Chronic Ethanol Consumption Lessens the Gain of Body Weight, Liver Triglycerides, and Diabetes in Obese ob/ob Mice

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ABSTRACT

Clinical studies suggest that moderate alcohol consumption can have beneficial effects, in particular regarding cardiovascular events, insulin resistance, and type 2 diabetes. In this study, lean and obese diabetic ob/ob mice were submitted or not to chronic ethanol intake via the drinking water for 6 months, which was associated with moderate levels of plasma ethanol. Plasma levels of alanine aminotransferase and aspartate aminotransferase were not increased by alcohol intake. Ethanol consumption progressively reduced the gain of body weight in ob/ob mice, but not in lean mice, and this was observed despite higher calorie intake. Increased plasma free fatty acids and glycerol in ethanol-treated ob/ob mice suggested peripheral lipolysis. Glycemia and insulinemia were significantly reduced, whereas adiponectinemia was increased in ethanol-treated ob/ob mice. Liver weight and triglycerides were significantly decreased in ethanol-treated ob/ob mice, and this was associated with less microvesicular steatosis. Hepatic levels of AMP-activated protein kinase and the phosphorylated form of acetyl-CoA carboxylase were higher in ethanol-treated ob/ob mice, suggesting better fatty acid oxidation. However, hepatic mRNA expression of several lipogenic genes was not reduced by ethanol consumption. Finally, mild oxidative stress was noticed in the liver of ethanol-treated mice, regardless of their genotype. Hence, our data are in keeping with clinical studies suggesting that moderate ethanol intake can have beneficial effects on type 2 diabetes and insulin sensitivity, at least in part through increased levels of plasma adiponectin. However, further studies are needed to determine whether long-term drinking of light-to-moderate amounts of ethanol is safe for the liver.

Heavy alcohol intake is associated with increased mortality, liver diseases such as steatohepatitis and cirrhosis, pancreatitis, hypertension, cardiomyopathy, and cerebrovascular events (Robin et al., 2005; Kloner and Rezkalla, 2007). However, although alcohol abuse is undoubtedly detrimental to human health, there is evidence that light-to-moderate alcohol consumption may have some beneficial effects, in particular regarding cardiovascular events, insulin resistance, and type 2 diabetes (Howard et al., 2004; Ting and Lautt, 2006; Kloner and Rezkalla, 2007). In addition, a recent study suggests that modest wine drinking could significantly reduce the prevalence of nonalcoholic fatty liver disease, which is closely related to obesity and metabolic syndrome (Dunn et al., 2008). Regarding ethanol consumption and diabetes, epidemiological data showed a U-shaped relationship between alcohol amount and the risk of type 2 diabetes. Indeed, whereas low-to-moderate alcohol intake decreases the risk of type 2 diabetes, abstinence and heavy alcohol drinking seem to be associated with an increased risk (Howard et al., 2004; Koppes et al., 2005). It is interesting...
that moderate alcohol intake could lower the risk of type 2 diabetes through an improvement of insulin sensitivity (Howard et al., 2004; Sierksma et al., 2004; Ting and Lautt, 2006). The mechanisms whereby moderate alcohol consumption ameliorates insulin sensitivity are currently poorly understood, but some studies pointed to a reduction of body weight (in particular in women) and increased adiponectin levels (Sierksma et al., 2004; Ting and Lautt, 2006; Imhof et al., 2009).

In a previous study, we showed that four repeated ethanol binges via gastric intubation (2.5 g/kg) induced hepatic apoptosis in obese ob/ob mice but not in lean animals (Robin et al., 2005). It is important that plasma levels of ethanol were approximately 1.2 g/l in the obese animals (Robin et al., 2005). In the present study, we used the same murine model of obesity to determine the hepatic effects of a chronic consumption of ethanol via the drinking water. It is interesting to understand, but some studies pointed to a reduction of body weight (in particular in women) and increased adiponectin levels (Sierksma et al., 2004; Ting and Lautt, 2006; Imhof et al., 2009).

