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RESEARCH ARTICLE

Histopathological features and metabolic disorders in Tunisian rodent *Psammomys obesus* fed high-caloric diets

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ABSTRACT

This study aimed at investigating the alteration of lipid serum profile and histopathological damage in *Psammomys obesus* fed different high calorie diets. Animals were randomly assigned to four groups. *P. obesus* of the control group were fed with a Low-Calorie natural Diet, the *Chenopodicae* plant (0.42 kcal/g). The three other groups were fed high calorie diets rich in carbohydrates and protein or rich in carbohydrates and fat (~3.5 - 4.7 kcal/g). Lipid serum profile was assessed bimonthly during seven-month diets. The recorded energy intake was significantly high in the groups fed high calorie diets compared with the control group. Body weight was significantly increased in animal groups fed high calorie diets. All *Psammomys obesus* fed high-calorie diets developed dyslipidemia with the distinction of different sub-groups developing or not obesity and diabetes. High calorie diets rich in carbohydrates and fat induced a remarkable increase in lipid serum biomarkers indicating a fast induction of dyslipidemia from the first month of the experiment with a significant increase in transaminase activities after two months revealing pronounced hepatotoxicity and nephrotoxicity which were confirmed by a significant increase in liver and kidney relative weight and adiposity index. Severe histological alterations were recorded in obese, diabetic and dyslipidemic *Psammomys obesus* with a noticeable hypertrophy of the adipocytes, glomeruli and islets of Langerhans, as well as increased hepatic lipid droplet accumulation, apoptosis, necrosis and inflammation. A significant decrease in the thickness of the whole retinal layer was also observed after seven-months diet. Animals fed Low-calorie natural diet don't show any signs of obesity, dyslipidemia or diabetes. The high calorie diets induced rapid and severe changes in body weight, severe metabolic syndrome and histopathological features causing organ structural and functional injuries. *Psammomys obesus* seems like an excellent model for studying nutritional pathophysiological-metabolic disorders including obesity, diabetes, dyslipidemia and their complications, particularly diabetic retinopathy, comparable to those of human metabolic processes.

Keywords: High calorie diet, *Psammomys obesus*, obesity, dyslipidemia, type 2 diabetes.

Abbreviations

AI Atherogenic index
ALAT Alanine amino-transaminase
ASAT Aspartate amino-transaminase
EI Energy intake
FC Food consumption
FI Food intake
GCL Ganglion cell layer
HCD High Calorie Diets
HCD 0 High Calorie standard laboratory granules rich in carbohydrate and protein
HCD 1 High Calorie Diet 1: formulation 1 developed at the laboratory rich in carbohydrate and fat
HCD 2 High Calorie Diet 2: formulation 2 developed at the laboratory rich in carbohydrate and fat
HDL High density lipoprotein
INL Inner nuclear layer
IPL Inner plexiform layer
LCD Low Calorie Diet
LDL Low density lipoprotein
NONDD Non-Obese Non-Diabetic, Dyslipidemic
NONDND Non-Obese, Non-Diabetic, Non Dyslipidemic
ODD Obese, Diabetic, Dyslipidemic
ONDD Obese Non-Diabetic, Dyslipidemic
ONL Outer nuclear layer
OPL Outer plexiform layer
P. obesus *Psammomys obesus*
POS Photoreceptor outer segment
RPE Retinal pigment epithelium
T2D Type 2 diabetes
TC Total cholesterol
TG Total triglycerides

Introduction

Food-related metabolic disease is an internationally major problem[1]. Poor quality diets and high consumption of high calorie diets (HCD) rich in fats and/or sugars, with low amounts of minerals, vitamins, antioxidants and other micronutrients are considered to be the root cause of the development of obesity[1,2] and its complications, mainly diabetes and diabetic retinopathy[3]. Obesity is also considered a major underlying risk factor for the development of various chronic pathologies including type 2 diabetes mellitus (T2D), dyslipidemia, diabetic nephropathy, cardiovascular diseases, cancer, hypertension and nonalcoholic fatty liver disease[4,5]. This risk is three to seven times higher in obese patients than in normal weight individuals and could play an important role in boosting the development of diabetic retinopathy[6]. Obesity and overweight result from an imbalance between food consumption, basal metabolism, and energy expenditure. They are characterized by excessive accumulation of fat in adipose tissue which can be harmful to health[1,7]. Furthermore, obese subjects are often characterized by a state of dyslipidemia in which plasma total triglycerides (TG) are increased, high density lipoprotein concentrations (HDL) are lowered, and LDL apolipoproteins (apo-B100) are increased. The central distribution of fat therefore plays an important role in lipid abnormalities[7]. On the other hand, diabetes mellitus is related to a lipid metabolic abnormality. The increase in diabetic cases has been related to the consumption of HCD, obesity, and sedentary lifestyles[8]. Understanding the physiopathology associated with diet related-chronic diseases requires the use of animal models close to human physiology and physiopathology. Several animal models have been used to study these chronic diseases such as obesity, diabetes and their complications[9]. The sand rat *Psammomys obesus* (*P. obesus*), is an arid-adapted gerbil with a low-calorie herbivorous diet consisting essentially of *chenopodiaceae*[10]. When subjected to a standard HCD for laboratory rodents, the animal becomes obese in a similar way to humans and develops hyperinsulinemia, dyslipidemia, hepatic metabolism dysfunction, myocardial anomalies and T2D[11]. Moreover, it may spontaneously develop a disabling complication of diabetes, diabetic retinopathy[12]. Therefore, *P. obesus* gerbil was used as a reliable model of diabetic complications including T2D when fed a HCD in captivity.

To our knowledge no study has been previously conducted to investigate the kinetics of biochemical blood markers in *P. obesus* fed different high-calorie diets for seven months. Besides, the histopathological alterations in the retina, liver, kidney, pancreas and adipose tissues at three-month and seven-month high-calorie diets were investigated and compared.

Methods

ANIMALS, DIET AND EXPERIMENTAL PROTOCOLS

In the present study, we have used 28 mature male *P. obesus* species (*Gerbillidae* family), aged from 2 to 3 months (98–107 g weight), trapped in BOUHEDMA Park in the south of Tunisia with the authorization of the Tunisian Agriculture Ministry (approval number: 2019/548). *P. obesus* were transferred to the laboratory, which was maintained under controlled conditions (22–25°C, 40 ± 10% hygrometry and 12:12 hours of light and dark cycles). Upon their arrival in the laboratory, the animals were numbered, weighed, and placed in a large and furnished space together. After a week of acclimation, animals were separated into individual cages and they were fed exclusively on the *Chenopodiaceae* plant (Low–Calorie natural

Diet: LCD) with water *ad libitum*. After an initial adaptation period of one week, *P. obesus* were randomly distributed into four groups as follows:

- The first group (LCD) (n=7): received the low-calorie diet of the *Chenopodiaceae* family (*Salicornia arabica*) with the following composition: water 81.63 %; ash 8.42 %; fats 0.48 %; proteins 3.09 %; carbohydrates 6.38 % and an energetic value (EV) of 0.42 kcal/g wb).
- The second group (n=7): fed with a High Calorie standard laboratory granule (EL BADR, Bizerte, Tunisia; HCD 0) rich in carbohydrate (61 %) and protein (19.5 %), EV (~3.5 kcal/g).
- The third group (n =7): fed with a High Calorie Diet 1 (HCD 1) rich in carbohydrate (58 %) and fat (19 %), EV (~4.5 kcal/g).
- The fourth group (n=7): fed with a High Calorie Diet 2 (HCD 2) rich in carbohydrate (55 %) and fat (23 %), EV (~4.7 kcal/g).

The experiment was conducted over a period of 7 months.

ETHICS STATEMENT

Animal treatment and experiment conformed to the "Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). The present protocol was approved by the local ethics committee. The Ethical Committee on Medical and Animal Research of the National Veterinary Medicine School, E.N.M.V of Tunisia (Approval Number: CEEA-ENMV 23/20). All efforts were made to minimize animal suffering and reduce the number of animals used.

BODY WEIGHT AND NUTRITIONAL MEASUREMENTS

Psammomys obesus, were weighed, once a week. The percentage of the *P. obesus* initial body weight, % Pi, is calculated as follows:

$$\% Pi = \frac{Pt \times 100}{Pi} \qquad Eq.01$$

Where Pt, is *P. obesus* weight at time t and Pi is its initial weight.

Animals are considered obese, O, when the weight change is equal or superior to 150%[13]. The nutritional estimation was determined by food consumption (FC), food intake (FI) and energy intake (EI)[13]. Food consumption was determined according to *Eq.02*. Food intake was defined as the ability of animals to convert feed energy consumed into body weight and was measured according to *Eq.03*. Energy intake was calculated according to *Eq.04*[14]. The daily food consumption was measured at the same time.

Formulas:

- $\text{FC (g)} = \text{quantity of food supplied} - \text{quantity of food remaining after 24h}$ *Eq. 02*
- $\text{FI } (\%/day) = \frac{\text{FC (g/day)}}{\text{Animal weight (g)}} \times 100$ *Eq. 03*
- $\text{EI } \left(\frac{\text{kcal}}{\text{day}}\right) = \text{FC } \left(\frac{\text{g}}{\text{day}}\right) \times \text{dietary metabolizable energy (kcal/g)}$ *Eq. 04*

Where dietary metabolized energy is calculated by using energetic value of each diet.

BLOOD SAMPLING AND SERUM BIOCHEMICAL PARAMETERS MONITORING

Blood was collected bi-monthly by retro-orbital sinus puncture using a capillary hematocrit first before starting high calorie diet feeding (day 0), glycemia was estimated using Accu-Chek Blood Glucose Meters (Accu-Chek® Active, Roche, Germany). Animals were considered diabetic when glycemia was more than or equal to 2 mg/dL[15,16]. Serum lipid parameters such as Total Cholesterol (TC), Total tri-Glycerides (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL) and serum activities of transaminases, Aspartate Amino-Transferase (ASAT) and Alanine Amino-Transferase (ALAT) were assessed on an Architect C8000 analyzer using the respective reagent kits (Abbott Laboratories, Abbott Park, IL, USA). Furthermore, the Atherogenic Index (AI), was determined according to Friedewald equation[13].

Animals are considered dyslipidemic when total cholesterol (TC) ≥ 2.00 g/L and/or low-density lipoprotein cholesterol (LDL) ≥ 1.60 g/L and/or triglycerides (TG) ≥ 1.50 g/L and/or high-density lipoprotein cholesterol (HDL) < 0.40 g/L[13].

$$\boldsymbol{AI} = \frac{(\boldsymbol{TC - HDL})}{\boldsymbol{HDL}} \qquad Eq.05$$

SACRIFICE AND ORGANS WEIGHTING

After seven months of experimentation, the animals were weighted and sacrificed by decapitation in order to minimize the handling stress and the trunk blood collected. The eyes, kidneys, liver and adipose tissue were removed, cleaned, and weighted, and the relative weights were determined.

$$\text{Relative organ weight (\%)} = \frac{\text{organ weight}}{\text{final body weight}} \times 10 \qquad \textit{Eq.06}$$

adiposity Index (Ia) was calculated as following[13]:

$$I_a = \frac{\text{AT (g)}}{\text{Animal weight (g)}} \times 100 \qquad \textit{Eq.07}$$

Where AT is the weight of adipose tissue

HISTOPATHOLOGICAL ANALYSES

Small pieces of liver, pancreas, adipose tissue, and kidneys were fixed in a 10% buffered neutral formalin solution and the eye of *P. obesus* was enucleated and conserved in a solution of buffered paraformaldehyde phosphate (4%). After embedding these organ pieces in paraffin, they were sectioned at 5 µm and stained with hematoxylin-eosin. Sections were examined using light microscopy (Leica, DM750, Leica Microsystems, Wetzlar, Germany). The thickness of retinal layers, the number of apoptotic cells, necrotic cells and lipid droplets in the liver and the size of each glomerular, adipocyte and islet were measured by the ImageJ software version 1.53 (Rasband, ImageJ, National Institutes of Health, Bethesda, MD, USA). Analyses were performed and compared between animals from different experimental groups.

STATISTICS

Statistical analysis was performed using SPSS system version 22 (SPSS Inc., Chicago, IL, USA). Results were presented as means ± SD for each group of animals. The results from each experimental group were compared using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test between the four diets (LCD, HCD 0, HCD 1 and HCD 2). A value of $p<0.05$ was considered to be statistically significant.

Results

EFFECT OF HIGH-CALORIE DIETS ON ANTHROPOMETRIC PARAMETERS

1. Effect of high-calorie diets on energy intake and food consumption

Figure 1 shows the average values of energy intake, EI (**Figure 1a**), food intake, FI (**Figure 1b**) and food consumption, FC (**Figure 1c**) for animals submitted to different diet groups (LCD: Low Calorie Diet and High-Calorie groups: HCD 0, HCD 1 and HCD 2) during 7 months. The EI (kcal/g/day) varied from 43.36 ± 5.63 (month 1) to 37.12 ± 4.01 (month 7) in HCD 0, from 49.92 ± 6.45 (month 1) to 36.88 ± 5.62 (month 7) in HCD 1 and from 38.40 ± 4, 70 (month 1) to 30.16 ± 2.67 (month 7) in HCD 2. The values of EI are significantly ($p<0.05$) higher than those of the control group, LCD ranging from 23.10 ± 2.1 (month 1) to 25.20 ± 2.42 (month 7) (**Figure 1a**).

Food consumption values (FC) (**Figure 1b**) of animals fed high calorie diets (HCD 0, HCD 1 and HCD 2) were lower than those of animals fed the LCD (ranging from 46.67 ± 5.77 g bh/day (1 month) to 56.67 ± 5.77 g bh/day (7 months).

The dietary attractiveness of the LCD appears to be higher than that of the high-calorie diets (HCD 0, HCD 1 and HCD 2) (**Figure 1b**). Due to the higher caloric values of HCD 0 (3.48 kcal/g), HCD 1 (4.47 kcal/g) and HCD 2 (4.64 kcal/g) compared with the LCD (0.42 kcal/g), animals fed the high-calorie diets have a higher EI than animals fed the LCD ($p<0.05$).

On the other hand, after 7 months of experimentation, the FC of HCD 1 and HCD 2 groups decreased (**Figure 1c**) and the EI increased (**Figure 1a**) compared to *P. obesus* fed the HCD 0.

