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# Exogenous Leptin Increases Lipid Peroxidation in the Mouse Brain

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Kutlu, S., Canpolat, S., Aydin, M., Yasar, A., Tuzcu, M. and Baydas, G. *Exogenous Leptin Increases Lipid Peroxidation in the Mouse Brain*. Tohoku J. Exp. Med., 2005, **206** (3), 233-236 —— Leptin, a hormone produced by the adipose tissues, reduces appetite and food intake, and increases energy expenditures by sending signals to the brain cells. As human obesity is associated with hyperleptinemia and increased systemic oxidative stress, we investigated whether leptin affects lipid peroxidation and antioxidant status in the brain. Leptin was intraperitoneally administered to adult male BALB/c mice ($n$ = 6) at a dose of 40 $\mu$g/animal for 5 days, while control mice ($n$ = 6) received phosphate buffered saline. All animals were decapitated one hour after the last injection, and the brain tissues were removed. Total brain tissues were homogenized with phosphate buffered saline. Lipid hydroperoxide and glutathione levels were measured by enzyme immunoassays. Data were statistically analysed by using Mann Whitney's U-test. Lipid hydroperoxide levels were significantly higher in the brain tissue of leptin-treated mice (3.44 ± 0.36 nmol/g tissue, mean ± S.E.M.) than those of the control mice (2.20 ± 0.38 nmol/g tissue, $p <$ 0.01). In contrast, leptin-treated mice had significantly lower glutathione levels in the brain tissue compared to the control (12.97 ± 1.32 and 17.91 ± 0.82 nmol/g tissue, respectively, $p < 0.05$). These results indicate that exogenous leptin increases lipid peroxidation and inhibits antioxidant system in the mouse brain. We therefore suggest that leptin may augment oxidative stress in the brain. ———— leptin; lipid peroxidation; brain and mouse
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It is well established that leptin, an adipocyte-derived hormone, is an important circulating satiety factor, which regulates body weight and food intake by acting on specific hypothalamic nuclei. In addition to its effects on satiety, leptin has been suggested to be involved in reproduction (Smith et al. 2002), hematopoiesis (Bennett et al. 1996), lactation (Mounzih et al. 1998), inflamation of some tissues (Yavuz et al. 2004) and homeostasis of fatty acid (Unger 2003). Initially, leptin was thought to be exclusively produced by adipocytes, however, it is now evident that leptin is present, in lesser amounts, in many fetal and adult tissues, including placenta (Hoggard et al. 2001), stomach (Bado et al. 1998), skeletal muscle (Wang et al. 1998), pancreas (Emilsson et al. 2001), bone (Reseland et al. 2001) and pituitary cell types in rodents (Jin et al. 2000). It has been demonstrated that leptin receptors are widely expressed throughout the brain of mouse (Huang et

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al. 1996; Li and Kane 2003), rat (Shioda et al. 1998) and human (Couce et al. 1997). In fact leptin has regulatory effects on the neuroendocrine function in the brain (Casanueva and Dieguez 1999). It also plays a modulating role in controlling hormone secretion in the anterior pituitary (Yu et al. 1997). Furthermore, leptin has been shown to have hyperalgesic effects in mice (Kutlu et al. 2003).

Leptin augments alcohol-induced lipid peroxidation in liver by lowering the levels of enzymatic and non-enzymatic antioxidants (Balasubramaniyan et al. 2003). There have been very few studies examining the effects of leptin on oxidative stress. Therefore, we have investigated the effects of exogenous leptin administration on lipid peroxidation and glutathione (GSH, an antioxidant) levels in the mouse brain.

## Materials and Methods

The protocol of this study was approved by the local Ethics Committee of Firat University, Faculty of Medicine. Adult male BALB/c mice (30 – 35 g) were divided into two groups ($n = 6$ per each group). The animals were obtained from the Firat University Medical School Experimental Research Unit where they were housed in a temperature-controlled environment (21 ± 1°C) with lights on between 07.00 and 19.00. Food and water were supplied *ad libitum*. Leptin was dissolved in phosphate buffered saline (PBS) (1 mg/ml) and intraperitoneally administered group at a dose of 40 $\mu$g/animal for 5 days. Control group received PBS. The volume of injected leptin solution or PBS was 100 $\mu$l.

All animals were decapitated one hour after the last injection. Brain tissues were rapidly removed and kept in dry ice. Total brain tissues were homogenized in PBS. Lipid hydroperoxide (which reflects the degree of lipid peroxidation better than other peroxidation products such as malondialdehyde) and GSH levels were measured by enzyme immunoassay using specific kits (Cayman Chemical Co., MI, USA).

Data were expressed as mean ± S.E.M. and statistically analyzed by using Mann Whitney's U-test. $P < 0.05$ was considered statistically significant.

