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Basic nutritional investigation

Modulation of cytokines, resistin, and distribution of adipose tissue in C57BL/6 mice by different high-fat diets

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ABSTRACT

Objective: To investigate whether changing the lipid source induces metabolic changes and/or modulates the adipose tissue distribution in mice fed with a high-fat (HF) diet.
Methods: C57BL/6 mice were subjected to a 10-wk control diet (10% fat) or an HF diet (60% fat) containing lard (HF-L), olive oil (HF-O), sunflower oil, or canola oil. Food intake and body weight were measured. At euthanasia, blood was collected and adipose tissue was dissected. Serum hormones and cytokines were determined.
Results: The plasma insulin levels were higher in the HF-L and HF-O groups than in the other three groups ($P < 0.0001$). The levels of resistin were highest in the HF-L and HF-O groups ($P < 0.0001$). Leptin expression was also highest in these two groups ($P < 0.0001$). Of the four groups, interleukin-6 was expressed at the highest level in the HF-L group ($P < 0.0005$), whereas adiponectin was expressed at the lowest level ($P < 0.0001$). The accumulation of subcutaneous and visceral adipose tissues was higher in the HF-L group compared with the other groups. This group was hypertrophic because of excess subcutaneous fat and epididymal fat in the adipocytes. However, the ratio of subcutaneous to visceral fat was significantly lower in the HF-L and HF-O groups compared with the other groups.
Conclusion: In mice fed fat-rich diets, the level of adipokines, the distribution of adipose tissue, and the metabolism of carbohydrates are more significantly influenced by the lipid content rather than the absolute amount of lipid.

Introduction

The primary role of adipose tissue was traditionally thought to serve as a temporary storage site for energy in the form of triacylglycerol, but adipose tissue is recognized currently as the largest endocrine organ of the human body [1]. Adipose tissue is known to express and secrete a variety of products known as *adipokines*, which include leptin, adiponectin, resistin, visfatin [2,3], vaspin [4], and omentin [5,6] in addition to proinflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-6, and monocyte chemoattractant protein-1 [7].

Studies have reported that C57BL/6 mice develop obesity, insulin resistance (IR), diabetes mellitus, hypertriglyceridemia [8,9], advanced fatty liver, and fatty pancreatic diseases when fed a high-fat (HF) diet (rich in saturated fat) [10], and the diseases these mice develop closely resemble common forms of disease in humans after developing obesity [11,12]. Compared with other animal models, such as Zucker obese rats, *ob/ob* or *db/db* mice, or other gene-deletion animal models, these HF diets serve as a model of exogenous obesity, which arises by a higher dietary caloric intake (fat overload) [13]. One study has shown that C57BL/6 mice carry a genetic trait that predisposes them to store fat when the dietary fat content is high [12]. However, these results cannot be generalized to human populations, because the obesity in humans responds more to a level of dietary carbohydrates and not to a level

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of lipid. In the human, the carbohydrate restriction has a more favorable effect on the metabolic syndrome than a low-fat diet [14]. This can be explained by the metabolic adaptations induced by carbohydrate restriction [15] and less stimulation of insulin. Lower insulin levels result in increased lipolysis and fatty acid oxidation and simultaneously decreases the activity of key enzymes in de novo lipogenesis [16]. To confirm this, one work studied overweight but otherwise healthy women with normal lipid profiles [14]. In this population, the results of this study demonstrated that a short-term, hypocaloric, very low-carbohydrate, and low-fat diet had a similar effect on cardiovascular risk as shown by similar changes in the ratio of total cholesterol to high-density lipoprotein cholesterol and fasting and postprandial triacylglycerol levels. However, a very low-carbohydrate diet was more effective than a low-fat diet at improving insulin sensitivity as measured by the homeostasis model assessment (HOMA) using fasting glucose and insulin levels, but the significance of this small decrease probably was not physiologically relevant in glucose clearance or insulin effectiveness [14].

In rats, an HF diet not only accelerates the filling of preexisting adipocytes but also stimulates the proliferation of adipocyte precursor cells [17]. Therefore, the hypothesis that obesity may be evaluated as an endocrine tumor has been approached from a genetic perspective in considering hyperplasia and hypertrophy of adipocytes, neovascularization, and increased functional activity in obesity [18].

An indicator for metabolic alterations is the "regional distribution of adipose tissue." Obesity is not a homogeneous condition, and the regional distribution of adipose tissue is important for understanding the relation among obesity, glucose metabolism, and lipid metabolism [19]. Although an increase in total adiposity promotes a higher risk of metabolic diseases, visceral adipose tissue is more closely related to obesity-associated pathologies and complications compared with total adiposity or the quantity of subcutaneous adipose tissue [20,21].

This study was undertaken to increase the understanding of the effect of specific lipid sources from HF diets on glucose metabolism, the distribution of adipose tissue, and the structure and secretory functions in C57BL/6 mice.

