EFFECT OF CAFETERIA DIET ON LIPID METABOLISM AND LIPASE ACTIVITIES IN WISTAR RATS

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Nowadays, diseases associated with lipid accumulation in the human body such as obesity are becoming very important health issues. The aim of this study is to evaluate the impact of cafeteria diet feeding by Wistar rats, used as an experimental model of nutritional obesity, during 8 weeks, on lipid metabolism. Thus, we determined the levels of total cholesterol (TC) and triglycerides (TG) in plasma, lipoproteins and organs (liver, adipose tissue, muscle), and the activities of lipoprotein lipase (LPL) in organs, and hormone-sensitive lipase (HSL). The results show that cafeteria diet causes increased accumulation of lipids in adipose tissue leads to obesity with ectopic accumulation of lipids in other organs as liver, and induce lipoproteins metabolic disorders. Our results also show a disruption in the pathway of lipid storage enzyme (LPL) and lipid mobilization enzyme (HSL). Cafeteria diet is not only a primary risk for obesity, but also acts indirectly by adversely affecting other primary risk factors to serious chronic disease.

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Introduction

The prevalence of obesity has increased significantly in developed countries, and also, although less rapidly, in developing ones.[1,2] Both genetic and environmental factors have been identified as potential causes of obesity,[3,4] but the relative significance of each or the interplay between the two may vary. The rise in the prevalence of obesity might result from the increasingly sedentary lifestyle of western civilization associated with a reduction in daily physical activity and/or from changes in eating behavior, both quantitatively and qualitatively. Whatever the complexity of risk factors, excess body weight and obesity always result from an imbalance between energy intake and energy expenditure with a positive energy balance due to either excessive calorie intake, decreased calorie expenditure or both. Other scientists have looked at the composition of the diet and reported that excessive consumption of dietary fat may be a more important determinant of obesity than excessive consumption of either carbohydrate or protein.[5,6]

Obesity is progressing in epidemic proportions, and demonstrates no signs of reduced incidence. As weight loss is not only difficult to achieve but more difficult to sustain in long term,[7,8] there must be mechanisms in body to defend the expanded fat mass. Following weight reduction, increases in energy intake, decreases in energy expenditure, and modifications of energy partitioning, storage and oxidation all contribute towards regaining of weight.[9,10] Therefore, it becomes increasingly important to understand how body weight and adipose tissue are regulated including the role of macronutrient partitioning.

Lipid partitioning is important for insulin action, energy balance and the regulation of body weight and composition. The normal physiology of lipid and lipoprotein fuel partitioning is controlled by the transport and uptake of adipose tissue-derived free fatty acids and lipoprotein-derived triglyceride fatty acids. As previously stated, lipoprotein lipid partitioning is largely dependent on the enzymatic action of lipoprotein lipase (LPL) [EC 3.1.1.34], and free fatty acids mobilization is largely dependent on the enzymatic action of hormone-sensitive lipase (HSL) [EC 3.1.1.79].

The aim of this study is to evaluate the impact of high fat and high caloric diet feeding by Wistar rats, used as an experimental model of nutritional obesity, during 8 weeks, on the metabolism of lipids and lipoproteins. Thus, we determined the levels of total cholesterol (TC) and triglycerides (TG) in plasma, lipoproteins and organs (liver, adipose tissue, muscle), and the activities of lipoprotein lipase (LPL) in organs, and those of hormone-sensitive lipase (HSL).

Experimentals

Adult Wistar rats were housed in wood-chip-bedded plastic cages at constant temperature (25 °C) and humidity (60 ± 5 %) with a 12-hour light-dark cycle. They had free access to water and were assigned to two dietary groups, with one group (control, *n*=6) fed a control commercial diet (O.N.A.B), whereas the second group (experimental group, *n*=6) was fed a fat-rich hypercaloric diet "cafeteria diet" during 8 weeks. The control diet was composed of 19 % of energy as protein, 8.50 % of energy as lipids and 56 % of energy as carbohydrate by dry weight. The components of the cafeteria diet were pate, cheese, bacon, chips, cookies and chocolate (in a proportion of 2:2:2:1:1:1, by weight) and control diet (mix/control diet), was given to each rat daily as published previously.[11,12]

The composition of the cafeteria diet, by dry weight, was 21.50 % of energy as protein, 33.50 % of energy as lipids and 33.50 % of energy as carbohydrates. The study was

conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the Regional Ethical Committee.

