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Effect of exogenous leptin administration on high fat diet induced oxidative stress

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The objective of the present study was to explore the tissue lipid peroxidation and antioxidant status in mice receiving exogenous leptin along with high fat diet for a period of 6 weeks. Significantly elevated levels of tissue thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and significantly lowered levels of glutathione and its related enzymes were observed in the liver, heart and kidney of mice fed with a high fat diet as compared with the control mice fed with a standard pellet diet. Subsequent to the treatment with high fat diet (ie., after the initial period of 30 days) exogenous leptin (230 μg kg^{-1} body weight) was simultaneously administered along with the regular high fat diet every alternate day for 15 days. Leptin administration significantly lowered the tissue levels of TBARS, CD and elevated the activities of reduced glutathione (GSH), glutathione peroxidase (GP_X) and glutathione S-transferase (GST) in both the control and high fat diet fed mice. Thus leptin supplementation was found to be effective in attenuating high fat diet induced oxidative stress.

1. Introduction

Lipid peroxidation probably plays an important role in the pathogenesis of coronary artery disease. The peroxidation process involves the oxidative conversion of polyunsaturated fatty acids to lipid hydroperoxides as the primary product, together with the formation of a variety of secondary metabolites (Kannel and Larson 1993). Free radicals are toxic to biomembranes and lead to peroxidation of lipids, unless they are removed by free radical scavengers such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) vitamin E and vitamin C (Esterbauer et al. 1989). In atherosclerosis, lipid peroxidation results in macrophage stimulation of foam cell formation, leading to endothelial damage, which is thought to initiate the formation of atherosclerotic plaque (Güler K et al. 1998). Increased lipid peroxidation in coronary artery diseases has also been reported (Ross 1986).

The *ob* gene protein, also known as leptin is a 16-kDa protein synthesised and secreted by adipose tissue in proportion to fat stores. Administration of recombinant leptin reduces food intake leading to weight loss, increases energy expenditure (Soriano et al. 1998) and has a negative role in lipid peroxidation (Unger and Orci 2001). There are no earlier studies on the role of leptin on high fat diet induced oxidative stress. The aim of our present study was to investigate the role of exogenous mouse recombinant leptin on lipid peroxidation and the antioxidant status of high fat diet fed mice.

2. Investigations and results

The effect of high fat diet and leptin on body weight is summarized in Table 1. The weight gain was significantly higher in high fat diet fed mice (group 3) as compared with the control animals (group 1) until the 30th day of the experiment. Exogenous leptin administration to the control and high fat diet fed mice (Groups 2 & 4) resulted in a sudden decline in the weight gain both in the control and high fat diet fed mice.

Table 1: Effect of high-fat diet and leptin on body weight

Groups	Body weight (g)		
	Day 1	Day 30	Day 45
Control	20.17 ± 1.07	23.3 ± 1.06	25.17 ± 1.77^{ae}
Control + leptin	25.0 ± 1.29	28.1 ± 1.24	20.33 ± 1.69^{b}
High-fat diet	20.16 ± 1.3	26.25 ± 1.19	31.83 ± 2.19^{c}
High-fat diet + leptin	30.83 ± 1.77	35.20 ± 1.72	25.67 ± 1.69^{de}

Values are mean ± SD of six mice from each group
Values sharing a common superscript are not significant with each other at $P < 0.05$ (DMRT)

Table 2: Effect of high-fat diet and leptin on food intake in mice

Groups	Time point		
	Day 1	Day 30	Day 45
Control	7.16 ± 0.09	7.48 ± 0.08	7.50 ± 0.10^{a}
Control + leptin	7.50 ± 0.08	7.84 ± 0.08	6.34 ± 0.08^{b}
High fat diet	7.22 ± 0.16	7.62 ± 0.10	8.42 ± 0.09^{c}
High fat diet + leptin	7.76 ± 0.10	8.18 ± 0.12	6.88 ± 0.11^{d}

Values are mean ± SD of six mice from each group
Values sharing a common superscript are not significant with each other at $P < 0.05$ (DMRT)

Table 3: Effect of leptin administration on tissue thiobarbituric acid reactive substances and conjugated dienes