Materials and Methods

Animals and Ethanol Administration. Sixteen 7-week-old male C57BL/6J-ob/ob mice (henceforth referred to as obese mice), weighing 35 to 43 g, and 16 C57BL/6J/+/+ mice (wild-type, referred to as lean mice), weighing 21 to 25 g, were purchased from Janvier (Le-Genest-St-Isle, France). Mice were accommodated in an animal care facility accredited by the French veterinary authorities. Mice were fed ad libitum on a normal diet bringing 2820 kcal/kg food (A04 biscuits; UAR, Villemoisson-sur-Orge, France). After 1 week of acclimatization, the groups of lean and ob/ob mice were further split into two subgroups of eight animals (equally distributed into two cages) that subsequently drank water or an ethanol solution for 6 months. The daily ethanol consumption was 3 g/kg body weight for the first 10 days. Thereafter, the daily intake of ethanol was regularly incremented by 1 g/kg to finally reach 21 g/kg after 6 months. To achieve this progressive augmentation, we adjusted the percentage of ethanol in the drinking water every 10 days, taking into account the body weight and the liquid intake of each mouse. Indeed, consumption of food and liquid was evaluated twice a week, whereas body weight was measured every week. It is important that the liquid intake remained stable during the experiment in lean mice (between 5 and 6 ml/day) but not in obese mice (from 9 ml/day at the beginning to 4 ml/day at the end of the treatment). Thus, whereas the percentage of ethanol in the drinking water was 1.5% for both genotypes at the beginning of the treatment (corresponding to 3 g/kg), it was 15 and 30% for lean and obese mice, respectively, to reach 21 g/kg in both genotypes. At the end of the treatment, all mice were sacrificed by cervical dislocation. This sacrifice was performed in the morning (approximately 10:00 AM), hence, in the postabsorptive state because food intake occurs essentially during nighttime in mice. The liver was quickly removed and immediately frozen in liquid nitrogen. All experiments were performed according to national guidelines for the use of animals in biomedical research, with the approval of the French National Medical Research Institute (Institut National de la Santé et de la Recherche Médicale).

Plasma Studies. Blood was collected from the retro-orbital sinus each month to monitor alanine aminotransferase (ALT) activity and just before the sacrifice for the measurement of a larger set of plasma parameters. A sample of blood was also taken 1 day before the initiation of the treatment to measure plasma glucose, insulin, and adiponectin. Blood was always collected in the postabsorptive state. Blood ethanol concentration was assessed using the NAD-ADH reagent kit from Sigma-Aldrich (St. Louis, MO). Plasma ALT and aspartate aminotransferase (AST) activities, iron, total cholesterol, triglycerides, glucose, nonesterified fatty acids (NEFAs), glycerol, β-hydroxybutyrate, and total antioxidant status (expressed as Trealox equivalents) were measured on a simultaneous analyzer AU400 (Olympus Diagnostics, Rungis, France). Plasma ALT, AST, iron, total cholesterol, triglycerides, and glucose were measured with the Olympus commercial kits OSR6107, OSR6109, OSR6186, OSR6116, OSR6133, and OSR6121, respectively. NEFAs, glycerol, β-hydroxybutyrate, and total antioxidant status were measured with kits FA115, GY105, RB1007, and NX2332, respectively, purchased from RANDOX Laboratories (Montpellier, France). Plasma tumor necrosis factor (TNF)-α was assessed with the KRC 3012 ELISA kit (Tago BioSource International, Camarillo, CA). Insulin was determined with the INSKR020 ELISA kit from Crystal Chem (Downers Grove, IL), and adiponectin was determined with the MRP300 mouse ELISA kit from R&D Systems (Lille, France).

Liver Histology, Total Lipids, and Triglycerides. Liver specimens were fixed with 10% neutral formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Inflammation (polymorphonuclear cell infiltration) was assessed on 10 different fields at 200× magnification. Steatosis was evaluated as the percentage of hepatocytes containing vacuoles of fat on 10 different fields at 200× magnification. The total surface of the vacuoles also was measured with an image analyzer (ImageJ; National Institutes of Health, Bethesda, MD) in five different fields by slide.

Total lipids in liver were measured gravimetrically as described previously (Robin et al., 2005). Lipids were subsequently resuspended in isopropanol (approximately 10 mg/ml). After removal of phospholipids with aluminum hydroxide hydrate, triglycerides were determined colorimetrically using the Nash’s reagent (containing acetylacetone, ammonium acetate, and isopropanol) and peroxidase (Robin et al., 2005).

Hepatic Glutathione and Enzyme Activities. Reduced glutathione levels and the activities of glutathione transferase, glutathione peroxidase, and glutathione reductase were assessed in liver homogenates, as reported previously (Robin et al., 2005). The activity of aconitase, an enzyme highly sensitive to oxidative damage and located both in cytosol and mitochondria (Missirlis et al., 2003), was assessed as described previously (Begriche et al., 2008a). CYP2E1 activity was determined by the hydroxylation of aniline into p-aminophenol (Robin et al., 2005). To assess caspase activities, livers were homogenized in 1 mM EDTA, 5 mM dithiothreitol, 50 mM HEPES, 0.1% 3-(3-cholamidopropyl)dimethylammonio-1-propane-sulfonate, 4 mg/ml leupeptin, and 4 mg/ml pepstatin, pH 7.4. The supernatant was recovered after centrifugation at 14,000 g and 4°C for 10 min. Caspase-2, -8, -9, and -3 activities were measured with the fluorescent ApoAlert Caspase Assay plate (Clontech, Mountain View, CA) following the manufacturer’s instructions.

