

# Food Restriction and the Accumulation of Fat in the Livers of Wistar Rats

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**Abstract:** Laboratory rats, when kept in conditions regarded as "standard", are physically inactive, do not have proper environmental stimulation and have free access to food, which can lead to metabolic syndrome and fat accumulation in the liver. This study aimed to analyse the susceptibility of "control animals", which have free access to food, to metabolic syndrome and fat accumulation in the liver compared to animals that were subjected to food restriction. Ninety-day-old Wistar rats were divided into two groups, fed ad libitum (free access to food) and food restricted (60% of the amount of food consumed by the ad libitum group), according to a protocol of alimentation for four weeks. To analyse the effects of food restriction on the nutritional status, serum albumin and total protein levels were analysed and showed no differences between groups, which indicated an absence of malnutrition. The retroperitoneal, subcutaneous and mesenteric fat tissues were weighed, and it was found that the mesenteric fat tissue showed a greater reduction from the food restriction. There was no difference in the insulin sensitivity and the fat accumulation in the liver between groups. However, it was shown that for every 1% decrease in weight due to the food restriction, insulin sensitivity increased by 0.67% and hepatic fat decreased. Therefore, we can conclude that caloric restriction leads to weight loss. Although no significant differences in insulin sensitivity and hepatic fat content existed between the groups at the end of the experiment, weight loss modulated these variables.

**Keywords:** Food restriction, Nonalcoholic fatty liver disease, Rats.

## INTRODUCTION

The accumulation of fat in the liver is a disease characterized by the presence of fat droplets in the organ [1] and discovered by Ludwig *et al.*, in 1980 [2]. This accumulation can vary from a simple steatosis without evidence of inflammation to necro-inflammatory manifestations, including nonalcoholic hepatitis that may progress to fibrosis in 50% of cases, to cirrhosis in 15% of cases and to liver failure in 3% of cases [3-7]. This framework is found in 57.5 to 75% of obesity cases [5] due to changes such as increased consumption of fat and decreased secretion of lipids by the liver [8] or by other mechanisms such as oxidative stress, an inflammatory response and insulin resistance (IR) [9, 10].

Increased caloric intake is directly related to the development of insulin resistance [11]. This imbalance in energy consumption leads to obesity, and in 1988, Reaven [12] was among the first to associate the hypertrophy of adipose tissue, commonly found in obese patients, with IR. One of the ways obesity causes IR is through the action of the fraction of the saturated fatty acids that impairs the transduction of the insulin signalling pathway causing protein kinases to be

serine-phosphorylated [13]. For an improved quality of life, the main changes include regular participation in physical activity and calorie restriction [14-16].

Because there are limitations on research using humans, animal models have become very important study tools in several areas of science. Mice and rats are widely used as animal models in biomedical research, including the field of nutrition, which uses about 90% of all research animals. When housed in conditions regarded as "standard" in most laboratories, these rodents are sedentary, have continuous access to food and do not have adequate environmental stimulation [17]. Therefore, it is questionable whether these animals are adequate "controls" for studies involving altered nutritional states.

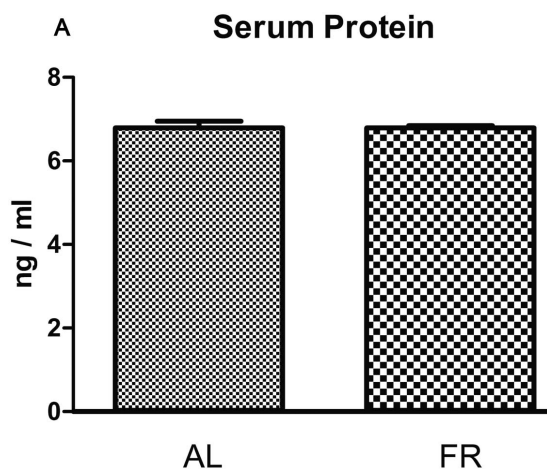
Compared with animals that exercise and are properly stimulated, these animals used as a "control" are relatively overweight, insulin-resistant and hypertensive [18, 19]. In fact, a simple reduction of daily food intake to 20- 40% below that of ad libitum consumption or by feeding intermittently rather than continuously was effective in significantly reducing the risk of chronic degenerative diseases such as cancer, type 2 diabetes and kidney disease and prolonged the life span of laboratory rats and mice by 40% without causing malnutrition [20-24].

Food restriction is an important tool to minimise the incidence of obesity and its consequences [25, 26], and there are few studies in the literature analysing the liver responses of Wistar rats subjected to caloric restriction; therefore, this

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**Fig. (2).** The serum concentrations of the total protein (A) and albumin (B) at the end of the experiment. Results are expressed as means  $\pm$  standard deviation (10 animals/group).