Groups	TBARS (nM/g tissue)			CD (μM/g tissue)		
	Liver	Heart	Kidney	Liver	Heart	Kidney
Control	23.81 ± 0.86^{a}	26.88 ± 2.57^{a}	46.47 ± 1.81^{a}	104.04 ± 4.17^{ae}	97.47 ± 5.92^{ae}	99.66 ± 6.84^{aef}
Control + leptin	19.95 ± 0.91^{b}	19.50 ± 1.24^{b}	16.17 ± 1.53^{b}	93.78 ± 3.03^{be}	95.93 ± 5.72^{be}	100.83 ± 6.89^{be}
High fat diet	46.38 ± 3.51^{c}	48.39 ± 2.45^{c}	51.79 ± 1.93^{c}	221.53 ± 11.59^{c}	246.88 ± 16.30^{c}	172.86 ± 14.65^{c}
High fat diet + leptin	36.51 ± 1.27^{d}	33.78 ± 2.14^{d}	38.9 ± 2.27^{d}	133.75 ± 12.15^{d}	112.99 ± 6.59^{d}	104.24 ± 7.22^{def}

Values are mean ± SD of six mice from each group
Values sharing a common superscript are not significant with each other at $P < 0.05$ (DMRT)

Table 4: Effect of leptin administration on glutathione peroxidase and glutathione S-transferase

Group	GP_X (mg of GSH consumed/min/mg protein)			GST (μM of CDNB-GSH conjugate formed/min/mg protein)		
	Liver	Heart	Kidney	Liver	Heart	Kidney
Control	7.22 ± 0.54^{ae}	7.37 ± 0.24^{a}	7.5 ± 0.41^{a}	19.83 ± 1.49^{ae}	14.3 ± 1.55^{a}	8.34 ± 0.75^{a}
Control + leptin	7.70 ± 0.64^{be}	7.88 ± 0.44^{b}	8.47 ± 0.29^{b}	18.89 ± 1.07^{be}	8.72 ± 1.05^{be}	5.88 ± 0.33^{b}
High fat diet	5.30 ± 0.29^{c}	3.18 ± 0.22^{c}	3.73 ± 0.29^{c}	8.28 ± 0.61^{c}	8.27 ± 0.25^{ce}	4.22 ± 0.30^{c}
High fat diet + leptin	6.04 ± 0.23^{d}	6.85 ± 0.54^{d}	5.81 ± 0.38^{d}	5.19 ± 0.37^{d}	10.49 ± 0.34^{d}	3.51 ± 0.17^{d}

Values are mean ± SD of six mice from each group
Values sharing a common superscript are not significant with each other at $P < 0.05$ (DMRT)

Effect of high fat diet and leptin on food intake by the mice is represented in Table 2. The food intake gradually increased in all the groups until the 30th day. Administration of leptin resulted in a sudden decrease in the food intake both by the control as well as the high fat diet fed mice.

The concentration of TBARS and CD in the tissues of control and experimental animals are shown in Table 3. Significantly elevated levels of TBARS and CD were observed in the liver, heart and kidney of mice fed with the high fat diet (group 3) as compared with the control mice (group 1). Administration of exogenous leptin to high fat

