Impact of high-fat diet on serum adiponectin and leptin level in streptozotocin-induced diabetes mellitus type 2

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ABSTRACT

Objectives: Alternations in adipokines secretion associated with obesity could play an important role in diet-induced diabetes. The aim of our study was to estimate the impact of high-fat diet on serum adiponectin and leptin levels in streptozotocin (STZ) induced type 2 diabetes mellitus (T2DM).

Methods: The study included 40 adult male Wistar rats were divided into four groups: Standard food control group (C-Non-HF)(n=10), standard food STZ group (STZ-NonHF)(n=10), high-fat diet control group (C-HF)(n=10) and high-fat diet STZ group (STZ-HF)(n=10). C-NonHF and STZ-NonHF group was fed with regular chow, and other two groups were given high-fat diet for 5 weeks. Type 2 DM was induced by single intra-peritoneal STZ injection (60 mg/kg). All the rats were fasted for 12 hours; when blood samples were taken for the measurement of serum leptin and adiponectin level by ELISA.

Results: Mean serum adiponectin level was significantly lower in STZ-HF (1.34±0.57 ng/mL) compared to STZ-NonHF (2.61±0.79 ng/mL), C-NonHF (3.13±0.74 ng/mL) and C-HF group (3.04±0.63 ng/mL) (p<0.01). Mean serum leptin level was significantly higher in STZ-HF (1792.0±1378.8 pg/mL) compared to STZ-NonHF (634.0±149.1 pg/mL), C-NonHF (671.5±164.0 pg/mL) and C-HF group (593.8±200.8 pg/mL) (p<0.05). In STZ-HF group, a significant positive correlation between leptin and glucose level was observed (r=0.71; p=0.048).

Conclusion: Our study results show that high fat diet induces an increase in serum leptin and the decrease in adiponectin levels in STZ diabetic rats and suggests that high fat diet impairs glucose control by increasing leptin secretion.

Key words: Diabetes mellitus, leptin, adiponectin, obesity

INTRODUCTION

High-fat intake leads to obesity and contributes to the development of many diseases, including diabetes mellitus (DM) [1]. Potential mechanisms by which mediate in this disease are still not clear. It is know that obesity alters adipose tissue metabolic and endocrine function and leads to an increased release of fatty acids, hormones, and proinflammatory molecules that contribute to obesity associated complications. Alterations in adipokines secretion associated with obesity could play an important role in diet-induced diabetes. Two major adipokines, leptin and adiponectin, are thought to play important roles in the regulation of cardiovascular and metabolic homeostasis [2].

Leptin is a 16-kDa protein hormone, which is secreted by adipocytes. Plasma leptin concentration in order to maintain body fat stores regulate food intake and energy expenditure and increases in proportion to body fat mass. Circulating leptin reaching to the brain through the brain-blood barrier (BBB) and cerebrospinal fluid (CSF) barrier leptin is secreted into the blood-stream. Leptin act in the hypothalamus, where leptin inhibits neuropeptide Y (NPY) neurons and causes anorexia [3]. Peripheral leptin acts to regulate the function of immune cells, adipocytes, muscle cells and pancreatic β cells, whereas the central action of leptin encompasses regulation of hemodynamics, bone mass, and immune function [2, 4].

Plasma leptin concentrations are significantly elevated in obese subjects in proportion to the degree of adiposity, suggesting that hyperleptinemia may play a role in the pathogenesis of obesity-related complications. Therefore, an association between plasma leptin and diabetes mellitus may be mediated by body fat. Higher leptin levels, conjunct with obesity and weight gain are probably involved in the subsequent development of diabetes [5].
Previous studies have shown inconsistent results regarding the influence of HF on leptin levels [6, 7]. Many studies have found an independent positive correlation between leptin concentration and insulin resistance (IR) among non-diabetic subjects [8, 9]. A few reports have examined the relationship between leptin concentration and IR in type 2 diabetic patients and the results from these studies were inconsistent [10, 11]. Rajkovic et al. [10] found that leptin concentration was not significantly associated with IR in type 2 diabetic patients. In contrast, Gulturk et al. [11] found that leptin was associated with IR, insulin and body composition parameters in patients with type 2 diabetes mellitus (T2DM). It has been demonstrated that leptin concentration in type 2 diabetic patients, compared with healthy subjects, is different among different ethnic groups [12].