RNA Isolation and Gene Expression. Total hepatic RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction. RNA integrity was assessed with the RNA 6000 nano LabChip kit (Agilent, Waldbronn, Germany). cDNAs were prepared by reverse transcription of 2 μg of total RNA using the Moloney murine leukemia virus enzyme from Invitrogen (Cergy-Pontoise, France), as described previously (Begriche et al., 2008a). cDNAs were thus amplified with specific primers using the Master SYBR Green mix (Sigma-Aldrich), in a Chromo IV light cycler apparatus (Bio-Rad Laboratories, Marnes-La-Coquette, France). The PCR conditions were one cycle at 94°C for 3 min, followed by 40 cycles at 94°C for 30 s and 60°C for 1 min. Amplification of specific transcripts was confirmed by melting curve profiles generated at the end of each run. Moreover, PCR specificity was further ascertained with an agarose gel electrophoresis by checking the length of the PCR products. Expression of the mouse ribosomal protein

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S6 (S6) was used as reference, and the 2^-ΔΔCT method was used to express the relative expression of each selected gene. Sequences of primers used for real-time quantitative PCR have been reported recently for sterol response element-binding protein (SREBP)-1c, peroxisome proliferator-activated receptor (PPAR)-α, stearoyl-CoA desaturase (SCD)-1, fatty acid synthase (FAS), ACC-1, phosphoenolpyruvate carboxikinase (PEPCK), liver-type pyruvate kinase (L-PK), medium-chain acyl-CoA dehydrogenase (MCAD), and S6 (Begriche et al., 2008a). Sequences of primers for carbohydrate response element binding protein (ChREBP), glucose-6-phosphatase (G6Pase), and insulin-like growth factor-binding protein (IGF)-1 were 5'-CTGGGGACCTAAACAGGAGC-3' (forward) and 5'-GAAGCCACCTATAGCTCCC-3' (reverse), 5'-CACACACCT-TCTCTATCAC-3' (forward) and 5'-GGTGGCTTACAGCAGACAG-3' (reverse), and 5'-AAATGGAAGAGCCTGGG-3' (forward) and 5'-CCATTCTGTGGCGAGTGGGC-3' (reverse), respectively.

Western Blot Analyses. Liver homogenates were prepared from 100 μg of frozen fragments in a sucrose/Tris-HCl buffer containing 0.1% SDS, 1% Nonidet P40, and protease inhibitors. After protein determination, homogenates underwent 8% SDS-polyacrylamide electrophoresis for phospho-ACC/ACC and FAS, 10% for phospho-AMPKα/AMPKα, 12% for cytochrome P450 E1 (CYP2E1), and 14% for SCD1 and manganese superoxide dismutase (MnSOD). Immunoblotting was thus carried out with rabbit polyclonal antibodies against CYP2E1 (Oxford Biomedical Research, Oxford, MI), MnSOD (Assay Designs, Ann Arbor, MI), phospho-AMPKα (Thr172), AMPKα, SCD1 (Cell Signaling Technology Inc., Danvers, MA), phospho-ACC (Ser79), and ACC (Millipore, Billerica, MA), FAS (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Blots were incubated with appropriate secondary antibodies, and protein bands were revealed by enhanced chemiluminescence (GE Healthcare, Orsay, France). To normalize protein loadings, strips were blotted and incubated with antibodies against β-actin (Sigma-Aldrich).

Statistical Analyses. Data are presented as means ± S.E.M. When four groups were compared, two-way analysis of variance (ANOVA) with the factors of genotype (+/− or ob/ob) and ethanol consumption (absent or present) was performed to assess statistical significances. When the ANOVA indicated a significant interaction between factors, individual means were compared with least significant differences (LSD) post hoc test. In experiments with only two sets of data, the Student’s t test was used.

Results

Regular Monitoring of Plasma ALT, Body Weight, and Energy Intake. Chronic ethanol administration was well tolerated by lean and obese mice. In contrast, two naive ob/ob mice died during the experiment after 10 and 22 weeks, respectively. Unfortunately, an autopsy was not performed on these obese animals.

Plasma ALT activity was measured each month as an index of hepatic cytolysis. Plasma ALT was higher in obese mice compared with their lean counterparts (Fig. 1A), reflecting liver damage as reported previously (Robin et al., 2005; Begriche et al., 2008b). However, ethanol administration did not change ALT values in both groups of mice (Fig. 1A).