## Results

Leptin administration did not cause any significant change in the body weight of the animals (data not shown). Lipid hydroperoxide levels were 3.44 ± 0.36 and 2.20 ± 0.38 nmol/g tissue in the leptin-treated and control group, respectively. Leptin treatment resulted in an increase in the level of lipid hydroperoxide ($p < 0.01$, Fig. 1). In contrast, leptin-treated mice had significantly lower GSH level ($p < 0.05$) in the brain tissue compared to the control group (12.97 ± 1.32 and 17.91 ± 0.82 nmol/g tissue, respectively, Fig. 2).

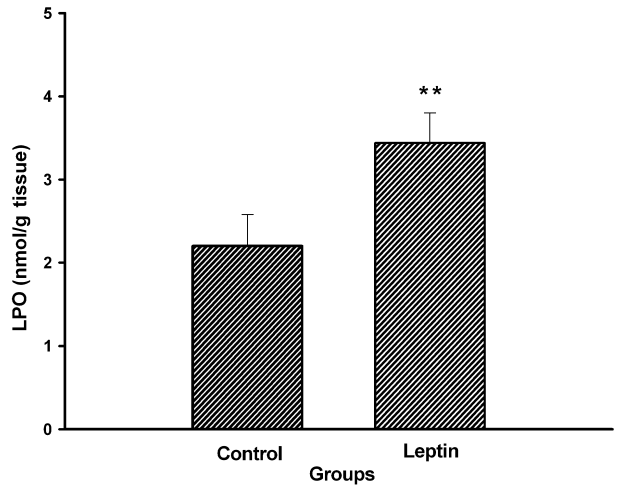



Fig. 1. Effect of leptin on lipid hydroperoxide (LPO, mean ± S.E.M.) levels in control and leptin administered groups.
** $p < 0.01$ compared to the control group, using Mann Whitney's U-test.

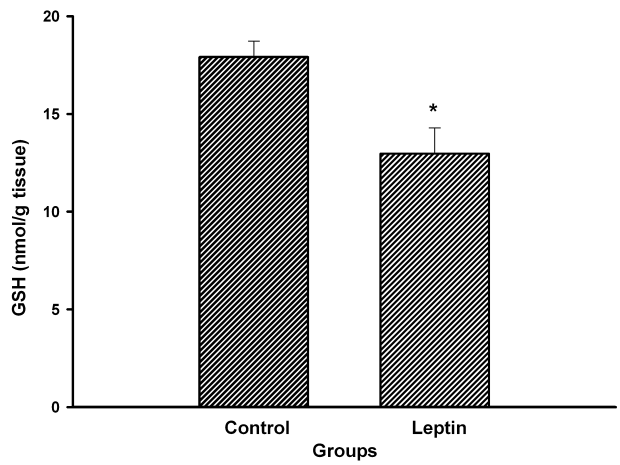



Fig. 2. Effect of leptin on glutathione (GSH, mean ± S.E.M.) levels in control and leptin administered groups.
* $p < 0.05$ compared to the control group, using Mann Whitney's U-test.

## Discussion

Free radical-induced lipid peroxidation is considered to be a primary mechanism of cell membrane destruction and cell damage. The brain is more susceptible to peroxidation since it is one of the most metabolically active organs in the body. Furthermore, the neural tissue is rich in polyunsaturated fatty acids and its antioxidant capacity is lower than that in other tissues. Leptin can cross the blood brain barrier and enter all regions of the rat brain; the distribution of leptin varies in the arcuate nucleus, thalamus, frontal cortex, choroid plexuses, and cerebellum (Banks et al. 2000; Kurrimbux et al. 2004).

Although Balasubramaniyan et al. (2003) found that leptin did not affect oxidative status in Swiss mice, the present study has demonstrated that leptin increase lipid peroxidation and decreased antioxidant GSH level in the mouse brain. This difference may due to the fact that we used the higher concentration of leptin than that used in the previous study. Alternatively, the genetic background of mouse strains might also be responsible for the different results.

To our knowledge, we have shown for the first time that exogenous leptin elevates lipid peroxidation in the brain tissue. However, the mechanism underlying this effect remains to be elucidated. One possibility is that leptin decreases antioxidant GSH levels and consequently increase lipid peroxidation in the brain. Another possibility is that increased peroxy nitrite level as free radical in brain may elevate lipid prexidation. Fruhbeck (1999) has shown that intravenous injection of leptin increased the plasma nitrite/ nitrate concentration in a dose-dependent manner in rats. The observed increase in lipid peroxidation in leptin-treated group might be due to elevated peroxy nitrite level in the brain that may result from the increase in the nitrite level.

Another possibility is that increased metabolic activity may cause cell damage via generating reactive oxygen species in the brain since leptin stimulates sympathetic nerve activity, which in turn increases metabolic rate (Diamond and Eichler 1999).

It has been demonstrated that leptin has neuroprotective effects in the mouse brain (Dicou et al. 2001). In addition, leptin is required for normal neuronal and glial maturation in the mouse nervous system (Ahima et al. 1999), although leptin appears to augment oxidative stress in the brain tissue. Further studies are needed for a conclusive statement on its effect on lipid peroxidation and antioxidant status in brain.

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