Bulletin UASVM, Veterinary Medicine 68(1)/2011
pISSN 1843-5270; eISSN 1843-5378

Quantification of iNOS Expression in Adipose Tissue From Human Obese Patients

Gabriel BORZA, Cornel CĂTOI, Adrian GAL, Pompei BOLFĂ, Marian TAULESCU, Andras NAGY, Flaviu TĂBĂRAN, Cosmina CUC, Raouad MOUSSA

University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Mănăştur street, 400372, Cluj-Napoca, Romania, e-mail: gabi_borza@yahoo.com

Abstract. Studies in recent years have led to a better understanding of the mechanisms by which obesity is closely linked to metabolic syndrome and its complications. Biomarkers of inflammation such as tumor necrosis factor α (TNF-α), leukocyte counts, interleukin 6 (IL-6), and C-reactive protein, have increased values in obesity and are used in prognosis of insulin resistance, type 2 diabetes and cardiovascular diseases. It is known that obesity is associated with a chronic inflammatory response, characterized by abnormal production of cytokines and activation of inflammatory signaling pathways. Recent studies in rats have shown that in obesity the adipose tissue is infiltrated by macrophages and thus activation of several inflammatory response genes. iNOS expression may be one's response to this activation. iNOS expression is regulated at a transcriptional level, and once activated, the enzyme can generate large amounts over a long period of time. Thus, in the early stages of obesity has been an increase in the amount of NO, not a decrease. The purpose of this paper is on determining the quantitative iNOS expression in macrophages and endothelial cells from adipose tissue harvested from two areas: subcutaneous fat and the greater omentum. It will also be evaluated the correlation between the amount of iNOS and the number of iNOS positive cells and the correlation between the degree of obesity and the quantitative expression of iNOS.

Keywords: obesity, adipocyte, iNOS, macrophage, immunohistochemistry.

INTRODUCTION

Obesity is responsible for growth in excess of oxidative stress that contributes to a large number of cardiovascular diseases (Keaney JF col, 2003). Obesity itself is considered a chronic oxidative stress and inflammation even in the absence of other factors that could cause cardiovascular diseases (Higdon JV et al., 2003). Animal studies have shown that obesity leads to increased oxidative stress in the myocardium (Vincent A. et al., 1999) and increased lipid peroxidation (Dobrian D. et al., 2000). Blood plasma, responsible for transporting LDL, has a wide range of antioxidant defense mechanisms (Stocker et al., 1991), including a number of antioxidant proteins, mainly metal chelation proteins and enzymes. Oxidative stress can be followed by biological makers (antioxidants and oxidants) that are found in plasma and erythrocytes (Pass et al., 2001).Overweight and obese animals have a high rate of mortality due to cardiovascular diseases such as atherosclerosis (Eckel et al., 2002). There are at least three ways in which obesity plays an individual can produce lipid peroxidation. Obesity increases metabolic activity and myocardial contraction, thereby increasing oxygen consumption at this level (Olusi et al., 2002). A negative effect of oxygen

consumption at this level is the production of reactive oxygen species such as superoxide, hydrogen radicals and hydrogen peroxide resulting from mitochondrial respiration (Turrens, 1997). The second mechanism by which obesity induces increased stress oxidaiv is the repeated insults to cells caused by the pressure exerted on them. Lesions in cellular tissue causes the release of cytokines, particularly tumor necrosis factor alpha responsible for, which generates reactive oxygen species (Lechieitner col, 2000). A third possible mechanism is through diet, hyperlipidemia, lipids are involved in oxygen metabolism. If these reactive oxygen species production is beyond the capacity of the cell antioxidant, oxidative stress resulting from lipid peroxidation process can lead to atherosclerosis.

MATERIALS AND METHODS

The biological material consisted of 10 biopsies (samples of 5-6 cm3) of adipose tissue taken intraoperatively from two regions, namely subcutaneous and adipose tissue from the omentum. Adipose tissue samples were collected during various surgical procedures, most being done for gastroplasty.