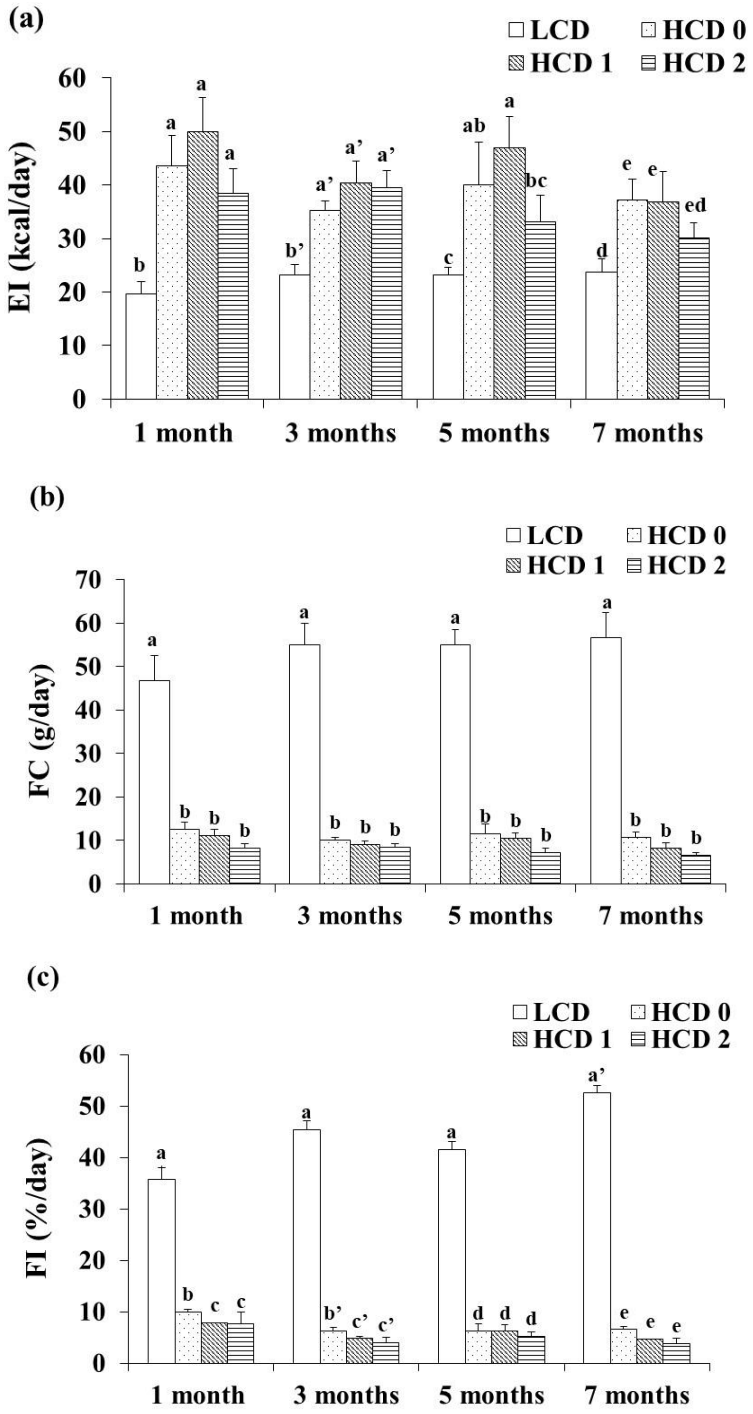


Figure 1. *Estimation of energy intake (EI) (a), food consumption (FC) (b) and food intake (FI) (c) evaluated in the 1st, 3rd, 5th and 7th months for animals subjected to different diets. The results are expressed as means ± SD; Histograms with different letters are significantly different between animal groups (Tukey's post hoc test, p <0.05). N=28 total.*

2. *Psammomys obesus* sub-groups distinction according to weight gain and biochemical parameters dysfunction

According to the percentage of their initial body weight, glycemia and biochemical parameters evolution, different groups and subgroups of animals are distinguished:

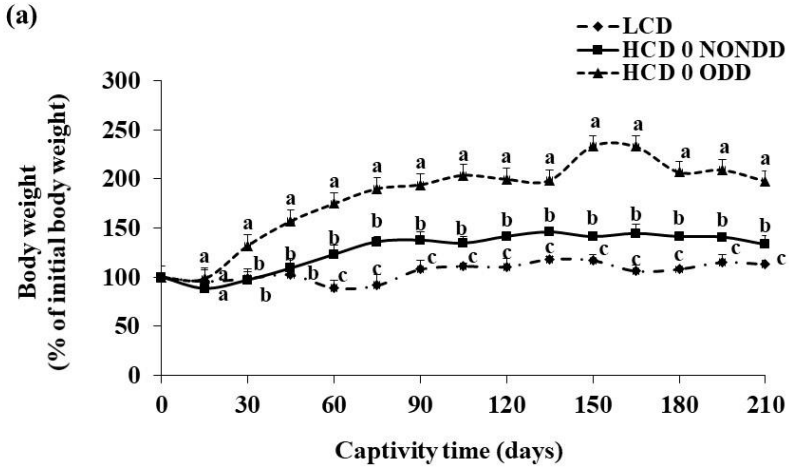
-Group 1, LCD, animals remain during seven months of monitoring, Non-Obese, Non-Diabetic, Non Dyslipidemic: NONDND,

-Group 2, submitted to standard high–calorie diet HCD 0, is divided into 2 subgroups: Non-Obese Non-Diabetic, Dyslipidemic (NONDD) and Obese, Diabetic, Dyslipidemic (ODD),

-Group 3, submitted to high–calorie diet HCD 1, is divided into 2 subgroups: Obese Non-Diabetic, Dyslipidemic (ONDD) and Obese Diabetic Dyslipidemic (ODD),
-Group 4, submitted to high–calorie diet HCD 2, is divided into 2 subgroups: Obese Non-Diabetic Dyslipidemic (ONDDa) and Obese Non-Diabetic Dyslipidemic (ONDDb).
62% of animals receiving HCD 0 rich in carbohydrate and protein remained Non-Obese and Non-Diabetic but became Dyslipidemic (HCD 0 NONDD) after three months, whereas 38% of animals developed Obesity, T2D and Dyslipidemia (HCD 0 ODD) after one-month. 100% of animals receiving HCD 1, rich in carbohydrates and fat developed Obesity and Dyslipidemia within month one. Overall, 33% of the animals contracted Obesity and Diabetes (HCD 1 ODD) and 67% developed Obesity with Dyslipidemia but Non-Diabetes (HCD 1 ONDD). 75% of animals fed HCD 2, rich in carbohydrate and richer in fat than HCD 1, developed Obesity and Dyslipidemia (HCD 2 ONDD) from the first month, whereas 25% of animals were Dyslipidemic but remained Not Obese and Non-Diabetic (HCD 2 NONDD) during three months. This subgroup developed Obesity after three months until the end of the experiment (seven months) (HCD 2 ONDD).

3. Effect of high-calorie diets on animals' body weight

At the beginning of the experiment, no significant differences were noted in body weight evolution (as a percentage of initial weight) between the four *P. obesus* groups (**Figure 2**), but these differences became noticeable during the second week of the diet. The body-weight decreased. This reduction in body weight is probably linked to the reduction in food consumption. Weight returned to normal and became significantly elevated up to 3 months between control animals and the different sub groups (HCD 0 ODD, HCD 1 ONDD, HCD 1 ODD and HCD 2 ONDD) ($p<0.05$), with the exception of subgroup HCD 2 ONDDb, for which there was no significant change ($p>0.05$) in weight at 3 months. HCD 2 ONDDb showed an increase in body weight (150%) after 3 months, and this remained stable up to 7 months. Obesity was established from day 30 in the HCD 2 ONDDa subgroup (**Figure 2c**), from day 60 in the HCD 0 ODD (Figure 2a), HCD 1 ONDD and HCD 1 ODD (**Figure 2b**) subgroups, and after day 90 in the HCD 2 ONDb subgroups. However, from five months to the end of the experiment (7 months), the weight of the HCD 0 ODD, HCD 0 NONDD and HCD 1 ONDD subgroups decreased considerably, with the exception of HCD 1 ODD, HCD 2 ONDDa and HCD 2 ONDDb (**Figure 2**). Throughout the 7 months of the different HCD, an extension of obesity, characterized by a sharp increase in body growth in the HCD 0 ODD, HCD 1 ONDD, HCD 1 ODD and HCD 2 ONDD subgroups, was observed. Average body weight gain increased in control animals, LCD (13%) and the HCD 0 NONDD (33.15%), HCD 0 ODD (97.73%), HCD 1 ONDD (11.66%), HCD 1 ODD (70.09%) and HCD 2 ONDDa (54.71%) and HCD 2 ONDDb (47.06%) subgroups respectively, ($p<0.05$) (**Figure 2**).



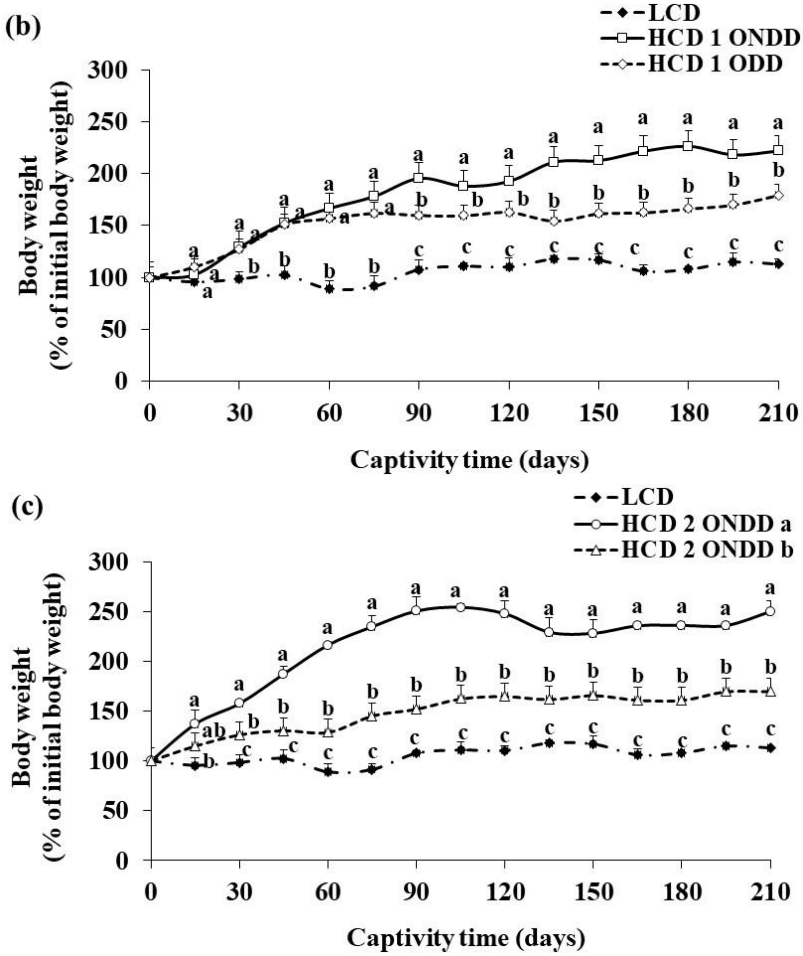


Figure 2. *Body weight (% of initial body weight) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

4. Effect of high-calorie diets on relative organ weights

The relative liver weights of the different animal groups are shown in **Figure 3a**. After 7 months, HCD 0, HCD 1 and HCD 2 induced hypertrophy of the liver. Indeed, there was a significant increase in relative liver weight in the subgroups receiving the HCD 0, HCD 1 and HCD 2 diets, with values of 4.61 ± 0.61% in HCD 0 NONDD; 6.01 ± 0.1% in HCD 0 ODD; 3.76 ± 0.28% in HCD 1 ONDD; 5.03 ± 0.08% in HCD 1 ODD; 2.71 ± 0.23% in HCD 2 ONDDa and 2.54 ± 0.21% in HCD 2 ONDDb, respectively, compared with 2.59 ± 0.03% in the LCD groups ($p< 0.05$).

As shown in **Figure 3b**, the relative weight of the kidney increased significantly in HCD groups after 7 months ($p<0.05$). Along with kidney hypertrophy, renal function was altered in obese and diabetic rats.

The weight of adipose tissue in each group is shown in **Figure 3c**. High-calorie diets rich in carbohydrates and protein (HCD 0) or rich in carbohydrates and fat (HCD 1 and HCD 2), induced an enlargement of the adipose tissue (**Figure 3c**). After 7 months, the adiposity index increased significantly in sub-groups subjected to HCD 0, HCD 1 and HCD 2 with values of 1.91 ± 0.24% in HCD 0 NOND; 3.88 ± 0.31% in HCD OD; 3.99 ± 0.43% in HCD 1 OND; 4.85 ± 0.05% in HCD 1 OD; 4.47 ± 0.13% in HCD 2 ONDa; 4.57 ± 0.14% in HCD 2 ONDb, compared with the LCD groups (0.15 ± 0.06%) ($p < 0.05$). The increase in body weight of *P. obesus* under a HCD is strongly associated with an increase in adiposity index (**Figure 3c**), confirming the obesogenic properties of HCD 1, rich in fats and richer in carbohydrates and HCD 2, rich in carbohydrates and richer in fats than in HCD 0, rich in carbohydrates and protein.

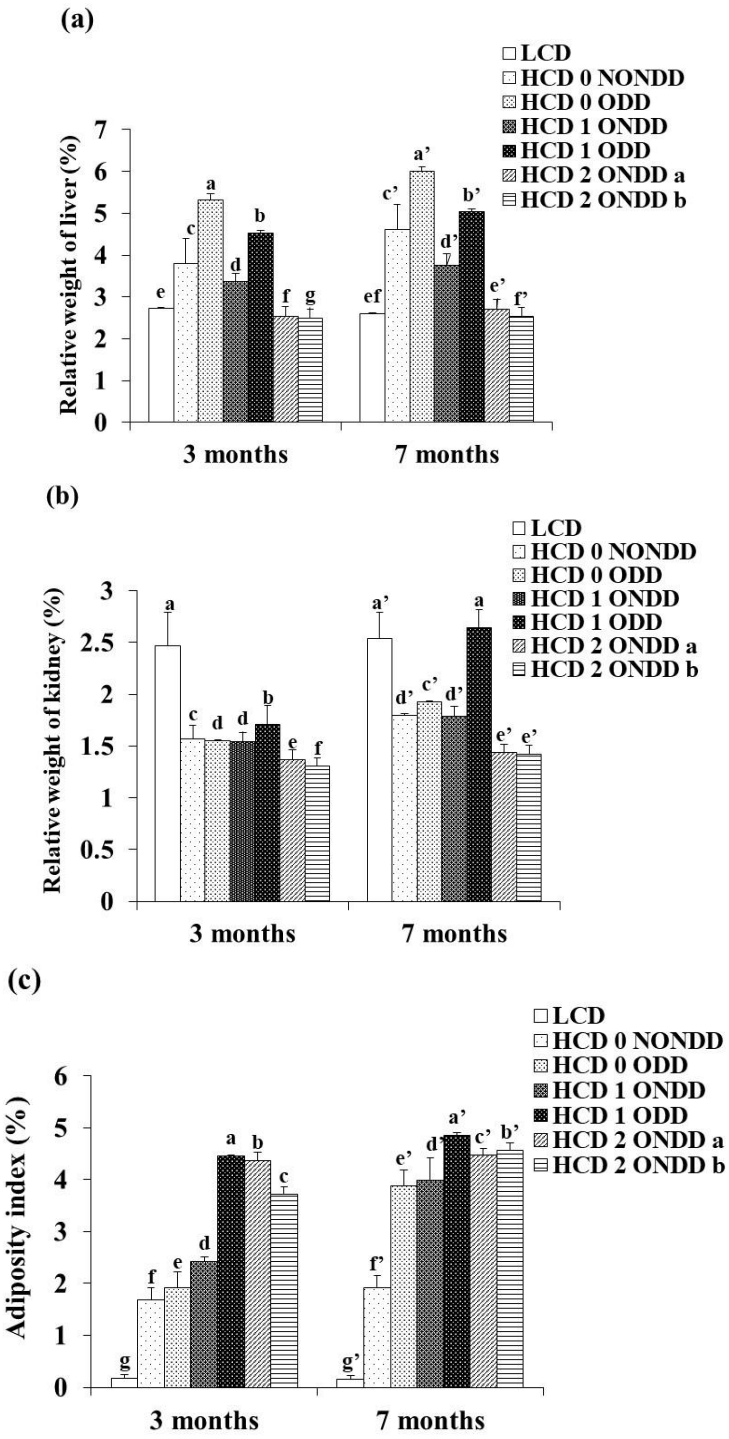


Figure 3. *Evolution of relative weight of liver (a), kidney (b) and adiposity index (c) for different subgroups of P. obesus during 3 and 7 months. The results are expressed as means ± SD; Histograms with different letters are significantly different between animal groups (Tukey's post hoc test, p <0.05). N=7 animals for each group.*

EFFECT OF HIGH-CALORIE DIETS ON BLOOD BIOCHEMICAL PARAMETERS

1. Impact of high-calorie diets on glycemia

Figure 4 shows no significant variation in glycemia levels throughout the experiment in the control (LCD) group during 7 months ($p>0.05$) (**Figure 4**). For the HCD 0 OD subgroup, mean glycemia values varied significantly, from 98 ± 20 mg/dL at the beginning of the experiment to 309 ± 76 mg/dL after 3 months and 370 ± 76 mg/dL after 7 months of the HCD ($p<0.05$), in comparison with the HCD 0 NONDD and LCD groups. Diabetes was established after one month of receiving HCD. Mean glycemia values in the HCD 0 ODD subgroup reached 501 ± 76 mg/dL at month 7, compared with 432 ± 16 mg/dL in the HCD 1 ODD subgroup.

