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The influence of obesity by a diet high in saturated fats and carbohydrates balance in the manifestation of systemic complications and comorbidities

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Abstract

Background: The aim of this study is to test the hypothesis that obesity induced by a diet rich in saturated fats and balanced in carbohydrates is associated with the development of systemic complications and comorbidities.

Methods: Thirty-seven 60-day-old male *Wistar* rats were randomized into two groups: control (C, $n = 18$, standard diet) and obese (OB, $n = 19$, high-saturated fat diet), for 33 weeks. Nutritional profile: food and caloric intake, feed efficiency, body weight, and adiposity index. Complications: in plasma were analyzed dyslipidemia, insulin resistance (HOMA-IR), glucose intolerance, hyperleptinemia, hyperinsulinemia, plasmatic C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α); in the myocardial and epididymal adipose tissue were assessed IL-6 and TNF-α. Comorbidities: diabetes mellitus and systemic blood pressure (SBP). Student's t test, ANOVA, and Bonferroni $P < 0.05$.

Results: The final body weight, feed efficiency, and adiposity index were higher in OB group than in control; although food intake was lower in OB group, caloric intake was similar in both groups. Specific parameters, such as LDL, cholesterol, triglycerides, HOMA-IR, CRP, TNF-α in epididymal adipose tissue, and IL-6 in the myocardium, were higher in obese rats than in controls. SBP, baseline glucose, and glucose after 2 h of overload were significantly increased in OB group; however, the severity was not enough to classify the animals as diabetic and hypertensive.

Conclusion: Obesity induced by a diet high in saturated fatty acids with balanced carbohydrates for 33 weeks in Wistar rats was effective in triggering complications but unable to develop comorbidities.

Keywords: Complications, Comorbidities, Obesity, Diet, Rat

Background

Obesity is a disease resulting from chronic energy imbalance and excessive accumulation of body fat [1–3]. This disease can lead to complications, such as inflammation, alteration of the redox system, hyperleptinemia, hyperinsulinemia, glucose intolerance, insulin resistance, and dyslipidemia, which may result in comorbidities as atherosclerosis [4–7], nonalcoholic steatohepatitis [8], diabetes mellitus [9], and systemic blood pressure [10].

Different types of high-fat diet have been used in animal models to develop complications and comorbidities observed in human obesity [11–13]. It is not well defined what would be the most appropriate diet to build up the triad in experimental models: obesity complications, and comorbidities. For this purpose, several authors use diets rich in fatty acids [14–18]. In diets using predominantly unsaturated fat, the rodents develop obesity and different types of complications associated, whereas comorbidities are not common manifestations [19, 20]. When there is a preponderance of saturated fatty acids, both abnormalities are more constant, by raising total cholesterol and LDL cholesterol, which

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causes deposition in the arteries in the heart and other parts of the body besides triggering obesity and overweight [14, 21, 22]. However, diets that use high fat content result in a decrement of other nutrients, such as carbohydrate, in order to maintain an appropriate proportion of dietary constituents [6, 17, 18]. Due to the reduction in energy availability from carbohydrates, the fat becomes the main source of energy, which could cause a decrease in fat deposits and attenuates the pathogenic events associated with obesity [23]. Considering this issue as well as the harmful role of saturated fat [21, 22], here we tested the hypothesis that the obesity induced by a diet rich in saturated fats and balanced in carbohydrates is associated with the development of systemic complications and comorbidities.

Methods

Animals and experimental protocol

Thirty-seven 60-day-old male Wistar rats, from Anilab Laboratory Animal Creation and Trade Ltd. (Paulinia, São Paulo, Brazil), were kept in rodent vivarium in Experimental Research Unit of Botucatu Medical School–UNESP under the following conditions: individual cages with free access to water and food ad libitum, controlled temperature (24 ± 2 °C), humidity (55 ± 5 °C), and light (12-h light/dark cycle). The experimental procedures were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the "US National Institutes of Health." The rats were randomized into two groups. The control group (C, $n = 18$) received standard diet (SD) and the obese group (OB, $n = 19$) was fed diet high in saturated fatty acids (SHFD), for a 33-week period. The body weight and food intake were measured weekly.

