

Effects of dietary fat content on adiposity during energy restriction in genetically obese rats

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Abstract — The effects of the amount of fat provided in a restricted diet on weight loss and body composition were studied in this work. Lean male (*Fa/?*) Zucker rats were fed a control diet ad libitum. Obese (*fa/fa*) Zucker rats were divided into three groups: one group was fed a control diet ad libitum and the other two groups were fed 75 % energy-restricted diets, which provided 10 or 50 % of calories as fat. After 4 weeks, energy restriction normalized body weight but not body composition in the genetically obese rats. Reductions in adipose tissue weights and adipocyte size, without changes in the cellularity, were observed. Differences only reached statistical significance in subcutaneous adipose tissue. A standard fat content in the diet induced the same fat-free mass reduction as a higher amount of this macronutrient, but a greater body fat reduction. This suggests that the restriction of dietary fat, as well as energy, is necessary to achieve dietary management in obesity. © Inra/Elsevier, Paris.

energy restriction / dietary fat / body weight / body composition / genetic obesity

Résumé — Effets de la quantité de lipides du régime sur l'adiposité pendant une restriction calorique chez des rats génétiquement obèses. Le travail a été consacré à l'analyse des effets de la teneur en lipides du régime sur la perte de poids et la composition corporelle. Des rats mâles Zucker maigres (*Fa/?*) ont été nourris avec un régime standard. Des rats mâles Zucker obèses (*fa/fa*) ont été répartis en trois groupes : un groupe nourri avec un régime standard et deux groupes nourris avec des régimes hypoénergétiques (75 % de restriction), qui fournissaient 10 ou 50 % de l'énergie sous forme de lipides. Les poids corporels des rats soumis à une restriction calorique pendant 4 semaines s'avèrent semblables à ceux des rats maigres, mais la composition corporelle reste modifiée. On observe des réductions du poids des tissus adipeux et de la taille cellulaire. Ces modifications n'atteignent la signification statistique que dans le tissu adipeux sous-cutané. En revanche, la cellularité n'est pas modifiée. La perte de masse maigre induite par les deux régimes hypoénergétiques est du même ordre, alors que le régime normolipidique produit une réduction de la graisse corporelle plus impor-

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tante. Ces résultats suggèrent que, pour le traitement nutritionnel de l'obésité, il est important de réduire la prise énergétique mais aussi la prise lipidique. © Inra/Elsevier, Paris.

restriction calorique / lipides alimentaires / poids corporel / composition corporelle / obésité génétique

1. INTRODUCTION

The genetically obese (*fa/fa*) Zucker rat is a good model which has several common characteristics with the human obese syndrome, such as an early hypertrophy of fat cells followed by severe fat cell hyperplasia [21], hypertriglyceridemia, hyperinsulinemia and insulin insensitivity [4]. These fatty rats show many metabolic and endocrine disturbances which lead to the accumulation of excessive quantities of fat in subcutaneous and intraperitoneal fat stores, even when hyperphagia is not developed [4]. Also, the genetically obese rats have an impaired protein metabolism which leads to a low protein utilization [9].

An energy imbalance is necessary to induce a net body weight gain or a net body weight loss. There is, however, convincing evidence that not only energy intake but also the macronutrient composition of the diet plays a major role in the development of such an energy imbalance [13, 35]. In this context, the importance of dietary fat in promoting weight gain and adiposity with ad libitum feeding is increasingly recognized. Thus, an increased proportion of fat in the diet is correlated with weight gain [14, 22, 24, 30, 33, 37]. In contrast, very little information concerning the role of dietary composition on body weight during energy restriction [3] or calorie-controlled refeeding [11] has been provided. Nowadays, many diets have been proposed for obesity treatment, some of which are based on an imbalance of diet macronutrients. The effects of these diets have rarely been studied and the available results are quite inconclusive. Scientific studies should be carried out in order

to shed light on the effectiveness of these diets.

