EFFECT OF LEPTIN ON CYTOCHROME P-450, CONJUGATION, AND ANTIOXIDANT ENZYMES IN THE OB/OB MOUSE

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ABSTRACT:

Leptin is a hormone that is secreted by adipocytes and regulates body weight through its effect on satiety and energy metabolism. The ob/ob mouse is deficient in this protein and is characterized by obesity and other metabolic disorders. This study investigated the alterations of several hepatic cytochrome P-450 (CYP), conjugation, and antioxidant enzymes in lean and ob/ob mice and the role leptin plays in the modulation of these enzymes. Lean and ob/ob male mice were injected with leptin (100 μg) or PBS for 15 days. Liver microsomes from ob/ob mice, when compared with lean controls, displayed significantly reduced chlorzoxazone 6-hydroxylation activity (27%); however, 7α- and 16α-testosterone hydroxylation and pentoxyresorufin O-dealkylation activities were significantly higher (47%, 22%, and 39%, respectively). Leptin administration corrected alterations seen with all P-450 activities. Dealkylation of ethoxyresorufin and ω-hydroxylation of lauric acid activities from ob/ob and lean mice were not statistically different; however, leptin exposure significantly increased ethoxyresorufin activity in lean mice (14%) and decreased the activity in ob/ob mice (36%). UDP-glucuronosyl-transferase and glutathione S-transferase activities were not altered. The antioxidant enzymes, catalase (11%) and glutathione peroxidase (26%), as well as glutathione reductase (17%), were lower in the ob/ob mice and leptin treatment corrected these alterations. The results of this study demonstrate alterations in constitutive expression of CYP2B, CYP2E, CYP2A, catalase, glutathione peroxidase, and glutathione reductase in ob/ob mice that were restored to lean control values following leptin treatment. Additionally, CYP3A activity was increased following leptin treatment in ob/ob mice. The mechanism for the observed alterations may be due to direct leptin effects or via indirect alterations in insulin, corticosterone, and/or growth hormone.

Leptin, a 167-amino acid peptide, is a newly discovered hormone that regulates food intake and energy balance (Zhang et al., 1994). The leptin mutation in the ob/ob mouse is characterized by obesity, hyperglycemia, hyperphagia, hyperinsulinemia, and infertility, which can be corrected by leptin replacement (Pellemounter et al., 1995). However, in the db/db mouse and the obese Zucker rat (fa/fa), there exists a leptin receptor mutation (Campfield et al., 1996). Although most human obesity is not related to leptin deficiency but to leptin resistance (Considine, 1996), the mechanisms by which leptin signals changes in these metabolic parameters in the ob/ob mouse may lend insight into these alterations with human obesity.

Leptin is mainly expressed in the adipose tissue with receptors located in the central nervous system and in peripheral tissues (Chen et al., 1996). The leptin receptor is a class I cytokine receptor, a family that includes interleukin-2 receptor, the interferon receptor, and the growth hormone (GH) receptor (Ihle, 1996). Leptin binding to receptors in the hypothalamus leads to activation of Jak kinases, a class of cytoplasmic tyrosine kinases and signal transducers and activators of transcription (STAT1), which results in the stimulation of transcription of responsive target genes.

The drug metabolizing and antioxidant enzyme system plays a major role in the detoxification and activation of many xenobiotics and reactive oxygen species. Therefore, changes in these hepatic enzymes associated with obesity is of great interest. A study by Zannikos et al. (1994) showed no alterations in cytochrome P-450 (CYP) 3A and CYP2C11 activities in the overfed rat, whereas Bandypadhyay et al. (1993) showed significant alterations in CYP2C11 expression in the obese Zucker rat. Roe et al. (1999), Barnett et al. (1992a), and Stengard et al. (1987) showed no significant changes in CYP1A, CYP2B, CYP2E, and CYP3A isoforms in the ob/ob mouse when compared with lean littermates. Additionally, Rouer and Leroux (1980) showed a reduction in amine hydroxylase activity (indicative of CYP2E1 activity) in the ob/ob mouse, which is in direct contrast to work by Raucy et al. (1990) in which enhanced catalytic activities associated with CYP2E1 was seen in obese overfed rats.

