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Impact of a high-calorie diet on liver and kidneys in Wistar rats

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Abstract

Obesity is a scourge of international public order and is accompanied by an impressive set of health issues, including pathologies related to the kidneys and liver. In our study, it was a question of setting up an experimental obesity model in order to evaluate the impact of the latter on the liver and kidneys of the Wistar rat. The rats were fed a high-calorie diet for 30 days. At the end of the trial, blood samples were taken for the determination of tissue lipids; the animals were then sacrificed, and their kidneys and livers were removed for histological examinations. The results revealed a significant increase in the biological markers of the liver (AST, ALT, PAL, and GGT) and those of the kidneys (uremia, uricemia, and creatinine) in the rats subjected to such a diet, thus highlighting the dysfunction of the liver and kidneys. Furthermore, histological examinations revealed no signs of damage to these organs. However, a significant increase in tissue lipids is noted. The high-calorie diet, therefore, caused an accumulation of fat in the liver and kidneys of the rats. This accumulation could reflect the onset of hepatic steatosis (fatty liver disease) in rats.

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Introduction

Metabolic diseases caused by a diet high in fats and/or sugars are nowadays a public health problem (Pan *et al.*, 2009). The active scientific research carried out has not made it possible to understand the mechanisms that lead to the appearance of metabolic disorders (Ziegler *et al.*, 2009). The link between food and health is more relevant than ever. Indeed, studies confirm that diet plays an essential role in the prevention of certain diseases, such as cancers, cardiovascular diseases, diabetes, hypertension, and obesity (Steinmetz *et al.*, 1996). According to the WHO, obesity is overweight due to excess fat that has harmful consequences for health (Nordoy *et al.*, 2001). Obesity could be responsible for morbidity and even mortality. It is considered an independent risk factor for the appearance of cardiovascular and hepatic pathologies (Lafontan *et al.,* 2013). Obesity is the 5th risk factor for death worldwide (Cole *et al.*, 2012); at least 2.8 million adults die from it each year. The IOTF has stated that approximately 1.5 billion adults are overweight or obese, with 500 million obese (OMS, 2008). Globalization and industrialization have negatively affected the dietary behavior of populations in general and those of developing countries in particular. The food resulting from this evolution leads to the appearance of chronic diseases, including liver and kidney diseases (OMS, 2002). These pathologies are today one of the main causes of mortality and are a public health problem in the world (Hamlat *et al.,* 2008). By 2030, liver and kidney diseases will be the leading cause of death with hyperlipidemia (OMS, 2011). The fundamental cause of overweight and obesity is a lack of physical activity, coupled with high consumption of high-calorie foods high in fats and sugars but low in minerals, vitamins, and other micronutrients (Lichtenstein *et al.,* 1998). However, obesity is not only a problem of overweight; it is also the cause of many alterations (Saltiel *et al.,* 2000). Diets containing more than 30% of energy intake in the form of lipids indeed lead to the development of obesity in rats due to an increase in caloric intake (West *et al.,* 1998). The objective of the present study was to evaluate the impact of a high-calorie diet on the liver and kidneys of Wistar rats.

Material and methods

Animal

The animal material consisted of normal Wistar rats with a weight between 100g and 120g, acquired at the Laboratory of Pharmacology and Improved Traditional Medicines (LPMTA) of the Faculty of Sciences and Techniques (FAST) of the University of Abomey Calavi (UAC). These rats were acclimatized in the laboratory animal house at a constant temperature with a photoperiod of 12h light and 12h dark. They were fed pelleted feed and water.

Induction of experimental obesity

In order to generate significant weight gain and thus constitute a good study model for obesity, the animals were subjected to a high-calorie diet. The latter is composed of 50g of granulated fattening feed (pre-mixed cottonseed and palm kernel meal, amino acids, limestone, dicalcium phosphate, wheat, and rice bran) and 50g of a mixture of sausage-dry biscuits, cheese, chips, chocolate, peanuts in the proportions 2:2:2:1:1:1. This diet induces obesity following hyperphagia (Darimont *et al.,* 2004).

