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IMPAIRED GLUCOSE TOLERANCE AND ADIPOSE TISSUE INFLAMMATION IN NUTRITIONAL INDUCED OBESITY IN RAT

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Summary

Intra-abdominal fat is closely associated with insulin resistance and, therefore, plays, an important role in the development of metabolic disorders including hyperglycemia, hypertension, hypertriglyceridemia, and low high-density lipoprotein (HDL)-cholesterol, all this are in direct relation with cardiovascular disease.

Data regarding obesity level, insulin sensitivity, the development of the intra-abdominal fat, histopatological aspect of intra-abdominal fat were collected from experimental obese rats comparative with normponderal rats. We want to see if there are any correlation between development of intra-abdominal adiposity and other metabolic and lesional modification.

Obesity is a component of the multiple risk factors that jointly constitute the metabolic syndrome and represents a major and increasing public health problem especially in the industrialized world.

Insulin-resistance represents an important factor linked to general indices of adiposity; the strongest association is with central abdominal fat. Intra-abdominal fat is a significant independent predictor for metabolic disorders like insulin sensitivity (16), impaired glucose tolerance (4), elevated blood pressure (5), hyperglycemia, hypertension, hypertriglyceridemia, and low HDL-cholesterol seen in the metabolic syndrome (1). Intra-abdominal fat accumulation causes dysregulation of adipocyte function, leading to oversecretion of tumor necrosis factor-α (TNF-α), free fatty acids, plasminogen activator inhibitor-1 (PAI-1), interleukin-6, and growth factors, as well as hyposecretion of adiponectin, all of which may participate in the development of metabolic dysfunction (11). Intraperitoneal adipocytes are more lipolytically active than subcutaneous adipocytes and potentially could contribute more to the overall flux of fatty acids and glycerol to the systemic circulation. These adipokines are directly or indirectly involved in the process of atherosclerosis, thus contributing to an increased cardiovascular risk.

Patients with metabolic syndrome have a 3- to 4-fold increased risk of mortality due to coronary heart disease (CHD) (8), and intra-abdominal fat is an important determinant of the risk of CHD (9, 13).

Also because of adipocyte secretion, obesity is often associated with a state of chronic low-level inflammation (2). Another reason for the inflammatory

status seems to be the macrophage infiltration in obese adipose tissue (17). Adipose tissue is a heterogeneous population of cells composed not only of adipocytes (30% to 60%) (6) but also non-adipocytes such as preadipocytes, fibroblasts, macrophages, and vascular cells. This implies that paracrine interactions between adipocytes and non-adipocytes such as macrophages influence the type of inflammatory response in adipose tissues in obesity-related inflammatory conditions. But the mechanisms by which this inflammatory response is triggered and maintained in obesity are still poorly understood, macrophage infiltration into the adipose tissue and cross-talk between adipocytes and interstitial macrophages in adipose tissue seem to represent central elements (10). Activated macrophages, which are indispensable for inflammatory responses, release a variety of proinflammatory mediators and, thus, play a pivotal role in inflammatory responses. The up-regulation of proinflammatory molecules, such as inducible NO synthase and TNFα, has been observed in adipose tissue-derived macrophages in obese mice (17), indicating that macrophage activation might occur in the adipose tissue.

Objective, material and method

In our experiment we want to obtain obese experimental models (whistar rats) especially with central (intra-abdominal) obesity. In these subjects we want to study general obesity, macroscopic and microscopic aspect of intra-abdominal adipose tissue, especially to observe inflammatory cell infiltration. Also we want to observe insulin resistance in obese subjects comparative with control.

For this we made 2 experimental lots of whistar rats:

- Control group (lot1) (n=8), female Whistar rats, age of aprox 100 days
- Obese group (lot 2) (n=8), female Whistar rats, age of aprox 100 days

Experimental group was housed in cages, 4 rats in a cage, on sawdust, 12 hours light and 12 hours dark, with water at discretion.

The control group was feed with the standard rat food with an approximate 25-30 g/animal. The obesity was induced by nutrition, using a hypercaloric diet. For this, the standard diet was supplemented with pig fat, sugar and vitamin-mineral premix; food was administrated *ad libidum*.

To appreciate obesity degree, weight, length (from mouth to base of the tail), abdominal and abdominal circumference were measured. Results of measurement were inserted in a adapted body mass index (BMIa) formula:

BMIa = [weight (g) + abdominal circumference (cm)]/length (cm)

At age of 300 days oral glucose tolerance test (OGTT) was made.