The present study was designed in order to determine the influence of dietary fat content in the effectiveness of energy restriction on weight loss, body composition and adipose cell size in genetic obesity.

2. MATERIALS AND METHODS

2.1. Animals and diets

Seven lean male (*Fa/?*) Zucker rats (11 weeks old) and 21 obese male (*fa/fa*) Zucker rats (11 weeks old) were purchased from Iffa-Credo (Barcelona, Spain). All animals were housed individually and maintained in a temperature (23 ± 2 °C) and humidity (50 %) controlled room with 12 h–12 h light–dark cycle, with lights on at 08.00 hours.

For 4 weeks, lean rats (AL) were fed a control diet ad libitum. Obese rats were divided into three groups: one group was fed a control diet ad libitum (AF) and the other two groups were fed energy-restricted diets, which provided a standard amount of fat (group RFS; 10 % of calories) or a high amount of fat (group RFH; 50 % of calories). Daily determination of food intake of the AL group enabled a precise control of the amounts fed to the restricted groups. Thus, RFS and RFH groups were restricted to 75 % of AL group intake. RFS and RFH diets were given in amounts which provided identical energy in both groups. The composition of all experimental diets is described in *table 1*. Concentrations of protein, vitamins and minerals of both restricted diets were adjusted to ensure the same intake of these nutrients with respect to rats fed ad libitum. All animals had free access to water. Casein was purchased from Sigma (Barcelona, Spain), starch from Vencasser (Bilbao, Spain) and cellulose from Fluka (Barcelona, Spain). Olive oil was obtained locally.

Table I. Composition of experimental diet.

Ingredients (g·kg ⁻¹ diet)	Experimental groups		
	AL and AF	RFS	RFH
Casein ¹	140	190	240
Methionine	5	7	9
Saccharose	350	310	160
Wheat starch	350	310	160
Olive oil	50	40	250
Cellulose	50	68	85
Mineral mix ²	45	61	77
Vitamin mix ³	10	13	17
Choline chloride	1	1	2

Experimental groups: AL, lean rats fed ad libitum; AF, fatty rats fed ad libitum; RFS, fatty rats fed a standard-fat energy-restricted diet; RFH, fatty rats fed a high-fat energy-restricted diet.

¹ Casein 90 %; ² mineral mix (mg·g⁻¹): NaCl 139.3, K₂HPO₄ 389.1, CaCO₃ 381.4, MgSO₄·7H₂O 57.3, FeSO₄·7H₂O 27.0, MnSO₄·H₂O 4.0, ZnSO₄·7H₂O 1.25, KI 0.8, CuSO₄·5H₂O 0.5, CoCl₂·6H₂O 0.02; ³ vitamin mix (µg·g⁻¹): retinol 120, cholecalciferol 2.5, α-tocopherol acetate 3 000, phyloquinone 5, thiamin 600, riboflavin 600, niacin 2 000, pyridoxine 600, folic acid 100, cyanocobalamin 5.4, calcium pantothenate 1 000 and excipient sufficient quantity for 1 g.

Body weight values were registered each day during the treatment period. After an overnight fast, rats were decapitated. Blood was collected in tubes containing EDTA. Blood samples were centrifuged and the plasma was frozen and stored (-80 °C) until the analysis of the biochemical parameters. Adipose tissue depots (subcutaneous, epididymal, perirenal and mesenteric) were dissected and weighed. Small samples (100 mg) of subcutaneous and perirenal adipose tissues were taken for fat cell size determination. Carcasses were weighed after removal of fat pads and internal organs, and frozen (-80 °C) until body composition analysis.

In our experimental design, two reference groups were considered. The AF group was used as a control to study the evolution of obese rats with dietary treatments. In relation to the studied parameters, the AL group was used to establish whether changes induced by dietary treatments allowed obese rats to reach the values of their lean litter mates.

