Serum levels of insulin and leptin in lipoic acid-treated and non-treated experimentally diabetic rats

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Diabetes is characterized by hyperphagia, and polydypsia. However, the mechanisms by which diabetes produces these effects are not clear. This study was conducted to examine changes in serum insulin and leptin levels in induced-type 1 diabetes mellitus in relation to concomitant changes in body weight, glycemic state and lipid profiles in rats. Moreover, we aimed to clarify that the treatment with lipoic acid (LA) is capable of reversing these effects or not. Ninety-six male rats were divided into 3 groups, control group (32 rats) was considered as normal non-diabetic, 64 rats were subcutaneously injected with alloxan (120 mg/kg b.wt) for induction of diabetes. Then the diabetic rats were divided into two equal subgroups, the first diabetic group that was not treated with LA, and the other is LA-treated diabetic group that was treated with LA at a dose 100 mg/kg b.wt / day for four weeks. Body weight, serum lipid profile, glucose, insulin, homeostasis model assessment—insulin resistance (HOMA-IR) and leptin were measured. The data showed significant increase in serum triacylglycerol, total cholesterol and glucose levels as well as HOMA-IR while significant decrease in the mean body weight gain, serum insulin and leptin levels in diabetic group in comparison with control group. The treatment with lipoic acid led to significant decrease in serum fasting and postprandial glucose, triacylglycerol and total cholesterol levels as well as slight decreased HOMA–IR with significant increased levels of serum insulin and leptin in comparison with diabetic group. It could be concluded that alloxan-induced diabetes led to hyperglycaemia, insulin resistance, hyperlipidaemia and hypoleptinemia. Moreover, treatment with lipoic acid ameliorates these changes and improves insulin sensitivity.

Diabetes is a metabolic disorder that is known to produce various dysfunctions in the body, and the central nervous system (CNS). Some of the diabetes-related CNS disturbances include hyperphagia, polydypsia and activation of the hypothalmo–pituitary–adrenal axis (Biessels et al., 1994). The sustained hyperglycemia leads to a further impairment of insulin production by β-cells, so called glucose toxicity (Del Prato and Marchetti, 2004). In addition, the elevated serum triacylglycerol and its accumulation in pancreatic islets during the development of diabetes have been associated with impaired β-cells secretory responses, so called lipotoxicity (Hirose et al., 1996) and are causally related to type 2 diabetes. Szkudelski et al., (1998) reported that the decrease in insulin concentration after alloxan injection was accompanied by a rise in blood glucose concentration and a decrease in the content of free fatty acids, suggesting that the use of lipids as a source of energy is enhanced. This assumption is additionally supported by a slight decrease of blood triacylglycerol in alloxan-treated rats. In contrast, Sheela and Augusti (1992) reported a significant increase of serum total cholesterol, triacylglycerol and total lipids in alloxan and streptozotocin-diabetic animals. Sobenin et al., (1994) also found an elevated total cholesterol level in plasma of diabetic patients.

The most important hormone produced by adipose tissue is leptin and adiponectin. leptin plays a significant role in the regulation of lipid and carbohydrate metabolism. Leptin is a cytokine that decreases appetite, increases energy expenditure, suppresses insulin synthesis and secretion and increases insulin sensitivity (Yildiz and Haznedaroglu, 2006). Changes in the secretion or sensitivity to leptin may contribute to the development of type 1 and type 2 diabetes (Huerta, 2006). Leptin is produced in proportion
to the amount of adipose tissue and acts in specific brain hypothalamic nuclei to reduce food intake and in rodents to activate thermogenesis (Friedman, 2000). Leptin also has actions outside the brain, one of which is the stimulation of fatty acid oxidation in muscles and liver, at least in part through AMP-activated protein kinase (AMPK) activation (Minokoshi et al., 2002). The secretion of Leptin hormone is affected by food consumption, insulin, fasting and cold exposure. It is known that the increased ATP and malonyl-CoA contents in adipocytes enhance secretion of leptin (Szkudelski, 2006). It was found that the insulin-induced rise in leptin secretion is accompanied by an initial decrease in the intracellular leptin content, probably due to its augmented release from fat cells (Barr et al., 1997). Glucose seems to be the most important source of ATP in adipocytes during leptin secretion. Insulin promotes the translocation of glucose transporter-4 (GLUT4) from the intracellular pool to the plasma membrane and thereby accelerates glucose transport into adipocytes (Khan and Pessin 2002). Insulin also shifts glucose metabolism from anaerobic to mitochondrial oxidation generating ATP and finally augmenting secretion of leptin (Levy and Stevens 2001). Compounds enhancing glucose uptake, but potentiating its metabolism to lactate (e.g. metformin) were found to restrict secretion of leptin (Mueller et al., 2000).

