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Original Article

Histopathological aspects of liver under variable food restriction: Has the intense one-week food restriction a protective effect on non-alcoholic-fatty-liver-disease (NAFLD) development?

Peter Makovicky[a,*], Eva Tumova[b], Zdenek Volek[c], Pavol Makovicky[d], Ludmila Vodickova[e], Jana Slyskova[e], Miroslav Svoboda[e], Alexandra Rejhova[e], Pavel Vodicka[e], Gabriel Samasca[f], Alena Kralova[g], Melinda Nagy[d], Marta Mydlarova-Blascakova[h], Jana Poracova[h]

[a] Laboratory of Veterinary Histopathology in Komarno, Slovak Republic
[b] Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Czech Republic
[c] Physiology of Nutrition and Quality of Animal Product, Institute of Animal Science in Prague – Uhrineves, Czech Republic
[d] Department of Biology, Pedagogical Faculty, Selye Janos University in Komarno, Slovak Republic
[e] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic in Prague, Czech Republic
[f] Department of Immunology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[g] Student of Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Czech Republic
[h] Department of Biology, University of Presov in Presov, Slovak Republic

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ABSTRACT

Non-alcoholic-fatty-liver-disease (NAFLD) is a clinicopathologic entity characterized by a variety of hepatic injury patterns without significant alcohol use. It has a close association with obesity, so treatment includes weight loss, control of insulin sensitivity, interventions directed at inflammation and fibrosis. There is a certain relationship between the grade and duration of food restriction and hepatic function. The objective of this work was to describe the relationship between biochemistry, autoantibodies, insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein 3 (IGFBP-3), and liver morphology in experimental rabbit groups with food restriction as compared to controls with ad libitum food (ADL) income. The experiment was performed on a total of 24 rabbits of a weaning age of 25–81 days. The first group (R1) was restricted between 32 and 39 days of age to 50 g of food per rabbit a day. The second group (R2) was also restricted between 32 and 39 days, but the rabbits received 65 g of food per rabbit a day. At the end of the experiment, the blood and liver samples were collected at necropsy. NAFLD has developed in all three groups. There was any autoantibody positivity in all three groups. IGF-I is moderately higher in R1 and R2 group, as compared to the control group ($P > 0.05$). IGFBP-3 is without statistical significance in all three groups. Alkaline phosphatase (ALP) is the only liver biochemical parameter that has significantly increased following food restriction ($P > 0.039$). Single one-week restriction has any protective effect on NAFLD development.

Introduction

Many independent studies proved that balanced, caloric intake limit is beneficial for health, and along with maintaining levels of trace elements, leads to the prolongation of active life [10,26]. These results have been historically verified under variable conditions [24,44]. Other sources refer to the differentiation of the metabolic profile [15], and this topic remains under worldwide research investigation [33]. In the literature, we can find references advocating a development of a universal model of caloric intake [8,13]. However, even today it is not generally accepted which limitations are beneficial in terms of quantity of nourishment and percentage of its components and which may already exhibit pathologic effects.

Liver represents the main central organ of metabolism with considerable energy capacity and a high proportion of mitochondria per cell. Due to the whole range of functions of liver parenchyma, changes that occur during food restriction may evoke different

* Corresponding author. Tel.: +420 77 6842 684.
E-mail address: pmakovicky@email.cz (P. Makovicky).

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degrees of hepatocyte damages that become apparent as discrepancies in laboratory markers [23,37]. Since different levels of food restrictions are correlated with liver metabolism, we can assume that parallel changes also occur in liver morphology and that these changes may result in an entire spectrum of liver diseases with wider clinical symptomatology. There is a lack of literature dedicated to the activity of liver enzymes and autoantibodies of growth hormones and liver histopathology in varying degrees depending on the food restrictions with furthermore ad libitum (ADL) channels. If causality would have been found between these changes, it would be confirmed that it is important to address those relationships with respect to the etiological aspects of hepatology. Liver diseases globally represent frequent pathologies [28,60]. The main tool for the diagnosis of liver diseases remains biopsy. One of the most common biopsy finding is liver steatosis with its etiology clearly related to nutrition. Etiopathogenic aspects and mechanisms are subject to an intense global research. Fat dystrophies have a different genesis and a different biological nature. They are mostly found as accumulated fat from food that hepatocytes cannot process. It was also confirmed that steatosis occurs as univacuolar fat from adipose tissue during starvation and that it can be induced experimentally [4,25,47,49,54]. This results from accumulation of triglycerides in hepatocytes, as a result of an imbalance between delivery of free fatty acids and endogenous lipogenesis, versus fatty acid. The whole process of generalization of non-alcoholic fatty liver disease (NAFLD) is often clinically asymptomatic and without any special findings by routine laboratory tests [42]. According to the literature, the elevation of liver enzymes may not occur even in the process of progredience to non-alcoholic-steatohepatitis (NASH) [2,17]. In this process, the various antibodies were positively tested [1,31,59]. Furthermore, the decrease of levels of growth hormones was observed as attributes of lipolytic function [55]. It was found out that insulin-like growth factor I (IGF-I) has a protective effect in preventing the development of NASH [39]. It is known that the IGF-I regulates cell growth [5,57] and that its activity is mediated by binding to insulin-like growth factor binding protein 3 (IGFBP-3) [48]. Its function is to extend the half-life of IGF-I growth factor in the circulation for several hours [29]. However, it is not clear whether the titers of IGF-I and IGFBP-3 in the post-restriction period are in relation to morphological changes in the liver. Our work focuses on the relationship between biochemical profile of liver autoantibodies, IGF-I, IGFBP-3 and liver histopathological aspects after food restriction with weekly consecutive ADL income. We believe that the results from this study could contribute to a better understanding of the relationship between food restrictions and liver function. Morphology of hepatocyte and changes in the liver parenchyma in the post-cleavage of ADL intake could enhance the protective effect of food restriction. Reaction of hepatocyte by secretion of IGF-I, IGFBP-3 with liver biochemistry and circulating autoantibodies reveal whether there are any relations between the time, the degree of food restrictions and ADL income.

The results would also be helpful for the histopathologic diagnosis of NAFLD and NASH. Other practical outcomes of present work would find its place in the early non-invasive therapeutic possibilities of liver dystrophy. Therefore, in our work, we verify a protective effect of food restrictions on the experimental models and control group. Furthermore, the aim of this work is to find the relationship between biochemistry, values of autoantibodies and activity of IGF-I, IGFBP-3 in the context of the histology of liver parenchyma after food restriction with weekly ADL income by using rabbit animal model.

Materials and methods

This study was approved by the Ethics Committee of the Institute of Animal Science and the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic and carried out according to the guidelines for applied nutrition experiments in rabbits [16].

Fig. 1. Macroscopic figure showing animals during experiment. This is timeline figure about the experimental design including young rabbits between 32 and 39 days old housed in cages (A) and one young rabbit, which is weighted as 39-day old (B). There are rabbits from R2 group after restriction feed ADL (C) and rabbits from ADL group before slaughtering at 81 day housed in cages (D).