Animal treatment

Two (2) batches of rats were formed. One control received a standard diet for 30 days of experimentation, and the other, a test batch, received a high-calorie diet and sugar water for the same duration of the experiment. Blood samples were taken on the D days indicated (on D0, D14, D21, and D30). At the end of the experiment, the animals were dissected. The kidneys and liver of the rats were removed, then ground using a homogenization ultrathurax in 10 mM Tris base (TBS) buffer, pH 7.4. The ground materials were centrifuged at 3000 rpm for 20 min, and supernatants were stored at -20°C. The removed organs were also the subject of a histological study.

Parameters evaluated

The biochemical examinations (ASAT, ALAT, PAL, Gamma GT, Urea, Creatinine, and Uric acid) were carried out by the kinetic method using the "Mindray-BS 240" automaton. Tissue lipids were assayed after

extraction in the presence of a chloroform-methanol (2:1, v/v) extraction solvent according to the method of Folch and Porter (Folch *et al.,* 1957). After centrifugation at 3000g/min, the lower chloroform phase containing the lipids is recovered using a Pasteur pipette, which is then dried in a water bath at 50°C.

Histological analyzes

The liver and kidneys are removed, fixed in a 10% buffered formalin solution, and embedded in paraffin. Sample sections (5 µm) are mounted on glass slides, deparaffinized and hydrated. For histological analysis, the sections are stained with hematoxylin and eosin (H&E) according to a standard protocol (Senou *et al.,* 2010). Photos are taken at 400X magnification.

Statistical analyzes

The data collected was entered into Excel 2013 software. Normality and homogeneity of variances were checked using R Studio software using the Shapiro test and Levene test respectively. Data comparison was performed using the paired two-sample parametric test with R Studio software. Significance is declared when the *p*-value is less than 0,05.

Results

Change in body weight of rats (g)

Fig. 1 shows the variation in the weight of the rats between D0 and D30 according to the diet to which they were subjected. The body weight of rats fed a high-calorie diet increased compared to those fed a standard diet. This variation is significant from D14 ($p<0.05$) and highly significant on the 30th day ($p<0.001$).

Impact of a high-calorie diet on liver function

Variation in ALT and AST levels in obese rats

Fig. 2 (a, b) presents the variation of the AST and ALT level of the rats during the experiment. The value of transaminases (ALT and AST) in Wistar rats given the high-calorie diet increased compared to those given the standard diet. The results of the analysis of variance showed no significant difference ($p >0.05$) for the data recorded on the 14th day. On the other hand, it is very significant ($p<0.01$) on the 30th day. No significant variation ($p >0.05$) was observed in rats fed the standard diet.

Table 1. Composition of standard and hypercaloric diets (g/100g).

Parameters	Standard diet	High-calorie diet
Lipids	5.44	35.56
Carbohydrates	11.26	27.33
Proteins	14.06	19.36

Variation in Gamma GT and PAL levels in obese rats

Fig. 3 (a, b) shows the variation in the level of Gamma GT and alkaline phosphatase of rats during the experiment. In Wistar rats given the high-calorie diet, Gamma GT levels increased compared to those given the standard diet.

The results of the analysis of variance showed no significant difference ($p >0.05$) for the data recorded on the 14th day. On the other hand, it is highly significant ($p <0.001$) on the 30th day. The different diets did not have a significant effect on alkaline phosphatase concentrations ($p > 0.05$).

Liver histology of rats subjected to a standard(a) and hypercaloric (b) diet

Fig. 4 presents the liver histology of rats subjected to a standard (a) and hypercaloric (b) diet at 400X magnification. The livers of rats fed a standard diet (a) showed no visible atypia. Normal-looking hepatocytes (arrows) are neatly arranged in radial cords around the centrilobular vein (CV). The venous sinusoids (S) are clearly visible. The livers of rats subjected to a high-calorie diet (b) showed no visible atypia. Normal-looking hepatocytes (arrows) are neatly arranged in radial cords around the centrilobular vein (CV). The venous sinusoids (S) are

clearly visible, as observed in the holy control rats.

Fat content in the liver

The results obtained show a very significant increase (p<0.01) in the fat content in the liver in the Wistar rats which received the high-calorie diet, compared to those which received the standard diet.

