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# Effect of food restriction on the insulin signalling pathway in rat skeletal muscle and adipose tissue

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**Abstract**

We tried to elucidate the effects of a brief and severe model of food restriction on insulin sensitivity in female rats, focusing on key proteins involved in the insulin signalling pathway in skeletal muscle and adipose tissue after 5, 10 and 15 days of food restriction. Using euglycemic clamp, we detected that food-restricted rats are significantly less sensitive to insulin action than control rats. However, the time of restriction promotes a progressive increase on insulin sensitivity. The analysis of the insulin signalling pathway showed a tissue-specific regulation of several proteins involved in insulin signalling. In skeletal muscle, insulin receptor substrate 1 and Glut4 are up-regulated at the end of the food restriction period, just the opposite of what we found in adipose tissue. In conclusion, a 50% reduction of food intake modulates insulin sensitivity through a tissue-specific regulation of the insulin signalling pathway in the main target tissues for this hormone.


*Keywords:* Food restriction; Insulin; Insulin receptor; IRS-1; Glut4

## 1. Introduction

Insulin is the main hormone that is involved in glucose, lipid and amino acid metabolism. It can be considered as an anabolic and anticatabolic hormone that promotes glucose uptake in muscle and fat and stimulates the storage of substrates in fat, liver and muscle by increasing lipogenesis, glycogen and protein synthesis and by decreasing lipolysis, glycogenolysis and protein breakdown [1].

Insulin binds to its receptor (IR), which has intrinsic tyrosine kinase activity; insulin binding promotes this activity, resulting in IR autophosphorylation. Next, the kinase phosphorylates several intracellular proteins, including those of the IR substrate (IRS) family, of which IRS-1 is the best characterized. IRS-1 lacks catalytic activity, but in order to affect the many biologic responses of insulin, it serves as a docking protein to which different signalling proteins such as phosphatidylinositol-3-kinase (PI3-K) bind through SH2 domains. PI3-K activation has a pivotal role in the metabolic actions of insulin, including insulin-stimulated glucose transport through the specific translocation of Glut4 transporters from intracellular storage to the plasma membrane (for a review, see Refs. [2–4]). The regulation of the insulin signalling pathway is of great interest. A significant increase in the Ser/Thr phosphorylation of IRS proteins has been shown to act as a negative feedback-control mechanism that uncouples IRS proteins from their upstream and downstream effectors and terminates signal transduction in response to insulin. Therefore, IRS-1 Ser/Thr phosphorylation is a potential molecular mechanism for insulin resistance [5,6].

A moderate reduction of food intake has important benefits on whole-body glucose homeostasis; this is characterized by low fasting blood glucose levels despite low insulin levels. Food restriction is a common treatment for insulin resistance, obesity and type 2 diabetes mellitus [7–9]. However, hyperglycemia and hyperinsulinemia are known to cause aging-like damage and the effects of glucose seem to be linked to glycation and/or glycoxidation while insulin itself is damaging because of its mitogenic action [10,11]. It has been demonstrated that restricting food intake to 50–70% of the level of ad libitum fed rats has important benefits on life expectancy and prevents age-associated diseases; this seems to be related to the effects of food restriction on plasma insulin and glucose levels (for a

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review, see Ref. [12]). The fact that food restriction can improve this situation might be related to the specific regulation of the insulin signalling pathway. Although there has been much research in this field over the past few years, most studies have considered the effects of moderate food restriction (30–40%) or protein deprivation on skeletal muscle [13–15]. To our knowledge, no previous study has investigated the adaptations of the insulin signalling pathway to food restriction in adipose tissue.

Previously, we studied the effects of a severe model of food restriction on glucose homeostasis and maternal adaptations in rats [16,17]. In the present work, we examined the influence of a brief and severe model of food restriction (50%) on some events in the insulin signalling cascade in both skeletal muscle and adipose tissue. The results of our study show that there are significant differences between food-restricted (R) and control (C) rats and that the adaptations to food restriction seem to differ between skeletal muscle and adipose tissue, thus suggesting a tissue-specific effect.