Adiponectin regulates glucose and lipid metabolism by targeting the liver and skeletal muscle. Adiponectin is the most abundant plasma protein synthesized for the most part in adipose tissue, and it is an insulin-sensitive hormone, playing a central role in glucose and lipid metabolism [13]. Adiponectin is an adipose tissue-specific cytokine. It has a protective role against insulin resistance and anti-inflammatory activity and seems to protect against metabolic diseases. There are different circulating forms of adiponectin complexes, low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW) adiponectin. Adiponectin is altered in obesity [14]. It was shown that the level of adiponectin is significantly lower in animals fed on a HF compared to control group [15].

High adiponectin levels were associated with a lowered incidence of T2DM [16]. In humans, plasma adiponectin levels were correlated negatively with adiposity, insulin resistance, T2DM, and metabolic syndrome, yet positively correlated with markers of insulin sensitivity in frequently sampled intravenous glucose tolerance testing and clamp studies [16].

The aim of our study was to estimate the impact of high-fat diet on serum adiponectin and leptin levels in streptozotocin (STZ) induced type 2 DM.

**MATERIALS AND METHODS**

The study included 40 adult male Wistar rats. Rats were kept in a temperature-controlled room (20°C) with constant humidity and a 12-h light–dark cycle. All animal procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved approved by the Local Committee of Science and Research Ethics, University Sarajevo, Bosnia and Herzegovina. Type 2 DM was induced by single intra-peritoneal STZ injection (60 mg/kg) in STZ-NonHF and STZ-HF groups. Age-matched controls were injected with an equivalent volume of citrate buffer solution (0.1 mol/l citrate, pH 4.5).

Rats were divided into four groups:

1. Standard food control group (C-NonHF) (n =10),
2. Standard food STZ group (STZ-NonHF) (n =10),
3. High-fat diet control group (C-HF) (n =10),
4. High-fat diet STZ group (STZ-HF) (n =10).

C-NonHF and STZ-NonHF groups were fed with regular chow and other two groups were given high-fat diet for 5 weeks. The composition of the studied diets was a follows: the standard chow diet (294 kcal/100g) contained 22.4 g/100 g diet fat, 20.5 g/100 g proteins and 57.1 g /100 g carbohydrates. High fat diet (576.8 kcal/100g) contained 46.8 g/100 g diet fat, 32.1 g/100 g proteins and 21.1 g /100 g carbohydrates. Water was available ad libitum. The body weight, fasting blood glucose and food intake were recorded every week. Blood samples glucose measurements were obtained from the tail veins. For this procedure, the rats were gently placed in a plastic restrainer and their tails were wrapped with a warm towel for ~ 10 s to facilitate blood flow. The tail vein was punctured with a 21G1 needle. Blood glucose levels were measured using a Glucocard Diameter (Arkray, Kyoto, Japan). After 5 weeks of DM induction, rats were sacrificed, under anesthesia with an intraperitoneal injection of sodium pentobarbital (65 mg/kg). A blood sample was withdrawn from the inferior vena cava and aliquots were stored at −80 °C until use.

**Leptin and adiponectin measurements**

Serum leptin concentration was determined by ELISA method (machine STAT FAX 2100, USA) at the Department of Physiology and Department of Biochemistry, Faculty of Medicine in Sarajevo, using commercial Millipore® Mouse Leptin ELISA kit.

Serum adiponectin concentration was determined by ELISA method (machine STAT FAX 2100, USA) at the Department of Physiology and Department of Biochemistry, Faculty of Medicine in Sarajevo, using Alpco ELISA kit (Alpco, Salem, NH, USA).

