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An update on the validity of irisin assays and the link between irisin and hepatic metabolism

1. Introduction

Common diseases (including obesity, type 2 diabetes mellitus [T2DM], cardiovascular disease and nonalcoholic fatty liver disease [NAFLD]) are complex traits resulting from environmental exposures acting, usually in association with multiple independent modifiers, on the basis of a susceptible polygenic background, which conveys a heritable component accounting for up to 30–50% of relative risk [1,2]. Candidate-gene studies and hypothesis-generating genome-wide association studies (GWAS) have provided key insights into the pathogenesis of NAFLD, with multiple genetic modifiers being described [2]. The patatin-like phospholipase domain containing 3 (*PNPLA3*) is the most validated gene associated with the full spectrum of NAFLD, being nonalcoholic simple steatosis (SS), nonalcoholic steatohepatitis (NASH), NASH-related cirrhosis and hepatocellular carcinoma [2,3]. The I148M (rs738409; C to G substitution encoding isoleucine to methionine) polymorphism of *PNPLA3* gene is a single nucleotide polymorphism (SNP), which is considered to be a key factor in the pathogenesis of NAFLD [2,3]. Despite the strong evidence linking I148M variant of *PNPLA3* gene with NAFLD, it remains to-date unclear why and how this gene is so strongly associated with hepatic lesions [2].

Irisin (named after the ancient Goddess, Iris, who served as a messenger among the Gods in Greek mythology) is a myokine, initially shown to be secreted by skeletal muscle in mice and humans [4]. Despite controversy generated around this molecule, irisin has been proposed to drive the brown-fat-like conversion ("browning") of the white adipose tissue, defined by the increased presence of thermogenic brown adipocytes in white adipose tissue [5]. By increasing energy expenditure, irisin may be associated with insulin resistance (IR) and systemic metabolism [6]. It seems that irisin represents another piece of the puzzle in the complex interactions between the skeletal muscle and other tissues implicated in energy homeostasis and metabolism [6]. If the proposed role of irisin is confirmed, this molecule or its analogues may emerge as appealing therapeutic target(s) for metabolic diseases and other disorders known to improve with exercise [6].

2. New Article in "Metabolism"

A new article linking irisin and NAFLD has been recently published in "Metabolism". Viitasalo et al. evaluated in a cross-sectional fashion circulating irisin levels in 454 children (6–8 years old) stratified according to *PNPLA3* gene I148M variants [7]. Baseline data were retrieved from the Physical Activity and Nutrition in Children (PANIC) study, which is an ongoing study carried out in Kuopio, Finland. They observed that the I148M polymorphism has a linear relationship with plasma irisin, even after adjustment for potential cofounders. More specifically, the carriers of 148M allele (G carriers) had higher irisin than the non-carriers, being gradually increased from I148I variant (CC homozygous) to I148M variant (GC heterozygous) and then to M148M (GG homozygous) variant [7]. The authors speculated that higher irisin levels in the carriers of 148M allele might be a compensatory anti-steatotic and anti-inflammatory mechanism, since the 148M allele is associated with more severe NAFLD. Since this study is limited by the relatively small number of 148M homozygous subjects and the absence of quantification of liver fat/inflammation/fibrosis (e.g., liver biopsy or magnetic resonance imaging or non-invasive indices for NAFLD, as elsewhere reviewed [8]), it has to be considered as hypothesis generating, and provides the first evidence for an association between circulating irisin and *PNPLA3* gene I148M polymorphism, a strong genetic factor for NAFLD and its severity. This hypothesis warrants further research.

3. *PNPLA3* Gene and Nonalcoholic Fatty Liver Disease

The *PNPLA3* represents to-date the only gene that has been consistently identified as a modifier of NAFLD pathogenesis

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; FNDC5, fibronectin type III domain containing 5; GWAS, genome-wide association studies; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain containing 3; SNP, single nucleotide polymorphism; SS, simple steatosis; SPECT/CT, single-photon emission computed tomography; T2DM, type 2 diabetes mellitus.

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across multiple GWAS examining both hepatic lipid content and biochemistry indices. The association between NAFLD and *PNPLA3* gene has been independently replicated in many studies of adult and pediatric NAFLD populations of different ethnicities [2]. It has been supported that the observed effect of the I148M variant on NAFLD is perhaps one of the strongest ever reported for a common variant modifying the genetic susceptibility of complex diseases [9].

There is also evidence from biopsy-proven studies that the polymorphism I148M (rs738409) of *PNPLA3* gene is associated with severity of steatosis, fibrosis and NASH [10,11]. In a meta-analysis of 16 observational studies, the I148M polymorphism was strongly associated not only with hepatic steatosis, but also with the susceptibility to more severe disease, since NASH is more frequently observed in GG than CC homozygous patients [9]. More specifically, GG homozygous have a 3.2-fold greater risk of necroinflammation and a 3.2-fold greater risk of fibrosis compared with CC homozygous subjects. Notably, the severity of NAFLD was similar between those carrying one or two G alleles, implying that the influence of this variant on disease severity follows a dominant model [9]. Another meta-analysis of 12 observational studies in Asian populations confirmed that the I148M variant is a risk factor for NAFLD [12]. Furthermore, G allele carriers were more likely to have higher level of serum alanine aminotransferase (ALT) and higher fibrosis score [12]. A third meta-analysis also indicated that the I148M polymorphism is associated with advanced fibrosis, but, most importantly, with greater risk of NASH-related hepatocellular carcinoma [13]. Notably, in a prospective cohort study, the *PNPLA3* GG genotype was significantly associated with underlying cirrhosis in patients with hepatocellular carcinoma and represented an independent risk factor for death [14]. Apart from NAFLD, the same SNP plays a role in other liver diseases, including chronic hepatitis C, in which it is associated with steatosis, cirrhosis, lack of response to antiviral treatment and possibly hepatocellular carcinoma [15].