Materials and methods

Animals and diets

All procedures were performed in accordance with the guidelines of the animal ethics committee at the State University of Rio de Janeiro and were conducted in accordance with the conventional guidelines for experimentation with animals (National Institutes of Health Publication No. 85-23, revised 1996). Animals were maintained under controlled conditions (21 ± 2°C, humidity 60 ± 10%, and 12-h dark/12-h light cycle). Male C57BL/6 mice (12 wk old) were randomly divided into five groups ($n = 15$ per group, total 75) and subjected to different dietary regimens (Table 1): the standard rodent chow (SC group; 10% of energy from fat, 3.8 kcal/g of chow) or an HF diet (60% of energy from fat, 5.4 kcal/g of chow) containing lard (HF-L group), olive oil (HF-O group), sunflower oil (HS-O group), or canola oil (HF-Ca group). The diets were manufactured by PragSolucoes (PragSolucoes, Jau, SP, Brazil; http://www.pragsolucoes.com.br) in accordance with AIN-93 recommendations [22]. Diets were administered over a 10-wk period.

Body mass, food intake, and feed efficiency

Mice had free access to food and water during the experimental period, and their intakes were monitored daily. In addition, their body mass was measured each week. Fresh chow was provided daily, and any remaining chow from the previous day was discarded. Food consumption was determined as the difference between the food supplied and the amount of food left in the grid. Energy intake was the product of food consumption by the energy content of the diet. Feed efficiency was calculated as the ratio between the body mass gain in grams and the food consumed in kilojoules per animal, multiplied by 100.

Table 1
Experimental diets

Ingredients (g/kg)	Groups				
	SC	HF-L	HF-O	HF-S	HF-Ca
Casein (≥85% protein)	140.0	190.0	190.0	190.0	190.0
Corn starch	620.692	250.692	250.692	250.692	250.692
Sucrose	100.0	100.0	100.0	100.0	100.0
Soy oil	40.0	40.0	40.0	40.0	40.0
Lard	—	320.0	—	—	—
Olive oil	—	—	320.0	—	—
Sunflower oil	—	—	—	320.0	
Canola oil	—	—	—	—	320.0
Fiber	50.0	50.0	50.0	50.0	50.0
Vitamin mix*	10.0	10.0	10.0	10.0	10.0
Mineral mix*	35.0	35.0	35.0	35.0	35.0
Cystine	1.8	1.8	1.8	1.8	1.8
Choline	2.5	2.5	2.5	2.5	2.5
Antioxidant	0.008	0.008	0.008	0.008	0.008
Total amount (g)	1000.0	1000.0	1000.0	1000.0	1000.0
Energy (kcal/g)	3807	5407	5407	5407	5407
Carbohydrate (%)	76	26	26	26	26
Protein (%)	14	14	14	14	14
Lipid (%)	10	60	60	60	60

HF-Ca, high-fat diet with canola oil; HF-L, high-fat diet with lard; HF-O, high-fat diet with olive oil; HF-S, high-fat diet with sunflower oil; SC, standard rodent chow

* Vitamins and minerals present in the mix were in accordance with AIN-93M specifications.

Blood analysis

A blood glucose test, an oral glucose tolerance test (OGTT), and an intraperitoneal insulin tolerance test (IPITT) were conducted before the initiation of the diet feeding and after 9 wk of diet feeding. For blood glucose levels, blood was obtained by milking the tail after a small incision was made to the animal's tail (Accu-Chek glucometer, Roche Diagnostic, Manheim, Germany). The OGTT was performed using a 25% solution of glucose in sterile saline (0.9% NaCl) at a dose of 1.0 g/kg. The glucose was administered by orogastric gavage after a 6-h fasting period. The blood glucose concentration was measured before glucose administration (0 min) and 15, 30, 60, and 120 min after administration. For the IPITT, fasting blood glucose levels were measured (after a 4-h fasting period, blood obtained from the tail vein) using a glucometer (Accu-Chek; 0 min). Insulin was subsequently injected intraperitoneally (1.0 U/kg) and blood glucose was measured again at 15, 30, 60, and 120 min.

On the day before euthanasia, animals were deprived of food for 6 h before being anesthetized (intraperitoneal sodium pentobarbital 150 mg/kg), after which blood samples were obtained by cardiac puncture through the right atrium. The blood plasma was obtained by centrifugation (120 × *g*, 15 min) at room temperature. Serum adipokines, inflammatory cytokines, and insulin were measured using Multiplex Biomarker Immunoassays for Luminex xMAP technology (Millipore, Billerica, MA, USA). The leptin, resistin, IL-6, monocyte chemoattractant protein-1, plasminogen activator inhibitor type 1, tumor necrosis factor-α, and insulin levels were assessed by MADPK-71K, and adiponectin levels were measured using SinglePlex adiponectin kits (mouse, EZMADP-60K). The HOMA-IR was calculated as fasting glucose (millimoles per liter) multiplied by the fasting insulin level (microunits per milliliter) divided by 22.5 [23].

