OPEN

Citation: Nutrition & Diabetes (2014) **4**, e110; doi:10.1038/nutd.2014.7

www.nature.com/nutd

SHORT COMMUNICATION

Higher baseline irisin concentrations are associated with greater reductions in glycemia and insulinemia after weight loss in obese subjects

P Lopez-Legarrea[1,2], R de la Iglesia[1], AB Crujeiras[3,4,5], M Pardo[3,4], FF Casanueva[4], MA Zulet[1,3] and JA Martinez[1,3]

Irisin is assumed to be a relevant link between muscle and weight maintenance as well as to mediate exercise benefits on health. The aim of this study was to assess the possible associations between irisin levels and glucose homeostasis in obese subjects with metabolic syndrome (MetS) following an energy-restricted treatment. Ninety-six adults with excessive body weight and MetS features underwent a hypocaloric dietary pattern for 8 weeks, within the RESMENA randomized controlled trial (www.clinicaltrials.gov; NCT01087086). After the intervention, dietary restriction significantly reduced body weight and evidenced a dietary-induced decrease in circulating levels of irisin in parallel with improvements on glucose homeostasis markers. Interestingly, participants with higher irisin values at baseline (above the median) showed a greater reduction on glucose ($P = 0.022$) and insulin ($P = 0.021$) concentrations as well as on the homeostasis model assessment index ($P = 0.008$) and triglycerides ($P = 0.006$) after the dietary intervention, compared with those presenting low-irisin baseline values (below the median). Interestingly, a positive correlation between irisin and carbohydrate intake was found at the end of the experimental period. In conclusion, irisin appears to be involved in glucose metabolism regulation after a dietary-induced weight loss.

Nutrition & Diabetes (2014) **4,** e110; doi:10.1038/nutd.2014.7; published online 24 February 2014

INTRODUCTION

Obesity is a worldwide health burden, accompanied by a number of comorbidities including glucose intolerance, insulin resistance and type 2 diabetes.[1] In this context, the myokine irisin,[2] which is a cleavage product of the type I membrane protein fibronectin type III domain-containing 5, has been hypothesized as a target to counteract obesity and type 2 diabetes.[3,4] Irisin is expressed in the muscle and the adipose tissue and has been associated with adiposity and body weight in animals[5,6] and humans.[7,8] However, the precise role and underlying mechanisms concerning irisin actions and signaling pathways remain incompletely understood.

The aim of this research was to assess changes on circulating irisin concentrations in obese subjects presenting metabolic syndrome (MetS) features after a treatment designed to lose weight and to analyze the potential relationships of this myokine with glucose homeostasis after dieting.

MATERIALS AND METHODS

Study protocol

This research reports the findings of the 8-week intervention period of the RESMENA randomized intervention trial (www.clinicaltrials.gov; NCT01087086), which was conducted following the CONSORT 2010 criteria. A full list of inclusion criteria, as well as a complete description of the study methodology can be found in earlier publications.[9,10] Briefly, participants were randomized into two intervention groups, with the same energy restriction (−30% E), but differing mainly in the carbohydrate/protein ratio and meal frequency: control group supplying 55% E from CHO and 15% E from proteins within a 3–5 meals per day pattern, and RESMENA group providing 40% E from CHO and 30% E from proteins within a 7 meals per day plan.

Subjects

Ninety-six adults (mean age = 50 years old; range 21–70 years old) with excessive body weight (mean body mass index = 35.9 kg m^{-2}; range 26.9–49.4 kg m^{-2}) suffering MetS according to the International Diabetes Federation criteria completed the intervention period. All the participants gave a written informed consent to participate as approved by the Ethics Committee of the University of Navarra (065/2009) and in accordance with the Declaration of Helsinki.

Participant's dietary intake was assessed by means of 48-h weighed records at baseline and at the end of the intervention and further analyzed using the DIAL software (Alce Ingenieria, Madrid, Spain). Subjects were asked to maintain their usual activity levels during the study, which was monitored at the beginning and endpoint with a validated 24-h physical activity questionnaire.[9]

Anthropometric measurements and body composition determinations were performed, as described elsewhere.[9] Overnight fasting plasma levels of glucose and triglycerides were measured in an autoanalyzer Pentra C-200 (HORIBA ABX, Madrid, Spain) with specific kits from this company. Insulin concentrations were determined with an enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden) in a Triturus autoanalyzer (Grifols SA, Barcelona, Spain) and the homeostasis model (homeostatic model assessment-insulin resistance (HOMA-IR)) was applied to estimate insulin resistance.