In summary, diabetic *P. obesus* subjected to HCD 0 and HCD 1 showed a markedly significant increase in glycemia levels compared with control as well as the HCD 0 NONDD, HCD 1 ONDD, HCD 2 ONDDa and HCD 2 ONDDb subgroups (**Figure 4**; $p<0.05$). In fact, both subgroups HCD 0 OD and HCD 1 OD became diabetic at day 30. High-calorie diet rich in fat and richer in carbohydrates (HCD 1), can be retained for a rapid induction of diabetes.

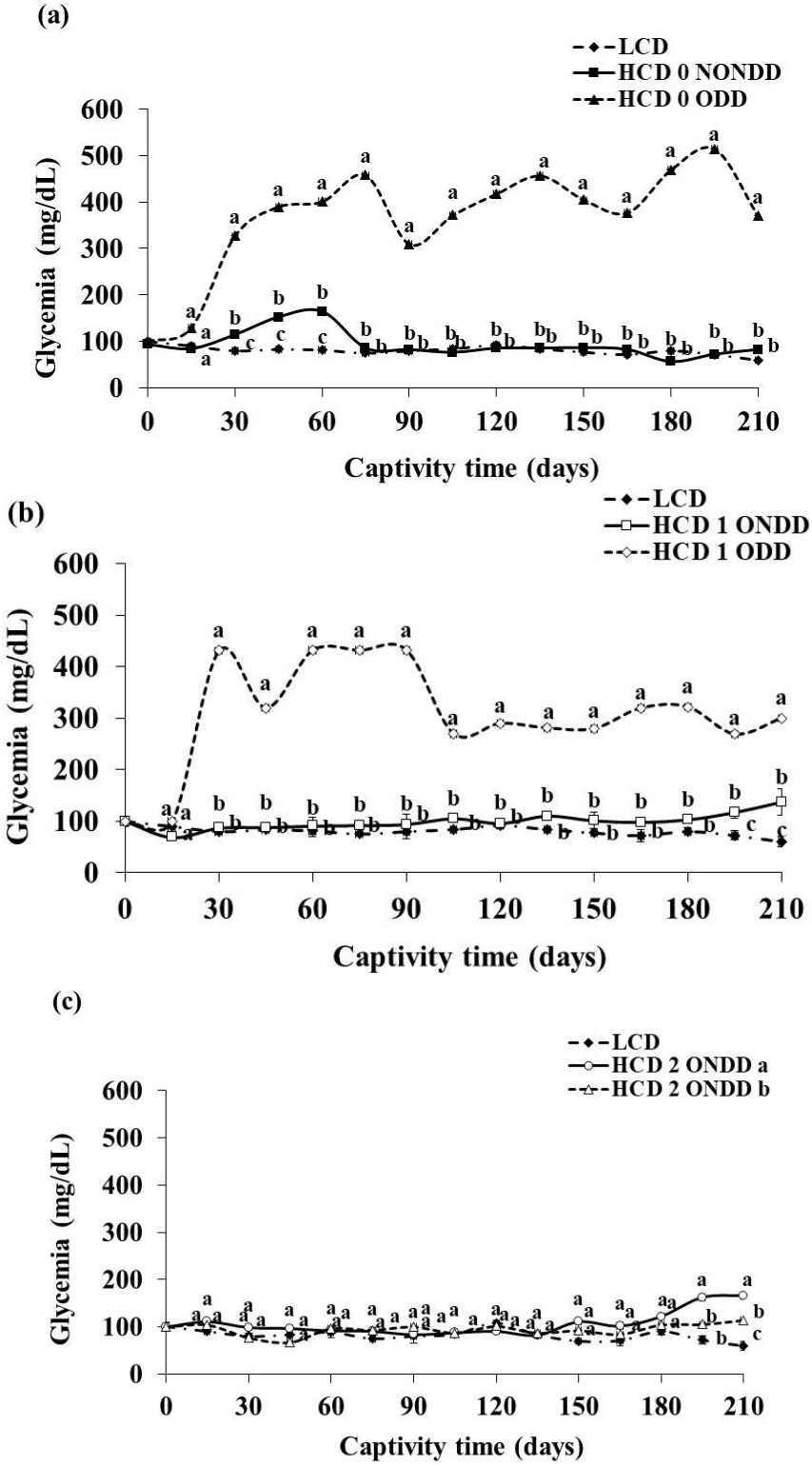


Figure 4. *Evolution of glycemia (mg/dL) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters ($^{a, b, c}$) for each captivity time are significantly different between groups (Tukey's post hoc test, $p< 0.05$). N=7 animals for each group.*

2. Lipidemia Kinetics

2.1. KINETICS OF TOTAL CHOLESTEROL

Figure 5 shows fluctuations in Total Cholesterol (TC) levels in all groups of *P. obesus*.

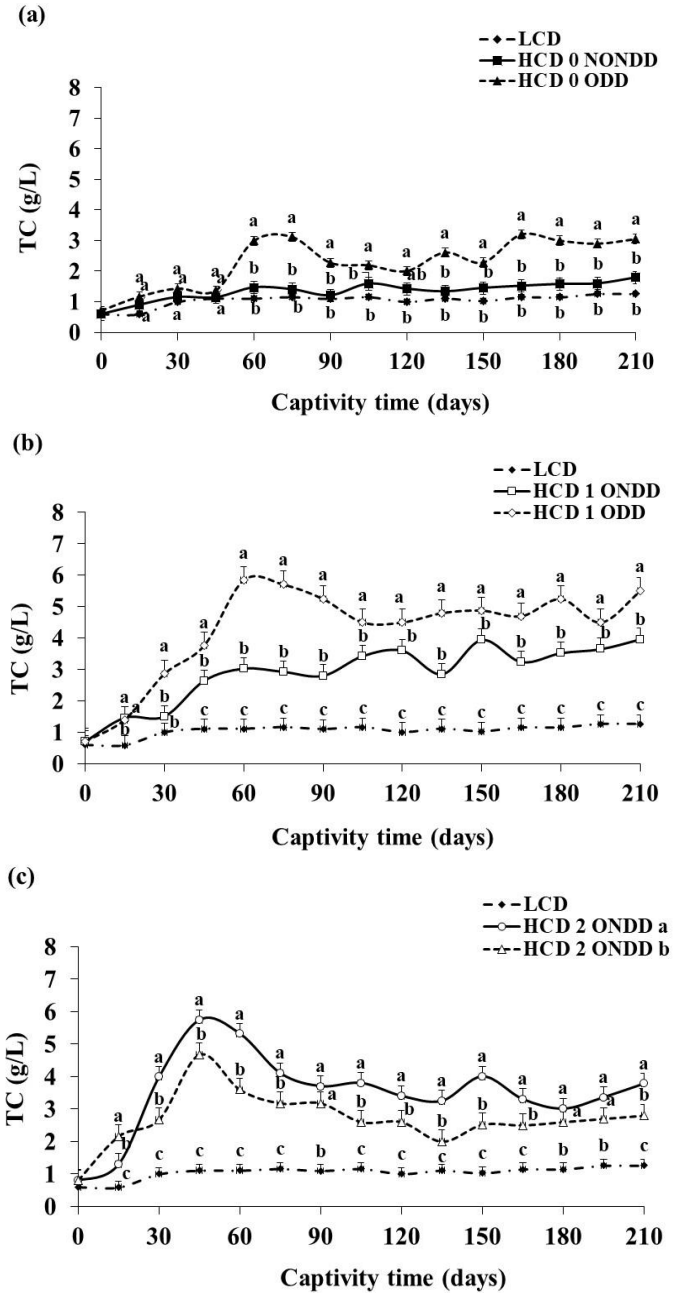


Figure 5. *Determination of Total Cholesterol (TC) (g/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

The Total Cholesterol (TC) values in diabetic rats HCD 0 ODD and HCD 1 ODD and obese rats HCD 2 ONDDa and HCD 2 ONDDb were significantly increased from month one to month seven. Diabetic rats, HCD 0 ODD, showed an increase in hypercholesterolemia (3.12 ± 0.45 g/L at day 90). This was followed by a slight decrease at month 4 (1.67 ± 0.32 g/L). The TC level then increased after month 4 and stabilized until the end of the study (2.05 ± 0.46 g/L). In diabetic rats (HCD 1 ODD), serum TC increased to reach a maximum value of 7.85 ± 0.15 g/L from day 60 to day 90. At 3 months, this increase is followed by a decrease until month 7 (2.25 ± 0.50 g/L). In contrast, obese HCD 2 ONDD sub-groups show an increase in TC, reaching a maximum value of 5.32 ± 2.01 g/L on day 60. This increase is followed by a decrease after 3 months. The TC level stabilizes up to 7 months, at 2.01 ± 0.35 g/L. *P. obesus* subjected to HCD 0, HCD 1 and HCD 2 showed a very significant increase in TC level, superior to 2 g/L in 60th days when compared with the control group ($p<0.05$).

2.2. KINETICS OF TRIGLYCERIDES

Figure 6 illustrates the variation in the triglycerides level (TG) of the four groups as a function of the duration of diet administration. The triglycerides level varies throughout the experiment starting on the 15th day. The triglycerides level of the control group is 0.93 ± 0.08 g/L. Triglycerides value undergoes an increase to reach 1.10 ± 0.37 g/L at the end of this study. At the second and fifth months, the TG level of the HCD0 OD sub-groups reaches its maximum (10.43 ± 1.06 g/L). The level of triglycerides (TG) in the sub-group HCD 0 NONDD is significantly lower than in the HCD 0 OD and LCD groups ($p<0.05$). Diabetic and obese rats, HCD 0 ODD (2.55 ± 1.06 g/L), HCD 1 ONDD (6.08 ± 0.92 g/L), HCD 1 ODD (2.40 ± 0.52 g/L), HCD 2 ONDDa (3.86 ± 0.14 g/L) and HCD 2 ONDDb (2.20 ± 1.12 g/L), subjected to a HCD showed a markedly significant increase in serum TG contents from month 3 to month 7 compared with LCD group and HCD 0 NONDD group ($p<0.05$) (**Figure 6a**).

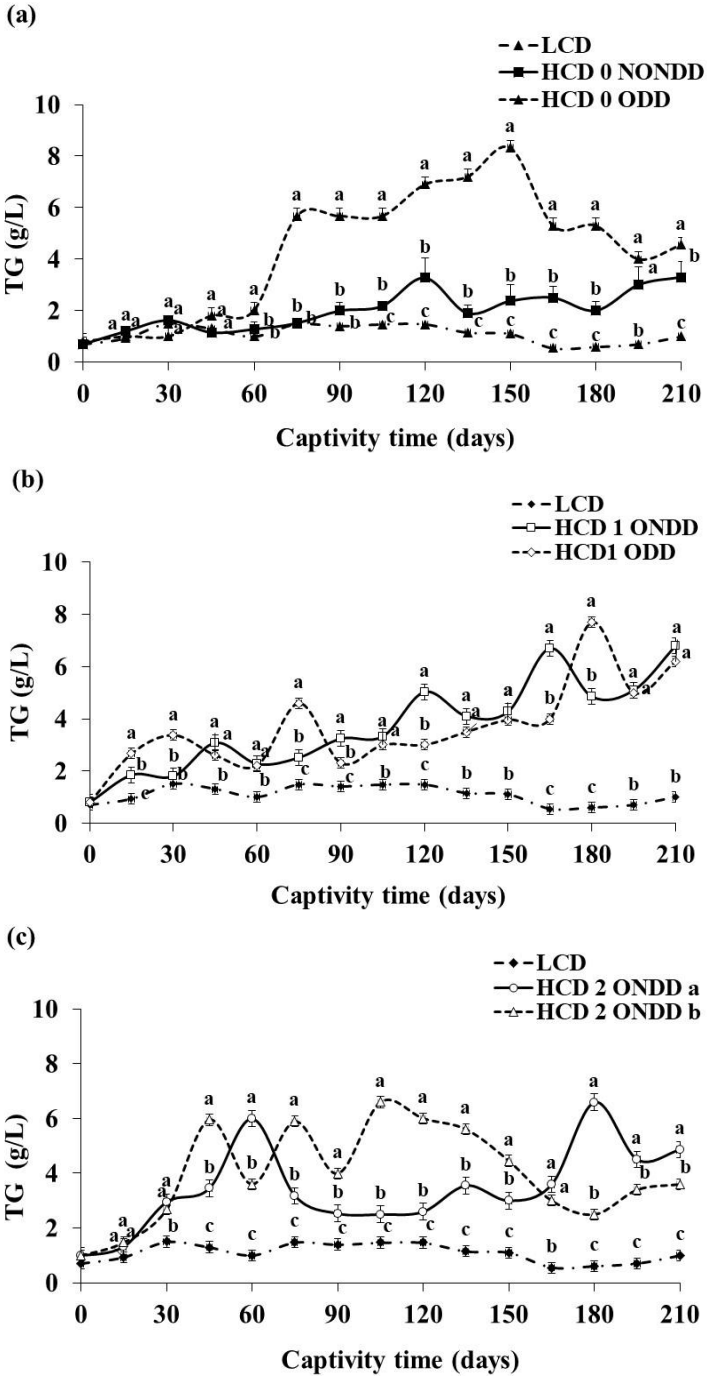
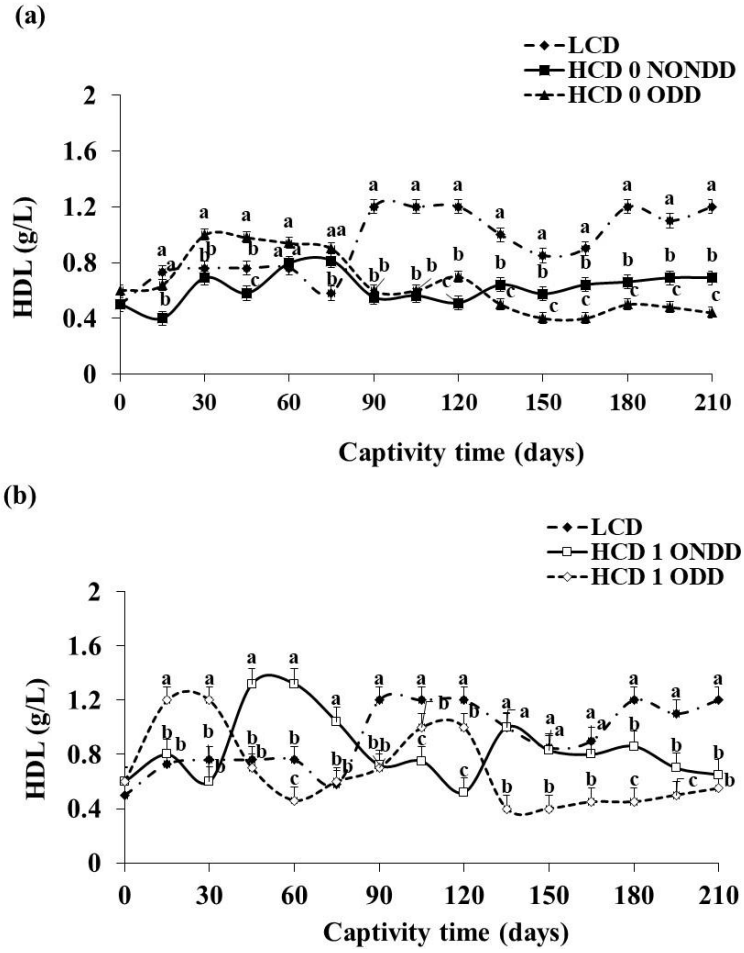


Figure 6. *Determination of triglycerides (TG) (g/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

2.3. KINETICS OF HIGH-DENSITY LIPOPROTEIN

As shown in **Figure 7**, the level of High-Density Lipoprotein cholesterol (HDL) in the control group (LCD) was 0.37 ± 0.01 g/L on the 15th day. Then, it undergoes an increase to reach 0.80 ± 0.30 g/L after 7 months. A similar result was obtained in the sub-groups HCD 0 NONDD (**Figure 7a**). The level of HDL in the HCD 0 ODD subgroup was significantly higher ($p<0.05$) than in LCD and HCD 0 ONDDb with a maximum value of 1.22 ± 0.09 g/L on day 60. This increase is followed by a decrease to reach 0.70 ± 0.33 g/L at month 7. The normal HDL level reached at the end of this study could be explained by a kind of stress adaptation.