## 2. Material and methods

### 2.1. Animals

Virgin female Wistar rats (from the biotery of the University of Oviedo, Spain) weighing 250–280 g were used in this study and were kept under standardized conditions: temperature (23±3°C), humidity (65±1%) and a 12-h light/dark cycle (8:00 AM–8:00 PM). The animals were fed a standard diet (Panlab A04). All experimental manipulations were performed between 9:30 AM and 12:30 PM. All the animal experiments were approved by the University of Oviedo Ethics Committee following the "Guiding Principles for Research Involving Animals and Human Beings: Recommendations from the Declaration of Helsinki" and the "Guiding Principles in the Care and Use of Animals" approved by the Council of The American Physiological Society.

The animals were separated randomly into two groups, C and R, with 27 animals per group, and were kept in metabolic cages (Tecniplast, Exton, PA, USA) throughout the experiment. The composition of the diet was as follows: 18% proteins, 3.5% lipids, 53% carbohydrates, 12.5% water, 8% ash, 1.3% minerals and 3.7% vitamins. The energetic value of the diet was 2.9 kcal/g. The C group had free access to food during the experiment, while the R group received approximately 50% of the average daily food intake of the C group on the corresponding day; as a result, the R rats received approximately 50% of each dietary component. All animals had free access to water.

The R and C groups were divided randomly into three subgroups with nine animals in each. The animals were sacrificed after 5, 10 and 15 days of the experiment in order to determine whether the length of food restriction affected the parameters that were studied in this work. Only rats in the diestrous phase were selected based on vaginal smears carried out on the morning of the day of sacrifice.

### 2.2. Euglycemic insulin-clamp studies

Clamp experiments were performed in anaesthetized rats using a procedure described previously [18]. Briefly, after 12 h of fasting on Days 5, 10 and 15, the animals were anaesthetized with sodium pentobarbital (50 mg/kg) and, after a stabilization period, the left saphenous vein was catheterized for insulin and glucose infusion. Biosynthetic human insulin (Actaprid, Novo Nordisk, Denmark) was infused at a constant rate of 20 μl/min (0.4 IU/kg/h) and the blood glucose level was clamped at the value measured in the basal state by a variable infusion of glucose (12% solution).

Plasma insulin was measured using a radioimmunoassay kit for rat insulin (DGR Instruments, Germany). The sensitivity of the assay was 0.1 ng/ml and the intra-assay coefficient of variation was 9.32%. The sample was assayed in duplicate. All samples were measured on the same day.

Finally, samples of skeletal muscle (flexor digitorum superficialis, extensor digitorum longus, soleus and extensor digitorum lateralis) and adipose tissue (retroperitoneal and epididymal) were collected and frozen immediately in liquid nitrogen for use in future experiments. The animals were sacrificed by bleeding.

### 2.3. Preparation of extracts from adipose and muscle tissues and Western blot analysis

The samples of skeletal muscle and adipose tissue (100 mg) were prepared as described previously [19,20]. Briefly, the samples were washed with ice-cold phosphate-buffered saline and homogenized immediately in 3 ml of lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P40 (Roche Diagnostics, Barcelona, Spain), 0.05% sodium deoxycholate and 1 mM sodium orthovanadate at 4°C. The extracts were centrifuged at 12 000×$g$ at 4°C for 10 min and the protein content was determined using the Bradford dye-binding method [21]. Similar-sized aliquots (30 μg for skeletal muscle and 60 μg for adipose tissue) were subjected to SDS-PAGE (8% Tris-Acri-Bis) and the proteins were electrotransferred from the gel onto nitrocellulose