Impact of a high-calorie diet on kidney function

Variation in urea (a), creatinine (b), and uric acid (c) levels

Fig. 6 (a, b, and c) shows the variation in the level of urea, creatinine, and uric acid in Wistar rats subjected to a standard diet and those subjected to a hypercaloric diet. The results of the analysis of variance show us that the high-calorie diet did not have a significant effect on the concentrations of urea, creatinine, and uric acid ((p > 0.05) on the 14th day. But on the 30th day, a highly significant increase (p <0.001) in blood creatinine and uric acid content was noted in Wistar rats given the high-calorie diet.

Renal histology of rats subjected to a standard (a) and hypercaloric (b) diet

Fig. 7 presents the Renal Histology of rats subjected to a standard (a) and hypercaloric (b) diet at 400X magnification. The renal parenchyma of rats submitted to a standard diet (a) retained its typical appearance. The glomeruli (G), the proximal tubes (TP), the distal tubes (TD), and the collecting ducts (CC) showed no visible atypia. The renal parenchyma of the rats subjected to a high-calorie diet (b) kept its typical appearance as observed in the healthy control rats. The glomeruli (G), the proximal tubes (TP), the distal tubes (TD), and the collecting ducts (CC) showed no visible atypia.

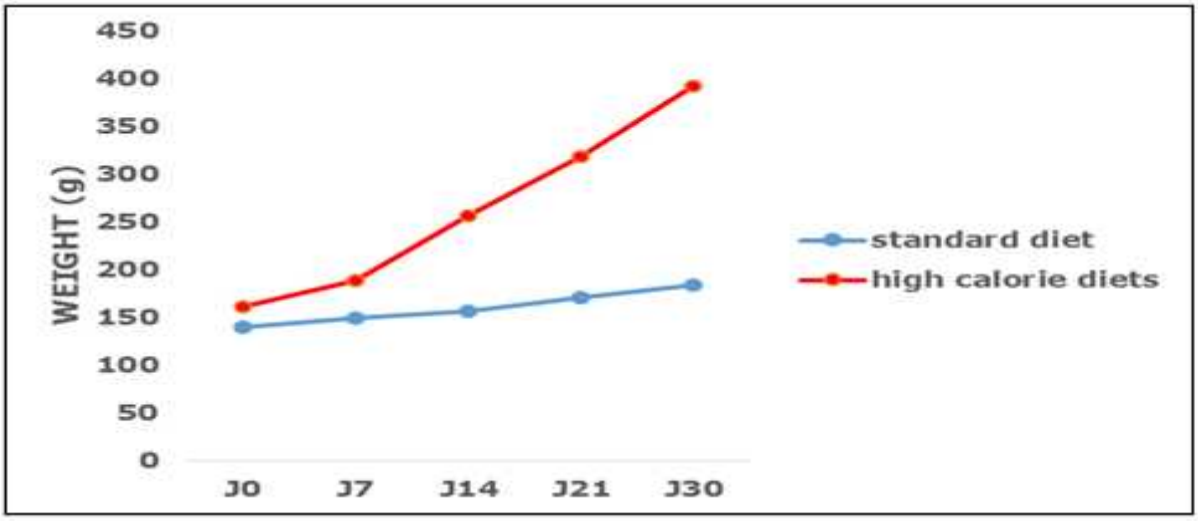

Fig. 1. Curve showing the variation in the weight of the rats between D0 and D30.

Fat content in the kidneys

The results obtained show a very significant increase (p<0.01) in the fat content in the kidneys in the Wistar rats which received the high-calorie diet, compared to those which received the standard diet.

Discussion

In our study, the high-calorie diet-induced, for 30 days, an increase in body weight in rats compared to rats subjected to the standard diet. Our results are in agreement with those of Milagro (Milagro *et al.*, 2006), who confirms that in Wistar rats, the consumption of a high-calorie diet increases body weight and promotes the accumulation of lipids in adipose tissue. This significant increase is observed statistically from the second week. The nature of macronutrients plays a key role in the accumulation of fats in both the liver and the kidneys (Okeke *et al.*, 2009). Indeed, dietary fats have a higher energy value than other macronutrients (Zimmet *et al.*, 2001), which may explain why a diet rich in lipids can lead to an increase in energy intake, and can lead to an increase in adipose mass in the long term. This is the reason why there is an increase in body weight and fat

content in the liver and kidneys of experimental rats. The liver participates in a variety of metabolic activities and secretes a large number of enzymes, including transaminases (ALT and AST), Alkaline Phosphatase, and Gamma GT. These parameters are used as biological markers highlighting liver dysfunction (Daum *et al.*, 1985). In the present study, a highly significant increase in these biological markers of the liver was noted on D30 in the blood of rats subjected to the high-calorie diet, thus highlighting liver dysfunction and possible liver damage that such a diet would cause.