Irisin plasma levels were determined using a commercial enzyme-linked immunosorbent assay kit following the manufacturer's instructions (Irisin ELISA kit EK-067–52; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA),

[1]Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain; [2]Faculty of Health Science, Universidad Autonoma de Chile, Santiago, Chile; [3]CIBERObn, Carlos III Health Institute, Madrid, Spain; [4]Laboratory of Molecular and Cellular Endocrinology, Health Research Institute (IDIS), University of Santiago Hospital Complex (CHUS) and Santiago de Compostela University (USC), Santiago de Compostela, Spain and [5]Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain. Correspondence: Professor JA Martinez, Department of Nutrition, Food Science and Physiology, University of Navarra, C/Irunlarrea 1, Pamplona 31008, Spain.
E-mail: jalfmtz@unav.es
Received 5 September 2013; revised 4 January 2014; accepted 18 January 2014

on a spectrophotometric reader at a wavelength of 450 nm (Versamax Microplate Reader, East Falmouth, MA, USA). This test provided a range of detection of 0.066–1024 ng ml^{-1} and exhibited a coefficient of variation of 6–10% inter- and intra-assay. The samples were kept at $-80\,^{\circ}C$ and were analyzed immediately after the experiment was ended.

Statistical analysis

The sample size of this secondary analysis was calculated for an $\alpha = 0.05$ and a power of 80% based on the waist circumference reduction, as described elsewhere.[9] Normality distributions of the measured variables were determined according to the Shapiro–Wilk test. Irisin plasma levels were not normally distributed, but based on the sample size ($n > 60$) a parametric test was performed. Indeed, after analysis with a log transformation of irisin values the statistical outcomes were maintained. Differences between baseline and endpoint values within groups were analyzed by a paired *t*-test. Analyses between dietary groups were performed with unpaired *t*-tests. A multiple linear regression analysis was applied in order to assess the potential relationships among irisin with anthropometric and biochemical measurements (95% confidence interval). The median value of irisin baseline concentrations was considered as the cutoff for analyzing the effect of high- or low-irisin levels on glucose regulatory factors, as previously applied.[11] This tool is based on the assignment of the studied population into two groups of disease risk. The association between irisin levels and carbohydrate intake was assessed using the parametric Pearson correlation. Specific statistical analyses (analysis of covariance) were performed after excluding outlier values in order to control the regression to the mean phenomenon. Statistical analysis was performed using SPSS15.1 software (SPSS Inc., Chicago, IL, USA). An alpha level of 0.05 was set up for determining statistically significant differences. Data are reported as mean ± s.e.

RESULTS

At the beginning of the intervention, there were no differences between groups in any of the anthropometric and routine biochemical markers ($P > 0.05$). After the intervention, an improvement (reduction) was observed on these measurements with apparently equal effectiveness between the two dietary treatments ($P > 0.05$, Table 1), except for adiponectin, which was increased in both groups, but without reaching statistical significance. Changes in irisin concentrations were similar ($P > 0.05$) in the control group (-87.3 ± 18.4 ng ml^{-1}) as compared with the RESMENA group (-59.8 ± 11.8 ng ml^{-1}), after following the energy-restricted treatment. Therefore, both groups were merged for subsequent analyses. Considering the whole sample, participant's mean body weight loss was -6.9 ± 3.0 kg and irisin plasma concentrations diminished (Figure 1a) in association with changes in body weight ($r = 0.21$; $P = 0.046$) and fat mass ($r = 0.22$; $P = 0.037$). As the main objective of this study was to evaluate the potential role of irisin on glucose homeostasis and given that some of the participants were diabetic, a preliminary analysis separating non-diabetic and diabetic participants was also performed. Differences were found for glucose concentrations and HOMA index between both groups after the nutritional intervention with energy restriction, but similar outcomes were found concerning irisin concentrations (data not shown).