Diabetics have increased levels of lipid hydroperoxides and protein carbonyls (Packer et al., 2001). α-Lipoic acid (ALA) is a naturally occurring short chain fatty acid with sulphydryl groups that has potent unique antioxidative activity in a wide variety of experimental systems and is clinically used to treat diabetic neuropathy (Biewenga et al., 1997, Packer et al., 2001; Wollin and Jones, 2003). Lipoic acid scavenges hydroxyl radicals, hypochlorous acid, nitric oxide, peroxy nitrite, hydrogen peroxide and singlet oxygen. It also chelates iron, copper and other transition metals (Packer et al., 1995). Therefore, lipoic acid and dihydrolipoic acid (DHLA) take central positions in the antioxidant network (Packer, et al., 2001). Lipoic acid may also increase nerve growth factors level (Hounsom et al., 1998) and promote nerve fibre sprouting (Dimpfel et al., 1990). In addition, it was recently reported that LA reduced the body weight gain of rodents by suppressing food intake and increasing energy expenditure (Lee et al., 2005; Song et al., 2005).

Leptin, insulin, glucose and alpha-lipoic acid have been shown to reduce food intake by lowering hypothalamic AMP-activated protein kinase activity (Lee et al., 2005). Short-term administration of LA at a high dose to normal and diabetic rats causes an inhibition of gluconeogenesis secondary to an interference with hepatic fatty acid oxidation. This may render LA an anti-hyperglycemic agent for the treatment of diabetic rats that display glucose overproduction as a major metabolic abnormality (Khamaisi et al., 1999).

In this study, we aimed to examine changes in serum insulin and leptin levels in induced-type 1 diabetes mellitus in relation to concomitant changes in body weight, glycemic state and lipid profiles in rats. Moreover, we aimed to clarify that the treatment with lipoic acid (LA) is capable of reversing these effects or not.

Materials and methods

Experimental animals. 96 male albino rats were involved in this study with (100-180 g) body weight and 10 weeks old. Rats were kept for two weeks on balanced ration and water ad libitum for acclimatization.

Alloxan (diabetogenic agent). 5,6-dioxouracil was purchased from Sigma Chemical Company USA. It was dissolved in citrate buffer (pH; 4.4) immediately before use.

Lipoic acid (LA). 1,2-dithiolane-3-pentanoic acid, marketed as thioctic acid® by EVA pharma for pharmaceuticals and medical appliances, Egypt. The tablets were crushed and suspended in distilled water.

Experimental diabetes. it was induced in over night fasted rats (16 h.) by subcutaneous injection of a single dose of alloxan (120 mg / Kg. b.wt.). Then after 4 – 5 days of alloxan injection, rats were screened for blood glucose levels. Rats with serum postprandial glucose level of (180 to 300 mg/dl) were considered as mild diabetic and were included in the experiment (cited by Abdel- Reheim,1997).

Animal grouping. control group was considered as normal non diabetic rats. It involved thirty-two non-diabetic rats and not treated with lipoic acid. Diabetic group was diabetic without treatment with lipoic acid, it involved thirty-two diabetic rats; the rats of diabetic group were orally given isotonic solution using stomach tube daily for 4 successive weeks. LA-treated diabetic group was diabetic treated with lipoic acid and it included thirty-two diabetic rats. The rats of this group were treated with LA (100 mg/Kg.b.wt daily) by
stomach tube for 4 successive weeks (Arivazhagan et al., 2003).

