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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 is 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, hyperlipideamia and hypoleptinamia. 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 hypothalamo–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 sulfhydryl 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 adlibitum for acclimatization.

Alloxan (diabetogenic agent). 5,6-dioxyuracil 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

Table (1): Mean body weight, body weight gain (gm), serum triacylglycerol and total cholesterol concentrations (mg %) in control, diabetic and LA- treated diabetic groups.

Groups	Mean Body Weight (gm)	Mean body weight gain (gm)	Triacylglycerol (mg %)	Total cholesterol (mg %)
Control group	180.37±6.75 a	29.32±1.77 a	81.69 ±2.32 a	80.42±2.26 a
Diabetic group	131.81±3.84 b	6.68±2.97 b	92.60±2.08 b	86.76±2.03 b
LA-treated diabetic group	172.04±5.75 a	16.49±1.15 c	78.48±5.001 a	80.98±0.90 a

Different letters indicate significant variation, while the same letters indicate non significant variation.

Table (2): Mean serum levels of fasting and postprandial glucose, insulin, leptin and HOMA-IR in control, diabetic and LA- treated diabetic groups.

Groups	Fasting Glucose (mg %)	Postprandial glucose (mg %)	Insulin (μIU/ml)	HOMA-IR (μIU/ml)	Leptin (ng/mL)
Control group	86.82 ±1.83 a	108.42±1.41 a	14.57 ± 0.99 a	3.12 ±0.16 a	4.04 ± 0.37 a
Diabetic group	259.19±19.17 b	355.67±11.72 b	7.4 ± 0.38 b	4.73 ±0.37 b	1.80 ± 0.16 b
LA-treated diabetic group	195.90 ±5.66 c	290.13±7.46 c	8.88 ± 0.45 c	4.05 ±0.36 ab	2.83 ± 0.25 c

Different letters indicate significant variation, while the same letters indicated non significant variation.

Table (3): Correlation coefficient between insulin, leptin and mean body weight.

Groups	Leptin (ng/mL)	Insulin (μIU/ml)	Mean body weight (gm)
Leptin	----	----	0.347
Insulin	0.504	----	0.4244
HOMA-IR	-0.5	-0.6937	-0.41811

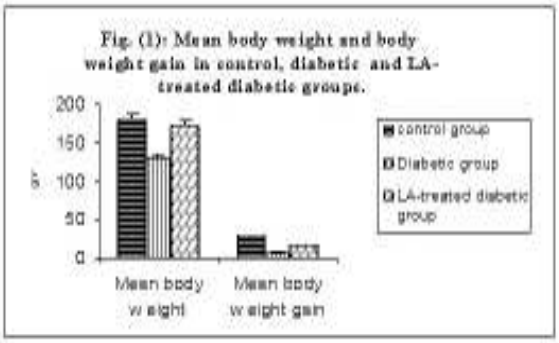
stomach tube for 4 successive weeks (Arivazhagan *et al.,* 2003).

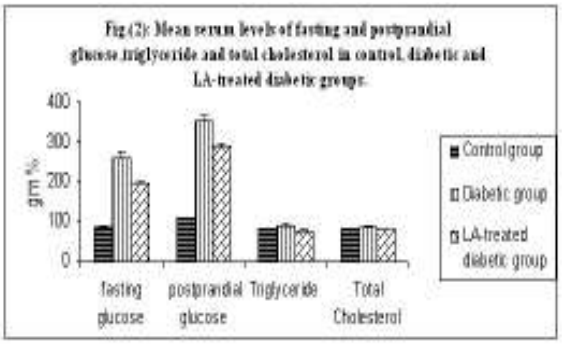
Sampling and preparations. at the end of experimental period, blood samples were collected from the rats in each group (fasting and postprandial) for determination of glucose level and other biochemical parameters.