Body weight was also monitored throughout the 6-month period of treatment. Ethanol consumption did not change body weight in lean mice (Fig. 1B). Indeed, after 6 months body weight gain was 9.9 ± 0.70 and 9.3 ± 1.2 g, respectively, in naive and ethanol-treated mice. In contrast, ethanol intake significantly decreased by 43% of the gain of body weight in obese mice as it was 26.2 ± 0.8 and 14.8 ± 0.5 g, respectively, in naive, and ethanol-treated ob/ob mice. Hence, at the end of the treatment, naive and ethanol-treated ob/ob mice weighted 65.5 ± 1.3 and 52.8 ± 1.0 g, respectively (Fig. 1B).

The significant reduction of body weight gain in ethanol-treated obese mice was already observable after 6 weeks (Fig. 1B).

Ethanol consumption involves a significant amount of calories (7 kcal/g) that must be taken into account in the daily energy intake. Hence, the energy intake was monitored through the estimation of food consumption for all mice and liquid intake for ethanol-treated animals. During the 6-month protocol, ethanol consumption was responsible for a reduced intake of food-derived calories in both lean and ob/ob mice, although this reduction was stronger in the obese animals during the last 2 months (Fig. 1C). However, when calories from ethanol were considered, the energy intake was higher in ethanol-treated animals, although this was significant in lean mice only for the last 2 months (Fig. 1C). Thus, lower body weight gain in ethanol-treated obese mice occurred despite higher energy intake throughout the treatment.

Initial and Final Plasma Levels of Glucose, Insulin, and Adiponectin. Three plasma parameters (namely, glucose, insulin, and adiponectin) were measured both before the initiation of ethanol administration and at the end of the treatment. The initial levels of plasma glucose were significantly augmented in ob/ob mice compared with lean animals, confirming the presence of type 2 diabetes (Fig. 2). However, plasma levels of glucose after 6 months were lower in naive obese mice, indicating an amelioration of the diabetic state, as described previously (Dubuc et al., 1982; Lindström, 2007). In contrast, plasma levels of glucose after 6 months were increased in the naive lean mice compared with the initial levels (Fig. 2A). Consequently, plasma glucose after 6 months was slightly but significantly lower in ob/ob mice compared with their lean counterparts (Fig. 2A). It id interesting that ethanol administration reduced plasma glucose in both genotypes, although the reduction was more pronounced in the obese animals (Fig. 2A).

Initial plasma levels of insulin were dramatically increased in ob/ob mice, reflecting severe insulin resistance. After 6 months, plasma insulin was less elevated in ob/ob mice, but it was considerably reduced in ethanol-treated obese mice (Fig. 2B). We also measured plasma adiponectin because this adipokine plays a major role in glucose homeostasis in particular by improving insulin sensitivity (Begriche et al., 2006; Kadowaki et al., 2006). Initial plasma levels of adiponectin were reduced in ob/ob mice compared with the lean animals (Fig. 2C), although the difference was not significant (P = 0.08). However, ob/ob mice after 6 months presented higher plasma adiponectin than lean mice (Fig. 2C), as reported in some studies (Ding et al., 2006; Itoh et al., 2007). In addition, plasma adiponectin levels were increased in both lean and obese mice after 6 months, suggesting an aging-dependent effect. Ethanol-administration further augmented adiponectin levels in both lean and ob/ob mice, although this was more pronounced in obese animals (Fig. 2C). It is interesting that a regression analysis showed a significant inverse relationship between plasma levels of adiponectin and glucose when all mice (n = 26) were considered (r = 0.40; P < 0.05).

Other Plasma Parameters. A larger set of plasma parameters were further measured at the end of the treatment. It is important that ethanol concentration was not different in lean and obese mice (Table 1). Plasma β-hydroxybutyrate...
and ferritin were unchanged regardless of the groups of mice (Table 1). Plasma TNF-α levels were similar between obese and lean mice, as already previously (Faggioni et al., 2000; Romics et al., 2004; Robin et al., 2005), and ethanol administration did not modify TNF-α (Table 1). Plasma ALT, AST, total cholesterol, antioxidant status, and iron were significantly increased in obese mice compared with lean mice, but ethanol did not change these parameters (Table 1). It is important that we previously reported higher plasma antioxidant status in ob/ob mice compared with wild-type mice, which could be a compensatory adaptation to chronic oxidative stress (Robin et al., 2005). The ob/ob mice were slightly hypotriglyceridemic, as reported in several studies (Lombardo et al., 1983; Camus et al., 1988; Lindström, 2007), and ethanol did not modify triglyceride levels (Table 1). Plasma levels of NEFAs and glycerol were higher in ob/ob mice, and ethanol intake further increased both parameters in obese animals (Table 1), whereas ethanol intake only enhanced plasma glycerol in lean mice, suggesting that lipolysis (i.e., the hydrolysis of adipose triglycerides and the subsequent release of glycerol and NEFAs into the blood) could have been more active in ob/ob mice. However, further investigations will be needed to support this hypothesis. Finally, plasma insulin-like growth factor (IGF)-I was significantly enhanced in ob/ob mice compared with the lean animals, and ethanol administration significantly lowered IGF-I in both lean and obese mice (Table 1).