2.2. Carcass composition analysis

For this analysis, the official methods of the Association of Official Analytical Chemists were used [1]. The frozen carcasses were minced in a grinder. Water and ash contents were determined gravimetrically, total protein by the Kjeldahl method and fat by ether extraction in a Soxhlet apparatus.

2.3. Adipose tissue preparations

Tissue fragments (100 mg) of perirenal and subcutaneous fat pads were incubated in 3 mL of Krebs-Ringer-bicarbonate solution, containing per millilitre: 3 mmol glucose, 40 mg defatted bovine serum albumin fraction V (Sigma, Spain) and collagenase from *Clostridium histolyticum* (Boehringer-Mannheim, Spain) at a concentration of 5–10 mg·g⁻¹ adipose tissue. The pH of the medium was adjusted to 7.4. The incubation was carried out at 37 °C for 30 min, at 60–80 strokes/min. The isolation, washing (in a medium lacking collagenase) and collection of fat cells were carried out following the procedure described by Rodbell [31].

2.4. Determination of adipocyte size

Adipocyte diameters were measured by direct microscopy using a 50 µL aliquot of each isolated fat cell suspension. The insertion of a micrometre disc in a focusing eyepiece placed in the phototube produced a projected caliper scale. The average adipocyte diameter was calculated from the diameter of 200 cells. The average volume was calculated for the average diameter, assimilating the adipocyte to a sphere; it was expressed in picoliters.

2.5. Plasma analyses

Plasma samples were analysed for glucose, total cholesterol, triglycerides and total protein levels in a BM/HITACHI™ 717 Autoanalyser (Boehringer Mannheim GmbH, Mannheim, Germany).

2.6. Statistics

Statistical analyses were performed using the Kruskal-Wallis test and a post hoc comparison

between group pairs was performed with the Mann-Whitney U test, after the Kruskal-Wallis test had established significant differences among groups. Differences were considered significant at $P < 0.05$.

3. RESULTS

Figure 1 shows growth curves for each group of rats during the treatment period. Fatty rats fed ad libitum (AF) gained an average of 47 ± 3 g body weight (+13.7 % versus initial body weight). Rats undergoing energy-restriction and fed a diet containing a standard amount of fat (RFS) showed a body weight reduction which represented -2.9 % of their initial body weight. Rats fed the high-fat energy-restricted diet (RFH) gained an average of 9 g body weight (+2.6 % versus initial body weight). Nevertheless, statistically significant differences in final body weight were not found between both restricted groups, despite a different composition of the diets used for feeding. All restricted rats showed a smaller average body weight than that of the AF group ($P < 0.001$) and reached similar values to those of lean rats fed ad libitum (AL).

Adipose depots differed in sensitivity to food restriction (figure 2). Energy restric-

tion resulted in significantly decreased subcutaneous adipose tissue weight (20.8 and 13.7 % in RFS and RFH groups, respectively, versus AF; $P < 0.05$). These values remained, however, clearly higher than those of lean rats (AL) ($P < 0.001$). Differences in epididymal, perirenal and mesenteric adipose depots did not reach statistical significance but there was a consistent trend towards smaller values for the RFS group. Therefore, taking these small differences into account, we found significant reductions in total mass of fat pads after energy restriction (RFS -17.0 %, $P < 0.01$ versus AF and RFH -9.6 %, $P < 0.05$ versus AF).

Central adiposity, which is defined as the ratio of perirenal plus mesenteric depots to total body fat [3], was assessed. It was observed that fat accumulation in obese rats took place mostly in a peripheral location because central adiposity was lower in ad libitum-fed fatty rats (9.6 ± 0.3 %) than in their lean litter mates (14.9 ± 0.7 %). The distribution of fat was not changed by the experimental diets during energy restriction (9.8 ± 0.3 % in the RFS group and 10.1 ± 0.3 % in the RFH group).

