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ORIGINAL CONTRIBUTION

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Effect of high-fat diet on lypolisis in isolated adipocytes from visceral and subcutaneous WAT

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Abstract Variations in total energy intake and composition of daily food play an important role in the regulation of metabolic processes and so, in the control of body weight. This study was designed in order to investigate the effect of a high-fat diet on lipolysis in isolated adipocytes. For this purpose, fourteen Wistar rats were divided into two groups and fed either a standard-fat diet or a high-fat diet *ad libitum* for 7 weeks. Adipocytes were prepared from fat pads by collagenase digestion and incubated *in vitro* in the absence or presence of various lipolytic agents. Lipolysis was measured by the release of glycerol into the medium during 90 min of incubation. We observed that a high amount of fat in the diet induced an enlargement of adipose tissue, which was accompanied by a reduction of β-adrenergic agonist-induced lipolysis, that could be due to a loss of β_1 and β_3-adrenoceptor number or to alterations of their coupling to adenylate-cyclase through the guanine nucleotide regulatory protein. New data about regional differences were provided by comparing two adipose locations (subcutaneous and visceral).

Key words Dietary fat – adipose tissue – lipolysis

Introduction

The regulation of body weight is complex because there are many factors involved in preventing or causing obesity. Some of them are related to dietary variables. Variations in total energy intake and composition of daily food play an important role in the regulation of metabolic processes (1, 2). It has been suggested that dietary fat promotes body accumulation more effectively than dietary carbohydrate (3-5). Thus, high-fat diets can induce body weight and adiposity increases in humans (6) and animals (7).

While carbohydrate and protein balances are closely adjusted, fat balance does not appear to be regulated accurately. Thus, fat added to a relatively normal diet is largely stored (5, 8).

Adipose tissue has a predominant role in the formation, storage and supply of energy reserves. Mobilization and storage of lipids take place simultaneously in this tissue, and the balance between these processes determinates whether there is net loss or net storage of fat (9). Diet, either amount or composition, can potentially influence lipolysis (10). Lipolysis is most sensitive to nutrient availability but dietary composition may also have an effect (11).

In adipose tissue, lipid mobilization can be initiated by stimulation of β-adrenergic receptors, which are coupled to adenylate-cyclase by a nucleotide binding protein (Ns). This stimulation induces cAMP production. cAMP activates a cAMP-dependent protein kinase that, in turn, activates the hormone sensitive lipase (HSL), leading to lipolysis (12).

The purpose of this work was to investigate the effect of a high-fat diet on adipose tissue lipolytic capacity. For this purpose we studied the step-wise regulation of β-adrenergic agonist-induced lipolysis in fat cells, by using some agents which act at well-defined steps of

lipolytic cascade. The work was carried out in two different fat pads (visceral: perirenal+parametrial and subcutaneous) in order to assess regional differences.

Methods

Animals and diets

Fourteen 5-week-old female Wistar rats (~115 g) purchased from Iffa-Credo (Barcelona, Spain) were divided into two groups and fed either a standard-fat diet (SF) or a high-fat diet (HF) during 7 weeks to induce an adiposity increase. The standard-fat diet (13) provided ~12 % of energy from fat and the high-fat diet ~60 % of energy from fat. The latter was formulated by replacing carbohydrate energy with coconut oil energy. The composition of the diets is given in Table 1. Casein was purchased from Sigma (St Louis, Mo), starch from Vencasser (Bilbao, Spain), cellulose from Sigma (St Louis, Mo), coconut oil from Acofarma (Barcelona, Spain) and vitamins from Roche (Barcelona, Spain).

Rats were housed individually in an animal room with constant temperature (20-22 °C) and humidity (50-60 %) and with a 12-h light: 12-h darkness cycle. All animals had free access to food and water throughout the experiment. Diets were provided in food pots that were tightly secured to one side of the cage with metal springs to prevent or minimize food spillage, which was measured. Food intake was measured daily.

