Metabolic programming of sirtuin 1 (SIRT1) expression by moderate energy restriction during gestation in rats may be related to obesity susceptibility in later life

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Abstract

In rats, 20 % gestational energy restriction programmes offspring for higher food intake, which in adulthood results in higher body weight in males but not in females. Here, we aimed to assess whether the effects of moderate energy restriction during gestation and the sex-related outcomes on adult body weight may be related to the metabolic programming of sirtuin expression in different tissues. For this purpose, 25-d-old offspring of control and 20 % energy-restricted (ER) rats (from days 1–12 of pregnancy) were studied. Body weight and the weight of white adipose tissue (WAT) depots and liver were recorded and mRNA expression of sirtuin 1 (*SIRT1*) and selected genes in the WAT, liver, muscle and hypothalamus were analysed. No differences were found in body weight or the weight of WAT and liver between the control and ER animals. A similar pattern of *SIRT1* mRNA expression was found in the WAT, liver and skeletal muscle of ER animals, but in a sex-dependent manner: ER males showed lower *SIRT1* mRNA levels than the controls, while no differences were found in females. A sex-different pattern was also observed in the hypothalamus. ER males, but not females, also showed lower mRNA levels of adipose TAG lipase (*ATGL*) and uncoupling protein 2 in WAT and of sterol response element binding protein 1c and stearoyl-CoA desaturase-1 in the liver. Both sexes of ER animals showed lower mRNA levels of 5′ adenosine monophosphate-activated protein kinase and *ATGL* in the liver. In conclusion, moderate maternal energy restriction during gestation programmes a particular, sex-dependent gene expression profile of *SIRT1* in different peripheral tissues, which may be related to obesity predisposition in adulthood; therefore *SIRT1* expression emerges as a potential early biomarker of obesity susceptibility.

Key words: Sirtuin 1: Energy restriction: Gestation: Early biomarker: Obesity

Obesity is a multifactorial disease with epidemic proportions. Its causes are mostly attributed to an excessive intake of energy together with low energy expenditure[1]. However, it is becoming increasingly clear that perinatal nutrition is also a key factor involved in the programming of later body weight. While moderate maternal energy restriction during lactation in rats has been associated in the offspring with lower body weight in adulthood[2,3], severe maternal undernutrition during gestation, in animal models and also in humans, has been related to a greater propensity to develop obesity and related metabolic alterations, such as CVD or diabetes[4,5]. Therefore, it becomes interesting to investigate the mechanisms occurring in early postnatal life that could underlie these substantial differences, even before the

appearance of differences in the phenotype, which may also be of great importance in the prevention of future health problems. In contrast to severe energy restriction, a moderate reduction of energy intake reflects better a more frequent condition in humans.

Concerning energy restriction during gestation and later obesity, we have described that moderate (20 %) maternal energy restriction during the first half of pregnancy in rats results in greater food intake in their offspring and this concludes in higher body weight in males but not in females[6]. These animals, at the early age of 25 d, showed alterations in the structure and function of the hypothalamus, associated with an impairment of the central insulin and leptin action which could, at least, be in part responsible for the

dysregulation of energy balance, leading to hyperphagia[7,8]. However, which mechanisms account for the different outcomes between males and females of this prenatal condition are not known, beyond the presence of reduced sympathetic innervation in the inguinal adipose tissue, which occurred only in male animals, and could be responsible, at least in part, for the greater body weight and adiposity displayed in adulthood[9].

Obesity is associated with an increased risk of morbidity and mortality as well as reduced lifespan[10]. The sirtuin family of NAD$^+$-dependent protein deacetylases and ADP-ribosyltransferases are one of the most potential molecular targets for having an impact on ageing events[11,12]. Sirtuin 1 (*SIRT1*) has acquired great importance due to its participation in the control of energy metabolism-related genes and its ubiquitous distribution in the organism; higher expression of *SIRT1* has been described to be advantageous as it activates catabolic metabolic pathways and increases longevity[13,14]. As with *SIRT1*, sirtuin 2 (*SIRT2*) is an ubiquitous, nuclear and cytoplasmic protein deacetylase which was first involved in the prevention of the instability of the chromosomes during mitosis division[15]. Later on, it has been shown to inhibit adipocyte differentiation, but its functions in the liver or muscle are not clearly elucidated[16]. All in all, the actions of *SIRT1* in different tissues together with the actions of *SIRT2* in adipose tissue might be able to stand up to obesity and the metabolic syndrome.

