

The Role of Irisin in Gestational Diabetes Mellitus: A Review

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Abstract

Irisin is a newly described myokine that improves insulin resistance via the browning of white adipose tissue. Irisin appears to be secreted in response to exercise, providing a hormonal link between exercise and improved insulin sensitivity. Irisin expression has been demonstrated to increase after acute exercise and cold exposure in animal and human studies. Circulating irisin is decreased in metabolic disease states characterised by insulin resistance, such as type 2 diabetes mellitus, non-alcoholic fatty liver disease, chronic kidney disease and metabolic syndrome. The role of irisin in normal pregnancy as well as gestational diabetes mellitus is yet to be fully elucidated. To date, there are inconsistent reports regarding the changes in circulating irisin that occur in pregnancy and Gestational Diabetes Mellitus (GDM). Overall, it appears that irisin levels increase with pregnancy, and that the placenta expresses low levels of irisin. There is evidence that irisin levels are decreased in GDM pregnancy. With further research, irisin may prove a future therapeutic target or risk marker for GDM as well as other disease states characterised by insulin resistance.

Keywords: Irisin; Gestational diabetes mellitus; Insulin resistance; Pregnancy; Exercise; Skeletal muscle

Introduction

Gestational Diabetes Mellitus (GDM) is defined as a disease of glucose intolerance, which is first identified in pregnancy. A diagnosis of GDM is given to women who were normoglycaemic prior to pregnancy, usually based on abnormal results of a 2 hr 75 g oral glucose tolerance test (OGTT) at 24-28 weeks of gestation [1]. GDM currently complicates 17.8% of pregnancies worldwide [2]. It is a common condition, with significant adverse impacts on the health of mother and child. Maternal hyperglycaemia is associated with higher rates of both birth weight and cord blood C-peptide levels above the 90th percentile, primary caesarean section, clinical neonatal hypoglycaemia, shoulder dystocia and birth injury, premature delivery, neonatal hyperbilirubinaemia, preeclampsia and neonatal intensive care admission [3].

GDM also has long-term impacts on the child. Intrauterine exposure to the diabetic environment places children born to GDM mothers at an increased risk of obesity, the development of altered glucose tolerance and eventually type 2 diabetes mellitus (T2DM) in later life [4]. It is thought that exposure to the abnormal metabolic environment during gestation leads to dysfunctional in utero programming of the offspring's metabolism [5]. Mechanisms by which this occurs include epigenetic modifications such as DNA methylation of genes involved in metabolism [6].

The mother is at significantly increased risk for both future T2DM and cardiovascular disease. Women with GDM have a 50% risk of developing type 2 diabetes in the 5 years following their pregnancy [4]. A prior diagnosis of GDM has been identified as an independent risk factor for cardiovascular morbidity [7].

The Pathophysiology of Gestational Diabetes Mellitus

GDM develops in women who cannot compensate for the normal metabolic changes of pregnancy. Normal pregnancy is associated with an approximate 60% decrease in insulin sensitivity in late pregnancy [8]. In order to ensure an adequate energy supply to the fetus, there is an increase in the levels of several hormones that oppose the effects of insulin, creating a state of insulin resistance. These hormones include human placental lactogen, placental growth hormone, oestrogen, progesterone, cortisol, human chorionic gonadotrophin and prolactin [9]. Normoglycaemia in pregnancy can only be maintained with a near doubling of basal insulin levels, as well as enhanced nutrient-stimulated insulin secretion [9]. In humans, it appears that enhanced function of existing beta cells accounts for the majority of the increase in insulin secretion, rather than hyperplasia [9,10]. The increased insulin resistance reverts with delivery of the placenta [11].

In GDM, an increased level of insulin resistance accompanied by an insufficient increase in insulin levels results in maternal hyperglycaemia. Insulin resistance in GDM women predates the pregnancy, and remains after delivery of the placenta, but results in overt diabetes in the face of the additional physiological insulin resistance of pregnancy. It is likely that enhanced compensatory insulin secretion is occurring in these women prior to gestation, leaving them with little reserve capacity to overcome the additional insulin resistance of pregnancy. This is especially true for obese women [12]: Obese women represented 13.7% of pregnant women in the general obstetric population but 25% of those diagnosed with GDM in the HAPO study [12]. Insulin resistance in obesity is likely partially caused by low-grade inflammation induced by white adipose tissue accretion [13].

The shared pathophysiology between GDM and T2DM is reflected in the shared genetic background of these conditions. It has been demonstrated that genetic variants in key genes lead to impaired beta

cell function, impaired glucose metabolism and insulin resistance which then confer increased risk of both T2DM and GDM [14].

