Lipid Metabolism in Ethanol-Treated Rat Pups and Adults: Effects of Folic Acid

INTRODUCTION

Lipid homeostasis is altered by chronic ethanol consumption leading to the development of a fatty liver as well as lipid alterations in other organs (Carrasco et al., 2002). The plasma triglyceride (TG) levels of alcoholic patients have been correlated with alcohol consumption (Baraona and Lieber, 1998). Pennington et al. (2002) reported an increase in fasting serum TG levels in rats whose mothers consumed ethanol. Hypertriglyceridaemia has been identified as an independent risk factor for cardiovascular disease (CVD) (Walldius and Jungner, 2006).

Studying ethanol-consumption-associated hyperlipidaemia is also relevant to CVD. In drinking populations, elevations in LDL cholesterol are correlated with CVD risk; however, increases in HDL cholesterol are associated with protection (Walldius and Jungner, 2006). In the adult population, alcohol consumption is independently and directly associated with circulating oxidized LDL (Schroder et al., 2006). Independently of the total cholesterol level (Chol), accumulating evidence suggests that certain lipid oxidation products, such as oxidized phospholipids (PL), may represent endogenously formed factors capable of triggering the vascular inflammation responsible for atherosclerosis (Walldius and Jungner, 2005; Leitinger, 2005).

Chronic alcoholism disturbs folate homeostasis (Fernandez-Borrachero et al., 1996), because it alters intestinal absorption, hepatic uptake and the renal conservation of folates, affecting the functional expression of folate transports in relevant tissue membranes: the jejunal brush border, the liver plasma membrane and the kidney brush border membrane (Hamid et al., 2007a, 2007b; Hamid and Kaur, 2007), even in suckling rats (Murillo-Fuentes et al., 2007).

Folic acid is recognized as possibly being an important element in the prevention of CVD. To date, most studies have focused on the homocysteine-lowering effect of folate; a supplementation with folic acid reduces serum homocysteine levels and in vitro LDL oxidation in patients with coronary artery disease (Bunout et al., 2000).

In endothelial cells, 5-methyltetrahydrofolate (5-MTHF), the active form of folic acid, has intrinsic antioxidant actions such as a reduction in superoxide anion production (Stroes et al., 2000) and it also reduces the human umbilical vein endothelial cells that induce LDL oxidation (Ronco et al., 2005). Ronco et al. (2007) have also shown for the first time that there is a specific mechanism between 5-MTHF and LDL that regulates endothelial function. Carnicer et al. (2007) have found that the administration of folic acid decreased atherosclerotic lesions independently of plasma homocysteine and cholesterol levels; it was associated with plasma levels of apolipoproteins A-I, A-IV and B, and decreased oxidative stress.

Folate deficiency might also contribute to atherosclerosis and vascular injury by modifying fatty acid metabolism in folic acid-deficient rats; Durand et al. (1996) have reported significant changes in the fatty acid composition.

Folic acid plays an important and recognized role in pregnant rats; alcohol consumption during pregnancy and lactation decreases milk and serum folate levels in both mothers and their pups (Garcia-Rodriguez et al., 2003; Tavares et al., 2000; Murillo-Fuentes et al., 2003). However, we did not find any studies on the effects of folic acid on lipid metabolism in adults and their offspring. Therefore, the present study was designed to study:

1. The effects of chronic alcohol consumption on serum and hepatic lipid levels, and whether administering folic acid to chronic alcohol-fed rats might contribute to preventing adverse ethanol-induced effects in the lipid metabolism of adults and pups.
2. The action of alcohol consumption and of folic acid upon cholesterol synthesis by measuring HMG-CoA reductase...
activity—the enzyme that limits Chol synthesis—and its action upon cholesterol catabolism by measuring bile acids (BA), whose task is to eliminate cholesterol.