## DISCUSSION

Laboratory rodents are widely used in scientific research and housed in conditions that are considered "standard" in most laboratories, even though these animals are sedentary, have continuous access to food and do not have adequate environmental stimulation [17]. This contributes to the development of hypertension, obesity and IR [18, 19], which directly interferes in research related to nutrition. Therefore, according to the literature [25, 26], dietary restriction is necessary to minimise the metabolic changes caused by obesity and IR, but little is known about its effects on the livers of eutrophic rats. In our study, it became clear that dietary restriction (40%), in addition to reducing the weight gain of animals without causing malnutrition, also contributed to greater insulin sensitivity and less fat accumulation in the liver.

According to Kraemer *et al.* [34], the main purpose of food restriction is to minimise the development of obesity. From our results, we note that a 40% food restriction promoted a 19.5% decrease of body weight when compared to the ad libitum control animals. According to the literature [33-35], high-energy intake contributes directly to an increased storage of triglycerides in the adipose tissue along with an increase of its final volume. Data related to the total increase in the adipose tissue from calorie availability [35-37] were more evident when the weights of adipose tissue from different regions were analysed. Food restriction reduced the weights of the mesenteric, retroperitoneal and subcutaneous adipose tissues by 57.35%, 54.63% and 29.16%, respectively.

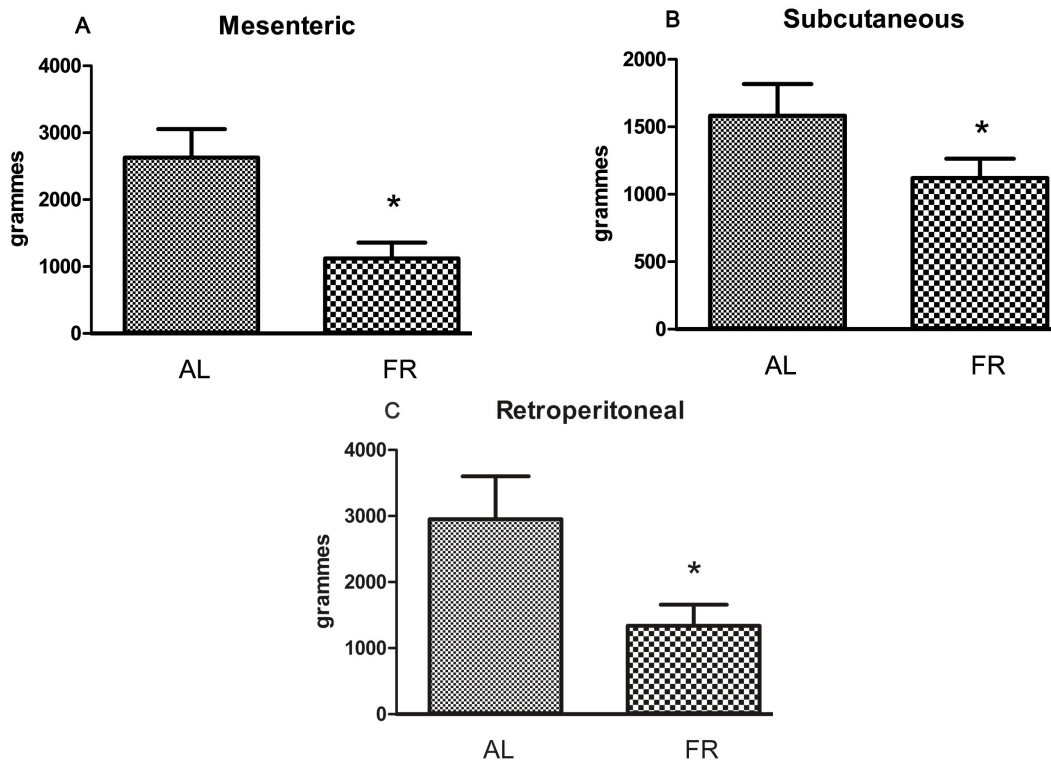
The mesenteric fat in our study was the of the most sensitive to the food restriction. Hermsdorff & Miller [38] showed that the greater susceptibility to a loss of fat tissue located in the visceral region was due to its high vascularity, and the size of their adipocytes indicated a greater response to catecholamines.

The animals with an ad libitum food supply had a higher amount of adipose tissue, specifically the fat tissue in the visceral region, and this increase can be related directly to IR. Various recent research shows that fat tissue has other functions in addition to storing fat [39,40]. White adipose tissue is considered an endocrine organ, producing active hormones such as tumour necrosis factor (TNF- $\alpha$ ), resistin, adiponectin and leptin [41]. TNF- $\alpha$  is directly linked to insulin resistance and leads to a reduced insulin-stimulated auto-phosphorylation of the insulin receptor and a subsequent inhibition of the phosphorylation of insulin receptor substrate-1 (IRS-1). In addition, TNF- $\alpha$  induces changes in the serine phosphorylation of IRS-1, which causes this molecule to inhibit the signalling of the insulin receptor [40]. In our study, we found that in the IR framework, there was a 0.67% improvement in peripheral insulin sensitivity for every 1% in weight loss. Our findings corroborate a study by Björntorp [41] that showed that decreases in total mesenteric fat due to dietary restriction can modulate the responses of peripheral tissues to insulin.