Considering the potential involvement of *SIRT1* in the protection from the metabolic syndrome, the aim of the present study was to ascertain whether the detrimental effects of gestational maternal energy restriction on later metabolic health and body weight maintenance also involve changes in sirtuin expression in different tissues involved in energy homeostasis, and contribute to explaining the sex-different outcome on adult body weight.

Methods

Animals and experimental design

The study was performed in male and female rats from twelve different litters, following the protocol below, as described previously[6,7,9]. All rats were housed under controlled temperature (22°C) and a 12 h light–dark cycle (lights on from 08.00 to 20.00 hours), and had unlimited access to tap water and a standard chow diet (13 kJ/g (3 kcal/g), with 2·9% energy from fat; Panlab) unless mentioned otherwise. Briefly, virgin female Wistar rats weighing between 200 and 225 g were mated with male rats (Charles River Laboratories). Day of conception (day 0 of pregnancy) was determined by examination of vaginal smears for the presence of sperm, and then female rats were single caged. Pregnant rats were divided into two groups: one with free access to a standard chow diet and the other one underwent a 20% restriction of energy intake from day 1 to day 12 of pregnancy. Energy restriction was performed by offering each dam a daily amount of food corresponding to 80% of the energy that should be eaten according to body weight and to the day of pregnancy. This amount was calculated considering the energy daily consumed by their pregnant controls under *ad libitum* feeding conditions. After the energy restriction period, rats were allowed to eat *ad libitum*, and food intake was measured. On day 1 after delivery, excess pups in each litter were removed to keep ten pups per dam (five males and five females, when possible). Weaning was conducted at 21 d of life.

On day 25 of life, animals from control dams and from energy-restricted (ER) dams (*n* 6–9 animals per group) were killed by decapitation under fed conditions, during the first 2 h at the beginning of the light cycle. Blood samples were collected in heparinised containers, then centrifuged at 700 **g** for 10 min to obtain the plasma, and stored at −20°C until analysis. Retroperitoneal and inguinal white adipose tissue (rWAT and iWAT, respectively), liver, skeletal muscle and hypothalamus were rapidly removed and frozen in liquid N$_2$ and stored at −80°C until ulterior studies. rWAT, iWAT and liver were weighed before freezing.

Although the retroperitoneal and the inguinal fat depots were sampled to be weighed, the retroperitoneal depot was selected as representative to be analysed for gene expression, based on the literature showing that this depot seems to be more sensitive to nutritional status, compared with other depots[17].

The animal protocol followed in the present study was reviewed and approved by the Bioethical Committee of our University and guidelines for the use and care of laboratory animals of the University were followed.

Measurement of circulating parameters

Blood glucose concentration was measured using an Accu-Chek Glucometer (Roche Diagnostics). Plasma insulin concentration was determined using a rat insulin ELISA kit (Mercodia AB) following standard procedures. Plasma leptin concentration was measured using a mouse leptin ELISA kit (R&D Systems).

RNA extraction

Total RNA was extracted from rWAT, iWAT, liver, muscle and hypothalamus by Tripure Reagent (Roche Diagnostic GmbH) according to the manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc.) and its integrity confirmed using 1% agarose gel electrophoresis.

Real-time quantitative PCR analysis

Real-time PCR was used to measure mRNA expression levels of *SIRT1* in rWAT, iWAT, liver, skeletal muscle and hypothalamus; *SIRT2* in rWAT; 5′ adenosine monophosphate-activated protein kinase (*AMPK*) and adipose TAG lipase (*ATGL*) in rWAT and liver; uncoupling protein 2 (*UCP2*) in rWAT; and sterol response element binding protein 1c (*SREBP1c*), stearoyl-CoA desaturase-1 (*SCD1*) and the free fatty acid transporter *CD36* in the liver. Briefly, 0·25 μg of total RNA (in a final