In addition to the changes in hepatic CYP-mediated reactions in obese rodent models, alterations in hepatic conjugative enzymes also occur. Barnett et al. (1992a), Roe et al. (1999), and Prohaska et al. 1
Changes in the antioxidant defense mechanisms have been seen in the obese rodent model. A study by Capel and Dorrell (1984) indicated lower levels of glutathione peroxidase (GSHPxs) activities in ob/ob mice when compared with their lean littermates, as well as lower glutathione reductase (GSHRed) activity and total GSH levels. Additionally, Prohaska et al. (1988) showed that hepatic GSHPxs activity of ob/ob mice was 70% of that in control lean mice and copper-zinc superoxide dismutase activity was 30% lower in obese mice.

Many of the drug metabolizing enzymes are altered in the obese rodent model; however, whether leptin plays a role in the regulation of these enzymes is unclear. Therefore, the objective of this study was to investigate whether leptin could alter or correct changes in the expression of several drug metabolizing and antioxidant enzymes in ob/ob mice.

Materials and Methods

Chemicals and Instrumentation. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All spectrophotometric analyses were done using a Shimadzu UV2100U spectrophotometer, an EL340 Bio Kinetic microplate reader or a Shimadzu HPLC.

Animals and Treatment. Twelve-week-old male ob/ob mice (57g) and their lean littermates (32g; obtained from Jackson Laboratories, Bar Harbor, ME) were acclimated to our laboratory conditions (12 h light/dark cycle) for several days before experiments. The animals had free access to laboratory rodent chow and tap water. Lean and ob/ob mice were injected with 100 µg leptin i.p. (Amgen Biologicals, Thousand Oaks, CA) in PBS for 15 days. Control mice (lean and ob/ob) received an equal volume of PBS. There were nine mice for each treatment group. Initial blood glucose levels were determined from blood obtained from tail sticks. Ending glucose, insulin, and corticosterone levels were determined by the method of Lowry et al. (1951) using BSA as the standard.

CYP Activity. CYP2E1 activity was determined using the 6-hydroxylation of chlorozoxazone according to the method of Peter et al. (1990). CYP1A and CYP2B activities were determined using the dealkylation of ethoxresorufin and pentoxyresorufin, respectively, to resorufin according to Burke et al. (1985). CYP4A activity was determined using the ω-oxidation of lauric acid according to Giera and van Lier (1991).

Analysis of Conjugation and Antioxidant Enzymes. GST activity was determined in liver cytosol by the method of Habig et al. (1974) using chloro-2,4-dinitrobenzene in ethanol as the substrate. UDP-glucuronosyltransferase activity was determined in liver microsomes by the method of Bock et al. (1979) using p-nitrophenol as the substrate. Total glutathione content was determined according to the enzymatic recycling method of Tietze (1969) using 5,5′-dithiobis (2-nitrobenzoic acid) as the substrate on a microtiter plate. GSHRed activity was determined in liver cytosol according to the method of Carlberg and Mannervik (1975). GSHPxs activity was assayed by the method of Lawrence and Burk (1976) using hydrogen peroxide as the substrate. Catalase activity was determined according to the method of Cohen et al. (1970) using potassium permanganate as the substrate.

The following denote the enzymes evaluated in this study followed by the substrates used to determine their activity. CYP2E1, chlorozoxazone 6-hydroxylation; CYP4A, lauric acid ω-hydroxylation; CYP1A, ethoxresorufin dealkylation; CYP2B, pentoxyresorufin dealkylation; CYP2A, testosterone 7α-hydroxylation; CYP3A, testosterone 6β-hydroxylation; GST, chloro-2,4-dinitrobenzene; UDPGT, p-nitrophenol; total glutathione, 5,5′-dithiobis(2-nitrobenzoic acid); GSHPxs, hydrogen peroxide; and catalase, potassium permanganate.