The response of peripheral tissues to insulin will interfere directly in the accumulation of fat in the liver because this

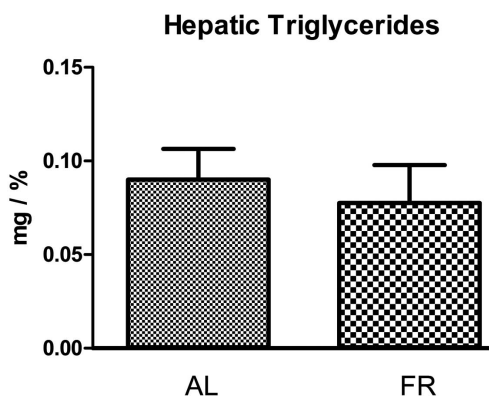
**Fig. (3).** The removal rate calculated by KITT serum glucose during the insulin tolerance test at the end of the experiment. Results are expressed as means  $\pm$  standard deviation (10 animals/group).

To evaluate the nutritional status of the animals, serum albumin and total protein levels were analysed [31-33]. According to the results, the animals subjected to food restriction in the present study showed no evidence of malnutrition because the concentrations of total protein and serum albumin did not decrease.



**Fig. (4).** The weight of adipose tissue from different regions, mesenteric (A), subcutaneous (B) and retroperitoneal (C), at the end of the experiment. Results are expressed as means  $\pm$  standard deviation (10 animals/group). \*  $\neq$  control group.

hormone, when in excess in the circulation (diabetes mellitus type 2), will play an important role by activating the lipogenic transcription factor SREBP (sterol regulatory element-binding protein) [42, 43]. The SREBPs directly activate the expression of approximately 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides and phospholipids and of NADPH, a cofactor required for the synthesis of these molecules [44, 45]. Though three SREBPs were found in the liver, SREBP-1c is preferentially more active by increasing the transcription of genes involved in fatty acid synthesis, including genes that encode for the enzymes acetyl-CoA carboxylase and fatty acid synthase. The first enzyme converts acetyl-CoA to malonyl-CoA and the second converts malonyl-CoA to palmitate [44] contributing to the accumulation of fat in the liver and consequently to the installation of nonalcoholic steatohepatitis (NASH).



**Fig. (5).** The concentrations of fat in the livers at the end of experiment. Results are expressed as means  $\pm$  standard deviation (10 animals/group).

NASH is characterised by fat accumulation in the liver and can lead to inflammation in the organ until fibrosis occurs [3-7]. IR will contribute to the development of NASH; in individuals resistant to insulin, the SREBP-1c levels are elevated due to high levels of the hormone [46]. Similarly, the SREBP-1c levels are elevated in the livers of ob/ob mice [47, 48]. Despite the presence of insulin resistance in peripheral tissues, insulin activates transcription of SREBP-1c in the livers of rats. The high levels of nuclear SREBP-1c increase the expression of lipogenic genes, fatty acid synthesis and an accumulation of triglycerides in the liver [48, 49]. In our study, four weeks were not sufficient for the development of IR, but it was seen that for a 1% decrease in body weight, insulin sensitivity increased 0.67% and hepatic fat accumulation decreased 0.67%. This may be due to a decreased supply of calories from the dietary restriction, which leads to a reduced production of insulin by the pancreas. There is a consequential decrease in the activation of SREBPs in the adipose tissue and liver, thus reducing the amounts of fat substrate in these tissues [42, 44, 48, 49, 50].

## CONCLUSION

From the observed results, we can conclude that four weeks were not sufficient for the development of IR and that dietary restriction, which does not cause malnutrition, improves insulin sensitivity and minimises the fatty accumulation on body and liver in both eutrophic rats.

## AUTHOR CONTRIBUTIONS

All of the authors contributed to the study not only with the sample collections but also with the preparation of this manuscript. All of the authors have read and approved of the final version of this manuscript.

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## CONFLICT OF INTEREST

None declared.

## ABBREVIATIONS

IR	=	Insulin Resistance
TNF- $\alpha$	=	Tumour Necrosis Factor
IRS-1	=	Insulin Receptor Substrate-1
SREBP	=	Sterol Regulatory Element-Binding Proteins
NAFLD	=	Nonalcoholic Fatty Liver Disease
ITT	=	Insulin Tolerance Test
Kitt	=	Removal Rate of Serum Glucose

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