Capítulo 1 Ingesta e indicadores bioquímicos Sricos en ratones adultos expuestos a una dieta alta en grasa por un periodo corto

Chapter 1 Food intake and biomedical serum indicators in mice adults exposed high fat diet for a short term

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Resumen

Poco se sabe acerca de los cambios que ocurren en los indicadores bioquímicos en suero asociados con la ingesta de una dieta isocalórica alta en grasa (DAG) y su relación con el comportamiento alimentario por un período de tiempo corto. El objetivo de esta investigación fue evaluar la ingesta, el peso corporal y marcadores en suero: triglicéridos, glucosa y colesterol, en ratones adultos expuestos a DAG por un periodo de tiempo corto. Se trata de un estudio experimental y longitudinal, con un total de 30 ratones (10 por grupo: Dieta Estándar (DE), Dieta Alta en Grasa (DAG), Dieta Baja en Grasa (DBG)), se expusieron durante 5 semanas a la dieta correspondiente. Además, el hígado fue evaluado por la técnica histológica de hematoxilina y eosina, para determinar el porcentaje de microvesículas y macrovesículas de grasa. Los resultados indicaron que en el grupo con DAG presentó una disminución del 37% y 48% en la ingesta calórica y alimento, respectivamente; un incremento del 13% en el peso corporal; además, de una disminución del 34% de glucosa en suero y un aumentó de 65% en el porcentaje de microvesículas de grasa en hígado en comparación con el grupo control. En conclusión, se resalta la importancia de considerar el contenido y la calidad de macronutrientes en la dieta. A pesar de que las dietas consumidas por los ratones en esta investigación fueron isocalóricas, la exposición a DAG por un período corto, evidenció una reducción en la ingesta de alimento y en los niveles séricos de glucosa, un incremento en el peso corporal y la presencia de esteatosis hepática. Se sugiere que este modelo experimental podría emplearse para evaluar el efecto de una DAG isocalórica sobre cambios en los niveles de glucosa y triglicéridos séricos, así como el desarrollo de esteatosis hepática y cambios en el comportamiento alimentario en adultos durante un período de tiempo corto. La evaluación de la ingesta de una DAG podría proporcionar una mejor comprensión de los mecanismos metabólicos relacionados con el desarrollo de enfermedades crónicas. Sin embargo, en el campo del comportamiento alimentario se requiere de más investigación, en aspectos fisiológicos, bioquímicos-moleculares y conductuales.

Dieta alta en grasa, ingesta, adulto, ratón, indicadores séricos, microvesiculas de grasa

Abstract

Little is known about the changes that occur in serum biochemical indicators associated with the intake of a high fat isocaloric diet (HFD) and its relationship with eating behavior for a short term. The objective of this study was to evaluate food intake and serum markers: triglycerides, glucose and cholesterol, in adult mice exposed to HFD for a short term. Experimental and longitudinal study, with 30 mice (10 per group: Standard Diet (DE), HFD Low Fat Diet (LFD), they were exposed for 5 weeks to corresponding diet. Furthermore, the liver was evaluated to determine the percentage of microvesicles and macrovesicles of fat by histological technique (hematoxylin and eosin). The results were: In HFD group, decreased 37% and 48% in caloric and food intake, respectively; 13% increased, body weight; in addition, a 34% decrease glucose and increased tryacylglyceride in serum and a 65% increase fat microvesicles in liver compared with control group. In conclusion, its importance considering the content and quality of macronutrients in diet Although the diets consumed by the mice in this research were isocaloric, exposure to HFD in short term evidenced a reduction in food intake and glucose triacylglicerides levels in serum, increase in body weight and hepatic steatosis. It is suggested this model could be used to evaluate the effect of an isocaloric HFD on changes in biomarker in serum, and development of hepatic steatosis in a short term. Assessing HFD intake could provide a better understanding of the metabolic mechanisms related to the development of chronic deseases.