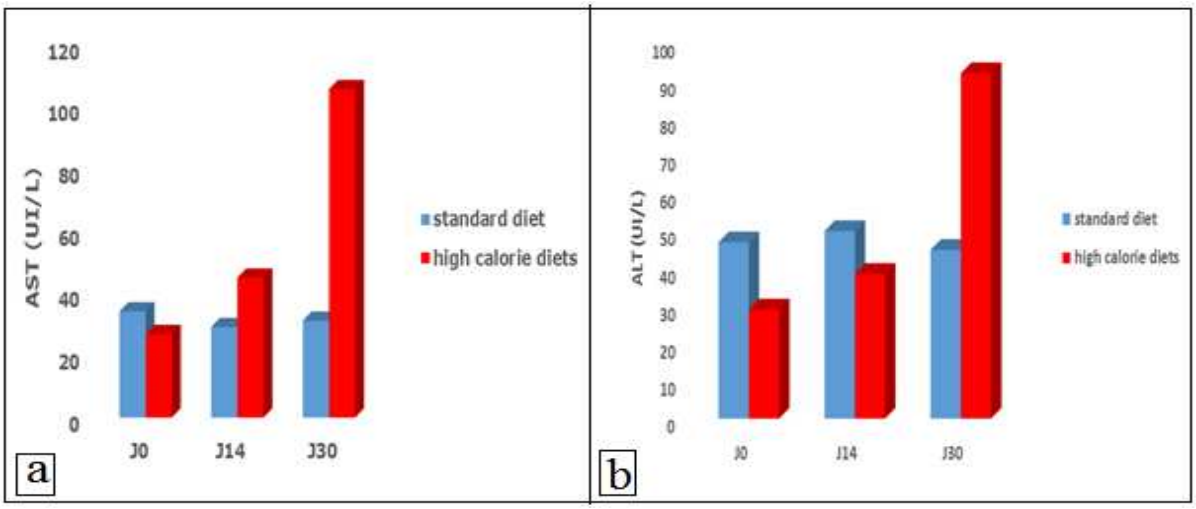

Fig. 2. Histogram showing the effect of a high-calorie diet on ALT(a) and AST(b) levels.

The increased blood content of AST, ALT, and Gamma GT could be attributed to the failure to safeguard the structural integrity of the liver, as these enzymes have cytoplasmic localization and are released into the circulation after damage (Recknagel *et al.,* 1989). The level of blood ALT content is an indicator of the degree of degradation of the cell membrane; that of the AST content is an indicator of mitochondrial damage since mitochondria contain 80% of this enzyme (Tang *et al.,* 2006). Obesity leads to a decrease in hepatic glutathione following the increase in free radicals.

