Efectos de una dieta con alto contenido de grasas sobre patrones conductuales alimentarios

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Recibido, marzo 27/2017
Concepto de evaluación, mayo 3/2017
Aceptado, junio 24/2017

Resumen

El consumo excesivo de alimentos con alto contenido de grasas se ha asociado con el incremento de la obesidad. Los efectos fisiológicos y metabólicos del consumo de dietas altas en grasa han sido estudiados extensamente; sin embargo, los mecanismos conductuales asociados al desarrollo de la obesidad por el consumo de estas dietas se han explorado en menor medida. Por tanto, el objetivo del presente estudio fue caracterizar los cambios en los patrones conductuales de la alimentación producidos por el consumo de una dieta alta en grasas durante diez días. Se utilizaron ratas macho Wistar con acceso libre al alimento, asignadas a uno de dos grupos, y durante diez días estuvieron bajo una dieta alta en grasa (45 % de calorías provenientes de grasas) o una dieta estándar de laboratorio. En los días 1, 5 y 10 se realizó un análisis detallado de la conducta alimentaria al inicio del periodo de oscuridad. Los resultados mostraron que los sujetos expuestos a la dieta alta en grasa acumularon más grasa corporal y tuvieron mayor eficiencia de la alimentación que el otro grupo, sin incremento del peso corporal ni alteraciones del patrón típico de la secuencia de saciedad conductual. Esto sugiere que la exposición a dietas con alto contenido de grasas puede producir cambios conductuales antes de que se presente una ganancia de peso excesivo, lo que afecta principalmente los mecanismos de control de eficiencia alimentaria.

Palabras clave: Conducta alimentaria, saciedad, peso corporal, tejido adiposo, dieta.

Effects of a high-fat diet on behavioral eating patterns

Abstract

Excessive consumption of high-fat food has been associated with increased prevalence of obesity. The physiological and metabolic effects of high-fat diets have been extensively studied. Nevertheless, the behavioral mechanisms associated with the development of obesity induced by consumption of these diets has been less explored. Therefore, the aim of the present study was to characterize the changes in the behavioral feeding patterns produced by the consumption of a high-fat diet during 10 days. Male Wistar rats with free access to food were assigned to one of two groups, and for 10 days, they had access to a high-fat diet (45 % calories from fat) or to a standard diet. Detailed analysis of feeding behavior was performed on days 1, 5 and 10 at the beginning of the dark period. The results showed that subjects exposed to the high-fat diet accumulated more body fat and showed increased feeding efficiency, in absence of excessive body weight increase or alterations in the behavioral satiety sequence pattern. These findings suggest that exposure to high-fat diets may produce behavioral changes before excessive gain of body weight occurs, primarily affecting control mechanisms of feeding efficiency.

Key words: Feeding behavior, satiety, body weight, adipose tissue, diet.

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INTRODUCTION

Fat is an important macronutrient in the “Western diet”, in fact it has been reported that 35% of the daily caloric intake comes from it (Hennink & Maljaars, 2013). A high-fat diet can easily lead to an excessive food consumption, which can eventually result in the development of obesity, due to the affectation of the homeostatic system. Previous studies with animal models have shown that high-fat diets affect components of the homeostatic system such as the circadian rhythm (Honma, Hikosaka, Mochizuki & Goda, 2016; Sherman et al., 2012) and spontaneous locomotor activity (Ludmilla et al., 2017). Some authors reported that animals exposed to a high-fat diet initially showed a significant increase of adipose tissue accumulation without any change in body weight However, after three weeks, it was observed increase in body weight and hypertrophy of adipocytes (La Fleur, Van Rozen, Luijendijk, Groeneweg & Adan, 2010; Woods, Seeley, Rushing, D'Alessio & Tso, 2003). Diet-induced obesity (DIO) models in rodents have shown that chronic consumption of obesogenic diets conducts to a body weight gain and adipose tissue accumulation leading to metabolic alterations (Sáinz, Barrenetxe, Moreno-Aliaga & Martinez, 2015).