Table 1
General characteristics of experimental animals

| | Group | Day 5 | Day 10 | Day 15 |
|---|---|---|---|---|
| Food intake (g) | R | 9.87±0.15[a] | 9.56±0.16[a] | 9.84±0.05[a] |
| | C | 18.27±0.61 | 19.27±0.77 | 20.25±0.89 |
| Body weight (g) | R | 229.50±4.11[a] | 225.17±4.65[a] | 206.92±3.57[a,b] |
| | C | 261.53±4.27 | 264.4±4.17 | 268.71±11.2 |
| Glucose (mg/dl) | R | 124.67±5.49[a] | 109.83±3.33[a] | 108.75±5.93[a] |
| | C | 141.73±6.4 | 142.34±5.94 | 143.02±5.57 |
| Insulin (ng/ml) | R | 1.27±0.21[a] | 1.49±0.5[a] | 1.17±0.23[a] |
| | C | 8.02±1.4 | 7.94±0.89 | 8.01±1.3 |

Values are mean±S.E.M. The number of cases was six for each group. Only significant differences are shown.

[a] C vs. R.

[b] R15 vs. R5 and R10.

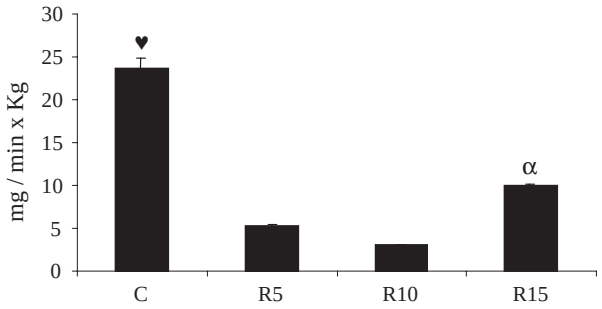


Fig. 1. Comparison of glucose infusion rates of C and R rats (R5, R10 and R15). Glucose infusion rate was assessed as the mean value from 40 to 60 min during euglycemic/hyperinsulinemic clamp experiments. C5, C10 and C15 animals are shown like a single group because we have not found significant differences in the glucose infusion rate. Values are mean±S.E.M. for six animals, except for the C group where there were 18 animals. Only significant differences are shown. (♥) C vs. R5, R10 and R15; (α) R15 vs. R5 and R10.

membranes (Hybond-ECL, Amersham Pharmacia Biotech, Barcelona, Spain) as described by Towbin et al. [22]. Nonspecific protein binding to the nitrocellulose membranes was reduced by preincubating the filter in blocking buffer (TNT; 7% BSA) and probing was carried out using a 1:75 000 dilution of anti-phosphotyrosine antibody (sc-7020, Santa Cruz Biotechnology, Santa Cruz, CA, USA) conjugated to horseradish peroxidase diluted in blocking buffer. The membranes were rinsed several times with blocking buffer without BSA. The protein bands were visualized using enzyme chemiluminescence (sc-2048, Santa Cruz Biotechnology) and quantified using a digital scanner (Nikon AX-110) and NIH Image 1.57 software. The membranes were then incubated in stripping buffer [50 ml 62.5 mM Tris–HCl (pH 6.8), 1 g SDS and 0.34 ml β-mercaptoethanol] at 60°C. Subsequently, another Western blot analysis was performed using a 1:5000 dilution of polyclonal antibody against the IR as the primary antibody, followed by alkaline phosphatase-conjugated anti-rabbit IgG antibody (TROPIX, Bedford, MA, USA) The same protocol was used to assay IRS-1 (dilution 1:5000, sc-559, Santa Cruz Biotechnology) and Glut4 (dilution 1:2000, sc-7938, Santa Cruz Biotechnology; 10% Tris-Acri-Bis). IR and IRS-1 tyrosine phosphorylation were expressed relative to the protein levels as the phosphorylation rate. All membranes were stripped and probed with a monoclonal anti-β-actin antibody (dilution 1:2500, sc-1615, Santa Cruz Biotechnology) to ensure equal protein loading. To facilitate intergel comparisons during the analysis, standards prepared from pooled skeletal heart samples were also run in each gel.