The HDL level in diabetic *P. obesus* (HCD 1 ODD) is 1.34 ± 0.59 g/L (**Figure 7b**); it decreased until the 90th day, this decrease is then followed by an increase to reach 1.05 ± 0.17 g/L at the end of the study. The level of HDL in HCD 1 ONDD subgroups showed a highly significant increase after the 4th month (2.50 ± 0.52 g/L). Besides, the HDL level decreases slightly to 1.48 ± 0.17 g/L on day 210. In fact, it can be noticed that the level of HDL in HCD 1 ODD was significantly different compared with the LCD group ($p<0.05$). Moreover, in obese rats, HCD 2 ONDDa, the level of HDL was significantly different compared to the HCD 2 ONDDb subgroup and the LCD group ($p<0.05$).



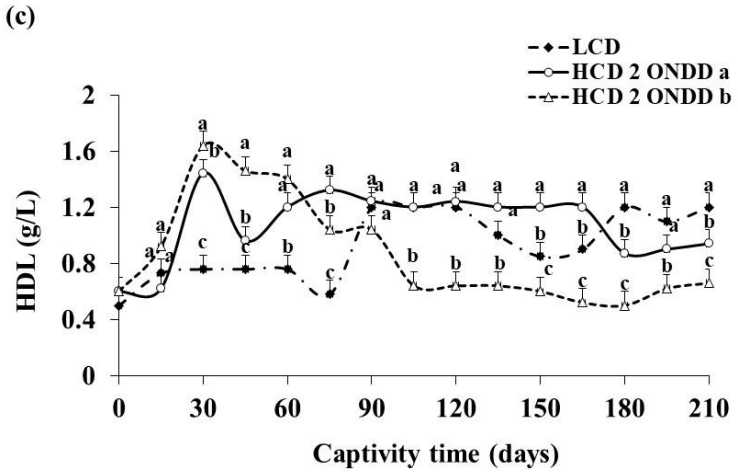
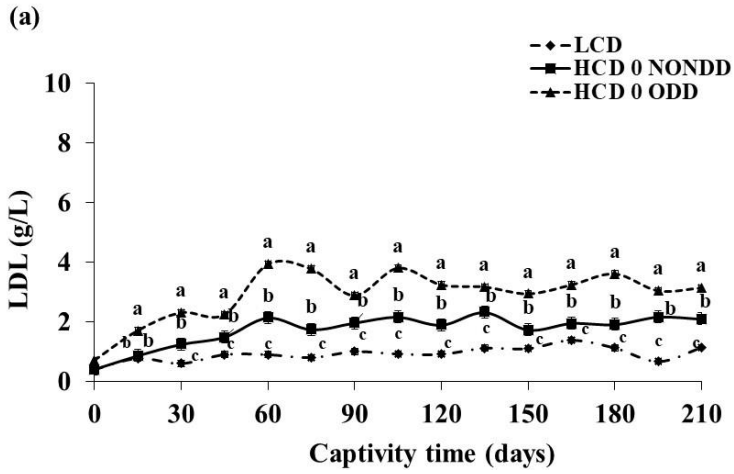


Figure 7. *Determination of High-Density Lipoprotein (HDL) (g/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

2.4.

2.5.KINETICS OF LOW-DENSITY LIPOPROTEIN

Figure 8 shows the variation of Low-Density Lipoprotein cholesterol (LDL) in the four groups. On day 15, the level of LDL in the control group (LCD) was 0.77 ± 0.07 g/L. It undergoes a slight increase to reach 1.53 ± 0.30 g/L after the 7th month of the HCD. In diabetic rats, HCD 0 ODD and HCD 1 ODD, the LDL levels at the beginning of the experimentation are 1.70 ± 0.22 g/L and 1.34 ± 0.84 g/L, respectively. After one month, these levels are significantly higher (2.30 ± 0.41 g/L and 3.52 ± 0.73 g/L, respectively) than those of rats in the LCD group (1.13 ± 0.55 g/L), HCD 0 NONDD (1.00 ± 0.48 g/L) (**Figure 8a**) and HCD 1 ONDD (0.78 ± 0.25 g/L) ($p<0.05$) (**Figure 8b**). The LDL level undergoes a decrease in HCD 0 ODD and HCD1 ODD, and then stabilizes until the end of the experiment. Low-Density Lipoprotein cholesterol levels in obese HCD 2 ONDDa rats were 0.35 ± 0.17 g/L at the beginning of the diet, compared to 0.77 ± 0.07 g/L in the LCD group and 1.89 ± 0.91 g/L in the HCD 2 ONDD b subgroup ($p<0.05$) (**Figure 8c**). HCD 2 ONDDa showed a significant increase in LDL level to reach a maximum on the second month (6.37 ± 2.22 g/L), this increase stabilized and decreased until the end of the experiment compared to HCD 2 ONDDb and the LCD groups ($p <0.05$) (**Figure 8c**).



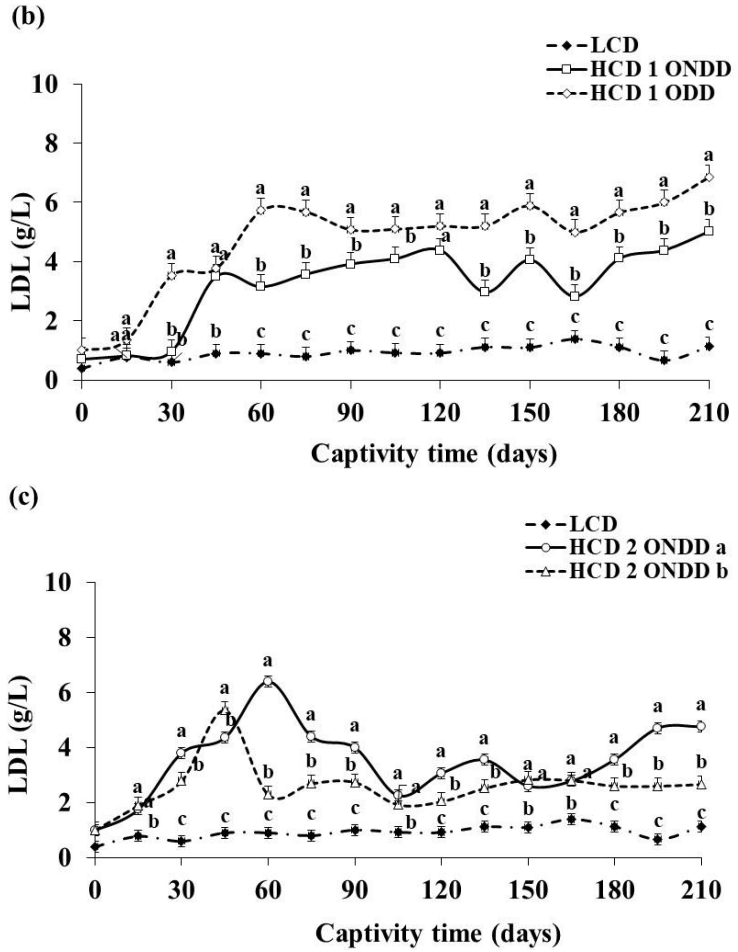


Figure 8. *Determination of Low-Density Lipoprotein (LDL) (g/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters ($^{a, b, c}$) for each captivity time are significantly different between groups (Tukey's post hoc test, $p< 0.05$). N=7 animals for each group.*

2.6. KINETICS OF ATHEROGENIC INDEX

The Atherogenic Index (AI) is defined as the ratio of LDL to HDL. **Figure 9** shows the variation of the AI in the animal subgroups as a function of time. On the 15th day, the AI of the control group was 0.59 ± 0.10. It undergoes an increase to reach a value of 0.99 ± 0.14 on the 75th day. Thereafter, there was a slight decrease until the end of this experiment to reach a value of 0.25 ± 0.11. AI in the HCD 0 ODD subgroup (**Figure 9a**) is significantly higher than in the LCD group and the HCD 0 ONDDb subgroup ($p<0.05$), it reaches a maximum value of 2.22 ± 0.35 on day 75 and then stabilizes from month 3 to month 7. The atherogenic index in obese rats (HCD 1 ONDD) was 0.83 ± 0.25 on the 15th day and showed an increase at the end of this experiment (1.65 ± 0.43) while in HCD 1 ODD, the rate of AI reached maximum values of 4.37 ± 0.82 after one month and increased after seven months (**Figure 9b**). It should be noted that the level of AI in HCD 1 ODD was significantly higher than the level of AI in the HCD 1 ONDD and LCD groups ($p<0.05$). As a consequence, AI in *P. obesus* subjected to the high-calorie diets, HCD 0, HCD 1 and HCD 2, increased significantly after 7 months ($p<0.05$).

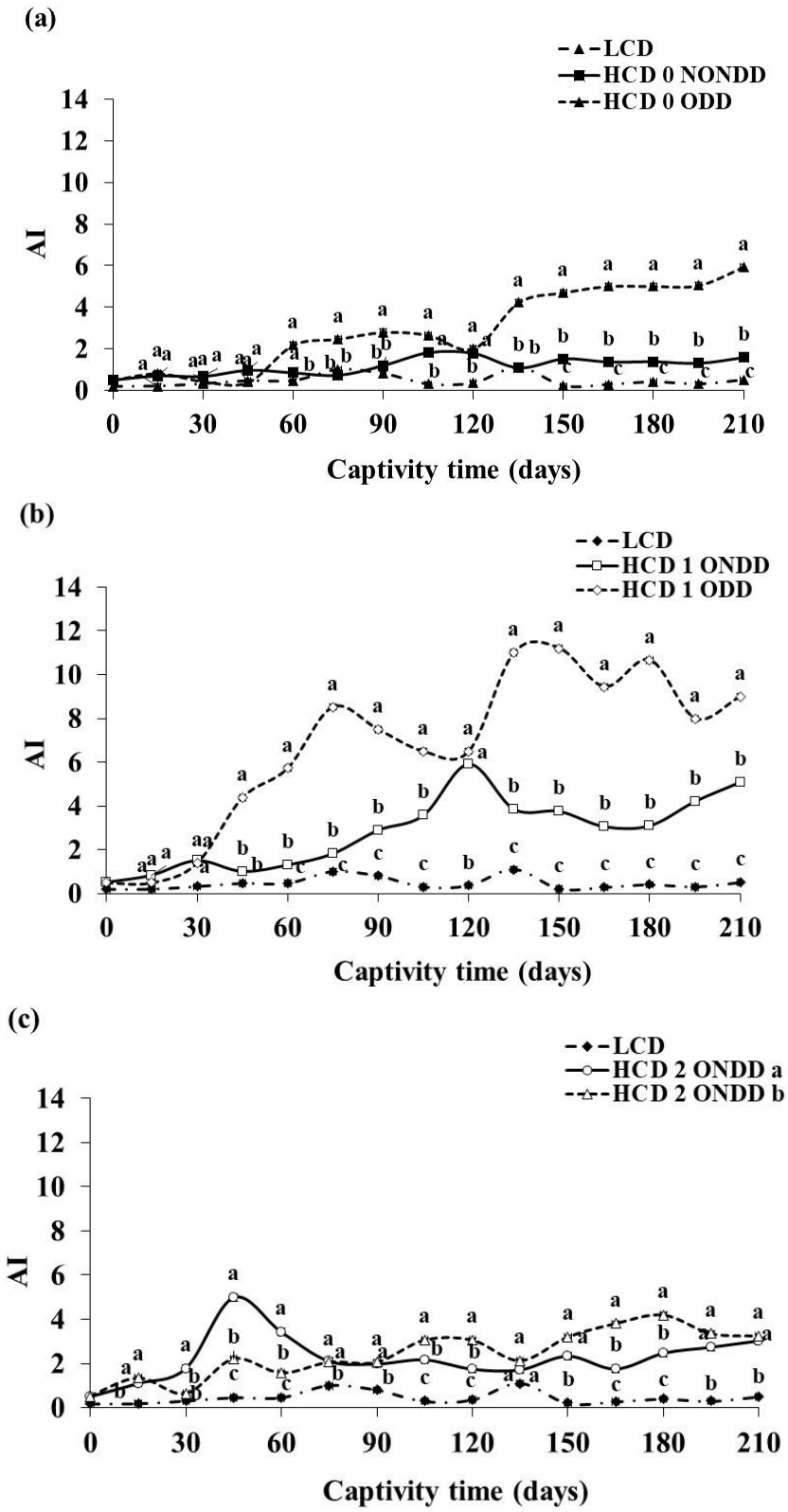


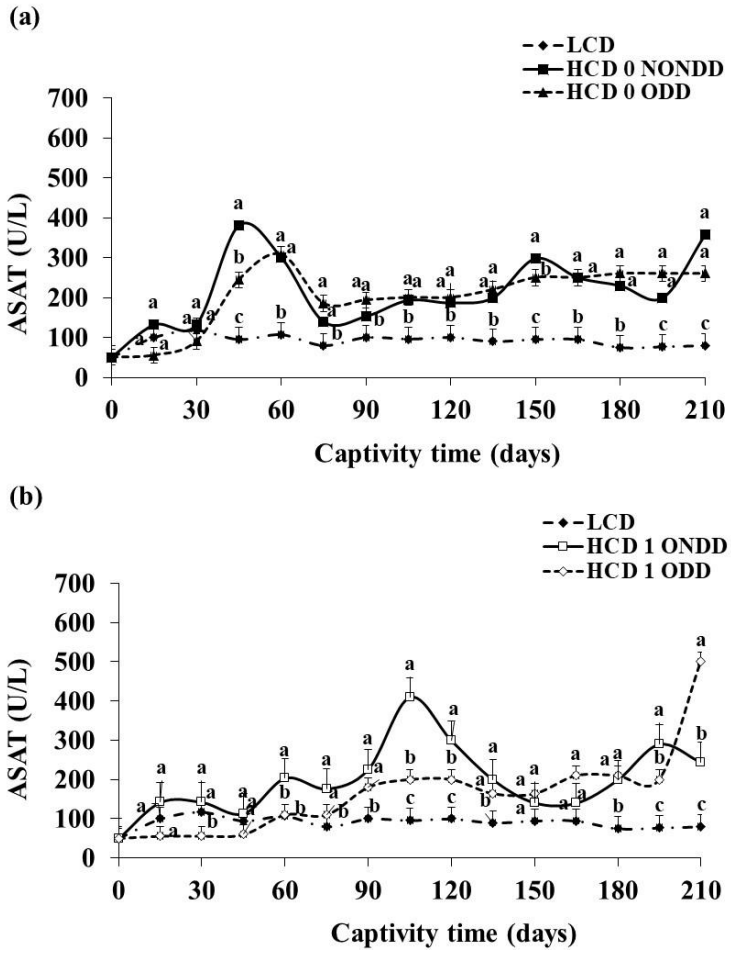
Figure 9. *Atherogenic index (AI) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

According to the results mentioned above, HCD 1 and HCD 2 have been confirmed as formulations inducing dyslipidemia in *P. obesus* (100%) on day 30. Whereas HCD 0 induces dyslipidemia in 40% of animals on day 30 and in 60% of animals on day 90.