Statistical Analysis. To establish statistical differences between control and leptin treated animals, a two-way ANOVA was performed. The Fischer least-significant difference (LSD) post hoc analysis was used to determine statistical differences for each time point. Percentages are reported as the change due to phenotype or to leptin treatment. For phenotype comparisons this percentage is calculated as the observed change divided by the lean untreated values × 100. For leptin treatment comparisons the percentage is calculated as the observed change divided by the untreated values × 100.

Results

Effects of Leptin Administration on Body Weight, Blood Glucose, Serum Insulin, and Corticosterone Levels. To verify the effect of leptin in the ob/ob mouse model, several parameters were monitored (Table 1). PBS-treated animals did not show any significant weight changes; however, leptin administration caused weight reduction in both the ob/ob mice and their lean littermates. PBS treatment caused no significant changes in glucose levels in the control animals. Before leptin treatment, the ob/ob mice were not hyperglycemic (>300 mg glucose/dl); however leptin administration caused a statistically significant decrease in this level. Leptin did not alter glucose levels in the lean mice. Circulating insulin was about 5-fold higher in ob/ob mice compared with their lean littersmates; however, following leptin administration, a decrease in insulin levels was seen in ob/ob mice. Corticosterone levels were about 6-fold higher in ob/ob mice. Leptin administration had similar effects on corticosterone levels in both the lean and ob/ob mice, lowering corticosterone levels 2-fold.

Effects of Leptin Administration on Total Hepatic P-450 levels and Microsomal CYP Activities. Total P-450 levels were statistically lower (22%) in ob/ob mice when compared with lean mice; however, leptin administration had no significant effect on total P-450 in hepatic microsomes from lean or ob/ob mice (data not shown). The dealkylation of ethoxresorufin and pentoxyresorufin were used as markers reflective of CYP1A and CYP2B activities, respectively. No significant change was seen in CYP1A activity between lean and ob/ob mice (Fig. 1A). There was a significant increase in this activity (14%) following leptin administration in lean mice; however, the ob/ob mice showed a significant decrease (36%) following leptin exposure. ob/ob mice displayed significantly greater CYP2B activity (39%) than the lean mice (Fig. 1B). Leptin treatment caused a significant decrease in this activity (63%) in ob/ob mice but no change in...
The mice but the ob/ob mice displayed a significant increase in activity. A statistically significant decrease was seen with the 6α-hydroxylation product (54%) in ob/ob mice when compared with the lean mice (48% and 22%, respectively); however, although higher, no statistically significant changes were seen with the 6β-hydroxylation product (Table 2). Conversely, a significantly lower activity was seen with the 6α-hydroxylation product (54%) in ob/ob mice when compared with lean controls. Leptin exposure caused no significant changes with any of the hydroxylation products in the lean mice. However, statistically significant increases were seen with the 6α- and 6β-hydroxylation of testosterone (33% and 16%, respectively) following leptin exposure in ob/ob mice but no change was seen in 16α-hydroxylation product.

Effects of Leptin Administration on Hydroxylation of Testosterone in Liver Microsomes. Several CYP activities can be monitored by the hydroxylation of testosterone. Higher activities of 7α- and 16α-hydroxylation of testosterone were seen in the ob/ob mice when compared with the lean mice (48% and 22%, respectively); however, although higher, no statistically significant changes were seen with the 6β-hydroxylation product (Table 2). Conversely, a significantly lower activity was seen with the 6α-hydroxylation product (54%) in ob/ob mice when compared with lean controls. Leptin exposure caused no significant changes with any of the hydroxylation products in the lean mice. However, statistically significant increases were seen with the 6α- and 6β-hydroxylation of testosterone (33% and 16%, respectively) following leptin exposure in ob/ob mice but no change was seen in 16α-hydroxylation product.