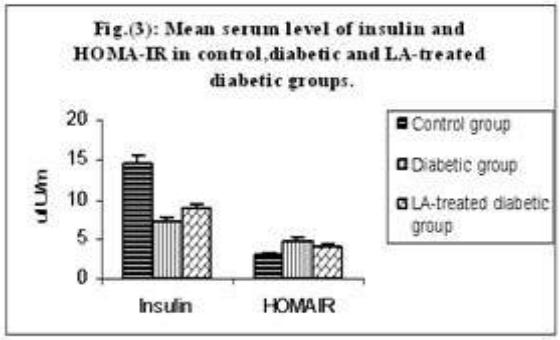
Biochemical assay. fasting serum triacylglycerol concentration was determined by enzymatic method according to Young and Pestaner (1975), serum total cholesterol concentration was determined by enzymatic method according to Richmond (1973), and serum glucose concentration was enzymatically estimated 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).

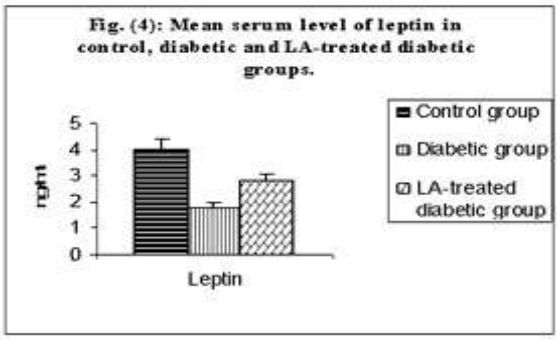
Results

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









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 triglyceredemia 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 hypertriglyceredemia 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 acetyle 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 streptozotocin 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 (Ohkuwa *et al*., 1995; Pusztai *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 authers 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 Haznedaroglu, 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

Abdel-Azim, S. A.; Bader, A. M. and Barakat, M. A. (2002): Effect of metformin, glyburide, and/or selenium on glucose homeostasis, lipid peroxidation, glutathione levels and changes in glutathione peroxidase activity in streptozotocin-induced diabetic rats. Egypt. J. Biochem., 20: 393-411.

Abdel-Reheim, E. S. (1997): The Effect of nigella sativa and on the glycemic state and immune response of diabetic albino rat. M.Sc. Thesis, Fac. Sci. Cairo Univ., Egypt.

Arivazhagan, P.; Panneerselvam, S. R. and Panneerselvam, C. (2003): Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and lipids in aged rats. J Gerontol A Biol Sci Med Sci., 58(9): B788-91.

Barber, M.; Kasturi, B. S.; Austin, M. E.; Patel, K. P.; MohanKumara, S. M. and MohanKumara, P. S. (2003): Diabetes-induced neuroendocrine changes in rats: role of brain monoamines, insulin and leptin. Brain Res., 964: 128–135.

Barr, V. A.; Malid, E. D.; Zarnowski, M. J.; Taylor, S. I. and Cushman, S. W. (1997): Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinol., 138: 4463-4472.

Beck-Nielsen, H. (2002): Insulin resistance: organ manifestations and cellular mechanisms. Ugeskr. Laeger, 15; 164(16): 2130-5.

Bhatti, F.; Mankhey, R. W.; Asico, L.; Quinn, M. T.; Welch, W. J. and Maric, C. (2005): Mechanisms of antioxidant and pro-oxidant effects of alpha-lipoic acid in the diabetic and non-diabetic kidney. Kidney Int., 67(4): 1371-80.

Biessels, G. J.; Kappelle, A. C.; Bravenboer, B.; Erkelens, D. W. and Gispen, W. H. (1994): Cerebral function in diabetes mellitus. Diabetologia, 37: 643–650.

Biewenga, G. P.; Haenen, G. R. and Bast; A. (1997): The pharmacology of the antioxidant lipoic acid. Gen. Pharmacol., 29: 315–331.

Bitar, M. S.; Wahid, S.; Pilcher, C. W.; Al-Saleh, E. and Al-Mulla, F. (2004): Alpha-lipoic acid mitigates insulin resistance in Goto-Kakizaki rats. Horm. Metab. Res., 36(8):542-9.

Caro, J. F. (1990): Effect of glyburide on carbohydrate metabolism and insulin action. Am. J. Med., 89 (20): 17S-24S.

Cho, K. J.; Moon, H. E.; Moini, H.; Packer, L.; Yoon, D. Y. and Chung, A. S. (2003): α-lipoic acid inhibits adipocyte differentiation by regulating pro-adipogenic transcription factors via mitogen-activated protein kinase pathways. c. J. Biol. Chem. 37(12): 34823–34833.

