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EVALUATION OF INDUCED METABOLIC SYNDROME OF OBESITY BY ADMINISTERING A PURIFIED DIET IN MICE

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Abstract

The worldwide prevalence of obesity has risen dramatically in the last decades. Obesity is associated with multiple medical conditions that appear in metabolic syndrome such as: type II diabetes, increased blood pressure, high triglyceride and cholesterol levels. The aim of this study was represented by the evaluation of diet-induced obesity, with the purpose of creating an experimental mouse model for testing food supplements and medication used in this syndrome. One group of C57BL/6 mice received an in-house purified hypercaloric diet, the second group received a standardized obesity diet and the third group received a control diet for a period of 60 days. The following aspects were assessed during the experiment: food intake, body weight, hematologic and biochemical parameters. On the final day, organ samples were collected (liver, kidneys and visceral adipose tissue) for necropsy and histopathologic examination. The obtained results showed that the administration of the in-house purified hypercaloric diet for a period of 60 days was optimal for installing obesity syndrome in mice. The use of unidirectional enriched diets presents an increased interest in current research for futher development of new therapeutic strategies in metabolic syndrome.

Key words: *metabolic syndrome, mice, obesity, purified diet.*

INTRODUCTION

Metabolic Syndrome (MetS) is characterized by the simultaneous occurrence of at least three of the following medical conditions: obesity, hyperglycemia, hypertension or dyslipidemia (Kaur, 2014).

Overweight and obesity are important clinical and public health concerns worldwide. The prevalence of obesity has increased dramatically during the last four decades and has reached epidemic proportions in both developed and developing countries (Hruby & Hu, 2015; Kelly et al., 2008). Globally, more than 1.9 billion adults aged 18 years and older are overweight, and of those, almost 700 million adults are obese (WHO, 2018).

Obesity is defined as an excessive or abnormal accumulation of adipose tissue in the body, associated with multiple medical conditions such as type 2 diabetes, hypertension,atherosclerosis, hyperlipidemia and arthritis (Alberti et al., 2006). Obesity is also associated with an important decrease in life expectancy and an increased risk of several cancer types (Engin, 2017). Obesity is considered a complex disease and has multifactorial etiology due to the interaction of both environmental and genetic factors and it is a result of the prolonged imbalance between caloric intake, basal metabolism and energetic consumption (Serra & Bautista, 2013; Lang et al., 2019).

In order to understand the pathophysiological basis of obesity and obesity-associated metabolic complications, it is imperative to develop animal models of MetS (Wong et al., 2016). Establishment of appropriate animal models mimicking MetS in humans is an

important concern for the biomedical research (Lutz & Woods, 2012).

Mice and rats are the most common animal models used in investigating obesity as they readily gain weight when provided with a high-fat diet (Speakman et al., 2008; Tristan et al., 2017). The aim of this study was represented by the evaluation of diet-induced obesity, with the purpose of creating an experimental mouse model for testing food supplements and medication used in this syndrome. Another purpose of the present study refers to the formulation, preparation and standardization of an in-house purified diet that might induce obesity in C57BL/6 mice, in comparison with an existing standardized obesity diet.

The following aspects were assessed during the experiment: weight gain, food consumption, hematological and biochemical parameters and total body lipid level. By analyzing the results, we showed that the administration of the in-house purified hypercaloric diet for a period of 60 days was optimal for installing obesity syndrome in C57BL/6 mice.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Cantacuzino National Medical-Military Development Research Institute and approved by the competent authority. The animals were provided by Băneasa SFP (Specific Pathogen Free) Animal Facility area for rats and mice of Cantacuzino National Medical-Military Development Research Institute, Bucharest.

All aspects related to animal housing and care were undertaken in accordance with the national and international regulations concerning animal testing. The animals were kept under standard conditions, temperature 18-24°C, humidity 35-75% and cycle of lighting 12/12 h. The food and the water were administered *ad libitum* during the entire experiment period.

C57BL/6 mice, males and females, 9 weeks old, weighing 18-21 g were used in this study. Mice were randomly divided into three groups, based on the received diet. Each group was divided into 2 sub-groups with an equal number of males and females. Group 1 received in-house purified diet with 32.81% fat (ICO), group 2 received standardized obesity diet with 47% fat (Altromin 1080), for comparison purposes, and group 3 was considered the control group, which received a maintenance diet with 12% fat (Altromin 1081). The purified diets were administrated for a period of 60 days. Chemical composition and nutritional values of the diets are presented in Table 1.

