The identification of irisin in human cerebrospinal fluid: influence of adiposity, metabolic markers, and gestational diabetes

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Piya MK, Harte AL, Sivakumar K, Tripathi G, Yovias PD, James S, Sabico S, Al-DaghrI NM, Saravanan P, Barber TM, Kumar S, Vatish M, McTernan PG. The identification of irisin in human cerebrospinal fluid: influence of adiposity, metabolic markers, and gestational diabetes. Am J Physiol Endocrinol Metab 306: E512–E518, 2014. First published January 7, 2014; doi:10.1152/ajpendo.00308.2013.—Peripheral action of irisin improves glucose homeostasis and increases energy expenditure, with no data on a central role of irisin in metabolism. These studies sought to examine 1) presence of irisin in human cerebrospinal fluid (CSF) and banked human hypothalamic tissue, 2) serum irisin in maternal subjects across varying adiposities with or without gestational diabetes (GDM), and 3) their respective neonate offspring. CSF, serum, and neonatal cord serum were collected from 91 pregnant women with and without GDM attending for an elective cesarean section [body mass index (BMI): 37.7 ± 7.6 kg/m2; age: 32 ± 8.3 yr]. Irisin was assessed by ELISA and correlated with biochemical and anthropometric data. Irisin expression was examined in human hypothalamus by immunohistochemical staining. Serum irisin in pregnant women was significantly lower in nonobese compared with obese and GDM subjects, after adjusting for BMI, lipids, and glucose. Irisin was present in maternal serum, cord serum, and maternal CSF. Irisin correlated positively with serum irisin levels from nonobese and obese pregnant women (P < 0.01), with CSF irisin significantly raised in GDM subjects (P < 0.05). Irisin was present in human hypothalamic sections in the paraventricular neurons, colocalized with neuropeptide Y. Irisin was detectable in CSF and in paraventricular neurons. Maternal serum irisin was lower in nonobese pregnant women after adjusting for BMI and a number of metabolic parameters. These studies indicate that irisin may have a central role in metabolism in addition to the known peripheral role. Further studies investigating the central action of irisin in human metabolic disease are required.

EMERGING DATA SUGGEST THAT a newly discovered polypeptide hormone, irisin, a cleaved secretion form of fibronectin type III domain containing 5 (FNDC5), has the potential to increase energy expenditure and improve glucose homeostasis in humans (4, 16, 31, 34). This is particularly significant, since irisin can induce the transformation of white adipocytes into “beige” or “brite” adipocytes, which can ultimately lead to increased mitochondrial respiration (4, 34), with implications for weight loss. Therefore, such studies suggest the potential therapeutic applications of irisin not only in use for weight loss but also to improve glucose metabolism (4). Subsequent research has also revealed that the function of irisin appears to fall beyond its original role noted in muscle (4, 9, 13, 21, 23), and the administration of exogenous irisin could theoretically increase energy expenditure during or after weight loss. Recent studies have shown that irisin may also act as an adipokine (21, 25) as well as a potential “neurokine” (9, 12). Although the role of irisin in the brain is unclear, analysis has revealed that FNDC5 knockout in murine embryonic stem cells reduces neurogenesis (11), whereas pharmacological doses of irisin increase proliferation of mouse hippocampal neuronal cells (20). Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) regulates FNDC5 expression, and recent studies have shown an increase in FNDC5 expression in mouse hippocampus neuronal cells in response to endurance exercise that is reduced in PGC1α knockout mice (33). Given that exercise is known to improve neurogenesis and slow the progression of neurodegenerative diseases (29), irisin may have both central and peripheral functions that could be used for therapeutic use in combination with exercise.

To determine the therapeutic potential of irisin, studies have subsequently focused on the peripheral changes in irisin in different clinical metabolic states. These studies have highlighted conflicting reports on changes in circulating irisin levels in patients with insulin resistance states such as obesity, type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus (GDM) (3, 5, 12, 17, 21, 22, 24, 28, 30). Specifically, some reports have noted an increase in circulating irisin, observing a negative correlation with hemoglobin A1c, body mass index (BMI), lipids, and irisin or no change in irisin with insulin sensitivity (12, 24, 28, 30). Serum irisin in children, especially girls, has been shown to have a significant negative correlation with fasting blood glucose, suggesting a possible link of irisin to glucose homeostasis from an early age (1).