The samples were fixed in 10% neutral buffered formaline and embedded in paraffin. Immunohistochemistry was done using a rabbit polyclonal antibody to human iNOS (ab15323, Abcam, UK). For immunohistochemistry sections of 4 mm were mounted on poly-L lysine coated slides and stored for a maximum of 48 hours at room temperature until use. The slides were deparaffinized using xylene. Antigen retrieval solution was realized using a pressurized cooker in citrate solution, pH=6. Endogenous peroxidase was inactivated using peroxidase blocking reagent (Dako) during 5 minutes at room temperature. Primary polyclonal antoibodies were maintained overnight at 4°C, using a dilution of 1:100 (Dako antibody diluent). The visualization of the immunological reaction was done using Universal LSAB+Kit/HRP, Rb/Mo/Goat (DAB+) system (Dako); counterstaining was performed using Mayer's hematoxylin.

The slides were examined under an Olympus BX 51 microscope, and the images were taken using an Olympus DP 25 digital camera and processed using the Olypmus Cell B image acquisition and processing software.

The quantitative expression of iNOS in macrophages and endothelial cells was determined using image program software DP 5, by measurement of color intensity for red, green and blue. The blue color intensity was not measured, because it was mainly due to hematoxylin counterstaining. Setting the coefficient of intensity was made by calculating the difference in intensity between red and green. To obtain the final results of intensity ratio was multiplied by the number of macrophages and endothelial cells that were iNOS positive.

RESULTS AND DISCUSSIONS

Macrophages iNOS expression increases with BMI only in patients with degrees of obesity from overweight to grade II. In the grade III obesity, morbid obesity,it decreases with the BMI value. Also in grade III obesity patients iNOS expression of macrophages is higher in the omentum, when patients with overweight or grade I, II obesity iNOS expression of macrophages is higher the subcutaneous fat. The differences are not statistically assured. The iNOS expression of macrophages is explained by much higher number of macrophages in adipose tissue than in other regions, so it is concluded that the intensity of iNOS expression in macrophages infiltrate is preserve the tissues. iNOS expression of macrophages can be seen in

fig. 1. There is no correlation between iNOS expression intensity and BMI, which is ensured statistically.

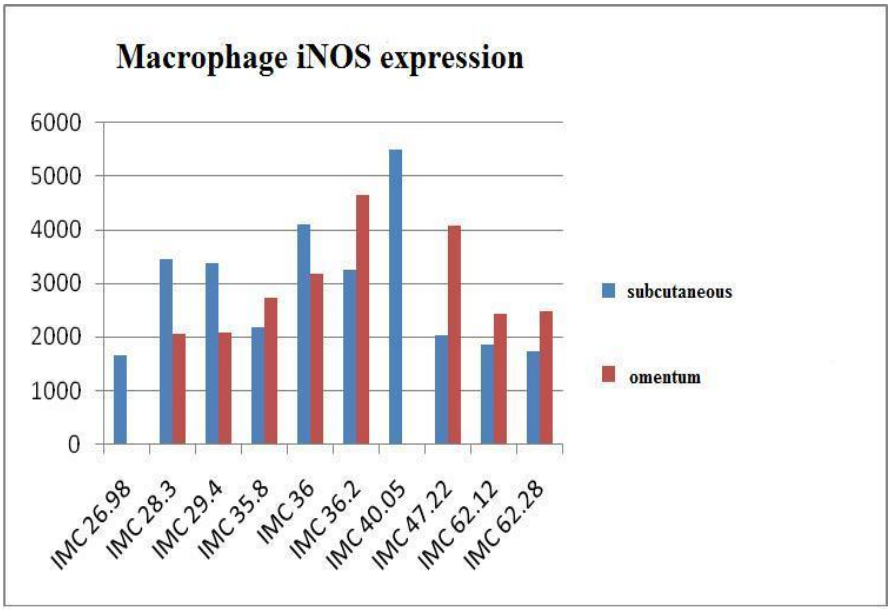

Fig.1. Quantification of iNOS positive macrophages according to BMI.