Table 1. Nucleotide sequences of primers used for PCR amplification

Gene	Forward primer (5′−3′)	Reverse primer (5′−3′)	Amplicon size (bp)
β-Actin	TACAGCTTCACCACCACAGC	TCTCCAGGGAGGAAGAGGAT	120
GDI1	CCGCACAAGGCAAATACATC	GACTCTCTGAACCGTCATCAA	210
SIRT1	AGCTGGGGTTTCTGTTTCCT	TGCTGAGTTGCTGGATTTTG	224
SIRT2	ACCTTCCTTCAGTCCCGTTT	AAGGGTTCACAGTGGTGGAG	173
AMPK	CCAAGTGATCAGCACTCCAA	CAACACGTTCTCTGGCTTCA	199
ATGL	TGTGGCCTCATTCCTCCTAC	AGCCCTGTTTGCACATCTCT	271
UCP2	CCTACAAGACCATTGCACGA	TGTCATGAGGTTGGCTTTCA	149
SREBP1c	AGCCATGGATTGCACATTTG	GGTACATCTTTACAGCAGTG	260
SCD1	ATCCCCTCCTCCAAGGTCTA	CGGGCCCATTCATATACATC	188
CD36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG	318

GDI1, guanosine diphosphate dissociation inhibitor 1; SIRT1, sirtuin 1; SIRT2, sirtuin 2; AMPK, 5′ AMP-activated protein kinase; ATGL, adipose TAG lipase; UCP2, uncoupling protein 2; SREBP1c, sterol response element binding protein 1c; SCD1, stearoyl-CoA desaturase 1; CD36, free fatty acid transporter CD36.

volume of 5 μl) were denatured at 65°C for 10 min and then reverse transcribed to complementary DNA using MuLV reverse transcriptase (Applied Biosystems) at 20°C for 15 min, 42°C for 30 min, with a final step of 5 min at 95°C in an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems). Each PCR was performed from diluted complementary DNA template, forward and reverse primers (1 μM each), and Power SYBER Green PCR Master Mix (Applied Biosystems). Primers were obtained from Sigma and sequences are described in Table 1. Real-time PCR was performed using the Applied Biosystems StepOnePlus™ Real-Time PCR Systems (Applied Biosystems) with the following profile: 10 min at 95°C, followed by a total of forty-two temperature cycles (15 s at 95°C and 1 min at 60°C). In order to verify the purity of the products, a melting curve was produced after each run according to the manufacturer's instructions. The threshold cycle (C_t) was calculated by the instrument's software (StepOne Software version 2.1) and the relative expression of each mRNA was calculated as a percentage of male control rats, using the $2^{-\Delta\Delta C_t}$ method[18]; β-actin and guanosine diphosphate dissociation inhibitor 1 (GDI1) were used as reference genes depending on the tissue, according to their better suitability.

Statistical analysis

Data are expressed as means with their standard errors (n 6–9). Two-way ANOVA with the factors of sex (S) and energy restriction (R) was performed to assess statistical significances. Individual means were compared with a Student's t test. The analyses were performed with SPSS for Windows (SPSS). Threshold of significance was defined at $P < 0.05$. Data were log-transformed for statistical analysis if they were found not to be normally distributed, but were expressed as original values in the text and tables for ease of interpretation.

Results

Weight-related parameters and blood parameters

As previously described in the same cohort of animals[7,9], control and ER animals exhibited no significant differences in their body weight at the age of 25 d, either in the weight of rWAT, iWAT or liver (Table 2). Blood glucose and plasma insulin levels were not significantly different in ER animals compared with their controls. Circulating leptin concentration was lower in ER male rats, but not in females, with respect to their controls ($P < 0.05$, Student's t test; Table 2).

Table 2. Weight-related and blood parameters at the age of 25 d†

(Mean values with their standard errors)

	Males				Females			
	Control		ER		Control		ER	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)	61·4	2·8	59·3	2·0	58·6	2·3	55·7	2·8
rWAT weight (mg)	113	11	95·7	7·8	95·4	16·5	92·2	18·4
iWAT weight (mg)	632	69	684	49	689	98	739	53
Liver weight (g)	2·86	0·11	2·60	0·11	2·79	0·07	2·69	0·12
Glucose (mg/l)	1300	60	1370	30	1240	40	1310	60
Insulin (ng/l)	170	38	122	36	154	41	167	35
Leptin (ng/l)	959	119	608*	51	984	164	841	40

ER, energy-restricted dams during gestation; rWAT, retroperitoneal WAT; iWAT, inguinal WAT.

* Mean value was significantly different from its respective control group ($P < 0.05$; Student's t test). No significant differences were found by two-way ANOVA.