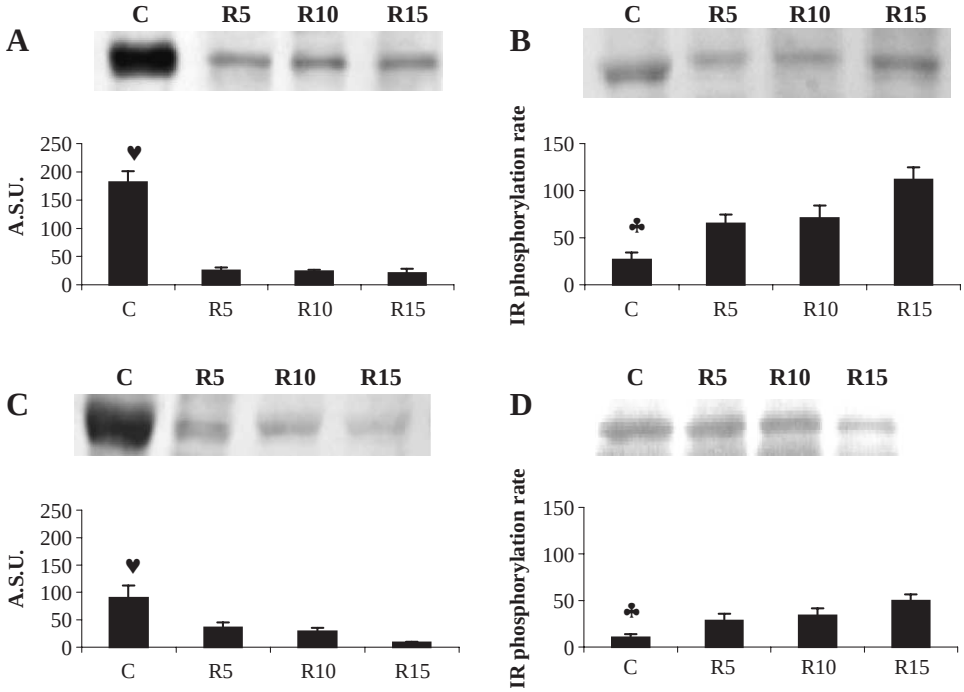


Fig. 2. IR protein levels and phosphorylation in the skeletal muscle (A and B) and adipose tissue (C and D) of the C and R rats (R5, R10 and R15). C5, C10 and C15 animals are shown like a single group because we have not found significant differences. The proteins were isolated with lysis buffer, as described in Material and Methods, and after centrifugation, aliquots of the supernatant containing equal amounts of protein (30 μg for skeletal muscle and 60 μg for adipose tissue) were separated by electrophoresis and immunoblotted with antiphosphotyrosine antibody. Later, the membranes were incubated with stripping buffer and immunoblotted with anti-IR antibody. Scanning densitometry was performed on six experiments. IR tyrosine phosphorylation was expressed relative to protein levels as phosphorylation rate. Results displayed on top panels represent typical inmunoblots. Data are expressed as mean±S.E.M. for six experiments, except for the C group where there were 18 animals. Only significant differences are shown. ASU indicates arbitrary scanning units. (♥) C vs. R5, R10 and R15; (♣) C vs. R15.

### 2.4. Statistics

The data are expressed as mean±S.E.M. Intergroup comparisons for each day of experimentation were performed using the Student–Newman–Keuls test. Intergroup comparisons for the length of food restriction were performed using analysis of variance. A $P$ value of .05 or less was considered statistically significant. All statistical analyses were performed using SPSS (v.6.01) for Windows. In some cases, the C group measurements at 5, 10 and 15 days are shown as a single value because no significant differences were detected between these time points in the parameters tested during the experimental period.

## 3. Results

### 3.1. General characteristics of experimental animals

Table 1 shows the food intake values, total body weight and glucose and insulin levels of the study animals. The food intake values remained relatively constant throughout the experimental period in both groups and, as expected, were significantly higher in the C group than in the R group.

The mean body weight of the C group was significantly higher than that of the R group. Considering body weight gain during the experimental period, a significant decrease was observed at the end of the food restriction period (R15) in the R group, whereas a progressive but nonsignificant increase was detected in the C group during the same period.

The values of glucose were significantly higher in the C group than in the R group throughout the experiment. During the food restriction period, the glucose levels seemed to decrease in the R group, although the differences were not statistically significant. No significant changes were observed in the C group during the experimental period. The insulin levels were always significantly higher in the C group than in the R group and no significant changes were noted within either group during the experiment.