Adipose tissue

The subcutaneous fat between the lower part of the rib cage and the midthigh was considered inguinal fat, whereas the fat connected to the posterior abdominal wall in the vicinity of the kidneys and the abdominal portion of the ureters was considered retroperitoneal fat. The abdominal fat in the lower part of the abdomen and connected to the epididymis was considered epididymal fat. Therefore, after the animals were euthanized, the inguinal (subcutaneous), epididymal, retroperitoneal, and perirenal fat pads were carefully dissected out (both sides of the animal) and weighed.

The adipose tissues from the subcutaneous and epididymal depots were fixed in freshly prepared 4% (w/v) buffered formalin (pH 7.2) and embedded in Paraplast plus (Sigma-Aldrich Co., St. Louis, MO, USA), sectioned (5 μm thick), and stained with hematoxylin and eosin. Digital images were obtained from the histologic sections and at least 500 adipocytes per group were randomly analyzed. The cell diameters were measured by imaging analysis using Image-Pro

Plus 7.0 (Media Cybernetics, Inc., Bethesda, MD, USA). The adiposity index was estimated as the weight of the visceral plus subcutaneous fat pads multiplied by 100 and then divided by the total body mass [24].

Data analyses

The resulting data were tested for normality and homogeneity of variances and then comparisons among groups were performed using one-way analysis of variance and the Tukey post hoc test. Correlations were assessed using the Pearson correlation test to investigate correlations among fat masses, adipokines, resistin, leptin, and adiponectin plasma levels. The differences within the same group as a function of time were analyzed using the paired *t* test (GraphPad Prism 5.03, GraphPad Software, La Jolla, CA, USA). $P \leq 0.05$ was considered statistically significant.

Results

Food intake and feed efficiency

Food intake was not different among the four groups. However, the feed efficiency in mice receiving the HF-L diet was significantly higher compared with the other three groups (+64%, $P < 0.01$). There were no statistically significant differences among the three other groups (HF-O, HF-S, and HF-Ca) in feed efficiency (Table 2).

Body mass

All animals started the experiment with similar body masses. Lard feeding induced a significant body mass gain in the HF-L mice that was observed at week 6, and this gain continued until the end of the experimental period, resulting in the greatest body mass among the four experimental groups. The HF-L group had a final body mass that was 18% greater than the SC control group and 15% greater than the other three experimental groups ($P < 0.001$; Fig. 1).

Carbohydrate metabolism

At the start of the experiment, the OGTT and IPITT measurements did not show any statistically significant difference among the groups. However, at the end of the experiment, the blood glucose levels and the OGTT and IPITT measurements remained unaffected by the diets, whereas the plasma insulin levels were increased by 225% and 156% in the HF-L and HF-O groups, respectively, compared with the SC control group ($P < 0.0001$). Moreover, the HOMA-IR indexes, which reflect whole-body IR, were 309% and 209% higher in the HF-L and the HF-O groups, respectively, compared with the SC control group ($P < 0.0001$). Notably, the serum insulin levels and the HOMA-IR index were slightly higher in the HF-S and HF-Ca groups compared with the SC control group; however, these differences were not statistically significant (Figs. 2, 3, Table 2).

Adipokines

In agreement with the results of carbohydrate metabolism, the levels of resistin were consistent with the results of the HOMA-IR index (Table 2). The resistin expression levels were 177% and 86% higher in the HF-L and HF-O groups, respectively, compared with the SC control group ($P < 0.0001$). Resistin levels were 150% higher in the HF-L group and 68% higher in the HF-O group compared with the HF-S group ($P < 0.0001$). The levels of resistin were 247% and 133% higher in the HF-L and HF-O groups compared with the HF-Ca group ($P < 0.0001$). The differences in the resistin levels were not statistically significant compared with the HF-S, HF-Ca, and SC groups.

The HF-L and HF-O groups showed the highest levels of leptin of all the experimental groups. The leptin levels were 252% and 358% higher in the HF-L and HF-O groups, respectively, compared

Table 2
Various lipid sources induce different responses in mice fed high-fat diets