We examined cerebrospinal fluid (CSF), serum, and cord blood levels of irisin in obese patients with and without GDM undergoing cesarean section under spinal anesthesia to test the...
These studies also examined the changes in serum irisin be-
establish the presence of irisin in human CSF as well as banked
that metabolic disease status may affect the central and periph-
metabolic regulation of energy homeostasis and, moreover,
thesis that irisin has both a peripheral and central role in

- **LDL**, mmol/l: 4.0
- **HDL**, mmol/l: 1.7
- **TG**, mmol/l: 2.6
- **Total cholesterol**, mmol/l: 6.9
- **Insulin**, pmol/l: 4.3
- **Glucose**, mmol/l: 1.8
- **Age**, yr (means ± SD): 32.0

Baseline clinical and metabolic data of pregnant and nonpregnant women

30 kg/m². GDM was diagnosed based on World Health Organiza-
for pregnant women at 12 wk gestation, and obese was defined as BMI
or current or recent use of systemic high-dose corticosteroids, antibi-
patients with a C-reactive protein level above 10 mg/dl were also excluded. BMI was calculated
AJP-Endocrinol Metab (n 34), obese women (n 39), and GDM women (n 18) (Table 1). Corresponding cord blood was taken from the neonates upon delivery (n 91; Table 2), and all samples were processed. In the GDM group, 5% were treated with diet alone, 39% each were on metformin or insulin alone, and 17% were on a combination of metformin and insulin. Blood samples were also obtained from a cohort of nonpregnant lean (BMI <25 kg/m²) (n 16) and obese (BMI >30 kg/m²) (n 18) women (Table 1).

**Biochemical analysis.** Serum samples were centrifuged 30 min after collection and stored at −80°C, whereas CSF samples were snap-frozen and stored at −80°C. Fasting blood samples were subsequently assessed for lipid profiles and fasting plasma glucose, using routine laboratory methods, undertaken in the biochemistry laboratory at University Hospital Coventry and Warwickshire (UHCW). In brief, these routine blood tests included serum glucose and full lipid profile [total cholesterol, triglycerides (TG), high density lipoprotein (HDL), and low density lipoproteins (LDL)], as noted in Table 1. Insulin measurements were performed by a solid-phase enzyme-amplified sensitivity multiplex immunoassay (Millipore, Hertfordshire, UK), and glucose was measured by a glucose oxidase method (YSI 200 STAT plus). For analysis of CSF levels, samples were used undiluted, following a serial dilution test. For serum evaluation, irisin manufac-
turers’ guidelines were followed, whereas for cord blood a 1:5 dilution was used because of the noted lower levels compared with serum. Hemocytometer analysis of the CSF samples did not show any nucleus-bearing cells. CSF samples were also screened for blood using the hemocult test (Hema-screen; Immunostics). The hemocult test was negative for CSF samples, whereas the test remained positive with spiking of full blood at a concentration of 1:10,000, as previously described (14). The homeostasis assessment model insulin resistance (HOMA-IR) index was calculated using the formula

\[ \text{HOMA-IR} = (\text{glucose (mmol/l)} \times \text{insulin (mU/l)})/22.5 \]

Samples for GDM patients were excluded from the insulin assay and HOMA-IR calculation.

Assessment of irisin by ELISA. Maternal serum, cord serum, and CSF were assayed using an irisin ELISA kit (Phoenix Europe, Karlsruhe, Germany). Spiking and recovery of human irisin in serum and CSF was examined to further validate the ELISA kit, using a commercially bought recombinant human (rh) irisin. The irisin ELISA kit was validated for recovery of CSF irisin by adding known concentrations of rh irisin (25, 50, and 100 ng/ml; Cayman Chemic-