### 3.2. Euglycemic/hyperinsulinemic-clamp studies

To investigate insulin sensitivity in rats at different stages of the experiment, glucose-clamp experiments were carried out under euglycemic and hyperinsulinemic conditions. Fig. 1 shows the comparison of glucose-infusion rates in the C and R groups. The amount of glucose infusion required to maintain euglycemia was significantly lower in the R group than in the C group. However, when the effect of the duration of restriction was considered, it was clear that the glucose infusion rate was significantly

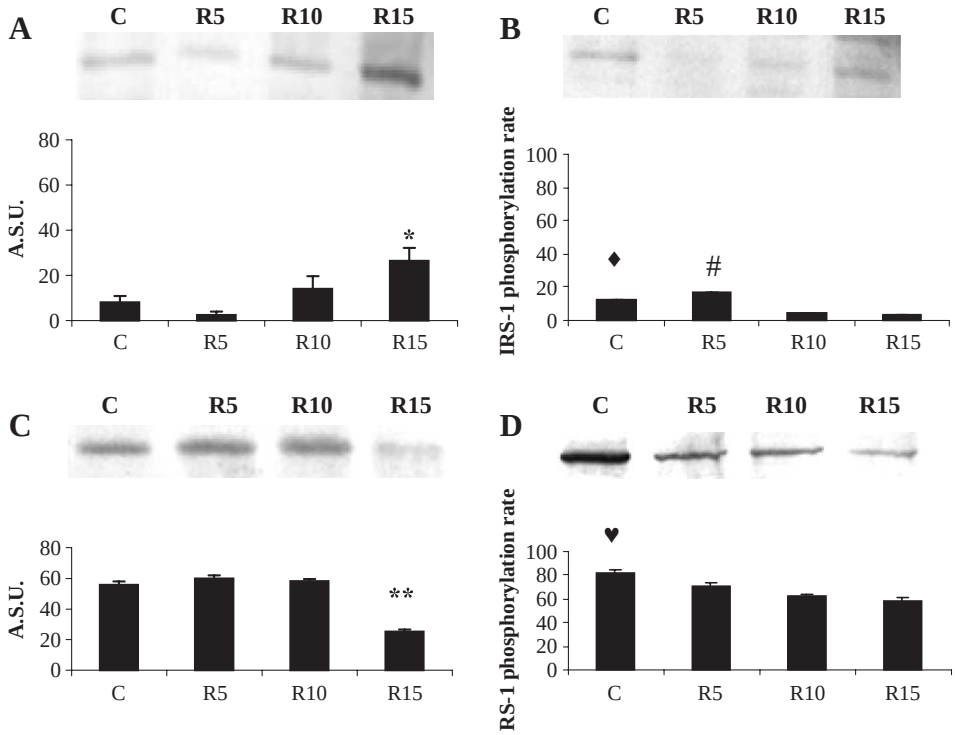


Fig. 3. IRS-1 protein levels and phosphorylation in the skeletal muscle (A and B) and adipose tissue (C and D) of the C and R rats (R5, R10 and R15). C5, C10 and C15 animals are shown like a single group because we have not found significant differences. The proteins were isolated with lysis buffer, as described in Material and Methods, and after centrifugation, aliquots of the supernatant containing equal amounts of protein (30 μg for skeletal muscle and 60 μg for adipose tissue) were separated by electrophoresis and immunoblotted with antiphosphotyrosine antibody. Later, the membranes were incubated with stripping buffer and immunoblotted with anti-IRS-1 antibody. Scanning densitometry was performed on six experiments, except for the C group where there were 18 animals. IRS-1 tyrosine phosphorylation was expressed relative to protein levels as phosphorylation rate. Results displayed on top panels represent typical inmunoblots. Data are expressed as mean±S.E.M. for six experiments. Only significant differences are shown. (*) R15 vs. C and R5; (♥) C vs. R5, R10 and R15; (♦) C vs. R10 and R15; (#) R5 vs. R10 and R15; (**) R15 vs C, R5 and R10.