Data	Groups				
	SC	HF-L	HF-O	HF-S	HF-Ca
Dietetic					
Food intake (g)	2.7 ± 0.3	2.8 ± 0.1	3.0 ± 0.2	3.1 ± 0.1	3.1 ± 0.2
Feed efficiency ($\times 10^3$; g/kcal)	5.5 ± 1.0	9.0 ± 1.3[a]	5.3 ± 1.1[b]	4.3 ± 1.1[b]	5.4 ± 1.1[b]
Carbohydrate metabolism					
Baseline glucose (mg/dL)	132.0 ± 8.5	158.2 ± 9.8	153.4 ± 9.2	127.4 ± 2.3	129.4 ± 4.3
Insulin (pg/mL)	423.1 ± 92.3	1376.0 ± 135.1[a]	1082.0 ± 69.2[a]	809.6 ± 51.7[b]	725.3 ± 71.5[b]
HOMA-IR	3.3 ± 0.6	13.5 ± 2.0[a]	10.2 ± 1.0[a]	6.3 ± 0.4[bc]	5.8 ± 0.7[bc]
Inflammatory cytokines					
IL-6 (pg/mL)	3.2 ± 0.6	17.6 ± 3.8[a]	5.5 ± 0.7[b]	6.0 ± 0.7[b]	7.8 ± 0.6[b]
PAI-1 (pg/mL)	1706.0 ± 338.2	1688.0 ± 240.1	1474.0 ± 132.2	1700.0 ± 188.9	1366.0 ± 318.5
MCP-1 (pg/mL)	40.7 ± 7.8	42.8 ± 7.7	54.2 ± 5.9	50.2 ± 4.3	37.1 ± 7.1
TNF-α (pg/mL)	3.6 ± 0.8	3.9 ± 0.4	2.9 ± 0.3	3.0 ± 0.2	3.7 ± 0.1
Adipose tissue					
Subcutaneous mass (g)*	0.09 ± 0.01	0.13 ± 0.01[a]	0.05 ± 0.01[ab]	0.09 ± 0.0[bc]	0.08 ± 0.0[bc]
Visceral mass (g)†	0.75 ± 0.03	1.33 ± 0.13[a]	0.53 ± 0.05[b]	0.49 ± 0.03[b]	0.57 ± 0.05[b]
Subcutaneous/visceral fat	0.14 ± 0.01	0.08 ± 0.01[a]	0.07 ± 0.02[a]	0.17 ± 0.01[bc]	0.14 ± 0.01[bc]
Adipocyte diameter (μm)					
In subcutaneous fat	57.4 ± 4.3	79.2 ± 4.8[a]	59.0 ± 3.5[b]	59.5 ± 3.2[b]	56.0 ± 2.7[b]
In inguinal fat	60.3 ± 4.4	95.8 ± 5.0[a]	58.3 ± 2.7[b]	60.3 ± 2.5[b]	57.7 ± 3.0[b]
Adipokines					
Resistin (pg/mL)	357.4 ± 52.12	990.6 ± 85.83[a]	664.8 ± 51.70[a]	396.4 ± 25.19[bc]	285.2 ± 55.06[bc]
Leptin (pg/mL)	598.6 ± 159.0	2105.0 ± 377.5[a]	2742.0 ± 337.7[a]	506.3 ± 133.8[bc]	153.5 ± 20.49[bc]
Adiponectin (pg/mL)	975.2 ± 53.40	544.0 ± 25.26[a]	734.6 ± 41.44[ab]	734.8 ± 53.82[ab]	733.5 ± 51.06[ab]

HF-Ca, high-fat diet based on canola oil; HF-L, high-fat diet based on lard; HF-O, high-fat diet based on olive oil; HF-S, high-fat diet based on sunflower oil; HOMA-IR, homeostasis model assessment for insulin resistance; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor type 1; SC, standard rodent chow; TNF-α, tumor necrosis factor-α

Values are presented as mean ± SEM. Statistical symbols ([a]SC group, [b]HF-L group, [c]HF-O group) represent statistical differences at $P < 0.05$

* The inguinal fat pad.

† The sum of the retroperitoneal, epididymal, and perirenal fat pads.

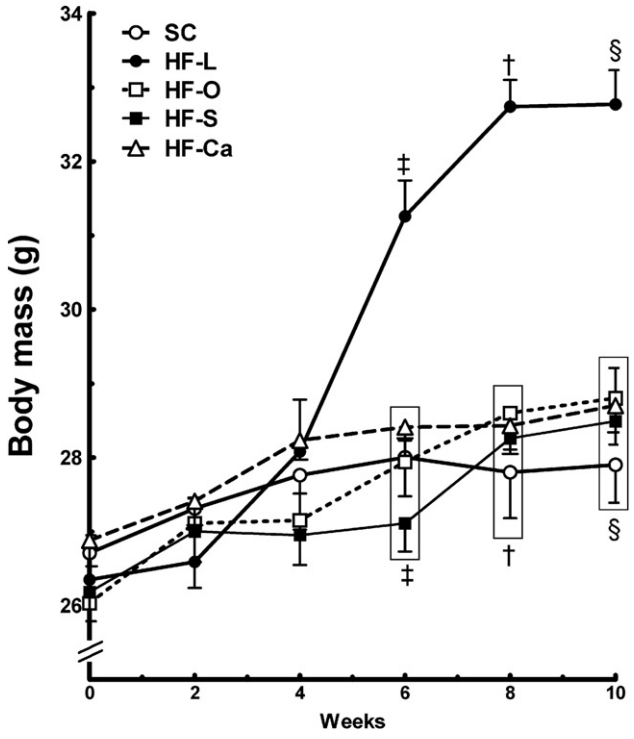

Fig. 1. Body mass evolution. Starting in the sixth week, the HF-L group gained more body mass than the other three groups. This mass difference was maintained until the end of the experiment. The values are reported as mean ± SEM. The statistical difference among the groups is indicated by the same symbol ($P < 0.01$). HF-Ca, high-fat diet based on canola oil; HF-L, high-fat diet based on lard; HF-O, high-fat diet based on olive oil; HF-S, high-fat diet based on sunflower oil; SC, standard chow.