High fat diet, food intake, adult, mice, serum indicators, fat microvesicles

1. Introduction

High fat diet (HFD) represents "*western diet*", which is associated with the development of metabolic disorders such as obesity and mellitus diabetes (Myles, 2014). Being exposed to HFD influences eating behaviors(Villamil et al., 2018). In a recent study in rats exposed to a long-term HFD induced impairments in glucose tolerance (la Fleur et al., 2011). The western diet and HFD intake also has been showed to modify the biochemical composition and function of high-density lipoproteins (HDL) in mice (Lewis et al., 2004; Mirmiran et al., 2014). Indeed, rats offered a free-choice high-fat, high-sucrose diet exhibited hyperphagic behavior, rapid onset of obesity, and impaired glucose tolerance (la Fleur et al., 2011). Is little evidence evaluating short-term consequences of isocaloric HFD intake and its relationship with peripheral markers such as glucose, triglycerides and cholesterol.

During development of obesity and changes in insulin levels, the liver can suffer modifications that are marked by the deposition of triglycerides in macro and microvesicular fat. Moreover, hypercaloric diet intake increases lipolysis in adipose tissue, modifying triglyceride levels (Blundell et al., 1995).

Nevertheless the mechanism to induce obesity by HFD is still not completely clear. Studies suggest that exposure to high-fat diets can produce behavioral changes before excessive weight gain occurs, mainly affecting food efficiency control mechanisms (Melhorn et al., 2010). Studies show that the intake of high fat diets are related to changes in insulin, with a consequent increase in the score for feeling of hunger and prospective desire to consume food, contributing to increased hyperphagia (Labayen et al., 1999). There is a lack of research-based evidence regarding isocaloric diets which modify the content of macronutrients such as fat and its relation with the consumption of food, is unknown. In this work, adult mice were exposed for short term to an isocaloric diet with HFD, LFD and a standard diet in order to evaluate food intake and development of damage signals at the hepatic level. Its experimental and longitudinal study, where a total of 30 mices (10 per group) were divided into the following groups and treated during 5 weeks: standard diet (SD) group, HFD group and low fat diet (LFD) group. In all groups, food and water intake, serum triglycerides, cholesterol and glucose were determined. In addition, liver was histologically evaluated.

1.2 Description of the method

1.3 Materials and Methods

1.4 Study design

The methodological procedure for this experimental and longitudinal study is shown in Table 1.1 All procedures were carried out according to the Official Mexican Norm NOM-062-81 ZOO-1999.

Table 1.1 Experimental Design.

Group	Diet	Registration of food intake	Decapitation
1 (n=10)	Standard		
2 (n=10)	HFD		
3 (n=10)	LFD		
Length of study Scheduled registration of food intake and water (every 12 hours dark-light cycle)		35days 7am (dark) 7pm (light)	Tissue samples from the hippocampus and liver were obtained in order to determine the genetic expression of the insulin receptor in the hippocampus and for the histological analysis of the liver

Three study groups were included: Group 1 mice with a standard diet, Group 2 mice with a HFD and Group 3 with a LFD. The length of the study period was 35 days and the scheduled registration of food intake and water was every 12 hours in a dark-light cycle (7am/7pm). Tissue samples of liver for histological analysis respectively.

1.5 Subjects

The animal subjects were 30 male Bald/c mice, which were divided into three groups:

Group 1 (standard diet) was formed by n=10 mice with an average weight of (23±4g); Group 2 (HFD) was formed by n=10 mice with an average weight of (22±3g) and exposed to HFD that was composed of 26.2 % protein, 26.3 % carbohydrate and 34.9 % fat (D12492) Research diet; Group 3 LFD was formed by n=10 mice with an average weight of (22±3g) and exposed to a LFD composed of 19.2 % protein, 67.3 % carbohydrate and 4.3 % fat (D12450B) Research diet. All of the study groups were exposed to their corresponding diets ad libitum during the days (length of the experiment).

In table 1.2, the macronutrient and micronutrient content of diets for each study group is described.

Table 1.2 Composition of diets

Diet	Kcal/gr	Macronutrients		
		Carbohydrates	Fats	Proteins
Standard	4.07	57.99%	13.49%	28.50%
High Fat Diet (HFD) (D12492) Research diet	4.07	26%	34.9%	26%
Low Fat Diet (LFD) (D12450B2) Research diet	4.07	63.3%	19.3%	4.3%

Note: The Standard (SD), Low Fat Diet (LFD) and High Fat Diet (HFD) content based on kilocalorie per gram of meal and macronutrients percentage

1.6 Materials

Three boxes measuring 21cm height, 23.5 cm width, and 38cm length with a metal grid to cover the box and divide food and water were used to feed the Babl/c mice. The bottom of the box was covered with sawdust, which was replaced every three days.