2.7. KINETICS OF ASPARTATE AMINO-TRANSFERASE, ALANINE AMINO TRANSFERASE AND LIVER WEIGHT OF *PSAMMOMYS OBESUS*

Figure 10 and Figure **11** present Aspartate Amino-Transferase (ASAT) and Alanine Amino-Transferase (ALAT) activities, which are considered the primary indicators of hepatocellular damage. The results of the time-dependent ASAT and ALAT assays revealed that obese and diabetic *P. obesus* fed the different high-calorie diets, HCD 0 and HCD 1, showed a significant increase in ASAT and ALAT levels from the 30th day of the experiment. In the control group (*P. obesus* fed Low-Calorie Diet, LCD, *chenopodiaceae*), ASAT and ALAT levels ranged from 100 to 120 U/L and 99 to 100 U/L, respectively. ASAT levels ranged from 55 to 280 U/L in the HCD 0 ODD subgroup and from 56 to 680 U/L in the HCD 1 ODD subgroup, while ALAT levels ranged from 64 to 210 U/L in the HCD 0 ODD subgroup and from 64 to 180 U/L in the HCD 1 ODD subgroup.

These results revealed that the different high calorie diets (HCD 0, HCD 1, HCD 2) induced a significant increase in ASAT and ALAT activities, compared with LCD *P. obesus* whose high levels could be explained by stress due to captivity (**Figure 10 and Figure 11**).



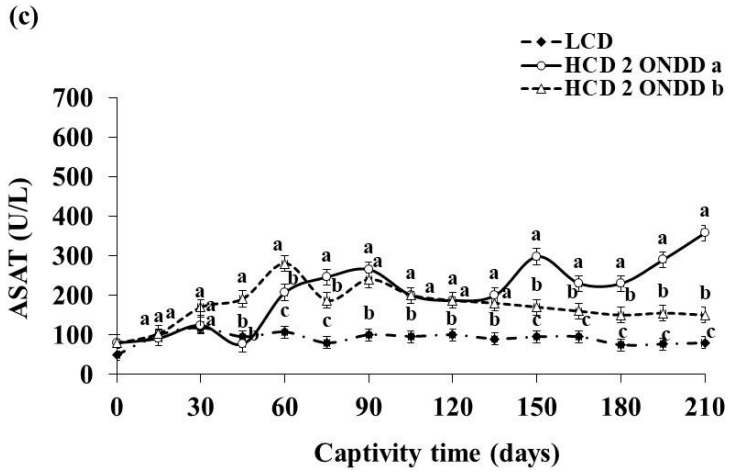
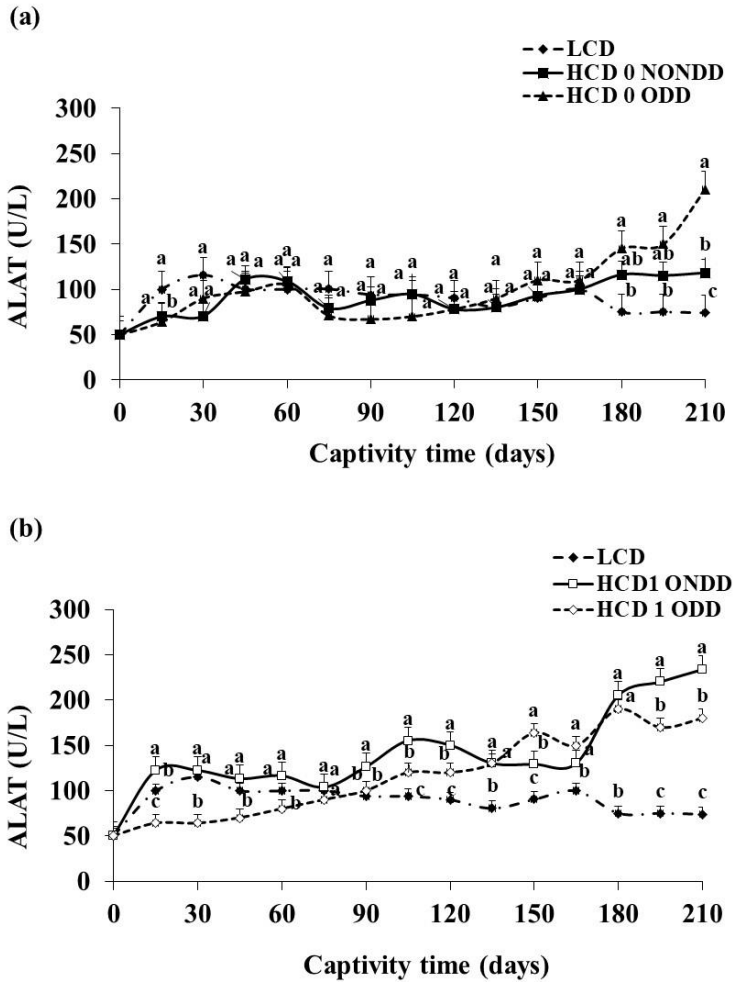


Figure 10. *Enzymatic activities of Aspartate Amino-Transferase (ASAT) (U/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*



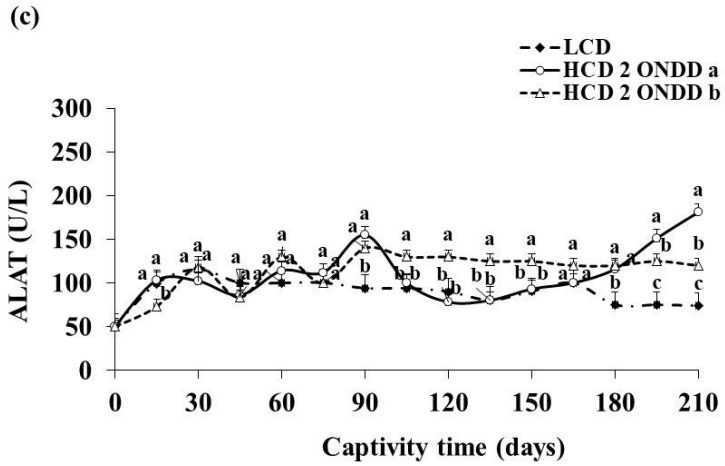
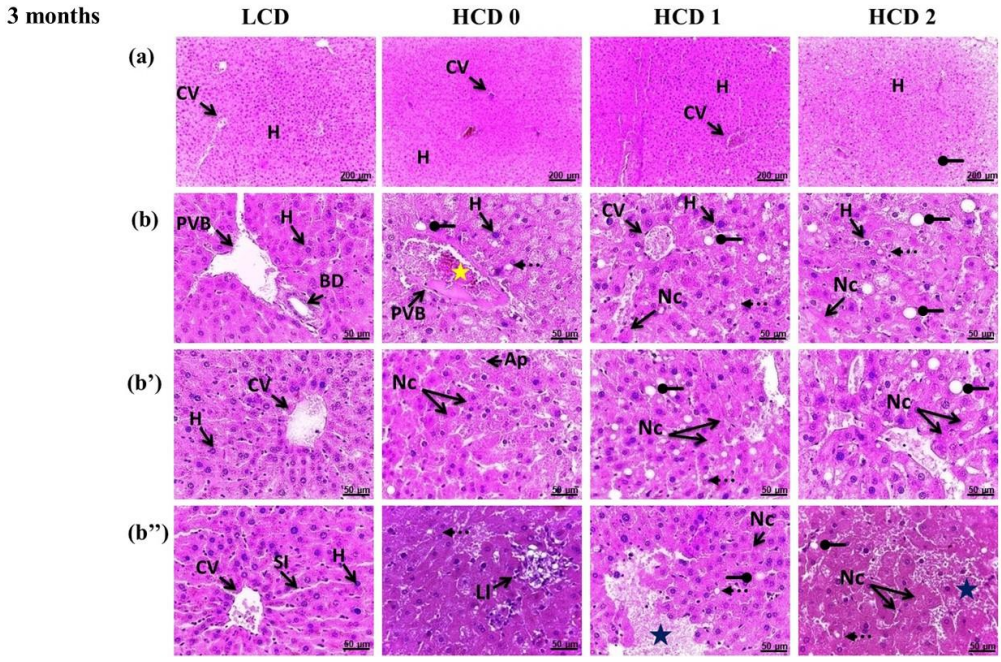


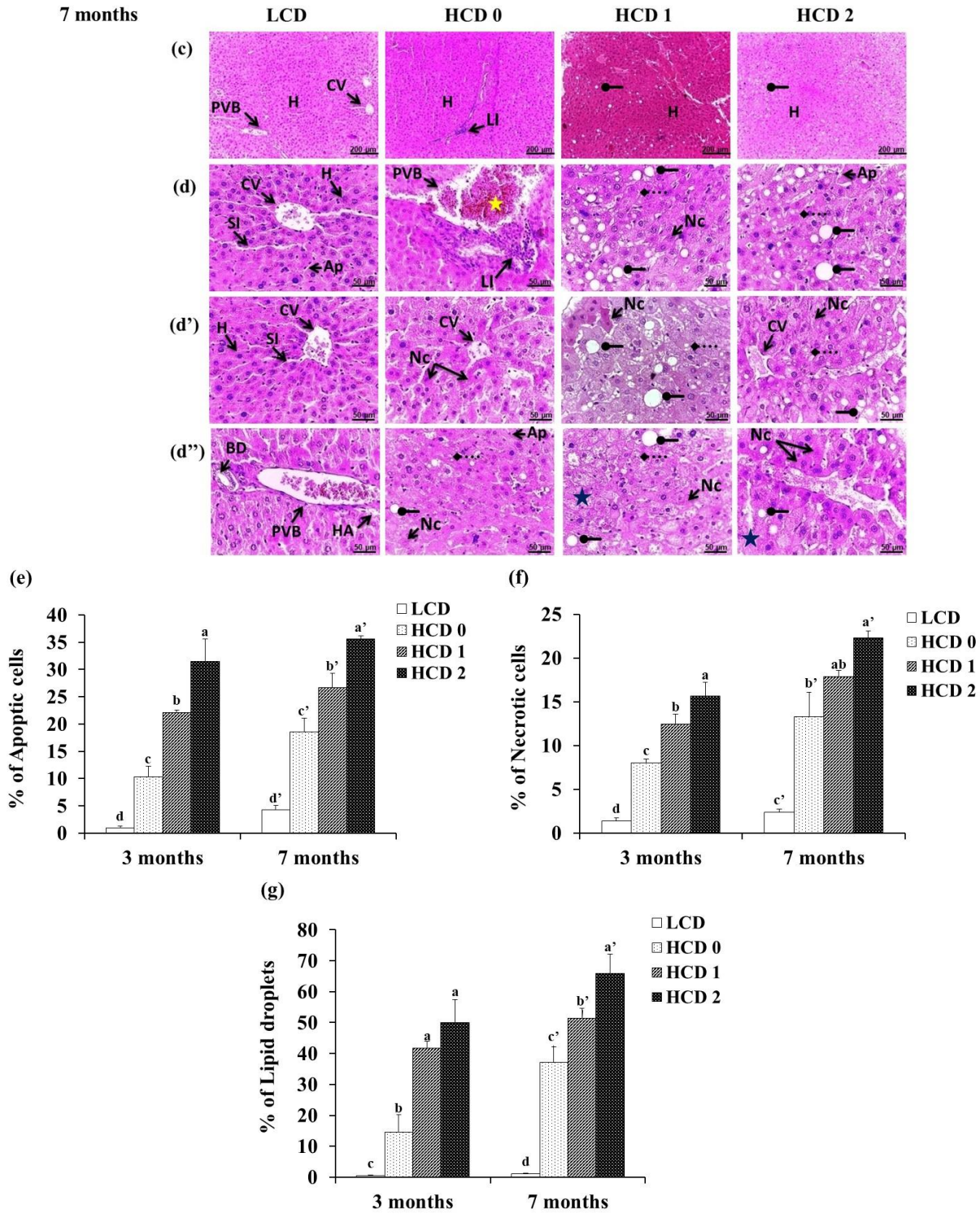
Figure 11. *Enzymatic activities of Alanine Amino-Transferase (ALAT) (U/L) in P. obesus subject to different diets for seven months. The results are expressed as means ± SD. Means not sharing the same superscript letters (a, b, c) for each captivity time are significantly different between groups (Tukey's post hoc test, p< 0.05). N=7 animals for each group.*

HISTOPATHOLOGICAL CHANGES

1. Effect of high calorie diets on histopathological changes in the liver

The histological study carried out on liver tissue at months 3 and 7 in animals fed low-calorie (LCD) and high-calorie diets (HCD 0, HCD 1, HCD 2) confirms the biochemical results observed above. Indeed, the histology of *P. obesus* livers from the LCD group showed normal hepatic cell architecture, a clearly visible centro-lobular vein, the presence of normal bile ducts and the absence of any cellular necrosis or inflammation (**Figure 12 a-b"**). Structural alterations were observed in the liver tissue of animals fed HCD. The microscopic analysis of histological sections from HCD rats revealed significant changes after 3 months, including the appearance of apoptotic and necrotic cells, significant cytoplasmic vacuolization with a major accumulation of lipid droplets, and damage to hepatic sinusoids with remarkable vascular congestions (**Figure 12 b-b"**). At month 7 of the different HCD (**Figure 12 d-d"**), severe alterations were observed in the liver tissue of groups HCD 0, HCD 1 and HCD 2, characterized by severe dilatation of the centrolobular vein, intense dilatation of the portal space and inflammatory infiltrates. The appearance of fibrosis, chromatin condensation and dislocation of the centrilobular vein were also noted, along with significant sinusoidal enlargement and extensive cytoplasmic vacuolization. On the other hand, we detected increased apoptotic and necrotic cells, and the formation of hepatic steatosis characterized by excessive accumulation of lipid droplets, especially TGs in cytoplasmic vesicles.