Exercise is known to improve insulin sensitivity [15]. This is partly due to the increased energy expenditure that occurs with exercise, which helps maintain body weight and prevents fat accumulation. However, regularly exercising muscle also displays differential expression of key genes involved in glucose metabolism and insulin sensitivity. Physical inactivity leads to insulin resistance in skeletal muscle through decreased GLUT4 levels. GLUT4 is the glucose transporter that is regulated by insulin. Lower GLUT4 expression results in decreased insulin stimulated glucose uptake [15,16]. Physical inactivity further alters the expression of over 4500 genes involved in glucose metabolism, which normalise after 4 weeks of retraining [16]. Some argue that the modern sedentary lifestyle, characterised by long periods of sitting, may in fact be the key risk factor for metabolic and cardiovascular disease [17,18].

Myokines are circulating hormones released from muscle. Some myokines are secreted at enhanced levels in response to muscle contraction and physical activity. They represent a hormonal mechanism by which exercise may alter body insulin sensitivity [15]. Current evidence suggests that dysregulation of myokines could play a role in the development of insulin resistance that underlies GDM. Myokines implicated in insulin sensitivity include IL-6, IL-13 and FGF21 [15]. Irisin is a newly identified myokine that improves insulin sensitivity [19]. Whilst its exact role in human metabolism is yet to be elucidated, current evidence suggests that irisin may have a role in the pathophysiology of a number of diseases characterised by insulin resistance.

Irisin: Initial Discovery

Bostrom et al. [19] first identified irisin as a novel myokine capable of inducing a brown adipose pattern of gene expression in white adipose tissue *in vitro*. In a mouse model, overexpression of irisin resulted in the browning of fat, a rise in metabolic rate and decreased insulin resistance [19]. Irisin secretion is induced by peroxisome proliferator coactivator 1 alpha (PGC1 α). PGC1 α is a key exercise inducible regulator of muscle metabolism. Increased PGC1 α expression confers resistance to metabolic disease. Irisin was discovered in the search for mediators that would explain how muscle PGC1 α levels affect whole body metabolism [18]. Irisin is secreted from muscle as the proteolytically cleaved product of FNDC5, and is present in human and mouse serum [19]. Specifically, irisin was demonstrated to increase uncoupling protein 1 (UCP1) levels in adipose tissue of mice, resulting in the production of heat rather than ATP. Such a pattern of gene expression is characteristic of brown adipose tissue. By dissipating excess energy intake as heat, metabolic rate is raised and provides resistance to obesity and related metabolic problems in mice.

Brown adipose tissue protects mice from diet induced obesity and insulin resistance [20]. Previously, brown adipose tissue was thought to be absent and physiologically unimportant in humans, but interest has been renewed by recent reports of brown and beige fat deposits in humans [21]. Such deposits were discovered through correlation of PET-CT scan images in combination with UCP1 stained tissue biopsies. Beige adipocytes are white adipocytes capable of being recruited to display some aspects of brown adipose gene expression [22]. It is important to note that less than 8% of humans appear to have active brown fat [21].

Bostrom et al. [19] demonstrated that overexpression of irisin at 3-4 fold of normal serum levels through the use of adenoviral delivery to murine liver could decrease fasting insulin and improve oral glucose tolerance in mice fed a high fat diet. This was accompanied by an increase in UCP1 expression in the adipocytes of these mice, as well as an increase in metabolic rate as evidenced by increased oxygen consumption. Lee et al. [23] confirmed the *in vitro* effects of FNDC5 using human cervical white adipocytes. This fat depot is rich in beige adipocytes.

They found that treatment with irisin or FNDC5 resulted in UCP1 protein staining becoming strongly positive, and that this was associated with a rise in the metabolic rate of the adipocytes. The effect was most marked in adipocytes treated with irisin in combination with FGF21, which is another inducer of brown adipose tissue, and further increased with exposure to increasing noradrenaline concentrations, as would occur with cold exposure *in vivo* [23]. The effect was not as marked in subcutaneous fat tissue derived adipocytes, and not present in omentally derived adipocytes. Bostrom et al. [19] additionally demonstrated that irisin is required for the exercise-induced increase in UCP1 in adipose tissue that occurs in both mice and humans (REF).