**EXPERIMENTAL PROCEDURES**

**Animals**

Male and female Wistar rats (Centro de Producción y Experimentación Animal, Vicerrectorado de Investigación, Universidad de Sevilla), weighing ~150–200 g, were randomized into four groups: control (C), alcohol (A), alcohol + folic acid (AF) and control + folic acid (CF). Drinking water (with or without ethanol) and diet (supplemented or not) were given *ad libitum* during 8 weeks. Male (*n* = 6) and female (*n* = 6) rats were mated to obtain the first generation offspring for each group. After reproduction, the male rats were divided into four groups of six and used as adult male groups. Pregnant rats were housed individually in plastic cages, and continued with their alcoholic treatment. The day of parturition was designated as Day 1 of lactation, the number of offspring being reduced to 10 per mother at parturition, and Day 21 as the final day of the lactation period. The experiments were performed on the offspring of all four groups 21 days postpartum (CO: control group; AO: alcohol group; AFO: alcohol folic acid group and CFO: control folic group) and in the four groups of male adult rats after 8 weeks of treatment (C, A, AF, CF). Due to their hormonal disorders, the adult female rats were not considered.

The animals were kept at an automatically controlled temperature (22–23°C) and a 12-h light–dark cycle (9:00 to 21:00 hours). Animal care complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996).

**Ethanol treatment**

Ethanol treatment has been described previously by our group (Fernández-Borrachero et al., 1998). Alcohol-fed adult animals were started on tap water containing 5% v/v alcohol in the first week (initial time). The alcohol concentration was increased to 10% in the second week, 15% in the third week and 20% in the fourth week; this solution was maintained for 4 weeks. Male rats finished their treatment after reproduction. Pregnant female rats were again assigned 20% ethanol in drinking water for another 6 weeks.

**Diets**

The diets were prepared according to the Council of the Institute of Laboratory Animal Resources (ILAR, 1979) (g/kg of diet): casein, 200; glass sucrose, 510; cornstarch, 140; fibre, cellulose, 50; corn oil, 50; AIN-76 mineral mix, 35; AIN-76 vitamin mix, 10; choline bitartrate, 2; DL-methionine, 3. The diet ingredients were mixed and homogenized in a double-cone blender (Rest, Haan, Germany). The supplemented diet contained 8 ppm folic acid (Acofarma) versus the control diet which contained 2 ppm. The diets were monitored by previously weighing the amount of food and weighing it again at the end of every week.

**Ethanol and lipid intakes measurements**

The amount of ethanol consumed was estimated by subtracting the initial volume provided in the bottle at the beginning of the week, from the volume at the end of the week. We have done it during all the experimental procedure. Lipid consumption was measured by weighing the initial amount of food and weighing it again at the end of every week. Diet lipid composition is known.

**Samples**

At the end of the experimental period, the rats were fasted for 24 h and anaesthetized with intraperitoneal urethane 28% v/v (0.5 ml/100 g of body weight). The abdomen was opened by a midline incision and whole livers were removed, debrided of adipose and connective tissue in ice-cold saline and weighed. Samples were immediately stored at −80°C prior to biochemical determinations. Blood was collected by heart puncture and then centrifuged.

**Analysis**

Serum ethanol was determined by a commercial enzymatic-colorimetric test (Sigma) according to the technique described by Bucher and Redetzki (1951) with alcohol dehydrogenase (ADH), at a wavelength of 340 nm. Serum folate levels were obtained by means of a Waxman and Schreiber radio assay kit (1980). TGs were determined by an enzymatic procedure described by Young and Pestaner (1975). HDL cholesterol was determined using the method described by Grove (1979). LDL and VLDL-cholesterol was evaluated using Friedewald’s formula (Friedewald et al. 1972). The LDL/HDL ratio was calculated as a ratio between the LDL and HDL values obtained by the cited methods. Total serum and liver cholesterol were determined by an enzymatic procedure based on the oxidase/peroxidase cholesterol method with commercial kits from Attom (Allain, 1974; Svensson, 1982). Serum and liver PL were determined by an enzymatic-colorimetric technique (Trinder, 1969; Takeyama et al., 1977). Bile acid in the serum and liver

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**Figure 1.** Alcohol treatment schedule. Alcohol-fed adult animals were started on tap water containing 5% v/v alcohol in the first week, the alcohol concentration was increased to 10% in the second week, 15% in the third week and 20% in the fourth week (this solution was maintained for 4 weeks). Male rats finished their treatment after reproduction. Pregnant female rats were again assigned 20% ethanol in drinking water for another 6 weeks.
was determined by using an enzymatic procedure with commercial kits (Sigma Diagnostic) (Svensson, 1982; Roda et al., 1982).

