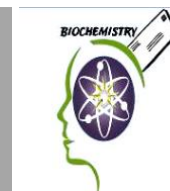




Scientific Research & Studies Center-Faculty of Science- Zagazig
University- Egypt

Biochemistry Letters

Journal home page:



The effects of weight loss intervention program on serum leptin level, histological and molecular alterations of adipose tissue in rat model with obesity.

Nearmeen M. Rashad¹, Sally E. Sayed², Mohamed H. Sherif³, Mahmoud Z. Sitohy⁴,

¹ Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

² Biochemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt

³Organic Chemistry Department, Faculty of Science Zagazig University, Zagazig, Egypt.

⁴ Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig Egypt.

ARTICLE INFO

ABSTRACT

Background: Obesity is a resilient and complex chronic disease.

Leptin is an adipocyte-derived hormone acts as a key regulator of feeding and energy expenditure.

The aim of our study was to assess the level of serum leptin as well as leptin immuno-histochemistry expression in adipose tissue in obese and non-obese rats.

Leptin was markedly detected in young preadipocytes during lipogenesis (before weight loss (regime) in contrast to adipocytes undergoing lipolysis (after weight loss (regime) in the later weak positive.

Corresponding author: Mobile:+201093052248,

E-mail address: sallyelsayeds@yahoo.com

INTRODUCTION

Obesity is a resilient and complex chronic disease. The prevalence of obesity, is now recognized worldwide as a major health problem, reaching epidemic proportions probably as a consequence of changes in food composition and exacerbated by sedentary lifestyles in Western societies [1,2]. An increasing body of evidence supports the evolving concept that obesity is associated with increased mortality mostly caused by augmented risk of cardiovascular death [3].

Obesity induces a complex remodeling of adipose tissue, which expands to accommodate the excessive caloric intake and markedly changes its structure and cellular composition. It is widely accepted that this obesity-associated remodeling generates a systemic proinflammatory state, which is mediated by an imbalanced production of adipocyte-derived cytokines (adipokines) [4]. Leptin, a product of the obesity gene (or ob gene) is a key regulator of feeding and energy expenditure [5]. This adipocyte-derived hormone was once heralded to be an antiobesity agent. While leptin is effective in certain individuals bearing congenital leptin deficiencies [6]. or lipodystrophies [7] as a monotherapy, leptin has been disappointing in humans and rodents with common obesity, that is, obesity associated with elevated serum leptin under a normal genetic background [8]. Leptin production increases proportionally with adiposity, and leptin levels are high in rodent and human models of diet-induced or adult-onset obesity. Yet,

the increased leptin fails to curtail the progression of obesity. This apparent leptin ineffectiveness is identified as leptin resistance [9, 10]. Emerging evidence suggests that leptin resistance predisposes the animal to exacerbated diet-induced obesity (DIO). Elevation of central leptin in young, lean rats induces a leptin resistance that precludes obesity on a chow diet but accelerates high-fat (HF)-induced obesity. Similarly, chronic dietary fructose consumption evokes a leptin resistance that causes obesity only upon HF exposure. Thus, the aim of our study was to assess the level of serum leptin as well as leptin immunohistochemistry expression in adipose tissue in obese and non-obese rats. Also, we aimed to examine the impact of weight loss on serum leptin and its immunohistochemistry expression in adipose tissue in obese rats.

MATERIAL AND METHODS

Experiment design

20 adult male albino rats weighing (190-210 g), which used throughout this study were purchased from Veterinary medicine faculty, Zagazig University. They were housed in stainless steel rodent cages at the animal house, Faculty of Medicine, Zagazig University and allowed one week as adaptation period at room temperature with a 12 hours dark / light cycle before beginning the experimental work. They were kept under environmentally controlled conditions and were fed with standard rodent chow (SCD) (EL-Nasr pharmaceutical Chemicals Company, Egypt) and allowed free access of tap water. All the experiments and animal handling were approved by the ethical committee of the Faculty of Medicine, Zagazig University.

After one week acclimatization, rats were divided into 2 main group (10 rats,each) the first, control group, the second was rendered obese by feeding a high fat diet. Water and feed were available all the time during the experimental period.

Blood samples

Blood samples were collected from the retro-orbital venous plexus under light ether anesthesia after fasting 12 hours to estimate for serum lipid profiles (serum TC, TG, HDL-c, and LDL-c)

Tissue sampling

At the end, rats were killed by decapitation; liver was collected from rats and fixed in 10% buffered formalin solution for histological examination.