Table 1 Composition of Diets with Different Fat Content

	Standard-Fat Diet	High-Fat Diet
	(*g/kg*)	
Casein[a]	180	180
DL-Methionine	3	3
Sucrose	335	190
Wheat starch	335	180
Olive oil	50	50
Coconut oil	–	300
Cellulose	50	50
Mineral mix[b]	NRC	NRC
Vitamin mix[b]	NRC	NRC
Choline chloride salt	2	2
Dietary energy (J/kg)	16205	22482
% energy from fat	11.6	58.6

[a] Casein 98 %.
[b] Mineral and vitamin mixes were formulated to provide 100 % of NRC requeriments for rats; the composition of mixes has been described in detail elsewhere (13).

At the end of the experimental period, all animals were fasted overnight with free acces to water and then killed by decapitation. Plasma was collected and frozen and adipose tissue from different locations (subcutaneous and visceral) were quickly dissected and weighed. Subcutaneous adipose tissue comes from dorsal, abdominal and inguinal regions. The adipose tissues surrounding both reins (perirenal) and both ovaries (parametrial) were pooled and used as representative of visceral fat.

Preparation of isolated adipocytes. Isolated fat cells were obtained according to the method of Rodbell (14) by collagenase digestion (1 mg/mL; 37° C) from visceral and subcutaneous adipose tissues in Krebs Ringer Bicarbonate buffer, containing 3.5 g/100 mL of bovine serum albumin (BSA V) and 0.6 mmol/100 mL of glucose at pH 7.4 (KRBA). Under our experimental conditions, isolated fat cells were obtained after 60 minutes of incubation. Fat cells were filtered through nylon mesh and washed twice with the same incubation buffer (KRBA).

Measurements of lipolysis. Measurements of lipolytic activity were performed by incubating isolated adipocytes (20-30 mg of total lipid) in 1 mL of KRBA buffer. After 90 minutes of incubation with dobutamine, a selective β_1-adrenergic agonist (10^{-8} M to 10^{-4} M), BRL 37344, a selective β_3-adrenergic agonist (10^{-8} M to 10^{-4} M), forskolin (10^{-5} M) and dibutyryl-cAMP (10^{-3} M) at 37° C, the reaction was stopped with ice, and an aliquot (200 µL) was taken to determine glycerol release in the incubation buffer by the method of Wieland (15).

The metabolic activity was expressed as micromoles of glycerol released per 10^6 cells. In order to determine the cell number, adipocyte size was measured according to the method of Di Girolamo (16). Basal rates of lipolysis were defined as the glycerol release in the absence of lipolytic agents.

Plasma free fatty acid concentration. This measurement was carried out by using a commercial kit: Free fatty acids, Half-micro test from Boehringer-Mannheim (Mannheim, Germany).

Drugs and chemicals. Collagenase (0.52 U/mg) and enzymes for glycerol assays were obtained from Boehringer-Mannheim (Mannheim, Germany). Bovine serum albumin (fraction V), forskolin and dibutyryl-cAMP were purchased from Sigma Chemical Co (St Louis, MO) and dobutamine from Lilly (Indianapolis, USA). BRL 37344 was a generous gift from Smitkline Beecham (Surrey, UK). All other chemicals were reagent grade.

Statistical methods. Values presented in tables and figures are given as mean ± SEM (standar error of the mean). The Mann-Whitney U test was used for comparisons between the two groups. *P* values less than 0.05 were considered as statistically significant.

Table 2 Body Weight, Food Intake and Adipose Tissue Weights[a]

	Standard-Fat Diet (n = 7)	High-Fat Diet (n = 7)
Initial body weight (g)	113 ± 2	117 ± 1
Final body weight (g)	217 ± 4	247 ± 5*
Food intake (g)	735.6 ± 23.5	589.2 ± 10.9**
Energy intake (kJ)	11.9 ± 0.4	13.2 ± 0.3**
Energy utilization (g/kJ)	8.8 ± 0.3	9.8 ± 0.4*
Adipose tissue weights		
subcutaneous WAT (g)	4.5 ± 0.9	8.1 ± 1.0**
visceral WAT (g)	10.9 ± 1.4	19.7 ± 1.9***

[a] Values are expressed as means ± SEM for seven rats; Statistical significance at *P<0.05, **P<0.01, ***P<0.001.

Results

Body weight, food intake and adiposity. As shown in Table 2, during the 7 weeks of the study, animals fed the high-fat diet gained an average of 26 g more than those fed the standard-fat diet (P <0.05).