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<th>Table 1. Baseline clinical and metabolic data of pregnant and nonpregnant women</th>
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<td>Age, yr (means ± SD)</td>
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<td>BMI, kg/m² (means ± SD)</td>
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<th>Table 2. Baseline comparison of neonate cord blood parameters across groups of pregnant women</th>
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<td><strong>Nonobese</strong></td>
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<td>Birth wt. kg</td>
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<td>Cord glucose, mmol/l</td>
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<td>Cord insulin, pmol/l</td>
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<td>Cord cholesterol = 16.1 ± 0.07</td>
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<td>Cord leptin, ng/ml</td>
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<td>Cord irisin, ng/ml</td>
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Values expressed as means ± SE; n, no. of subjects. *P < 0.05 and **P < 0.01 compared with GDM.
Body mass index (BMI) that were added to pooled CSF (46 ng/ml). The percentage of irisin recovery from CSF ranged from 70 to 103%, with a mean of 81% in CSF, similar to the range noted by the manufacturer, with a spiked irisin recovery ranging from 82 to 104%, and a mean spiked recovery of 91%.

For serum analysis, known concentrations of rh irisin (25, 50, and 100 ng/ml; Cayman Chemicals) were added to pooled serum (1,447 ng/ml), based on a 1:20 dilution, to ensure the spiking remained within the linear range. The percentage of irisin recovery ranged from 93 to 97%, with a mean of 94% in serum, which highlighted a similar percentage recovery to the manufacturer (spiked irisin recovery range from 82 to 104% and a mean spiked recovery of 91%). In accordance with the manufacturer’s guidelines, assay limits were noted between 0.1 and 1,000 ng/ml with an intra-assay coefficient of variation <10% and interassay variability <15%.

Further comparison of the irisin ELISA kit with another commercially available kit (Aviscera Biosciences, Santa Clara, CA) was undertaken on serum and CSF samples. Although the absolute levels of detected irisin in CSF, cord blood, and maternal serum were around threefold lower than the levels detected on the Phoenix kit, the ratio of CSF irisin to cord irisin and maternal serum irisin remained the same.

Immunohistochemistry. Anonymized, human tissue samples for immunohistochemical staining were obtained from the Human Tissue Bank at UHWC for both human hypothalamic brain sections as well as sternum skeletal muscle. For immunohistochemistry, sections of tissue were incubated with primary polyclonal irisin antibody (Phoenix Europe) in a dilution of 1:200 and dual stained with neuropeptide Y (NPY) in a dilution of 1:500. Sections were developed using a peroxidase substrate kit VIP (Vector Laboratories Peterborough) for irisin and dianminobenzidene for NPY (BioGenex). To demonstrate specific binding, the primary antibody was blocked with rh irisin (Cayman Chemicals) in a 1:50 dilution and stained for negative control for both hypothalamus and skeletal muscle.

Statistical analysis. Data analysis was performed using SPSS version 21 (SPSS, Surrey, UK). Variables are expressed as means ± SE unless otherwise specified. Non-Gaussian variables such as BMI, glucose, insulin, HOMA-IR, TG, leptin, and irisin were log-transformed before parametric comparisons. ANOVA was used to compare serum lipids or glucose.

Analysis of covariance (ANCOVA) was carried out to determine associations of detected irisin in CSF, cord blood, and maternal serum were around 100 ng/ml; Cayman Chemicals) were added to pooled CSF (46 ng/ml). The percentage of irisin recovery ranged from 70 to 103%, with a mean of 91%.

Irisin serum levels in women. Serum irisin levels were similar in pregnant women from the nonobese (805 ± 53 ng/ml), obese (791 ± 52 ng/ml), and GDM (802 ± 88 ng/ml; Table 1) groups. Serum irisin levels were similar in a separate cohort of nonpregnant lean (781 ± 49 ng/ml) and obese (795 ± 83 ng/ml) women. Analysis of the pregnant subjects revealed that there was a significant inverse correlation of BMI with serum irisin (r = −0.25, P = 0.026) (Fig. 1A). In the pregnant cohort, serum irisin levels were also positively correlated with glucose (r = 0.40, P < 0.001), insulin (r = 0.26, P = 0.048), HOMA-IR (r = 0.32, P = 0.013), total cholesterol (r = 0.35, P = 0.002), TG (r = 0.33, P = 0.003), LDL (r = 0.24, P = 0.036), and HDL (r = 0.27, P = 0.017). Analysis using ANCOVA revealed that serum irisin was significantly lower in nonobese pregnant women compared with the obese pregnant and GDM women after adjusting for BMI, serum lipids, and glucose (P < 0.01), whereas there was no significant difference between serum irisin levels across groups when only adjusting for serum lipids or glucose.