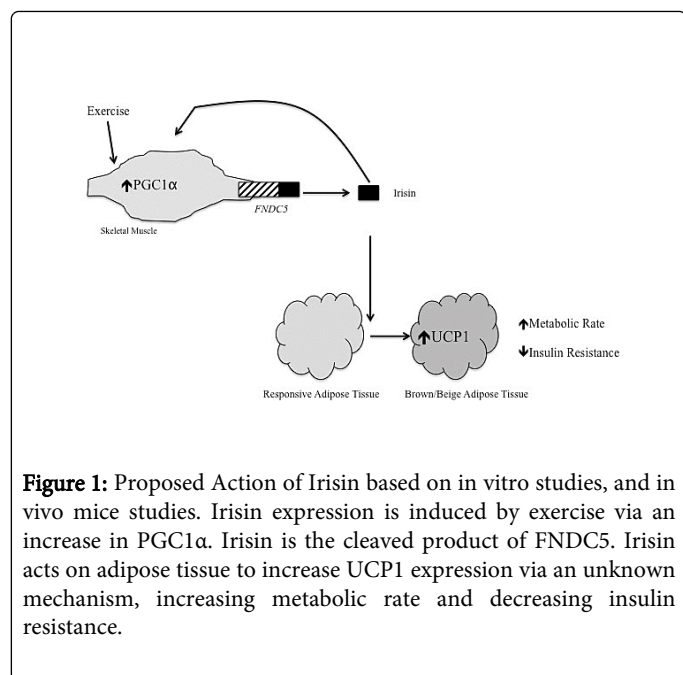
The effect of irisin on myocytes has also been investigated *in vitro* [24]. In myocyte culture, irisin increases oxidative metabolism and mitochondrial uncoupling in myocytes, as well as increasing PGC1 α levels and GLUT4 mRNA and protein levels. Such a discovery suggests a positive feedback loop whereby increased circulating irisin increases PGC1 α levels, which in turn induces further irisin secretion. PGC1 α is in fact the master regulator capable of increasing UCP1 protein in brown adipose tissue [25].

Although no downstream irisin receptor has been since identified, the effects of irisin are hypothesised to be mediated by increased peroxisome proliferator-activated receptor alpha (PPAR α) [19]. PPAR α is a transcription factor that is involved in enhanced β -oxidation of fatty acids and anti-inflammatory effects [26]. Subsequent research has looked at the role of irisin deregulation in metabolic disease, and its role in normal human metabolism. Figure 1 summarises the proposed action of irisin.

Irisin: After Exercise

A key role of irisin as suggested by Bostrom et al. [19] is in mediating the beneficial effects of exercise on human metabolism. A 2-fold increase in serum irisin levels in human subjects after 10 weeks of aerobic exercise was reported [19]. However, efforts to replicate these results have been inconsistent, muddling irisin's role in mediating the benefits of exercise on insulin resistance in humans.

The great heterogeneity in study protocols and choice of subjects could possibly explain the variability in results. In general, serum irisin levels increase acutely after exercise in young and otherwise healthy individuals [23,27-30]. There is little evidence that mean circulating irisin levels change in response to extended protocols of regular exercise. Table 1 summarises animal studies looking at exercise-induced changes in irisin, and Table 2 outlines human studies.



Irisin in response to Exercise: Animal Studies

Several studies have used animal models to examine changes in irisin with exercise. Bostrom et al. [19] demonstrated that 7 days of swim training could induce UCP1 mRNA expression in inguinal white adipose tissue when compared to 7 days of rest in mice (n=10). However, injection of an anti-irisin antibody prior to exercise blocked this increase in UCP1. This implies that irisin is a critical signal to white adipose tissue to increase UCP1 expression after exercise [19]. Brenmoehl et al. demonstrated a near doubling of serum irisin levels in mice after acute submaximal treadmill exercise [31]. Interestingly, although 3 weeks of exercise increased skeletal muscle PGC1 α mRNA over 30 fold, there was no change in FNDC5 mRNA or protein. Fain et al. also demonstrated that exercise training could increase serum irisin levels by 42%, but not skeletal muscle or adipose FNDC5 levels after a 16-20 week exercise intervention in pigs [32]. This study demonstrates an effect of chronic exercise. However, by only measuring 24hr after the final training session and not comparing to changes that occurred after the initial training session, it cannot be excluded that this is an effect of acute exercise.

Study	Participants	Design	Outcome
Animal studies demonstrating changes in irisin after exercise			
Bostrom et al. [19]	5 12-week old B6 mice	1. 3 weeks of free wheel running 2. 10 days of swim training	1. Irisin increases by 65% in mice after exercise 2. Injection of anti-FNDC5 antibodies prior to exercise reduces induction of UCP1
Brenmoehl et al. [31]	Acute: 12 70-day old male DUhTP mice Long-Term: 12 DUhTP and 11 Coeval male mice	Acute: Submaximal treadmill test, with animals sacrificed immediately after. Long-Term: Three weeks of free wheel running or kept sedentary (coeval mice), sacrificed at 70 days old.	Acute: Mice had 1.9-fold higher serum irisin after treadmill test, with higher irisin protein in femoral muscle homogenate. No effect on FNDC5 mRNA Long: no effect on serum irisin, muscle FNDC5 mRNA/protein
Fain et al. [32]	5 exercised and 8 sedentary Rapacaz FHM pigs (defective LDL receptor) 8 exercised and 8 sedentary normal Yucatan pigs	Exercised pigs did 16-20 weeks exercise training of daily moderate treadmill training. Samples taken 24hrs after final exercise.	Plasma irisin rose by 42% in exercised FHM pigs, but not in normal pigs. No effect on muscle FNDC5 mRNA
Animal studies showing no change in irisin after exercise.			
Seo et al. [33]	Male Sprague Dawley Rats (n=5-7/group)	Mice fed either high or low fat diet. Exercised rats performed 4 weeks of 5day/week treadmill exercise. Age garlic extract (AGE) groups received 4 weeks AGE injections	AGE and exercise alone and in combination decrease weight and improve insulin resistance. Neither intervention alters serum irisin or skeletal muscle FNDC5.