RESULTS

Clinical and laboratory characteristics of studied groups at base line and obese group

Our study shown that, serum leptin, rat weights, total cholesterol, triglycerides, LDL cholesterol, epididymal fat pad mass and body fat % were significantly lower in obese group after 8 weeks of weight loss compared to obese rats at baseline compared obese group.

Immunohistochemical staining procedures.

Experimental animals were sacrificed at week six post treatment and tissue samples from the adipose tissue were fixed in 10% Neutral Buffer Saline (NBS) for 24-48 hours. They were dehydrated in ascending concentration of Absolute alcohol, cleared in xylene,dipped in Paraffin wax and sections of 5µ thick were stained in Hematoxylin and Eosin (H&E) for light microscope examination. The standard immunohistochemical method were adopted for the detection of marker of obesity in

adipose tissue sections (leptin). The tissue sections were routinely microwave-treated to unmistak the epitopes of antigen. The Biotin-Streptavidin (BSA) system was used to visualize the obesity marker . Diaminobenzidine (DAB) was used as chromogen since it allows a permanent preparation.Hematoxylin counterstain was done.

Assessments of lean mass and fat mass

At the end of 20 weeks study, the lean and fat mass were determined by dual energy x-ray absorptiometry (DEXA) with small animal software (Lunar Prodigy, General Electric). Standard values for animals were used for compliance with the small animal software. Standard DEXA values included height of 10 inches, and weight of 0.3 lbs. Removal of the epididymis fat pads was conducted following the DEXA scan, and was immediately weighted.

Detection of Serum leptin by ELISA Method

Serum leptin levels were assessed using commercial ELISA (Enzyme-Linked Immunosorbent Assay) kit (Human Leptin ELISA kit, (Biovendor [Cat No: RD191001100], USA).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed using “t” test. We considered P to be significant at <0.05 with a 95% confidence interval (CI).

Results of Immuno-histochemistry:

1- Obese rats. (I) before weight loss(regimen)

Examined sections from collected **adipose tissue** of this group revealed normal histomorphological characteristics represented by rounded regular fatty cells with peripherally situated nuclei. leptin expression becomes apparent after differentiation of preadipocytes to adipocytes coinciding with early stages of lipid accumulation in the cells(lipogenesis). Fully mature cells lack leptin expression. (plate 1).

Plate 1. Photomicrograph, adipose tissue of (obese rats) showing normal rounded regular fatty cells with peripherally situated nuclei. leptin expression is seen in preadipocytes coinciding with early stages of lipid accumulation (B, red arrow). Fully mature cells lack leptin expression (A, red arrow).X 100(A),400(B)

2- Control rats (II).

Sections from fat of this group revealed apparently normal histomorphology of the corresponding tissues. Leptin expression couldn't be detected in control group (Plate 2).

Plate 2. Photomicrograph of adipose tissue of control group showing apparently normal histo-morphology of the corresponding tissues (a red arrows).

3- Obese rats. (III) after weight loss(regimen)

Examined sections from adipose tissue of this group revealed weakly and moderately expressed leptin, lipid metabolic marker respectively. It was seen in full

organized adipocytes undergoing lipolysis as a weakly stained material. (Plate 3)

Plate 3. Photomicrograph, adipose tissue after weight loss(regimen) showing weakly and moderately expressed leptin (lipid metabolic marker) respectively. It is seen in full organized adipocytes undergoing lipolysis as a weakly stained material (A, red arrow).

Discussion

The increasing rates of obesity and consequent morbidity represent a major epidemic worldwide and threaten to bankrupt health care systems [11-13]. Friedman *et al.*, While prevention is of great importance, it is medically relevant to identify biological pathways with the potential to treat obesity and related disorders, particularly in adults with fully established obesity and comorbid conditions, such as type 2 diabetes mellitus. Leptin, the product of the ob gene, plays a central role in the regulation of food intake and energy expenditure [14] Zhang *et al.*,

The notion of leptin resistance conjures different interpretations, and its complex nature gives way to several definitions. In the most general terms, leptin resistance is described as the failure of elevated circulating leptin to reduce common obesity. This resistance may be due to an inability of leptin reaching target sites within the brain (resistance to peripherally administered leptin) [15] Banks *et al.*, and/or impaired cellular responses within selected neurons in defined brain regions (central leptin resistance) [16] Enriori *et al.*,

In rodents, leptin resistance is often noted as the reduced sensitivity with respect to the anorectic response to exogenous leptin introduced either peripherally or centrally. the aim of the current study was to assess the level of serum leptin as well as leptin immuno-histochemistry expression in adipose tissue in obese and non-obese rats. Also, we aimed to examine the impact of weight loss on serum leptin and its immuno-histochemistry expression in adipose tissue in obese rats.