**Sampling and preparations.** at the end of experimental period, blood samples were collected from the rats in each group (fasting and postprandial) for determination of glucose level and other biochemical parameters.

**Biochemical assay.** fasting serum triacylglycerol concentration was determined by enzymatic method according to Young and Pestaner (1975), serum total cholesterol concentration was determined by enzymatic method according to Richmond (1973), and serum glucose concentration was enzymatically estimated according to the method of Trinder (1969). Serum leptin concentration was estimated according to Considine et al., (1996) using DSL (Diagnostic system Laboratory, Inc.) active Leptin ELISA Kit, Catalog No. DSL-10-24100. Serum insulin was measured according to Turkingto et al., (1982) using Insulin AccuBind ELISA Microwells from Monobind Inc., Costa Mesa, CA 92627 (USA). HOMA-IR score was calculated to show the insulin resistance by using the following formula: (insulin ([μIU/ml] X fasting glucose [mmol/L] /22.5) (Haffner et al., 1997), higher HOMA-IR scores denote lower insulin (insulin resistance). The obtained data during the period of experiment were statistically analyzed by T-test as well as the correlation between variables was evaluated using correlation coefficient (Snedecor and Cochran 1980).

**Results**

The obtained data revealed that, there was a significant decrease in the mean body weight and body weight gain in the rats of diabetic group compared with those of control group (Table 1, Fig. 1). LA was found to significantly raise the mean body weight gain in LA-treated diabetic group as compared with diabetic one. Data of Table (1) and Fig. (2) showed a significant increase in serum triacylglycerol and total treated diabetic group, significant lower levels of both triacylglycerol and total cholesterol were recorded as compared with diabetic group (Table1, Fig. 2). Comparison of diabetic group cholesterol concentrations in

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<th>Table (1): Mean body weight, body weight gain (gm), serum triacylglycerol and total cholesterol concentrations (mg %) in control, diabetic and LA- treated diabetic groups.</th>
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Different letters indicate significant variation, while the same letters indicate non significant variation.

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<th>Table (2): Mean serum levels of fasting and postprandial glucose, insulin, leptin and HOMA-IR in control, diabetic and LA- treated diabetic groups.</th>
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<th>Table (3): Correlation coefficient between insulin, leptin and mean body weight.</th>
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diabetic group when compared with control group. In LA- versus control group showed a significant increase in both fasting and postprandial glucose levels in diabetic group which was significantly decreased after treatment with LA (Table 2, Fig. 2). There was a significant decrease in the serum levels of insulin and leptin hormones in diabetic group compared with control group. LA administration significantly increased the serum levels of insulin and leptin hormones (Table 2, Fig. 3, 4). On the other hand, there was a significant increase of HOMA-IR in diabetic group compared with control one, but LA treatment could increase the insulin sensitivity as reflected by low HOMA-IR levels in LA-treated diabetic group (Table 2, Fig. 3).

The present study revealed a positive correlation coefficient between leptin and mean body weight as well as a positive correlation coefficient between leptin & insulin and between insulin & mean body weight (Table 3). HOMA-IR was found to be negatively correlated with leptin, insulin and body weight (Table 3).

**Discussion**

The present study showed a significant decrease in mean body weight and body weight gains in diabetic group compared with control one. Schedl and Wilson (1971) explained the loss in body weight by enhanced transport of sugar and amino acids associated with alloxan-induced diabetes. In addition, brush border enzymes namely disaccharidases, are increased in chemically induced diabetes (Younsozai and Schedl, 1972). Thus, it appears that both the absorptive and digestive functions of small intestinal mucosa are altered in diabetes.

Our results showed a significant increase in mean body weight gain in the diabetic rats treated with LA compared with diabetic group but still lower than control group. LA is an insulin sensitizer, where it can improve insulin-stimulated glucose uptake and improve insulin sensitivity (Moini et al., 2002). Since, LA by its ability to improve the insulin sensitivity and accelerate recovery of pancreatic β-cells can nearly restore the metabolic alterations associated with diabetes.