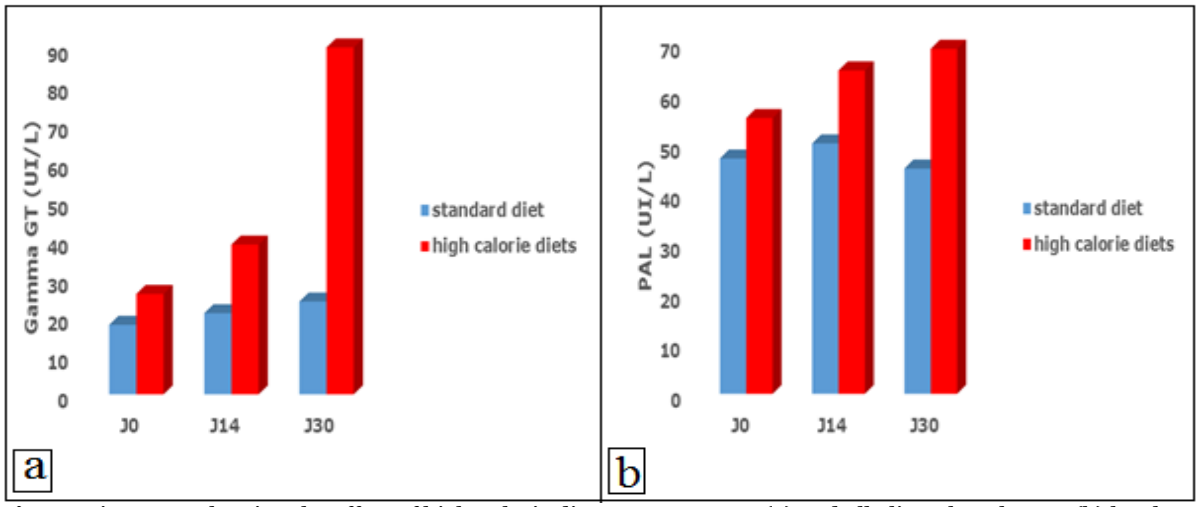

Fig. 3. Histogram showing the effect of high-calorie diet on Gamma GT (a) and alkaline phosphatase (b) levels.

These oxidants induce necrosis in liver cells, leading to an increase in AST and ALT levels (Saxena *et al.,* 2004). In addition, ALT is an enzyme synthesized by hepatocytes and released in the event of hepatocellular injury or necrosis; it is expressed in very low concentrations in other tissues and is

considered specific for hepatocellular injury (Saxena *et al.,* 2004). Based on the previous information, we performed a histological study to confirm the results. The appearance of the hepatocytes revealed by histological examination of the liver shows that the hepatic parenchyma did not present any atypia; no visible lesion in the hepatic cells is therefore observed. Our results also showed a very significant increase (p<0.01) in the fat content in the liver of obese Wistar rats.

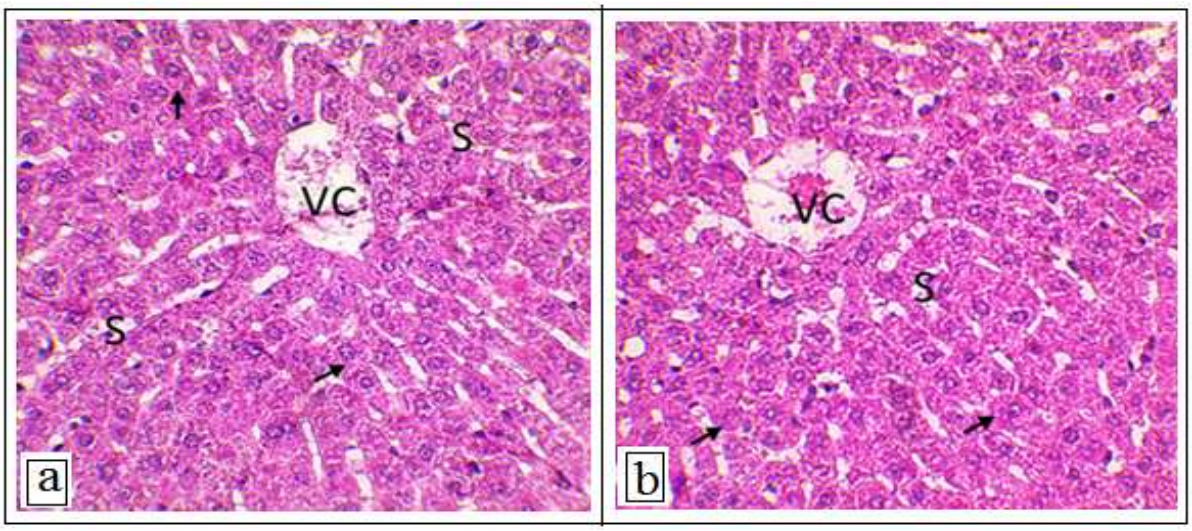

Fig. 4. Liver histology of rats fed a standard (a) and high calorie (b) diet at 400X magnification.