Considine, R. V.; Sinha, M. K.; Heiman, M. L.; Kriauciunas, A.; Stephens, T. W.; Nyce, M. R.; Ohannesian, J. P.; Marco, C. C.; McKee, L. J.; Bauer, T. L. and Caro, J. F. (1996): Serum immunoreactive-leptin concentrations in normal-weight and obese human sy. N Engl J. Med 334:292 –295.

Cusin, I.; Sainsbury, A.; Doyle, P. et al., (1995): The ob gene and insulin: A relationship leading to clues to the understanding of obesity. Diabetes, 44:1467-1470.

Del Prato, S. and Marchetti, P. (2004): Beta- and alpha-cell dysfunction in type 2 diabetes. Horm. Metab.Res., 36(11-12):775-81.

Dimpfel, W.; Spüler, M.; Pierau, F. K. and Ulrich, H. (1990): Thioctic acid induces dose-dependent sprouting of neurites in cultured rat neuroblastoma cells Dev. Pharmacol. Ther.; 14(3):193-9.

Frederich, R. C.; Hamman, A.; Anderson, S.; et al. (1995): Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nat Med 1:1311-1314,

Friedman, J. M. (2000): Obesity in the new millennium. Nature, 404: 632–634.

Gold, A. H. (1970): The effect of diabetes and insulin on liver glycogen synthetase activation. J. Biol. Chem., 245: 903-905.

Haffner, S. M.; Miettinen, H. and Stern, M. P. (1997): The homeostasis model in the San Antonio Heart Study. Diabetes Care.; 20:1087–92.

Hathout, E. H.; Sharkey J.; Racine, M.; Ahn, D.; Mace, J. W. and Saad, M. F. (1999): Changes in plasma leptin during the treatment of diabetic ketoacidosis. J. Clin. Endocrinol. Metabol. 84 (12): 4545-4548.

Hirose, H.; Lee, Y. H.; Inman, Y.; Nagasawa, J. H. J. and Unger, R. H. (1996): Defective fatty acids-mediated beta-cell compensation in zucker diabetic fatty rats. Pathogenic implications for obesity dependent diabetes. J. Biol. Chem., 271: 5633-5637.

Hounsom, L.; Horrobin, D. F.; Tritschler, H.; Corder, R. and Tomlinson, D. R. (1998): A lipoic acid-gamma linolenic aci,d conjugate is effective against multiple indices of experimental diabetic neuropathy. Diabetologia., 41(7):839-43.

Huerta, M. G. (2006): Adiponectin and leptin: Potential tools in the differential diagnosis of pediatric diabetes? Rev. Endocr. Metab. Disord., 7(3):187-96 .

Jacob, S.; Streeper, R. S.; Fogt, D. L.; Hokama, J. Y.; Tritschler, H. J.; Dietze, G. J. and Henriksen, E. J. (1996): The antioxidant insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. Diabetes, 45(8): 1024-9.

Khamaisi, M.; Rudich, A.; Potashnik, R.; Tritschler, H. J.; Gutman, A. and Bashan, N. (1999): Lipoic acid acutely induces hypoglycemia in fasting nondiabetic and diabetic rats. Metabolism, 48(4): 504-10.

Khan, A. H. and Pessin, J. E. (2002): Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia, 45: 1475-1483.

Kirel, B.; Dogruel, N.; Korkmaz, U.; Kilic, F. S.; Ozdamar, K. and Ucar, B. (2000): Serum leptin levels in type 1 diabetic and obese children: relation to insulin levels. Clin. Biochem., 33(6):475-80.

Kocak, G.; Aktan, F.; Canbolat, O.; Ozogul, C.; Elbeg, S.; Yildizoglu-Ari, N. and Karasu, C. (2000): ADIC study group-antioxidants in diabetes-induced complications. Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes. Diabetes Nutr. Metab., 13(6): 308-18.

Lauszus, F. F.; Schmitz, O.; Vestergaard, H.; Klebe, J. G. and Pedersen, O.(2001): Serum leptin levels in pregnant women with type 1 diabetes mellitus. Acta Obstet Gynecol Scand., 80(7):596-601.