with the SC control group ($P < 0.0001$). Furthermore, the leptin levels were 316% and 442% higher in the HF-L and HF-O groups, respectively, than in the HF-S group ($P < 0.0001$); compared with the HF-Ca group, the leptin levels were 1,272% and 1,687% higher in the HF-L and HF-O groups, respectively ($P < 0.0001$; Table 2).

Interestingly, the levels of adiponectin were lowest in the HF-L group. In general, these levels were low in mice fed HF diets compared with mice fed standard chow (HF-L, 44% less, $P < 0.001$; HF-O, 25% less, $P < 0.01$; HF-S, 25% less, $P < 0.01$; HF-Ca, 25% less, $P < 0.01$). In the HF-L group, the levels of adiponectin were lower than in the HF-O, HF-S, and HF-Ca groups (26% less, $P < 0.05$). No statistically significant differences were found among the HF-O, HF-S, and HF-Ca groups (Table 2).

Inflammatory cytokines

The IL-6 levels were the highest in the HF-L group. This level was 450% higher than that observed in the SC group ($P < 0.001$), 220% higher than in the HF-O group ($P < 0.01$), 193% higher than in the HF-S group ($P < 0.01$), and 126% higher than in the HF-Ca group ($P < 0.01$). No statistically significant difference was found in the IL-6 levels among the SC, HF-O, HF-S, and HF-Ca groups. The remaining inflammatory cytokines, plasminogen activator inhibitor type 1, monocyte chemoattractant protein-1, and tumor necrosis factor-α, did not change as a function of the different diets (Table 2).

Adipose tissue

The subcutaneous and visceral fat masses were greatest for the HF-L group (Table 2, Fig. 4). The subcutaneous fat mass in the HF-L group was 44% greater than the corresponding masses in the SC and HF-S groups ($P < 0.001$). The subcutaneous fat mass was also 160% greater than that observed in the HF-O group ($P < 0.001$) and 62% greater than the mass in the HF-Ca group ($P < 0.001$). However, the subcutaneous fat pad in the HF-O group was 44% less than that observed in the HF-S group ($P < 0.05$) and 37% less than in the HF-Ca group ($P < 0.05$). The HF-L group also showed the greatest visceral fat pad, which was 77% greater than in the pad for the SC group ($P < 0.001$), 151% greater than in the HF-O group ($P < 0.001$), 171% greater than in the HF-S group ($P < 0.001$), and 133% greater than in the HF-Ca group ($P < 0.001$). No statistically significant difference was observed for the masses of the subcutaneous fat pad and visceral fat pad among the HF-S, HF-Ca, and SC groups.

The ratio of subcutaneous to visceral fat ratio was more than 40% smaller in the HF-L and HF-O groups compared with that

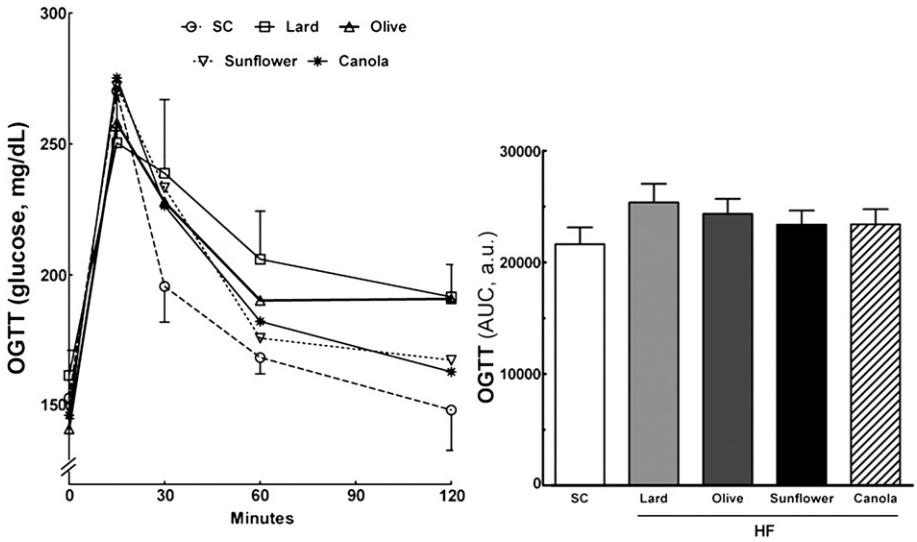

Fig. 2. The OGTT included the curves (left) and the bar graph for the AUCs (right). The values are reported as mean ± SEM. No statistical differences were found among the groups. a.u., arbitrary unit; AUC, area under the curve; HF, high-fat diet; OGTT, oral glucose tolerance test; SC, standard chow.