**Preparation of hepatic microsomes**

The microsome preparation involved the livers being excised, weighed and washed in an ice-cold homogenization medium. All subsequent operations were carried out at 4°C. The livers were homogenized in a Potter-Elvehjem homogenizer with an ice-cold homogenization medium containing 0.25 M NaCl, 50 mM imidazol (pH 7.4), 20 mM ethylenediamine tetra-acetic acid (EDTA), 5 mM DTT and 50 mM NaF. In the presence of NaF (a dephosphorylation inhibitor), the measured activity of the enzyme reflected the activity that was present initially in the tissue (active form). Each homogenate was centrifuged in a Beckman centrifuge J2–21 class, JA-17 rotor to remove particulate matter, for 20 min at 15,000 g. The supernatant was collected and the centrifugation (15,000) was repeated. The 15,000 g supernatant was centrifuged at 105,000 g for 60 min in a Sorvall ultracentrifuge, model OTD 50 B. The resulting microsomal pellets were immediately frozen in liquid N₂ and stored at −80°C until assaying. The storage time did not result in a significant loss of enzyme activity (results not shown). HMG-CoA reductase activity was measured as described by Shapiro et al. (1974) with slight modifications by Molina et al. (1997). This method measures the formation of radioactive mevalonate from labelled HMG-CoA reductase. One hundred microlitres of pre-incubated medium containing 0.2–0.4 mg mucosal protein, in addition to the components present in the homogenization medium, was incubated at 37°C for 15 min. Then, 50 μl of solution containing 0.25 M NaCl, 50 mM imidazol (pH 7.4), 20 mM EDTA, 5 mM DTT, 110 mM glucose-6-phosphate dehydrogenase and 1 mM DL-3-14C-HMG-CoA reductase (specific activity 4000–8000 dpm/nmol) was added to the pre-incubated mixture and then incubated for 30 min at 37°C. The reaction was stopped by adding 25 μl of 5 N HCl. The 14C mevalonate formed was converted into the labelled lactone, isolated by thin-layered chromatography and counted by liquid scintillation using an internal standard of ³H-mevalonate to correct for incomplete recovery.

**Statistical analyses**

The results are expressed as a mean ± SEM. The data were analysed using a statistical program (GraphPad InStat 3) by analysing the ANOVA parametric variance test followed by Tukey–Kramer tests. A P value of <0.05 was considered to be statistically significant.

**RESULTS**

As Table 1 shows, the lipid intake in adult male rats was significantly lower in groups treated with ethanol (A, AF), than in the control groups (C, CF) throughout the whole experimental period (8 weeks). This decrease was especially significant between the fifth and eight weeks of treatment, when alcohol concentration was 20%. The supplementation with folic acid did not alter the lipid intake.

The ethanol-fed dams’ offspring’s birth weight and body weight at 21 days postpartum (AO) were lower than control group (CO), control folic group (CFO) and alcohol folic group (AFO) (Table 2).

In Table 3, ethanol intake and serum ethanol levels were similar in the A and AF groups. Folic acid intake and serum folate levels were significantly lower in ethanol rats (A) than in the rest of the groups. In the folic acid supplement groups (AF and CF), higher serum folate concentrations were observed, confirming that the supplementation was effective. However, the groups treated with ethanol (A, AF) presented lower serum folate levels than control with or without folate supplementation (C, CF).