1.7 Experimental period

The experimental study was performed for a 7-day period to allow adaptation and 35-day (5 weeks) non-stop period to conduct the registration of the data.

1.8 Procedure

Ten mice were located per box (three groups) and they were kept inside for 7 days to allow the adaptation period; providing water and food to each group. Once the adaptation period was over, for 35 days the quantities of food and liquids during the consumption period were recorded as well as the body weight by using the *A&D Weighing serie GF-3000* scale. Food intake was registered twice a day (7pm and 7am), and body weight of each animal subject was registered every 24 hours. Afterwards, the mice were decapitated to obtain the brain and the liver. Once the organs were properly prepared to be stored, their weight was recorded so that the evaluation of the relationship between the organ and body weight could be drawn.

1.9 Food intake and body weight

To register body weight and food intake a precision scale (A&D Weighing GF-3000) was used. The high and low fat content diets were taken from the Research Diet Laboratory, and Rodent Laboratory Chow 5001 by Purina was defined to be the standard diet. Table 2 shows the nutritional content of the three diets included in this study. All mice were given purified water ad libitum. The diet specifications for Group 2, mice exposed to a HFD, included the (D12492) Research diet and Group 3, mice exposed to a LFD, included the (D12450B) Research diet for 35 days. In addition to peripheral markers, an extraction of body fat and tissues (liver) was performed with dissection materials. In order to maintain liver samples, a *Revco Thermo Fisher Scientific ULT1786-4-A47* container was needed as well as plastic containers with 4% formaldehyde, respectively. In order to prepare the formaldehyde, dibasic sodium phosphate anhydrous, sodium phosphate monobasic monohydrate, distilled water and paraformaldehyde were used. Sodium hydroxide (NaOH) was used to adjust the pH.

1.10 Determination of serum levels of triglycerides, glucose, total cholesterol, and high and low density lipoproteins

To determine levels of glucose, cholesterol and triglycerides, serum samples were stored at -80°C after the extraction and centrifugation of the blood.

To determine the values of glucose and the lipid profile, the following commercial kits from Spinreact were used: Glucose-LQ Ref: 41010, Cholesterol-LQ Ref: 41020, Triglycerides-LQ Ref: 41030 Ctra. Santa Coloma, Spain; the procedures given by the manufacturer were followed, and the readings were obtained by spectrophotometry using a colorimetric method.

1.11 Histological analysis

Once the livers extracted and weighed, the organs were fixed with 4% formaldehyde by applying a hematoxylin eosin technique for the histological analysis. Briefly, livers were embedded in paraffin and 3 micron coronal slices were made with a rotation microtome *(Leica DSC1).* These layers of liver were placed on slides to perform a staining of hematoxylin and eosin; paraffin sections were deparaffinized in xylol and rehydrated in ethanol in decreasing concentrations and then immersed in distilled water for 3 minutes; the staining process took 10 minutes and the dehydration in ethanol took place in increasing concentrations. The final clearing stage was performed with xylol. In order to determine hepatic damage by morphologic changes, cells were analyzed and the number of macrovesicular and microvesicular fat in liver cells as well as necrosis and inflammation values were obtained.

These results are shown in Table 1.3. Histological System Scoring System, and date are expressed as the percentage of cells with normal and abnormal morphology.

Table 1.3 Histological damage scoring system

	Standard Diet %	HFD (D12492)g	LFD (D12450B)g
Casein		200	200
L Cystine		3	3
Corn Starch	31.9%	0	315
Maltrodextrin		125	35
Sucrose	3.7%	68.8	350
Cellulose		50	50
Soybean Oil		25	25
Lard		245	20
Mineral Mix	9.05%	10	10
Calcium Phosphate	0.95%	13	13
Calcium Carbonate		5.5	5.5
Potassium Citrate	1.18%	16.5	16.5
Vitamin Mix	293ppm	10	10
Choline Bitartrate	2250ppm	2	2
Cholesterol (mg/kg)	200ppm	300.8	51.6
Glucose	0.22%		
Fructose	0.30%		
Sucrose	3.70%		
Lactose	2.01%		
Fatty Acids	1.60%		