Similar values were found concerning physical activity assessments at the beginning and at the end of the intervention in both dietary groups. Moreover, the regression analysis showed no relationships between physical activity factor and irisin levels changes ($P = 0.736$). An association of circulating glucose ($B = -0.134$, 95% confidence interval: -0.245 to -0.024; $P = 0.018$) and irisin concentrations changes was found, irrespective of confounding factors: gender, age, diet, body weight loss and irisin baseline values.

Interestingly, after adjusting for gender, age and weight loss, participants belonging to the high-irisin group at baseline (> 308.0 ng ml^{-1}) evidenced significantly greater reductions (Figure 1b) on glucose ($P = 0.022$), insulin ($P = 0.021$), HOMA index ($P = 0.008$) and triglycerides ($P = 0.006$), compared with those belonging to the low-irisin group at baseline (< 308.0 ng ml^{-1}). Furthermore, the decrease in irisin concentrations was significantly greater ($P < 0.001$) within the group with high-irisin values at baseline (-126.6 ± 15.9 ng ml^{-1}) than within the lower irisenemia group (-18.2 ± 9.1 ng ml^{-1}). After 8 weeks of nutritional intervention, irisin concentrations were positively correlated with carbohydrate intake (cereals, pulse, fruits and vegetables; $r = 0.234$, $P = 0.023$; Figure 1c).

DISCUSSION

This study evidenced that irisin *per se* may exert an effect on the reduction of glucose, insulin and triglycerides concentrations after prescribing an 8-week nutritional intervention to obese subjects with MetS traits.

Irisin is a recently discovered muscle-derived hormone, whose secretion is induced by exercise.[2] This myokine has been shown to be able to increase energy expenditure, and therefore, it has been proposed to have a potential role in obesity and diabetes treatments.[2,12–14] Since discovery, a number of original studies have addressed various aspects of the biology of irisin.[15] However, the regulation and specific role of irisin in human's glucose metabolism remain still unclear. Thus, the main objective of the current research was to investigate the potential relationships between irisin concentrations and glucose homeostasis, after dieting.

Table 1. Changes in selected anthropometric and biochemical parameters within each dietary group (control and RESMENA) after the 8-week intervention and comparison between groups

	Control group			*RESMENA group*			*Difference between diet groups* (P-*value*)
	Baseline	*Endpoint*	P-*value*	*Baseline*	*Endpoint*	P-*value*	
Body weight (kg)	99.5 ± 2.8	92.7 ± 2.7	< 0.001	100.0 ± 2.4	92.9 ± 2.3	< 0.001	0.555
BMI (kg m^{-2})	36.2 ± 0.7	33.7 ± 0.7	< 0.001	35.6 ± 0.6	33.0 ± 0.6	< 0.001	0.732
Fat mass (%)	39.1 ± 1.1	36.2 ± 1.1	< 0.001	39.2 ± 0.9	36.4 ± 1.0	< 0.001	0.854
Fat mass (kg)	39.0 ± 1.6	33.7 ± 1.5	< 0.001	39.2 ± 1.4	33.8 ± 1.3	< 0.001	0.886
Glucose (mg dl^{-1})	121.0 ± 5.0	108.0 ± 2.0	0.006	123.8 ± 5.5	110.2 ± 3.8	0.016	0.939
Insulin (μU ml^{-1})	15.3 ± 1.7	9.3 ± 1.1	< 0.001	14.4 ± 1.2	9.1 ± 0.9	< 0.001	0.557
HOMA	4.7 ± 0.6	2.6 ± 0.3	< 0.001	4.5 ± 0.4	2.6 ± 0.3	< 0.001	0.686
Triglycerides (mg dl^{-1})	176 ± 13	145 ± 10	0.005	194 ± 18	151 ± 14	< 0.001	0.421
Irisin (ng ml^{-1})	412.3 ± 31.6	326.7 ± 22.6	< 0.001	299.4 ± 16.3	239.6 ± 8.8	< 0.001	0.234
Leptin (ng ml^{-1})	22.4 ± 2.3	14.8 ± 1.8	< 0.001	20.2 ± 2.1	12.8 ± 1.6	< 0.001	0.883
Adiponectin (ng ml^{-1})	13.6 ± 1.5	13.8 ± 1.3	0.863	12.1 ± 1.3	17.6 ± 3.3	0.127	0.152

Abbreviations: BMI, body mass index; HOMA, homeostasis model assessment.