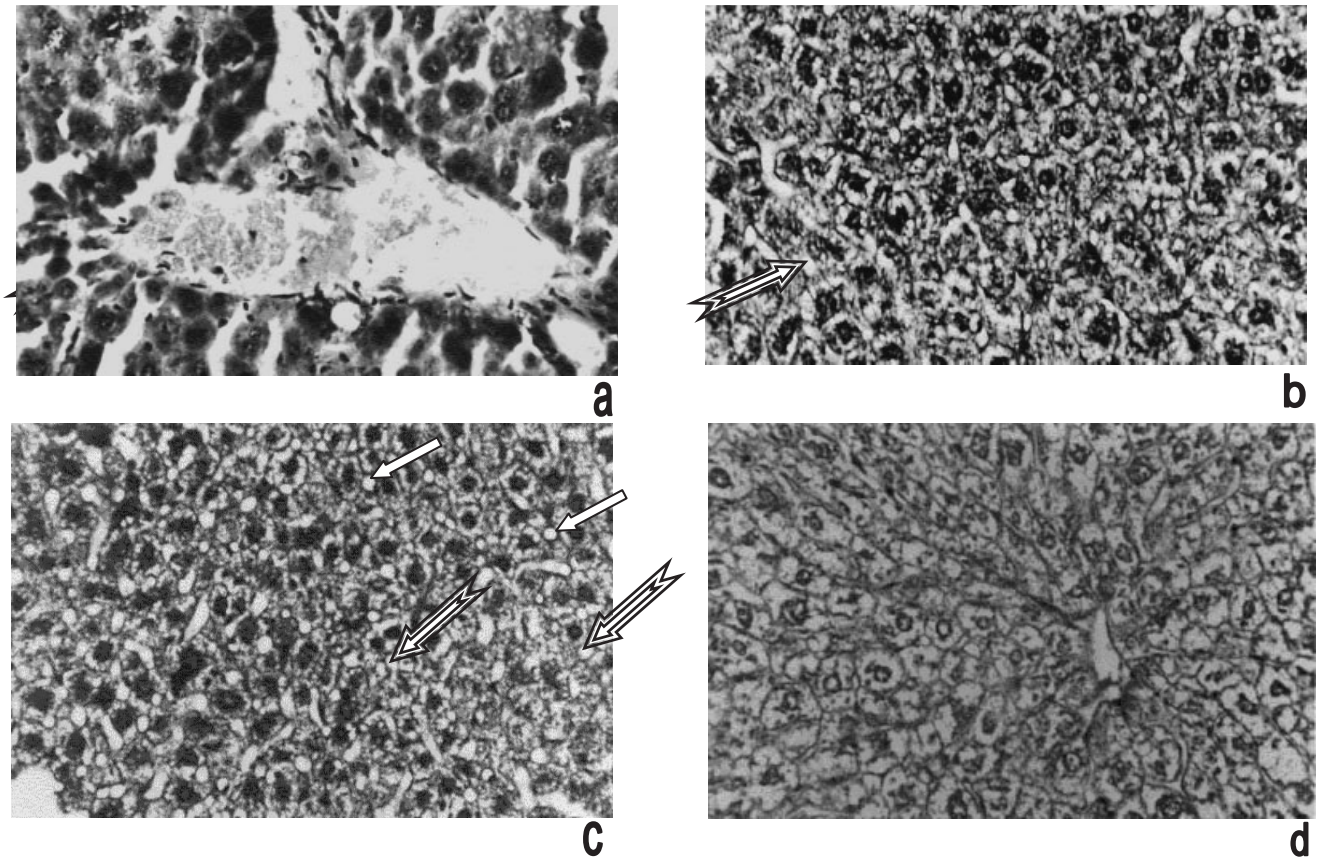

Fig.: a: Control animal liver showing (⟹) central vein and hepatocytes arranged in the form of cords H&E X 20; b: Leptin treated animal liver: H&E X 20. Mild feathery degeneration (⟹); c: High fat diet treated animal liver: H & E X 20. Macrovesicular type (⟹) and microvesicular type (⟹). of fatty change involving entire parenchyma of the liver; d: High fat diet + leptin treated animal liver: H & E X 20. Reduced number of fat vacuoles are observed, however the individual hepatocytes show feathery degeneration

Table 5: Effect of leptin administration on tissue reduced glutathione

Groups	GSH (mM/g tissue)		
	Liver	Heart	Kidney
Control	0.48 ± 0.02^{ae}	0.75 ± 0.027^{ae}	0.29 ± 0.026^{a}
Control + leptin	0.49 ± 0.03^{be}	0.78 ± 0.07^{be}	0.33 ± 0.024^{b}
High fat diet	0.22 ± 0.02^{c}	0.62 ± 0.012^{c}	0.17 ± 0.016^{c}
High fat diet + leptin	0.41 ± 0.021^{d}	0.69 ± 0.033^{d}	0.22 ± 0.017^{d}

Values are mean ± SD of six mice from each group
Values sharing a common superscript are not significant with each other at $P < 0.05$ (DMRT)

diet fed mice (group 4) significantly lowered the TBARS and CD levels in all the tissues as compared with the untreated high fat diet fed mice (group 3).

The activities of GP_X and GST (Table 4) were significantly lowered in tissues such as liver, heart and kidney of animals receiving the high fat diet as compared to those of the control mice. Administration of exogenous leptin to the high fat diet fed mice (group 4) significantly elevated the activities of glutathione peroxidase and glutathione S-transferase in all tissues as compared with the untreated high fat diet fed mice (group 3).

Table 5 shows significantly lowered levels of glutathione in the liver, heart and kidney of mice fed with the high fat diet as compared to control mice. Administration of exogenous leptin to the high fat diet fed animals (group 4) significantly elevated the glutathione values in the liver, heart and kidney as compared with the untreated high fat diet fed mice (group 3).