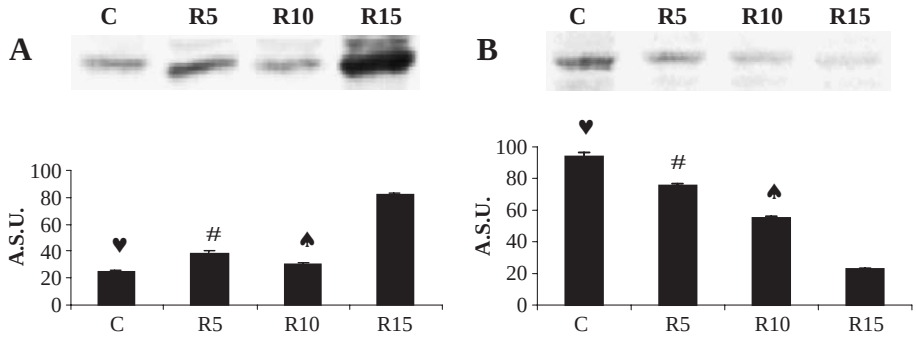


Fig. 4. Glut4 protein levels in the skeletal muscle (A) and adipose tissue (B) of the C and R rats (R5, R10 and R15). C5, C10 and C15 animals are shown like a single group because we have not found significant differences. The proteins were isolated with lysis buffer, as described in Material and Methods, and after centrifugation, aliquots of the supernatant containing equal amounts of protein (30 μg for skeletal muscle and 60 μg for adipose tissue) were separated by electrophoresis and immunoblotted with anti-Glut4 antibody. Scanning densitometry was performed on six experiments, except for the C group where there were 18 animals. Results displayed on top panels represent typical inmunoblots. Data are expressed as mean±S.E.M. for six experiments. Only significant differences are shown. (♥) C vs. R5, R10 and R15; (#) R5 vs. R10 and R15; (♠) R10 vs. R15.

increased throughout the restriction period, suggesting that our model of restriction promotes a progressive increase in insulin sensitivity.

### 3.3. Tyrosine phosphorylation and the protein content of IR

Initially, we investigated whether our model of food restriction influenced the amount or the insulin-induced tyrosine phosphorylation of IR using immunoblot analysis of skeletal muscle and adipose tissue. In both tissues, the amount of IR (Fig. 2A and C) was significantly lower in the R group than in the C group. The food restriction period did not seem to be significantly associated with changes in the amount of IR in either skeletal muscle or adipose tissue. The IR tyrosine phosphorylation rate (Fig. 2B and D) was significantly higher in R15 rats than in the C group in both tissues.

### 3.4. Tyrosine phosphorylation and the protein content of IRS-1

Next, we determined whether food restriction had an effect on a subsequent protein in the insulin signalling pathway, IRS-1. Our results show that this model of food restriction modulates the amount of IRS-1 in a tissue-dependent manner. In skeletal muscle (Fig. 3A), the amount of IRS-1 was significantly higher in R15 rats than in R5 rats or the C group, whereas in adipose tissue (Fig. 3C), the R15 rats showed the lowest level of this protein. The IRS-1 tyrosine phosphorylation rate was modulated by our model of food restriction; in both skeletal muscle (Fig. 3B) and adipose tissue (Fig. 3D), this parameter was reduced in the R group compared with the C group, with the exception of the skeletal muscle of the R5 rats, in which the differences did not reach statistical significance.

### 3.5. Glut4 content in skeletal muscle and adipose tissue

Glut4 showed similar tissue-specific changes to those described for IRS-1 in skeletal muscle and adipose tissue (Fig. 4). Thus, in skeletal muscle, our model of food restriction showed a significant increase in Glut4 content at the end of the experimental period, which was the opposite of the pattern observed in adipose tissue.