† Body weight, rWAT, iWAT and liver weight, and circulating glucose, insulin and leptin levels at 25 d of life (n 6−9) of male and female offspring from controls and ER animals, under *ad libitum* feeding conditions.
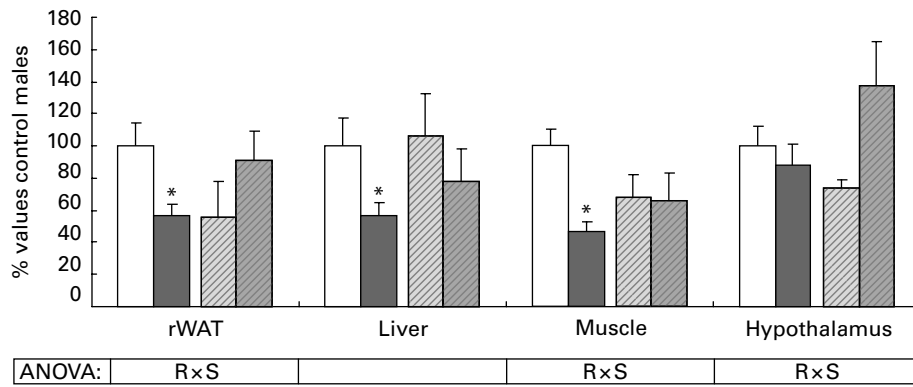
Fig. 1. Sirtuin 1 mRNA expression levels in the retroperitoneal WAT (rWAT), liver, skeletal muscle and hypothalamus of 25-d-old male and female offspring of controls and energy-restricted dams during gestation (ER). mRNA levels were measured by real-time PCR and expressed as a percentage of the value of control male rats. Values are means, with their standard errors represented by vertical bars (*n* 6−9). R × S, interactive effect between energy restriction and sex (*P*<0·05; two-way ANOVA). *Mean value was significantly different for the controls from that of ER animals (*P*<0·05; Student's *t* test). □, Control males; ▪, ER males; ▨, control females; ▨, ER females.

Sirtuin 1 mRNA expression in white adipose tissue, liver, hypothalamus and muscle

Fig. 1 shows mRNA expression levels of *SIRT1* in the rWAT, liver, skeletal muscle and hypothalamus of controls and ER animals. Interestingly, a similar pattern of *SIRT1* mRNA expression was found in the tissues studied. In rWAT, liver and skeletal muscle, ER male rats showed lower *SIRT1* mRNA levels with respect to their controls (*P*<0·05, Student's *t* test), whereas no significant differences were observed in females, and an interaction between the effect of energy restriction during gestation and sex was found in rWAT and skeletal muscle (*P*<0·05, two-way ANOVA). An interaction between these factors was also found in the hypothalamus (*P*<0·05, two-way ANOVA), but was attributed in this case to a tendency to higher mRNA levels in ER females compared with the controls (*P*=0·067, Student's *t* test), with no differences in males.

To ascertain whether changes in *SIRT1* expression found in rWAT could be representative for the WAT in general, *SIRT1* expression was also measured in the subcutaneous inguinal depot. Interestingly, this depot showed a similar pattern to that of the retroperitoneal one, with a significant decrease in ER males with respect to their controls (100 (SEM 20) and 33·0 (SEM 3·7), controls and ER animals, respectively, *P*<0·05, Student's *t* test), and no changes in females (61·2 (SEM 8·3) and 61·8 (SEM 8·1), controls and ER animals, respectively) (interaction between the effect of energy restriction during gestation and sex; *P*<0·05, two-way ANOVA).

5′ Adenosine monophosphate-activated protein kinase, adipose TAG lipase and uncoupling protein 2 mRNA expression in retroperitoneal white adipose tissue