Diet

The diets were developed in Experimental Research Unit of Botucatu Medical School–UNESP in partnership with Biotron Zootecnica® (Rio Claro, SP, Brazil) and adapted from the dietary model used previously by our research group [18]. The following ingredients were used: soybean bran, soybean hulls, corn bran, dextrin, soybean oil, palm kernel oil (as source of saturated fatty acid), and vitamin and mineral complex (adjusted in both diets for all animals receive similar amounts). The SD consisted of 24% of its kilocalorie from protein, 40% from carbohydrates, and 6% from fat; the SHFD 18% from protein, 40% from carbohydrate, and 17% from fat. The SHFD contained a higher amount of calories (SHFD = 3.85 kcal/g vs SD = 3.10 kcal/g) due to increased content of energy from fat. The proportion of saturated/unsaturated fatty acids was 61.5/38.5% in SD and 64.8/35.2% in SHFD (Table 1).

Table 1 Composition of diets

Components	Diet	
	Normolipidic	Hiperlipidic
Proteín (%)	24	18
Carbohydrate (%)	40	40
Fat (%)	6	17
Fibers (%)	12	9
Others[a] (%)	18	16
Calories (kcal/g)	2.93	3.64
Saturated fatty acids	61.5	64.8
Unsaturated fatty acids	38.5	35.2

[a]Others: humidity, vitamins, and minerals

Euthanasia of animals and biological material collection

At the end of the experimental protocol, the animals were fasted for 12 h and the euthanasia was performed by decapitation after anesthesia via intraperitoneal with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). Then, the samples were collected and stored at –80 °C for further analysis.

Nutritional profile

The nutritional status was determined by food and caloric intake feed efficiency, body weight, and adiposity index. Dietary intake and body weight were measured weekly. The caloric intake was determined by the following formula: weekly food intake multiplied by the energy value of each diet (g × kcal). To analyze the animal's capacity to convert consumed food energy in body weight, feed efficiency was calculated by dividing the total body weight gain (g) by the total energy intake (kcal). The amount of total body fat was obtained by the sum of epididymal, retroperitoneal, and visceral deposits; the adiposity index was calculated by the total body fat normalized by the final body weight and multiplied by 100.

Complications

The presence of complications was divided into two sections: (1) metabolic/hormonal which were assessed by dyslipidemia, insulin resistance, glucose intolerance, hyperleptinemia, hyperinsulinemia, and (2) inflammatory, analyzed by C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). The plasmatic triglyceride, total cholesterol, and high- (HDL) and low-density lipoprotein (LDL) were determined using specific kits (BIOCLIN®, Belo Horizonte, MG, Brazil) and analyzed by automated enzymatic colorimetric method (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China); the nonesterified fatty acids (NEFA) levels were evaluated by colorimetric kit (WAKO Pure Chemical Industries Ltd.,

Osaka, Japan). Insulin and leptin were analyzed by enzyme-linked immunosorbent assay (ELISA) method (EMD Millipore Corporation, Billerica, MA, USA). The reading was performed using a microplate reader (Spectra MAX 190, Molecular Devices, Sunnyvale, CA, USA). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) calculated by the formula: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [8]. The glucose intolerance was assessed through the glucose tolerance test and area under the 2-h blood glucose response curve (AUC). After a period of 6 h fasting, the blood samples were collected in the caudal artery at baseline and 15, 30, 60, 90, and 120 min after intraperitoneal administration of glucose 30% (Sigma-Aldrich, ®St Louis, MO, USA), equivalent to 2.0 g/kg body weight. The measurement of blood glucose level was performed using a handheld glucometer (Accu-Chek Go Kit, Roche Diagnostic Brazil Ltda, São Paulo, Brazil). TNF-α and IL-6 (R&D Systems Inc., Minneapolis, MN, USA) levels were analyzed in the plasma and myocardial and epididymal adipose tissue and C-reactive protein (CRP) (Linco Research Inc., St. Louis, MO, USA) in the plasma by ELISA according to manufacturer's instructions. The reading was performed using a microplate reader. The cytokine (TNF-α and IL-6) results in adipose tissue and myocardium were corrected per gram of total protein.