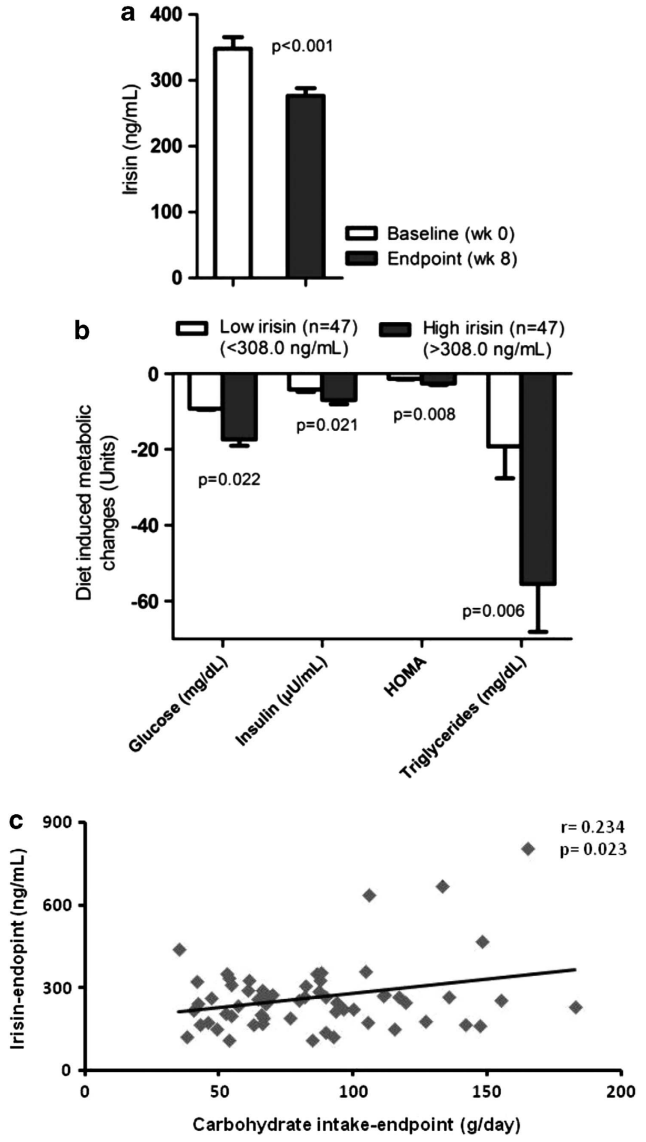

Figure 1. Irisin changes from baseline (week 0) to the end (week 8) of the intervention (**a**); changes in glucose, insulin, HOMA index and triglycerides, depending on irisin baseline levels after the intervention of 8 weeks duration (**b**); and irisin correlation with carbohydrate intake (cereals, pulse, fruits and vegetables) at the endpoint of the intervention (**c**).

The study was designed as a randomized controlled nutritional intervention comparing two energy-restricted dietary treatments.[9] Both control and RESMENA dietary strategies proved to be effective for improving MetS disturbances by lowering anthropometric and biochemical markers, being these outcomes in agreement with other studies concerning hypocaloric diets.[16] However, no differences between treatments were observed for any of the studied variables including irisin. For that reason, the sample was merged and considered as a whole for the subsequent analyses regarding irisin concentrations and its potential associations with glucose metabolism.