Table 1: Animal studies investigating the effect of exercise on serum irisin and FNDC5/irisin expression

Study	Participants	Design	Outcome
Human Studies demonstrating an increase in serum irisin after acute exercise			
Huh et al. [27]	15 young moderately trained lean healthy males	8-week exercise intervention of 3 sprint sessions/week. Blood samples before and 30 mins after first and last session.	At first session, irisin was 630 min after sprinting exercise.

			After 8 weeks, exercise induction effect was lost.
Kraemer et al. [28]	7 young healthy lean males 5 young healthy lean females	Exercise test at 60%VO ₂ max for 90min with samples taken at 0,54,90 min, and after 20 min rest. Females completed protocol during mid-follicular and then mid-luteal phase of cycle	Irisin é by ~20% in first 54min of exercise, declined to baseline by 20 mins of rest, regardless of gender, or phase of menstrual cycle.
Norheim et al. [29]	13 normglycaemic lean men age 40-65 years. 13 overweight pre-diabetic men age 40-65 years.	12-week intervention of 4x weekly strength and endurance training. Exercise test before and after intervention at 70%VO ₂ max.	In lean normoglycaemic men, circulating irisin é1.2 fold immediately after acute exercise. This only occurred in the overweight group after 12 wks training. 12-weeks training tended to decrease plasma irisin. 12 weeks of exercise éexpression FNDC5 mRNA in skeletal muscle.
Lee et al. [23]	4 females and 6 males; lean, young and otherwise healthy	Maximal exercise test at VO ₂ max. Submaximal 1 hr exercise test at 40% VO ₂ max.	1hr submaximal exercise éserum irisin 2.8-3.1 fold, with lesser increase after maximal exercise
Human studies demonstrating no increase in serum irisin after acute or long term exercise			
Kuridova et al. [34]	Study 1: described in Table 3. Study 2: 16 older (~36.5yrs) overweight individuals	Study 2:12-week intervention of 3x weekly 1hr strength and endurance training. Acute bout of 1hr exercise at 70% VO ₂ max, serum sample 1 hr later	Neither 3 months of exercise or acute bout of exercise resulted in altered skeletal muscle FNDC5 mRNA or circulating irisin.
Pekkala et al. [35]	Study 1: 17 overweight middle aged men Study 2: 10 young lean men and 11 old lean men Study 3: 9 older normal weight men/group	Study 1: 1hr acute exercise at 50% VO ₂ max. Study 2: acute resistance exercise Study 3: 21 weeks of 2 or 4 times weekly endurance alone or also strength training	Study 1 and Study 3: No significant change in skeletal muscle PGC1 α / FNDC5 mRNA or serum irisin Study 2: No alternations in serum irisin before, during or after exercise bout. Skeletal muscle FNDC5 mRNA é in young men only.
Bostrom et al. [19]	8 male non-diabetic obese individuals	10-week intervention of 4-5x weekly aerobic training at 65% VO ₂ max. Blood samples and skeletal muscle biopsy obtained 1-2 weeks before and 48 hours after intervention	2 fold é in serum irisin after exercise.
Human Studies demonstrating no increase in irisin after long term exercise			
Hecksteden et al. [36]	RCT in 112 healthy individuals, ages 30-60 with BMI<30	Intervention groups completed either 45 min aerobic training or strength training 3x weekly exercise for 26 weeks. Control group remained sedentary.	No difference between circulating irisin.
Hofmann et al. [37]	19 moderate activity and 19 high activity individuals with anorexia nervosa	Physical activity was measured over 3 days. Fasting serum samples collected.	No difference in circulating irisin.
Human Studies demonstrating an increase in irisin after long term exercise			
Bluher et al. [38]	65 obese children age 7-18 years	Intervention of a 1 year obesity therapy program of ~150min/week exercise + diet counselling and psychological education and support.	Circulating irisin é by 12 % after intervention.
Norheim et al. [39]	As described above. 12 weeks of exercise é expression of PGC α and FNDC5 mRNA in skeletal muscle.		