There was no correlation between iNOS expression of endothelial cells and BMI. However you can see a sharp decrease in the expression of iNOS in proportion to the increase of BMI, but only in patients with grade III obesity, and only in the omentum adipose tissue. Also there was no correlation between iNOS expression by endothelial cells of the two areas studied. The results are shown in *fig.2.*

Quantification of iNOS expression by all iNOS positive cells revealed a higher value at the omentum than the subcutaneous fat, except one case, on which the amount of subcutaneous adipose tissue iNOS expression was higher than that of the omentum. In contrast, there was no correlation between the iNOS positive cells expression andn BMI. The values obtained can be seen in *fig. 3.*

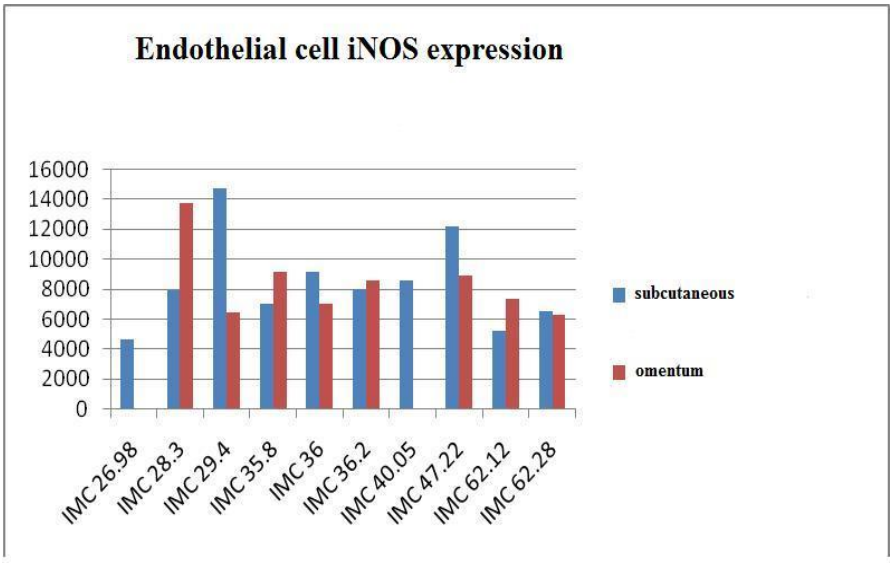

Fig. 2. Quantification of iNOS positive endothelial cells according to BMI.

To establish the total quantity of iNOS for each individual, we used a coefficient that was the quotient of each individual BMI over the BMI mean for normal-weight people, which is 21.7.

Total iNOS expression revealed a significant increase in both areas studied, increase which was proportional to the BMI value. This fact demonstrates that the intensity of iNOS expression is directly proportional with BMI, and hence the degree of obesity. Total iNOS expression is higher in the omentum adipose tissue, compared with subcutaneous fat. The results can be seen in f*ig. 4.*

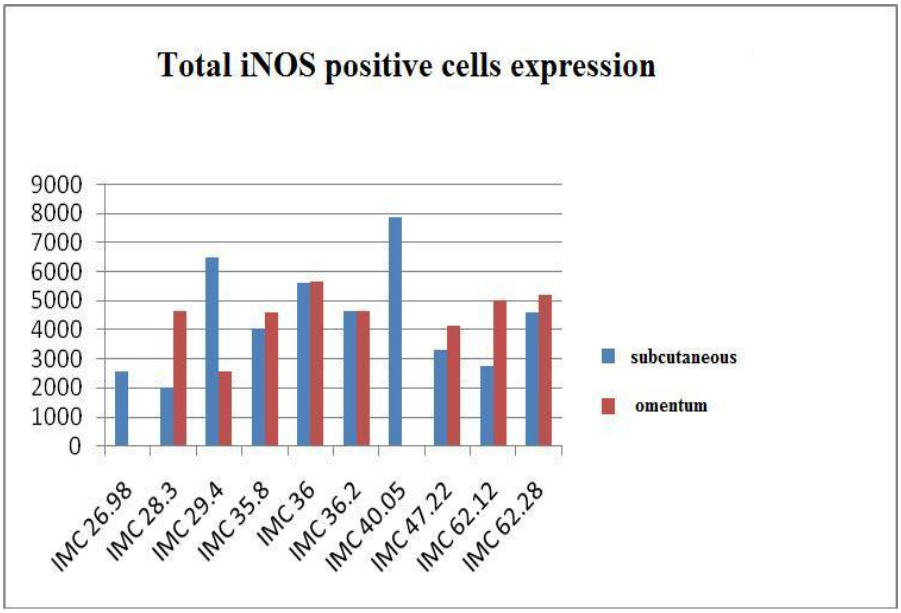

Fig. 3. Quantification of total iNOS positive cells according to BMI.