At age of 400 days all subjects were euthanized and samples from various types of adipose tissue were isolated from different fat depots (e.g., mesenteric, epididymal, renal, and subcutaneous adipose tissues) from obese and non-obese rats.

Ingathered samples of intra-abdominal obese adipose tissue was processed by paraffin technique and colored Hematoxiline-Eosine (HE). The macrophages infiltration in obese adipose tissue was quantified.

Results and discussions

After the administration of a hyper-caloric diet we obtained obese rats, with an elevated BMIa comparatively with control lot. It can be observed that obese group has a statistically significant higher obesity degree comparatively with control group (Tab. 1.).

Many studies have reported that obesity is consistently related to unfavorable lipid profiles and cardiovascular disease. Body mass index formula, kilograms per meters squared, how is determined in human does not include waist circumference (WC) although each, but especially intra-abdominal obesity, are associated with the risk of developing cardiovascular disease (CVD). Therefore, a combination of the two may be more effective in identifying subjects at risk than either alone. Because of this we made a new formula for BMI to combine these two parameters.

Table 1

Medium weight and obesity degree in control and obese group; p – control statistic difference signification comparative with control lot (fd- without statistical signification)

Lot	Weight (g)	Length (cm)	abdominal circumference (cm)	BMIa
1	305	21,375	15,625	10,26
2	414	22,70	17, 875	18,52
p	< 0,01	f.d.	< 0,01	< 0,01

However, body fat distribution is a more powerful predictor than BMI for cardiovascular risk factors, morbidity, and mortality. How it can be observed in pictures below (Fig. 1. and Fig. 2) abdominal adiposity, especially mesenteric and also renal and dorsal adiposity is very developed in obese rats comparatively with control. It is known that abdominal and body obesity is important determinants of glucose intolerance, hypertension, intima media thickness (12).

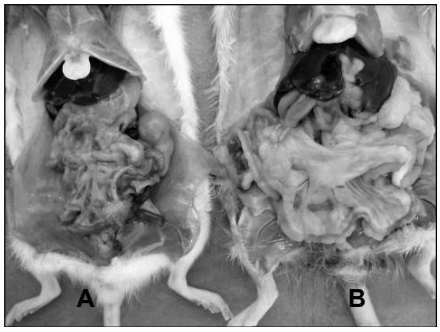

Fig.1. Mesenteric adiposity in control (A) and obese rat (B)

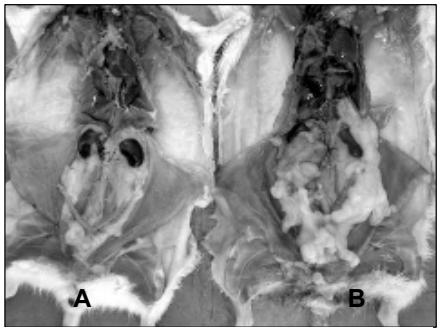

Fig. 2. Renal and dorsal adiposity in control (A) and obese rat (B)

Oral glucose tolerance test (OGTT) was made in order to demonstrate insulin resistance. It can be observed that glycaemia *a jeun* is rise in obese lots comparative with control. At 1h after glucose administration glycaemia level is rising in both lots respectively 131 mg/dl in control lot and 146 mg/dl in obese lot. At 2 h in control lot glycaemia rich the initial level (70 mg/dl) during in obese lot remains elevated respectively 126 mg/dl, substantially hire then initial value of 95,4 mg/dl (Fig 3).

Type 2 diabetes is associated with insulin resistance and impaired insulin secretion. Insulin resistance occurs early in the development of the disease and can predict the risk of future diabetes.

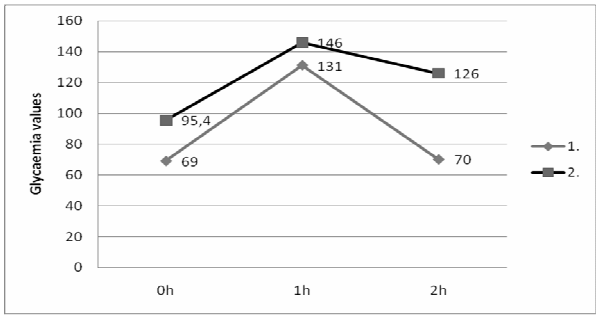

Fig. 3. Glycaemia evolution in control (1) and obeselots (2) after OGTT determianation

Histological, intra-abdominal adipose tissue has characteristic adipocytes, with ring shape, but we also observed an inflammatory infiltrate, representated by macrophages, some of them with an abundant foam cytoplasm (Fig. 4).