Table 2: Evidence from human studies investigating the effect of exercise of irisin

Study	Participants	Design	Outcomes
T2DM			
Choi et al. [40]	681 men and 1092 women recruited for health survey in rural Korea	All Participants subjected to glucose tolerance testing and venous blood samples taken? identified 104 subjects with newly diagnosed T2DM and 104 age, sex and BMI matched controls for study population	Irisin has a significant inverse correlation with new onset T2DM
Liu et al. [41]	96 T2DM individuals and 60 non-matched, non-diabetic healthy controls recruited from hospital in Singapore	Venous blood samples collected for irisin levels from participants.	Serum irisin levels were lower in those with T2DM, significant after adjusting for age and gender differences
Liu et al. [42]	365 T2DM subjects recruited from Singapore hospital	Venous blood samples for lipids, irisin and eGFR and morning spot urine for albumin levels, as well as measurement body composition from each participant.	Irisin was lowest in those with Stage 5 CKD. No correlation was found between irisin and non-fat lean body mass.
Morreno-Navarrete et al. [46]	Study 1: 35 non-obese, 56 obese, 6 non-obese T2DM and 28 obese T2DM recruited from hospital in Spain. Study 2: 76 Caucasian men from northern Spain	Study 1: Adipose tissue and Myocytes from participants were cultured and examined for mRNA and protein levels including FNDC5 and PGC1 α . Serum irisin samples obtained from 29 obese participants. Study 2: participants underwent OGTT and anthropometric measurements	Study 1: white adipose tissue FNDC5 expression decreased with obesity. In obese T2DM, white adipose FNDC5 was decreased significantly. Study 2: Circulating irisin was decreased significantly in those with T2DM.
Kuridova et al. [33]	Study 1: 29 lean healthy controls, 29 healthy overweight/obese individuals, 25 individuals with impaired glucose tolerance, 16 with new onset T2DM Study 2: described in Table 1.	Subcutaneous adipose tissue collected from abdominal fat biopsy and cultured. Myocytes collected from vastus lateralis biopsy, and cultured with 100 μ palmitate and 5.5-20mM glucose. Circulating irisin from venous blood samples.	Circulating irisin decreased by 40% in T2DM, unaltered in other states. In T2DM, Adipose tissue, but not muscle displayed a ~40% decrease in FNDC5 expression. T2DM myocyte FNDC5 expression in vitro was decreased by palmitate and glucose.
Metabolic Syndrome			
Yan et al. [47]	1115 community recruited Chinese individuals with central obesity	Venous blood samples, clinical measurements and lifestyle information obtained	Serum irisin levels significantly reduced in those with metabolic syndrome and raised fasting plasma glucose.
Hee Park et al. [48]	151 Caucasian and African American subjects	Fasting venous blood samples, clinical measurements and lifestyle information obtained	Irisin levels were significantly higher in those with the metabolic syndrome
NAFLD			
Polyzos et al. [49]	24 lean controls, 28 obese controls, 15 NAFL, 16 NASH	Fasting venous blood samples, liver biopsy from NAFLD/NASH individuals, clinical information and measurements including BMI were obtained	Serum irisin levels were significantly lower in the NASH, NAFL and obese group compared to lean. Lower irisin was associated with higher intrahepatic triglyceride content, and higher irisin with portal inflammation
Zhang et al. [50]	296 obese Chinese adults	Venous blood samples, clinical information were obtained. OGTT and intrahepatic triglyceride content were determined.	Serum irisin levels decreased with increasing intrahepatic triglyceride content.
Chronic Kidney Disease			
Wen et al. [44]	38 patients with Stage 5 CKD (excluding those with other health	Morning fasting venous blood samples were collected.	Irisin levels are lower in subjects with Stage 5 CKD

	conditions including diabetes), 19 age and sex matched healthy controls		
Ebert et al. [51]	532 patients with Stage 1-5CKD	Venous blood samples were collected, and clinical information and characteristics obtained.	Irisin levels decrease with increasing severity of CKD.

Table 3: Summary of Studies examining irisin levels in various Metabolic Disease States

In contrast, Seo et al. [33] used a rat model to demonstrate that 4 weeks of exercise and aged garlic extract (AGE) supplementation could both individually reduce body weight and insulin sensitivity, regardless of a high or low fat diet. This occurred without alterations in serum irisin or FNDC5 mRNA/protein levels in visceral adipose tissue, skeletal muscle or liver. Interestingly, serum irisin was significantly lower in the high fat diet non-exercise control group [33], indicating that the regulation of serum irisin levels is complex.

Irisin in response to Exercise: Acute Exercise in Human Subjects

In humans, irisin levels reportedly increase after an acute bout of exercise, but not with long-term exercise. Huh et al. subjected 15 young healthy lean males to 8 weeks of sprint training. They demonstrated that serum irisin levels increased 30 minutes after completion of the first session, but the exercise induction was lost when measured after the final session 8 weeks later [27]. Similarly, Kraemer et al. found an over 20% increase in serum irisin levels in the first 54 minutes of treadmill exercise in healthy young individuals. Irisin levels then reverted to baseline levels despite a further 36 minutes of treadmill exercise and remained steady through 20 minutes of rest [28]. Norheim et al. subjected a group of 13 lean and 13 overweight older men with abnormal glucose tolerance to a 12 week training intervention. In agreement with the previous two studies, they found a 1.2 fold increase in circulating irisin levels immediately after the acute exercise test, which was only apparent in the pre-diabetic group after the 12-week training protocol. Skeletal muscle FNDC5 expression was increased after the 12-week training protocol, but not after the acute exercise. Interestingly, baseline FNDC5 mRNA expression was higher in adipose tissue of the control group compared to the overweight, abnormal glucose tolerant men, potentially indicating a higher metabolic rate in the control group [29]. Lee et al. [23] demonstrated an approximate 3-fold increase in serum irisin levels when measured immediately following 1hr of moderate exercise in a group of healthy young lean individuals [23].