mRNA levels of the selected genes related to lipid metabolism in rWAT are shown in Fig. 2. ER male rats showed lower
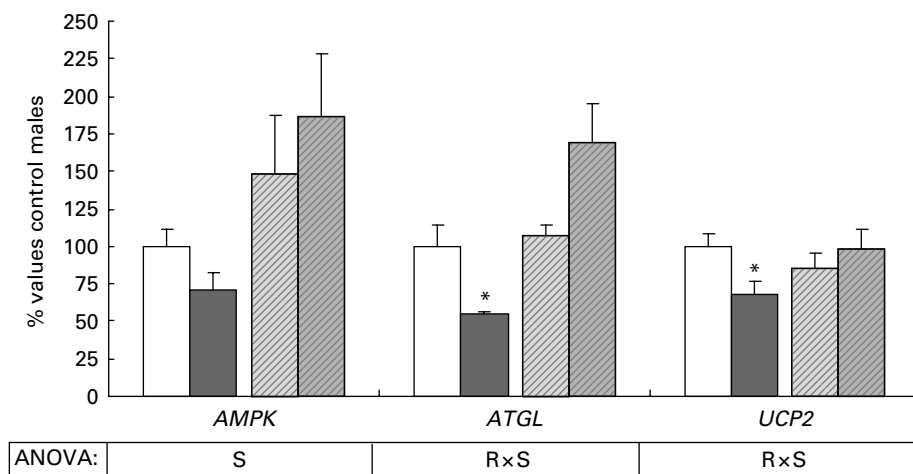

Fig. 2. mRNA expression levels of the selected genes related to lipid metabolism in the retroperitoneal WAT of 25-d-old male and female offspring of controls and energy-restricted dams during gestation (ER). mRNA levels were measured by real-time PCR and expressed as a percentage of the value of control male rats. Values are means, with their standard errors represented by vertical bars (*n* 6−9). The genes determined were 5′ adenosine monophosphate-activated protein kinase (*AMPK*), adipose TAG lipase (*ATGL*) and the uncoupling protein 2 (*UCP2*). S, effect of sex; R × S, interactive effect between energy restriction and sex (*P*<0·05; two-way ANOVA). *Mean value was significantly different for the controls from that of ER animals (*P*<0·05; Student's *t* test). □, Control males; ▪, ER males; ▨, control females; ▨, ER females.
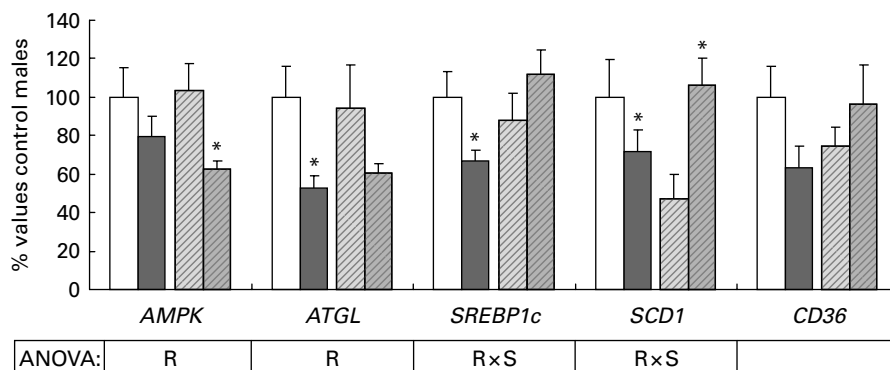
Fig. 3. mRNA expression levels of the selected genes related to lipid metabolism in the liver of 25-d-old male and female offspring of controls and energy-restricted dams during gestation (ER). mRNA levels were measured by real-time PCR and expressed as a percentage of the value of control male rats. Values are means, with their standard errors represented by vertical bars (n 6–9). The genes determined were 5′ adenosine monophosphate-activated protein kinase (AMPK), adipose TAG lipase (ATGL), sterol regulatory element binding protein 1c (SREBP1c), stearoyl-CoA desaturase-1 (SCD1) and the free fatty acid transporter CD36 (CD36). R, effect of energy restriction; R × S, interactive effect between energy restriction and sex ($P < 0.05$; two-way ANOVA). * Mean value was significantly different for the controls from that of ER animals ($P < 0.05$; Student's t test). □, Control males; ■, ER males; ▨, control females; ▨, ER females.

mRNA expression of the lipolysis-related gene ATGL compared with the controls ($P < 0.05$, Student's t test), while a tendency to higher mRNA levels was found in ER females ($P = 0.071$, Student's t test) with respect to their controls (interaction between the effect of energy restriction during gestation and sex; $P < 0.05$, two-way ANOVA). A similar tendency, but not significant, was also found for AMPK. Higher mRNA expression levels of AMPK were observed in females compared with males ($P < 0.05$, two-way ANOVA).