Irisin cord serum levels in neonates. Baseline biochemical data were collated as described in Table 2. Irisin levels were significantly lower in cord serum samples compared with adult irisin levels (neonate irisin: 237 ± 8 ng/ml; maternal irisin: 799 ± 35 ng/ml; P < 0.001; Tables 1 and 2). Maternal serum irisin levels appeared to correlate with cord serum irisin levels (r = 0.28, P = 0.015) (Fig. 2A). No further correlations were noted between neonatal serum irisin, taking into account neonatal biochemical data as well as birth weight or maternal BMI. Cord serum leptin levels were also noted to have a positive correlation with cord serum irisin levels (r = 0.24, P = 0.035; Fig. 2B).

Irisin CSF levels in women. Cerebrospinal irisin levels were 32 ± 1.5 ng/ml. The levels in the CSF were therefore ~20- to 25-fold lower than the maternal serum. CSF irisin did not correlate with BMI, lipids, or HOMA-IR. GDM status significantly increased CSF irisin levels compared with nonobese women (39 ± 2.8 ng/ml) (P < 0.05).
From the entire cohort, CSF irisin levels positively correlated with serum irisin levels ($r = 0.32, P = 0.006$) (Fig. 3A). This positive correlation was significant in the nonobese and obese groups (Fig. 3, B and C), but the correlation was lost with GDM status (Fig. 3D). In contrast to irisin serum data, there was no correlation between CSF irisin levels and BMI ($r = 0.06$, not significant) (Fig. 1B).

Expression of irisin in the human hypothalamus. Immunohistochemistry showed the presence of irisin in the neuronal cells in the human hypothalamus from donated samples obtained from a human tissue bank (Fig. 4A). Irisin was observed to be concentrated in the paraventricular nucleus (PVN) within the neurons, which were dual stained with NPY (Fig. 4D). Skeletal muscle was used as a positive control for irisin expression (Fig. 4E).

**DISCUSSION**

From this study, we have identified that irisin is detectable in CSF, and CSF levels are higher in GDM mothers compared with nonobese and obese pregnant mothers. We have also identified that irisin was detectable in human hypothalamic sections, concentrated around the PVN region. Serum irisin in the pregnant women was positively correlated with glucose, insulin, HOMA-IR, total cholesterol, TG, LDL, and HDL, and the serum irisin levels in the nonobese women were significantly lower than the obese and GDM women after correction for BMI, serum lipids, and glucose. Irisin was also detectable in neonatal cord serum, with no differences in levels between groups.
On the basis of this study, CSF irisin appears ~20- to 25-fold lower than circulating serum levels. This still remains relatively high, particularly since previous CSF-to-serum ratios for human adipokines have shown a 1:100-fold ratio for leptin, 1:1,000-fold ratio for adiponectin, and 1:100-fold ratio for resistin (14, 15). The irisin CSF levels, although much lower than concentrations in the periphery, are sufficient for activation of neural pathways and signaling in the hypothalamic nuclei for proteins like leptin (7, 35).

To address whether irisin may influence energy expenditure, immunohistochemical analysis was undertaken to detect the presence of irisin in banked human brain tissue. From the immunohistochemistry, irisin was observed to be present in the human hypothalamus, with concentrated expression of irisin in neuronal cells of the PVN, which also express NPY. There may be a cross-reactivity between irisin and FNDC5, but the presence in these neurons is particularly relevant, since the PVN receives input from the arcuate nucleus, a region of the brain containing key appetite-regulating pathways via NPY neurons (8).

Analysis of irisin levels also observed a positive linear correlation between serum and CSF irisin, with this correlation being lost in the GDM group. However, in the GDM group, CSF irisin levels were significantly higher when compared with the nonobese group. This increase in irisin CSF could suggest a compensatory mechanism, in GDM subjects, with a metabolically compromised state, as suggested previously for serum irisin (12). Furthermore, the positive linear correlation between serum and CSF irisin suggests that the detected CSF irisin may be derived from the periphery and that the blood-brain barrier (BBB) limits access of irisin in the lean and obese states.