Note: Adapted from (Morgan et al., 2008)

Morgan, K., Uyuni, A., Nandgiri, G., Mao, L., Castaneda, L., Kathirvel, E., & Morgan, T. R. (2008). Altered expression of transcription factors and genes regulating lipogenesis in liver and adipose tissue of mice with high fat diet-induced obesity and nonalcoholic fatty liver disease. *European journal of gastroenterology & hepatology*, *20* (9), 843-854

1.12 Ethical considerations

The animals used in this experiment where held under controlled dark/light environmental conditions with ventilation and in cages that were suitable and clean. The procedures were carried out under the Official Mexican Norm NOM-062-ZOO-1999, which points out techniques for their reproduction, care and use of laboratory animals.

1.13 Statistical analyses

Statistical analyses were carried out using a SPSS software (version 19) and the values of $p<0.05$ were considered significant. Finally, to prove the normality of the chemical variables, the Kolmogorov-Smirnov test was used *($p>0.05$*). The relationship between biochemical parameters (the levels of glucose, cholesterol and triglycerides) and eating parameters was carried out by the F-Fisher distribution with a post-hoc test.

1.14 Results

Food intake

Food intake was recorded during light/dark cycles, as well as every 24 hours. The group that was provided with a HFD [Group 2] (29.8±1.9g) consumed less food when compared to the group with a standard diet [Group 1] (47±1.9g) (*$p>0.05$*) and also when compared with the LFD group (Group 3) (33.3±11g) (*$p>0.05$*). (Figure 1, A). During both time periods (light/dark cycles), the group that was exposed to a standard diet (Group 1) showed the highest food intake (Table 4) shows the recorded data per mice (data are expressed as mean values) regarding food intake in both time periods.

Table 1,4. Food intake (g) during light/dark cycle: The length of the study period was 35 days and the scheduled registration of food intake and water was every 12 hours in a dark-light cycle (7am/7pm). Data are expressed as mean ± standard deviation in day 35. A p value of <0.05 was considered statistically significant. HFD= High fat diet, LFD= Low fat diet

Table 1.4 Food intake (g) during light/dark cycle

	Standar	HFD	LFD	p=
Light	1.95±0.07	1.51±0.09	1.62±0.05	0.001
Dark	3.11±0.10	1.42±0.10	1.90±0.11	0.005

A lower food intake was observed in the group that was exposed to a HFD when compared the other groups (low fat and standard diet) ($p>0.05$). Moreover, the group that was exposed to a HFD showed a reduction in food intake and Kcal intake (Figure 1) (A and B). Regarding water intake, the study group exposed to a HFD group showed a lower intake, however these results were not significant (Figure 1C), $p= 0.060$.

Figure 1.1 Food and water intake

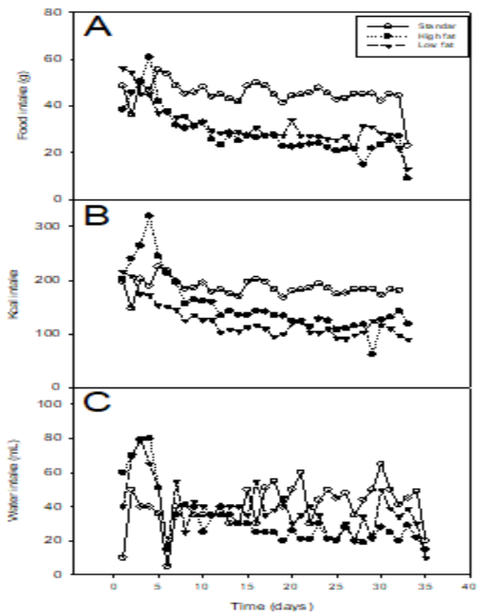


Figure 1.1 Food and water intake. The food intake observed in groups. HFD group showed a reduction in food intake and Kcal intake (A and B), *p value of* <0.05 was considered statistically significant. C) Water intake, the study groups, no significant differences were.