At 12 weeks of age, rats were anaesthetized with intraperitoneal injection of 10 % chloral (0.3 ml per 100 g of body weight). The abdominal cavity was opened and blood was drawn from the abdominal aorta into EDTA and sec tubes. Blood samples were centrifuged to obtain the plasma for determination of glucose and lipids parameters, and serum for determination of total proteins and lipoproteins composition.

Liver, gastrocnemius muscle and fat tissue were removed, washed with ice-cold saline, and quickly blotted and weighed. An aliquot of each tissue was used immediately to measure the activities of lipoprotein lipase; an aliquot of adipose tissue was used to measure the activity of hormone-sensitive lipase. The rest was stored at –20 °C for determination of lipids tissues content.

Glucose, triglycerides and total cholesterol, were determined using colorimetric enzymatic assays, in plasma and in different lipoprotein fractions after separation by precipitation according to the method of Burstein *et al.*[13] Protein contents of lipoprotein fractions were determined by the method of Lowry *et al.*,[14] and total proteins serum contents were determined by the method of Biuret,[15] with BSA as the standard.

Liver, muscle and fat tissue triglycerides and total cholesterol levels were measured using colorimetric enzymatic essays after homogenization an aliquot of each tissue in phosphate/EDTA buffer, pH = 7.2, containing sodium dodecyl sulfate (SDS 1 %) (1/1, V/V), in an Ultraturax homogenizer, and centrifugation at 3000 g for 10 min.

Lipase activity (LPL, EC 3.1.1.34; LHS, EC 3.1.1.79) was measured by pH-stat by titrimetric measurement of fatty acids released after hydrolysis of triglycerides of synthetic substrate with NaOH 0.05 M at pH 8 and at 25 °C. Enzyme activity was expressed in international units (IU). One unit corresponds to the release of a micro-equivalent of fatty acid per minute.

Results are expressed as means ± standard deviation (SD). The significance of differences between experimental and control rats was assessed using Student's *t* test. The calculations were performed using STATISTICA, version 4.1 (Statsoft, Tulsa, OK). Differences were considered statistically significant at $p < 0.05$.

Results

Body weight, relative weight, triglycerides and total cholesterol contents of organs

The cafeteria diet was associated with increased body weight and weight gain compared to control diet (Figure 1). Relative liver and muscle weight did not differ between animals fed the cafeteria diet and control diet (Table 1); however, cafeteria-diet-fed rats had a higher relative adipose tissue weight compared with control rats.

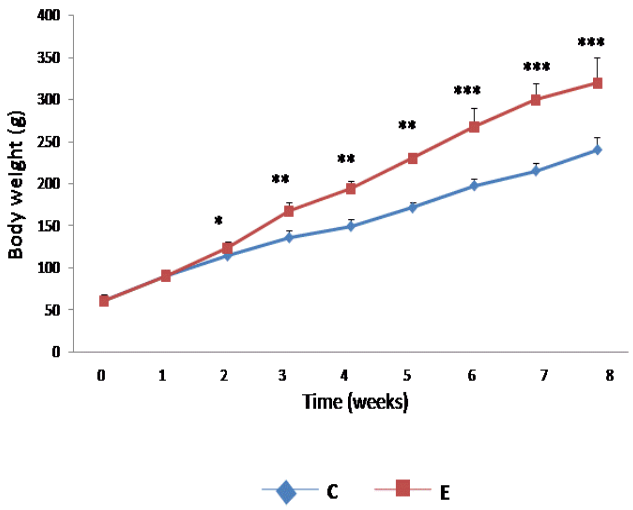

Figure 1. Changes in body weight in control and experimental rats during the 8 weeks of diet. Values are means ± SD. Significant differences between control and experimental rats, at each week, are indicated as follows: *p <0.05; **p< 0.01; ***p<0.001.