Not all studies have supported an increase in irisin after acute exercise. Kuridova et al. [34] investigated the effects of 1 hr of vigorous exercise as well as a 12-week exercise program in older, overweight obese individuals on serum irisin levels [34]. They found that serum irisin and skeletal muscle FNDC5 mRNA was not altered by either intervention. However, serum samples were obtained 1 hour after the acute bout, which may be too late to detect the acute rise in irisin. Acute resistance, but not aerobic, exercise in young men also induced a 1.4 fold increase in skeletal muscle FNDC5, but not circulating irisin [35]. They found no effect of chronic exercise, however where previous studies reported substantial (~6 fold increases) in PGC1 α levels after exercise, this study only reported a 2-4 fold increase. This may indicate that the exercise intensity was not sufficient to stimulate changes in

muscle gene expression. Additionally, the 3 hr timeframe was likely too long to detect changes in circulating irisin.

In summary, the balance of the evidence suggests that an acute increase in circulating irisin levels occurs during and immediately on completion of high intensity acute exercise in young healthy lean and physically active people. In older people, those with baseline insulin resistance, or sedentary people, it appears that a degree of re-training is required before serum irisin increases can be observed after exercise. There is minimal evidence that FNDC5 mRNA expression is altered in skeletal muscle, suggesting mechanisms other than increased transcription account for the rise in circulating irisin.

Irisin in Response to Exercise: Long Term Changes in Human Subjects

In general, long-term exercise programs have failed to raise serum irisin levels (references Kuridova, Pekkala). Further to this, a randomised control trial of 6 months aerobic or strength training in 102 healthy individuals age 30-60 had no effect on serum morning fasting irisin levels [36]. Of note, the participants in these studies were older than those in the acute exercise studies. Additionally, serum irisin levels do not differ between patients with anorexia nervosa who complete high compared to moderate levels of daily exercise [37]. However, it is hard to extrapolate findings from this population to the general population given their low BMI (~15 kg/m²).

In contrast, Bluhner et al. demonstrated a 12% increase in serum irisin levels in 65 obese children after a 1-year obesity intervention, including exercise [38]. This was independent of the changes in BMI and age that occurred, although there was no lean control group for comparison. This discrepancy might be explained by the lower age of the participants and the addition of a dietary intervention.

Overall, it is difficult to draw conclusions in terms of the role of irisin in human metabolism in response to exercise. There is evidence that circulating irisin increases acutely after exercise. Nevertheless, since mean serum irisin does not simply increase in proportion to long term physical activity, it appears likely that other factors regulate its secretion. Further research into the exact role of irisin in human metabolism and a better understanding of the factors other than exercise that alter irisin secretion is called for.

Irisin: Thermoregulation

There is currently renewed interest in the role of brown adipose tissue in humans. Although humans have very low levels of brown adipose tissue, there is a current appreciation of the existence of 'beige' adipocytes that can be recruited to allow for non-shivering thermogenesis in response to cold exposure. Lee et al. [23] measured circulating irisin levels in young lean individuals whilst exposing them to graded stepwise cold exposure from 24-12°C. Serum irisin levels

rose in subjects in correlation with the extent of shivering. They postulated that irisin is secreted from shivering muscle as a signal to white adipose tissue to increase thermogenesis, and thus increase the capacity for non-shivering thermogenesis [23]. In addition to this, serum irisin levels have been demonstrated to increase by over 25% during the luteal phase of the menstrual cycle, when a rise in core body temperature in women occurs [39]. These results suggest irisin may act as a signal to adipocytes to increase heat production in response to various stimuli.

Irisin: Metabolic Disease

Knowledge of the mechanism of action of irisin suggest that low levels may be a risk factor for developing insulin resistance and metabolic disease. Indeed, low levels have been demonstrated in various metabolic disease states, including type 2 Diabetes Mellitus (T2DM), chronic kidney disease (CKD), non-alcoholic fatty liver disease (NAFLD), obesity and the metabolic syndrome. However, there have again been inconsistencies in these results.