Ethanol adult fed rats (A) had higher serum TG levels than controls and supplemented animals with or without alcohol ingestion (Table 4). However, there were no significant differences in hepatic TG levels among the experimental groups (Table 6). Serum TG levels in pups did not present differences between the groups (Table 5). Hepatic pups’ TG levels were significantly lower when their mothers were treated with alcohol; however, alcohol intake plus folic acid supplementation has no effect on these values (Table 7). Serum TG levels were significantly higher in adult rats (P < 0.001).

We did not find differences in serum Chol between adult groups. However, chronic alcohol consumption resulted in higher serum LDL and VLDL and lower HDL, and therefore the LDL/HDL ratio was higher in A (Table 4). Ethanol-fed rats supplemented with folic acid had no differences to the control group. Curiously, the results in pups were different: only HDL serum levels presented differences between the groups, and in this case HDL was significantly higher in AO, presenting a better LDL/HDL ratio than in the rest of the groups (Table 5). Ethanol folic acid-supplemented rats have similar values to control levels. Serum HDL levels in AO pups were significantly higher than in A adults. Serum LDL in control pups (CO and CFO) were higher than those in adults (P < 0.001); however, serum VLDL values were higher in adults (P < 0.001).

Hepatic Chol values in both ethanol groups (A and AO) were significantly higher than controls groups. However, alcohol intake plus folic acid supplementation had no effect on these values (Tables 6 and 7).

HMG-CoA reductase activity was similar in all pup groups (Table 7), but was higher in A adults than the others adult groups (Table 6). Ethanol-treated adult rats supplemented with folic acid (AF) had similar levels to control rats. Adult A rats showed higher values of hepatic HMG-CoA reductase activity than pups (AO).

Both in pups and in adult ethanol rats (A, AO), serum PL presented higher levels (Tables 4 and 5) than control and supplemented animals with or without alcohol ingestion. However, despite the hepatic PL, the values did not present differences between groups in adults (Table 6). AO pups had lower hepatic PL; however, AFO animals had similar values to control group (CO) (Table 7). Hepatic PL levels were significantly higher in pups (P < 0.001); however, serum PL was only higher in AO pups with respect to the adults (A) (P < 0.001).

Serum BA values did not show differences between the eight experimental groups (Tables 4 and 5). In both pups and adults, hepatic BA levels were higher in ethanol groups with respect to control and control folic groups; however, AF showed the highest BA hepatic levels (Tables 6 and 7). Hepatic BA in A and AF adults was higher than in pups (P < 0.001).
The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by Tukey’s test. n indicates the number of animals in each group. Groups: C, control group; A, alcohol group; AF, alcohol folic acid group; CF, control folic acid group. Signification: A versus C, ***P < 0.001, **P < 0.01; A versus CF, ***P < 0.001, **P < 0.01, *P < 0.05; A versus AF, ***P < 0.001, **P < 0.01, *P < 0.05; AF versus C, ***P < 0.001, **P < 0.01; CF versus C, **P < 0.01, *P < 0.05.

Table 2. Birth weight and body weight of the offspring at day 21 postpartum (g)

<table>
<thead>
<tr>
<th>CO (n = 10)</th>
<th>AO (n = 10)</th>
<th>AFO (n = 10)</th>
<th>CFO (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>6.71 ± 0.07</td>
<td>5.9 ± 0.2 <strong>aa</strong></td>
<td>6.5 ± 0.08</td>
</tr>
<tr>
<td>Body weight at 21-day postpartum (g)</td>
<td>36.2 ± 0.6</td>
<td>19.3 ± 0.9 <strong>aa</strong></td>
<td>29.6 ± 0.7 <strong>aa</strong></td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by Tukey’s test. n indicates the number of animals in each group. Groups: C, control group; A, alcohol group; AO, alcohol oleic acid group; CFO, control folic acid group. Signification: AO versus CO, ***P < 0.001, **P < 0.01; AO versus CFO, ***P < 0.001; *P < 0.05; A versus AF, ***P < 0.001, **P < 0.01; AF versus C, ***P < 0.001; AF versus CF, **P < 0.01; AO versus CFO, **P < 0.01; AFO versus CFO, **P < 0.001.

