that treatment with recombinant irisin prevented and restored bone loss and muscle atrophy in hind-limb suspended mice [150]. More specifically, micro-computed tomography analysis of femurs showed that recombinant irisin preserved both cortical and trabecular BMD, while preventing the dramatic decrease of the trabecular bone volume fraction [150].

In humans, irisin levels were also found to be inversely correlated with serum sclerostin levels among adults of variable ages in both genders [151]. Moreover and with regard to mechanical loading in the human skeleton, irisin levels in athletes were reported to be positively associated with BMD and strength [152], although it seems that acute strenuous rather than long-term exercise induces the incremental changes in serum irisin levels both in children and adults [30]. We have recently reported that irisin levels are inversely associated with previous osteoporotic fractures, independently from other factors affecting bone fragility, such as BMD, BMI, smoking, 25(OH)-vitamin D levels, and bone markers [153]. Regarding the effect of anti-osteoporotic treatment, irisin levels were not affected by a short-course treatment with either denosumab or teriparatide [153]. The inverse correlation of serum irisin levels and fragility fractures has been also confirmed in two recent studies among post-menopausal women [154, 155], suggesting that this myokine could potentially be a useful marker for the assessment of disorders of the muscle-bone unit and metabolic bone diseases [148].

In conclusion, more specifically designed studies are required to explore the possible exercise-mimetic potential of irisin against bone loss and to further determine whether this myokine could serve as a diagnostic marker and/or a novel medication for several disorders of the muscle-bone unit, including osteoporosis [148, 149]. If the results from mice are confirmed in human studies, one could envision an irisin-based treatment for physically disabled or bedridden patients.

Closing remarks

The main findings on irisin in metabolic diseases are summarized in Fig. 1, in which best evidence or currently predominant suggestive evidence are presented. It is highlighted that all clinical data are to date derived from observational studies, thereby lying lower at the pyramid of evidence.

Since the identification of irisin [2], great expectations have been arisen for the diagnosis and treatment of metabolic diseases, at least those known to improve with exercise [7]. Ideally, if irisin represented the link between the beneficial effects of exercise and improvement in metabolic diseases, treatment with recombinant irisin or with medications possibly able to upregulate the endogenous irisin would be a beneficial treatment, at least for those that are not able to regularly exercise, e.g., morbidly obese, physically disabled or bedridden handicapped patients. However, as with the identifications of other adipokines and myokines, for which there are initially high expectations for the diagnosis and treatment of metabolic diseases [156], deeper knowledge of the pathophysiology of irisin may subside our initial expectations. For example, irisin may finally prove to play a role in the pathogenesis of some but not all metabolic diseases, so the irisin treatment may not be a panacea for all metabolic diseases. The rational next step is the identifications of irisin receptor, which will shed light into its pathophysiology, but will also open a new window to related novel pharmacology. In this regard, it has been proposed that FNDC5 and irisin is subjected to glycosylation and dimerization, and therefore it remains largely unknown which is the functional isoform [88, 157]. It has been recently reported that N-glycosylation is required for FNDC5 stabilization and irisin secretion [88]. Although this remains to be further elucidated, we should cautiously consider studies with recombinant irisin use. Another issue needing cautious interpretation is the extrapolation of FNDC5 mRNA expression or circulating FNDC5 levels to irisin levels. There is weak association between mRNA and secreted proteins [158, 159], and, moreover, the enzyme(s) and the dynamics for the excision of irisin from FNDC5 remain largely unknown. For these reasons, we tried to distinguish evidence referring to circulating irisin levels from that referring to circulating FNDC5 levels or FNDC5 expression in this review.

Strong evidence favors that irisin is a true circulating protein [160], though owing to the aforementioned skepticism on the quality of commercially available ELISA kits for circulating FNDC5 and irisin [9, 10], current and future