Kilocalorie consumption

Due to the differences in the data recorded from the study groups, a difference was found in kilocalories that were consumed. The group that showed the highest consumption of kilocalories was the group exposed to a standard diet with 94 Kcal/day. Mean while, group exposed to HFD consumed 34.72 Kcal/day and the group exposed to a LFD group consumed 69.46 Kcal/day (Figure 1, B). The study group exposed to HFD showed a lower consumption of kilocaries from carbohydrates with respect to the study group exposed to a LFD and the standard diet group (*p<0.05*). The group exposed to a HFD showed a high consumption of kilocalories from lipids with respect to the group exposed to a low fat content diet and the standard diet group *(p<0.05*), as well as a lower kilocalorie consumption from proteins with respect to the group exposed to standard diet (*p<0.05*), (Figure 2, A,B,C).

Figure 1.2 Kilocalorie, food and macronutrient intake

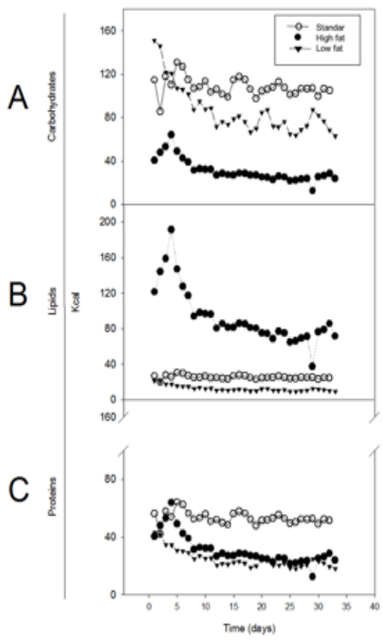


Figure 1.2 Kilocalorie, food and macronutrient intake (carbohydrates, lipids and proteins). The study group exposed to a high fat content diet showed a lower consumption of kilocaries from carbohydrates with respect to the study group exposed to a low fat content diet and the standard diet group. The group exposed to a HFD also showed a lower intake of kilocalories from lipids with respect to the group exposed to a LFD group and the standard diet group, as well as a lower kilocalorie consumption from proteins with respect to the group exposed to a LFD and the group exposed to the standard diet *p* value of <0.05 was considered statistically significant.

Body weight

Body weight was registered every 24 hours. The study group weighing (13%) more was the one exposed to the HFD (group 2) (30.49±1.30g) when compared to the standard diet group (26.87±0.80g) (group1); and LFD (group 3) (28.87±1.36g) (Figure 3).

All study groups increased body weight after 35 days of exposure to each particular diet. The group with a HFD increased its body weight significantly after day-8 (9g) while group exposed to a standard diet increased its body weight 3g; and the group exposed to a LFD increased 6g. Regarding individual body weight, the group HFD content diet showed the highest body weight increment (3.04±0.13g vs 2.68±0.08g), and lowest was the group exposed to a LFD (3.04±0.13g and 2.8±0.13g, respectively) [Figure 1.3].

Figure 1.3 Body weight

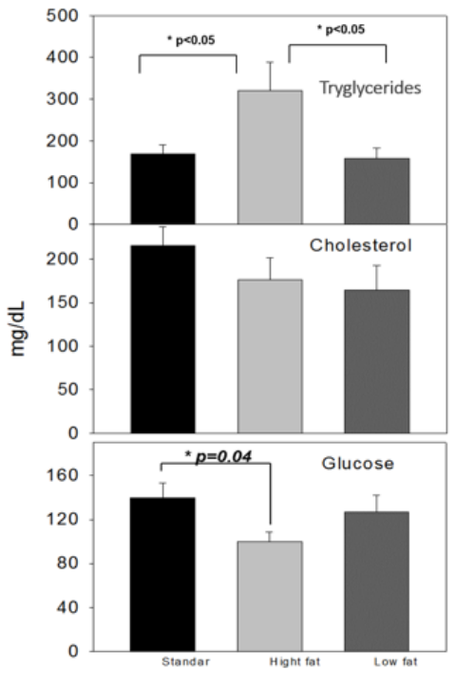


Figure 1.3. Body weight. The high fat diet (Group 2) compared to the standard diet group (Group1); and the low fat diet (group 3). Increase of body weight in HFD group in comparison with and LFD and diet group and standard group. Data are expressed as mean ± standard deviation. *p* value of <0.05 was considered statistically significant.