Fig. 1 shows the histopathologic changes in the liver of control (Fig. 1a), the leptin treated (Fig. 1b), high fat diet treated (Fig. 1c) and the high fat diet + leptin treated groups (Fig. 1d) of mice respectively. Liver of control animals showed central vein and hepatocytes arranged in the form of cords (Fig. 1a). The liver samples of animals treated with high fat showed the macrovesicular type of fatty change (Fig1. c). Reduced macrovesicular type fatty change was observed on leptin administration to high fat diet fed mice (Fig. 1d). Leptin *per se* had no significant effect on liver histology (Fig. 1b).

3. Discussion

The presented results indicate that administration of exogenous mouse recombinant leptin brings about profound alterations in the tissue lipid peroxidation and antioxidant status of mice fed with a high fat diet. In our study, high fat diet fed mice showed a significantly higher weight gain than control mice as expected but there was a significant weight loss on administering exogenous leptin in both the control and high fat diet fed mice. Leptin is known to inhibit the release of neuropeptide Y, an appetite stimulator in the hypothalamus and this effectively reduced food intake. Leptin also decreases appetite by stimulating the production of corticotropin releasing hormone. Additionally, leptin stimulates sympathetic nerve activity which increases the metabolic rate and leptin inhibits insulin secretion from the β-cells of the pancreas. Insulin is one of the primary regulators of energy levels in the body as it stimulates glucose uptake and glycogenesis while also stimulating TG synthesis and inhibiting lipolysis. Thus by inhibiting insulin secretion and inhibiting appetite, leptin may reduce energy intake and energy storage while promoting energy usage (Guyton, Hall 2000). Earlier studies in our laboratory also revealed that exogenous leptin injections significantly reduce weight gain (Balasubramaniyan et al. 2003a, 2003b). Moreover, the weight loss observed in leptin treated mice may be due to decreased food intake and increased basal metabolic rate (BMR) with selective promotion of fat metabolism (Levin et al. 1996). Recent evidence suggests that hypercholesterolemia, a major risk factor for atherosclerosis could increase oxidative stress to the cells by producing reactive oxygen species [ROS] such as OH and O_2^- (Sheela Augusti 1995). We observed significantly elevated levels of TBARS and CD, the markers of lipid peroxidation, in the liver, heart and kidney of high fat diet fed mice. These results are in agreement with the observations of previous researchers (Jeyakumar et al. 1999; Khan et al. 1997; Sheela and Augusti 1995; Thampi et al. 1991).

Leptin administration to high fat diet fed mice significantly reduced the level of TBARS and CD in the liver, heart and kidney as compared to the untreated high fat diet fed animals. Normally fatty acid delivery to non adipose tissue is tightly coupled to their need for fuel. Plasma free fatty acid levels rise during exercise and fasting to meet the metabolic requirements, leaving little or no unoxidised fatty acids in those cells. During calorie excess the fatty acid supply to non-adipose tissues may exceed their oxidative needs; however upregulation of peroxisome proliferator – activated receptors α, (PPARα), the transcription factor for enzymes of fatty acid oxidation, carnitine palmitoyl transferase −1 (CPT-1), acyl CoA oxidase (ACO) and uncoupling protein (UCP-2) will promote compensatory oxidation of the surplus fatty acid, and the unneeded energy will be dissipated as heat. This compensatory system requires leptin and a normal leptin receptor (OB-R) (Keller et al. 1993; Kim et al. 1998; Kliewer et al. 1994 and Schoonjans et al. 1996). But chronic overfeeding or prolonged high fat diet consumption leads to leptin and insulin resistance (Wang et al. 2001). During those circumstances fatty acid storage occurs not only in adipocytes, resulting in obesity, but also in non adipocytes causing lipotoxicity. With PPARα expression reduced due to leptin resistance, surplus fatty acid influx presumably binds to the high levels of PPAR γ, which upregulates the lipogenic enzymes acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS). This causes ectopic accumulation of TG, increased non-oxidative fatty acid metabolism, lipid peroxidation and lipoapoptosis (Unger and Orci 2001). So it can be hypothesized that on exogenous leptin administration, the condition is reversed. As leptin stimulates the expression of PPARα, the activities of CPT-I and ACO are elevated. Thus excess fatty acids do not accumulate to undergo lipid peroxidation or lipoapoptosis. This could be the reason for the decreased lipid peroxidation observed in our study on administering exogenous leptin to high fat diet fed mice.

Glutathione peroxidase and glutathione S-transferase are enzymes that use glutathione as cofactor. Activities of these antioxidant enzymes and GSH were significantly lowered on treatment with high fat diet. Our results are inline with previous studies (Jeyakumar et al. 1999). But administration of exogenous leptin to high fat diet fed mice elevated the levels of GP_X, GST and GSH. Under normal physiological conditions cellular homeostasis is incessantly challenged by stressors arising from both internal and external sources (Yu 1994). The endogenous antioxidants are thus subjected to homeostatic control in animal tissues (Lopez-Torres et al. 1993; Sohal et al.

