SUMMARY

of the PHD Thesis entitled

Inducing of some morbid metabolic entities in laboratory animals using special diets

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Key words: diabetes mellitus, atherosclerosis, special diets, mouse, rat.

The Doctoral thesis is classic composed of Part I, allowed to the theoretical presentation and Part II, allowed to the personal researches. Part I is preceded by Introduction that presents the issue in the proposed theme, that of experimental animal models for the study of diabetes and atherosclerosis.

In Part I, the doctoral student do an exhaustive presentation about the current state of the literature data on:

- metabolism and physiopathtology of fructose and glucose metabolic disorders;
- diabetes;
- general characterization, absorption and lipid metabolism of lipids;
- physiopathology of lipid metabolism in animals;
- physiopathology of insulin resistance and type 2 diabetes caused by obesity.

Part I ends by a chapter regarding the animal models use and diet models use (natural and purified diets) in the study of the different metabolic disorders.

Part II of the Thesis, allowed to the personal researches, starts by Chapter material and methods, in which they are described the used animal models, composition of the used diets, working methods and the management of the experiments.

Part II continues by Chapter Results and discussion, which comprise two large subchapters:

1. Results and discussion regarding experimental acquirement of the hiperglycemic syndrom using a special diet in mice and rats

In the frame of this chapter, they are presented the results and their discussion following the experimental feeding of two groups, of mice and rats, respectively, for 93 days by a purified

60%-fructose-enriched diet containing 68% carbohydrates, vs. two corresponding control groups, fed by a standard diet (AIN-93M).

They were monitored the food consumption, the weight gain, blood morphology parameters (erythrocyte count, hemoglobin content, hematocrit, MCV, MCH and MCHC), blood biological parameters (blood glucose concentration, lipid concnetration, cholesterol concentration), blood metabolic enzymes (ALT, AST, γGT, ALP și LDH), the weight evolution of some internal organs and the effects on histology structure of some internal organs.

The mean food consumptions were lower vs. afferents control group, the decrease being greater in rats vs. mice. The reason of this decreased consumption could be the palatability of the forages and a consequence of the metabolic disorders induced by the fructose excess of the diet.

The weight evolution of the animals presented a descendent evolution, foreseeable taking into account the food consumption. The differences between the experimental and control groups became significant from statistic point of view later in mice (27 days) vs. rats (21 days) of experimental feeding). The negative weight evolution of the experimental groups could be explained by the decreased food consumption and by the disorders of the intermediate metabolism induced by the excess of fructose, centered firstly on the mobilization and consumption of the fat depots, which can subordinate the lipogenic effects of high fructose content.

The experimental use of the fructose-enriched diet didn't significantly influenced the erythrocyte parameters, excepting the decrease of the hemoglobin content (and the subsequent modification of the erythrocyte parameters (MCH and MCHC), which could be explained by the general metabolism disorders induced by the excess of fructose in the diet.

Leukocyte count increased too, both in mice and rats. This increase of the leukocyte count is performed from the increase of the neutrophil percent. These effects on the leukocyte count could be induced by the metabolism disorders.

The use of the fructose-enriched diet induced an increase of the blood glucose concentration. This increase became statistically significant later, in the second part of the experiment, next to the first 39 days, respectively. The final increase of the blood glucose concentration was 46.89% in mice and 62.29% in rats.

The analysis of the evolution of the triglyceride levels relieved significant increases soon after the 39 days of experimental feeding, greater in rats ($P < 0.001$) vs. mice ($P = 0,011$). The fact that in our experiment it was obtained a significant increase of the serum triglyceride level both in mice and rats could be allowed to an intensified de novo hepatic synthesis both in mouse and rat by the excess of fructose.

The blood plasma level of cholesterol increased significantly too, from the zero moment of the experimental feeding to the 93 days of experimental feeding by comparing to the control groups

The serum transaminase activity doubled and even tripled for the majority of the analyzed enzymes, the increases being greater in mouse vs. rat. The graph evolution of the ALP begun to increase over the control in the period of the first 39 days of experimental feeding: this fact relieves o greater sensibility of ALP in the monitoring of the liver health. LDH activity presented species particularities, being more elevated in rat vs. mouse.