Table 3. Effects of ethanol and folic acid treatment on ethanol and folic acid intake and serum ethanol and folic acid in male adult rats

<table>
<thead>
<tr>
<th>C (n = 6)</th>
<th>A (n = 6)</th>
<th>AF (n = 6)</th>
<th>CF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol intake (g/kg b.w./day)</td>
<td>–</td>
<td>15.9 ± 1.4</td>
<td>16.7 ± 1.7</td>
</tr>
<tr>
<td>Folate intake (μg/rat/day)</td>
<td>38.6 ± 1.53</td>
<td>22.48 ± 0.95 <strong>aa</strong></td>
<td>103.34 ± 4.23 <strong>aa</strong></td>
</tr>
<tr>
<td>Serum ethanol (mg/dl)</td>
<td>–</td>
<td>60.03 ± 22.2</td>
<td>57.17 ± 11.4</td>
</tr>
<tr>
<td>Serum folate (ng/ml)</td>
<td>11.5 ± 0.2</td>
<td>7.31 ± 0.23 <strong>aa</strong></td>
<td>21.2 ± 0.63 <strong>aa</strong></td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by the Tukey’s test. n indicates the number of animals in each group. Groups: C, control group; A, alcohol group; AF, alcohol folic acid group; CF, control folic acid group. Signification: A versus C, ***P < 0.001, **P < 0.01; A versus CF, ***P < 0.001; A versus AF, ***P < 0.001; AF versus C, **P < 0.01; AF versus CF, **P < 0.01; CF versus C, **P < 0.01.

Table 4. Serum lipid values in male adult rats

<table>
<thead>
<tr>
<th>Adult rats serum values</th>
<th>C (n = 6)</th>
<th>A (n = 6)</th>
<th>AF (n = 6)</th>
<th>CF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol (mg/dl)</td>
<td>96.7 ± 8.7</td>
<td>112.17 ± 9.5</td>
<td>111.95 ± 9.5</td>
<td>94.78 ± 8.3</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>37.8 ± 2.62</td>
<td>22.65 ± 1.05 ****</td>
<td>43.89 ± 2.73 <strong>bb</strong></td>
<td>40.08 ± 2.2 <strong>aa</strong></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>46.74 ± 1.7</td>
<td>66.9 ± 4.41 **</td>
<td>52.56 ± 4.38</td>
<td>44.86 ± 2.3 <strong>aa</strong></td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>12.1 ± 1.05</td>
<td>23.14 ± 1.86 <strong>bb</strong></td>
<td>15.5 ± 1.48 <strong>bb</strong></td>
<td>9.84 ± 2.3 <strong>aa</strong></td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>1.23 ± 0.15</td>
<td>2.9 ± 0.26 ****</td>
<td>1.19 ± 0.13 <strong>bb</strong></td>
<td>1.11 ± 0.12 <strong>aa</strong></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>60.7 ± 5.25</td>
<td>105.7 ± 9.22 <strong>bb</strong></td>
<td>77.94 ± 7.45</td>
<td>49.2 ± 3.2 <strong>aa</strong></td>
</tr>
<tr>
<td>PL (mg/dl)</td>
<td>104 ± 13.08</td>
<td>147 ± 9.5 *</td>
<td>134 ± 7.68</td>
<td>101 ± 13.08 a</td>
</tr>
<tr>
<td>BA (mmol/l)</td>
<td>37.7 ± 5.6</td>
<td>32.9 ± 3.7</td>
<td>26.2 ± 2.6</td>
<td>33.7 ± 4.6</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by the Tukey’s test. n indicates the number of animals in each group. Groups: C, control group; A, alcohol group; AF, alcohol folic acid group; CF, control folic acid group. Signification: A versus C, ***P < 0.001, **P < 0.01; A versus CF, ***P < 0.001; A versus AF, ***P < 0.001; AF versus C, **P < 0.01; AF versus CF, **P < 0.01; CF versus C, **P < 0.01.