1984). Increased oxidative stress observed in high fat fed mice can be correlated with decreased antioxidant levels (Thampi et al. 1991). Previous studies in our laboratory also showed similar findings (Jeyakumar et al. 1999). In the present study, on administering exogenous leptin to high fat diet fed mice we observed elevated levels of GSH and glutathione related enzymes in the liver, heart and kidney. This may be a compensatory mechanism and may be one of the reasons for the lowered lipid peroxidation observed in the tissues of these mice. In this context, studies have shown that decreased lipid peroxidation in biological membranes is associated with increased GP_X activity (Michiels et al. 1994).

Liver histology showed macrovesicular type of fatty change in high fat diet fed mice, which was significantly reduced on administering leptin. This proves that elevated systemic leptin levels can inhibit the accumulation of fat in the liver, thus reducing lipid peroxidation and lipoapoptosis.

In summary administration of exogenous mouse recombinant leptin to high fat diet fed mice significantly lowered the levels of TBARS and CD and optimised the activities of antioxidant enzymes to near normal levels. Our findings indicate that leptin can markedly reduce the high fat diet induced oxidative stress in mice. Further research is needed to ascertain the exact mechanism of action of leptin.

4. Experimental

4.1 Animals

Sixty healthy male Swiss mice (4 weeks old) in the weight range of 20–25 g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University and maintained at 25 °C with a 12 : 12 h light: dark cycle. They were housed 3 per cage in plastic cages (47 × 34 × 18 cm) lined with husk renewed every 24 h and had free access to drinking water and food. The animals were cared for as per the principles and guidelines of the ethical committee for Animal care of Annamalai University in accordance with the Indian National Law on animal care and use (National Institute of Health 1985).

2.2. Mouse recombinant leptin

Mouse recombinant leptin (purity ≥ 97% as determined by SDS-PAGE) was purchased from Sigma Chemical Co., St. Louis, MO, US. Leptin was reconstituted by adding 0.5 ml of 0.2 μm-filtered 15 mM HCl. After the protein was dissolved, 0.3 ml of 7.5 mM 0.2 μm-filtered NaCl was added. The dissolved protein was then stored in a refrigerator. The hormone was diluted with phosphate buffered saline just before use.

4.3. Experimental induction of hyperlipidemia

Hyperlipidemia in mice was induced by administering a high fat diet comprising 2% cholesterol, 0.125% bile salts and 5% peanut oil mixed with the powdered standard pellet diet.

4.4. Experimental design

The animals were divided into four groups of 15 each. Water was given *ad libitum.* Animals in groups 1 and 2 received a standard pellet diet (Lipton Lever Ltd., Mumbai) and animals in groups 3 and 4 received high fat diet during the initial period of 30 days. At the end of this period, the dietary protocol of the animals in the various groups were unaltered, and in addition, animals in groups 2 and 4 received exogenous mouse recombinant leptin (230 μg kg^{-1} body weight) intraperitoneally every alternate day at the beginning of the light phase for the next 15 days.

The animals were monitored closely and weighed every day. At the end of the total experimental period of 45 days after an overnight fast, the animals were sacrificed by cervical dislocation after ether anesthesia.

Liver, heart and kidney were cleared of adhering fat, weighed in a torsion balance and used for the estimation of thiobarbituric acid reactive substances (TBARS) (Ohkawa et al. 1979), conjugated dienes (CD) (Rao and Recknagel 1968), glutathione peroxidase (GP_X, EC 1.11.1.9) (Rotruck et al. 1973), glutathione S-transferase (GST, EC 2.5.1.18) (Habig et al. 1974) and reduced glutathione (GSH) (Ellman 1959). Protein was estimated by the method of Lowry et al. (Lowry et al. 1951).

4.5. Histological investigations

Liver slices fixed for 48 h in 10% formalin were processed for paraffin embedding following the standard microtechnique (Galighar and Kozloff 1971). Sections (5 μm) of liver stained with hematoxylin and eosin were evaluated for histopathological changes under a light microscope.

2.6 Statistical analysis

The results obtained were expressed as mean ± S.D of 6 mice in each group. One way ANOVA was done followed by Duncan's multiple range test (Duncan BD 1957) and the level of statistical significance was set at $P < 0.05$.

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