Of interest, UCP2 mRNA expression was also decreased in ER males with respect to their controls ($P < 0.05$, Student's t test), whereas females did not show differences between the groups (interaction between the effect of energy restriction during gestation and sex; $P < 0.05$, two-way ANOVA).

5′ Adenosine monophosphate-activated protein kinase, adipose TAG lipase, sterol response element binding protein 1c, stearoyl-CoA desaturase-1 and CD36 mRNA expression in liver

Fig. 3 shows the mRNA expression levels of the selected genes related to lipid metabolism in the liver. Both male and female ER rats showed lower mRNA levels of AMPK and ATGL in comparison with the controls ($P < 0.05$, two-way ANOVA). It was observed that ER male animals exhibited lower mRNA levels of SREBP1c and SCD1 than the controls, unlike female ER rats which showed higher mRNA levels compared with the controls (interaction between the effect of energy restriction during gestation and sex; $P < 0.05$, two-way ANOVA). A similar pattern to that of SREBP1c and SCD1 was also observed concerning CD36 mRNA expression levels, although differences did not reach statistical significance.

Sirtuin 2 mRNA expression in retroperitoneal white adipose tissue

Fig. 4 shows mRNA expression levels of SIRT2 in rWAT. ER animals showed a tendency to lower SIRT2 mRNA levels compared with their controls ($P = 0.072$, two-way ANOVA),

but differences were more marked and statistically significant only in females ($P < 0.05$, Student's t test).

Discussion

Here we show that a moderate maternal energy restriction of 20 % during the first half of pregnancy programmes a characteristic profile of SIRT1 mRNA expression in different key tissues involved in the control of energy metabolism in male and female offspring, which may be related to the different predisposition of these animals to overweight development in later life[6,8].

The thrifty phenotype hypothesis proposes that undernutrition during early life may induce lasting alterations in tissue and organ function to increase efficiency using available nutrients when they are in short supply, preserving glucose for the development of main organs in the fetus, particularly the brain[19].
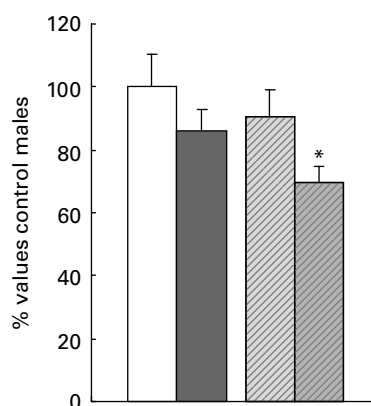

Fig. 4. Sirtuin 2 mRNA expression levels in the retroperitoneal white adipose tissue of 25-d-old male and female offspring of controls and energy-restricted dams during gestation (ER). mRNA levels were measured by real-time PCR and expressed as a percentage of the value of control male rats. Values are means, with their standard errors represented by vertical bars (n 6–9). * Mean value was significantly different for the controls from that of ER animals ($P < 0.05$; Student's t test). A tendency to lower sirtuin 2 mRNA levels in ER rats was found with two-way ANOVA ($P = 0.072$). □, Control males; ■, ER males; ▨, control females; ▨, ER females.

These prenatal alterations to a poor nourishment may be detrimental in a positive nutritional environment, because the balance setpoint for nutrient availability has been changed, leading to storage at the expenses of energy expenditure, therefore increasing the risk of obesity development and associated diseases in adulthood[4,5,19].

In accordance with this hypothesis, we have previously described that moderate undernutrition during fetal life (achieved by 20% maternal energy restriction during the first part of gestation) was associated with hyperphagia in postnatal life, which resulted in higher body weight in adulthood in males but not in females[6,8]. The hyperphagia could, in part, be explained by alterations in the hypothalamic structures related to the control of food intake[7], and by a lower capacity to respond to insulin and leptin action at the central level, which was already present at the premature age of 25 d of life[8]. However, the reasons for the sex-dependent consequences in later body weight have not yet been clearly elucidated.