**RESULTS**

In our study, mean body weight significantly decreased, while mean serum glucose significantly increased during the study period in STZ treated rats regardless of the diet (p<0.001) (Table 1).

Mean serum adiponectin level was significantly lower in STZ-HF (1.34±0.57 ng/mL) compared to STZ-NonHF (2.61±0.79 ng/mL), C-NonHF (3.13±0.74 ng/mL) and C-HF group (3.04±0.63 ng/mL) (p<0.01) (Figure 1).
Mean serum leptin level was significantly higher in STZ-HF (1792.0±1378.8 pg/mL) compared to STZ-SF (634.0±149.1 pg/mL), SFC (671.5±164.0 pg/mL) and HFC group (593.8±200.8 pg/mL) (p<0.05) (Fig. 2).

In STZ-HF group, a significant positive correlation between leptin and glucose level was observed (r=0.71; p=0.048).

**Discussion**

Obesity is generally recognized as an increasingly important cause of human morbidity worldwide and is a contributer to chronic diseases such as type 2 diabetes mellitus (T2DM) and Coronary Heart Disease [17]. High fat diet administration exerts a direct effect on pancreatic beta-cell failure, resulting in the onset of IR and T2DM. The severity of beta-cell failure depends on the progenitor’s metabolic state during HF diet administration and on the duration of injury [19].

Obesity is defined as an excessive growth of adipose tissue. White adipose tissue is recognized as a dynamic endocrine organ able to produce and release several bioactive polypeptides such as adipokines. It is likely that adipokines could play an important role in the development of diseases associated with obesity including insulin resistance, inflammation, hypertension, cardiovascular risk and metabolic disorders [2].

In humans, the tissue expression and circulating concentration of many adipokines increase with increasing adiposity, as is the case for leptin. Obesity in both humans and in rodents placed on a HF diet are characterized by hyperleptinemia, and the ability of leptin to suppress food intake and reduce body weight is attenuated [20].

Our results showed that serum leptin level was significantly higher in STZ-HF compared to STZ-SF and HFC group.

Handjieva-Darlenska T et al. [6] found that rats fed on a high-fat diet showed significant increase in total body weight compared to control group. The long-term intake of high-fat diet caused hyperleptinemia. Authors found a significant positive correlation between plasma leptin levels and epididymal fat mass, liver and heart. Compared to a low-fat diet, high fat diet led to increased body weight and glucose intolerance. Leptin is a potent insulin sensitizer and in leptin-deficient, insulin insensitive, Lep(ob/ob) mice, leptin improves glucose tolerance, indicating that leptin resistance may link obesity to insulin insensitivity. Both hyperleptinaemia and inflammation have been proposed as causative mechanisms due to leptin resistance occurs in response to a high-fat diet [22].

In contrast, Ainslie et al. [7] showed that short-term, high-fat diets are associated with reduced leptin secretion. They observed that in the animals fed the high-fat diet this reduced leptin secretion may contribute to the subsequent weight gain. It has been shown that decreases in insulin-stimulated glucose metabolism is associated with decreases in leptin expression and secretion in isolated adipocytes [22]. It may explain the
lower leptin concentrations authors observed after high fat diets because high-fat feeding can decrease insulin-stimulated glucose uptake into adipocytes and it may explain why fasting decreases leptin concentrations [7].

Some studies have reported unchanged or decreased or increased serum leptin levels in diabetic patients so reports regarding the role of leptin in diabetes are inconsistent [23]. Previous studies examining the association between serum leptin levels and diabetes mellitus were restricted to specific racial/ethnic groups and were not consistent in their findings [23]. While some studies reported significant positive associations between plasma leptin levels and diabetes only in men [25]; Sun et al. [26] reported an inverse relation. Still some studies reported that there is no association between plasma leptin levels and diabetes [24].

As a consequence of obesity, hyperleptinemia however may affect the pathogenesis of obesity-related complications. Leptin resistance, with resultant dysregulation of leptin action, is related to processes that produce diabetes and other related diseases in humans, such as impaired insulin secretion and decreased whole-body glucose utilisation, insulin resistance, lipotoxicity, ectopic fat deposition, altered hepatic metabolism [27].