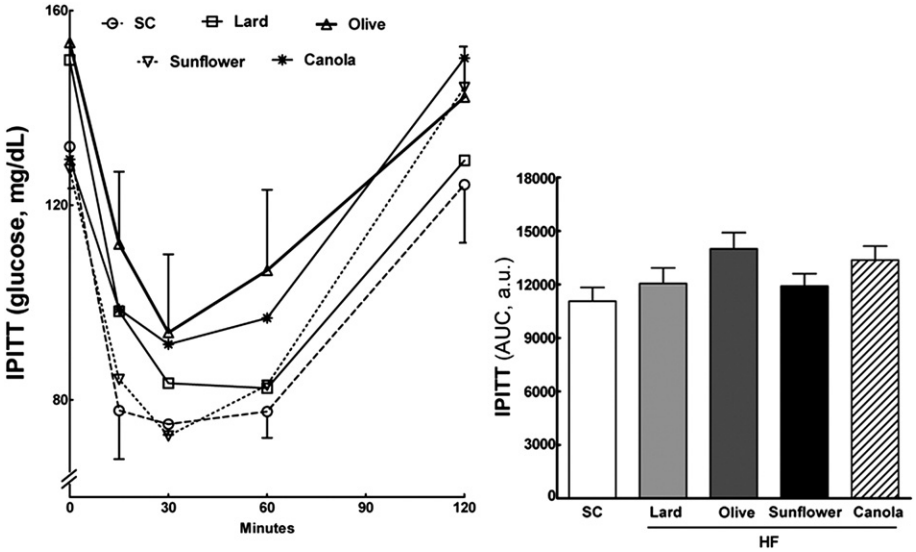

Fig. 3. The IPITT included the curves (left) and the bar graph for the AUCs (right). The values are reported as mean ± SEM. No statistical differences were found among the groups. a.u., arbitrary unit; AUC, area under the curve; HF, high-fat diet; IPITT, intraperitoneal insulin tolerance test; SC, standard chow.

calculated for the other groups ($P < 0.001$; Table 2). No statistically significant differences were reported for this ratio among the SC, HF-S, and HF-Ca groups.

The adipocytes were hypertrophic in the epididymal and subcutaneous fat pads of the HF-L group. The epididymal adipocyte in the HF-L group was more than 30% larger than in