Effects of Leptin Administration on Cytosolic GST Activity and Microsomal UDP Glucuronidation. Figure 2A shows the changes in cytosolic GST activity following leptin exposure in lean and ob/ob mice. No change was seen in GST activity between the lean and ob/ob mice. Leptin exposure caused no change in GST activity with the lean mice; however, there was a trend toward a decrease with the ob/ob mice. Figure 2B shows the effect leptin has on microsomal UDP-glucuronidation activity. No differences were seen between control lean and ob/ob mice and leptin treatment had no effect.

Effects of Leptin Administration on Antioxidant Enzyme Activities. Figure 3 shows the effect leptin had on several antioxidant enzymes and GSHRed. Catalase activity from ob/ob mice was statistically lower than activity (11%) from lean mouse (Fig. 3A). Leptin exposure had no effect on this activity in lean mice but there was a specific correction (to lean control levels) in this activity (10%) in ob/ob mice. Leptin can alter the observed defect in GSHPx activity in ob/ob mice (Fig. 3B). GSHPx activity from ob/ob mice was statistically lower (26%) than activity from lean mice. A significant increase in this activity in ob/ob mice (to control levels) was seen following leptin exposure but no change was seen with the lean mice. GSHRed activities from ob/ob mice was statistically lower (17%) than activity from lean mice (Fig. 3C). In contrast to GSHPx, leptin exposure caused a significant increase in GSHRed activity in both lean and ob/ob mice. Figure 3D represents the changes in total glutathione

Data are expressed as means ± S.E. of nine replications for each treatment. Numbers in parentheses represent S.E.M. For weight and glucose levels, similar lowercase letters represent means within a treatment that were not statistically different by LSD (P ≤ .05). For insulin and corticosterone levels, similar lowercase letters represent means within a parameter that were not statistically different by LSD (P ≤ .05). Mice were i.p. injected with PBS (100 μl/mouse) or leptin (100 μg/mouse) for 15 days before euthanasia. LC and OC represent lean and ob/ob mice injected with PBS, and LL and OL represent lean and ob/ob mice injected with leptin.
levels in control lean and ob/ob mice following leptin exposure, where there were no significant changes between control lean and ob/ob mice.

Discussion

The metabolism of many xenobiotics by phases I and II enzymes and antioxidant enzymes depends on the nature of these enzymes at the time of exposure. Therefore, any factor, such as obesity, can modulate the activities of these enzymes that could ultimately affect the rate of xenobiotic metabolism. This study demonstrated that the activity of several drug metabolizing and antioxidant enzymes are altered in the ob/ob mouse and that leptin replacement effectively corrects some of these alterations. Specifically, leptin treatment produced alterations in CYP2B, CYP2E, CYP2A, CYP3A, catalase, GSHPx, and GSHRed. Leptin treatment produces alterations in several endogenous factors including insulin, corticosterone, and GH.

Any of these factors may alter the regulation of the affected enzymes. The remainder of this discussion will focus on these results in relation to known regulators of expression for each of the aforementioned enzymes.

Several CYP-related activities were monitored in the ob/ob mouse and their lean littermates using substrates for specific CYP isofoms. Presently, as a result of a lack of specific antibodies and inhibitors for mouse P-450, it is not known whether the activities for the individual isofoms are the same as found in the rat model. However, activities in mice will be assigned the same P-450 designations as the rat model.

### Table 2

**Effect of leptin on hydroxylation of testosterone in liver microsomes in obese (ob/ob) and lean (Ob/?) mice**

Data are expressed as means of nine replications for each treatment. Activities are expressed as pmoles per minute per milligram protein. Numbers in parentheses represent S.E.M. Similar lowercase letters represent means within an activity that are not statistically different by LSD ($p \leq .05$). Mice were i.p. injected with PBS (100 µl/mouse) or leptin (100 µg/mouse) for 15 days before euthanasia. LC and OC represent lean and ob/ob mice injected with PBS and LL and OL represent lean and ob/ob mice injected with leptin.