The sirtuin family of NAD-dependent protein deacetylases and ADP-ribosyltransferases regulates the activities of a variety of transcription factors, co-regulators and enzymes improving obesity resistance and expanding lifespan[20]. Here we found that ER male animals, but not females, displayed lower mRNA expression levels of *SIRT1* in comparison with the controls in WAT, liver and skeletal muscle, before any difference in body weight, but in accordance with their later overweight when adults[6]. Hence, it could be hypothesised that programmed changes in *SIRT1* expression may, to a certain extent, account for the sex-dependent outcomes on the body weight of adult ER animals. Holness *et al.*[20] have also recently propounded a link between the thrifty phenotype, programmed changes in *SIRT1* expression and energy supply. In agreement, other studies have also described changes in *SIRT1* expression depending on the perinatal nutritional conditions; while maternal protein restriction during the lactation period resulted in increased *SIRT1* mRNA expression levels in the kidney of the offspring, together with improved insulin sensitivity[21], the offspring of protein-restricted mice during gestation showed lower *SIRT1* protein expression in the skeletal muscle[22]. Therefore, the sex-related differences found here in ER animals concerning *SIRT1* mRNA levels and the similar expression profile found in different tissues, namely WAT (including one internal and one subcutaneous depot), liver and skeletal muscle, increase the power of these findings suggesting a main role of *SIRT1* expression in peripheral tissues in the programming of adult body weight. It could be speculated that the maintenance of *SIRT1* expression in peripheral tissues of ER females could explain or contribute, at least in part, to the capacity of these animals to maintain their body weight in adulthood despite their higher energy intake.

Unlike the potential anti-obesogenic actions of *SIRT1* in peripheral tissues, hypothalamic *SIRT1* expression increases by fasting in rats[23] and mice[24], and fasting-induced hyperphagia has been shown to require hypothalamic *SIRT1* activity[23]. *SIRT1* at the central level has been proposed to sense the nutritional status of the body and regulate the hypothalamic central melanocortin signalling and S6K pathway to govern food intake and body weight[23]. Therefore, it is interesting to highlight that *SIRT1* mRNA levels in the hypothalamus were not different between the control and ER animals; in fact, an opposite tendency ($P=0\cdot067$, Student's *t* test) to higher mRNA levels was even found in ER females with respect to their controls, which is in accordance with their higher food intake. However, we have to take into account that the results presented here are based on gene expression and further analyses are needed to determine whether changes in *SIRT1* mRNA levels are reflecting changes in its protein amount or activity.

SIRT1 is frequently defined as a master metabolic regulator since it is able to influence several transcription factors involved in energy homeostasis; in fact, it has been described to regulate glucose and lipid metabolism in the liver, WAT, pancreas and muscle, increasing lipid catabolism and energy expenditure[25]. Thus, to ascertain whether changes in *SIRT1* mRNA expression could be accompanied with changes in lipid metabolism and energy expenditure, and in order to explain the sex differences in adult body weight, the expression of the selected genes in rWAT and liver was also studied. Lower mRNA levels of *AMPK* in the liver of both male and female ER rats and a tendency to lower levels in the rWAT of ER males were found compared with the controls. *AMPK* stimulates catabolic pathways in order to generate ATP and inhibits non-essential anabolic processes that consume ATP when energy status is decreased[26], and it has recently been described that *SIRT1* and *AMPK* regulate each other and share several common target molecules[26]. In fact, AMPK activity is reduced in most genetic models of rodent obesity in peripheral tissues such as heart, skeletal muscle and liver[27]. Interestingly, the changes observed here in *AMPK* mRNA levels at this early stage of life are early events before changes in body weight. Leptin has been described to activate *AMPK* expression[28,29], therefore changes in *AMPK* mRNA levels could be partially explained by changes in circulating leptin levels, which were lower in ER male controls compared with their controls. However, the decrease in *AMPK* mRNA levels also occurring in the liver of ER female animals does not appear to be explained by changes in circulating leptin levels, which remained unchanged in these animals. Nevertheless, considering that leptin present in breast milk can be absorbed by the immature stomach of suckling rats and affect later development[30–32], the mentioned changes in *AMPK* expression levels could be reflecting changes in the amount of leptin ingested during lactation, more than a direct effect of their circulating levels after weaning. In fact, as previously described in the same model of animals[8], the offspring of 20% ER animals during gestation did not show the expected peak in circulating leptin levels during the neonatal period, a process termed as 'neonatal leptin surge'[29], which occurred in control animals at the age of 9 d.