Carcass weight and composition are shown in table II. Although lean and fatty rats fed ad libitum had similar body carcass

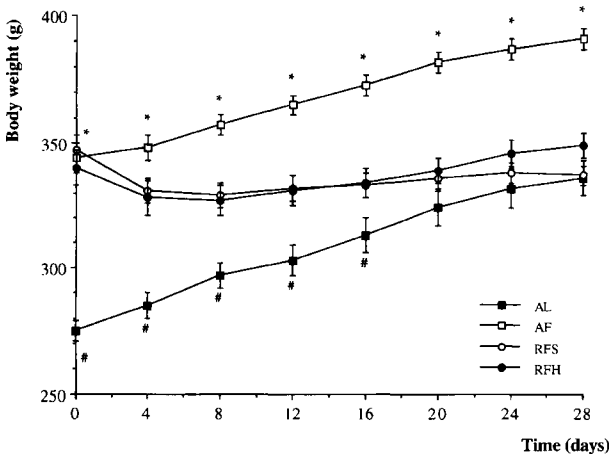
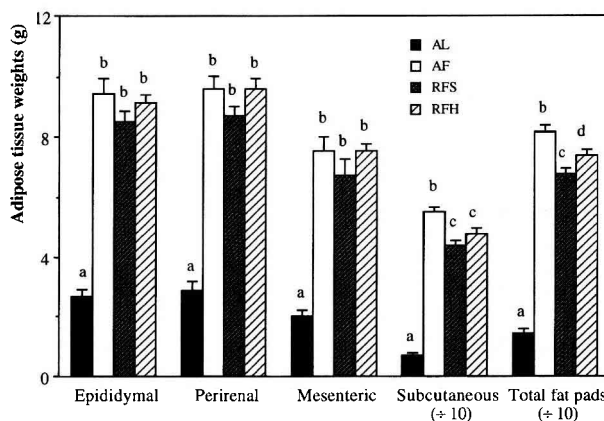


Figure 1. Body weight changes in lean rats fed ad libitum (AL) and in fatty rats fed ad libitum (AF), a standard-fat energy-restricted diet (RFS) or a high-fat energy-restricted diet (RFH) for 4 weeks. Each value is the mean \pm SEM of seven animals. Statistical significance for $P < 0.05$ is indicated as follows: * both restricted groups versus fatty rats fed ad libitum; # both restricted groups versus lean rats.

Figure 2. Adipose tissue weights. Values are the mean \pm SEM of seven animals. Means sharing the same letter are not significantly different, $P < 0.05$. Experimental groups: lean rats fed ad libitum (AL), fatty rats fed ad libitum (AF), fatty rats fed a standard-fat energy-restricted diet (RFS) and fatty rats fed a high-fat energy-restricted diet (RFH) for 4 weeks. Values for subcutaneous adipose tissue and total fat pads must be calculated as follows: data on vertical axis $\times 10$.



weights, carcass composition was significantly different. Fatty rat carcasses had more fat and less protein, water and ash ($P < 0.001$) than their lean litter mates. Feeding fatty rats energy-restricted diets resulted in significant loss of body carcass weight, 11 and 8 % in RFS and RFH, respectively, versus unrestricted fatty rats (AF) ($P < 0.001$), although the differences between both restricted groups in carcass weights were not significant. It is important to emphasize that in animals fed the RFS diet, carcass weight loss was due to fat, protein and water content decreases. In contrast, the RFH diet produced similar reductions in protein and

water but a very small reduction in fat carcass content, which was not statistically significant.

By taking fat pad weights and carcass composition modifications into account, the contribution of fat mass and fat-free mass to body weight reduction for each restricted diet was calculated (figure 3). Regardless of the dietary fat content, energy restriction produced a loss of about 33 g of fat-free mass, which represented a 16 % reduction when compared with 212 g of fat-free mass found in fatty rats fed ad libitum ($P < 0.001$). However, only the RFS diet significantly

Table II. Carcass weight and composition of animals fed the experimental diets.