7 months
LCD
HCD 0
HCD 1
HCD 2
(c)
(d)
(d')
(d'')
PVB
H
CV
LI
SI
Ap
Nc
BD
HA
(e)
% of Apoptic cells
(f)
% of Necrotic cells
(g)
% of Lipid droplets
3 months
7 months
LCD
HCD 0
HCD 1
HCD 2

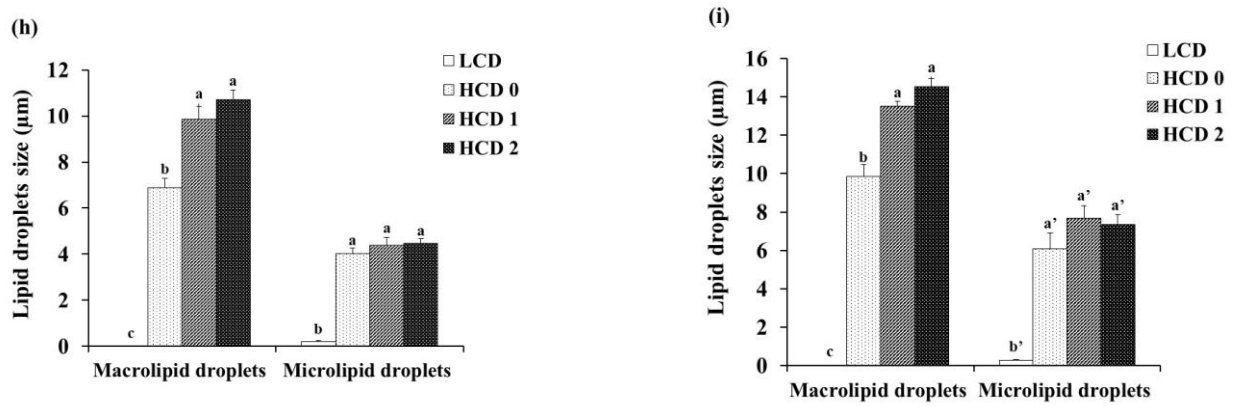
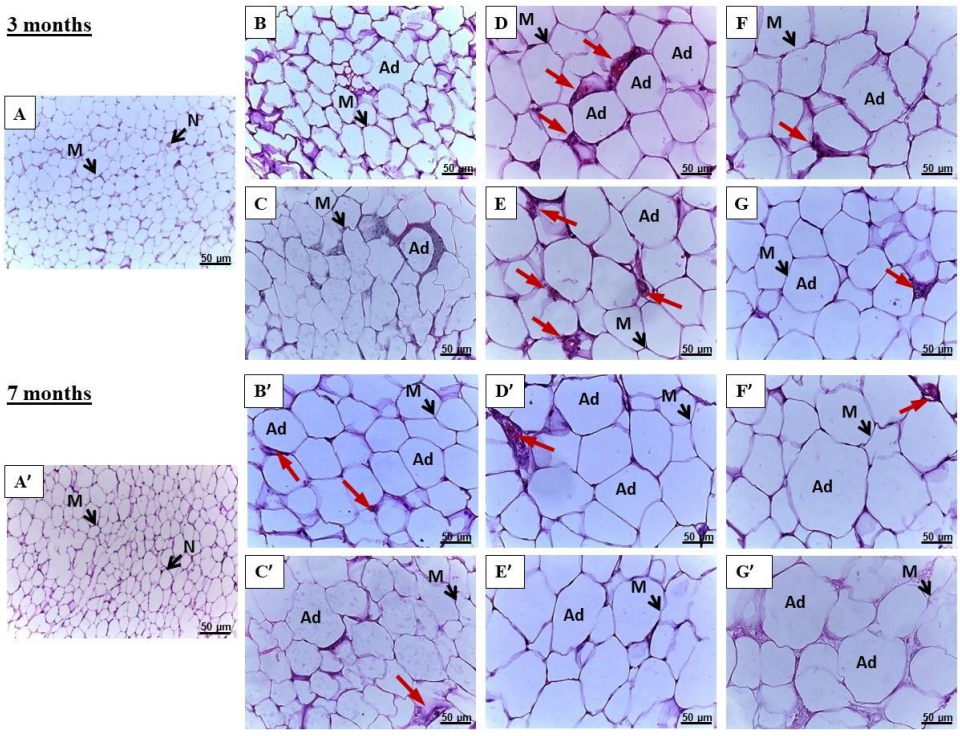


Figure 11. *Histological analysis of liver tissues by hematoxylin-eosin (H&E) staining during 3 months (a: magnification, 10×; scale bar = 200 µm), (b, b', b'': magnification, 40×; scale bar = 50 µm) and 7 months (c: magnification, 10×; scale bar = 200 µm), (d, d', d'': magnification, 40×; scale bar = 50 µm). CV: centrolobular vein, SI: sinusoids, H: hepatocyte, Ap: apoptic cell, Nc: necrotic cell, PVB: portal vein branch, PT: portal triad, HA: hepatic artery, BD: bile duct, LI: inflammatory leukocyte infiltrations. The continuous spherical heads arrows indicate: macrovesicular droplets; the discontinuous arrows indicate: microvesicular droplets. (e) Apoptic cells, (f) Necrotic cells and (g) Lipid droplet quantification expressed in percentage of the number of hepatic cells. Lipid droplet size quantification expressed in squared micrometer (µm²) during 3 months (h) and 7 months (i). Means ± SD for triplicate analyses. Values not sharing a common superscript differ significantly (Tukey's post hoc test, p< 0.05). N=28 total.*

2. Effect of high calorie diets on histopathological changes in adipose tissue

The histological architecture of the adipose tissue was analyzed in the different groups of *P. obesus* at 3 and 7 months. Sections from the LCD group (**Figure 12A, A'**) showed no abnormalities in adipocytes even after 7 months. Histological evaluation of the adipose tissue after 3 months in the different high-calorie diet groups, HCD 0, HCD 1 and HCD 2, revealed an abnormal architectural observation (hypertrophied adipose cells, inflammation). By the 7th month of HCD, adipose damage was extensive, with numerous foci of inflammation (**Figure 12 B'- G'**). Adipocyte size was significantly greater after 7 months than after 3 months of the various hypercaloric diets in the HCD 2 group (70.61 ± 4.44 µm²), followed by the HCD 1 (49.15 ± 2.42 µm²) and HCD 0 (35.09 ± 0.89 µm²) groups, compared with the LCD group (13.74 ± 0.54 µm²) (**Figure 12 a**).



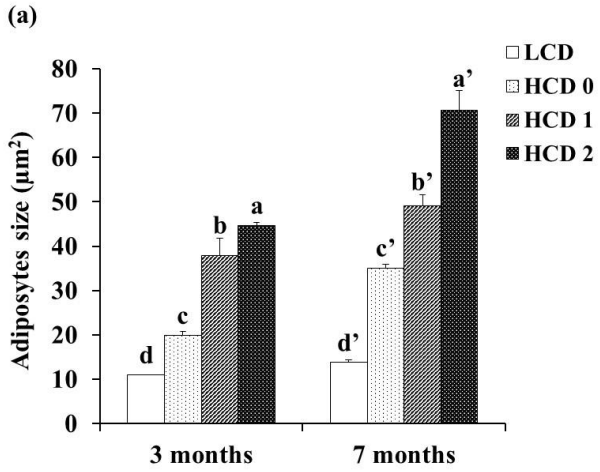
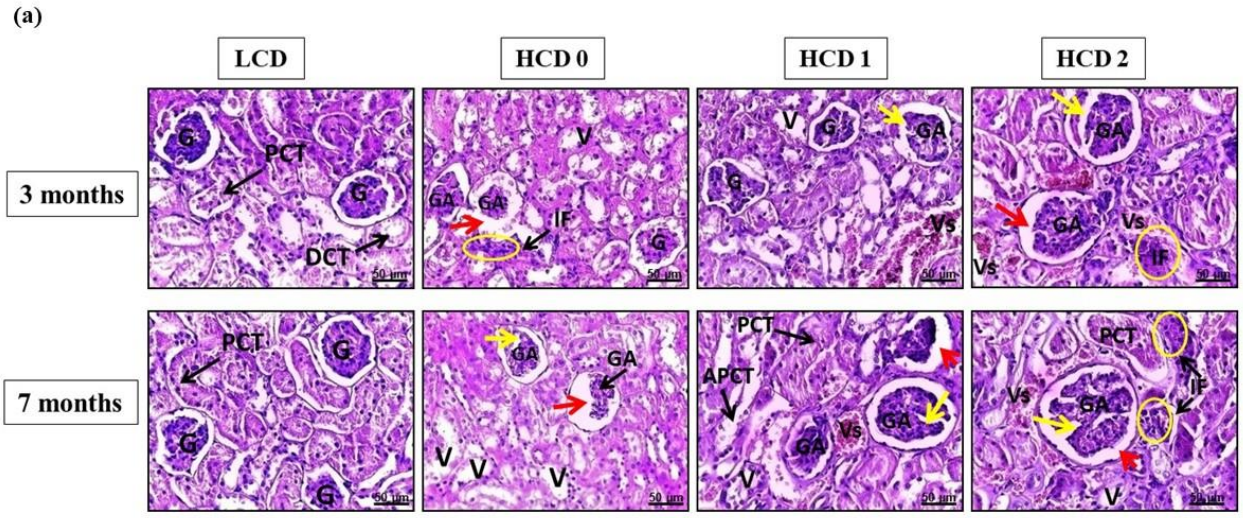


Figure 12. *Histological analysis of adipose tissue by hematoxylin-eosin (H&E) staining (40× magnification; scale bar = 50 µm). Adipose sections of LCD (A-A'), HCD 0 (B-C, B'-C'), HCD 1 (D-E, D'-E') and HCD 2 (F-G, F'-G'). Ad: Adipose cell, M: Cell membrane, N: Nucleus, C: Cytoplasm, Red arrows: Inflammation. (a) Size of adipocytes are expressed in squared micrometer (µm²). Means ± SD for triplicate analyses. Values not sharing a common superscript differ significantly (Tukey's post hoc test, p< 0.05). N=28 total.*

3. Effect of high calorie diets on histopathological changes in kidneys

The architecture of kidney tissue in control *P. obesus* (LCD group) shows the presence of the same glomeruli with clearly marked membranes (**Figure 13 a**). In fact, after 7 months, there were severe structural alterations in the cortical zone where Bowman's capsules adhered to the glomeruli, leading to an enlargement of Bowman's space, as shown by the red arrows. Granular degeneration of distal and proximal tubules, vascular congestion, inflammation (circle) and glomerular fragmentation (yellow arrows), were observed in the different HCD (**Figure 13 a**). These histological changes in kidney tissue were more marked after 7 months in the kidneys of the HCD 1 and HCD 2 groups than in those of the HCD 0 group (**Figure 13 a**).

The HCD 2 (70.61 ± 4.44 µm2), HCD 1 (49.15 ± 2.43 µm²) and HCD 0 (35.09 ± 2.43 µm²) groups showed a significant increase ($p< 0.05$) in glomerular size compared with the LCD group (13.74 ± 0.54 µm²) (**Figure 13 b**).



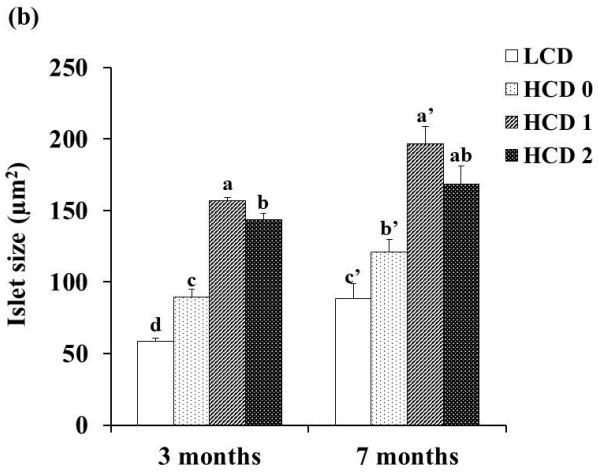
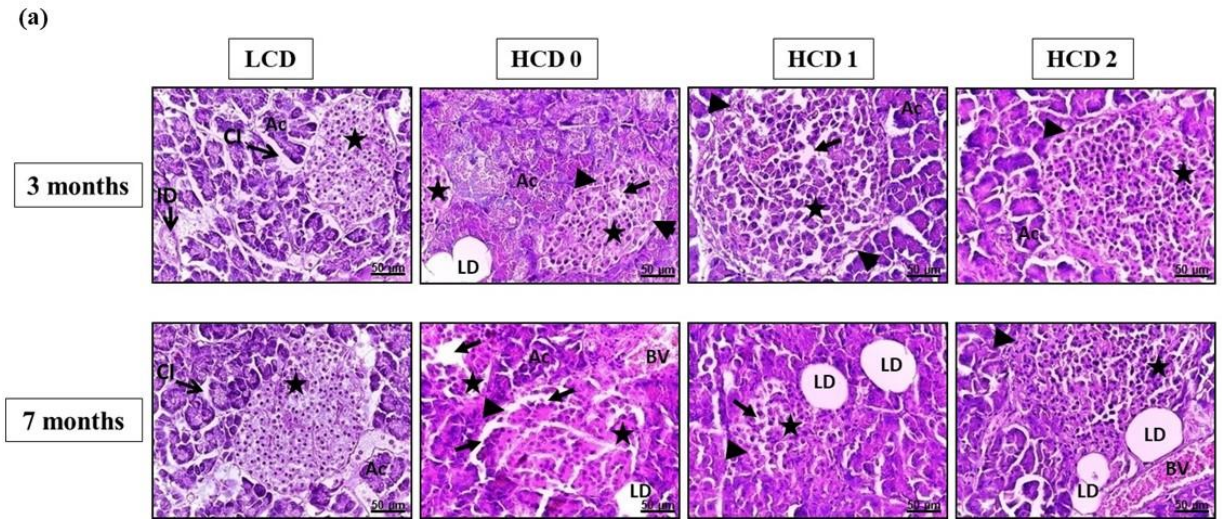


Figure 13. *Histological sections of kidney in control (LCD) and treated (High Calorie Diet: HCD 0, HCD 1 and HCD 2) P. obesus during 3 and 7 months. (a) Sections were stained with hematoxylin–eosin (40× magnification; scale bar = 50 µm). G: Normal Glomeruli, GA: Glomeruli atrophy, PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, ADCT: Atrophy distal convoluted tubules, V: Vacuolization, Vs: Vascular congestion, IF: Inflammation (circle), Yellow arrows indicate: glomeruli fragmentation, red arrows indicate: Bowman's space enlargement. (b) Size of glomerular are expressed in squared micrometer (µm²). Means ± SD for triplicate analyses. Values not sharing a common superscript differ significantly (Tukey's post hoc test, p< 0.05). N=28 total.*

4. Effect of high calorie diets on histopathological changes in the pancreas

Histopathological examination of the pancreas of rats subjected to the different high-calorie diets (HCD 0, HCD 1 and HCD 2) revealed degenerative changes after 3 months such as morphologically remarkable lesions of islets and acinar cells and reduction of β-cells, apoptosis and necrosis in islet cells (**Figure 14a**). In addition, there is the accumulation of lipid droplets, congested blood vessels, vacuolated cells and dilated interlobular ducts. These changes are marked when compared with pancreatic sections from the LCD group, which show a typical histological organization. In terms of pancreatic tissue, the various high-calorie diets (HCD 0, HCD 1 and HCD 2 groups) induced at month 7, generalized pancreatic damage, with degeneration of the β-cells, which became atrophied, disappearance or shrinkage of the islets of Langerhans, numerous necrotic cells and numerous lipid droplets (**Figure 14a**). A significant increase ($p<0.05$) in pancreatic islet size was observed after 7 months in the HCD 2 (168.70 ± 12.21 µm²) and HCD 1 (196.48 ± 12.02 µm²) groups compared with the LCD (88.28 ± 10.45 µm²) and HCD 0 (120.98 ± 9.02 µm²) groups (**Figure 14b**).



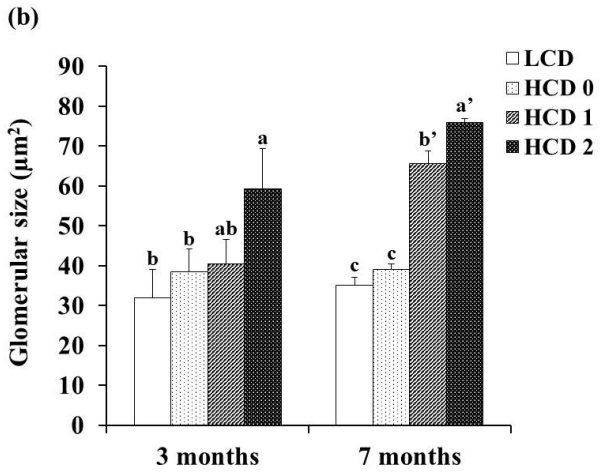


Figure 14. *Histological analysis of pancreas by hematoxylin-eosin (H&E) staining (40× magnification; scale bar = 50 µm) during 3 and 7 months (a). Stars indicate: Islet of Langerhans, IS: Interlobular septa, Ac: Acinar cells, BV: Blood vessels, LD: Lipid droplets, arrows indicate: reduced, dilated and congested islets cell with vacuolated cytoplasm, arrowheads indicate: Shredded islets with irregular contour, arrows with spherical heads indicate: dilated interlobular duct. (b) Size of islets are expressed in squared micrometer (µm²). Means ± SD for triplicate analyses. Values not sharing a common superscript differ significantly (Tukey's post hoc test, p< 0.05). N=28 total.*

5. Effect of high calorie diets on histopathological alterations in *p. Obesus* retina

The four animal groups (LCD, HCD 0, HCD 1 and HCD 2) have distinct morphological modifications in the *P. obesus* retina. Low Calorie Diet (LCD) retinas showed regular layers with a thin inner limiting basement membrane (**Figure 15a**), whereas HCD 0 retina slices showed a reduced thickness of retinal layers and thin irregular nuclear layers after 3 and 7 months of hyperglycemia in comparison with LCD groups. This was also seen more reduction in HCD 1 and HCD 2 retinas. The results of this study show that *P. obesus* retina has a thinned and scalloped appearance in the HCD 0 group and more in the HCD 1 and HCD 2 groups after 3 and 7 months of different HCD animal groups developing obesity, hyperglycemia and dyslipidemia. This study also indicates a larger cell density and a clear reduction in the number of cells in the ONL, INL and GCL in HCD 0 and more in HCD 1 and HCD 2 groups as compared with the LCD group (**Figure 15a**). This observation was confirmed by a quantitative measurement of retinal layer thickness at months 3 and 7 (**Figure 15 b-c**). A significant decrease in the retinal pigment epithelium (RPE: ~ -59%, -38%, -46% / -58%, -69%, -52%), photoreceptor outer segment (POS: ~ -35%, -68%, -57% / -7%, -62%, -34%), outer nuclear layer (ONL: ~ -27%, -41%, -33% / -58%, -77%, -82%), outer plexiform layer (OPL: ~ -40%, -60%, -43% / -29%, -50% -43%), inner nuclear layer (INL: ~ -13%, -67%, -63% / -36%, -53%, -32%), inner plexiform layer (IPL: ~ -57%, -70%, -64% / -23%, -59%, -54%) and retinal ganglion cell layer (GCL: ~ -27%, -55%, -43% / -74%, -82%, -66%) was noticed in HCD 0, HCD 1 and HCD 2 versus LCD animals, respectively.