Table 5. Serum lipid values in offspring rats

<table>
<thead>
<tr>
<th>Offsprings serum values</th>
<th>CO (n = 10)</th>
<th>AO (n = 10)</th>
<th>AFO (n = 10)</th>
<th>CFO (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol (mg/dl)</td>
<td>121.7 ± 13.89</td>
<td>131.7 ± 10.77</td>
<td>102.7 ± 14.3</td>
<td>113.7 ± 10.89</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.28 ± 2.1</td>
<td>58.64 ± 4.3 ****</td>
<td>36.63 ± 3.3 <strong>bb</strong></td>
<td>35.28 ± 1.9 <strong>aa</strong></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>77.9 ± 6.2</td>
<td>66.25 ± 5.7</td>
<td>60.6 ± 6.2</td>
<td>72.9 ± 5.9</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>5.58 ± 0.45</td>
<td>6.22 ± 0.99</td>
<td>5.5 ± 0.43</td>
<td>5.53 ± 0.48</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.02 ± 0.2</td>
<td>1.13 ± 0.11 **</td>
<td>1.65 ± 0.17 **</td>
<td>2.06 ± 0.18 ** aa</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>27.93 ± 2.28</td>
<td>31.11 ± 3.13</td>
<td>27.5 ± 2.46</td>
<td>27.65 ± 2.3</td>
</tr>
<tr>
<td>PL (mg/dl)</td>
<td>156.1 ± 6.7</td>
<td>273.8 ± 27 <strong>bb</strong></td>
<td>154.5 ± 15 <strong>bb</strong></td>
<td>155.6 ± 8.7 <strong>aa</strong></td>
</tr>
<tr>
<td>BA (mmol/l)</td>
<td>40.3 ± 1.89</td>
<td>44.17 ± 3.4</td>
<td>34.8 ± 3.4</td>
<td>38.3 ± 2.89</td>
</tr>
</tbody>
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Table 6. Hepatic lipid values and HMGCoA reductase activity in male adult rats

<table>
<thead>
<tr>
<th>Adult rats hepatic values</th>
<th>C (n = 6)</th>
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<th>AF (n = 6)</th>
<th>CF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG-CoA reductase activity (nmol/mg/min)</td>
<td>92.3 ± 6.9</td>
<td>148.3 ± 16.8***</td>
<td>137.9 ± 12.3b</td>
<td>90.6 ± 5.7***</td>
</tr>
<tr>
<td>Chol (mg)</td>
<td>17.24 ± 1.8</td>
<td>31.8 ± 3.4***</td>
<td>14.9 ± 0.9bbb</td>
<td>15.8 ± 1.2***</td>
</tr>
<tr>
<td>PL (mg)</td>
<td>63 ± 4.2</td>
<td>59 ± 3.9</td>
<td>55 ± 3.8</td>
<td>61 ± 4.7</td>
</tr>
<tr>
<td>TG (mg)</td>
<td>113.96 ± 7.05</td>
<td>96.20 ± 9.14</td>
<td>84.7 ± 8.44</td>
<td>109.6 ± 9.05</td>
</tr>
<tr>
<td>BA (µmol/g)</td>
<td>1.96 ± 0.4</td>
<td>5.4 ± 0.55**</td>
<td>8 ± 0.9b,cc</td>
<td>2.13 ± 0.22ad,d</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by Tukey’s test. n indicates the number of animals in each group. Groups: C, control group; A, alcohol group; AF, alcohol folic acid group; CF, control folic acid group. Signification: A versus C, ***P < 0.001, **P < 0.01; A versus AF, ***P < 0.001, **P < 0.01; A versus CF, ***P < 0.001, bP < 0.05; AF versus C, **P < 0.01;