Lee, W.J.; Song, K. H.; Koh, E. H.; Won, J. C.; Kim, H. S.; Park, H. S.; Kim, M. S.; Kim, S. W.; Lee, K. U. and Park, J. Y. (2005): a-Lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. Biochem. Biophys. Res. Communi., 332: 885–891.

Levy, J. R. and Stevens, W. (2001): The effects of insulin,

glucose, and pyruvate on the kinetics of leptin secretion. Endocrinol., 142: 3558-3562.

Malmstroem, R.; Taskinen, M. R.; Karonen, F.; et al., (1996): Insulin increases leptin concentrations in normal subjects and patients with IDDM. Diabetologia, 39: 993-996.

Maha, M.; El Sawalhy and Raffat, M. (2007): Effect of alpha lipoic acid on oxidative stress status in rat kidney subjected to ischemia/reperfusion injury. Egypt. J. Biochem. Mol. Biol., 25: 1, 59-76.

Mathe, D. (1995): Dyslipidemia and diabetes: animal models. Diabete. Metab., 21(2): 106-111.

Mazzone, T.; Foster, D. and Chait, A. (1984): In vivo stimulation of low-density lipoprotein degradation by insulin. Diabetes, 33: 333-338.

Melhem, M. F.; Craven, P. A.; Liachenko, J. and DeRubertis, F. R. (2002): α-Lipoic acid attenuates hyperglycemia and prevents glomerular mesangial matrix expansion in diabetes J. Am. Soc. Nephrol., 13:108-116.

Minokoshi, Y.; Kim, Y.; Peroni, O.; Fryer, L.; Muller, C.; Carling, D. and Kahn, B. (2002): Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature, 415: 268–269.

Miyanaga, F.; Ogawa, Y.; Ebihara, K.; Hidaka, S.; Tanaka, T.; Hayashi, S.; Masuzaki, H. and Nakao, K. (2003): Leptin as an adjunct of insulin therapy in insulin-deficient diabetes. Diabetologia. 46(10):1329-37. Epub

Moini, H.; Packer, L. and Saris, N. E. (2002): Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. Toxicol. Appl. Pharmacol., 182(1): 84-90.

Monnier, L.; Colette, C.; Percheron, C. and Descomps, B. (1995): Insulin, diabetes and cholesterol metabolism. CR Seances Soc. Biol. Fil., 189 (5): 919-931.

Mueller, W. M.; Stanhope, K. L.; Gregoire, F.; Evans, J. L. and Havel, P. J. (2000): Effects of metformin and vanadium on leptin secretion from cultured rat adipocytes. Obes. Res., 8: 530-539.

Ohkuwa, T.; Sato, Y. and Naoi, M. (1995): Hydroxyl-radical formation in diabetogenic rats induced by streptozotocin. Life Sci., 56: 1789-1795.

O` Meara, N. M.; Devery, R. A.; Owens, P. B.; Johnson, A. H. and Tomkin, G. H. (1990): Cholesterol metabolism in alloxan- induced diabetic rabbits. Diabetes, 39: 626-633.

Packer, L.; Kraemer, K. and Rimbach, G. (2001): Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition, 17: 888–895.

Packer, L.; Witt, E. H. and Tritschler, H. J. (1995): Alpha-lipoic acid as a biological antioxidant. Free Radic Biol. Med., 19: 227-50.

Pusztai, P.; Prechl, J.; Somogy, A.; Szaleczky, E. and Feher, J. (1996): Experimental models in research of the pathomechanism of diabetes mellitus. Orv. Hetil., 137 (34): 1865-1869.

Quaschning, T.; Schomig, M.; Keller, M.; et al., (1999): Non-insulin dependent diabetes mellitus and hypertriglyceridemia impair lipoprotein metabolism in chronic hemodialysis patients. J. Am. Soc. Nephrol., 10 (2): 332-341.

Raju, J.; Gupta, D.; Rao, A. R.; Yadava, P. K. and Baquer, N. Z. (2001): Trigonellafoenum graecum (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol. Cell. Biochem., 224(1-2): 45-51.