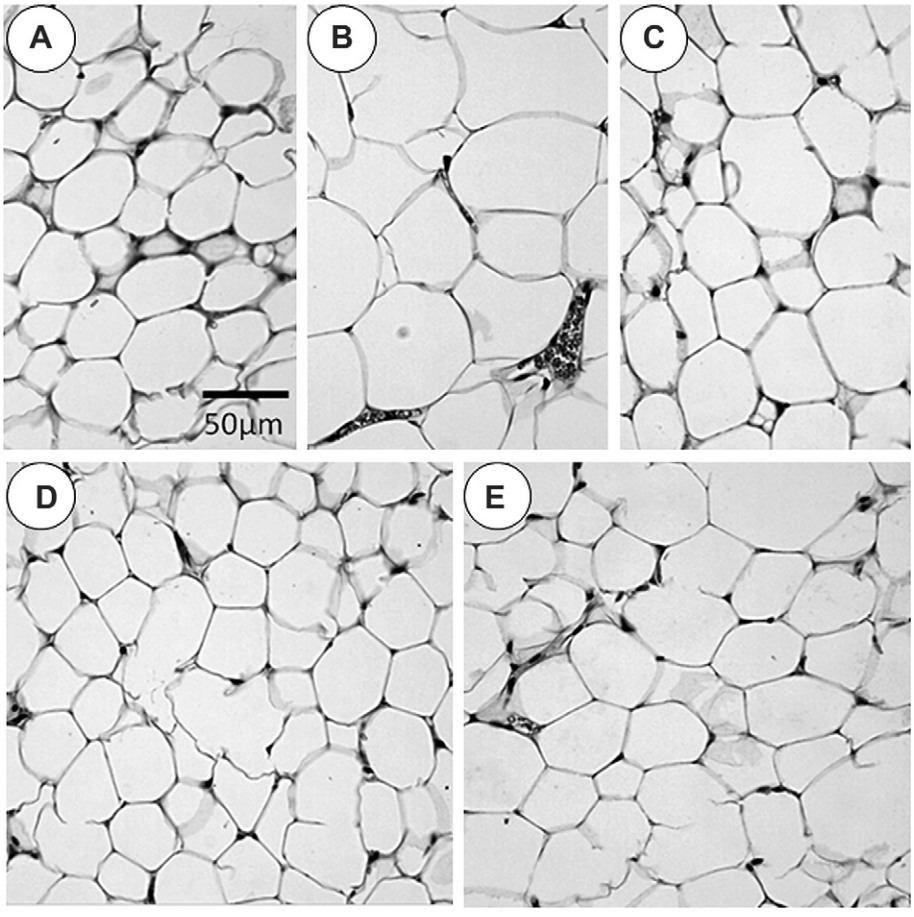

Fig. 4. The adipocyte diameter of the epididymal adipose tissue in (A) the group fed standard chow, (B) the group fed a high-fat diet based on lard, (C) the group fed a high-fat diet based on olive oil, (D) the group fed a high-fat diet based on sunflower oil, and (E) the group fed a high-fat diet based on canola oil.

the pads for the SC, HF-O, HF-S, and HF-Ca groups ($P < 0.05$). In addition, the subcutaneous adipocytes of the HF-L group were more than 60% larger than in the SC, HF-O, HF-S, and HF-Ca groups ($P < 0.05$; Table 2, Fig. 4).

Correlation among adipokines, adipose tissue, and adiposity index

There was a significant positive correlation between resistin and visceral fat masses ($R = 0.72$, $P < 0.00005$) and between resistin levels and the adiposity index ($R = 0.72$, $P < 0.00006$). Interestingly, the levels of leptin and adiponectin were not correlated with visceral or subcutaneous fat masses or with the adiposity index.

Discussion

In the present study, the diets rich in saturated (HF-L) and monounsaturated (HF-O) fatty acids induced IR by increasing the level of inflammatory adipokines and decreasing the ratio of subcutaneous to visceral fat, regardless of the animal's body mass. In contrast, diets that were rich in polyunsaturated fatty acids (HF-S and HF-Ca) did not alter any of the factors involved in the genesis of IR and obesity.

In the present study, the HF-L mice were heavier than the other mice on the HF diets, but HF-L mice actually consumed the same amount of diet per gram of body weight as the other groups. This point is emphasized by the finding of a much higher feed efficiency (weight gain divided by caloric consumption) in HF-L mice compared with the other mice on HF diets. This means that HF-L mice can store more fat without consuming more calories. Therefore, total calories alone do not account for the greater weight gain of HF-L mice, and this suggests that the body fat gain in HF-L mice despite the same diet intake is tightly linked to the source of dietary lipid. The ability to store fat seems to be more important in the development of obesity in C57BL/6 mice than the increase in caloric content of an HF diet [12].

It has been argued that because the rate of carbohydrate and protein oxidation is determined by intake of these nutrients, whereas the rate of fat oxidation is determined largely by the gap between total energy expenditure and energy intake, obesity is the natural result of a diet rich in fat [25]. From the other studies cited earlier, it would seem that the metabolic rate can be altered as a consequence of altering the fatty acid profile of the diet. Some classic studies have directly addressed this possibility. In animal studies comparing isocaloric HF diets containing saturated (edible beef tallow) with ω-6 fat (safflower oil), larger amounts of body fat were accumulated in rats that consumed saturated fat [26]. The expired air from the rats was trapped and the oxygen and carbon dioxide concentrations analyzed by a gas mass analyzer. The increased body fat was associated with lower oxygen consumption compared with the safflower oil–fed animals, indicating a decreased diet-induced thermogenesis of the saturated fat compared with the safflower fat, suggesting that the metabolic rate is altered in conjunction with the modification of membrane phospholipids using the dietary intervention. Another work using open-circuit respirometry; the rodents consuming diets with a relatively high-unsaturated fat profile had significantly greater metabolic rates than those consuming isocaloric high-saturated fat diets [27,28]. Thus, the phospholipid fatty acid composition of membranes seems to be a direct determinant of the metabolic rate in rodents. Higher levels of saturated fatty acids in the diet affect membrane fatty acid composition, leading to a decreased metabolic rate and thus increased susceptibility to obesity.

There are few intervention studies in humans addressing the dietary fatty acid profile and metabolic rate. Perhaps the classic study is that by Jones and Schoeller [29]. In this 2-wk crossover design study, consisting of two consecutive 7-d dietary treatment periods, subjects consumed diets differing only in the dietary ω-6 polyunsaturated-to-saturated fatty acid ratio. The basal metabolic rate and the thermogenic effect of food were measured in each subject on days 1 and 7 of each diet period using open-circuit respiratory gas exchange. Consistent with the animal data, the more saturated diet resulted in the lowest basal metabolic rate, thus favoring weight gain. However, that study was limited because a high-carbohydrate diet was not compared with an HF diet.

High-fat diet–induced obesity has been well understood for more than a century [30]. Further studies have shown that HF diets promote hyperglycemia and increase body mass and IR [9,10]. Therefore, it can be used as an experimental model for studying the metabolic syndrome of IR in rodents [31–33].