Liver Weight, Lipids, and Histology. Liver weight was dramatically increased in ob/ob mice compared with lean mice (Fig. 3A). It is interesting that ethanol administration was associated with a significant reduction of liver weight only in obese mice (Fig. 3A). Because body weight was also reduced by ethanol in obese mice, the liver/body weight ratio remained virtually unchanged in the obese animals (data not shown).

Next, total lipids and triglycerides were measured in
mouse liver. Hepatic lipids and triglycerides were consider-
able enhanced in ob/ob mice compared with lean mice, re-
reflecting massive steatosis (Robin et al., 2005; Ding et al.,
2006). Whereas ethanol administration slightly augmented
liver lipids and triglycerides in lean mice, it decreased sig-
nificantly liver triglycerides in ob/ob mice (Fig. 3). However,
liver lipids were not significantly reduced in ethanol-treated
ob/ob mice (Fig. 3B), possibly due to a compensatory increase
in nontriglyceride lipids.

A histological examination of the livers was subsequently
performed in 12 wild-type mice (including six naive and six
ethanol-treated animals) and 14 obese mice (including six
naive and eight ethanol-treated animals). In lean mice, eth-

![Fig. 2. Plasma glucose (A), insulin (B) and adi-
opnectin levels (C) before the onset of the ex-
periment (initial) and after 6 months of water
or ethanol drinking. For initial levels, results
are mean ± S.E.M. for 16 mice in each group (∗,
* P < 0.05, significantly different from lean mice;
Student's t test). After 6 months, results are
mean ± S.E.M. for six to eight mice in each
group. Letters above the graphs indicate an
effect of the genotype (G), ethanol treatment
(E), or an interaction between genotype and
ethanol (GxE) (* P < 0.05; two-way ANOVA). In
case of interaction, statistical significance be-
tween groups was calculated (* P < 0.05; LSD
post hoc test). ∗, different from lean mice; †,
different from naive mice.](https://jpet.aspetjournals.org/doi/10.1124/jpet.116.241242)
vacular in one mouse and both macrovacular and microvacular in the other ethanol-treated obese animals. It is important that ethanol intake did not aggravate the moderate necroinflammation observed in the obese mice (data not shown). Finally, the total area of the lipid vacuoles was shown (Fig. 6). We also measured the expression of hepatic AMPK. It is interesting that AMPK expression was significantly augmented in ob/ob mice treated with ethanol compared with the naive animals, although this was not associated with increased levels of its phosphorylated form (Fig. 6). In ethanol-treated and naive wild-type mice, hepatic AMPK levels were similar to those found in naive ob/ob mice (Fig. 6).

**Oxidative Stress and Caspase Activity in Liver.** Finally, several parameters related to hepatic oxidative stress and apoptosis were measured in lean and obese mice. Ethanol intake was associated with increased CYP2E1 protein and activity, as well as enhanced MnSOD protein (Table 2). Aconitase activity, but not reduced glutathione, was lowered by ethanol intake (Table 2). Ethanol administration also reduced the activity of glutathione peroxidase but not that of glutathione reductase and glutathione transferase (Table 2). It is interesting that both aconitase and glutathione peroxidase can be inactivated by reactive oxygen species and free radicals (Rouach et al., 1997; Missirlis et al., 2003). The activity of caspase-2, -3, -8, and -9 was furthermore determined, but no difference was found between the four groups of mice (data not shown).