Comorbidities

The following comorbidities were evaluated: diabetes mellitus (DM) and systemic arterial hypertension (SAH). The diagnoses of DM and SAH were determined using the same criteria used by the World Health Organization (WHO) [24] to humans, i.e., DM: fasting blood glucose higher than 126 and ≥200 mg/dL 2 h after a glucose load and SAH: arterial pressure ≥140/90 mmHg. The glycemic values were determined during the glucose tolerance test. In this experiment, only the systolic blood pressure was measured by tail plethysmography using electro-sphygmomanometer, Narco Bio-System®, model 709-0610 (International Biomedical, Austin, TX, USA); this method does not assess diastolic blood pressure. These two criteria of comorbidities were established in human references by the facts that criteria for rats are close to those for humans [25].

Statistical analysis

All results are presented as mean ± standard deviation or median (minimum and maximum value) and were subjected to Student's *t* test for independent samples or Mann-Whitney. The level of significance for all variables was 5%.

Results

Nutritional profile

Table 2 shows the nutritional profile in C and OB animals. The final body weight was greater in OB group as well as the adiposity index. The caloric intake was similar in both groups; however, the obese rats had higher feed efficiency, even with a lower food intake.

Complications

The complications due to obesity are presented in Table 3. The obese group showed a significant increase in the concentrations of cholesterol triglycerides, LDL, HOMA-IR, plasma CRP, TNF-α in the epididymal adipose tissue, and IL-6 in the myocardium when compared to control group.

Comorbidities

The levels of systolic blood pressure baseline glucose and glucose after 2 h of overload were significantly higher in the OB group. However, the levels were not high enough to classify the animals as diabetic and hypertensive according to the established criteria (Table 4).

Discussion

The aim of this study was to determine whether the obesity induced by a diet rich in saturated fatty acids and appropriated (balanced) amounts of carbohydrates is associated with the development of complications and comorbidities. We observed that despite the SHFD-fed animals having eaten a lower amount of food and a similar amount of calories to control, they showed a significant increase in body weight and adiposity index, whereas they were classified as obese. The effectiveness of dietary model is attributed to the greater feed efficiency in obese animals probably due to the thermal effect of fat, since this nutrient requires less energy (2–3%) to be absorbed and digested, making it easily stored as triglycerides in adipocytes [6, 17, 26].

The current study showed that obese animals had numerous complications and absence of comorbidities. These results are in agreement with some

Table 2 Nutritional profile

	C	OB	*P* value
FBW (g)	467 ± 52	541 ± 68	0.001
Adiposity index (%)	4.5 ± 1.4	9.2 ± 1.5	0.001
Food intake (g)	24.1 ± 2.1	19.7 ± 1.9	0.002
Caloric intake (kcal)	71.2 ± 6.2	71.9 ± 6.9	>0.05
Feed efficiency (%)	1.3 ± 0.2	1.6 ± 0.2	0.005

Data expressed as mean ± standard deviation. Student's *t* test for independent samples
C control *n* = 18, *OB* obese *n* = 19, *FBW* final body weight

Table 3 Metabolic, hormonal, and inflammatory complications

	Groups		*P* value
	C	OB	
Total cholesterol (mg/dL)	68.0 ± 13.4 (*n* = 18)	78.2 ± 14.5 (*n* = 19)	0.037
Triglycerides (mg/dL)	46.7 ± 18.2 (*n* = 18)	72.7 ± 25.4 (*n* = 19)	0.001
HDL (mg/dL)	25.1 ± 3.9 (*n* = 18)	27.1 ± 4.7 (*n* = 19)	0.173
LDL (mg/dL)	24.0 ± 4.3 (*n* = 18)	27.7 ± 5.4 (*n* = 19)	0.033
NEFA (mmol/L)	0.42 ± 0.08 (*n* = 14)	0.52 ± 0.12 (*n* = 15)	0.016
HOMA (IR)	29.30 ± 10.6 (*n* = 14)	42.90 ± 18.70 (*n* = 15)	0.025
Glucose (AUC)	18387 ± 3955 (*n* = 18)	24356 ± 4640 (*n* = 19)	<0.001
Insulin	4.46 ± 1.39 (*n* = 14)	6.08 ± 2.27 (*n* = 15)	0.030
Leptin	5.59 ± 2.16 (*n* = 14)	16.90 ± 8.89 (*n* = 11)	<0.001
PCR plasma (mg/dL)	0.23 (0.20–0.40) (*n* = 9)	0.41 (0.30–0.56) (*n* = 7)	0.008
IL-6 plasma (pg/mL)	940 ± 337 (*n* = 9)	1010 ± 234 (*n* = 7)	0.648
IL-6 epididymal (pg/g protein)	2.29 ± 0.92 (*n* = 12)	3.33 ± 2.19 (*n* = 12)	0.141
TNF-α epididymal (pg/g protein)	872 (630–1345) (*n* = 12)	1584 (894–3074) (*n* = 12)	0.014
TNF-α myocardial (pg/g protein)	1.80 ± 0.27 (*n* = 12)	2.03 ± 0.41 (*n* = 12)	0.251
IL-6 myocardial (pg/g protein)	5.52 ± 2.78 (*n* = 12)	7.65 ± 1.25 (*n* = 12)	0.089