Triglycerides, cholesterol and glucose serum levels

For serum biochemical indicators, the concentration of triglycerides showed to increase in the group exposed to a HFD compared to the LFD group and the standard group 319.70±68 mg/dL *vs* 172.50±28mg/dL, 185.87±25 mg/dL, respectively) (p<0.05) and the lowest concentration of glucose was shown significantly (99.70±27mg/dL vs, 134±51mg/dL, 149.6±42 mg/dL, respectively) HFD vs standard group (p<0.05). Serum cholesterol values for each group were: 185.11±78mg/dL (HFD group) and 181.12±93mg/dL (LFD group) and 228.22±59mg/dL (standard group), (p=0.386) [Figure 4].

Figure 1.4 Differences between groups in triglycerides, cholesterol and glucose serum levels

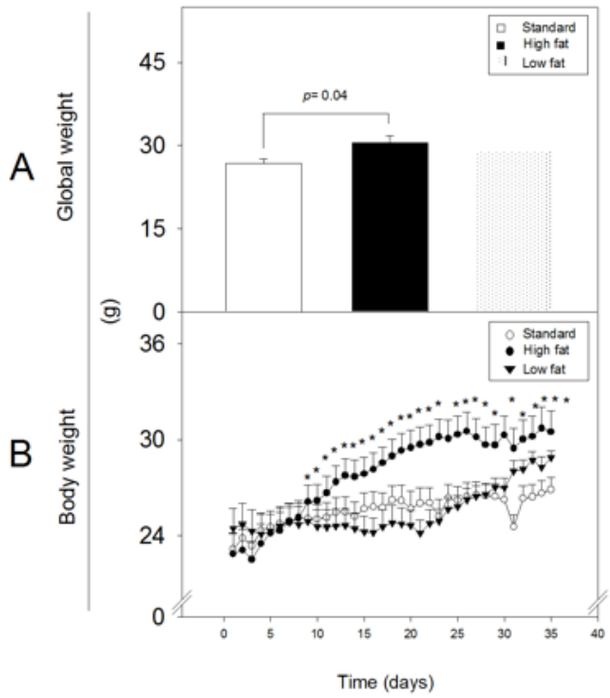
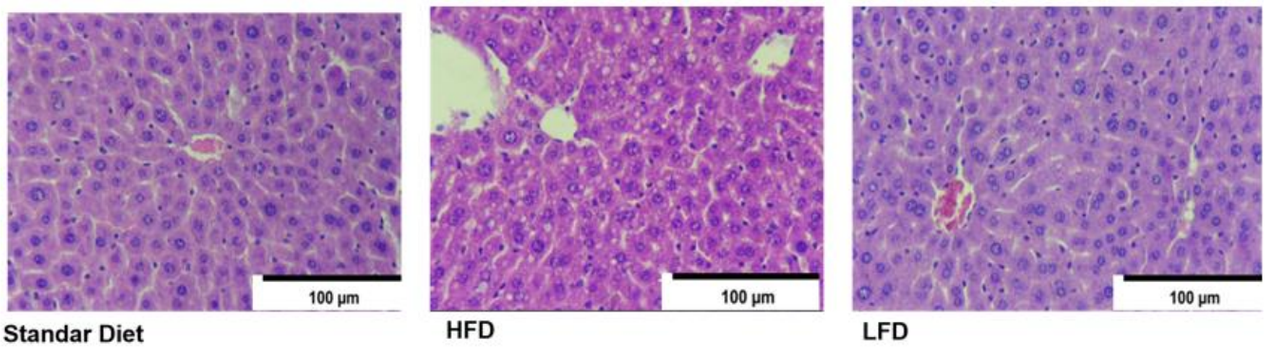


Figure 1.4. Differences between groups in triglycerides, cholesterol and glucose serum levels. Black bar, represent standar diet, light gray bar shows HFD and last dark gray bar shows LFD. The highest concentration of triglycerides founded in the group exposed to a HFD group compared to LFD group and the standard group and the lowest concentration of glucose. Serum cholesterol values for each group. p value of <0.05 was considered statistically significant.