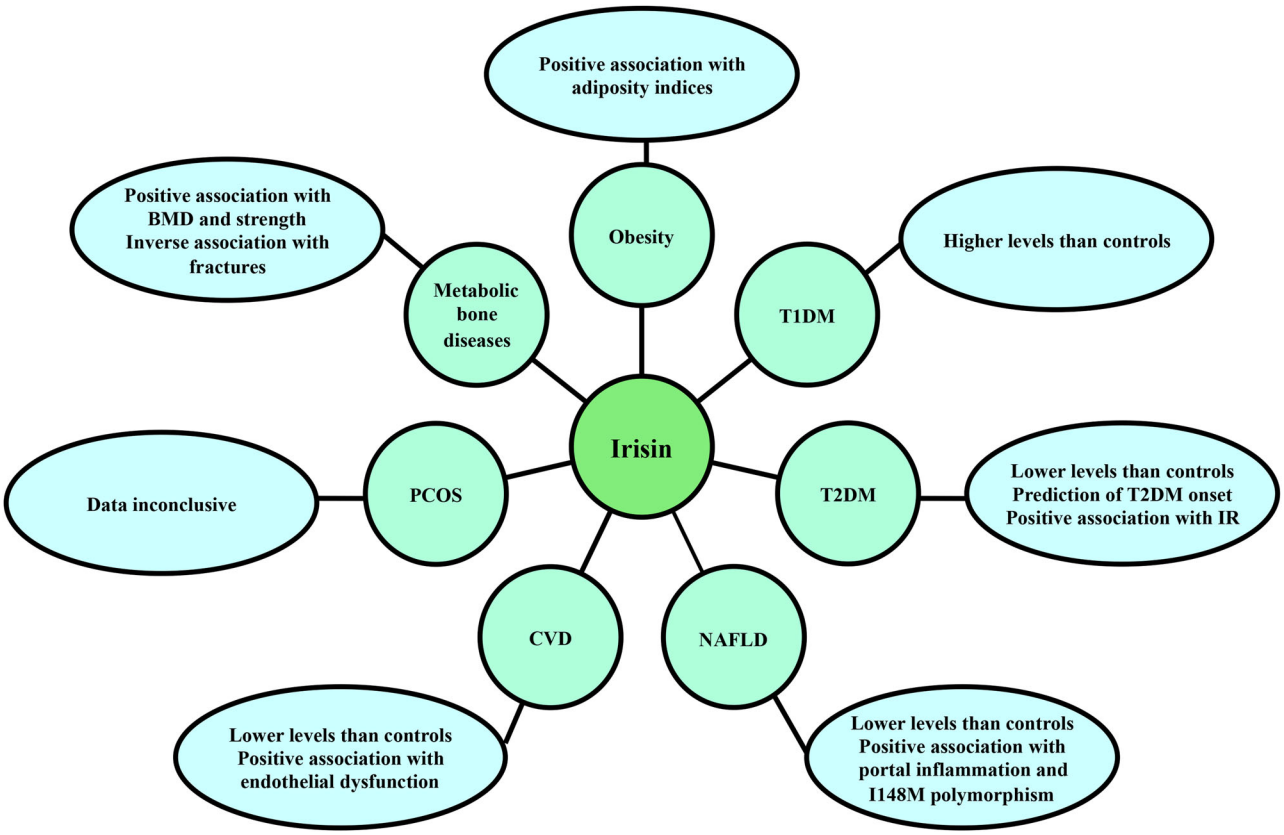

Fig. 1 The main findings on irisin in metabolic diseases as derived from human studies. Data represent best evidence or predominant tendency. All data are derived from observational studies. *BMD* bone mineral density, *CVD* cardiovascular disease, *IR* insulin resistance, *NAFLD* nonalcoholic fatty liver disease, *PCOS* polycystic ovary syndrome, *T1DM* type 1 diabetes mellitus, *T2DM* type 2 diabetes mellitus

irisin ELISA kits need validation toward tandem mass spectrometry, considered to be the gold standard method [11]. As reviewed elsewhere, EK-067-52 (Phoenix Pharmaceuticals), EK-067-29 (Phoenix Pharmaceuticals), and AG-45A-0046EK-k101 (Adipogen) have been used in more than 90% of irisin studies [6]. EK-067-52 has been proposed as the best validated ELISA kit to date; nevertheless, owing to the limited availability of the former, EK-067-29 is regarded as the best currently available alternative [6]. However, for the gild to advance, most accurate antibody against irisin is needed.

Much are still needed to know about irisin and other myokines; this class of molecules remains appealing with a potential to bridge our knowledge gaps between exercise and the beneficial effects on metabolic diseases, and, most importantly, a potential to provide new weapons in our armamentarium to fight metabolic diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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