**Discussion**

In this study, we showed that chronic administration of ethanol significantly reduced the gain of body weight and alleviated type 2 diabetes in obese ob/ob mice. These beneficial effects were associated with increased plasma adiponec- tin, lower liver triglycerides, and higher hepatic levels of AMPK and phospho-ACC. It is important that plasma levels

### TABLE 1

**Plasma parameters in lean and ob/ob mice after 6 months of water or ethanol drinking.**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Lean, Ethanol</th>
<th>Obese</th>
<th>Obese, Ethanol</th>
<th>Factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (g/l)</td>
<td>N.D.</td>
<td>0.27 ± 0.06</td>
<td>N.D.</td>
<td>0.35 ± 0.04</td>
<td>G</td>
</tr>
<tr>
<td>AST (UI/l)</td>
<td>94 ± 13</td>
<td>115 ± 15</td>
<td>234 ± 51</td>
<td>291 ± 33</td>
<td>G</td>
</tr>
<tr>
<td>ALT (UI/l)</td>
<td>40 ± 8</td>
<td>39 ± 8</td>
<td>280 ± 24</td>
<td>303 ± 46</td>
<td>G</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>11.1 ± 0.8</td>
<td>10.0 ± 0.9</td>
<td>9.6 ± 0.7</td>
<td>9.7 ± 0.7</td>
<td>G</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>1.81 ± 0.18</td>
<td>2.18 ± 0.08</td>
<td>5.79 ± 0.50</td>
<td>6.28 ± 0.49</td>
<td>G</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>1.07 ± 0.11</td>
<td>1.12 ± 0.03</td>
<td>0.90 ± 0.06</td>
<td>0.87 ± 0.05</td>
<td>G</td>
</tr>
<tr>
<td>NEFAs (mM)</td>
<td>0.39 ± 0.05</td>
<td>0.36 ± 0.04</td>
<td>0.45 ± 0.01</td>
<td>0.71 ± 0.08*</td>
<td>G, E, GxE</td>
</tr>
<tr>
<td>Glyceral (μM)</td>
<td>546 ± 33</td>
<td>715 ± 69</td>
<td>690 ± 71</td>
<td>841 ± 49</td>
<td>G, E</td>
</tr>
<tr>
<td>β-Hydroxybutyrate (mM)</td>
<td>0.14 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>G</td>
</tr>
<tr>
<td>Antioxidant status (mM)</td>
<td>0.93 ± 0.07</td>
<td>0.85 ± 0.08</td>
<td>1.36 ± 0.20</td>
<td>1.37 ± 0.16</td>
<td>G</td>
</tr>
<tr>
<td>Iron (μM)</td>
<td>21.2 ± 1.7</td>
<td>25.7 ± 1.2</td>
<td>33.6 ± 5.8</td>
<td>31.7 ± 2.5</td>
<td>G</td>
</tr>
<tr>
<td>Ferrititin (ng/ml)</td>
<td>41.2 ± 2.6</td>
<td>39.8 ± 2.4</td>
<td>43.0 ± 6.6</td>
<td>40.4 ± 3.3</td>
<td>G</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>294 ± 28</td>
<td>213 ± 25*</td>
<td>395 ± 51*</td>
<td>167 ± 18*</td>
<td>E, GxE</td>
</tr>
</tbody>
</table>

N.D., not determined.

* G, effect of genotype; E, effect of ethanol administration; GxE, interaction between genotype and ethanol administration. In case of interaction, symbols indicate statistical differences between groups.

† Significant difference from lean mice (P < 0.05).

* Significant difference from naive mice (P < 0.05).
of ethanol were moderate in our murine model of chronic alcohol intake.

Although heavy alcohol intake is detrimental for human health (Robin et al., 2005; Kloner and Rezkalla, 2007), there is increasing evidence that light-to-moderate alcohol consumption could have beneficial effects, in particular regarding insulin resistance and type 2 diabetes (Howard et al., 2004; Ting and Lautt, 2006; Kloner and Rezkalla, 2007), and possibly nonalcoholic steatohepatitis as well (Dunn et al., 2008). The mechanisms whereby moderate alcohol drinking could afford these favorable effects are still hypothetical but could involve an augmentation of adiponectin levels and a reduction of body weight, at least in some individuals (Sierksma et al., 2004; Ting and Lautt, 2006; Imhof et al., 2009). A beneficial effect of ethanol consumption on plasma adiponectin levels is an attractive hypothesis because this adipokine is known to improve insulin sensitivity and fatty acid oxidation in different tissues (e.g., skeletal muscle and liver), at least in part through an AMPK-dependent pathway (Begriche et al., 2006, 2009; Kadowaki et al., 2006). Thus, our murine model could recapitulate some of the favorable metabolic effects that have been observed in individuals drinking reasonable amounts of alcohol.