The cafeteria diet significantly increased triglycerides and total cholesterol adipose tissue contents (Table 1). Liver triglycerides and total cholesterol contents were significantly higher in experimental rats compared to the control rats, muscle triglycerides and total cholesterol contents did not change significantly between the two groups of rats (**Table 1**).

Table 1. Body weight, organ relative weights and lipids composition in control and experimental rats.

Parameter	Animals	
	Control	**Treated**
Body weight (g)	241.00±15.23	320.00±29.99***
	Adipose tissue	
Relative weight (g)	2.6±0,69	6.17±1.05***
Total cholesterol (mg g^{-1} of tissue)	9.46±0,67	11.29±1.02*
Triglycerides (mg g^{-1} of tissue)	33.70±0.96	52.16±2.89***
	Liver	
Relative weight (g)	9.7±0.82	12.00±2.42
Total cholesterol (mg g^{-1} of tissue)	11,47±0.52	13.06±0.82*
Triglycerides (mg g^{-1} of tissue)	23.29±0.77	36.34±4.71***
	Muscle	
Relative weight (g)	2.48±0.20	2.55±0.17
Total cholesterol (mg g^{-1} of tissue)	5.90±0.82	6.09±1.30
Triglycerides (mg g^{-1} of tissue)	17.75±0.49	17.89±0.71

Values are means ± SD. Significant differences between control and experimental rats are indicated as follows: *p <0.05; **p<0.01; ***p<0.001.

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Table 2. Serum and lipoproteins compositions in control and experimental rats

Parameter	Animals	
	Control	Treated
Serum		
Glucose (mg dL^{-1})	120.13±6.10	152.05±5.83***
Total cholesterol (mg dL^{-1})	124.19±5.14	179.51±3.65***
Triglycerides (mg dL^{-1}l)	82.44±2.88	124.07±3.01***
Total proteins (mg dL^{-1})	777.28±13.16	766.43±12.91
VLDL		
Total cholesterol (mg dL^{-1})	24.33±1.54	60.10±3.15***
Triglycerides (mg dL^{-1})	40.82±1.06	75.50±1.19***
Total proteins (mg dL^{-1})	88.09±1.26	93.50±1.51*
LDL		
Total cholesterol (mg dL^{-1})	34.70±2.10	66.82±4.01***
Triglycerides (mg dL^{-1})	28.22±2.35	32.19±2.20
Total proteins (mg dL^{-1})	248.10±2.86	256.01±3.01*
HDL		
Total cholesterol(mg dL^{-1})	65.11±3.12	52.03±1.81**
Triglycerides (mg dL^{-1})	16.22±0.33	13.01±1.41**
Total proteins (mg dL^{-1})	430.72±1.60	419.01±2.03*

Values are means ± SD. Significant differences between control and experimental rats are indicated as follows: *p <0.05; **p< 0.01; ***p<0.001.

Table 3. Lipoprotein lipase and hormone-sensitive lipase activities in control and experimental rats.

Parameter	Animals	
	Control	**Treated**
LPL (mol g^{-1} min^{-1})		
Adipose tissue	0.049±0.0015	0.0632±0.0011**
Liver	0.0543±0.002	0.0827±0.0013**
Muscle	0.0321±0.003	0.0386±0.002*
HSL (mol g^{-1} min^{-1})		
Adipose tissue	0.0294±0.0014	0.0555±0.0018***

Values are means ± SD. Significant differences between control and experimental rats are indicated as follows: *p <0.05; **p< 0.01; ***p<0.001.