Table 1. Chemical composition and nutritional values for each diet

Diet	ICO	1080 (Altromin)	1081 (Altromin)
Energy (Kcal/kg)	4821,17	4553	3501
Protein (%)	18.47	18	23
Fat (%)	32.81	47	12
Moisture (%)	3.83	5.1	7.8
Fiber (%)	4.08	4.7	4.6

Animals were daily inspected and food consumption was recorded for each group once a week. Weight measurements were performed for each mouse every 14 days during the entire feeding period.

Blood collection from the retro-orbital sinus was performed on days 0, 30 and 60, under general anaesthesia, using a cocktail of acepromazine (5 mg/kg) and ketamine (100 mg/kg). For hematological tests, blood was sampled in EDTA pre-conditioned tubes and IDEXX ProCyte Dx 5 Diff analyzer was used. For biochemistry, blood was sampled in lithium–heparin pre-conditioned tubes and tests were performed on VetTest 8008 Chemistry Analyzer.

On the final day, animals were euthanized using anaesthetic overdose. Liver, kidneys and visceral adipose tissue were collected for necropsy, weighing and histopathological examination. Total body lipid content was determined from the animal carcass by using petroleum ether extraction.

Statistical analysis

All data are shown as mean values for each group. Statistical comparisons were performed using the Microsoft Excel T-test for independent groups and one-way analysis of variance for comparison of means of parameters within the same group. P-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Body Weight

Compared to the maintenance diet (1081) and the standardized obesity diet (1080), mice fed with in-house purified diet (ICO) recorded the greatest weight gain, 48.9% in males and 41.2% in females. The results of the weight measurements during the study are graphically represented in Figure 1.

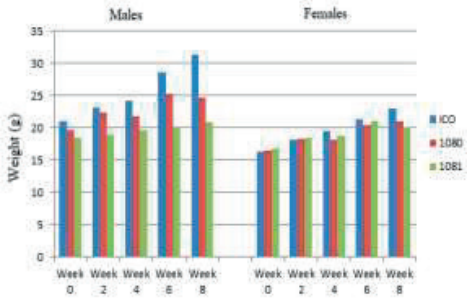

Figure 1. Weight measurement in males and females along 60 days study

Food Consumption

Food intake was determined weekly for each diet and an average consumption/animal/day was calculated for the entire feeding period. Group 1 recorded the highest food consumption, with an average of 2.68g/mouse/day in males and 3.91g/mouse/day in females. The results of the food intake during the entire study are graphically represented in Figure 2.

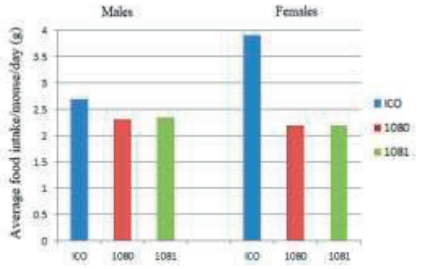

Figure 2. Average food consumption/mouse/day (g) for each diet

Biochemical Results

We focused on the most relevant biochemical parameters in obesity syndrome: cholesterol (CHOL), glucose (GLU), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The mean GLU value for ICO diet was significantly increased compared to 1080 and 1081 in both genders, reaching a maximum value on day 30. GLU level slightly decreased by the end of the study for ICO diet but remained higher compared to the other two groups (Figures 3 and 4).

The mean CHOL values were significantly increased for ICO group compared to control group, reaching the maximum value in day 30 in males and maintaining the high value during the entire period of the experiment. In females, CHOL level increased continuously until the end of the study (Figures 5 and 6).

The aminotransferases values were variable and did not entirely reflect the changes induced by the obesity diet compared to control diet. In males, mean ALT value on day 60 was higher in ICO fed group compared to the other 2 groups. In females, ALT value was higher for ICO group compared to 1080 group but lower compared to control group (Figure 7). Mean AST value was similar for ICO fed group and control group on day 60 (Figure 8).