<table>
<thead>
<tr>
<th>Hydroxylation</th>
<th>LC</th>
<th>LL</th>
<th>OC</th>
<th>OL</th>
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<tbody>
<tr>
<td>6α</td>
<td>282.33 (11.34)</td>
<td>312.15 (18.63)</td>
<td>129.46 (8.12)</td>
<td>193.20 (12.25)</td>
</tr>
<tr>
<td>6β</td>
<td>790.12 (31.66)</td>
<td>832.10 (71.64)</td>
<td>929.08 (51.26)</td>
<td>1105.87 (58.36)</td>
</tr>
<tr>
<td>7α</td>
<td>419.90 (11.0)</td>
<td>411.80 (28.28)</td>
<td>799.15 (36.61)</td>
<td>501.58 (16.46)</td>
</tr>
<tr>
<td>16α</td>
<td>217.37 (9.91)</td>
<td>256.74 (24.44)</td>
<td>278.19 (11.51)</td>
<td>237.21 (9.83)</td>
</tr>
</tbody>
</table>

### Figure 2

**GST (A) and UDP-glucuronosyltransferase (B) activities from lean and ob/ob mice following leptin treatment.**

Data are expressed as means ± S.E. of nine replications for each treatment. Activities are expressed as nanomoles per minute per milligram protein. Bars followed by a different letter are significantly different by LSD ($P \leq .05$). Mice were i.p. injected with PBS (100 µl/mouse) or leptin (100 µg/mouse) for 15 days before euthanasia. LC and OC represent lean and ob/ob mice injected with PBS and LL and OL represent lean and ob/ob mice injected with leptin.

### Figure 3

**Catalase (A), GSHPx (B) and GSHRed (C), and total glutathione levels (D) from lean and ob/ob mice following leptin treatment.**

Data are expressed as means ± S.E. of nine replications for each treatment. A–C, catalase, GSHPx, and GSHRed activities are expressed as nanomoles per minute milligram protein. D, glutathione activity is expressed as nanomoles per milligram protein. Bars followed by a different letter are significantly different by LSD ($P \leq .05$). Mice were i.p. injected with PBS (100 µl/mouse) or leptin (100 µg/mouse) for 15 days before euthanasia. LC and OC represent lean and ob/ob mice injected with PBS and LL and OL represent lean and ob/ob mice injected with leptin.
Many CYPs are influenced by a variety of factors, including inducers and endogenous substances such as insulin, GH, testosterone, and triglycerides. It is well known that ob/ob mice have higher levels of insulin (Harris et al., 1998) when compared with their lean littermates and insulin has been shown to differentially regulate CYP1A1/2 and CYP2E1 isoforms. Barnett et al. (1992b) suggest that hyperinsulinemia causes an increase in CYP1A activity in male Wistar albino rats. In our study, CYP1A activity was not influenced by phenotype; however, leptin treatment, lowering insulin levels in ob/ob mice, caused a decrease in CYP1A activity. These results suggest that insulin, in addition to other factors, may influence the activity of this isoform.