Those studies suggest that the increase in both, body weight and adipose tissue is the result of fats overconsumption, but also some changes in behavioral feeding patterns may contribute to this effect. To illustrate, Melhorn et al. (2010) found that subjects on a high-fat diet had less frequent but longer-lasting feeding episodes. In addition, the increase in adipose tissue correlated significantly with the duration of the feeding episodes. Those results suggest an alteration of a neurotransmitter system that participates in energy homeostasis, as these behavioral parameters are regulated centrally. Recent evidences have revealed that chronic intake of high-fat diets produced changes in some neurotransmitter systems involved in the central regulation of hedonic and homeostatic properties of feeding, including dopaminergic (Carlin et al., 2016; Fordahl, Locke & Jones, 2016), serotonergic (Yu et al., 2013) and melanocortin system (Chandler, Viana, Oswald, Wauford & Boggiano, 2005; La Fleur et al., 2010). Consequently, these neurotransmission systems have been associated with specific components of the regulation of feeding behavior (Leibowitz & Alexander, 1998; Mul, Spruijt, Brakkee & Adan, 2013; Terry, Gilbert & Cooper, 1995).

From a physiological perspective, the mechanisms that control food consumption and satiety are complex. The process of satiety (inhibition of food ingestion) begins in the gastrointestinal tract through two pathways. In the first one, the mechanical distension of the stomach sends signals through the nucleus of the solitary tract to the satiety centers in the central nervous system. In the second, the intake of food received by the small intestine stimulates the secretion of peptides that function as satiety signals in brain regions such as the hypothalamus. These peptides travel through the bloodstream or activate vagal afferents, transmitting signals of satiety to the brain (Hennink & Maljaars, 2013).

In a DIO model, the vagal response to gastric distention is reduced and the effects of ghrelin, a known orexigenic peptide, are increased. Similarly, the vagal activity in response to the entry of nutrients into the intestine is also diminished, and alteration of peptides secretion reduces the

Resumo

O consumo excessivo de alimentos com alto conteúdo de gordura tem sido associado com o aumento da obesidade. Os efeitos fisiológicos e metabólicos do consumo de dietas altas em gordura têm sido estudados extensamente, contudo os mecanismos comportamentais relacionados com o desenvolvimento da obesidade pelo consumo dessas dietas têm-se explorado em menor medida. Portanto, o objetivo deste estudo foi caracterizar as mudanças nos padrões comportamentais da alimentação produzidas pelo consumo de uma dieta alta em gordura durante dez dias. Utilizaram-se ratos de laboratório machos Wistar, com acesso livre ao alimento, designados a um de dois grupos, e durante dez dias estiveram sob uma dieta alta em gordura (45 % de calorias provenientes de gorduras) ou uma dieta padrão de laboratório. Nos dias 1, 5 e 10, realizou-se uma análise detalhada do comportamento alimentar ao início do período de escuridão. Os resultados mostraram que os sujeitos expostos à dieta alta em gordura acumularam mais gordura corporal e tiveram maior eficiência da alimentação do que o outro grupo, sem aumento do peso corporal nem alterações do padrão da sequência de saciedade comportamental. Isso sugere que a exposição a dietas com alto conteúdo de gordura pode produzir mudanças comportamentais antes de apresentar um ganho de peso excessivo, o que afeta principalmente os mecanismos de controle de eficiência alimentar.

Palavras-chave: Comportamento alimentar, dieta, peso corporal, saciedade, tecido adiposo.
signals of satiety, promoting the increase of food intake and body weight gain (Kentish et al., 2012; Maljaars, 2013).

Alternatively from studies focused on analyze the total food intake (g) or the energy intake (kcal), from the behavioral perspective it becomes essential to perform the measurement of different behavioral parameters (latency, local feeding rate, frequency and duration of the episodes, among others). These parameters describe the feeding behavioral patterns and determine the causes of the increase or diminution of food ingestion due to the manipulation of a variable. These patterns have been characterized by the use of techniques such as the analysis of the feeding microstructure and the behavioral satiety sequence (BSS, López-Alonso, Mancilla-Díaz, Rito-Domingo, González-Hernández & Escartín-Pérez, 2007; Mancilla-Díaz, Escartín-Pérez, López-Alonso, Floran-Garduño & Romano-Camacho, 2005; Tallett, Blundell & Rodgers, 2009; Tejas-Juárez et al., 2014). The mentioned techniques provide information on specific behavioral aspects (e.g. the satiation process or the termination of a feeding interval and the development of the satiety or post-ingestive inhibition of food ingestion) or unspecific aspects (e.g. ending of feeding due to nausea, pain, sedation, hyperactivity) of the feeding behavior (Halford, Wanninayake & Blundell, 1998). In this way, it has been determined that naturally food ingestion induces satisfaction, which is expressed by the presence of a highly stereotyped behavioral sequence, where feeding is followed by activity (e.g. rodent grooming) and concludes with the resting (Halford et al., 1998).