	Experimental groups			
	AL	AF	RFS	RFH
Carcass wet weight (g)	252 \pm 4 ^a	249 \pm 4 ^a	221 \pm 3 ^b	228 \pm 4 ^b
Carcass composition				
Fat (g)	17.9 \pm 1.1 ^a	96.6 \pm 2.4 ^b	90.1 \pm 1.9 ^c	95.7 \pm 2.1 ^b
Protein (g)	60.1 \pm 0.9 ^a	40.7 \pm 1.3 ^b	33.5 \pm 1.2 ^c	34.5 \pm 1.1 ^c
Water (g)	162.6 \pm 1.9 ^a	102.3 \pm 1.7 ^b	86.4 \pm 1.3 ^c	87.5 \pm 1.6 ^c
Ash (g)	11.8 \pm 1.2 ^a	7.6 \pm 0.2 ^b	7.2 \pm 0.4 ^b	7.4 \pm 0.4 ^b

Values are mean \pm SEM of seven animals. Values sharing the same letter are not significantly different, $P < 0.05$. Experimental groups: AL, lean rats fed ad libitum; AF, fatty rats fed ad libitum; RFS, fatty rats fed a standard-fat energy-restricted diet; and RFH, fatty rats fed a high-fat energy-restricted diet. Carcasses were deprived of fat pads and internal organs.

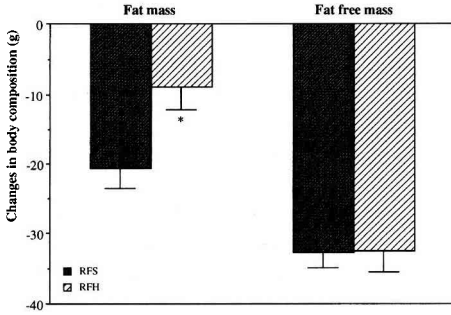


Figure 3. Contribution of fat mass and fat-free mass to body weight loss induced by restricted diets. Values are the mean \pm SEM of seven animals. Statistical analysis represents comparisons between both restricted groups, * $P < 0.05$. Experimental groups: fatty rats fed a standard-fat energy-restricted diet (RFS) and fatty rats fed a high-fat energy-restricted diet (RFH) for 4 weeks.

reduced total body fat (fat depots + carcass fat) ($P < 0.01$) when compared with 178 g of total body fat of AF rats.

Two important markers of obesity, total body fat expressed as the percentage of body

weight and the fat mass/fat-free mass ratio are shown in *figure 4*. In obese rats, the standard-fat energy-restricted diet did not modify these parameters (versus AF group). In contrast, the high-fat energy-restricted diet induced a significant increase ($P < 0.01$).

Fat cell size was significantly increased in fatty rats fed ad libitum versus lean rats in perirenal and subcutaneous adipose tissues, indicating hypertrophy. When fatty rats were fed restricted diets, reductions of adipocyte size were observed. Differences only reached statistical significance in the subcutaneous adipose tissue from the RFS group (*figure 5*). The adipose tissue weight/adipocyte volume ratio was calculated and used as an indicator of adipocyte number. No differences in this parameter ($\text{mg}\cdot\text{pL}^{-1}$) were observed after energy restriction: 221 ± 69 (AF), 228 ± 46 (RFS) and 217 ± 52 (RFH) in subcutaneous adipose tissue and 78 ± 18 (AF), 92 ± 17 (RFS) and 83 ± 19 (RFH) in perirenal adipose tissue.