High-fat feeding induced a significant decrease in total food intake after 7 weeks (-20 %; P <0.01). Taking the composition of diets into account, the high-fat fed group consumed 11 % more energy (P <0.01) and 4 times more fat than standard-fat fed group. The efficiency of energy utilization (body weight gained per kJ consumed) was 11 % greater for the high-fat fed animals than for controls (P <0.05).

When rats were fed the high-fat diet, there was a significant enlargement of fat pads. Thus, subcutaneous and visceral fat adipose tissues were increased by about 80 % and 81 % respectively.

Lipolysis in isolated adipocytes and plasma FFA concentrations. In order to express lipolysis as µmol glycerol released by 10^6 cells, adipocyte size was measured. In rats fed the high-fat diet adipocytes showed an increase in cell diameter whathever the adipose tissue considered (7.7 % visceral; 6.3 % subcutaneous). The basal lipid mobilization was greater in adipocytes from subcutaneous adipose tissue of animals fed a high-fat diet (0.492 ± 0.044 µmol glycerol/10^6 cells) than in control rat adipocytes (0.349 ± 0.039 µmol glycerol/10^6 cells) (P=0.009). Moreover, small differences between the two groups were found in adipocytes from visceral adipose tissue (0.220± 0.02 µmol glycerol/10^6 cells in controls *vs* 0.261 ± 0.025 µmol glycerol/10^6 cells in treated rats; P=0.07). Figure 1 illustrates the values for stimulation of

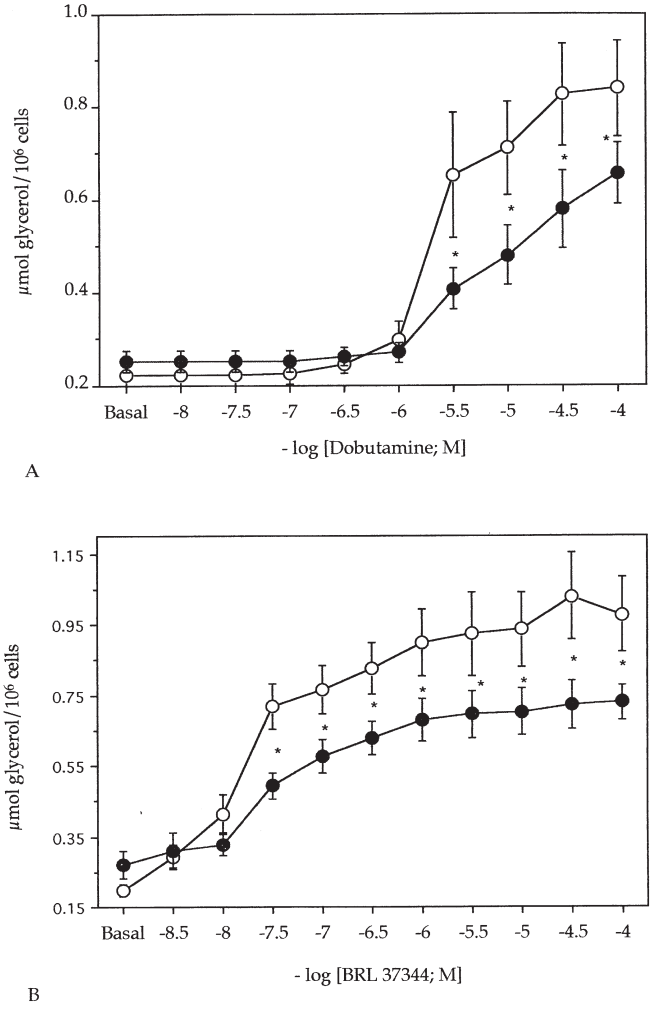

Fig. 1 Dobutamine (A) and BRL 37344 (B)-induced glycerol release in adipocytes from rats fed a standard-fat diet (open symbols) or a high-fat diet (closed symbols). Adipocytes were isolated from visceral adipose tissue. Data are the mean of seven animals per group and SEM are represented as vertical lines. Statistical significance of differences between groups is *P<0.05.

glycerol release by the β_1-adrenergic agonist dobutamine and the β_3-adrenergic agonist BRL 37344 from visceral adipocytes. It could be observed that the ability of increasing concentrations of each β-adrenergic agonist to stimulate glycerol release was blunted in rats fed the high-fat diet. Maximal dobutamine and BRL 37344 responses were significantly lower (P <0.05) (Table 3). In contrast, significant differences were not observed in subcutaneous adipocytes (Figure 2, Table 3).