Monitoring of some organ weight (liver, kidney, spleen, heart, pancreas, and brain) showed that in both, mouse and rat experimental groups the liver weight was increased vs. the corresponding control groups. The weight of the other analyzed organs didn't significant modify vs. controls, some times being more reduced than in controls.

Regarding the glucose tolerance test, following the first 39 days of experimental feeding, it was found individual different reactions to the fructose-enriched diet. The maximal blood glucose levels (210 - 215 $mg \cdot dL^{-1}$) were reached at T30 (minutes from glucose administration) later in rats vs. mice. Resilience of blood glucose levels was kept every time in 120 min from the administration of the glucose solution.

At the end of the experimental period, the glucose tolerance test presented the following particularities:

- the levels of blood glucose concentration à jeune were over the normal limits: about 50 $mg \cdot dL^{-1}$);

- the hyperglycemic peak-ul was reached at T30: 360 – 400 $mg \cdot dL^{-1}$;

- resilience of glicaemia was done following a slowly curve, at T120, the values being between 275 and 300 $mg \cdot dL^{-1}$, more decreased in mice;

- at T150, the values of glicaemia are still over those of à jeune values, more decreased in mouse (225 $mg \cdot dL^{-1}$) and more elevated in rats (280 $mg \cdot dL^{-1}$).

Microscopic modifications were searched on kidney, cardiac, splenic, and pancreatic tissues, both in mice and rats. In majority, they were found minimal lesions, scattered and of low amplitude. With a higher frequency and amplitude, they were found: steatotic liver degeneration, hyperemia, pancreatic, renal and hepatic micro bleedings, pancreatic interlobular edema and infiltrates, ectasia and formation of hyaline renal cylinders, without significant species differences;

2. Results and discussion regarding experimental acquirement of atherosclerosis syndrom in mouse and rat using special diets

In the case of the diets supplemented by cholesterol 1% and cholic acid 0.5%, food consumption of the experimental groups evolved again under those of control groups. Incriminated reasons: lack of palatability of food and possibly effects induced by cholesterol excess on intermediary metabolism and consequently on the whole health of the animals.

Both, experimental mouse group weight and experimental rat group weight evolved under those of the corresponding control. Practically, the animals have weakened while the animals from the control groups gained.

Erythrocyte count decreased during experimental feeding period. This decrease was more accentuated during 0 - 39 days of feeding vs. the next interval, 40 - 93 days.

Leukocyte count decreased also during the experimental feeding period, based on increase of neutrophile percent. The increase of the leukocyte coint on the base of neutrophile is in some measure physiological, but it was accelerated by the cholesterol-enriched diet.

Total cholesterol was already increased in 39 days from the beginning of the experimental feeding, and increased further, up to 93 experimental days, showing the efficacy of the experimental diet. The rat was found to be a more sensitive species than mice, achieving greater blood cholesterol values than mice, and larger differences compared to the control.

Total blood lipids increased in both, mice and rat species.

Transaminase activity (ALT, AST, and γGT) also significantly increased during the experimental period. They were found species differences (between mouse and rat) regarding γGT activity. The greatest increasing activity presented GPT in mouse, and the lowest, γGT in rats.

ALP activity also significantly increased ($P < 0,001$) yet from the first 39 days of experimental period by comparing to its initial values. The great sensibility of this enzyme level makes its level as a marker of the preatheromatosis state.

The application of a experimental cholesterol-enriched diet induced after 93 days of feeding a significant increase ($P < 0.001$) of liver weight in both, mice and rats. There were no found significant increases in weight neither heart weight and nor kidney weight ($P > 0.05$).

Histological analysis revealed lesions in experimental animals both in large vessels (aorta) and at the level of parenchymal organs (liver, pancreas, spleen, heart and kidneys) as it follows.

In aorta wall uneven deformation prevailed, with thickening of the intima and media due to blocking cholesterol absorption.

In liver, predominant lesions were severe lipid degeneration, hyperemia, exolobular steatosis and vacuolation.

In the heart, subepicardic, intercellular and intrefibriar lipid infiltration were found, with redness and hyalinisation of myocardiocytes.

In the kidneys, they were found tubular hyalinizations, interstitial hemorrhage, degeneration and lipid vacuoles in the epithelial cells of the convoluted tubule structure

The next chapter in Part II is allowed for general conclusions.

The thesis ends by 182 references which are cited in the text.