A high-fat diet as a model of fatty liver disease in rats

Dieta hiperlipídica como modelo de esteatose hepática em ratos

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ABSTRACT
PURPOSE: The objective of the present study was to analyze the physiological and metabolic changes occurring in rats subjected to high-fat diet for one month.

METHODS: The animals received a modified AIN-93 diet with increased lipid content and decreased carbohydrate content, while the control group received the normal AIN-93 diet.

RESULTS: It was observed that the high-fat diet did not induce weight gain but led to greater gain of hepatic fat compared to control. Biochemical parameters, glycemia, total cholesterol and serum protein did not differ between groups. In parallel, rats receiving the high-fat diet consumed less feed.

CONCLUSION: The development of obesity through high-fat diet is associated with increased energy intake and time of exposure to the diet, while the metabolic syndrome is more associated with the combination of a diet rich in fat and carbohydrates.

Key words: Obesity. Lipids. Hyperphagia. Weight Gain. Rats.

RESUMO
OBJETIVO: Analisar modificações fisiológicas e metabólicas em ratos submetidos à dieta hiperlipídica por um mês.

MÉTODOS: Os animais receberam a dieta AIN-93 modificada, com aumento do teor de lipídeos e diminuição do teor de carboidratos, enquanto o grupo controle recebeu a normal AIN-93.

RESULTADOS: Foi observado que a dieta hiperlipídica não induziu o ganho de peso, porém levou a um maior ganho de gordura hepática, em comparação ao grupo controle. Os parâmetros bioquímicos, glicemia, colesterol total e proteína sérica não diferiram entre os grupos. Ao mesmo tempo os ratos alimentados com dieta hiperlipídica, apresentaram uma menor ingestão de alimentos.

CONCLUSÃO: O desenvolvimento da obesidade com a dieta hiperlipídica está associado com aumento da ingestão de energia e tempo de exposição à dieta, enquanto a síndrome metabólica está mais associada a dietas com alto teor de gordura e carboidratos, concomitantemente.

**Introduction**

The prevalence of obesity has been increasing all over the world. This increase is frequently attributed to changes in the life style of western societies, particularly important among them the consumption of high-fat diets. Fat is the dietary nutrient with the greatest energy density since it provides 9 kcal per gram, while carbohydrate and protein provide only 4 kcal. Thus, and increased fat intake can promote a high energy consumption and for this reason dietary fat is considered to be one of the environmental factors that most contribute to the current epidemic of obesity. While the consumption of a high-fat diet is only a hypothesis for the cause of obesity, genetic factors also play an important role.

Obesity predisposes to a variety of metabolic diseases, most of the times leading to the development of metabolic syndrome, which can be characterized by a groups of metabolic risk factors such as central obesity, insulin resistance, increase arterial pressure, and non-alcoholic steatohepatitis (NASH). In addition, metabolic syndrome increases the risk to develop type 2 diabetes and cardiovascular diseases. Epidemiological studies have documented that nutritional factors such as a high consumption of saturated fat and cholesterol can influence the prevalence of metabolic syndrome.

Studies on animals have indicated that a high-fat (HF) diet is an important component of the etiology of obesity. According to West and York, HF diets lead to excess body fat in monkeys, dogs, swine, squirrels, hamsters, and rats. The mechanisms responsible for the correlation between dietary and body fat are unclear. Hyperphagia may be an important mechanism, although high energy density, palatability and other metabolic effects may also contribute to this correlation. According to Hill et al., HF diets containing 30% or more of total energy from fats cause obesity in rats, mice, dogs and primates as a result of increased energy intake and efficient energy storage. In contrast, obesity is rare in animals receiving a control fat diet but steatosis can occur in rats receiving a low-protein diet.

The objective of the present study was to analyze the physiological and metabolic changes resulting from the administration of HF diet to rats.

**Methods**

The study was conducted on 14 male Wistar rats aged on average 21 days and with initial mean weight of 59 g, provided by the Central Animal Facilities of Faculty of Medicine of Ribeirão Preto (FMRP-USP). The animals were housed in individual cages on a 12 hour light/dark cycle under controlled temperature, with free access to water and diet. All procedures were approved by the Ethics Committee for Animal Experimentation of FMRP-USP.

The animals were divided into two groups: control group consisting of 7 rats fed the AIN-93 diet containing 4.17 kcal/g ready diet, and the group high-fat (HF+) consisting of 7 rats fed a modified AIN-93 diet with an increased lipid content and a reduced carbohydrate content, containing 6.57 kcal/g. The groups received the respective diets for one month. The composition of the control and HF diets is given in Table 1.

**TABLE 1 - Composition of the diets (g/kg total) administered to control animals (AIN-93) and to test animals (HF+)**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control (AIN-93)</th>
<th>High fat (HF+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (q.s.p)</td>
<td>635</td>
<td>155</td>
</tr>
<tr>
<td>Protein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Lipids</td>
<td>70</td>
<td>550*</td>
</tr>
<tr>
<td>Fibers</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Choline</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Energy value (kcal/kg)</td>
<td>4170</td>
<td>6570</td>
</tr>
</tbody>
</table>

*350g animal fat and 200g soy oil.