Taken together, the current serum/CSF data would suggest a transport mechanism for irisin crossing the BBB, rather than simple osmosis, but it is unknown what may mediate this mechanism. Ideally, for an active transport mechanism, it would be important to establish irisin receptor presence on human endothelial cells of the choroid plexus. This region controls entry of proteins through the BBB and is where prior studies have identified functional leptin receptors (19). If irisin could be transported from blood to CSF by receptor-mediated transcytosis, similar to a mechanism proposed for leptin, this would support the concept that CSF entry may be facilitated at lower irisin levels in nonobese subjects, which may support body energy homeostasis and enhance thermogenesis, again, in
a similar fashion to leptin (27, 32). In the GDM state, one may speculate that such a mechanism may become dysfunctional and, as a consequence, lead to “irisin resistance.” However, to illustrate the functional role of irisin in a central nervous system, it would be important to identify irisin receptors and study the effects of intracerebroventricular administration of irisin on energy expenditure in animal studies as noted for leptin (14, 15).

Specific maternal analysis of serum leptin showed an anticipated increase with adiposity and GDM status and was positively associated with BMI, insulin, and HOMA-IR. Independent of BMI, serum leptin remained correlated with insulin and HOMA-IR. These leptin data emphasized this cohort to be typical based on previous studies and current literature (6, 27, 32). Interestingly, maternal serum irisin directly correlated with cord irisin, although at significantly lower levels, and cord irisin levels positively correlated with cord leptin. Such an association suggests the possibility that irisin levels could be predetermined in utero by maternal influences, as previously noted for leptin and adiponectin (10, 26). Our data also highlighted that the level of serum irisin in pregnancy was not solely related to this state, since findings were comparable in the nonpregnant state, affirming previous findings using the same irisin ELISA kit from Phoenix Europe (1, 30).

Assessment of serum irisin levels highlighted that there was no significant difference between nonobese, obese, and GDM groups, although a negative correlation between BMI and serum irisin was noted. Further analysis using ANCOVA was undertaken and demonstrated that the serum irisin levels were significantly larger in the nonobese pregnant group compared with the obese pregnant and GDM groups, after adjusting for a combination of BMI, serum lipids, and serum glucose. Furthermore, in addition to supporting the emerging concept of irisin resistance in metabolic disease, this may partly explain the conflicting reports on the serum levels of irisin in different metabolic disease states (3, 5, 12, 17, 21, 24, 28, 30).

This is the first study to show presence of irisin in human CSF as well as in the human hypothalamus. Serum samples have been collected from three different groups of pregnant women: GDM, obese, and nonobese groups. The strength of this study is that CSF samples and neonatal cord serum have also been collected from the corresponding mothers, allowing for a detailed study of the possible role of irisin in humans as well as the possible influences of metabolic state. The main weakness of this study is that the serum and CSF samples were obtained from a cohort of pregnant women, which may not be representative of a nonpregnant population. The BMI of the women was calculated at 12 wk gestation to avoid the effect of weight gain in pregnancy on the BMI, whereas the serum and CSF samples were collected at the time of delivery. However, given that serum irisin levels from nonpregnant women were found to be similar to the levels in pregnant women, and serum leptin levels were positively correlated to the calculated BMI, we believe that these data may be extrapolated to different populations. In summary, irisin is present in human CSF and appears colocalized with NPY in the neuronal cells of the PVN in the hypothalamus. Irsin CSF levels appeared correlated with serum levels and influenced by metabolic markers and GDM status. As such, these findings strengthen the concept that irisin has both peripheral and central functions. Further studies are required in nonpregnant populations, as well as to examine the direct influence of irisin on central metabolism, as part of understanding whole body irisin metabolism, and whether obesity-coupled T2DM status leads to irisin resistance in a similar fashion noted for leptin.

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DISCLOSURES

M. Vatish and P. G. McTernan are the guarantors of this work, had full access to all the data, and take full responsibility for the integrity of data and the accuracy of data analysis.

AUTHOR CONTRIBUTIONS


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