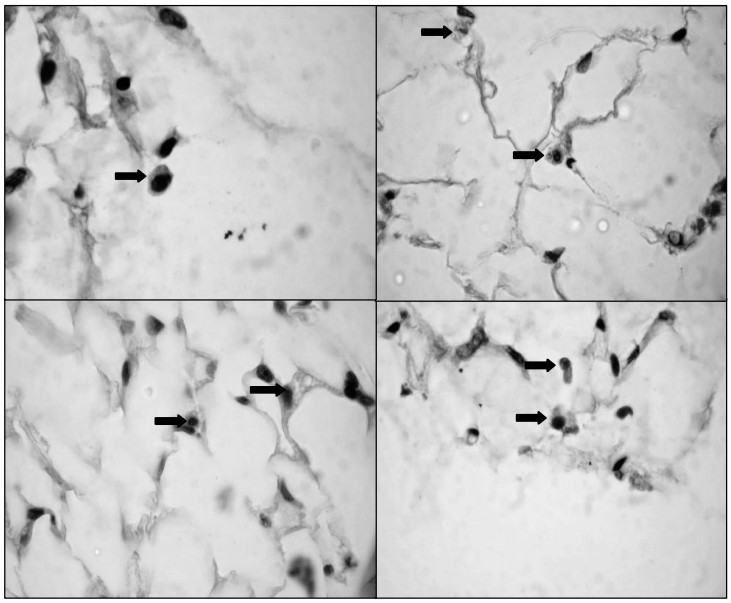

Fig. 4 Macrophages infiltration in abdominal adipose tissue (Black arrow) HE

In some area of intra-abdominal adipose tissue we observed a plasmocytes infiltration (Fig. 5).

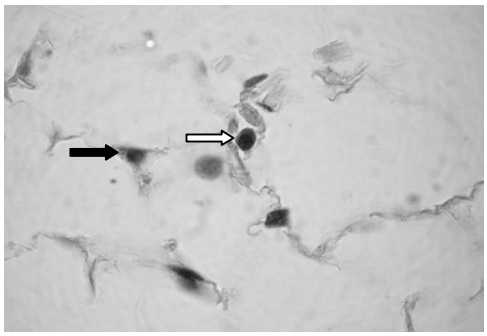

Fig. 5. Macrophages (black arrow) and plasmocytes (white arrow) infiltration in abdominal obese adiposity HE x1000

The mechanism of macrophage recruitment to adipose tissue has not been fully elucidated. Adipose tissue resident macrophages could originate from either blood monocytes or from preadipocytes (3). It seems more likely that the macrophage population found in adipose tissue originates as a consequence of the recruitment of blood monocytes. Tissue infiltration of blood monocytes is a complex phenomenon that involves several steps, including the activation of the capillary endothelium and the increased expression of adhesion molecules, such as

intercellular adhesion molecule – 1 (ICAM-1), and the adhesion of blood monocytes, followed by their transmigration across the endothelium and differentiation into macrophages. Macrofaigic tissue infiltration involves the adipose tissue-derived chemotactic molecules. One such candidate is monocyte chemoattractant protein-1 (MCP-1), synthesized in and secreted from preadipocytes and mature adipocytes (15). Another candidate molecule involved in the recruitment of macrophages in obese adipose tissue is macrophage-colony stimulating factor (M-CSF) (15). Also increased plasma concentrations of soluble cell adhesion molecules (E-selectin, Vascular cell adhesion molecule 1 [VCAM-1], ICAM-1, and von Willebrand factor) have been reported (7 in 3) in overweight and obese individuals, suggesting that increased fat mass is associated with an early systemic endothelial activation.

Conclusions

An important conclusion that can be made from this work is that central adiposity (intra-abdominal adiposity) is an important predictor for metabolic and lesional disorders that appears in obesity and furthermore in metabolic syndrome. Because of this we think that is important to include in BMI formula measurement of abdominal circumference.

How it can be observed BMIa calculated by us has a positive correlation with values from OGTT, so a positive correlation with insulin resistance.

Also the presence of macrophages and plasmocytes in adipose tissue indicate that there we have an inflammatory status, situation that can lead to vascular lesions, how we showed in a previous work (14).

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