Plasma and lipoproteins biochemical parameters

The cafeteria diet induced a significant increase in plasma glucose in experimental rats compared to control rats (Table 2).

A significant increase in the total cholesterol level was found in the plasma, LDL and VLDL of experimental rats compared to the control rats; however, HDL total cholesterol was low in experimental rats compared with control rats values (Table 2).

Plasma and VLDL triglycerides contents were significantly higher in experimental rats than in control rats (Table 2). There were no significant differences between experimental and control rats concerning LDL triglycerides contents. On the other side, HDL triglycerides contents were significantly decreases in experimental rats compared with control rats (Table 2).

Higher VLDL and LDL apoprotein levels were observed in experimental rats compared with controls (Table 2). However cafeteria-diet-fed rats had a lower HDL apoproteins levels compared with standard-diet-fed rats (Table 2). There were no significant differences between experimental and control rats concerning totals proteins serum contents (Table 2).

Lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activity

Adipose and hepatic LPL activities was significantly higher in cafeteria-diet-fed rats compared with control rats (Table 3), also a higher muscular LPL activity was observed in experimental rats compared with controls rats (Table 3). The cafeteria diet induced a significant increase in HSL activity compared to control diet (Table 3).

Discussion

The present study has evaluated the role of cafeteria diet in induction of lipids and lipoproteins metabolic abnormalities.

The rats that received the cafeteria diet had an increase in body weight, as described previously in the literature with animals fed cafeteria diet.[16,17] The enhancement of body weight in cafeteria-diet-fed rats was strongly associated with the increase in weight of adipose depots, confirming the obesigenic properties of the cafeteria diet, these results are in agreement with previous studies.[17,18,19]

In the other hand, our results show that liver and muscle relative weight did not change between cafeteria-diet-fed rats and control-fed-rats. Similar observations have been made in previous studies.[17] However, analysis of the lipids contents of organs suggests the existence of alterations in experimental rats compared to controls rats; triglycerides and total cholesterol contents of liver and adipose tissue were higher in rats feeding cafeteria diet compared with controls. Rats fed cafeteria diet exhibited a markedly increased adiposity. The increased adiposity was characterized both by an increase in fat pad weight as well as intrahepatic lipid deposition.[20]

Adipose tissue is the site of safe storage of fat and is indispensable for normal metabolic function. A lack of adipose tissue leads to insulin resistance[21] and is responsible for accumulation of fat in the "wrong places".[22,23] The built up of fat in organs other than adipose tissue is believed to alter the normal function of these organs and leads to insulin resistance.

The analysis of blood lipids yields information about the predominant metabolic pathway (carbohydrate utilizing or fat utilizing) active in the body. Blood lipids values are also risk markers for obesity, diabetes, and coronary heart disease. Our results show alterations in plasma lipid and lipoproteins levels. These dyslipidemia manifested as high plasma and VLDL triglycerides levels, low total cholesterol-HDL and high total cholesterol-LDL in experimental rats compared with control, these results are in agreement with previous studies.[24,25,26] Disturbances in lipoproteins metabolism in visceral obesity may be attributable to insulin resistance,[27] insulin resistance increase hepatic synthesis of lipid substrates and the secretion of VLDL apo B-100,[28] it also down regulates LDL receptors.[32] These effects

potentially increase the plasma concentrations of remnant lipoproteins containing apo B-100 and increase competition for hepatic uptake between chylomicron and VLDL remnants.[29]

Arshag *et al.*[26] suggested that the low plasma HDL cholesterol concentrations in obese people could be the result of an increased fractional clearance of HDL secondary to reduced cholesterol content, and reduced production of the main cardioprotective apoprotein, notably apo A-I. Although, low HDL cholesterol levels in obese people are commonly a concomitant of hypertriglyceridemia, it can occur independently of elevated serum triglyceride levels.

Lipoprotein lipase plays a major role in the metabolism and transport of lipids; it is the rate-limiting enzyme for the hydrolysis of the triglyceride core of circulating triglyceride-riche lipoproteins, chylomicrons and very low density lipoproteins.