The amount of diet ingested by the rats was determined by regular weekly weighing of the troughs and of the animals. At the end of 30 days, the two groups were sacrificed by decapitation and blood samples were collected for the determination of total serum cholesterol, serum protein and glycemia. The liver was then weighed and placed in liquid nitrogen (-196°C) for later determination of hepatic fat by Bligh and Dyer extraction.

Glycemia, total cholesterol and protein were determined by a colorimetric enzymatic method using a commercial kit (Labtest®, Minas Gerais, Brasil). Data were analyzed statistically by the unpaired t-test corrected by the Welch test. The level of significance was set at \( p < 0.05 \) for all analyses.

**Results**

Regarding the weight evolution of the animals, no significant difference in mean weekly weight variation was observed between groups (Figure 1). However, there was a statistically significant difference in mean weekly diet consumption between groups \( p < 0.05 \), with the control group ingesting more ration than the HF+ group throughout the four weeks of the study (Figure 2). In the last week of the study, control animals ingested a mean amount of ration of 114.6 ± 21.6 g, corresponding to 478 kcal, as opposed to 73.4 ± 13.14 g for the HF+ group, corresponding to 482 kcal. Thus, even though the diets offered to the two groups differed in energy density, the difference in diet consumption resulted in equal calorie intake.
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Controle HF+ p valor
Glycemia (mg/dl) 95.46 ± 20.02 120.3 ± 39.32 p>0.05
Serum protein (g/dl) 4.97 ± 0.38 5.07 ± 1.41 p>0.05
Total cholesterol (mg/dl) 116.12 ± 19.96 79.04 ± 27.94 p>0.05

Discussion

In the present study, the rats that consumed the high-fat (HF) diet ingested a smaller amount of food than the controls, in contrast to the hypothesis that diets with a high fat content promote hyperphagia in rodents20. However, since the energy density of the HF diet was higher than the energy density of the control diet, i.e., 6.57 kcal/g versus 4.17 kcal/g, both groups consumed the same amount of calories. The present data agree with those reported by Chen et al.21, who also observed a reduced food consumption in animals receiving an HF+ diet compared to control.

Both the rats receiving the AIN-93 diet and the HF diet presented a discrete weight gain during the first two weeks, with a more marked weight gain starting in the third week, with no significant difference between. Also, Akagiri Akagiri et al.22, in a 14 week study, did not detect significant differences in weight gain between animals fed the HF diet and controls, although the first one tended to gain more weight. In contrast, Chen et al.23, after six weeks of study, observed that rats receiving a high-calorie HF diet (22.4% more energy than the control diet) gained more weight than rats receiving a normal diet. The same result was obtained by Matsuzawa-Nagata et al.23, after 24 weeks of administration of the HF diet.
Buettner et al.\textsuperscript{24} observed that prolonged offer of a lipid-rich diet induces weight gain in susceptible rats of the order of 10 and 20\% compared to control. According to these authors, the induction of obesity is more effective when the high-fat diet is started at a young age and is continued for several weeks. The weight gain is gradual, increasing discretely during the first two weeks and becoming more marked after the fourth week, corroborating the data presented in this study. However, some authors have suggested that rats fed an HF diet maintain metabolic homeostasis for approximately six weeks, when they start to develop obesity and insulin resistance.

A possible explanation for the influence of time of exposure to the HF diet on weight gain is that diet induces a positive fat balance on a short-term basis due to the loss of adjustment between fat oxidation and consumption. In the long term, this positive accumulation can lead to weight gain\textsuperscript{24} and therefore, the longer the duration of the diet, the greater the gain of body weight and fat\textsuperscript{25}. However, there is controversy about the definition of a long term.

Another variable that may influence the weight gain of the animals is the type of lipid used in the formulation of high-fat diet. In human studies there are the influence of type of fat and other factors, as exercise\textsuperscript{26}. In a prospective study, Colditz et al.\textsuperscript{26} observed that weight gain showed a slight positive association with animal fat (usually seen as harmful) and negative association with vegetable fat (usually seen as beneficial). On this basis, even though the difference may be small, these two types of fat may be metabolically different in terms of their effect on weight gain\textsuperscript{26}.

Literature data show that the fat content of the diet influences the amount of body fat only when the energy consumption is also increased\textsuperscript{27}. In a study of two groups of rats receiving an HF diet and a low-fat (LF) diet, both isocaloric, it was observed that the weight gain of the animals of the two groups was similar, but that visceral fat and adipose tissue were significantly increased in the first one. In contrast a high-calorie HF diet led to greater weight gain than in the two groups cited above\textsuperscript{28}, in agreement with the findings of the present study showing that a high-fat content, but with an isocaloric content compared to the control diet, did not induce weight gain.

It has been suggested that energy density, rather than a simple increase in the percent fat of the diet is the actual factor predisposing to weight gain in many animal studies\textsuperscript{28}. Since high-fat diets are characterized by increased energy density and palatability, they frequently lead to higher energy intake. Thus, the HF diet is expected to be able to increase body weight\textsuperscript{29,30}.