Hepatic histology

The animals from the three groups underwent a histological liver analysis in order to evaluate the morphologic changes. The results are shown in Figure 6. The mean values for each study group regarding the presence of micro-vesicles of fat were: 5-33% for the standard group, >67% for the HFD group and 33-66% for the LFD group; the mean value for each study group regarding the presence of macrovesicles of fat was: 5% for the standard group, 33-66% for the HFD group, and 0% for LFD group. The mean values for the presence of necrosis per group were the following: standard diet group showed 2-3 per field (score of 2), in HFD group 4-5 per field (score of 3), and LCD group >5 per field (score of 4). The inflammation mean values per group were: standard diet 1-2 per field (score of 1), HFD group 3-4 per field (score of 2) and in LFD group 5-8 per field (score of 3). The standard diet did not show significant changes in architecture of the liver and neither in the observed hepatocytes, the cytoplasmic accumulation of cell infiltration or changes in cell nuclear morphology were also not observed. In addition, necrosis and sinusoidal dilatation or vascular congestion were also not observed. The LFD showed slight changes in the cytoplasmic accumulation of fat through a microvesicular type histology, but generally preserved tissue architecture, cell and vascular morphology. However, there was a slight nucleomegaly and binucleation. The HFD group showed liver damage. Presence of liver steatosis. In hepatocytes, fat accumulation was observed with macro and microvesicular fat, with sinusoidal dilation and a swelling cytoplasm and slightly edematous tissue; nucleomegaly and sinusoidal congestion were also observed. In some cuts, necrotic cells were also observed, Figure 1.6.

Figure 1.5 Histologic liver injury scores



	Standard Diet				HFD				LFD				
Score	1	2	3	4	1	2	3	4	1	2	3	4	*p*=
Microvesicular fat (%)	2	7	1	0	1	0	2	7	4	1	5	0	0.001
Macrovesicular fat(%)	10	0	0	0	6	3	1	0	10	0	0	0	0.056
Necrosis(%)	9	1	0	0	5	4	0	0	8	2	0	0	0.269
Inflammation (%)	9	1	0	0	6	4	0	0	8	2	0	0	0.271

Figure 1.5. Histologic liver injury scores The mean values for each study group regarding the presence of micro-vesicles of fat, macrovesicles of fat, necrosis and inflammation, mean values per group were: standard diet 1-2 per field (score of 1), HFD group 3-4 per field (score of 2) and in LFD group 5-8 per field (score of 3), *p value* of <0.05 was considered statistically significant.

1.15 Discussion

Food intake

The excessive consumption of HFD is related to the development of chronic diseases such as obesity and type 2 diabetes mellitus (Myles, 2014). There is little research analyzing the effect of high-fat is isocaloric diets intake on changes in serum biomarkers that are related to the development of chronic diseases. Based on this, the aim of this study was to evaluate the effect of a isocaloric HFD in food intake, body weight and biomarkers like triglycerides, glucose and cholesterol. All groups had free access to food diet (standard, HFD and LFD, as well as the control group) and a significant decrease of food intake in HFD group was shown when compared to the standard diet group. The harmful effects of a HFD could be different, depending on dietary fat quality. In fact, high fat diets rich in unsaturated fatty acids are considered less deleterious for human health than those rich in saturated fat (Crescenzo et al., 2015). Eating a high-fat diet is known to alter gut-brain communication and attenuate subsequent satiety signaling (Duca et al., 2013; Farley et al., 2003; Melhorn et al., 2010). The changes associated with HFD intake may be related to the fact that overfeeding of fats causes reduced carbohydrate oxidation and no change in fat oxidation (Schutz et al., 1989). The food intake observed during the light/dark cycles every 24 hours in group 1 (standard diet) was constant with patterns characteristic of murine models (Cripps & Williams, 1975). In addition, reports have shown that a high fat diet induces changes in metabolism regarding lipids since there is an imbalance between the lipogenesis and the lipolysis (Jiang et al., 2005). The reduction of food intake in HFD group suggests that there is a physiological reaction that adapts to diet composition. It is important to point out that diets applied in this study contained an isocaloric energetic portion with an alteration in the macronutrient distribution. However, it is essential to consider that the characteristics of diet provided such as caloric content, flavor, texture, color, temperature or size might have also determined the quantity of food intake. Nevertheless, in this research such characteristics were not taken into consideration, but there is a possibility that other factors (flavor and texture) may have altered the quantity ingested by the animal subjects. A report states that the energetic density is also related with food intake and that an energetic imbalance and increased body weight have been associated with a risk of developing obesity (Arroyo & Méndez, 2007).