Several studies have demonstrated lower irisin in T2DM. Serum irisin levels were significantly lower in 104 individuals with newly diagnosed and untreated T2DM as compared to age, gender and BMI matched controls [40]. Decreased serum irisin levels were also reported in 96 individuals with T2DM receiving various medications when compared to 60 individuals with normal glucose tolerance [41] as well as in obese T2DM patients. Decreases in irisin levels in T2DM subjects were amplified by renal insufficiency, with the reduction most marked in those with stage 5 CKD [42]. This is in keeping with reports regarding those with renal disease [43,44]. Some have postulated that muscle wasting in end stage kidney disease accounts for this effect, as muscle mass is the main determinant of circulating irisin levels in humans [27]. Interestingly, no correlation exists between irisin levels and percentage of non-fat lean body mass [40]. The question of the importance of muscle will be explored further in a study on the effects of sarcopenia on circulating irisin [45]. A 40% decrease in circulating irisin levels in T2DM subjects (n=6) was associated with a similar decrease in white adipose tissue but not muscle irisin expression [34]. Indeed, it was demonstrated that muscle from T2DM patients actually had the highest levels of irisin secretion in cell culture, but that irisin secretion decreased with increasing glucose concentration from 5.5 to 20 mM in combination with 100uM palmitate. They postulated that diabetic environment inhibited irisin secretion in vivo.

Irisin levels in pre-diabetic states of insulin resistance such as metabolic syndrome, are less clear. In 1115 Chinese adults with central obesity, circulating irisin levels were inversely correlated with the metabolic syndrome, particularly impaired fasting glucose [46,47]. Irisin levels were negatively correlated with waist circumference, but not with BMI. In contrast with these results, in a community study of 151 Caucasian and African American individuals, serum irisin was significantly increased in those with the metabolic syndrome [48]. Key differences between the two studies are the level of central obesity, diagnosis of metabolic syndrome and ethnicity: in the Chinese study all individuals had central obesity and over 60% met the criteria for the metabolic syndrome, whereas only 27.8% of participants in the Caucasian/African American study did.

Irisin levels have also been studied in NAFLD, which is also characterised by insulin resistance. Serum irisin levels were lower in

NAFLD, those with non-alcoholic steatohepatitis and obese individuals when compared to lean controls [49,50].

Chronic Kidney Disease is associated with altered metabolism. Irisin levels are significantly decreased in individuals with stage 5 CKD compared to age matched controls [44]. Similarly, decreased levels of irisin were reported in 532 subjects with CKD, with serum irisin levels correlating with disease severity [51]. As previously discussed, it is unclear as to the reason behind this decrease.

It can be seen there is a clear association between decreased serum irisin levels and metabolic disease states characterised by insulin resistance. Whilst laboratory studies have established a causative role for irisin in metabolic regulation, evidence from human studies is correlational only. Further research will be required to determine whether the decreased irisin levels indeed have a causative role in metabolic disease.

Irisin: Pregnancy and Gestational Diabetes Mellitus

In normal pregnancy, serum irisin levels are altered. Garces et al. found serum irisin levels to be higher in pregnancy. Kuzinicki et al. reported a significant decrease in irisin levels 3 months postpartum in both women with gestational diabetes mellitus (GDM) and controls with normal glucose tolerance. These two studies suggest that the placenta and the pregnant state may contribute to an increase in circulating irisin levels. Irisin has been localised via immunohistochemical staining to the cytoplasm of decidual, cytotrophoblast and syncytiotrophoblast of the placenta [39].

Placental expression of irisin raises questions as to whether it is secreted into the fetal circulation, where it could have important effects on the growth and development of the fetus. This needs further exploration. However, Ebert et al. found no decrease in serum irisin immediately after delivery or 4.3 years later, and calculated that the irisin concentration in the placenta was only 53.3 µg/g total protein.

It is important to note that in the long follow up time of 4.3 years, other influences may contribute to the regulation of irisin levels. However, Piya et al. also reported no significant differences in serum irisin levels between pregnant and a non-pregnant women [52]. Given that muscle and adipose tissue are likely to be key contributors to the pool of circulating irisin in these women, it would be interesting for future studies to include an analysis of irisin levels in muscle and fat biopsies.

GDM is a metabolic disease characterised by insulin resistance, and as such, alterations in irisin levels may occur. Three studies have found a decrease in irisin levels in GDM pregnancies. A description of study design and findings for studies looking at irisin in pregnancy can be found in Table 4. Yuksel et al. reported lower mean serum irisin levels in GDM compared to controls. These results were supported by Kuzmicki et al. who demonstrated lower maternal serum irisin concentrations at time of oral glucose tolerance testing in women with GDM compared to age, BMI and gestational age matched women. As mentioned, irisin levels had decreased 3 months post-partum in both groups, with no persisting differences [53]. It has also been demonstrated that in addition to serum, breast milk from mothers with GDM has significantly lower irisin levels, representing another possible mechanism by which GDM has long term effects on the metabolism of the offspring [54,55].