First, changes on irisin concentrations after the 8 weeks of nutritional intervention were evaluated. This study evidenced that irisin plasma concentrations decreased after the energy restriction program and the subsequent weight loss, independently of the dietary group. This finding is in agreement with a previous study that reported a reduction in irisin levels after surgically induced weight markdown.[8]

Then, the potential role of irisin on glucose homeostasis-related parameters was analyzed in order to reach the main objective of the research. The prime finding of the current investigation was that higher irisin concentrations at the beginning of the intervention were associated with greater reductions on glucose and insulin concentrations as well as on the HOMA index, independently of body weight loss. Although this outcome should be carefully examined, similar results have been reported in children by Al-Daghri *et al.*[17] where a crucial role for irisin in glucose homeostasis was suggested. On the other hand, those individuals with higher irisin concentrations at the beginning of the intervention also achieved higher beneficial effects regarding the lowering of triglycerides concentrations. This effect could be explained by the fact that triglycerides levels have been revealed to positively correlate with glucose levels.[18] Thus, the effects of irisin on the changes of glucose concentrations may have been subsequently reflected on triglycerides. In addition, taking into account that irisin has been evidenced to increase energy expenditure,[19] the greater reduction observed in triglycerides according to the high-irisin levels at baseline may be also due to a higher utilization of triglycerides as energy substrate. Previous studies have also evidenced an inverse association of irisin levels with triglycerides concentrations.[20] Taking together these outcomes, it can be suggested that irisin may be involved in the regulation of glucose homeostasis in obese subjects presenting MetS features. Thus, irisin could mean a physiological feedback to counteract potential glucose metabolism-related disturbances associated to an excessive body weight state. Irisin would seem to be increased in unfavorable metabolic situations acting as a compensatory triggering mechanism. Other authors have likewise claimed that the increase in irisin under obesity conditions may indicate a physiological adaptation to improve glucose tolerance, which is often impaired in obese subjects.[3] Indeed, this behavior has been observed predominantly in individuals with metabolic disease[21] as it is the condition of our study population. However, other studies reported associations between plasma irisin levels and important metabolic factors in non-diabetic subjects, but not in individuals with type 2 diabetes.[4,22] Our suggested corollary would be that irisin is increased in metabolically altered situations and may diminish as a consequence of the weight loss, as irisin is then 'less' needed to restore the altered metabolic state. Thus, the theory about a possible irisin resistance appears similar to the well-known leptin insensitivity in obesity and cannot be discarded[21] as has been reported for leptinemia and insulinemia after dieting.[11]

The association between irisin concentrations and carbohydrate intake was related to the consumption of some sources of carbohydrates (cereals, pulse, fruits and vegetables). This outcome may be explained because the dietary modifications during the hypocaloric intervention evolved with shifts in carbohydrate consumption within the energy restriction. Thus, irisin could be increased in response to the dietary pattern, depending on the carbohydrate content, in order to prevent/improve the rise on glucose, insulin or HOMA index values, linked to latter damage on multiple organs.[23] This finding is interesting given that modifying the macronutrient distribution is a recurrent approach for treating obese and MetS patients.[24]

The observed results appear to be irrespective to the physical activity, as patients in this study maintained the same physical activity level along the intervention. The statistical adjustments for sex did not revealed specific differences between males and females concerning the analyzed irisin outcomes. A limitation of this study is that it demonstrated an association but not evidenced causation. Moreover, the methods to assess the dietary intake and physical activity were based on questionnaires, which could bias the results interpretation. Also, some other relevant measurements in relation to glucose metabolism, such as OGTT or Clamp-test would be appropriate. However, the design of the current trial based on a

nutritional intervention involving a quite large human sample is indeed a valuable feature enabling pre- and post-test comparisons within subjects. An effect of regression to the mean could not be attributed since pertinent statistical procedures were performed in order to control this phenomenon.

This research concerns the investigation of a potential role of irisin on impaired glucose homeostasis associated to obesity and, consequently, the metabolic interplay on glucose metabolism and insulin secretion control. Indeed, the search of predictive laboratory markers is of value for clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Government of Navarra (48/2009), the LE Nutrition, Obesity and Health (University of Navarra LE/97) and CIBERobn/RETICS, ISCIII initiatives. PL-L is funded by the Government of Navarra (233/2009) and ABC and MP by the ISCIII (Sara Borrell C09/00365 and Miguel Servet schemes). This research is collaborative study of the CIBERobn program on Fisiopatologia de la Obesidad y la Nutricion funded by the Institute Carlos III of the Spanish Ministry of Health, Madrid.