In addition to insulin’s effects on CYP1A, it has also been shown to affect CYP2E1. A study by De Waizier et al. (1995) showed down-regulation of CYP2E1 expression by insulin at the post-transcriptional level in a rat hepatoma cell line. Additionally, a study by Woodcroft and Novak (1997) also showed decreased expression of CYP2E1 in primary rat hepatocytes following insulin treatment. A study by Roe et al. (1999) showed increased CYP2E1 activity in diabetic ob/ob mice, which is in contrast to results seen in this study in which the ob/ob mice were not diabetic. These results support insulin’s role in the decreased expression of the CYP2E1 isoform because insulin levels were elevated in ob/ob mice. Additionally, it is known that the hyperglycemic state (Yamazoe et al., 1989), high-energy diets (Raucy et al., 1990) in obese rodents, and human obesity (O’Shea et al., 1994) cause an increase in the expression of the CYP2E1 isoform. This has been attributed to the presence of increased levels of ketone bodies secondary to the hyperglycemic state. Although the levels of ketone bodies were not measured, it is assumed that the ob/ob mice were not ketotic because they were not hyperglycemic; therefore, the ketogenic state of the mice may not have played as great a role in altering CYP2E1 activity. Leptin administration decreased insulin levels in the ob/ob mice, which resulted in an expected increase in CYP2E1 activity. These results suggest that insulin and not the ketogenic state of the ob/ob mice played a greater role in the observed CYP2E1 alterations in this study.

7α-Hydroxylation of testosterone (reflective of CYP2A activity), was elevated 2-fold in the ob/ob mouse, which is in agreement with the work of Thummel and Schenken (1990) in streptozotocin-induced diabetic rats. In Thummel’s study, GH administration did not affect CYP2A activity but testosterone replacement reversed the changes seen in this protein. In our study, leptin administration corrected the 2-fold increase in 7α-hydroxylation of testosterone in ob/ob mice. It is possible that leptin caused an increase in the secretion of testosterone in the sterile ob/ob male mouse, which could have affected the expression of CYP2A activity.

The ob/ob mouse model is characterized by a reduced level of GH and this reduction in GH has been implicated in the changes seen with several of the CYP isoforms. Waxman et al. (1995) showed that the CYP genes CYP2C11 and CYP2C12 are dependent on GH and that GH can activate several STAT proteins including 1, 3, and 5 (mainly 5) in the rat liver (Ram et al., 1996). These STAT-dependent signaling pathways can target distinct genes and contribute to the diverse effect of GH. GH has been shown to cause alterations in both the CYP2B and CYP3A isoforms. In our study, there was a 2-fold greater activity of CYP2B in ob/ob mice as evidenced by the dealkylation of pentoxyresorufin. Yamazoe et al. (1989) suggest that lower GH levels may trigger the synthesis of CYP2 family of proteins, which is supportive of the results seen in our study with ob/ob mice. However, following leptin administration, we observed a substantial decrease in pentoxyresorufin activity to below control level, indicating a reduction in CYP2B expression, possibly via increased GH levels (Carro et al., 1997).

Subramanian et al. (1998) suggest that GH mediates the up-regulation of CYP3A transcription by a STAT factor, which is not consistent with our results. In our study, the ob/ob mice, characterized by lower GH levels, exhibited a trend toward greater 6β-testosterone hydroxylase activity (reflective of CYP3A activity). Irizar et al. (1995) and Barnett et al. (1992a) showed greater CYP3A activity in obese Zucker rats and ob/ob mice, respectively. ob/ob mice, following leptin treatment, showed an increase in CYP3A activity (6β-testosterone hydroxylation), which may be a result of increased GH levels in treated ob/ob mice. Increased GH levels may have caused activation of the STAT protein necessary for transcription of CYP3A protein, resulting in higher activity. The different patterns of activity for these isoforms suggest that they may be regulated by different STAT proteins.