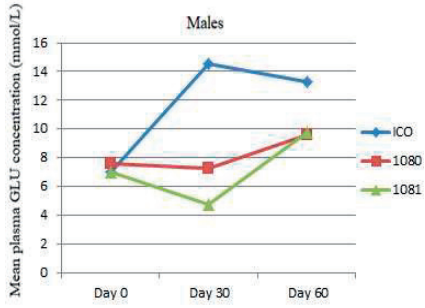

Figure 3. Glucose (GLU) measurements in males during the study

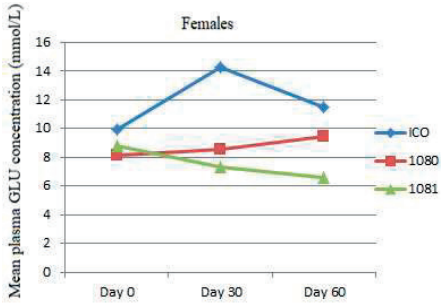

Figure 4. Glucose (GLU) measurements in females during the study

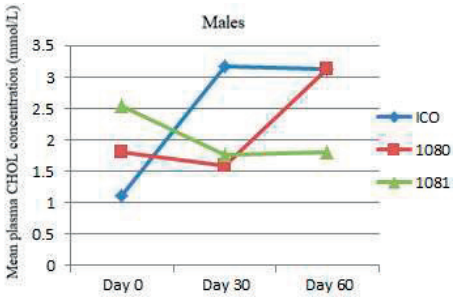

Figure 5. Ccholesterol (CHOL) measurements in males during the study

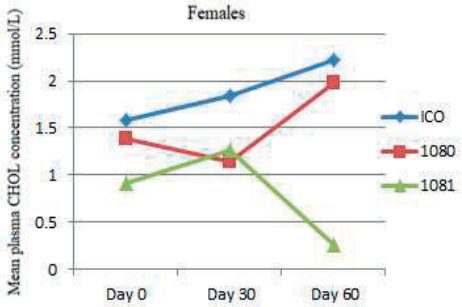

Figure 6. Glucose (GLU) measurements in females during the study

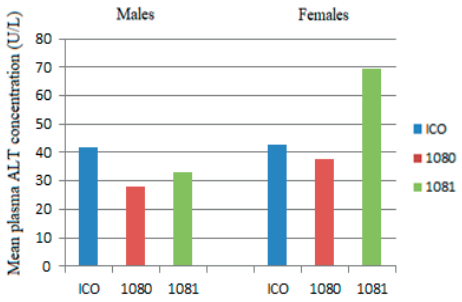

Figure 7. Alanine aminotransferase (ALT) measurements in males/females on day 60

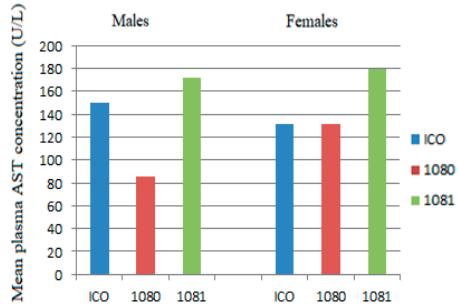

Figure 8. Alanine aminotransferase (AST) measurements in males/females on day 60

Hematological Results

The reference interval for hematology was based on the average values obtained in a previous experiment on C57BL/6 mice fed with a high fat diet, in animals provided by the same animal facility and the use of the same analysis laboratory (Popoiu et al., 2020).

Our study revealed a higher total leukocytes count (WBC) in ICO fed group for males compared to control group. For the same high-fat diet, lymphocyte count (LYM) and platelet count (PLT) were increased in males, compared to control group. In females, these values were similar for all groups. RBC count showed slight variations in the case of animals fed with diets for inducing obesity compared to control group mice.

Other hematologic parameters registred low degree variations and there were not considered relevant for the present study.

The mean hematological parameters on the final day are presented in Table 2.

Table 2. Hematological parameters on day 60 for both genders

Diet	ICO		1080 (Altromin)		1081 (Altromin)	
Parameter	♂	♀	♂	♀	♂	♀
RBC 10^12/L	8.83	8.82	8.84	8.31	8.89	8.6
WBC 10^9/L	3.59	1.57	2.26	1.17	2.06	1.10
LYM 10^9/L	1.77	0.87	1.67	0.49	1.04	0.81
PLT K/µL	735	570	468	509	426	538

Total Body Lipid

Petroleum ether extraction (Soxhlet technique) is a commonly used method of isolating lipids and determining the total body lipid content.

ICO fed group recorded the highest average body lipid/mouse (g) compared to 1080 group and control group. In males, body lipid percentage was significantly increased for ICO diet compared to the other two groups (Table 3).

Table 3. Average body lipid content (g) and total body lipid percentage (%) for both genders

Diet	Body lipid content (g)		Body lipid percentage (%)	
	♂	♀	♂	♀
ICO	2.95	0.98	10.23	4.89
1080	1.22	0.79	5.43	3.98
1081	0.78	0.40	3.66	2.3

Necropsy

On the final day, liver and kidneys were collected for necropsy, weighing and histopathological examination.

Organ examination revealed enlarged liver and dystrophic liver appearance in animals fed with obesity diet compared to control group. No pathological changes were observed in kidneys examination.

Animals in ICO group had increased organs and carcass weight compared to groups 2 and 3 in both genders. Values obtained by weighing the organs and the carcass for each group are presented in Table 4 and graphically represented in Figure 9.

Histopathological results will be reported in a future paper, as data will be available.