REFERENCES

1 Straughen JK, Trudeau S, Misra VK. Changes in adipose tissue distribution during pregnancy in overweight and obese compared with normal weight women. *Nutr Diabetes* 2013; **3**: e84.
2 Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC *et al.* A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481**: 463–468.
3 Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity—Correlation with body mass index. *Peptides* 2013; **39**: 125–130.
4 Liu JJ, Wong MD, Toy WC, Tan CS, Liu S, Ng XW *et al.* Lower circulating irisin is associated with type 2 diabetes mellitus. *J Diabetes Complications* 2013; **27**: 365–369.
5 Roberts MD, Bayless DS, Company JM, Jenkins NT, Padilla J, Childs TE *et al.* Elevated skeletal muscle irisin precursor FNDC5 mRNA in obese OLETF rats. *Metabolism* 2013; **62**: 1052–1056.
6 Roca-Rivada A, Castelao C, Senin L, Landrove M, Baltar J, Crujeiras A *et al.* FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One* 2013; **8**: e60563.
7 Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F *et al.* Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab* 2013; **98**: E769–E778.
8 Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE *et al.* FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism* 2012; **61**: 1725–1738.
9 Lopez-Legarrea P, de la Iglesia R, Abete I, Bondia-Pons I, Navas-Carretero S, Forga L *et al.* Short-term role of the dietary total antioxidant capacity in two hypocaloric regimes on obese with metabolic syndrome symptoms: the RESMENA randomized controlled trial. *Nutr Metab (Lond)* 2013; **10**: 22.
10 Lopez-Legarrea P, De la Iglesia R, Abete I, Navas-Carretero S, Martinez JA, Zulet MA. The protein type within a hypocaloric diet affects obesity-related inflammation: the RESMENA project. *Nutrition* 2013; doi:10.1016/j.nut.2013.09.009.
11 Crujeiras AB, Goyenechea E, Abete I, Lage M, Carreira MC, Martinez JA *et al.* Weight regain after a diet-induced loss is predicted by higher baseline leptin and lower ghrelin plasma levels. *J Clin Endocrinol Metab* 2010; **95**: 5037–5044.
12 Castillo-Quan JI. From white to brown fat through the PGC-1alpha-dependent myokine irisin: implications for diabetes and obesity. *Dis Model Mech* 2012; **5**: 293–295.
13 Kelly DP. Medicine. Irisin, light my fire. *Science* 2012; **336**: 42–43.
14 Sanchis-Gomar F, Lippi G, Mayero S, Perez-Quilis C, Garcia-Gimenez JL. Irisin: a new potential hormonal target for the treatment of obesity and type 2 diabetes. *J Diabetes* 2012; **4**: 196.
15 Bostrom PA, Fernandez-Real JM. Metabolism: irisin, the metabolic syndrome and follistatin in humans. *Nat Rev Endocrinol* 2014; **10**: 11–12.
16 Katcher HI, Legro RS, Kunselman AR, Gillies PJ, Demers LM, Bagshaw DM *et al.* The effects of a whole grain-enriched hypocaloric diet on cardiovascular disease risk factors in men and women with metabolic syndrome. *Am J Clin Nutr* 2008; **87**: 79–90.
17 Al-Daghri N, Alkharfy K, Rahman S, Amer O, Vinodson B, Sabico S *et al.* Irisin as a predictor of glucose metabolism in children: sexually dimorphic effects. *Eur J Clin Invest* 2013; e-pub ahead of print 5 November 2013; doi:10.1111/eci.12196.
18 Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes* 2011; **60**: 2441–2449.
19 Swick AG, Orena S, O'Connor A. Irisin levels correlate with energy expenditure in a subgroup of humans with energy expenditure greater than predicted by fat free mass. *Metabolism* 2013; **62**: 1070–1073.
20 Zhang HJ, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW *et al.* Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. *J Hepatol* 2013; **59**: 557–562.
21 Park KH, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE *et al.* Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab* 2013; **98**: 4899–4907.
22 Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK *et al.* Serum irisin levels in new-onset type 2 diabetes. *Diabetes Res Clin Pract* 2013; **100**: 96–101.
23 Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* 2011; **378**: 169–181.
24 Makris A, Darcey VL, Rosenbaum DL, Komaroff E, Vander Veur SS, Collins BN *et al.* Similar effects on cognitive performance during high- and low-carbohydrate obesity treatment. *Nutr Diabetes* 2013; **3**: e89.