The current data showed that, serum triacylglycerol concentrations were elevated in diabetic group compared to control one. These results come in accordance with Sheela and Augusti (1992); Abdel-Azim et al., (2002). The observed triglyceremia in diabetic rats resulted from hepatic overproduction of triacylglycerol that is probably a consequence of increased flux of glucose and free fatty acids (FFA) to the liver (Abdel-Azim et al., 2002) and impaired clearance of triacylglycerol-rich lipoproteins resulted from lowered lipoprotein lipase activity (LPL) (Quaschning et al., 1999). LPL is an enzyme on the surface of endothelial cells lining the vessels. The activity of this
enzyme requires insulin and in its absence a hypertriglyceridemia results.

Our results showed that serum total cholesterol levels increased significantly in diabetic group than control one. These results agreed with Monnier et al., (1995). They attributed the increase in total cholesterol level to the increased β-oxidation of long chain FA and increased oxidation of ketogenic amino acids producing excess of hepatic acetylo coA that is used for cholesterol synthesis (Abdel-Azim et al., 2002). It could be also due to depressed hepatic phenol 2-mono-oxygenase activity, the key enzyme responsible for the catabolism of cholesterol to bile acids (O.Meara et al., 1990). Moreover, decreased LDL receptors activity with a consequent delayed clearance of the glycated cholesterol LDL particles could be another cause of the increased total serum cholesterol level (Mazzone et al., 1984; Monnier et al., 1995).

The decrease in serum triacylglycerol and total cholesterol after administration of LA comes in agreement with Kocak et al., (2000); Song et al., (2005). This result could be attributed to the effect of LA in reducing triacylglycerol accumulation in skeletal muscles, pancreatic islets as well as in adipose tissue where, LA increases fatty acid oxidation by activating the AMPK in skeletal muscles (Lee et al., 2005). The anti-oxidative action of LA may suggest its preventive effect on LDL-cholesterol oxidation and thus, enhancing its catabolism. From the previous results, it could be concluded that, LA has an anti-hyperlipidemic effect on diabetic rats.

The present work revealed that serum insulin levels significantly decreased in diabetic group compared to control group. These results are in agreement with Szkudelski et al., (1998), Kocak et al., (2000); Melhem et al., (2002). Alloxan and streptozocin are widely used as inducers of diabetes mellitus in experimental animals. Both chemicals cause selective destruction of pancreatic islet β-cells and can induce chronic or permenant diabetes in these animals (Mathe, 1995).

The current results showed that, both fasting and postprandial serum glucose concentrations were significantly increased in diabetic group compared to control rats. The chronic hyperglycemia and glucose intolerance could arise from a defect in insulin secretion as in case of IDDM (Caro, 1990). In experimental diabetes that may represent a model of IDDM, alloxan generates some types of oxygen radicals that attack DNA inducing DNA-strand breaks in β-cells. The breaks induce DNA repair involving the activation of poly (ADP-ribose) polymerase (PARP), which uses NAD+ as a substrate. As a result, the intracellular levels of NAD+ fall. The fall in NAD+ inhibits ATP synthesis and cellular functions including insulin synthesis and secretion, and thus the beta cell ultimately dies (Okuwa et al., 1995; Pusztaï et al., 1996). This will lead to reduced uptake of glucose by peripheral tissues like muscles and adipose tissue (Beck-Nielsen, 2002), glycogenolysis (Gold, 1970) and increased gluconeogenesis and hepatic glucose production (Caro, 1990; Raju et al., 2001).