Despite its definitive association between *PNPLA3* gene and NAFLD, the mechanistic basis for this association remains enigmatic. It is neither through changes in insulin sensitivity nor by influencing the severity of the broader features of the metabolic syndrome such as body mass index (BMI), dyslipidemia and T2DM [2]. In an effort to elucidate the interplay between *PNPLA3* gene and hepatic steatosis, Li et al. generated transgenic mice that overexpress similar amounts of wild-type *PNPLA3* or mutant *PNPLA3* [*PNPLA3(I148M)*] either in liver or adipose tissue [16]. Overexpression of the transgenes in adipose tissue did not affect liver fat content. Expression of *PNPLA3(I148M)*, but not of the wild-type, in liver recapitulated the fatty liver phenotype. Subsequently, metabolic studies provided evidence for three distinct alterations in hepatic triacylglycerol metabolism in *PNPLA3(I148M)* transgenic mice: increased synthesis of fatty acids and triacylglycerol, impaired hydrolysis of triacylglycerol and relative depletion of triacylglycerol long-chain polyunsaturated fatty acids [16]. These data suggest that *PNPLA3* may affect the remodeling of triacylglycerol in hepatic lipid droplets, as they accumulate after food intake. More recently, Smagris et al. introduced a knock-in mouse model by inserting a methionine codon at position 148 of the mouse *PNPLA3* gene [17]. Normal levels of hepatic fat were observed in knock-in mice, when on a chow diet, whereas liver fat content increased 2- to 3-fold compared to wild-type littermates when on a high-sucrose diet. The increased liver fat in the knock-in mice was accompanied by a 40-fold increase in *PNPLA3* on hepatic lipid droplets, with no increase in hepatic *PNPLA3* mRNA, and did not have any association with changes in glucose homeostasis [17]. These findings indicate that the I148M substitution is not equivalent to loss of protein expression, but *PNPLA3*-associated hepatic steatosis requires the presence of the catalytically inactive protein, not simply the absence of *PNPLA3* activity [17].

Although progress has been recently made on the interplay between *PNPLA3* gene and hepatic steatosis, further research is required to clarify how *PNPLA3* is associated with hepatic inflammation and fibrosis, as well as NASH-related cirrhosis and hepatocellular carcinoma, in which no clear mechanism has yet been identified.

4. Existing Literature on the Potential Interplay Between Irisin and Liver Physiology

4.1. *Experimental Studies*

Fibronectin type III domain containing (FNDC)5 mRNA/irisin is not exclusively produced by skeletal muscle in humans and animals, but also by other tissues, including the liver, albeit in lower amounts [18–21]. Specifically for the liver, it seems that irisin is produced by hepatocytes, Kupffer cells and sinusoidal endothelial cells [20,21]. The role of the liver-derived irisin is not currently known, and it might be local, through autocrine and paracrine pathways, but also endocrine through the circulation. In the liver, irisin might be associated with lipid and carbohydrate metabolism, but also with hepatic IR [6]. Early evidence showed that higher irisin expression caused a significant improvement in glucose tolerance and decrease in fasting insulin in FNDC5 overexpressing mice compared to controls [4], but further studies are needed.

The hepatic distribution of irisin following intravenous administration of ^{125}I-labeled irisin in mice is also of interest [22]. The highest level of radioactivity (ID per gram of tissue), as assessed by single-photon emission computed tomography (SPECT/CT), was observed in the gallbladder, followed by the liver and kidney [22]. The radioactivity was gradually reduced at 60 and 120 min in the liver, whereas it was reduced at 60 min, but remained unchanged at 120 min in the kidney. The radioactivity in adipose tissue was minimal and remained essentially unchanged at 60 and 120 min. This study may imply that the liver is a target tissue for irisin and/or that it participates, together with the kidneys, to the metabolic clearance of irisin; further investigations along these lines are warranted.

Notably, serum irisin, as well as skeletal muscle and hepatic malondialdehyde were increased, whereas muscle and hepatic glutathione decreased in rats after acute exercise [23]. Serum irisin was positively correlated with muscle and hepatic malondialdehyde and negatively with skeletal muscle and hepatic glutathione [23], a finding possibly implying a compensatory irisin increase to limit the oxidative stress induced by acute exercise. Although hepatic FNDC5 mRNA/irisin was not measured in this study, it would be of interest for future studies to evaluate this, so as to specifically elucidate whether hepatic FNDC5 mRNA/irisin may increase in response to oxidative