In this study, ethanol intake lowered the gain of body weight in obese mice, but not in their lean counterparts, although the total energy intake was enhanced in both groups of animals. The mechanism whereby ethanol reduced the gain of body weight in ob/ob mice was not investigated.
However, our data suggested lipolysis in ethanol-treated obese mice as reflected by increased plasma glycerol and NEFAs. It is interesting that a recent study in rats indicated that chronic ethanol intake enhanced triglyceride degradation in adipose tissue through a reduction of the antilipolytic action of insulin (Kang et al., 2007). Alternatively, other mechanisms could have contributed to lower body weight gain in obese mice. Indeed, several studies suggest that ethanol calories are less efficiently used than calories derived from other nutrients and that ethanol consumption increases resting energy expenditure and thermogenesis and favors lipid use (Klesges et al., 1994; Addolorato et al., 1998). Finally, the significant reduction of plasma insulin and glucose could have significantly reduced de novo lipogenesis, in particular in the adipose tissue (Wang et al., 2004; Begriche et al., 2006).

Reduction of body weight is generally associated with improved insulin sensitivity and increased plasma levels of adiponectin (Takahashi et al., 2004; Bruun et al., 2006). Hence, reduced plasma insulin and glucose (suggesting better insulin sensitivity) and higher adiponectin in ethanol-treated ob/ob mice could be an indirect consequence of lower body weight gain. However, ethanol administration induced a significant augmentation of adiponectin in lean mice, although they did not lose body weight. Thus, the amelioration of type 2 diabetes in ethanol-treated ob/ob mice could be due to the concomitant action of reduced body weight gain and enhanced plasma levels of adiponectin. It is interesting that, several in vitro and in vivo investigations showed that ethanol can augment adiponectin expression and secretion in adipocytes, as well as its plasma levels (Wandler et al., 2008; Buechler et al., 2009; Imhof et al., 2009). Furthermore, amelioration of insulin sensitivity and reduced risk of type 2 diabetes in individuals drinking moderate amounts of alcohol were associated with increased adiponectin levels (Sierksma et al., 2004; Englund Ogge et al., 2006; Ting and Lautt, 2006; Beulens et al., 2008). Thus, one important mechanism whereby moderate ethanol consumption ameliorates insulin sensitivity could be through increased adiponectin secretion and plasma levels.

Plasma IGF-I levels were augmented in obese mice compared with lean animals. Increased circulating IGF-I could be an adaptive mechanism in the context of insulin resistance because this hormone favors insulin sensitivity (Ezzat et al., 2008). It is interesting that ethanol administration dramatically reduced plasma IGF-I in obese mice and to a lesser extent in lean mice. Thus, lower IGF-I levels in ethanol-treated ob/ob mice could be, at least in part, the consequence of better insulin sensitivity in these animals.

Adiponectin is able to induce AMPK activation in liver, which in turn decreases gluconeogenesis and increases fatty acid oxidation through ACC phosphorylation (Begriche et al., 2006; Kadowaki et al., 2006). Indeed, phosphorylation of ACC leads to the inactivation of its activity, reducing hepatic levels of malonyl-CoA, the only endogenous inhibitor of CPT-1 (Begriche et al., 2008b, 2009). In this study, hepatic phospho-ACC was significantly augmented in ethanol-treated ob/ob mice, and this was associ-
Fig. 5. Hepatic mRNA expression in lean and obese mice after 6 months of water or ethanol drinking. The mRNA expression of several transcription factors (SREBP1c, ChREBP, and PPARα), lipogenic enzymes (ACC1, FAS, and SCD1), gluconeogenetic (G6Pase and PEPCK) and glycolytic (L-PK) enzymes, fatty acid oxidation enzymes (MCAD and CPT1), and IGFBP-1 was assessed by quantitative PCR. Expression of S6 was used to normalize mRNA levels of the different genes. Results are mean ± S.E.M. for six to eight mice in each group. Letters above the graphs indicate an effect of the genotype (G), ethanol treatment (E), or an interaction between genotype and ethanol (GxE) (P < 0.05; two-way ANOVA). In case of interaction, statistical significance between groups was calculated (P < 0.05; LSD post hoc test). *, different from lean mice; †, different from naive mice.
Fig. 6. Protein expression of lipogenic enzymes and AMPK in liver homogenates from naive and ethanol-treated lean and ob/ob mice. Liver expression of SCD1, FAS, total ACC, the phosphorylated form of ACC (P-ACC), total AMPKα and the phosphorylated form of AMPKα (P-AMPKα) were assessed by Western blot analysis. β-Actin was used as a loading control. Data showed in the graphs are mean ± S.E.M. for six to eight mice in each group. Relative protein expressions are represented in percentages of naive lean animals. Letters above the graphs indicate an effect of the genotype (G), ethanol treatment (E), or an interaction between genotype and ethanol (GxE) (P < 0.05; two-way ANOVA). In case of interaction, statistical significance between groups was calculated (P < 0.05; LSD post hoc test). *, different from lean mice; †, different from naive mice.
ated with enhanced total AMPK levels. These data suggested higher hepatic fatty acid oxidation, although plasma β-hydroxybutyrate measured in the postabsorptive state was unchanged. Lower insulin and glucose levels and inactivation of hepatic ACC could also have contributed to decrease liver triglycerides through reduced de novo lipogenesis.