Concerning plasma parameters (*table III*), the restricted diets induced no changes in

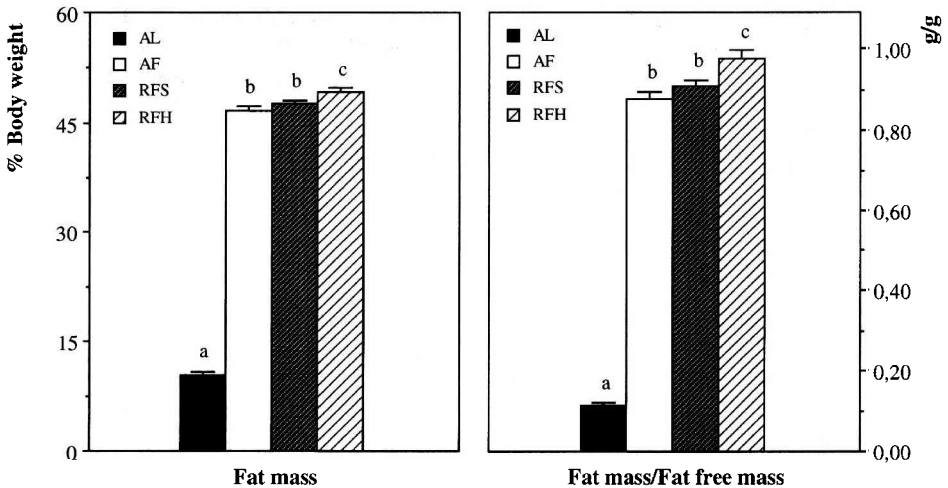


Figure 4. Total body fat mass and fat mass/fat-free mass ratio. Values are the mean \pm SEM of seven animals. Means sharing the same letter are not significantly different, $P < 0.05$. Experimental groups: lean rats fed ad libitum (AL), fatty rats fed ad libitum (AF), fatty rats fed a standard-fat energy-restricted diet (RFS) and fatty rats fed a high-fat energy-restricted diet (RFH) for 4 weeks.

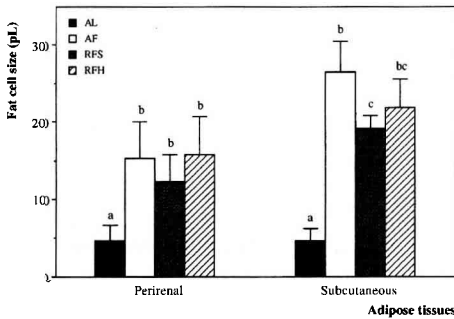


Figure 5. Cell size of adipocytes from perirenal and subcutaneous adipose tissues. Values are the mean \pm SEM of seven animals. Means sharing the same letter are not significantly different, $P < 0.05$.

Experimental groups: lean rats fed ad libitum (AL), fatty rats fed ad libitum (AF), fatty rats fed a standard-fat energy-restricted diet (RFS) and fatty rats fed a high-fat energy-restricted diet (RFH) for 4 weeks.

glucose, total cholesterol and total protein levels. Plasma triglycerides were reduced by energy restriction, but they remained higher than those of lean rats in both restricted groups.

4. DISCUSSION

The genetically obese (*fa/fa*) Zucker rat is frequently used to investigate metabolic alterations associated with the development

and maintenance of obesity. In this study, the genotype of control animals was not defined because accurate information was not available. Nevertheless, Cleary and Phillips [7] showed that the *fa* gene, which had been documented [25] to be expressed during suckling in the heterozygous (*fa/fa*), was not apparent in young adult male rats. Therefore, both homozygous (*fa/fa*) and heterozygous (*fa/fa*) genotypes are useful as controls.

Because there is convincing evidence that not only energy intake but also the macronutrient composition of the diet plays a major role in the development of an energy imbalance, the aim of our work was focused on the study of the influence of the amount of dietary fat on the effectiveness of energy restriction in relation to genetic obesity.

Growing rats were chosen because they are known to be in a dynamic state of fat accumulation [20]. It was thought that the influence of dietary fat and energy restriction would be more evident in this period. As well as in other works, RFS and RFH groups were restricted to 75 % of the AL group intake [2]. In this case, the AL group was used as a control because it has been reported that the magnitude of hyperphagia shown by growing obese Zucker rats varies with age and that the duration of hyperphagia is influenced by dietary composition. When maintained on a standard diet, as in

Table III. Plasmatic parameters of animals fed the experimental diets.