The present data demonstrated that serum glucose levels significantly decreased while serum insulin levels significantly increased in diabetic group administrated lipoic acid in comparison with the diabetic group. These data are in agreement with Kocak et al., (2000); Packer et al., (2001) who reported that LA increases glucose uptake through translocation of the glucose transporter to plasma membranes, a mechanism that is shared with insulin-stimulated glucose uptake. In experimental and clinical studies, LA markedly reduced the symptoms of diabetic pathologies, including cataract formation, vascular damage, and polyneuropathy (Packer et al., 2001), indicating the role of LA as glucose lowering agent. In this context, Moini et al., (2002) reported that the insulin receptor is a potential cellular target for LA action and they explained the mechanism of action of LA as an insulin sensitizer. This mechanism occurs by autophosphorylation of insulin receptors and oxidation of thiol groups present in insulin receptors β-subunits by the oxidized form of LA, which in turn may be required for insulin-stimulated glucose transport. In addition, Cho et al., (2003) ; Bitar et al., (2004) stated that LA activates the insulin-signaling pathway and exerts insulin-like actions in adipocytes and muscle cells by the phosphorylation of insulin receptor (IR). Schroeder et al., (2005) recently attributed the mechanism of action of LA to its blocking effect on interleukin-1beta that is secreted by activated macrophages in response to immune-mediated process causing islet cell death in IDDM. In addition to this action of LA in the protection of pancreatic β-cells from death in IDDM, Song et al., (2005) reported another role of LA in protecting pancreatic β-cell by reducing...
triacylglycerol accumulation in such cells. This accumulated metabolite is considered as a factor contributing to insulin resistance in obesity and is causally related to NIDDM. So, it can be concluded that LA can improve insulin-stimulated glucose transport, reduce insulin resistance (Jacob et al., 1996) and protect pancreatic islet cells from destruction.

Over the past few years, our understanding of leptin biology has significantly expanded. A more detailed clarification of the mechanisms of both leptin and insulin interaction in the setting of diabetes with hypoleptinemia may ultimately provide a novel therapeutic target for treating this disease.

One of the results of this current study was the significant decrease of serum leptin level in diabetic group compared with the control one. These results come in agreement with Hathout et al., (1999), Kirel et al., (2000); Barber et al., (2003). These authors indicated that diabetes causes marked decrease in serum leptin level. Our data support a direct relationship between the circulating insulin concentration and leptin secretion. Patients with type1 diabetes (during insulinopenia) had significant lower leptin concentration compared with normal ones (Soliman et al., 2002). Also streptozotocin-treated mice had low leptin levels (Cusin et al., 1995). Decreased leptin secretion is expected to stimulate appetite and contribute to hyperphagia in those patients. This was confirmed by a study of Sindelar et al., (1999) who showed that administration of leptin in diabetic animals could indeed reverse diabetes-induced hyperphagia. In other words, the beneficial effects of insulin on diabetes-induced hyperphagia may be mediated through leptin (Barber et al., 2003); thereby these observations provide an insight into the therapeutic implication of leptin as an anti-diabetic agent (Miyanaga et al, 2003).

The observed low serum leptin levels in type 1 diabetic rats under the present study may be due to insulin deficiency.

The present data demonstrated that treatment with lipoic acid significantly increased serum leptin levels in LA-treated diabetic group when compared with diabetic group.

Overall, insulin and glucose appear to increase leptin secretion. In turn, leptin increases peripheral insulin sensitivity while it decreases insulin secretion from pancreatic beta cells. Leptin increases skeletal muscle glucose uptake and oxidation, and suppresses hepatic glucose output. Effects of leptin on lipid metabolism might reduce lipotoxicity and therefore contribute to the improvement of hepatic, skeletal and whole body insulin sensitivity (Yildiz and Haznedaroğlu, 2006).

This work showed a positive correlation coefficient between leptin and body weight. Previous studies reported a strong positive correlation between leptin and body mass index in type 1 diabetes mellitus (Lauszus et al., 2001), and in patients with insulin resistance (Rudzka-Kocjan et al., 2006). Human obesity is suggested to be, in part, due to desensitization of leptin receptors within the hypothalamus resulting in hyperphagia (Frederich et al., 1995; Smith, 1996). Leptin, which has a dual nature as a hormone and cytokine, plays an important role in the regulation of body weight and energy balance. Interestingly, serum leptin and triacylglycerol levels were independently associated with insulin resistance (Taniguchi et al., 2002). Leptin and insulin may have complementary roles in maintaining a stable body weight (Kirel et al., 2000).

In addition, a positive correlation coefficient was found between insulin and leptin concentrations. These data come in agreement with the results obtained by Malmstroem et al., (1996); Hathout et al., (1999).

The present study indicates that alpha-lipoic acid has an effective protective role in diabetic rats. This role has been concluded from its reduction to plasma glucose level and an accelerated recovery of pancreatic insulin-producing cells which is deduced from the significantly increased serum level of insulin in LA-treated diabetic group.