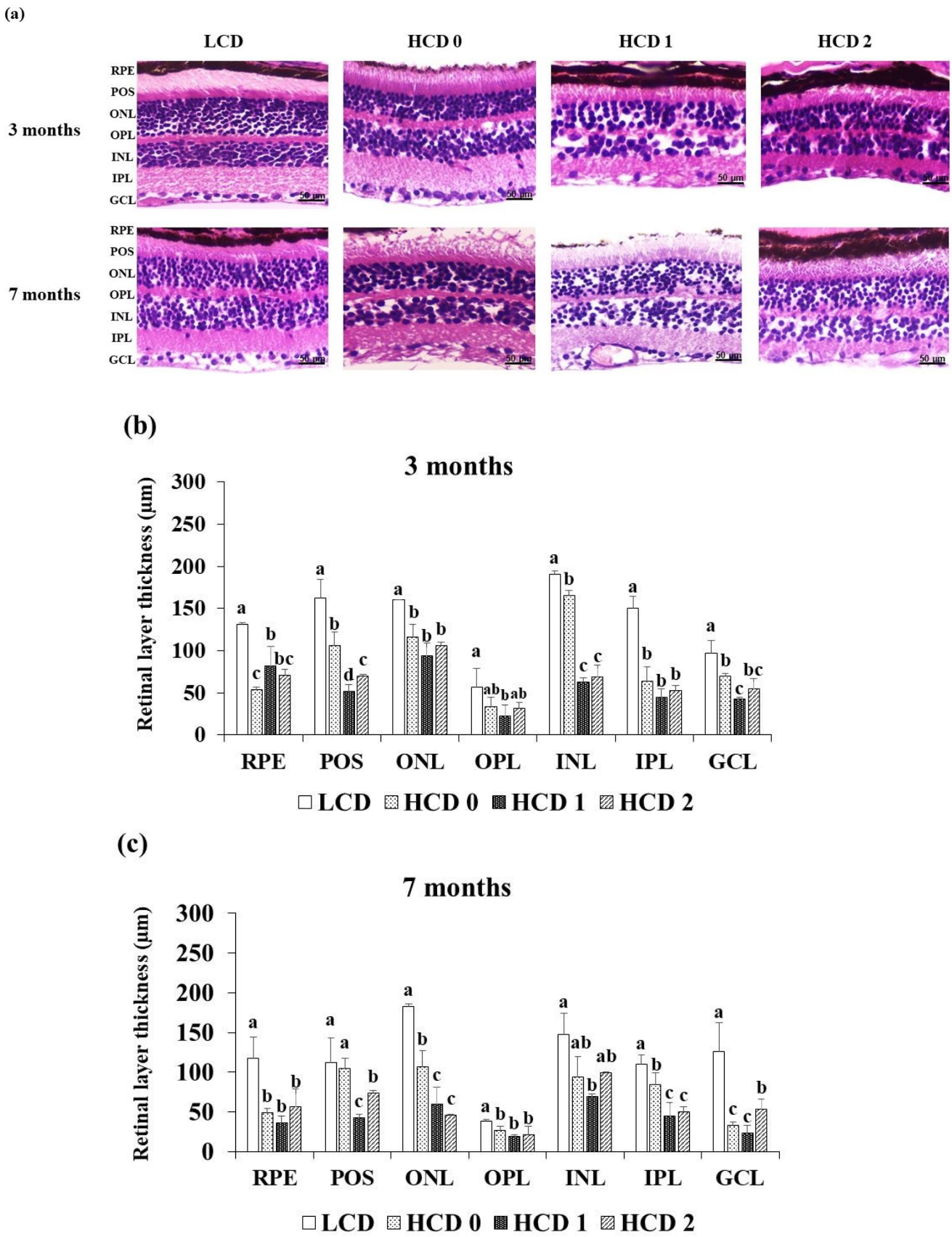


Figure 15. *(a) Retinal morphology and (b-c) Quantitative measurements of retinal thickness in control (LCD) and treated (High Calorie Diet: HCD 0, HCD 1 and HCD 2) P. obesus during 3 and 7 months. Sections were stained with hematoxylin–eosin (40× magnification; scale bar = 50 µm). Data are expressed as means ± SD. Values not sharing a common superscript differ significantly (Tukey's post hoc test, p< 0.05). N = 28 total.*

Discussion

The present study aims to investigate the influence of different high-calorie diets (HCD) on biochemical blood markers and histological organ damage in the Tunisian rodent model *P. obesus* over three and seven months. The animals fed high-calorie diets rich in carbohydrate and richer in fat, showed less food consumption than the other high calorie diet groups. This may be explained by the fact that a high-fat diet could induce food rejection in rats[17]. This result is similar to that observed in Wistar rats[18]. However, other studies report that a high-fat diet induces less satiety and therefore leads to higher levels of food intake[19]. These contradictory findings may be linked to the variable feeding behavior of animals under stressful conditions. During the second week of the different high-calorie diets rich in carbohydrates and fat, a significant reduction in body weight was observed in the different animal groups. This is probably due to a stage of adaptation to living in captivity and/or stressful conditions [20].

After three and seven months of receiving the high calorie diets, *P. obesus* developed obesity by increasing lipid storage in metabolic organs such as adipose tissue and the liver. Similar studies showed that *P. obesus* develops obesity after 3 months of HCD (3.25 and 3.85 kcal/g, respectively)[11, 12, 21]. Several studies carried out on Wistar rats confirmed that the consumption of HCD (3.65 kcal/g) increases body weight and induces an accumulation of lipids in adipose tissue after 6 months of HCD[22]. The increase in body weight as a consequence of the increased storage of body fat has been observed in many studies investigating the relationships between diets supplemented with either fats or carbohydrates and induced metabolic disorders[18,22, 23].

Excessive fat and carbohydrate consumption can affect the liver[24], leading to increased relative liver weight in *P. obesus* fed a HCD compared with a low-calorie diet based on *chenopodiaceae*, as triglycerides accumulate in adipose tissue and liver. Similar results were observed in Wistar rats fed a high-fat diet[25]. In contrast to its inhibitory effect on cholesterol biosynthesis, cholesterol from food has been shown to stimulate hepatic fatty acid biosynthesis. Subsequently, these metabolic disorders can lead to serious diseases, including T2D and non-alcoholic fatty liver disease[26]. Rats fed HCDs showed an increase in TC and TG levels, which eventually triggered the development of lipo-toxicity and lipid accumulation in the liver[25]. However, this accumulation of fat is considered non-alcoholic fatty liver disease and evolves into steatosis[27].

After three and seven months, the adiposity index increases because of the increase in body weight in animals fed high calorie diets. Several studies suggest that hypertrophy of adipose tissue is found as soon as overweight appears and precedes hyperplasia[28]. In contrast, hyperplasia is a characteristic of severe or morbid obesity. Actually, adipose hypertrophy is an independent risk factor for developing T2D[29]. According to previous studies, animals subjected to HCD rich in sugars and oils exhibited hyperglycemia. In fact, the saturated fats in high calorie diets. are responsible for the increase in carbohydrate profiles[30]. The high calorie diets were effective in promoting the development of metabolic disorders in different animal groups that are manifested by an increase in body weight and body fat accompanied by hyperglycemia which are the major characteristics of T2D qualified by diabesity. Similar results were found when *P. obesus* was subjected to a high calorie diet highlighting the appearance of the same physiological disorders[31]. Hyperglycemia is linked not only to hyperinsulinemia due to the high calorie diet but also to a decrease in the activity of insulin receptors especially since *P. obesus* naturally has a lower number of insulin receptors at the level of the liver and muscle[32]. In addition, hyperglycemia is linked to an increase in the proportion of pro-insulin in the bloodstream following hyper-stimulation of pancreatic β cells[33].

According to the literature, hypercholesterolemia is strongly associated with metabolic dysfunction and T2D, which confirms our results. Moreover, the oleic acid in corn oil used in food formulation (37%) can be converted to acetate and then to cholesterol in the presence of glucose[34]. Sugars associated with fats have also been shown to increase cholesterol biosynthesis or facilitate metabolic pathways leading to hypercholesterolemia[34]. During a seven-month period of high calorie diet, the hepatic tissue can convert glucose into fatty acids, from which TG is manufactured and transported to the blood stream as very low-density lipoprotein cholesterol (VLDL) and stored as fat in the adipose tissue[35].

Metabolic syndrome and dyslipidemia, seem to be the result of the development of insulin resistance in peripheral tissues leading to an enhanced hepatic flux of fatty acids from dietary sources, intravascular lipolysis and adipose tissue resistance to the antilipolytic effects of insulin[36]. High levels of serum TG observed in high-calorie groups are generally associated with increased VLDL secretion, through which lipolysis could produce HDL[34]. This may explain the high levels of HDL observed in HCD 1 ONDD after 7 months. The saturated fatty acids (14%) present in the corn oil could increase the

production of TG and TC by the liver and could also decrease the catabolism of LDL by repressing their receptors[37], resulting in the high levels of LDL observed in HCD 1 ODD rats. HCD 1 ODD and HCD 2 ONDD sub-groups had increased TC and LDL compared with the LCD group, and HCD 1 ODD sub-groups had the highest LDL concentrations. This fact was explained by the decreased HDL observed in HCD 1 ODD rats after 7 months, thus decreasing the reverse cholesterol transport from the bloodstream to the liver[37].

High-calorie diets rich in lipids and carbohydrates induced the disturbances in the *P. obesus* lipid profile. These metabolic disturbances could be due to diabetic stress after a sudden change in diet[11]. When *P. obesus* was fed a low-calorie natural diet composed of halophyte plants (*Chenopodiaceae*), it never developed any pathology[11]. After seven months in *P. obesus* fed high calorie diets, hyperglycemia and weight gain were observed in different subgroups (HCD 0 ODD, HCD 1 ONDD, HCD 1 ODD and HCD 2 ONDD) and were associated with dyslipidemia which is closely associated with obesity.

The results of this study indicate that high calorie diets rich in lipids and carbohydrates induce glucose intolerance. Furthermore, the two subgroups HCD 0 ODD and HCD 1 ODD showed a loss of body weight and T2D complications with hyperglycemia and dyslipidemia at month seven. These results are due to the adverse effect of high glucose levels on the pancreas, which induces apoptosis and pancreatic B-cell necrosis and consequently a considerable decrease in insulin levels[38]. Our results are similar to those reported by Sihali-Beloui et al. (2016)[11]. Another study showed that chronic exposure of pancreatic islets to high amounts of nutrients (glucose, fatty acids), induces beta cell dysfunction and cell death, which results at the systemic level in a decrease in insulinemia[39]. The present study demonstrates that seven months of HCD enriched with sugar and oil were associated with an increase in circulating TC, TG, HDL and LDL levels, strongly suggesting a major dyslipidemia feature in *P. obesus* gerbils. Hyperlipidemia is also closely associated with the development of diabetic retinopathy[6]. The results of the present study are similar to those of Spolding et al. (2014)[40]. The serum ASAT and ALAT levels are also affected by stress and cortisol released by the adrenal glands. Adrenal insufficiency has been shown to correlate with the progression of liver disease and elevated ASAT and ALAT levels[41]. Moreover, similar results have already been reported by other researchers. Increased activity of aminotransferases (ALAT and ASAT) is an indicator of cellular leakage and failure of membrane functional integrity resulting from liver damage[42].

The increase in serum lipid profile and hepatic enzymes such as ASAT and ALAT activity levels is in agreement with the analysis of the histology of the various tissues such as the liver, adipose tissue, kidney, pancreas and retina. After seven months of high-calorie diets rich in different concentrations of carbohydrates and fats, liver damage was confirmed through increased apoptotic and necrotic cells, and the formation of hepatic steatosis characterized by excessive accumulation of lipid droplets. Spolding et al. (2014) reported that after 4 weeks of the standard laboratory HCD enriched with 2% cholesterol, *P. obesus* contributes to the development of hepatic steatosis, comparable to that of humans[40].

High-calorie diet rich in fat and in carbohydrate induced severe alterations in adipose tissue that correlate with the development of obesity. Our results are in agreement with those of Jung & Choi (2014) who demonstrated that excess adipose tissue contributes to the development of metabolic diseases and leads to adipokine secretion and adipose tissue deregulation[43].

The effects of a high-calorie diet rich in fat on the renal structure of rats are responsible for obesity and may lead to renal deformities as a result of histopathological changes. Our study highlighted the association between high-calorie intake, obesity, and type 2 diabetes, which are known causes of renal disease. It is reported that obesity and type 2 diabetes can influence the progression of chronic kidney disease due to their direct effects on renal hyperfiltration, increased glomerular pressure, and podocyte damage[44]. These findings underscore the complex interplay between diet, metabolic disorders, and renal health, and they emphasize the potential role of HCD in the pathogenesis of diabetic nephropathy in rat models. For the pancreatic tissue, our results suggest that all alterations indicate physiological and metabolic alterations associated with diabetes and its complications. This result is in accordance with the findings of Madić et al. (2020)[45]. Moreover, it has been shown that high-calorie diets, especially those high in fat and carbohydrate, are associated with early impairments in the retina, which are linked to glucose metabolism deregulation[46]. Additionally, high-fat diets have been found to profoundly affect vision, retinal function, and various ocular tissues through a variety of mechanisms[47]. Three and seven months of obesity, diabetes and dyslipidemia induced by high calorie diets (HCD 0 richer in carbohydrates and protein than HCD 1 and HCD 2,

HCD 1 richer in fats and richer in carbohydrates than HCD 2, and HCD 2 richer in carbohydrates and richer in fats than HCD 1), caused major damage to the retina in *P. obesus*, affecting photoreceptors. These histological abnormalities of the retinal layers indicate diabetic retinopathy. These results are in agreement with those found in seven months diabetic *P. obesus*[15] and Ins2Akita mice[48]. Many studies have shown that hyperglycemia leads to a significant decrease in the number of GCL cells, causes a loss of 20% to 25% of GCL in the peripheral retina in C57BL/6J mice at four months[49] and 44% of GCL in *P. obesus* at five to six months[15], accompanied by a significant decrease in the thickness of the INL. Other studies in mice and rats demonstrate no loss of different cell layers, such as GCL during one year of diabetes[50].

The current study provides a baseline for a better understanding of nutritional pathophysiological-metabolic disorders. Elucidating the morphological changes in liver, renal, retinal, and adipose tissue, would facilitate the design of new strategies to prevent hepatic, renal, adipose and visual dysfunction associated with both nutritional and metabolic pathologies. The effect of high-calorie diets on other biomarkers produced by the target organs will be explored in future studies.