	Experimental groups			
	AL	AF	RFS	RFH
Glucose (mg·dL ⁻¹)	113 \pm 5 ^a	133 \pm 3 ^b	140 \pm 8 ^b	146 \pm 4 ^b
Total cholesterol (mg·dL ⁻¹)	88 \pm 6 ^a	293 \pm 24 ^b	312 \pm 23 ^b	292 \pm 22 ^b
Triglycerides (mg·dL ⁻¹)	61 \pm 4 ^a	395 \pm 25 ^b	319 \pm 28 ^c	323 \pm 30 ^c
Total protein (mg·dL ⁻¹)	6.2 \pm 0.1 ^a	7.3 \pm 0.1 ^b	7.1 \pm 0.1 ^b	7.2 \pm 0.2 ^b

Values are mean \pm SEM of seven animals. Values sharing the same letter are not significantly different, $P < 0.05$. Experimental groups: AL, lean rats fed ad libitum; AF, fatty rats fed ad libitum; RFS, fatty rats fed a standard-fat energy-restricted diet; and RFH, fatty rats fed a high-fat energy-restricted diet.

our study, male obese rats are hyperphagic until 15 weeks of age, when hyperphagia reaches a 'breakpoint' and intake by obese rats begins to decline to lean levels [39].

Important body weight losses were not observed in restricted rats when final body weights were compared with initial body weights. The reason that may explain this is that our study was conducted on growing rats. The correct way to analyse the results of this study is to compare body weight values reached by restricted rats with that of fatty rats with free access to food at the same age. Energy restriction resulted in a lower rate of body weight gain and allowed fatty rats to reach the same values of body weight as their lean litter mates. No significant differences were observed between both restricted diets for this parameter. However, rats consuming the high-fat energy-restricted diet retained significantly more body fat than did rats fed the standard-fat energy-restricted diet as shown in *figures 2 and 3*.

It must be pointed out that not only fat reduction was involved in body weight changes. An important loss of protein and water occurred despite the macronutrient proportions in the diets. This fact is especially negative in genetically obese rats because they showed, prior to treatment, a reduced fat-free mass as compared with lean litter mates [9]. This effect has also been observed by other authors. Indeed, Glore et al. [15] showed strong reductions in muscle protein content and, obviously, of muscle weight in obese Zucker rats during prolonged food restriction.

Despite the reduction in total fat mass induced by energy restriction, the values concerning total body fat (expressed as percentage of body weight), as well as the fat mass/fat-free mass ratio, were maintained in the RFS group and increased in the RFH group, as compared with the AF group. This could be due to the important fat-free mass losses observed in both restricted groups.

This study supports the view that the proportion of dietary fat affects adiposity during food restriction. This can be related to the fact that the cost of incorporating dietary triglycerides into adipose tissue is considerably less ($0.16 \text{ kJ}\cdot\text{kJ}^{-1}$ gained) than the cost involved with de novo lipogenesis from dietary carbohydrates ($0.36 \text{ kJ}\cdot\text{kJ}^{-1}$ gained) [11]. Our results agree with those presented in the study of Boozer et al. [3]. They found a poor effect of high-fat restricted diets on body weight reduction using a diet-induced obesity model. In their study, differences between standard-fat diet and two high-fat diets were more evident than those in our study. This fact may be due to the duration of the feeding period: they treated rats for 10 weeks and our study was conducted for 4 weeks. It could also be due to a greater difficulty in treating genetic rather than diet-induced obesity.

Similar results were also obtained by Dulloo and Girardier [11] when they examined the extent to which dietary composition can alter the elevated efficiency of food utilization and modulate energy partitioning during refeeding. In this work they observed a greater body fat content on high-fat compared with low-fat diets.