CYP4A activity was not altered significantly by phenotype but was changed in the lean mice following leptin exposure but not in the obese animals. The induction of CYP4A expression has been shown to be a result of transcriptional activation, mediated possibly by a peroxisome proliferator activated receptor (PPAR; Simpson, 1997). The ob/ob mouse has elevated fatty acid levels (Enser and Ashwell, 1983), which has been suggested to be an activator of PPAR. Studies by Robertson et al. (1999) showed that CYP4A10 mRNA was expressed at higher levels and CYP4A1 was lower in the ob/ob mice. They also showed that in obese Zucker rats, CYP4A1 was unchanged, CYP4A2 was elevated, and CYP4A3 was decreased when compared with controls. In our study, the trend toward higher levels of CYP4A activity in the ob/ob mouse may be a result of increased transcription of this protein via fatty acids. Leptin treatment has been shown to decrease triglyceride content in pancreatic islet from Wistar rats in the presence of free fatty acids (Shimabukuro et al., 1997), which suggests a decrease in transcription rate of CYP4A. However, in our study, there was an increase in CYP4A activity in the lean mice following leptin exposure and no change in the ob/ob mice. This suggests that, in addition to the activation of PPAR, other factors may influence the regulation of the CYP4A gene.

With obesity comes changes in the CYP-mediated reactions as well as conjugative enzymes such as GST that detoxify reactive substances produced by CYPs. In our study, GST activity in ob/ob mice was not significantly different when compared with lean controls. This is in contrast to studies by Barnett et al. (1992a) who demonstrated a 75% decrease in GST activity. This discordance may be explained by the lower glucose levels in our study compared with those obtained by Barnett and coworkers, who implicated hyperglycemia in the down-regulation of GST.

Changes in the antioxidant defense mechanisms, which detoxify reactive oxygen species, were also evaluated in the ob/ob mice. In our study, similar decreases were seen in both GSHPx and catalase activities in the ob/ob mice when compared with the lean mice. These results are consistent with results reported by Capel and Dorrell (1984). They suggest in their study that the decrease in GSHPx activity in ob/ob mice may be caused by a deficiency in selenium, which is needed for GSHPx when using H2O2 as the substrate. It has also been suggested that increased β-oxidation in the peroxisomes causes elevated peroxides (Murphy et al., 1979), which could also result in decreases in these antioxidant enzymes. Some researchers suggest that decreases in these antioxidant enzymes may be attributed to an increase in CYP-mediated reactions, namely CYP2E1 (Lieber, 1997). However, in our study CYP2E1 activity was reduced in the ob/ob mouse and therefore, may not have contributed substantially to the observed decrease in these enzymes. It has been shown that hyperinsulinemia also may play a role in intracellular H2O2 generation (Mukherjee and Lynn, 1977). It is possible that the increased level of insulin in the ob/ob mouse may have contributed to the
increased production of H₂O₂ and therefore, a reduction in these antioxidant enzyme activities. Leptin administration caused both catalase and GSH-Px activities in ob/ob mice to normalize (lean control levels) but had no effect on activities in lean mice. Leptin administration causes a decrease in body weight by an increase in fat oxidation resulting in the loss of body fat (Pellemounter et al., 1995). This loss in body fat could potentially reverse the above-mentioned reactions occurring in the ob/ob mice, causing these activities to return to normal. The normalization of insulin levels by leptin may have also played a role in returning the antioxidant enzymes to normal levels.

Total GSH levels and GSHRed activity were monitored in lean and ob/ob mice following leptin exposure. Total GSH levels were not significantly altered with phenotype or with leptin administration; however, the GSHRed activities were affected by both parameters. The lower level of GSHRed activity found in the ob/ob mouse in our study is supported by Capel and Dorrell (1984). It is possible that reduced GSHRed reflects lower levels of reduced GSH, which are needed for protection against reactive oxygen compounds.

In conclusion, the present study demonstrated that obesity does play a role in altering some of the CYP-mediated reactions as well as antioxidant enzymes in the ob/ob mouse that can be corrected by leptin replacement. However, it is not known whether these changes occur as a result of leptin’s effect on weight reduction, hormonal changes, changes in the amount of nutrients as a result of decreased food intake, or by activation of secondary messenger systems and transcription factors. Therefore, further in vitro studies are necessary to determine whether leptin treatment alterations occur as a result of a direct second messenger event or whether the alterations are due to indirect changes in insulin, cortisoli, and/or GH.

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