Despite the knowledge of the physiological and metabolic effects of the consumption of high-fat diets is extensive, the behavioral mechanisms associated with the development of obesity and overweight induced by the consumption of these diets have been less explored. Thus, the study of the high-fat diet effects on behavioral patterns of food intake may provide relevant information for motivational processes (hunger, appetite, satisfaction and satiety) that are related to the development of the abnormal weight gain. Consequently, the aim of the present study was to characterize the changes in the behavioral patterns of feeding produced by the consumption of a high-fat diet during 10 days.

METHOD

Subjects
For the present study it was used 17 male adult Wistar rats (provided by UNAM FES Iztacala vivarium, n= 17), weighting 200-220 g at the beginning of the investigation. All the experimental procedures complied with the Official Mexican Norm (NOM-062-ZOO-1999), entitled Technical Specifications for the Production, Care and Use of Laboratory Animals.

Experimental situation
Animals were individually housed in clear acrylic home-cages (27×37×15 cm) under a 12/12 h light-dark cycle automatically controlled (lights out at 12:00 pm) and temperature of the experimental room at 22±1°C. Food and water were available ad libitum.

Diets
Two diets with different energy density were used, the control group had access to standard chow pellets (18% fat, 24% protein and 58% carbohydrates; 3.1 kcal/g, Harlan Laboratories, Mexico) and the experimental group had access to high-fat pellets (45% fat, 20% protein and 35% carbohydrate; 4.77 kcal/g food for rodents D12451, Research Diet Inc., New Brunswick, NJ).

Experimental Design
A multiple time-series design without pretest was employed. This design assesses through time several observations that can be made on one or more variables, under the consideration that the effect of the manipulation of the independent variable takes time to express its effects. This design can evaluate the effect of the independent variable in a short, medium and long term (Campbell & Stanley, 1995). In this case the independent variable was the access for ten days to the high fat diet, making the observations on days 1, 5 and 10.

Apparatus
A camera for low-intensity light was used to record the behavioral tests; a DGT-TBS1 digital video recorder and a HICOM monitor were also used.

Procedure
After a habituation period to the vivarium (3 days), the animals were weighed daily; in this way the portion of eaten food was determined for 24 h (the spilt food was collected) for 10 days, at the end of the light phase. Pre-weighed and fresh food was placed each day at the beginning of the dark phase (12:00 h). In addition to measuring the intake of food in grams (g), the intake of calories (kcal/g) and local feeding rate (g/min and kcal/min) were also calculated. On days 1, 5 and 10, the behavioral test was performed at the beginning of the dark phase. The three tests were videotaped (60 min duration) with the low-intensity light camera, which was placed in front of the home-cages. From video
Data analysis

Data presented in this study are expressed as means ± S.E.M. Since the data complied with the assumptions of normality and homoscedasticity, parametric statistical tests were used. Food intake and body weight per day were analyzed with a two way ANOVA (diet x time). Data from ingestion of accumulated food, energy intake and weight of adipose tissue during the 10 days were compared between groups with the Student’s t test for unpaired samples. The criteria for statistical significance was set at \( p < .05 \). Statistical analysis was performed using the software GraphPad Prism Version 5.01 (GraphPad Software, San Diego, CA, USA).

RESULTS

In this section are presented the results of the evaluation of food (g per day and accumulated) and energy consumption (kcal / g per day and accumulated), body weight (g accumulated) and adipose tissue (g at the end of the protocol) during the ten days of exposure to diets control and High-Fat. The detailed results of behavioral analyses (local eating rate, BSS and areas under the curve of durations of the BSS) conducted specifically at the beginning of the dark phase of the light/dark cycle on days 1, 5 and 10 of exposure to diets. All data were expressed in terms of means ± standard error of mean (SEM).