Further studies should be carried out in order to establish the mechanisms underlying these results. Differences in diet-induced thermogenesis [16], lipolysis stimulation [40] and fat oxidation [36] have been proposed in order to explain adiposity increase induced by high-fat feeding. It could be possible that they were also involved in the effects that were observed when high amounts of fat were included in an energy-restricted diet.

By comparing values of central adiposity between fatty rats (AF) and their lean litter mates (AL), it can be concluded that fat accumulation induced by a genetic syndrome is greater in peripheral anatomical locations than in abdominal depots. Thus, subcutaneous adipose tissue seems to be more affected by metabolic alterations

related to genetic obesity. Other authors have also observed this phenomenon in Zucker rats [17]. In their work, obese rats showed 21-fold higher subcutaneous adipose tissue, 9-fold higher retroperitoneal adipose tissue and 7-fold higher mesenteric adipose tissue compared with lean rats.

A good explanation for this feature was proposed by Shimomura et al. [34]. They found fatty Zucker rats to have greater mRNA levels of lipoprotein lipase and greater acyl-CoA synthetase activity in abdominal subcutaneous fat than in mesenteric fat. They suggested that these rats are a good model of subcutaneous obesity.

It has been established that the lipolytic capacity of subcutaneous adipose tissue is weaker than that of deeper depots such as the retroperitoneal [26, 27, 38]. In fact, in the above-mentioned article by Hashimoto et al. [17], the beta₃-adrenergic agonist BRL 35135A showed greater lipolytic activity in the visceral than in the subcutaneous fat of obese Zucker rats. Data from our laboratory also showed that subcutaneous adipose tissue is less sensitive to lipolytic agents (dobutamine and CGP 12177) than a pool of epididymal and perirenal adipose tissues, in young obese and lean Zucker rats [28].

When we analysed the effects of energy-restriction, we observed that subcutaneous adipose tissue was the most sensitive, because significant weight reductions were only observed in this anatomical location. This effect can not be explained in terms of lipolytic capacity differences, as is shown above. However, a higher diminution of lipid deposition in subcutaneous depot than in the deeper adipose tissues could be proposed.

When the effects of the amount of fat in the diet on fat accumulation and fat distribution are assessed, it is important to consider the possible influence of the lipid source. In our study, the high-fat diet was constructed with olive oil, which provided a high amount of monounsaturated fatty acids. The results could have been different if other

fat types were used [5, 8, 12, 18, 19].

Changes in adipose cell size induced by dietary treatments are concordant with adipose tissue weight modifications. This adipocyte size reduction was also observed in fatty Zucker rats by Hirsch and Han [20] after a period of starvation, and by York and Bray [41] after 8 weeks of food restriction. In our work, no differences in the adipose tissue weight/adipocyte volume ratio, an indicator of adipocyte number, were observed after energy restriction. In contrast, Cleary [6] observed a lower fat cell number in adipose tissue with no effect on fat cell size in obese Zucker rats after four periods of restricted feeding/refeeding. The discrepancies can be explained by differences in some important variables such as the level of food restriction [23], the age of animals at the beginning of the experiment and the duration of the feeding period.

It is important to point out that the precise number of adipocytes was not calculated because the usual value of adipocyte density used for this purpose ($0.915 \text{ g}\cdot\text{mL}^{-1}$; triolein density) does not seem to be very appropriate in the case of obese Zucker rats [21]. This fact is due to the particular composition of the adipocyte triglycerides [29] and to their high adipocyte size [10].

In conclusion, although energy intake restriction can induce body weight loss, allowing obese rats to reach the body weight of their lean litter mates, it does not have the effect of normalizing their altered body composition. However, despite the reduction induced by energy restriction in plasma triglycerides, the levels remained too high, as did those of glucose, total cholesterol and total protein.

A standard fat content in the diet promotes greater body fat reduction and leads to a lower fat mass/fat-free mass ratio than a higher amount of this macronutrient. Under the experimental conditions of this study, the subcutaneous adipose tissue was more sensitive to dietary manipulations than the perirenal depot.

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