Energetic density, diversity of diets and familial income in rural and urban households. On the other hand, isocaloric diets provide the same amount of grams, however, the alteration of the macro and micro-nutrient composition could affect the food regulation process. Flavor and post digestive consequences have been proposed as the main elements which influence the selection of food, the most common example is the preference for sweet choices, as a possible effect to an adaptation based on a basic survival instinct (Martínez Moreno et al., 2009). It has been suggested that the regulation of food consumption is carried out based on the energetic diet content and not based on palatability differences.

Body weight

In group 1 (HFD), a steady body weight increase was observed versus 2 and 3 group. A possible explanation of these findings could be attributed to an adaptive mechanism metabolic of fats which influences weight development. Others studies showed that HFD intake for 10 weeks in rodents increased 10% in body weight compared to a LFD (Kennedy et al., 2007; Woods et al., 2003). In addition to energy density, the composition of macronutrients in the diet plays an important role in determining the type and magnitude of adaptive metabolic responses caused by diet in the body (So et al., 2011). These results could be related to macro-nutrient acquisition, studies about metabolic efficiency and eating behavior could help clear out the topic.

Peripheral biomarkers

The increase in the lipid profile and food intake are main factors that lead to cardiovascular diseases. Diverse factors have been related to the increase of lipid serum such as high density lipoproteins, the increase in caloric intake, obesity and diabetes (de Oya, 1998). The evidence suggests that the consumption of HFD could influence change of glucose and cholesterol levels and decrease HDL, hence, these three biomarkers are associated to a higher risk of acquiring cardiovascular diseases (Adams et al., 2010; Estrany et al., 2013). In this study we pointed out these alterations, for example, to glucose levels. We showes that glucose levels decrease in animals that consumed the high fat diet and triglyceride levels increased. HFD is known to show high efficacy in inducing obesity in mice and rats (Cripps & Williams, 1975; Morgan et al., 2008), however excessive accumulation of adiposity caused by this approach in rodents is not necessarily accompanied by overfeeding (Arroyo & Méndez, 2007; Jiang et al., 2005; Martínez Moreno et al., 2009), although the absolute and relative energy intake of the HFD diet-induced obesity could be associated with changes in the eating pattern (larger food size and reduced frequency of eating) instead of overfeeding, since the total number of calories ingested by day was no different between animals on a control diet and HFD (Furnes et al., 2009; Woods et al., 2003).

Hepatic histology

The excess of adipose tissue, which affects health negatively, has been defined by the *World Health Organization* as the main cause of obesity. The quantification of adipose tissue and its percentage of fat body are risk indicators of suffering diseases related to feeding behavior such as DM2 (García-García et al., 2008). In this work the group exposed to a HFD presented microvesicles of fat in 67% of hepatocytes, and 4 to 5 characteristics of necrosis per layer. Several authors have pointed out the relationship between high fat intake and increase of adipose tissue. The concentrations of insulin could be implicated in the decrease of insulin sensitiveness in diana tissues, for example adipose tissues, which are also associated with an increase of fat deposits in the organism. Therefore, it is suggested that the consumption of fat could be related directly with the increase of adipose tissue, less insulin sensitiveness and higher levels of serum insulin during fasting periods. The mechanisms possibly implicated in a greater fat body accumulation can be related to the development of microvesicles of fat. The analysis of the accumulation of liver lipids might be caused by the activation of nonalcoholic fatty disease in the liver. The mechanism of hepatic steatosis recent studies suggested persistent hepatic lipogenesis (Diraison et al., 2002). Fat synthesis is a dynamic process could that responds to dietary conditions.

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1.17 Conclusions

In conclusion, the content and quality of macronutrients in the diet is highlighted, despite the fact that those consumed by the mice in this research were isocaloric, exposure to HFD for a short period, evidenced a reduction in food intake and blood glucose and hepatic steatosis. This experimental model could be used to evaluate to effect an isocaloric HFD on development of hepatic steatosis and changes in eating behavior in short period of time. Assessing the intake of a HFD could provide a better understanding of the metabolic mechanisms that are connected to the development of chronic diseases. However, in the field of eating behavior, more research is required, related to physiological and biochemical-molecular behavioral aspects.

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