In order to study the temporal course of food and energy consumption, these variables were evaluated for 10 days in two groups of rats, a group with access to a standard diet and another group with a high-fat diet. As expected, food intake differed between groups as a function of time (diet x time interaction), \( F_{(9,150)} = 4.07, p < .001 \). Figure 1 (left panel [above]) shows that subjects in the control diet consumed increasing amounts of food while experimental subjects maintained relatively stable consumption of diet with high fat content (factor time), \( F_{(1,150)} = 199.5, p < .001 \). In contrast, the accumulated ingestion of food (g) during the 10 days of observation was significantly lower in animals fed with the high-fat diet, \( t_{(15)} = 8.15, p < .0001 \) (see Figure 1, right panel [above]).

At the same time, energy intake was assessed and it was expressed in terms of calories consumed per gram of food (kcal/g). It was found that animals exposed to the high-fat diet had a significantly higher intake in the first days of access to food (days 1-5, see Figure 1, left pane [below]) and subsequently decreased (diet interaction x time), \( F_{(9,150)} = 4.03, p < .01 \). This result indicates that animals tended to consume less food (days 6-10) to compensate the increase in energy intake. However, the accumulated caloric intake (kcal/g) was significantly higher in the high-fat group, \( t_{(15)} = 4.17, p < .001 \) (see Figure 1, right panel [below]).

After the exposure to the diets, the animal’s body weight did not showed significant differences between groups (see Figure 2 left panel [above]). However, subjects with access to the high-fat diet accumulated significantly more total adipose tissue, \( t_{(15)} = 8.15, p < .001 \) (see Figure 2 right pane [above]). The adipose tissue increased significantly in animals fed with the high-fat diet (see Figure 2 panel below) in the gonadal, retroabdominal and visceral regions, \( t_{(15)} = 6.43, p < .0001, t_{(15)} = 4.31, p < .0006 \) and \( t_{(15)} = 7.43, p < .0001 \), respectively.

In order to characterize the behavioral feeding pattern produced by the high-fat diet, the BSS and the local feeding rate were analyzed on days 1, 5 and 10 of dietary exposure. The results show that the consumption of food per unit of time (g/min) did not differ between the groups, but the energy intake (kcal/min) was higher in the high-fat diet group (see table 1), this increase reached statistical significance the day 10 of dietary exposure, \( F_{(1,34)} = 25.89, p < .001 \).

The temporal pattern of feeding behavior of the control group on the first day showed a typical BSS, characterized by the presence of feeding episodes interleaved with activity during the first periods of the registry, the transition between the feeding and resting occurred in the 5th period, indicating that satiety was expressed from minutes 25-30 of the behavioral registry (see figure 3 left panel [above]).
Figure 1. Temporal pattern of food (g) and energy intake (kcal/g) during 10 days of exposition to the control (n=9) or high-fat diet (n=8) (left panel, upper and lower). Overall food and energy intake (10 days of cumulative consumption) after the recording period (right panel, upper and lower). Data are expressed as means ± S.E.M. * p < .05, ** p < .01, *** p < .001 control diet vs high fat diet.

Table 1

Local feeding rate (g/min, kcal/min) at the onset of the dark phase (60 min) in the days of record 1, 5 and 10

<table>
<thead>
<tr>
<th>Category</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>High-Fat diet</td>
<td>Control</td>
</tr>
<tr>
<td>Local feeding rate (g/min)</td>
<td>.30 ± .04</td>
<td>.40 ± .07</td>
<td>.42 ± .05</td>
</tr>
<tr>
<td>Local feeding rate</td>
<td>.93 ± .12</td>
<td>1.89 ± .33</td>
<td>.93 ± 12</td>
</tr>
</tbody>
</table>

Legend: ***p < .001 vs same day control group.
On the other hand, the group with access to the high-fat diet on the first day (see Figure 3 right panel [above]) presented a BSS similar to the control group, developing an orderly transition from feeding to resting. However, postprandial satiety occurred after the seventh registration period (minutes 35-40), the animals also had more activity and tended to rest less time in the complete registry, indicating that the BSS progressed slower in the control group, delaying the process of satiation.

On the 5th day of the protocol, the control group developed the typical satiety sequence (see figure 3 left panel [middle]), similar to that observed on the 1st day, although with a faster progression, the transition from feeding to resting is presented in 4th period of the behavioral test (20 min).