It is interesting that the reduction of liver triglycerides in ethanol-treated ob/ob mice was associated with a change in the morphology of steatosis. Indeed, none of the ethanol-treated ob/ob mice presented microvesicular steatosis, whereas this liver lesion was predominant in half of the naive obese mice. It is noteworthy that microvesicular steatosis reflects mitochondrial dysfunction (Fromenty and Pessayre, 1995) and that ob/ob mice present some mitochondrial alterations (Melia et al., 1999; García-Ruiz et al., 2006), although different studies have shown several metabolic and mitochondrial adaptations in ob/ob liver such as increased PPARα expression, cytochrome c, and mitochondrial DNA levels (Robin et al., 2005; Begriche et al., 2008b, 2009). Thus, the lack of microvesicular steatosis in ethanol-treated ob/ob mice could reflect improved mitochondrial function although further investigations will be necessary to confirm this hypothesis.

Oxidative stress is a major mechanism whereby ethanol abuse is able to damage the liver and other tissues (Mansouri et al., 2001; Hoek and Pastorino, 2002). However, despite the presence of some oxidative stress in ethanol-treated mice, there was no evidence of liver injury as reflected by unchanged plasma ALT and AST. Enhanced hepatic mRNA levels of IGFBP-1 in ethanol-treated mice could have involved c-Jun NH2-terminal kinase activation or metabolic stresses (Magne et al., 2007). Hence, further investigations are needed to determine whether the maintenance of moderate ethanol consumption over extensive periods cannot eventually damage the liver despite an amelioration of glucose homeostasis.

### References


Lindström P (2007) The physiology of obesity-hyperglycemic mice (ob/ob mice). *Scien-


Lombardo YB, Chicco A, Mochiutti N, de Rodi MA, Nusimovich B, and Gutman R

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Lean, Ethanol</th>
<th>Obese</th>
<th>Obese, Ethanol</th>
<th>Factor&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2E1 expression (% of lean, untreated)</td>
<td>100.0 ± 6.9</td>
<td>155.6 ± 9.1</td>
<td>103.8 ± 11.5</td>
<td>146.3 ± 3.8</td>
<td>E</td>
</tr>
<tr>
<td>Aniline hydroxylase activity (pmol/min/mg protein)</td>
<td>0.57 ± 0.05</td>
<td>0.81 ± 0.04</td>
<td>0.74 ± 0.11</td>
<td>0.97 ± 0.07</td>
<td>E</td>
</tr>
<tr>
<td>MnSOD expression (% of lean, untreated)</td>
<td>100.0 ± 6.0</td>
<td>175.8 ± 8.1</td>
<td>120.5 ± 4.7</td>
<td>160.0 ± 7.7</td>
<td>E</td>
</tr>
<tr>
<td>Aconitase (mU/mg protein)</td>
<td>8.55 ± 0.42</td>
<td>7.82 ± 0.56</td>
<td>7.2 ± 0.68</td>
<td>4.90 ± 0.17</td>
<td>E</td>
</tr>
<tr>
<td>Glutathione (nmol/mg protein)</td>
<td>57.3 ± 6.9</td>
<td>41.3 ± 3.4</td>
<td>78.3 ± 4.9</td>
<td>74.5 ± 4.5</td>
<td>G</td>
</tr>
<tr>
<td>Glutathione peroxidase activity (pmol/min/mg protein)</td>
<td>320 ± 16</td>
<td>297 ± 13</td>
<td>301 ± 13</td>
<td>215 ± 12</td>
<td>G,E</td>
</tr>
<tr>
<td>Glutathione reductase activity (pmol/min/mg protein)</td>
<td>57.1 ± 4.4</td>
<td>54.8 ± 2.6</td>
<td>59.3 ± 2.0</td>
<td>54.8 ± 2.0</td>
<td></td>
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<tr>
<td>Glutathione transferase activity (pmol/min/mg protein)</td>
<td>1.74 ± 0.12</td>
<td>1.50 ± 0.09</td>
<td>1.62 ± 0.14</td>
<td>1.37 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

* G, effect of genotype; E, effect of ethanol administration.
Effect of sucrose diet on insulin secretion in vivo and in vitro and on triglyceride storage and mobilisation of the heart of rats. Horm Metab Res 15:69–76.


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