AMPK has been described to activate the lipolytic pathway by regulating hormone-sensitive lipase (HSL) activity[27]. However, *ATGL* has recently been identified as an important TAG lipase that regulates lipid hydrolysis upstream of *HSL*[27]. Here, both male and female ER animals displayed a lower

mRNA expression of *ATGL* in the liver, in accordance with the lower *AMPK* mRNA levels; yet only ER males showed lower *ATGL* mRNA levels in rWAT, a similar pattern to that found for *AMPK* in this tissue. It could be suggested that reduced lipid mobilisation in adipose tissue is early programmed only in males, whereas hepatic lipid metabolism, particularly catabolic pathways, is altered in both male and female ER animals. In fact, dysregulation of adipose tissue and hepatic lipid metabolism has been described in the same cohort of animals in adulthood at the age of 6 months[8]. On the other hand, ER males, but not females, also showed lower *UCP2* mRNA levels in rWAT with respect to their controls. Although the primary role of *UCP2* is not clearly established, considerable evidence suggests that *UCP2* plays a physiological role in regulating fatty acid oxidation, mitochondrial biogenesis, substrate utilisation and reactive oxygen species elimination[33]. Hence, the lower expression levels found in ER male animals could be related to lower lipid mobilisation and fatty acid oxidation. Moreover, although a direct association between *UCP2* and obesity or energy expenditure is not well established[34], higher mRNA levels of *UCP2* have been described in the WAT of obesity-resistant mice compared with obesity-prone mice[35], and lower expression levels have been reported in the WAT of obese human subjects[36]; therefore, the decrease in the expression of this gene occurring only in male ER animals and not in females, as occurring with *AMPK* and *ATGL*, may also be related to the different outcomes of these prenatal conditions on later body weight between males and females.

In addition to *AMPK* and *ATGL*, the expression of the other genes involved in lipid metabolism, in particular lipogenic genes such as *SREBP1c*, *SCD1* and *CD36*, was also studied in the liver. Of interest, energy restriction during gestation resulted in a different pattern of expression between males and females concerning *SREBP1c* and *SCD1*. Their expression levels decreased in ER males but increased in ER females. A similar pattern was also found for *CD36*, although differences did not reach statistical significance. These changes could be reflecting impaired insulin signalling, as the expression of SREBP1c is up-regulated by insulin[37], and SREBP1c regulates fatty acid biosynthetic genes such as *SCD1*[38]. In this sense, we have previously described that this condition during gestation programmes the offspring, both males and females, for a lower capacity to respond to insulin action, which is already present at early ages[8]. Therefore, although insulin resistance in the liver seems to be early programmed in both male and female animals, other factors occurring in females may protect and counterbalance this malprogramming effect due to energy restriction on energy homeostasis. The results of the present study support a potential role of *SIRT1* in these differences.

To explore other members of the sirtuin family as potential markers of later obesity, mRNA expression levels of *SIRT2* in rWAT were analysed. Unlike *SIRT1*, the role of *SIRT2* has not been explored as much and its action in some tissues needs to be clarified. Nevertheless, in WAT, *SIRT2* is known to inhibit adipocyte differentiation by regulating Forkhead box protein O1 (*FOXO1*) acetylation[39]. Here, we found a tendency to lower *SIRT2* mRNA levels in rWAT in ER animals compared

with the controls ($P=0.072$), although it was more pronounced and only significant by Student's *t* test in ER females. We previously described that the accumulation of an excessive amount of fat in ER males was related to hyperplasia in the iWAT and to hypertrophy in the rWAT[9]. Thus, the tendency to lower *SIRT2* mRNA levels in ER males, together with the lower mRNA levels of *SIRT1*, *ATGL* and *UCP2* in the rWAT, agrees with later hypertrophy development in this depot when rats were exposed to a high-fat diet in adulthood[9]. However, unlike males, changes occurring in *SIRT2* mRNA levels in the rWAT of females do not directly agree with their capacity to maintain body weight in adult life, supporting a major role for *SIRT1* in the regulation of energy homeostasis.

In conclusion, the present study shows that a moderate maternal energy restriction of 20 % during the first half of gestation programmes a particular, sex-dependent expression profile of *SIRT1* in different peripheral tissues, which may be associated with obesity susceptibility and related metabolic disorders in adulthood; this suggests that *SIRT1* mRNA expression levels in particular tissues may be considered as potential early biomarkers of obesity susceptibility.

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