Likewise, the animals with the high-fat diet showed a typical BSS, although faster than on the 1st day of registry, the transition of the feeding to resting was presented in the periods 3-4 of the registry (15-20 min, Figure 3 right panel [middle]). Since both groups tended to decrease the time of activity on the 5th day of registration, it can be suggested that animals presented a greater feeding efficiency than the 1st day as they developed the satisfaction process in less time.

On the 10th day of the exposure to the diets, the control group presented the typical pattern of the behavioral satiety sequence in the first 4 periods (minutes 20-25), giving rise to the natural development of the satisfaction process (see Figure 3 left panel [below]). The group with the high-fat diet again presented a satiety pattern similar to the 5th day

*Figure 2. Body weight (left panel [upper]) of animals exposed to the control (n=9) and high fat diet (n=8), total cumulative adipose tissue (right panel [upper]) and cumulative adipose tissue in specific regions (lower panel), after the 10 days of exposition. Data are expressed as means ± S.E.M. ***p < .001 control diet vs high fat diet, NS = non significant.*
Figure 3. Temporal pattern of the behavioral satiety sequence of the animals exposed to the control (n=9) or the high-fat diet (n=8) on the 1st, 5th and 10th days of the feeding protocol. The 60 min of the videotapes were divided into 12 periods of 5 min bins. Data are expressed as means ± S.E.M for each behavioral category. Vertical line indicates the transition from feeding to resting.
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(see Figure 3 Right panel [below]), with a rapid satiety development, as the transition from feeding to rest is established in third period of registration (minutes 15-20).

The quantitative analysis of the BSS was carried out by calculating the area under the curve of each behavioral category (feeding, grooming, activity and resting), thus evaluating the average duration of the categories in each group (see Table 2). Consequently, it was found that the duration of the feeding was differentially affected depending on the number of days under the diet (diet interaction x time), $F_{(2,36)} = 3.28, p < .05$. Animals in the high-fat diet reduced the duration of feeding as the days elapsed. According to the Bonferroni’s post-hoc, the reduction in the duration of feeding behavior was statistically significant on the 5th day ($p < .05$), in the group with the high-fat diet. Furthermore, the duration of grooming behavior differed significantly (diet factor) $F_{(1,35)} = 4.41, p < .05$, specifically increased the duration of grooming in rats with high-fat diet. Finally, the behavioral categories of activity and resting showed no significant differences between the groups.

DISCUSSION

This research characterized the behavioral and temporal patterns of feeding on animals exposed to a high-fat diet for 10 days. Initially it was found that exposure to the high-fat diet only increased the accumulation of adipose tissue and the efficiency of the feeding behavior (higher energy consumption per unit of time), without increasing the body weight excessively and without altering the typical pattern of the behavioral satiety sequence. These results suggest that exposure to high-fat diets can lead to behavioral changes, specifically in feeding efficiency.

In addition, it was observed that animals exposed to the high-fat diet decreased food intake (g) voluntarily, suggesting the intervention of regulatory systems that detect the increase of calories ingested and respond to maintain the homeostasis. It has been reported that duration of food intake episodes are primarily controlled by homeostatic processes through the measure of calories consumed (Cavanaugh, Schwartz & Blouet, 2015). At the beginning of the protocol, the increase of food intake was accompanied by the increase of the energy ingestion, which probably induced the intensification of the satiety signals, decreasing progressively the amount (g) of food consumed to prevent the weight gain. It is known that food intake stimulates the release of satiety signals, which can be act through two ways. The first is the stomach distention by ingesting food, which is merely a volumetric phenomenon (mechanical signals). The second is more complex and involves an increase in chemical signals, including the release of peptides such as cholecystokinin (CCK, Paulino, Darcel, Tome & Raybould, 2008; Savastano & Covaa, 2005), glucagon-like peptide 1 (GLP-1, Williams, Baskin, & Schwartz, 2009) and peptide YY (PYY, Batterham et al., 2002). These signals travel from the stomach to the brain, and specifically the hypothalamus receives and integrates this information to promote both satisfaction and satiety, these physiological signals regulates the duration of food intake episodes (amount of food ingested).