## 4. Discussion

Altered energy availability is of fundamental biologic importance and reduced caloric intake has profound effects on glucose homeostasis and insulin action. A 50% food restriction has systemic consequences, including reduced glycemia accompanied by a relative decrease in insulinemia and weight loss (Table 1). In the present study, the R rats showed significantly lower fasting glucose levels despite having lower insulin levels compared with the C rats, suggesting an improvement in glucose homeostasis. In order to confirm this, we developed a euglycemic/hyperinsulinemic clamp (Fig. 1), which has become the standard reference method for the study of glucose metabolism [23,24]. Surprisingly, we found that the rate of glucose infusion that was needed to maintain euglycemia was significantly lower in the R group than in the C group. Therefore, our model of food restriction showed a significant decrease in insulin sensitivity, which seemed to refute the hypothesis that calorie restriction improves whole-body glucose homeostasis. This is not the first study that has reported an impairment of insulin sensitivity following a food restriction protocol. Andreelli et al. [25] showed that lean subjects on an extremely low hypocaloric diet displayed a significant decrease in insulin levels measured using the clamp methodology.

We suggest that this discrepancy is related to the food restriction protocol and the sex of the animals. We used a severe restriction protocol (50% food intake) for 15 days, while most other authors [13–15] used moderate restrictions of food intake (30–40%) for longer periods. We reported a significant increase in insulin sensitivity at the end of the restriction period; however, we did not follow the changes

in this parameter beyond 15 days of restriction. Therefore, it is unclear whether the differences in insulin sensitivity described here were due to the severity or the length of the food restriction protocol or even to both. In addition, the present work involved female rats in the diestrous phase and, curiously, this model of food restriction promoted a significant decrease in plasma 17β-estradiol levels, while plasma progesterone did not change [16]. The influence of female sex steroids on insulin sensitivity is well established and we have shown that the relative plasma levels of 17β-estradiol and progesterone play a pivotal role in the regulation of insulin sensitivity [18–20]. Moreover, Gazdag et al. [26] showed that a reduction in food intake had a different effect on insulin-mediated glucose uptake in male and female mice: glucose transport was increased by food restriction in male rats but was unaffected in female rats. Therefore, we propose that our model of food restriction could promote a special hormonal milieu that might be involved in the regulation of insulin sensitivity and glucose homeostasis.

The R rats in our study showed a contradictory association between hypoglycemia, insulin deficiency and insulin resistance, suggesting that our model of food restriction was associated with an impairment of glucose homeostasis, as has been reported previously [27]. R rats in a fasting state have shown a decrease in plasma glucagon levels due to a reduction in α-cell secretion and a diminished ability to mobilize glucose in response to glucagon [27,28]. We believe that the hypoglycemia reported in animals in our R group could be explained in this way.

Conversely, the model of food restriction used showed a significant increase in insulin sensitivity at the end of the experimental period (Day 15). As explained above, plasma insulin and glucose levels did not change during the experiment in the R group rats, suggesting that the adaptation to this model of restriction led to an improvement in the insulin signalling pathway. For this reason, the next step in our research was to study the changes in key proteins involved in the insulin pathway in skeletal muscle and adipose tissue (namely, IR, IRS-1 and Glut4). We selected these particular tissues in which to study insulin signalling for two major reasons: (1) they are the main targets of insulin and (2) their different metabolic responses to this hormone (lipogenic vs. nonlipogenic) might promote specific adaptations to food restriction.

In both skeletal muscle and adipose tissue, we found that the amount of IR was significantly lower in the R group than in the C group (Fig. 2A and C). However, the relative phosphorylation of IR increased significantly at the end of the experimental period (Fig. 2B and D), suggesting that this activity might be related to the increase in insulin sensitivity observed on Day 15 of food restriction (Fig. 1). These results are in agreement with those of Dean and Cartee [29], who showed that a 40% food restriction significantly increased the amount of IR tyrosine phosphorylation in insulin-treated muscles. The reason for this increase in phosphorylation remains unclear. Most previous studies have shown that food restriction is not associated with a significant increase in IR kinase activity, and it has been suggested that reduced food intake might be related to decreased phosphotyrosine phosphatase activity. We are currently testing this possibility. However, the results of Dean and Cartee and those of the present work should be compared with caution because we used a shorter and more restrictive protocol (50% food intake) than the said authors (40% food intake).