There is much controversy about the weight gain of rats fed an HF diet and a possible explanation for this wide variety of results could be the absence of standardization of the HF diets used in the various studies. Many of these studies do not describe the exact quantity of lipids of the HF diet, its energy density (hypercaloric or isocaloric), the time of exposure to the diet, or the types of fat used (animal or vegetable fat)\textsuperscript{30}.

The groups did not differ significantly regarding the other biochemical parameters such as glycemia, total cholesterol and serum protein. Akagiri et al.\textsuperscript{30}, also did not detect significant differences in glycemia between groups and concluded that, even though there were no significant differences between groups regarding various parameters, many characteristics of metabolic syndrome, including the accumulation of visceral adipose tissue and the reduced expression of adiponectin, occurred earlier in the HF+ group. In contrast, Xie et al.\textsuperscript{32} demonstrated that rats fed an HF diet had a discrete increase in fasting glycemia and a significant increase in serum cholesterol compared to control, with no significant increase in hepatic fat levels. In another study, no changes in metabolic parameters occurred in rats receiving an HF diet for six weeks compared to control. In contrast, after 24 weeks HF+ rats had a greater quantity of visceral fat and accumulation of fatty acids in the liver. The authors also observed impaired glucose tolerance and insulin resistance\textsuperscript{35}.

According to Buettner et al.\textsuperscript{24}, the HF diet may induce hepatic steatosis and signs of hepatic insulin resistance in the animals, as also observed in obese humans. These authors believe that the mechanism by which the diet induces fat deposition and NASH in rats could be the activation of hepatic inflammatory cycles.

There is discrepancy in the literature about the effects of the HF diet on glycemia. However, Buettner et al.\textsuperscript{24}, on the basis of a literature review, concluded that the prolonged consumption (a minimum of 2 weeks) of HF diets will eventually lead to hyperglycemia in most rats. According to these authors, the type of fat used in an HF diet does not influence the development of insulin resistance, even though some poorly conducted studies have reported this relation.

Shiraev et al.\textsuperscript{33} offered three types of diet to Sprague-Dawley rats: control (14\% lipids), ad libitum HF (35\% lipids) and HF isocaloric to the control diet, for 11 weeks and observed increased body fat, glucose intolerance and elevation of serum insulin in the groups receiving the HF diet. The authors concluded that the presence of large amounts of fat in the diet, even with low calorie content, can cause an increase in adiposity and hyperlipidemia, which may lead to insulin resistance on a long-term basis.

To elaborate an HF diet, part of one of two macronutrients (carbohydrate and protein) must be replaced with fat. The change in the equilibrium of these macronutrients can have different metabolic effects. In a 10 week study on rats, the food intake of three groups was compared: control group receiving a low-fat diet (CG) and two HF+ groups, one of them receiving a high carbohydrate diet (HC) and the other a low carbohydrate diet (LC). The body weight of rats fed the HC diet was greater due to their greater increase in body weight and greater food consumption. Postprandial glycemia was higher in the HC group than in the LC group\textsuperscript{2}. According to this investigator, the combination of a diet rich in fat and carbohydrate is responsible for the development of some peculiarities related to metabolic syndrome in rats.

In contrast, low-carbohydrate and high-fat and protein diets have attracted attention in the treatment of obesity in humans\textsuperscript{34,35}, with the reduction of weight being mainly attributed to the reduced energy consumption possibly caused by the greater ability of protein to satiate\textsuperscript{36} and also to the increase thermogenesis induced by the high protein content of the diet\textsuperscript{36}.
Conclusions

Rats fed an HF diet did not show a greater weight gain because they ingested smaller amounts of this diet compared to rats fed the control diet, so that both diets provided the same quantity of energy to both groups. The reduction of the amount of carbohydrates in the diet in order to increase the amount of lipids may explain the smaller dietary consumption of the HF diet. However, this increase in fat content of the diet can lead to an increase in the risk factors for metabolic syndrome, including an increase in hepatic fat and a tendency to higher glycemia values. In man, a prolonged time of exposure to an HF diet may lead to an increased formation of atheroma plaques since dietary fat first reaches the circulation before accumulating in the liver.

An increased energy intake is needed for an HF diet to induce obesity since the development of obesity is characterized by an imbalance between energy consumption and caloric expenditure. The time of exposure to this diet is also responsible for weight gain and for the development of some characteristics related to metabolic syndrome. In fact this syndrome is more associated with diets with simultaneously increased lipid and carbohydrate that favor an increase in adipose tissue, but not in body weight, which in the long term may lead to the development of insulin resistance.

High-fat diets normally promote an increased caloric intake since they are more palatable and contain high energy density. For this reason, high-fat diets should be avoided since their long-term use can result in weight gain, increased adipose tissue, glucose intolerance, elevation of circulating serum lipids, and hepatic steatosis.

References


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Conflict of interest: none
Financial source: none

1Research performed at Laboratory of Nutrition and Metabolism, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (FMRP-USP), Ribeirao Preto-SP, Brazil.
Presented at the XII National Congress on Experimental Surgery of the Brazilian Society for Development of Research in Surgery-SOBRAPEC, 2011 October 26-29 Ribeirao Preto-SP, Brazil.