Conclusion

Psammomys obesus subjected to seven-month high calorie diets showed an alteration of the serum lipid profile. The induced metabolic disorder leads to the accumulation of lipids and consequently the development of obesity, dyslipidemia (hypertriglyceridemia and hypercholesterolemia) with a subsequent increase in the activity of transaminases (ASAT and ALAT) associated with hyperglycemia. Histopathological examination of the liver, adipose tissue, kidneys and pancreas revealed the harmful effects of the different high-calorie diets, manifested by the increased size of adipocytes, glomeruli and islets of Langerhans, as well as increased hepatic lipid droplet accumulation, apoptosis, necrosis and inflammation. Induced nutritional dyslipidemia and diabesity in *P. obesus* confirms that it is an excellent animal model for studying pathophysiological-metabolic disorders and their complications. It can be used in testing compounds toxicity, validating therapeutic and/or preventive new bioactive compounds that cannot be carried out directly in humans.

Conflicts of Interest Statement

The authors have no conflict of interests

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References

1. Feng K, Zhu X, Chen T, Peng B, Lu M, Zheng H, Huang Q, Ho CT, Chen Y, Cao Y. Prevention of obesity and hyperlipidemia by heptamethoxyflavone in high-fat diet-induced rats. *J. Agric. Food Chem.* 2019; 67(9): 2476-2489. https://doi.org/10.1021/acs.jafc.8b05632.
2. Baker KD, Loughman A, Spencer SJ, Reichelt AC. The impact of obesity and hypercaloric diet consumption on anxiety and emotional behavior across the lifespan. *Neurosci. Biobehav. Rev.* 2017; 83: 173-182. https://doi.org/10.1016/j.neubiorev.2017.10.014.
3. Dow C, Mancini F, Rajaobelina K, Boutron-Ruault MC, Balkau B, Bonnet F, Fagherazzi G. Diet and risk of diabetic retinopathy: a systematic review. *Eur. J. Epidemiol.* 2018; 33(2): 141-156. https://doi.org/10.1007/s10654-017-0338-8.
4. Jiang B, Liang Y, Sun X, Liu X, Tian W, Ma X. Potent inhibitory effect of chinese dietary spices on fatty acid synthase. *Plant Foods Hum. Nutr.* 2015; 70(3): 257-262. https://doi.org/10.1007/s11130-015-0486-5.
5. Dharmalingam M, Yamasandhi PG. Nonalcoholic fatty liver disease and type 2 diabetes mellitus. *Indian J. Endocrinol. Metab.* 2018; 22(3): 421.https://doi.10.4103/ijem.IJEM_585_17
6. Kowluru RA. Retinopathy in a diet-induced type 2 diabetic rat model and role of epigenetic modifications. *Diabetes*. 2020; 69(4): 689-698. https://doi.org/10.2337/db19-1009.
7. Ronkainen J, Huusko TJ, Soininen R, Mondini E, Cinti F, Mäkelä KA, Kovalainen M, Herzig KH, Järvelin MR, Sebert S. Fat mass-and obesity-associated gene Fto affects the dietary response in mouse white adipose tissue. *Sci. rep.* 2015; 5: 9233. https://DOI: 10.1038/srep09233
8. Hebi M, Eddouks M. Hypolipidemic activity of *Tamarix articulata* Vahl. in diabetic rats. *J. Integr. Med.* 2017; 15(6): 476-482. http://dx.doi.org/10.1016/S2095-4964(17)60361-3.
9. Hammoum I, Mbarek S, Dellaa A, Dubus E, Baccouche B, Azaiz R, Charfeddine R, Picaud S, Chaouacha-Chekir RB. Study of retinal alterations in a high fat diet-induced type ii diabetes rodent: *Meriones shawi*. *Acta histochem.* 2017; 119(1): 1-9. https://doi.org/10.1016/j.acthis.2016.05.005.
10. Schmidt-Nielsen K, Haines HB, Hackel DB. Diabetes mellitus in the sand rat induced by standard laboratory diets. *Science*. 1964; 143(3607):689-90. https://doi.org/10.1126/science.143.3607.689.
11. Sihali-Beloui O, Aroune D, Benazouz F, Hadji A, El-Aoufi S, Marco S. A hypercaloric diet induces hepatic oxidative stress, infiltration of lymphocytes, and mitochondrial reshuffle in *Psammomys obesus*, a murine model of insulin resistance. *C R Biol.* 2019; 342(5-6): 209-219. https://doi.org/10.1016/j.crvi.2019.04.003.
12. Baccouche B, Benlarbi M, Barber AJ, Ben Chaouacha-Chekir R. Short-term administration of astaxanthin attenuates retinal changes in diet-induced diabetic *Psammomys obesus*. *Curr. Eye Res.* 2018; 43(9):1177-89. https://doi.org/10.1080/02713683.2018.1484143.
13. Chrigui S, Hadj Taieb S, Jemai H, Mbarek S, Benlarbi M, Feki M, Haouas Z, Zemmel A, Chaouacha-Chekir RB, Boudhrioua N. Anti-Obesity and Anti-Dyslipidemic Effects of *Salicornia arabica* Decocted Extract in Tunisian *Psammomys obesus* Fed a High-Calorie Diet. *Foods*. 2023; 12(6): 1185. https://doi.org/10.3390/ foods12061185.
14. Rocha VD, Claudio ER, Da Silva VL, Cordeiro JP, Domingos LF, Da Cunha MR, Mauad H, Nascimento TB, Lima-Leopoldo AP, Leopoldo AS. High-fat diet-induced obesity model does not promote endothelial dysfunction via increasing Leptin/Akt/eNOS signaling. *Front. physiol.* 2019; 10:268. https://doi:10.3389/fphys.2019.00268.
15. Saidi T, Chaouacha-Chekir R, Hicks D. Advantages of *Psammomys obesus* as an animal model to study diabetic retinopathy. *J Diabetes Metab.* 2012; 3(207): 2. https://doi.org/0.4172/2155-6156.1000207.
16. Benlarbi-Ben Khedher M, Hajri K, Dellaa A, Baccouche B, Hammoum I, Boudhrioua-Mihoubi N, Dhifi W, Ben Chaouacha-Chekir R. Astaxanthin inhibits aldose reductase activity in *Psammomys obesus*, a model of type 2 diabetes and diabetic retinopathy. *Food Sci Nutr*. 2019; 7(12): 3979-85. https://DOI: 10.1002/fsn3.1259.
17. Ji G, Zhao X, Leng L, Liu P, Jiang Z. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. *Lipids Health Dis*. 2011; 10: 1-0. https://doi.org/10.1186/1476-511X-10-23

18. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RA, Nogueira CR, Cicogna AC. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arq. Bras. Endocrinol. Metabol.* 2008; 52: 968-74. https://doi:10.1590/s0004-27302008000600007
19. Wali JA, Jarzebska N, Raubenheimer D, Simpson SJ, Rodionov RN, O'Sullivan JF. Cardio-metabolic effects of high-fat diets and their underlying mechanisms—A narrative review. *Nutrients*. 2020; 12(5):1505. https://doi.org/10.3390/nu12051505
20. Einat H, Kronfeld-Schor N, Eilam D. Sand rats see the light: short photoperiod induces a depression-like response in a diurnal rodent. *Behav. Brain Res.* 2006; 173(1): 153-7. https://doi.org/10.1016/j.bbr.2006.06.006
21. Dellaa A, Mbarek S, Kahloun R, Dogui M, Khairallah M, Hammoum I, Rayana-Chekir NB, Charfeddine R, Lachapelle P, Chaouacha-Chekir RB. Functional alterations of retinal neurons and vascular involvement progress simultaneously in the *Psammomys obesus* model of diabetic retinopathy. *J. Comp. Neurol.* 2021; 529(10): 2620-35. https://doi.org/10.1002/cne.25114
22. Benkalfat NB, Merzouk H, Bouanane S, Merzouk SA, Bellenger J, Gresti J, Tessier C, Narce M. Altered adipose tissue metabolism in offspring of dietary obese rat dams. *Clin. sci.* 2011; 121(1): 19-28. https://doi.org/10.1042/CS20100534.
23. Rodrigues L, Mouta R, Costa AR, Pereira A, e Silva FC, Amado F, Antunes CM, Lamy E. Effects of high-fat diet on salivary α-amylase, serum parameters and food consumption in rats. *Arch. Oral Biol.* 2015; 60(6): 854-62. https://doi:10.1016/j.archoralbio.2015.02.015.
24. Coate KC, Scott M, Farmer B, Moore MC, Smith M, Roop J, Neal DW, Williams P, Cherrington AD. Chronic consumption of a high-fat/high-fructose diet renders the liver incapable of net hepatic glucose uptake. *Am. J. Physiol. - Endocrinol. Metab.* 2010; 299(6): E887-98. https://doi.org/10.1152/ajpendo.00372.2010.
25. Ulla A, Alam MA, Sikder B, Sumi FA, Rahman MM, Habib ZF, Mohammed MK, Subhan N, Hossain H, Reza HM. Supplementation of *Syzygium cumini* seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats. *BMC Complement Altern Med.* 2017; 17: 1-3. https://doi.org/10.1186/s12906-017-1799-8.
26. Feng R, Luo C, Li C, Du S, Okekunle AP, Li Y, Chen Y, Zi T, Niu Y. Free fatty acids profile among lean, overweight and obese non-alcoholic fatty liver disease patients: a case–control study. *Lipids Health Dis.* 2017; 16(1): 1-9. https://doi.org/10.1186/s12944-017-0551-1.
27. Ragab SM, Abd Elghaffar SK, El-Metwally TH, Badr G, Mahmoud MH, Omar HM. Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. *Lipids Health Dis.* 2015; 14(1): 1-1. https://doi.org/10.1186/s12944-015-0087-1.
28. Arner E, Westermark PO, Spalding KL, Britton T, Rydén M, Frisén J, Bernard S, Arner P. Adipocyte turnover: relevance to human adipose tissue morphology. *Diabetes.* 2010; 59(1): 105-109. https://doi.org/10.2337/db09-0942
29. Lönn M, Mehlig K, Bengtsson C, Lissner L. Adipocyte size predicts incidence of type 2 diabetes in women. *The FASEB journal.* 2010; 24(1): 326-31. https://doi.org/10.1096/fj.09-133058
30. Timmers S, De Vogel-Van Den Bosch J, De Wit N, Schaart G, Van Beurden D, Hesselink M, Van Der Meer R, Schrauwen P. Differential effects of saturated versus unsaturated dietary fatty acids on weight gain and myocellular lipid profiles in mice. *Nutr Diabetes.* 2011; 1(7): e11-e11. https://doi.org/10.1038/nutd.2011.7
31. Bouderba S, Sanchez-Martin C, Villanueva GR, Detaille D, Koceïr EA. Beneficial effects of silibinin against the progression of metabolic syndrome, increased oxidative stress, and liver steatosis in *Psammomys obesus*, a relevant animal model of human obesity and diabetes. *J. Diabetes.* 2014; 6(2): 184-92. https://doi.org/10.1111/1753-0407.12083
32. Kanety H, Moshe S, Shafrir E, Lunenfeld B, Karasik A. Hyperinsulinemia induces a reversible impairment in insulin receptor function leading to diabetes in the sand rat model of non-insulin-dependent diabetes mellitus. *PNAS.* 1994; 91(5): 1853-7. https://doi.org/10.1073/pnas.91.5.1853.
33. Gadot M, Leibowitz G, Shafrir E, Cerasi E, Gross DJ, Kaiser N. Hyperproinsulinemia and insulin deficiency in the diabetic *Psammomys obesus*. *Endocrinology.* 1994; 135(2): 610-616. https://doi.org/10.1210/en.135.2.610.
34. Kamgang R, Mboumi RY, N'dillé GPRM, Yonkeu JN. Cameroon local diet-induced glucose intolerance and dyslipidemia in adult Wistar

rat. *Diabetes Res Clin Pract*. 2005; 69(3): 224-230. https://doi.org/10.1016/j.diabres.2005.02.005.

35. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA1c. *Diabetes care*. 2003; 26(3): 881-885. https://doi.org/10.2337/diacare.26.3.881.
36. Klop B, Elte JWF, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*. 2013; 5(4): 1218-1240. https://doi.org/10.3390/nu5041218.
37. Raveh O, Pinchuk I, Fainaru M, Lichtenberg D. Kinetics of lipid peroxidation in mixtures of HDL and LDL, mutual effects. *Free Radic. Biol. Med*. 2001; 31(11): 1486-1497. https://doi.org/10.1016/S0891-5849(01)00730-4.
38. Kaiser N, Leibowitz G, Nesher R. Glucotoxicity and β-cell failure in type 2 diabetes mellitus. *J. Pediatr. Endocrinol. Metab*. 2003; 16(1): 5-22. https://doi.org/10.1515/JPEM.2003.16.1.5.
39. Leibowitz G, Kaiser N, Cerasi E. β-Cell failure in type 2 diabetes. *J. Diabetes Investig*. 2011; 2(2): 82-91. https://doi.org/10.1111/j.2040-1124.2010.00094.x.
40. Spolding B, Connor T, Wittmer C, Abreu LL, Kaspi A, Ziemann M, Kaur G, Cooper A, Morrison S, Lee S, Sinclair A. Rapid development of non-alcoholic steatohepatitis in *Psammomys obesus*. *PloS One*. 2014; 9(3): e92656. https://doi.org/10.1371/journal.pone.0092656.
41. Kharb S, Garg MK, Puri P, Nandi B, Brar KS, Gundgurthi A, Pandit A. Assessment of adrenal function in liver diseases. *Indian J. Endocrinol. Metab*. 2013; 17(3): 465. https://doi.org/10.4103/2230-8210.111643.
42. Lin SC, Chung TC, Lin CC, Ueng TH, Lin YH, Lin SY, Wang LY. Hepatoprotective effects of *Arctium lappa* on carbon tetrachloride-and acetaminophen-induced liver damage. *Am. J. Chin. Med*. 2000; 28(02): 163-73. https://doi.org/10.1142/S0192415X00000210.
43. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int. J. Mol. Sci*. 2014; 15(4): 6184-6223. https://doi.org/10.3390/ijms15046184.
44. Esquinas P, Rios R, Raya AI, Pineda C, Rodriguez M, Aguilera-Tejero E, Lopez I. Structural and ultrastructural renal lesions in rats fed high-fat and high-phosphorus diets. *Clin. Kidney J*. 2021; 14(3): 847-54. https://doi.org/10.1093/ckj/sfaa009.
45. Madić V, Petrović A, Jušković M, Jugović D, Djordjević L, Stojanović G, Vasiljević P. Polyherbal mixture ameliorates hyperglycemia, hyperlipidemia and histopathological changes of pancreas, kidney and liver in a rat model of type 1 diabetes. *J. Ethnopharmacol*. 2021; 265: 113-210. https://doi.org/10.1016/j.jep.2020.113210.
46. Vidal E, Lalarme E, Maire MA, Febvret V, Grégoire S, Gambert S, Acar N, Bretillon L. Early impairments in the retina of rats fed with high fructose/high fat diet are associated with glucose metabolism deregulation but not dyslipidaemia. *Sci. Rep*. 2019; 9(1): 5997. https://doi.org/10.1038/s41598-019-42528-9.
47. Clarkson-Townsend DA, Douglass AJ, Singh A, Allen RS, Uwaifo IN, Pardue MT. Impacts of high fat diet on ocular outcomes in rodent models of visual disease. *Exp. Eye Res*. 2021; 204: 108-440. https://doi.org/10.1016/j.exer.2021.108440.
48. Hombrebueno JR, Chen M, Penalva RG, Xu H. Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse. *PloS one*, 2014; 9(5): e97970. https://doi.org/10.1371/journal.pone.0097970.
49. Sasaki M, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, Kobayashi S, Ishida S, Tsubota K. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia*. 2010; 53: 971-9. https://doi.org/10.1007/s00125-009-1655-6.
50. Kern TS, Barber AJ. Retinal ganglion cells in diabetes. *J physiol*. 2008; 586(18): 4401-4408. https://doi.org/10.1113/jphysiol.2008.156695