When we analyzed IRS-1 and Glut4 in our experimental animals, we observed tissue-specific differences between adipose tissue and skeletal muscle. The amount of IRS-1 (Fig. 3A) and Glut4 in skeletal muscle (Fig. 4A) increased significantly at the end of the food restriction period, while a significant decrease in these proteins was observed in adipose tissue (Figs. 3C and 4B). The IRS-1 tyrosine phosphorylation rate was decreased in both tissues (Fig. 3B and D) by our model of food restriction. To our knowledge, this is the first study to demonstrate a tissue-specific effect of food restriction on the insulin signalling pathway, showing that the pathway is up-regulated in skeletal muscle and down-regulated in adipose tissue. We suggest that the reason for this tissue-specific regulation is related to the different metabolic adaptations of these tissues to food restriction and the specific hormonal milieu that we discussed previously [16]. However, the roles of 17β-estradiol and progesterone in this activity remain unclear.

Under hyperinsulinemic conditions, as in clamp studies, skeletal muscle is the main tissue that is involved in glucose uptake [23,30] and, as discussed above, the restriction period is related to a significant increase in insulin sensitivity. Accordingly, in skeletal muscle, the insulin signalling pathway should be up-regulated and we showed that the IR phosphorylation rate, the amount of IRS-1 and the amount of Glut4 were increased at the end of the restriction protocol (Figs. 2B, 3A and 4A). However, the changes in the IRS-1 tyrosine phosphorylation rate (Fig. 3B) seemed to contradict this up-regulation. IRS-1 contains at least 14 potential tyrosine phosphorylation sites, only 9 of which are essential for insulin signalling [31]. In our experimental design, the amount of tyrosine-phosphorylated IRS-1 did not reveal which of these residues were phosphorylated and, as was proposed previously [29], it was possible that food restriction did not affect the phosphorylation of all of IRS-1 tyrosine residues uniformly. We are currently trying to resolve this issue.

To our knowledge, this is the first work that has studied the effects of a 50% food restriction on the insulin signalling pathway in adipose tissue. Our results clearly showed a down-regulation of the insulin signalling pathway in this tissue, which was the opposite of that described above for skeletal muscle. According to our results, the amount of IR, the amount and phosphorylation rate of IRS-1 and the amount of Glut4 were significantly reduced at the end of the restriction period (Figs. 2C, 3C and D and 4B). The

amount of white adipose tissue was greatly lowered in R rats, and, along with several other authors [32,33], we propose that its contribution to the overall response to insulin could be less important than that of the skeletal muscle under clamp experiments. It has been shown that under severe models of food restriction, triglycerides that are stored in adipose tissue, which are mobilized by lipolysis, are the major energy source [34]. We believe that the down-regulation of the insulin signalling pathway in adipose tissue reported here suggests that this tissue is less sensitive to the antilipolytic effects of insulin in R rats.

We do not know the precise reason for the diverse regulation of key proteins involved in the insulin signalling pathway. However, it was demonstrated recently that dietary deprivation decreased IRS-1 serine phosphorylation and increased IRS-1 tyrosine phosphorylation in skeletal muscle [35], suggesting that the reduction in serine phosphorylation might be related to the up-regulation of the insulin signalling pathway. We believe that this might reflect the specific regulation of serine phosphorylation in adipose tissue. However, further studies will be needed to identify the serine kinase that is involved in this process.

In summary, the main finding of this study is that a 50% food restriction promotes the tissue-specific regulation of the insulin signalling pathway. In skeletal muscle, which is the main organ involved in insulin-induced glucose uptake, we observed the up-regulation of several elements of this pathway such as Glut4, IRS-1 and IR phosphorylation, while in adipose tissue, we observed the opposite trend, suggesting down-regulation through a decrease in Glut4, IRS-1 and the tyrosine phosphorylation of IR. However, further studies will be necessary to elucidate the factors and the molecular mechanisms that are involved in this tissue-specific regulation.

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