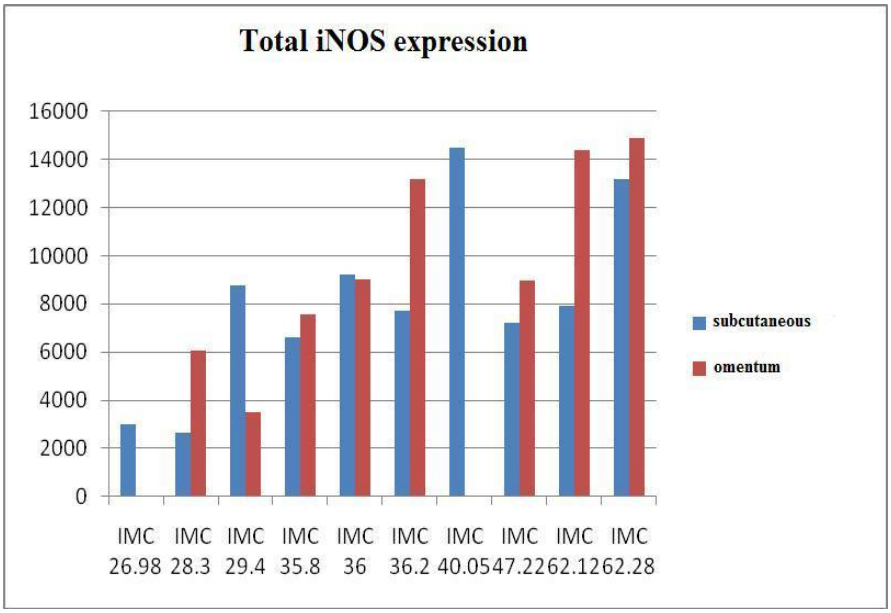

Fig. 4. Total iNOS expression according to BMI.

CONCLUSIONS

- iNOS expression of macrophages in adipose tissue is increased in the subcutaneous fat rather than in the omentum, but only in patients with degrees of obesity from overweight to grade II. In grade III obese patients iNOS expression of macrophages is higher in the omentum.
- There could not be established a correlation between endothelial cells iNOS expression and BMI. It also has not been established any correlation between iNOS expression in the endothelial cells of the two areas studied.
- iNOS expression of positive macrophages and endothelial cells revealed a significant increase at the omentum, rather than of subcutaneous fat tissue.
- total iNOS expression revealed a significant increase in adipose tissue from both regions (omentum, subcutaneously) proportional to BMI value, which means that in overweight people is iNOS expression is much higher.

REFERENCES

1. Eckel R.H., W.W. Barouch, A.G Ershow., 2002. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity associated cardiovascular disease. Circulation, 105: 2923–2928.
2. Higdon J.V., B. Frei, 2003, Obesity and oxidative stress: a direct link to CVD, Arterioscler Thromb Vasc Biol 23, 365–367.
3. Keaney J.F. Jr., M.G. Larson, R.S. Vasan, P.W. Wilson, I. Lipinska, D. Corey, J.M. Massaro, P. Sutherland, J.A. Vita, E.J. Benjamin, 2003, Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study, Arterioscler Thromb Vasc Biol, 23:434–439.
4. Lechieitner M., T. Koch, M. Harold, A. Dzien, F. Hoppiahler, 2000, Tumour necrosis factor-alpha plasma level in patients with type 1 diabetes mellitus and its association with glycaemic control and cardiovascular risk factors, J. Intern. Med., 248: 67-76.
5. Stocker R. B. Frei, 1991, Endogenous antioxidant defences in human blood plasma in rats