We attemped to see whether the lipid mobilization defect was proximal or distal to the activation of cAMP. For this purpose, forskolin and dibutyryl-cAMP were added to incubation medium at maximal effective concentrations (10^{-5} M and 10^{-3} M respectively). Forskolin is an agent that activates adenylate cyclase and dibutyryl cAMP is an

Table 3 Maximal lipolysis, expressed as % basal lipolysis, induced by dobutamine, BRL 37344, forskolin and dibutyryl cAMP in adipocytes from rats fed a standard-fat diet or a high-fat diet [a]

	Standard-Fat Diet (n = 7)	High-Fat Diet (n = 7)
Visceral WAT		
Dobutamine	386.9 ± 39.8	258.2 ± 30.3*
BRL 37344	514.6 ± 36.5	360.5 ± 30.6*
Forskolin	409.8 ± 32.4	379.9 ± 28.2
Dibutyryl cAMP	309.4 ± 30.3	285.8 ± 9.7
Subcutaneous WAT		
Dobutamine	346.3 ± 49.2	262.4 ± 27.7
BRL 37344	437.4 ± 41.2	380.0 ± 15.0
Forskolin	392.9 ± 44.0	362.1 ± 33.1
Dibutyryl cAMP	309.4 ± 30.8	312.6 ± 32.8

[a]Values are expressed as means ± SEM for seven rats; Statistical significance at $*P<0.05$.

analogue of cAMP that is not metabolizable by phosphodiesterase. The results indicated that maximal lipolytic responses induced by these agents were not significantly reduced in rats fed the high-fat diet whatever the adipose tissue locations considered (Table 3).

Finally, plasma FFA concentrations were measured. The mean level was 25 % higher in the high-fat group (100.3 ± 10 µmol/dL *vs* 80.0 ± 10 µmol/dL), although the difference was not statistically significant.

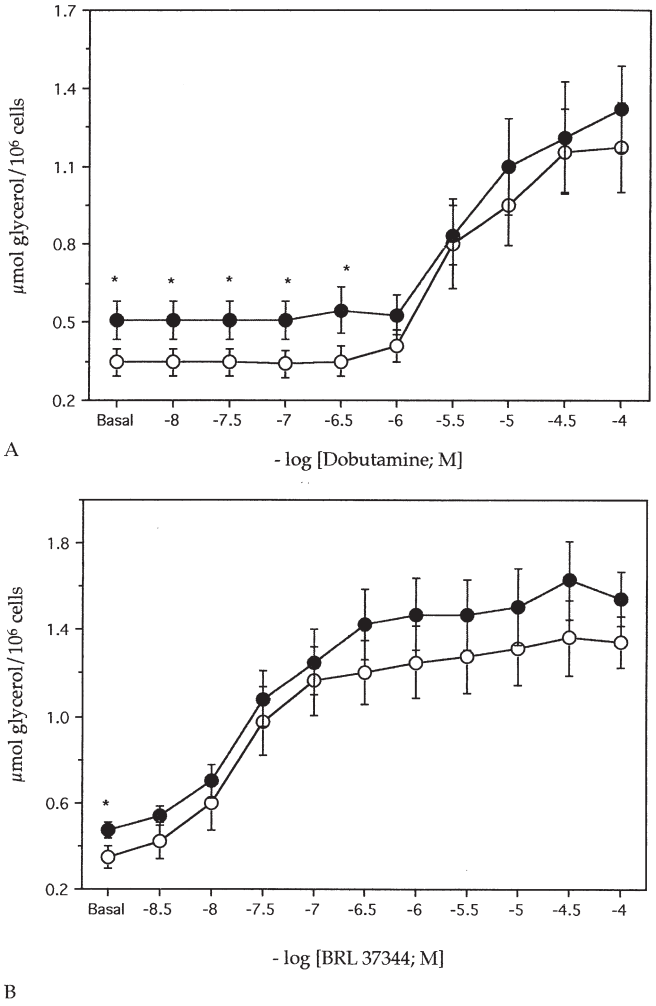

Fig. 2 Dobutamine (A) and BRL 37344 (B)-induced glycerol release in adipocytes from rats fed a standard-fat diet (open symbols) or a high-fat diet (closed symbols). Adipocytes were isolated from subcutaneous adipose tissues. Data are the mean of seven animals per group and SEM are represented as vertical lines. Statistical significance of differences between groups is $*P<0.05$.