In spite of reducing its food intake, animals with a high-fat diet consumed more calories. This phenomenon contributed to increased accumulation of gonadal, retroabdominal and visceral adipose tissue without significantly increasing body weight. Previous research emphasize that the increase in adipose tissue is a condition that precedes weight gain and the presentation of obesity-related symptoms (Bjursell et al., 2008). Although obesity is a multicausal condition in which environmental, genetic and behavioral factors are included, its etiology has been mainly associated with the consumption of foods with high energy density and the decrease of energy expense (Ludmilla et al., 2017). In this

### Table 2

<table>
<thead>
<tr>
<th>Category</th>
<th>Day 1</th>
<th></th>
<th>Day 5</th>
<th></th>
<th>Day 10</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>High-Fat diet</td>
<td>Control</td>
<td>High-Fat diet</td>
<td>Control</td>
<td>High-Fat diet</td>
</tr>
<tr>
<td>Feeding (s)</td>
<td>440.14 ± 52.51</td>
<td>529.42 ± 75.91</td>
<td>596.28 ± 60.90</td>
<td>345.833 ± 69.21*</td>
<td>351.94 ± 55.38</td>
<td>360.62 ± 81.41</td>
</tr>
<tr>
<td>Resting (s)</td>
<td>1416.57 ± 128.09</td>
<td>756.83 ± 102.37</td>
<td>1266.71 ± 303.83</td>
<td>1616.00 ± 277.64</td>
<td>2036.19 ± 187.95</td>
<td>1499.71 ± 323.36</td>
</tr>
<tr>
<td>Grooming (s)</td>
<td>571.00 ± 31.81</td>
<td>750.00 ± 66.57</td>
<td>741.50 ± 158.56</td>
<td>772.92 ± 181.74</td>
<td>507.14 ± 81.37</td>
<td>1023.87 ± 199.49*</td>
</tr>
<tr>
<td>Activity (s)</td>
<td>872.50 ± 88.46</td>
<td>1264.42 ± 125.28</td>
<td>695.64 ± 149.58</td>
<td>559.58 ± 96.51</td>
<td>428.12 ± 68.13</td>
<td>572.813 ± 115.68</td>
</tr>
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</table>

**Leyend:** *p < .05 vs same day control group.
way, the increase in energy intake and the accumulation of body fat could be the previous phase to gain weight and the development of obesity itself.

Contrary to the idea that high-fat diets promote the occurrence of feeding episodes with low frequency but long duration (Melhorn et al., 2010), in the present study it was found that animals did not increase the duration of food intake, but did increase the caloric intake in the second half of the exposure to the diet. Although rats with access to the high-fat diet decreased food intake in terms of grams and duration from day 6 to day 10, and the total food intake during the 10 days of exposure to the experimental condition was lower than in the control condition, the accumulated caloric intake was significantly higher.

The BSS has been used to characterize the effect of pharmacological and non-pharmacological manipulations on rodent feeding behavior. The preservation of the pattern (feeding, activity, grooming and resting) has been associated to the process of satiation, result of the post ingestion of food. In this study, the characterization of the behavioral patterns associated with the caloric overeating of the animals exposed to the high-fat diet was performed, and it was found no alteration in terms of its morphology (sequence of behavioral categories, Halford et al., 1998; Tallett et al., 2009), but affecting its efficiency. Hence, one of the most important findings of this study was the observation of the increase in food efficiency at the end of the exposure to the high-fat diet. Animals that fed with the high-fat diet ingested higher amounts of calories per unit of time, and the BSS although it was not altered in its basic pattern (ordered progression of feeding to rest), it was developed faster, and although the process of satisfaction occurred in less time, the animals consumed greater amounts of calories in the behavioral test at the beginning of the dark phase (record 60 minutes).

To summarize, exposure to the high-fat diet promoted the increase of adipose tissue accumulation without producing body weight changes. Although it was observed the reduction of food intake in animals exposed to the high-fat diet, it was not enough to prevent the increase in caloric intake, which was significantly higher in animals exposed to the experimental diet. The results of this research suggested that the intake of high-fat diets may contribute to changing behavioral patterns, particularly those involved in regulating feeding efficiency (energy intake per time unit). Future research should study the changes in satiety signals in specific brain regions and in the appetite control mechanisms (feeding efficiency) that result from exposure to high-fat diets, in order to have a better understanding of the neurochemical mechanisms that promote the development of overweight and obesity.

REFERENCES