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<th>CFO (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG-CoA reductase activity (nmol/mg/min)</td>
<td>78.38 ± 5.37</td>
<td>80.18 ± 9.87</td>
<td>73.45 ± 4.68</td>
<td>77.45 ± 6.7</td>
</tr>
<tr>
<td>Chol (mg)</td>
<td>21.49 ± 0.98</td>
<td>40.75 ± 3.75***</td>
<td>23.69 ± 1.95bbb</td>
<td>20.18 ± 1.2aaaa</td>
</tr>
<tr>
<td>PL (mg)</td>
<td>141.11 ± 6.21</td>
<td>108.46 ± 6.73**</td>
<td>151.20 ± 7.5b,bb</td>
<td>143.2 ± 5.21aa</td>
</tr>
<tr>
<td>TG (mg)</td>
<td>148.9 ± 12.44</td>
<td>76.31 ± 10.5***</td>
<td>104.7 ± 11.29</td>
<td>143.2 ± 13.5aa</td>
</tr>
<tr>
<td>BA (µmol/g)</td>
<td>0.67 ± 0.07</td>
<td>1.19 ± 0.12c</td>
<td>1.51 ± 0.14b,bb</td>
<td>0.75 ± 0.06</td>
</tr>
</tbody>
</table>

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**DISCUSSION**

The principal finding of this study was that folic acid, a potential antioxidant nutrient (Durand et al., 1996), could be associated for treatment for the lipid homeostasis alteration provoked by chronic ethanol consumption and could prevent CVD risk in adults and even in pups whose mothers drank alcohol during pregnancy and lactation. This action is clear when we supplement alcoholic pregnant rats with folic acid; in this case their pups' weights at birth are similar to control and higher than ethanol pups'; this effect of supplementation is not so great during lactation.

Following Pennington et al. (2002), who defended that maternal dietary ethanol consumption is associated with hypertriglyceridaemia in adult rat offspring, we have not found differences in TG pups levels, but we have found an increase in serum TG levels in adult A rats, despite a decrease of lipid intake in the alcohol group during the experimental period. Pups showed a significant decrease in hepatic TG levels when they were treated with alcohol; this fact might be due to the low-serum-TG concentrations in normal pups due to the biological variability of serum lipid parameters in children (Okada et al., 2002) and the poor TG level regulation shown at this early age. In any case, ethanol folic acid-supplemented pups had similar hepatic TG values to controls, and AF adult rats had similar serum TG levels to controls.

Brand-Herrmann et al. (2005) have found a genetic association in humans between alcohol and HDL. It appears that alcohol intake modulates the relationship between the peroxisome proliferator-activated receptor δ (a receptor that can affect plasma lipids) and HDL. This alteration in plasma lipids was also found in several studies with chronic ethanolics and ethanol adult rats (Kwon et al., 2005; Maneesh and Jayalekshmi, 2005; Choi et al., 2006). In line with these studies, our results showed a significant increment in serum VLDL and LDL, and a decrease in HDL levels in A adult rats; these values imply a significant increased risk for CVD (Walldius and Jungner, 2006).

Seeking new strategies against alcoholism, Lee (2004) found that Pueraria radix, a traditional oriental medicinal plant, could diminish plasma Chol in ethanol-treated rats; Maneesh and Jayalekshmi (2005) also found that exogenous ascorbic acid, alpha-tocopherol and other antioxidants exhibited an ability to counteract the alcohol-induced changes in lipid parameters. Kwon et al. (2005) reported the effects of traditional Chinese medicine on alcohol-induced fatty livers in rats, concluding that this treatment reduced plasma TG and Chol. Taking our data as our point of departure, we can report that folic acid supplementation in ethanol adult rats improves the serum lipid profile of these animals, diminishing TG, VLDL and LDL serum and increasing HDL levels. Folic acid intake in chronic ethanol adult rats therefore significantly reduced the LDL/HDL cholesterol ratio, probably due to changes in the HDL-cholesterol concentration. These effects of folic acid are similar to those studied by Mayer et al. (2006) in hypercholesterolaemic humans: folate co-administered with fenofibrate was followed by a significant decrease in plasma LDL and oxidized LDL.