The present study revealed a significant increase in adipose LPL activity, in agreement with previous studies reporting that, LPL activity has been reported to increase as a function of fat cell size [30,31,32,33], also, LPL is an important marker for adipocyte differentiation [34], and LPL expression increases in parallel with cellular triglyceride accumulation as preadipocytes differentiate[35]. Although, adipose tissue can synthesize free fatty acids *de novo*, free fatty acids for lipid storage are preferentially provided by LPL-mediated hydrolysis of plasma triglyceride-rich lipoproteins [36]. LPL is thus considered a gatekeeper enzyme to play an important role in the initiation and/or development of obesity.

Glucose also increases adipose tissue LPL activity. The glucose stimulatory effect appears to be mostly through the glucosylation of LPL, which is essential for LPL catalytic activity and secretion. Glucose also stimulates LPL synthetic rate and potentiates the stimulatory effect of insulin.[37,38] Our results show an increase in serum glucose level in cafeteria-diet-fed rats.

In our study, a higher activity of hepatic LPL was observed in experimental rats in agreement with Kim *et al.*[39] who reported that, when LPL is over expressed in the liver in mince, a 2-fold increase in liver triglyceride content and insulin resistance was observed. In those mince, increase in hepatic LPL activity impaired the ability of insulin to suppress endogenous glucose production in the liver, and the defect in insulin action and signaling in the liver is associated with increases in intracellular fatty acid-derived metabolites.

Skeletal muscle is a major site for LPL synthesis; it is also the major tissue responsible for whole-body insulin-stimulated glucose uptake/disposal. Arguably the most productive line of research related to the tissue-specific effects of LPL on lipid fuel partitioning, body weight regulation, and insulin action have come from genetic modifications of the LPL gene in skeletal muscle.

The present study revealed a higher muscular LPL activity in the cafeteria-diet-fed rats. Several studies have reported that mice transgenic for LPL overexpression in skeletal muscle are insulin resistant.[39,40] and have increased muscle triglycerides.[39,41,42] Decreases in insulin-stimulated glucose uptake in skeletal muscle and insulin activation of insulin receptor substrat-1 (IRS-1)-associated phosphatidylinositol (PI)3-Kinase activity are also associated with increases in intracellular fatty acid derived metabolites.[39] Pervious study showed that LPL deletion in skeletal muscle seems to reduce lipid storage and increase insulin signaling in skeletal muscle without change in body composition.[43]

Accumulating evidences have defined important functions for HSL in normal physiology affecting adipocyte lipolysis, it has been suggested that HSL is the rate limiting enzyme in intracellular lipolysis,[44] however, direct links between abnormal expression of HSL and human disorders, such as obesity, insulin resistance and hyperlipidemia, await clarifications. In our study a higher HSL activity was observed in experimental rats, these results are in agreement with previous studies reporting a positive relationship between fat cell size and HSL expression in rats with high fat feeding, where fat feeding was associated with an increase in adipocyte cell size and an increase in both basal and stimulated HSL activity.[45] The high HSL activity may be due to insulin resistance, in which insulin has little effect on lipolysis. Insulin stimulation of adipocytes prevents HSL activation, leading to a decrease in the release of free fatty acids and glycerol.[46]

Conclusion

In summary, the data presented in this paper show that rats fed cafeteria diet were characterized by lipid accumulation in adipose tissue leads to visceral obesity associated with insulin resistance and ectopic accumulation of lipids in other organs as liver. In addition cafeteria diet induces lipid and lipoproteins metabolic disorders, associated with abnormal expression of pathway enzymes lipid storage (LPL) and lipid mobilization enzyme (HSL). High fat- and caloric-diet is not only a primary risk factor for obesity, but also acts indirectly by adversely affecting other primary risk factors, such as lipid profile and glycemic control, to serious chronic disease.

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