Discussion

In this study, we considered the effect of a high proportion of fat in the diet on adiposity and lipolytic capacity of adipose tissue. The results of our study showed that rats fed a diet with a high percentage of fat had greater body weight and adiposity. This fact is related to the higher energy intake observed in those rats and is in good accordance with data presented by several authors (17, 18). Nevertheless, a direct effect of dietary fat could not be discarded because some studies have suggested that fat intake plays a role in obesity, regardless of total energy intake (19-22).

Recognizing the prominent role of adipose tissue in the formation, storage and supply of energy reserves, we focused on the degradating aspect of adipose tissue metabolism. It has been proposed that changes in the size of the adipose cells are a factor contributing to the regulation of adipocyte metabolism (23). Thus, when the amount of adipose tissue increases, the rate of fat turnover and the fasting free fatty acid levels rise progressively, promoting fat oxidation (24, 25). In this context, it could be likely a relationship between the elevated FFA concentrations found in rats fed the high fat diet, which presented greater adipose tissue weights, and the increased basal lipolysis. These results agree with the study of Calles-Escandon and Driscoll (1995), where the macronutrient composition of the diet, specially the content of fat as a percent of food intake, was proposed as a strong determinant of basal lipolysis (25). Previous reports have considered the effects of macronutrient proportions in the diet on lipid mobilization (27-30). Our work confirms the results provided by those reports and presents new data

about dose response curves for β_1- and β_3-selective adrenergic agonists and intertisular differences in response to the diet. The response to β-adrenergic agonists was markedly impaired in visceral adipose tissue from rats fed the high-fat diet. This reduction was not shown by the subcutaneous adipose tissue, indicating a different pattern of response between the two anatomical locations considered in our study. In agreement with our results, intertisular differences in lipid mobilization ability in different situations have been reported by several authors (31-33). Furthermore, there are several differences concerning innervation and blood supply in the adipose tissue microenvironment depending on the anatomical location (34-38). It has been reported that sympathetic activity in adipose tissue was diminished after high-saturated fat feeding and that this effect could contribute to the reduced lipolytic activity (39). The sympathetic innervation of each anatomical location could determine the sensitivity to the changes in lipolysis induced by the reduction of tonic activity of the sympathetic system.

In an effort to distinguish whether adrenergic lipolytic reduction was due to an effect of the high-fat diet on β-adrenoceptors or to a post-receptor effect, we studied lipolysis induced by forskolin and dibutyryl-cAMP. These agents were used at concentrations that allow maximal lipolysis stimulation (40).

Maximal lipolytic response to forskolin was not significantly affected. This supports the view that the decrease in lipolytic response observed in visceral adipose tissue was not due to a defect in cAMP production. On the other hand, the mild modification observed in maximal lipolytic response induced by dibutyryl-cAMP was not statistically significant. This fact argues against the phosphodiesterase as a major cause of the blunting of lipolysis with high-fat feeding.

In summary, these findings suggest that the reduced lipolysis induced by β-adrenergic agonists in rats fed the high-fat diet could be due to a loss of β_1 and β_3 adrenoceptor number or to alterations of their coupling to adenylate cyclase through the guanine nucleotide regulatory protein. The higher circulating concentrations of fatty acids that occured in high-fat fed animals could contribute to the impairment of the coupling between β-adrenoceptors and the catalytic unit of adenylate cyclase, resulting in reduced β-adrenergic responsiveness (27, 28). However, this is not the only mechanism underlying the observed effects because if so, differences between both anatomical locations could not be explained.

More studies are needed to assess if the decreased responsiveness to β-adrenergic agonists, observed in this work, is related to the high amount of fat into the diet or to the nature of dietary fatty acids (mainly saturated in coconut oil). These results have been obtained in isolated adipocytes, but it is important to be cautious with extrapolations of *in vitro* data to *in vivo* conditions (41).

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