Previous researchers found that leptin regulates energy homeostasis and reproductive, neuroendocrine, immune and metabolic; its concentration reflects the amount of energy stored in body fat. Circulating leptin levels are directly proportional to the amount of body fat [17] Considine *et al.*, and fluctuate with acute changes in caloric intake [18] Boden *et al.*, Leptin controls energy homeostasis and body weight primarily by activating Ob Rb in the hypothalamus [19] Chan *et al.*,

According to our results, there were significant higher levels of serum leptin, rat weights, total cholesterol, triglycerides and LDL cholesterol compared to control group. However, HDL levels were significantly lower in case group compared to control group.

Similar results obtained by previous studies, [20] Considine *et al.*, who declared that most obese individuals have higher leptin levels than lean individuals and are resistant or tolerant to the effects of leptin. Leptin resistance was first thought to be due to mutations of the leptin receptor and other rare monogenic obesity syndromes. Mutations of other genes downstream of leptin, including POMC and MC4R, also result in an obese phenotype with associated neuroendocrine dysfunction [21] Farooqi *et al.*,

However, the study done by Remsberg et al. [22] Karen *et al.*, found that leptin levels reflect total body fat and are less affected by fat distribution. Their results parallel those reported by other investigators in which leptin levels exhibit relatively stable associations across differing types of localized fat deposits [23] Chapman *et al.*,

The main finding of the present study is that immunohistochemical techniques of to localize leptin expression in adipose tissue of control group showing apparently normal histo-morphology of the corresponding tissues. However, adipose tissue of obese rats showing normal rounded regular fatty cells with peripherally situated nuclei. leptin expression is seen in preadipocytes coinciding with early stages of lipid accumulation and fully mature cells lack leptin expression. Regarding immunohistochemical of adipose tissue after weight loss(regimen) showing weakly and moderately expressed leptin (lipid metabolic marker) .It is seen in full organized adipocytes undergoing lipolysis as a weakly stained material

In the Cinti et al study, they used immunohistochemical techniques to localize leptin expression in adipose tissue of fed and fasted lean mice, as well as in genetically obese (db/db) mice. to characterize cell types expressing leptin, morphological features and they found that in white adipose tissue, leptin is present in adipocytes of all diameters of lean and obese animals, including adipocytes at a multilocular stage of differentiation, but not in any other cell types. classical brown adipocytes differ from white adipocytes, not only by their morphology and UCP expression, but also by their apparent lack of detectable leptin expression. db/db brown adipocytes, however, were unilocular and leptin-positive. The molecular mechanisms mediating expression of leptin were found in

white but not brown adipocytes of lean animals.

In conclusion

Obese rats had significantly higher levels of serum leptin and immunohistochemical techniques to localize leptin expression in adipose tissue revealed leptin expression is seen in preadipocytes coinciding with early stages of lipid accumulation and fully mature cells lack leptin expression. Regarding immunohistochemical of adipose tissue after weight loss (regimen) showing weakly and moderately expressed leptin (lipid metabolic marker). It is seen in full organized adipocytes undergoing lipolysis as a weakly stained material.

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Variables	Control group (mean \pm SD) (n=10)	Obese group (mean \pm SD) (n=10)	Obese group after 8 weeks (mean \pm SD), (n=10)	P
Rat weight (g)	188.01 \pm 4.26	397.01 \pm 71.5	287.01 \pm 61.54	<0.001 *
Food intake(gm/day)	42 \pm 7.16	66 \pm 6.5	42 \pm 3.2	<0.001 *
Fluid intake (ml/day)	33 \pm 3.06	46 \pm 3.3	29 \pm 1.8	<0.001 *
Total cholesterol (mg/dL)	144.4 \pm 12.37	222.3 \pm 44.4	104.6 \pm 3.3	<0.001 *
Triglycerides (mg/dL)	133.36 \pm 12.28	197.09 \pm 26.3	9.98 \pm 0.11	<0.001 *
LDL cholesterol (mg/dL)	91.92 \pm 0.168	141.68 \pm 34.2	180.3 \pm 22.4	NS
HDL cholesterol (mg/dL)	57.6 \pm 3.95	35.09 \pm 7.76	158.09 \pm 26.3	NS
Serum leptin (ng/ml)	4.23 \pm 1.95	11.43 \pm 3.85	139.68 \pm 24.2	<0.001 *