As evident from the literature, diets with varied fatty acid composition are broadly grouped under the term *high-fat diet*, even if most of the corresponding studies involved the administration of a formula with only one HF component for the purposes of comparison with standard chow and openly failed to analyze the influence of different fat components in the model. Thus, few studies have been performed by changing the components of these HF diets with the goal of a subsequent assessment of their metabolic effects.

It is generally believed that diets based on saturated fatty acids induce the typical HF diet phenotype, whereas diets containing polyunsaturated fatty acids have beneficial effects on body composition and insulin action [34,35]. However, the role of monounsaturated fatty acids in this context is unclear. In the present study, saturated and monounsaturated HF diets proved effective for inducing the inflammation response and IR commonly found in human metabolic syndromes.

Abnormal adipose tissue development leads to IR in non-adipose organs. Ectopic deposition and inflammation play a central role in the development of IR in the muscle, liver, and other tissues and establish a negative reverberating cycle [36]. There is a correlation between the accumulation of intrahepatic and intramuscular triacylglycerols and a low capacity to oxidize fatty acids in the presence of IR [37–39]. This is in agreement with the findings of the present study in the HF-L and HF-O groups that showed IR. This effect is clearly demonstrated when saturated fatty acids are used [9,10,40], but the data are scarce regarding the other sources of fatty acids. A previous study [31] and the present study have demonstrated a marked IR in mice fed saturated or monounsaturated fatty acids, demonstrating that the lipid source is as important as the quantity of the food consumed.

In the present study, we observed that the parameters of IR and the inflammatory response with the HF-L and HF-O diets led to the most pronounced manifestations. We also observed less subcutaneous fat mass deposition in HF-O mice compared with controls (this difference was not seen in the visceral fat mass deposition), but the inflammatory response and IR in these animals occurred despite this smaller fat deposition. In contrast, we observed a decrease of the ratio of subcutaneous to visceral fat mass by 50% in HF-O and HF-L mice. In this case, these results may suggest that the hyperinsulinemia and inflammatory response are more associated with body fat distribution (ratio of subcutaneous to visceral fat) than with total body fat deposition. For example, not all obese patients have the same risk of developing diabetes, cardiovascular disease, or hyperlipidemia. Individuals with peripheral obesity, i.e., fat distributed subcutaneously in the

gluteofemoral region, are at little or no risk of the common medical complications of obesity, whereas individuals with central obesity, i.e., fat accumulated in visceral depots, are prone to these complications [41–43]. Furthermore, obesity occurs with different degrees of fat accumulation in different depots, and these are associated with different metabolic consequences, with visceral accumulation of fat producing a much greater risk of diabetes, dyslipidemia, and accelerated atherosclerosis than subcutaneous fat and the patterns of fat distribution, and may have a developmental genetic origin [44]. In some cases, the metabolic consequences of fat distribution are different in rodents and humans. For example, leptin exhibits a higher expression in subcutaneous than in visceral adipose in humans [45,46], whereas in mice leptin expression is higher in visceral fat than in subcutaneous fat [47].

The release of adipokines has been shown to play a role in the development of IR and has been shown to increase the risk of obesity [7]. Among all adipokines, resistin has the highest correlation with IR [48]. Low levels of glucose present during periods of fasting improve glucose tolerance and increase insulin sensitivity in resistin knockout mice [49]. An increase in resistin levels has been described in several experimental studies using HF diets based on saturated fatty acids [50,51]. Interestingly, we found for the first time an increase in resistin levels not only in mice fed with saturated fat acids, but also in mice fed an HF diet rich in monounsaturated fatty acids (olive oil). Moreover, the correlation between resistin levels and visceral fat or the adiposity index was very strong, indicating a role of the visceral fat compartment in IR. Other studies demonstrating a causal relation between resistin and glucose homeostasis have been based on animal models with altered serum resistin [52]. In this study, we also showed that IL-6 circulation was drastically increased only in HF-L mice. IL-6 is an important component of obesity-related IR in the liver through impairment of insulin signaling [53,54]. IL-6 can induce IR in the liver. Senn et al. [55] showed that IL-6 can inhibit insulin receptor signal transduction in hepatocytes, indicating that IL-6 and insulin share at least some common signal transduction pathways. In fact, we observed a relation between IR and serum levels of IL-6 in HF-L mice, because these mice presented the highest values of serum insulin and HOMA-IR.

Our study showed that even in excess, polyunsaturated fatty acids do not affect the genesis of obesity and IR. In addition, it is interesting to note that excessive intake of monounsaturated fatty acids had similar deleterious effects compared with a diet rich in saturated fatty acids.

Conclusion

These results demonstrated that the type of the lipid provided in an HF diet is more important than its quantity, especially when considering IR, adipose tissue remodeling, and changes in the levels of proinflammatory adipokines.

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