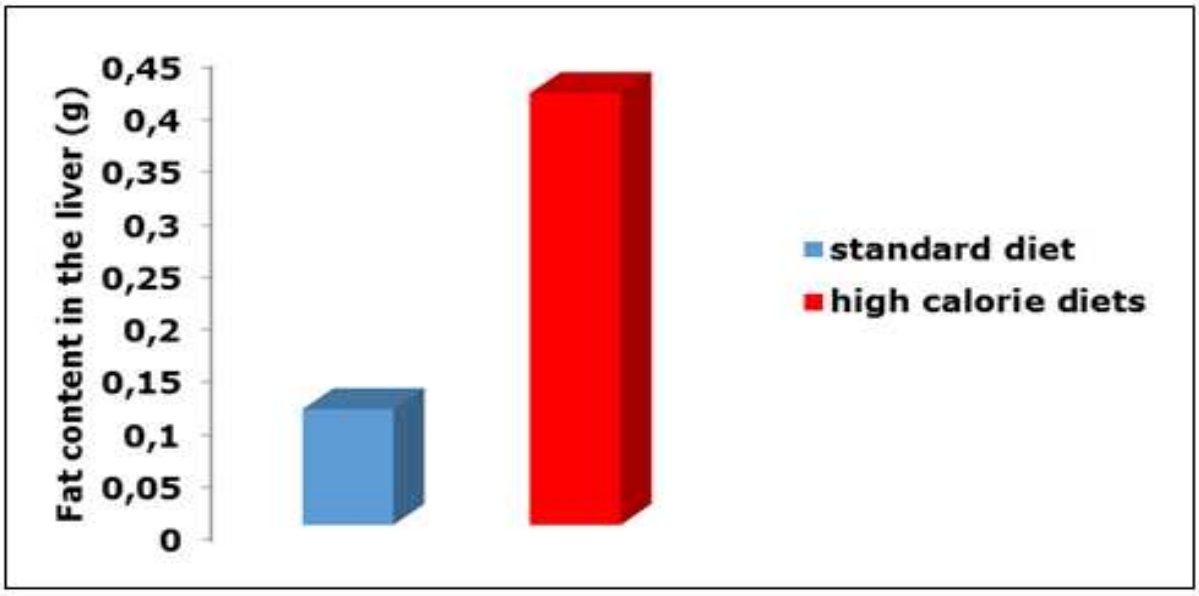

Fig. 5. Histogram showing liver fat content.

This high-calorie diet therefore caused an accumulation of fat in the livers of the rats. The latter reflects the onset of hepatic steatosis in these animals (Bragança *et al.,* 2017). Steatosis results from an imbalance between the breakdown of fatty acids and their synthesis and is favored in the liver by the influx of secondary fatty acids due to an increase in lipogenesis (de novo synthesis of fatty acids), the release of fatty acids by adipocyte lipolysis, the alteration of their export in the form of very low-density lipoproteins and their mitochondrial and paroxysmal oxidation (Angulo *et al.,* 2007). Very often, the histological lesions are much less marked from the beginning of the installation of the steatosis, which may explain our histological examination results.