Richmond, W. (1973): Enzymatic determination of cholesterol. Clin. Chem., 19: 1350-1356.

Rudzka-Kocjan, A.; Szarras-Czapnik, M. B. J.; and Ginalska-Malinowska, M. (2006): Estimation of the correlation of insulin resistance and selected adipocytokines in children with simple obesity-preliminary study. Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw.12(3):211-215.

Schedl, H. P. and Wilson H. D. (1971): Effects of diabetes on intestinal growth and hexose transport in the rat. Am. J. Physiol., 220: 1739.

Schroeder, M. M.; Belloto, R. J. Jr; Hudson, R. A. and McInerney, M. F. (2005): Effects of antioxidants coenzyme Q10 and lipoic acid on interleukin-1 beta-mediated inhibition of glucose-stimulated insulin release from cultured mouse pancreatic islets. Immunopharmacol. Immunotoxicol., 27(1): 109-22.

Sheela, C. G. and Augusti, K. T. (1992): Antidiabetic effects of S-ally cysteine sulphoxide isolated from garlic Allium sativum Linn. Ind. J. Exp. Biol., 30: 523-526.

Sindelar, D. K.; Havel, P. J.; Seeley, R. J.; Wilkinson, C. W.; Woods, S. C. and Schwartz, M. W. (1999): Low plasma leptin levels contribute to diabetic hyperphagia in rats. Diabetes, 48: 1275–1280.

Smith, S. R. (1996): The endocrinology of obesity. Endocrinol. Metab. Clin. North. Am., 25:921-942.

Snedecor, G. W. and Cochran, W. G. (1980): Statistical Methods. Iowastate University Press, Ames, Iowa, U.S.A.

Sobenin, I. A.; Tertov, V. V. and Orekhov, A. N. (1994): Characterization of chemical composition of native and modified low-density lipoprotein occurring in the blood of diabetic patients. Inter. Angio., 13: 78-83.

Soliman, A. T.; Omar, M.; Assem, H. M.; Nasr, I. S.; Rizk, M. M.; El Matary, W. and El Alaily, R. K. (2002): Serum Leptin Concentrations in Children with Type 1 Diabetes Mellitus: Relationship to Body Mass Index, Insulin Dose, and Glycemic Control. Metabolism, 51 (3): 292-296.

Song, K. H.; Lee, W. J.; Koh, J. M.; Kim, H. S.; Youn, J. Y. Park, H. S.; Koh, E. H.; Kim, M. S.; Youn, J. H.; Lee, K. U. and Park, J. Y. (2005): Alpha-lipoic acid prevents diabetes mellitus in diabetes-prone obese rats. Biochem. Biophys. Res. Commun., 7; 326(1): 197-202.

Strödter, D.; Lehmann, E.; Lehmann, U.; Tritschler, H. J.; Bretzel, R. G. and Federlin, K. **(1995):** The influence of thioctic acid on metabolism and function of the diabetic heart. Diabetes Res. Clin. Pract. , 29(1):19-26.

Szkudelski, T. (2006): Intracellular mediators in regulation of leptin secretion from adipocytes. Physiol. Res., Dec 19.

Szkudelski, T.; Kandulska, K. and Okulicz, M. (1998): Alloxan in vivo does not only exert deleterious effects on pancreatic β-cells. Physiol. Res., 47: 343-6.

Taniguchi, A.; Nakai, Y.; Sakai, M.; et al., (2002): Relationship of regional adiposity to insulin resistance and serum triglyceride levels in nonobese Japanese type 2 diabetic patients. Metabolism, 51: 544- 8.

Trinder, P. (1969): Enzymatic method for glucose estimation. Ann. Clin. Biochem., 6, 24.

Turkingto, R. W.; Eslkowkski, A. and Link, M. (1982): Secretion of insulin or connecting peptid; a predictor of insulin dependence of obese diabetics: Archives of Internal Med., 142:1102-1105.

Wollin, S. D. and Jones, P. J. (2003): Alpha-lipoic acid and cardiovascular disease. J. Nutr., 133: 3327–3330.

Yi, X. and Maeda, N. (2006): {alpha}-Lipoic Acid Prevents the Increase in Atherosclerosis Induced by Diabetes in Apolipoprotein E-Deficient Mice Fed High-Fat/Low-Cholesterol Diet. Diabetes, 55(8):2238-44.