Study	Participants	Design	Outcomes
Gestational Diabetes Mellitus			
Yuksel et al. [55]	20 GDM women and 20 control women, all planning to deliver via Caesarean Section. No baseline difference in age or BMI between groups.	Venous samples maternal serum obtained prior to delivery, umbilical cord blood collected after delivery.	Lower mean maternal serum irisin in GDM. No difference in cord blood irisin.
Kuzmicki et al. [53]	130 GDM women and 140 normal glucose tolerance women, matched for age, gestational age and BMI.	Venous samples maternal serum collected at time of OGTT, then at 10-12 wks postpartum, and at 3 months postpartum.	Lower mean maternal serum irisin in GDM Irisin drops to same baseline in all women 3 months post-partum
Aydin et al. [54]	15 lactating GDM, 15 lactating control, 14 non-lactating women.	Breast milk and maternal serum sampled on days 1, 7 and 15 of lactation.	Irisin increased significantly with maturation of the milk Plasma and breast milk irisin levels were lower in GDM compared to healthy lactating and non lactating women
Ebert et al. [56]	Study 1: 74 GDM patients and 74 healthy gestational age matched controls Study 2: 40 healthy uncomplicated pregnancies	1: Maternal serum sampled after an overnight fast at OGTT, then at follow up ~1576 days post partum 2: Fasting sample maternal serum prior to and then 24hr after delivery, umbilical cord blood and placental tissue samples.	1: No significant difference between groups, however fasting insulin predicts irisin levels in GDM women 2: No significant changes in irisin 24hr post delivery. Irisin is 53.3µg/g placental tissue protein.
Piya et al. [52]	34 non-obese women, 39 obese women and 18 GDM women.	Maternal serum and CSF samples taken prior to caesarean section. Cord blood taken at time of delivery.	No significant difference in irisin between groups No alterations in umbilical cord irisin Significantly higher CSF irisin in GDM women
Preeclampsia			
Garces et al. [39]	Study 1: 40 healthy women and 10 women who developed preeclampsia taken from a larger longitudinal cohort study Study 2: 20 healthy menstruating women Study 3: Placental tissue from 38 weeks gestation	1: venous blood samples at various points through pregnancy 2: venous blood samples during follicular and luteal phase of menstrual cycle 3: Immunohistochemical staining performed	1: Serum irisin increases by 16% from mid to late pregnancy in all groups Serum irisin is 49% higher in third trimester in healthy compared to preeclamptic women 2: Serum irisin levels are 26% higher in the luteal phase of cycle. 3: FNDC5 stains to the cytoplasm of decidual, cytotrophoblasts and syncytiotrophoblasts.
Idiopathic IUGR			
Caglar et al. [57]	15 women with idiopathic IUGR at third trimester and 15 healthy controls	Maternal blood collected prior to delivery and fetal umbilical cord blood collected with delivery	No difference in maternal serum irisin level Umbilical artery irisin levels higher in normal pregnancy and correlated with birth weight

Table 4: Summary of Studies looking at changes in irisin levels in pregnancy related complications

However, the evidence has not been consistent. Ebert et al. [56] found no significant difference between maternal fasting serum irisin levels between GDM patients and gestational-age matched controls at both the time of oral glucose tolerance testing and at a follow up a mean of 1576 days (or 4.3 years) later [56]. In contrast with all previous studies, Piya et al. [52] found higher serum and CSF irisin

levels in both obese pregnant and GDM pregnant women compared to non-obese controls. This led to speculation as to whether irisin had a central role in metabolism, as irisin was found to be present in hypothalamic tissue where it co-localised with Neuropeptide-Y, a key appetite regulating hormone [52]. No studies have reported a difference between cord blood irisin levels in GDM [52,55].

Irisin has been studied in relation to both preeclampsia and intrauterine growth restriction. Garces et al. [39] found that serum irisin levels increased in a group of 40 women throughout pregnancy, but in 10 women who developed preeclampsia, third trimester levels were 49% lower [39]. No significant difference has been found between maternal serum irisin levels in idiopathic intrauterine growth restriction compared to uncomplicated pregnancy [57]. It was however demonstrated that umbilical artery irisin levels were significantly increased in the control group, possibly reflecting larger fetal body mass. Further research is required to confirm these results.

It can be seen that there is still conflicting evidence as to the exact changes in irisin that occur in GDM, and that further research in this area is warranted. Again, this research is of a correlational nature only, and a causative role for irisin in the pathogenesis of these diseases is yet to be established.

Conclusion

Irisin is a newly discovered myokine that acts to increase UCP1 expression in white adipose tissue. Such an alteration in gene expression 'browns' the adipose tissue, and protects against insulin resistance in *in vitro* experiments and animal models. Further research is required to determine the importance of irisin to human metabolism and the factors that regulate irisin expression in humans. Preliminary research indicates that irisin is decreased in human metabolic disease states characterised by insulin resistance including pregnancy complications such as GDM.

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