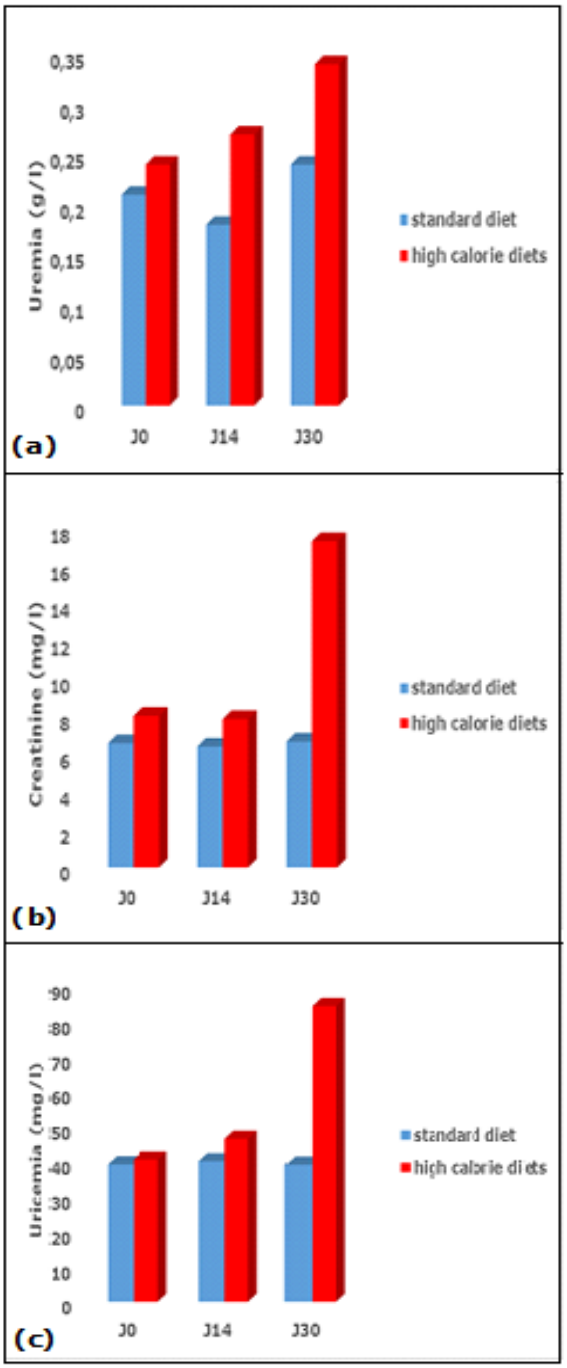

Fig. 6. Histogram showing the effect of high-calorie diet on urea (a), creatinine (b), and uric acid (c) levels.

It will be necessary to extend the duration of the experiment in order to highlight the visibility of the lesions. Vital confirm our results by stipulating that the high-calorie diet is a factor in the development of steatosis, mainly because the unbalanced consumption of energy-dense foods leads to obesity which, in turn, increases the risk of fatty liver disease. However, not only the quantity but also the quality of food could play an important role in the development or progression of steatosis (Angulo *et al.,* 2007). Diets high in cholesterol and saturated fatty acids and low in polyunsaturated fatty acids have been associated with steatosis (Vergniol *et al.,* 2011). Steatosis progresses to the onset of hepatic fibrosis and cirrhosis (Vial *et al.,* 2009). Thus, the disturbance in the level of transaminases during follow-up reflects the activity of the disease and its progression toward fibrosis (Martinez *et al.,* 2012). Lipid overload in the liver leads to the impairment of insulin signaling pathways, which are responsible for cellular and systemic resistance to insulin (Shulman *et al.,* 2006).

In a healthy individual, the lipid content of the liver is low since it represents only 5% of the total weight of the liver. On the other hand, in an obese individual, this limit is exceeded and leads to hepatic steatosis. Several studies in rodents and also in humans have shown that two weeks of high-fat diet can induce fatty liver (Postic *et al.,* 2008). However, its seriousness lies in its risk of progression to fibrosis-type lesions that can lead to cirrhosis and possibly become complicated in hepatocarcinoma, whereas simple steatosis is considered a benign condition (Yki-Järvinen *et al.,* 2005). Our results also showed a very significant (p <0.01) increase in fat in the kidneys of obese Wistar rats, thus preventing the kidneys from fully playing their roles. Indeed, a highly significant increase in uric acid and creatinine was noted in the blood of these rats. The measurement of urea, creatinine, and uric acid is used to explore renal function. An increase in the level of one of these parameters is considered a pathological indicator reflecting renal dysfunction (Coco *et al.,* 2007). The high level of fat in the kidneys of Wistar rats would have resulted in damage to them. Although the histological examinations showed no lesion after four (4) weeks, the inability of the kidneys to eliminate the waste products (urea, creatinine, and uric acids) produced by the body was highlighted. Urea, the end product of protein catabolism in the body, should be excreted by the kidneys (Kent *et al.,* 2014). Visceral obesity is accompanied by high cholesterol, TG, and GLA levels.