Data are expressed as mean ± standard deviation and median (minimum and maximum value). Student's *t* test for independent samples
C control, *OB* obese, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *NEFA* nonesterified fatty acids, *AUC* area under curve, *HOMA-IR* homeostasis model assessment of insulin resistance, *CRP* C-reactive protein, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-alpha

authors [9, 10, 20–25] that did not find comorbidities, only complications, and in disagreement with other studies that did not find such complications in obesity induced by diet with a predominance of saturated fatty acids [27–30]. Although blood glucose 2 h after the glucose tolerance test and blood pressure were significantly higher than those in the control group, they did not reach the levels considered to classify animals as having diabetes mellitus and systemic hypertension, according to the World Health Organization [1]. All changes, metabolic/hormonal/inflammatory, viewed in obese rats probably result from adipose tissue dysfunction; this imbalance in adipose tissue is characterized by adipocyte hypertrophy followed by reduced capacity of triglyceride storage and increased production of inflammatory adipokines, oxidative stress, and fatty acid releasing, by lipolysis, that cause systemic deleterious effects [31, 32]. Though, even with double fat index, obese animals have not reached a magnitude capable of triggering the comorbidities proposed in this experiment. One of the explanations for the absence of comorbidities was the use of complex carbohydrates in the hyperlipid diet, and literature reports that simple carbohydrates are more deleterious and frequently used in the induction of obesity, complications, and comorbidities [33, 34]. However, in this study, this type of carbohydrate was not used, since this would not allow knowing if the manifestation of the complications and comorbidities would be exclusively due to the increase of the amount of fat or its association with a carbohydrate with harmful capacity. It is believed that other factors may also have influenced this result as time of exposure to diet, fat percentage, and type (saturated or unsaturated) and source (animal or vegetable) of fatty acids and animal model [6, 17, 18, 30, 33, 35–37].

Conclusion

In conclusion, obesity induced by a diet rich in saturated fatty acids with equilibrated amount of carbohydrates was effective in triggering complications but unable to develop comorbidities analyzed. A relevant aspect, based on our results, would be that a diet rich in saturated fatty acids, with balance of other nutrients, does not trigger comorbidities. Another would be that this model of dietary obesity can be used in research involving only complications arising from the increase in fat mass.

Table 4 Comorbidities

	Groups		*P* value
	C	OB	
SBP (mmg/Hg)	121 ± 10	129 ± 7	0.009
Glucose (mg/dL)	86 ± 7	93 ± 8	0.021
Glucose after 2 h (mg/dL)	115 ± 20	137 ± 30	0.012

Data are expressed as mean ± standard deviation. Student's *t* test for independent samples
C control *n* = 18, *OB* obese *n* = 19, *SBP* systolic blood pressure

Authors' contributions
CSA conducted the experiment and participated in writing the paper, CRC participated in the outline of the experiment and writing the paper, DFV participated in the preparation of the experiment and interpretation of the results, DHSC participated in the implementation of diet and CRP performed the statistical treatment, IOM, participated in the study of the paper and ACC delineated the experiment and participated in writing the paper. All authors read and approved the final manuscript.

Ethics approval
The protocol was approved by the Ethics Committee on Animal Experiments of the Botucatu Medical School–UNESP (protocol: 993/2012).

Competing interests
The authors declare that they have no competing interests.

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