Yildiz, B. O. and Haznedaroglu, I. C. (2006): Rethinking leptin and insulin action: Therapeutic opportunities for diabetes. Int. J. Biochem. Cell Biol., 38: 820–830.

Young, D. and Pestaner, L. (1975): Enzymatic determination of triacylglycerol in serum. Clin. Chem., 21: 5.

Younsozai, M. K. and Schedl, H. P. (1972): Intestinal amino acid transport and tissue concentration in diabetes. Am. J. Physiol., 223: 828.

مستويات كل من الأنسولين و اللبتن فى الفئران المصابة تجريبيا بمرض البول السكرى المعالجة و غير المعالجة بحمض الليبويك

تهدف هذه الدراسة الى معرفة بعض التغيرات الكيميائية الحيوية فى مستويات الانسولين و اللبتن فى كل من الفئران المصابة بمرض البول السكرى التجريبى بواسطة الالوكسان و كذلك بعد العلاج بحمض الليبويك وأجريت التجربة على عدد ٩٦ فأرا حيث قسمت الى ثلاث مجموعات ، المجموعة الاولى و تشمل ٣٢ فارا تركت كمجموعة ضابطة طبيعية (بدون مرض البول السكرى التجريبى) و ٦٤ فأر قد استحدث بهم مرض البول السكرى تجريبيا باستخدام مادة الالوكسان بالحقن تحت الجلد (١٢٠ ملجرام/كجم وزن) ثم قسمت بعد ذلك الى مجموعتين: المجموعة الثانية (٣٢ فأر) مصابة بمرض البول السكرى فقط و المجموعة الثالثة (٣٢ فأر) مصابة بمرض البول السكرى و تم معالجتها بحمض الليبويك. استمرت التجربة اربعة أسابيع متتالية وتم تجميع عينات الدم من الفئران بكل مجموعة فى نهاية التجربة لتقدير مستويات الجلوكوز،الجليسريدات الثلاثية، الكوليسترول الكلى، هرمونى الأنسولين و اللبتن. كما تم تسجيل متوسط وزن الجسم ومتوسط وزن الجسم المكتسب للفئران بكل مجموعة و كذلك تم حساب (مقاومة الأنسولين)و معامل الأرتباط بين كل من اللبتن و الأنسولين و أوزان الجسم. وأوضحت النتائج بعد التحليل الآحصائى أن هناك ارتفاع معنوى فى مستوى الجليسريدات الثلاثية ، الكوليسترول الكلى ، الجلوكوز ، مقاومة الانسولين.و انخفاض معنوى فى متوسط وزن الجسم المكتسب و مستوى هرمونى الأنسولين و اللبتن بالفئران المصابة بمرض البول السكرى مقارنة بالمجموعة الضابطة. كما أوضحت النتائج تأثير حمض الليبويك فى تقليل مستوى كل من الجليسريدات الثلاثية ، الكوليسترول الكلى ، الجلوكوز،مقاومة الأنسولين بشكل معنوى. علاوة على ذلك فقد ادى حمض الليبويك الى زيادة معنوية فى مستوى هرمونى الأنسولين و اللبتن مقارنة بالمجموعة المصابة بمرض البول السكرى.

تخلص الدراسة الى أن مرض البول السكرى التجريبى بواسطة الألوكسان يؤدى الى ارتفاع سكر الجلوكوز بالدم ، زيادة مقاومة الأنسولين، ارتفاع الدهون بالدم و انخفاض مستوى اللبتن بالدم.و كذلك توضح الدراسة التأثير الفعال لحمض الليبويك فى تحسين استجابة الجسم للأنسولين و الذى يظهر من خلال انخفاض مستوى الجلوكوز و ارتفاع مستوى الأنسولين و اللبتن. ولقد ارتبط مستوى اللبتن ايجابيا مع وزن الجسم والانسولين، كما ارتبط مستوى الأنسولين ايجابيا مع وزن الجسم، بينما ارتبط مستوى مقاومة الأنسولين سلبيا مع كل من الانسولين واللبتن و وزن الجسم.