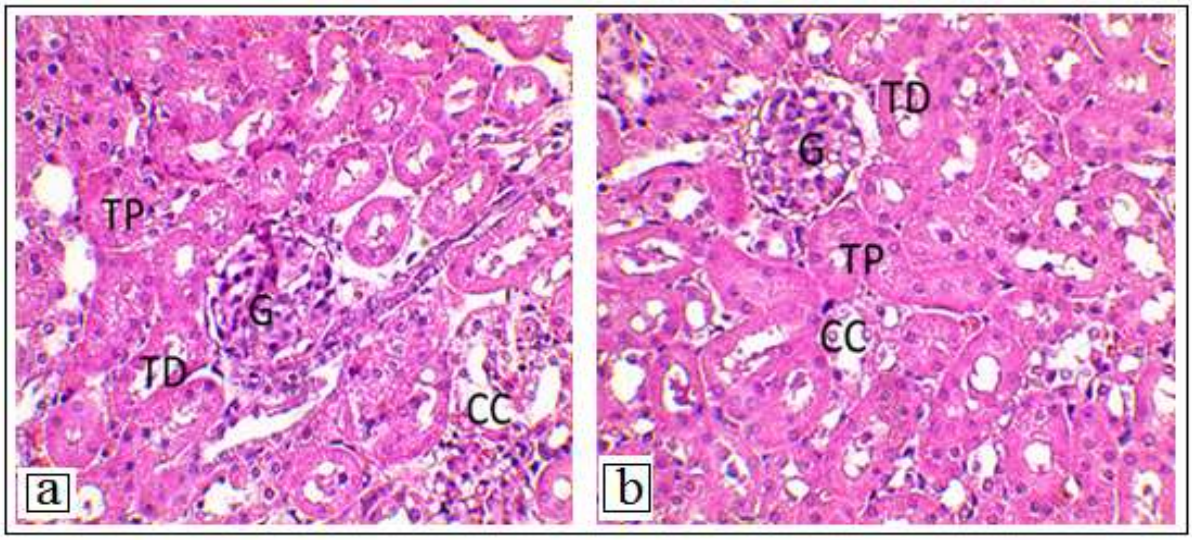

Fig. 7. Renal Histology of Rats Subjected to Standard (a) and High Calorie (b) Diet at 400X Magnification.

These lipids accumulate in the kidneys (ectopic storage), leading to cell dysfunction and cell death (lipotoxicity) (Wennberg *et al.,* 2006). Excess lipids affect the physiology of the proximal tubules through the excessive reabsorption of fatty acids, cholesterol, or phospholipids. This induces tubulointerstitial inflammation and foam cell formation (Kamijo *et al.,* 2002). Obese rats have been shown to develop glomerulosclerosis associated with tubulointerstitial fibrosis resulting from intra-renal accumulation of triglycerides (Wang *et al.,* 2005).

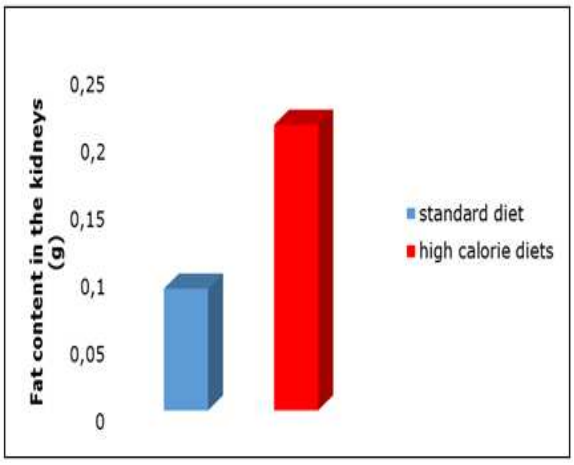

Fig. 8. Histogram showing fat content at kidney level.

The injection of AG into rats leads to the aggravation of tubulointerstitial fibrosis in an experimental proteinuria model (Thomas *et al.,* 2003). In vitro, GLA induces apoptosis of the epithelial cells of the proximal tubules (Arici *et al.,* 2003).

Conclusion

A diet rich in fat promotes the development of obesity; the use of an experimental model to evaluate the impact of the latter on the liver and kidneys of the Wistar rat was the objective of this study. The high-calorie diet, therefore, caused an accumulation of fat in the liver and kidneys of the rats. This accumulation of fat in the liver induces the installation of hepatic steatosis (fatty liver disease) in rats, which explains the increase in the level of the content of biological markers of the liver (AST, ALT, and GGT) noted in the blood of rats fed such a diet. On the other hand, the increase in fat in the kidneys of these animals prevents these organs from fully playing their roles, especially that of blood purification. Metabolic waste products such as uric acid, creatinine, and urea are eliminated less and less, and their content increases in the blood. The effects of the hypercaloric diet, although causing dysfunctions in the liver and kidneys, do not influence, in the short term, the structural integrity of these vital organs of Wistar rats.

Conflicts of interest

The authors declared no conflicts of interest.

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