Table 4. Average weight merasurments in organs and animal carcass on final day

Diet	Liver weight (g)		Kidneys weight (g)		Carcass weight (g)	
	♂	♀	♂	♀	♂	♀
ICO	1.47	1.23	0.45	0.34	28.69	20.46
1080	1.12	1.14	0.41	0.32	22.8	19.18
1081	1.08	0.95	0.38	0.29	21.36	17.53

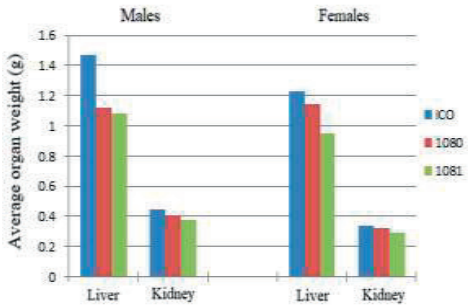

Figure 9. Average weight merasurments in organs and animal carcass on final day

In light of the rapid growth of the obesity rate and concerns over the health effects of obesity, diet-induced obesity animal models are precious resources for the biomedical research . This models allow us to create a controlled environment in order to study and understand the mechanisms involved in obesity development and as well as its effects.

For obesity animal models, unidirectional enriched diets with high fat content, play an important role.

Diverse high energy diets have been used to induce obesity and related metabolic disorders in rodent models, though the dietary mediation has not been absolutely standardized. (Sasidharan et al., 2013). These diets consist of a simple exchange of carbohydrate-derived calories with fat-derived calories and are being compared to a standard chow diet as control.(Lang et al., 2019; Sampey et al., 2011). Herein, we describe a diet-induced obesity model in C57BL/6 mice based on feeding with an in-house purified diet and a standardized obesity diet in comparison to a maintainance diet.

Our results showed significant changes in the body weight, caloric intake, glucose and cholesterol metabolism, inflammation indicators and adipose tissue, for in-house high-fat diet compared to control diet.

In another study, the body weight was increased after 2 weeks of high-fat diet feeding and the hyperglycemia reached the maximum level around the 4th week (Della Vedova et al., 2016). Fraulob et al. (2010) showed that mice fed with high-fat (60%) diet exhibited greatly increased body mass, fat pads and total plasma cholesterol.

Following the administration of the diet for obesity, the body weight of the mice was increased in both genders starting with day 30 of the study, and the glucose values were increased in males and had similar values as the control in females (Popoiu et al., 2020).

Toita et al. (2018) showed in their study that serum ALT levels were similar between high-fat diet and normal diet fed mice. However, a normal serum ALT value may not guarantee absence of hepatic inflammation. In our study, serum levels of hepatic enzymes were higher at the same time with the increase of the body weight only in males.

Total body mass and liver mass following 14 weeks of high fat diet were also significant increased, while kidney mass was not positively correlated to adipose tissue in C57BL/6 mice (Wooten et al., 2016).

Several studies relate inflammation to obesity, an indicator of inflammation being the increase of the number of leukocytes (WBC), as our results also showed. The WBC count was positively correlated with percentage of total body lipid.and fasting plasma leptin

concentration. Evidence suggests that leptin and the leptin receptor are part of a pathway which stimulates hematopoiesis (Wilson et al., 1997).

Panagiotakos et al. (2005) found higher rate of inflammatory markers, including a 17% higher WBC count in participants with a central obesity as compared with those whose body fat was distributed normally.

Jamshidi & Seif (2017) showed a relationship between central and general adiposity and WBC count as an inflammation factor, and higher count of platelets count in obese subjects.

The origin of inflammation during obesity and the underlying mechanisms that explain its occurrence are not yet fully understood, but pro-inflammatory cytokines play a central role, the adipose tissue being the main source of inflammatory cytokines. (Rodríguez et al., 2013).

Lipid infusion and a high-fat diet activate hypothalamic inflammatory signaling pathways, resulting in increased food intake and nutrient storage (Thaler & Schwartz, 2010).

In order to confirm the onset of inflammation at tissue level, there is a need for additional histopathological tests,which will be reported in a future paper, as data will be available.

CONCLUSIONS

The comparative analysis of the animal body weight, blood parameters and body lipid content showed better results in ICO purified diet then standardized obesity diet (1080) in inducind mice obesity. Data obtained in this study has shown that the changes in measured indicators during the experiment can be related to the metabolic syndrome of obesity in mice. We will further correlate this results to the data obtained in histopathologic examination.

ICO purified diet is a reliable diet in inducing mice obesity and can be used for standardizing animal models of metabolic syndrome as new preventive strategies and constant research is needed.

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The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

All authors have read and approved the final manuscript.

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