LA treatment effectively reversed body weight, blood glucose, plasma insulin, cholesterol, triacylglycerol and lipid peroxidation levels of streptozotocin-diabetic animals (Kocak et al., 2000). Moreover, Bhatti et al., (2005); Maha and Raafat (2007) stated that a potent antioxidant, LA improves renal function in diabetes by lowering oxidative stress. LA could be considered as an adjuvant therapy in diabetic cardiomyopathy (Strödter et al., 1995; Yi and Maeda, 2006).

It could be concluded that, alpha-lipoic acid treatment effectively reversed body weight, triacylglycerol, total cholesterol, blood glucose, plasma insulin, HOMA-IR and leptin suggesting a potential therapeutic approach. In conclusion, LA stimulates basal glucose transport and has a positive effect on insulin-stimulated glucose
uptake and improves insulin sensitivity. Moreover, dietary alpha-lipoic acid is a promising protective agent for reducing cardiovascular complications of diabetes.

References


مسوئيات كل من الأنسولين والليبتين في الفداران المصابة تجريبياً بمرض البول السكري المعالجة و غير المعالجة

بحمض الليبوتوك

تهدف هذه الدراسة إلى معرفة بعض التغيرات الكيميائية الحيوية في مسوايات الأنسولين والليبتين في كل من الفداران المصابة بمرض البول السكري التجريبي بواسطة الألوكسان وكذلك بعد العلاج بحمض الليبوتوك وأجريت التجربة على عدد 69 فئاً حيث سُمّرت إلى ثلاث مجموعات، المجموعة الأولى تم تقديم 22 مصل لكل فئتين لمدة 4 أسابيع، ثم تم نشر البول السكري للعلاج، و60 مصل تم استخدامه في مسواة الألوكسانcoal. (320 ملجم/كم ور) تم قص الباقي بعد ذلك إلى مجموعتين: المجموعة الثانية (32 فئاً) مصلية بمرض البول السكري فقط والمجموعة الثالثة (32 فئاً) مصلية بمرض البول السكري و تم علاجها بحمض الليبوتوك. استمرت التجربة 4 أسابيع مماثلة وتم تجميع عينات الدم من الفداران لكل مجموعة في نهاية التجربة للقيام بمسوايات الجلوكوز، الجلوكوزيات الثلاثية، الكوليستيرول الكل، هرمون الأنسولين والليبتين، كما تم تسجيل مسواة وزن الجسم ووزن الجسم المكعب للفران لكل ممثلة و كذلك تم حساب (مقاومة الأنسولين) عامل الارتباط بين كل من الليبتين و الأنسولين ووزن الجسم المكعب. وظيفتها التحليل الاحصائي أن هناك ارتفاع في مستويات الجلوكوزيات الثلاثية، الكوليستيرول الكل، هرمون الأنسولين وليبتين في الفداران المصابة بمرض البول السكري مقارنة بالمجموعة المضافة. كما أوضحت النتائج أن معدلات الليبوتوك في أقل من مستوى كل من الجلوكوزيات الثلاثية، الكوليستيرول الكل، الجلوكوز، هرمون الأنسولين بشكل ملحوظ. علاوة على ذلك فقد ادى حمض الليبوتوك إلى زيادة معنوية في مستويات هرمون الأنسولين والليبتين الممضية. مسواة بمرض البول السكري.

تتعرض الدراسة إلى أن مرض البول السكري التجريبي بواسطة الألوكسان يؤدي إلى ارتفاع نسبي للجسم بالدم، زيادة مقاومة الأنسولين، ارتفاع الدهون بالدم،خفض مستوى الليبتين بالدم، ذلك توضح الدراسة التأثير الفعال لحمض الليبوتوك في تحقيق استجابة الجسم للأنسولين والذي يظهر من خلال ارتفاع مستويات الجلوكوز، ارتفاع مستويات الأنسولين والليبتين، وقد ارتبط مستوي الليبتين إيجابياً مع وزن الجسم، بينما ارتبط مستوي مقاومة الأنسولين سلبياً مع كل من الأنسولين والليبتين ووزن الجسم.