From the limited literature available, it would appear that the biological variability of serum lipid parameters in children is similar to that reported for adults (Tolfrey, 2002). However, the studies that have focused on this issue are scarce, so Kwiterovich et al. (2004) determined that the gestational age in humans has a significant effect on both LDL and HDL. Bastida et al. (2002) suggested that when Chol levels are high in humans at birth, this is mainly due to an increase in LDL. Surprisingly, in our pup rats lipoprotein results were different; only serum HDL presented differences between the groups, and in this case HDL was significantly higher in AO, presenting a better LDL/HDL cholesterol ratio than the rest of the groups. It appears that chronic alcohol consumption by the pups’ mothers could have a protective effect on the pups’ lipid metabolism. This is not exactly the case, however; it must be borne in mind that in their normal status, offspring (CO and CFO) showed significantly higher LDL serum values than adults and a trend of higher serum Chol levels and therefore the basal status in pups is different to that of adults. LDL did not increase in AO pups,
and VLDL is lower than that in adults, so the only lipoprotein that could increase was HDL. Moreover, PL levels (higher in pups) are the principal components of HDL. Furthermore, it is important to remember that there is great intra-individual variation in children’s lipid-lipoprotein profiles (Cabrera et al., 1996; Tolfrey, 2002).

Serum PL (a new atherogenix risk factor (Zhang and Salomon, 2005)) presented higher levels in ethanol groups. Folic acid diminished PL values, showing its action against CVD risk factors. Pups presented higher values of serum PL in AO and hepatic PL in all of the groups, perhaps, because the presence of these lipids in newborn pups is essential for their development (Rump et al., 2001).

Despite the fact that alcoholic adults and pups did not present higher serum Chol values, they did present higher Chol hepatic values, yet increased HMG-CoA reductase activity was only present in adults. Hepatic BA values increased in ethanol groups, increasing Chol catabolism, so it seems to be an effective mechanism to control serum Chol. However, HMG-CoA did not increase in AO offspring. This might be because AO presented high serum HDL levels that returned Chol to the liver, and because hepatic BA in offspring was significantly lower than that in adults despite similar or slightly higher hepatic Chol levels.

Curiously, hepatic BA presented a significant increase in ethanol with respect to control and control-folic groups and when rats were supplemented with folic acid these values were even higher. These values are in concordance with other studies that present folinic acid as a good choleretic treatment (Kajiyama et al., 1998). Perhaps, because of this possible choleretic action, folic acid contributes to improving ethanol rats’ serum and hepatic Chol. However, serum BA levels were similar between adult and pup groups, perhaps BA are being eliminated in the faeces.

Our findings suggest that supplementary folic acid only benefits individuals with low-plasma-folate concentration (chronic ethanol treatment), as occurs with other folate actions (Martinez et al., 2006) due to the fact that control values were similar to the control-folic values in all of the experiments.

The proposed mechanism of folic acid on lipid metabolism is that it acts indirectly, reducing the homocysteine levels that are elevated during ethanol consumption (Bayerlein et al., 2005), and by a specific mechanism between 5-MTHF and LDL that regulate endothelial functions reducing oxidized LDL, thus improving lipid metabolism in ethanol-treated rats. This effect is more pronounced in the adult population, where alcohol consumption was independently and directly associated with circulating oxidized LDL (Schröder et al., 2006).

In summary, ethanol intake provokes lipid alterations in adult rats and in those offspring rats whose mothers drank ethanol, but it affects them in a different manner. Ethanol alters serum PL and HDL in offspring, and serum TG and lipoproteins in adults. This is not unusual because these parameters are significantly different in basal status (except HDL). Ethanol alters hepatic Chol, BA, TG and PL in pups, and Chol, BA and HMG-CoA reductase activity in adults. In the basal status, only PL values were significantly different in adults and pups. In all of these cases, folic acid contributes to alleviating the adverse effect of ethanol ingestion by decreasing HMG-CoA reductase activity and by restoring lipid values, except BA levels. This fact demonstrates that folic acid has a choleretic action.

We could therefore conclude that folic acid supplementation reduces alcohol-induced hypercholesterolemia by decreasing synthesis (in adults) and increasing lipid catabolism, but further evidence is necessary.

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