Also, leptin can affect processes such as promoting platelet aggregation, which could cause thrombosis; impairing endothelial function; fostering inflammation and angiogenesis; and increasing immune function, all of which could produce or worsen diabetic complications or other diseases [28].

On the other hand, Kusakabe et al [29] observed beneficial effects of leptin on glycaemic and lipid control in a mouse model of T2DM with increased adiposity induced by STZ and a high-fat human. In a study with model mouse mimicking human T2DM (STZ/HFD), authors showed that continuous leptin infusion improved lipid and glucose metabolism and reduced food intake with enhancement of insulin sensitivity. Accompanied by an increase of alpha2 AMPK activity in skeletal muscle leptin also decreased liver and skeletal muscle triacylglycerol content. In pair-feeding experiments authors showed that the independently of the food intake reduction leptin improved glucose and lipid metabolism. These results indicated the possible clinical usefulness of leptin as a new glucose-lowering drug in humans, by demonstrating beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity.

In STZ-HF group, a significant positive correlation between leptin and glucose level was observed (r=0.71; p=0.048).

Some authors showed that in adipocytes and muscle cells leptin can modulate insulin action (on glucose uptake and/or lipid synthesis), however other studies found no effect of leptin on peripheral glucose uptake [17]. Leptin may affect the transcription to membrane permeability to inhibit insulin synthesis as well as secretion and other different intracellular levels. Functional leptin receptors are also present on insulin-secreting pancreatic β-cells. Through these receptors the insulin-lowering effect of leptin administration could be mediated. Recent studies showed a direct effect of leptin on insulin gene transcription in pancreatic β-cells, with a reduction of preproinsulin mRNA by 50% [17].

A number of metabolic processes including those resulting in T2DM, coronary artery disease, metabolic syndrome and obesity are modulated by adiponectin. Contrary to expectations, adiponectin is decreased in obesity [30].

Our result showed that serum adiponectin level was significantly lower in STZ-HF compared to STZ-SF, SFC and and HFC group. Hou et al. [31] found that offspring born to dams fed high-fat chow (HF; 31% of calories from fat) had elevated body and adipose tissue weight and higher serum glucose levels after glucose challenge at three weeks (W3) and eight weeks (W8) of age. Compared to control group, offspring exposed to a high fat diet also showed higher serum adiponectin levels at W3. However, adiponectin levels were significantly decreased compared to controls by W8.

Moreover, in nonhuman primates, decreased adiponectin levels were seen in parallel with the progression of insulin resistance and T2DM. This suggests that maintaining high circulating adiponectin levels may be a potential approach to prevent the development of these disorders. Kandasamy et al. [32] have shown that adiponectin gene therapy results in a reduction in diet-induced weight gain. This corroborates with a previous report showing that similar reductions in body weight in mice fed a high-fat diet for 32 weeks was observed after continuous infusion of globular adiponectin. Authors have shown that adiponectin gene therapy results in increased energy expenditure and locomotor activity, although all of the mechanisms by which weight gain is reduced in adiponectin-treated mice are likely not known. These results are in accordance with earlier reports indicating adiponectin decreases body weight by increasing energy expenditure [33]. Treatment with adiponectin decreases fat content and body weight. Many evidence also suggest that adiponectin may also act via the hypothalamus to increase energy expenditure. This suggests that some of the observed effects of adiponectin gene therapy could be centrally mediated in the present study. Beside the effect such as reducing weight gain, increased circulating adiponectin levels improved peripheral insulin sensitivity and resulted in a significant reduction in insulin levels and fasting glucose [32].
Conclusion

Our study results show that high fat diet induces an increase in serum leptin and the decrease in adiponectin levels in STZ diabetic rats and suggests that high fat diet impairs glucose control by increasing leptin secretion.

Declaration of interest

The authors declare no conflict of interest for this study.

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References


