Chronic ethanol administration depresses fatty acid synthesis in rat adipose tissue

Jeremy S. WILSON,*† Mark A. KORSTEN,† Louise P. DONNELLY,* Peter W. COLLEY,* John B. SOMER* and Romano C. PIROLA*

*Division of Medicine, Prince Henry Hospital, N.S.W. 2036, Australia, and †Gastroenterology Section, V.A. Medical Center, Bronx, NY 10468, U.S.A.

Administration of ethanol as part of a nutritionally adequate liquid diet to female Wistar rats was found to depress markedly incorporation of labelled glucose into adipose-tissue acylglycerol fatty acids. Similar results with labelled pyruvate and acetate suggested inhibition of the fatty-acid-synthesis pathway at, or distal to, the acetyl-CoA carboxylase step. Activities of acetyl-CoA carboxylase and fatty acid synthetase were markedly lower in ethanol-fed animals. The activity of another lipogenic enzyme, phosphatidate phosphohydrolase, was not affected by chronic ethanol feeding. These findings suggest that chronic ethanol administration has marked effects on adipose-tissue lipogenesis.

INTRODUCTION

Chronic administration of ethanol is known to alter lipid metabolism in a number of tissues, including the liver (Lieber, 1982), pancreas (Wilson *et al.*, 1982, 1984) and heart (Somer *et al.*, 1981) and to promote hyperlipidaemia (Baraona & Lieber, 1979; Crouse & Grundy, 1984). Despite the prominent role played by adipose tissue as a site of clearance and storage of neutral lipid, relatively little is known about the effects of chronic ethanol administration on this tissue. In a previous report, we noted that incorporation of labelled glucose into rat adipose-tissue acylglycerol fatty acids was markedly depressed after chronic ethanol feeding (Wilson *et al.*, 1986). The present study was undertaken to determine the mechanism of this finding.

METHODS

Animals and experimental diets

Littermate female Wistar rats (100–150 g) were paired on nutritionally adequate liquid diets containing ethanol as 36 % of energy, or an isoenergetic amount of carbohydrate for 3 weeks (Lieber & DeCarli, 1982). Another group of rats was fed on liquid diets in which protein energy was substituted for that of ethanol (high-protein/low-carbohydrate controls) and energy intakes were individually matched to those animals receiving ethanol. To ensure equal duration of fasting before killing, the animals were given their diets in divided rations and then allowed water only overnight. The following morning, they were killed by cervical dislocation and exsanguination. Periovarian adipose tissue was then removed for radiolabel-incorporation studies, enzyme assays and determination of lipid content.

Radiolabel-incorporation studies

Adipose-tissue slices were prepared at 4 °C and then incubated at 37 °C for 1 h in 25 ml Ehrlenmeyer flasks containing 5 ml of Krebs–Ringer bicarbonate buffer (118 mM-NaCl/5 mM-KCl/2.5 mM-CaCl₂/0.25 mM-

KH₂PO₄/2.5 mM-MgSO₄/25 mM-NaHCO₃, pH 7.4) with 5.6 mM-glucose, 4 % (w/v) fatty-acid-free bovine serum albumin and one of the following labelled precursors: D-[U-¹⁴C]glucose (5 µCi/flask; final sp. radioactivity 0.18 mCi/mmol), [3-¹⁴C]pyruvic acid (5 µCi/flask; 25 mCi/mmol) or [1-¹⁴C]acetic acid (10 µCi/flask; 55.7 mCi/mmol). For studies with labelled pyruvate, unlabelled sodium pyruvate was added to each flask to a concentration of 0.6 mM, giving a final specific radioactivity of precursor of 1.5 mCi/mmol. Similarly, when labelled acetate was used as precursor, unlabelled acetate was added to a final concentration of 0.6 mM, giving a final specific radioactivity of 3.1 mCi/mmol. Preliminary experiments had established that incorporation of all three labelled precursors was linear for 2 h under the conditions described. At the end of the incubation, medium was decanted and tissue was rinsed in ice-cold buffer. Lipids were extracted with chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957) and washed with 0.73 % NaCl and pure solvent upper phase (chloroform/methanol/water, 3:43:47, by vol.) containing the appropriate unlabelled precursor (Somer *et al.*, 1974). Saponifications were performed as previously described (Somer *et al.*, 1974) by heating lipids in a 15 % (w/v) solution of KOH in 90 % (v/v) ethanol. After 4 h the reaction mixtures were cooled, and non-saponifiable lipid was extracted with hexane (3 × 10 ml). The remaining hydrolysate was acidified with 6 M-HCl, and fatty acids were extracted with diethyl ether (3 × 10 ml). The fatty acid extract was evaporated to dryness, scintillation fluid was added and radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer. Quench corrections were made by the external-standardization method.

Enzyme assays

Preparation of tissue. Adipose tissue (1.5–3.0 g) was homogenized in a Dounce tissue grinder in 1 vol. of ice-cold buffer (pH 7.4) containing 0.25 M-sucrose, 20 mM-Tris/HCl, 20 mM-Mops, 1 mM-dithiothreitol and 2 mM-EGTA, as described by Brownsey *et al.* (1979). Homo-

† To whom reprint requests should be addressed.

genate was then centrifuged at 10000 *g* for 10 min at 4 °C, after which fatty-acid-free bovine serum albumin (10 mg/ml) was added to the infranatant. The infranatant was then centrifuged at 100000 *g* for 1 h, and the resulting supernatant was used directly for assays of acetyl-CoA carboxylase and fatty acid synthetase activities. Phosphatidate phosphohydrolase activity was determined in both the 100000 *g* supernatant and the 100000 *g* pellet (microsomal fraction). To avoid high blank values caused by cytosolic P_i in the assay of phosphatidate phosphohydrolase, the enzyme was precipitated from the cytosol by adding an equal volume of 3.5 M-(NH₄)₂SO₄. After standing for 15 min at 4 °C and centrifugation at 10000 *g* for 20 min, the pellet was redissolved to the initial volume of cytosol with 0.25 M-sucrose containing 0.5 mM-dithiothreitol and 20 mM-Tris/HCl, pH 7.4 (Savolainen & Hassinen, 1978).

Acetyl-CoA carboxylase (EC 6.4.1.2). Acetyl-CoA carboxylase activity was determined by a modification of the ¹⁴CO₂-fixation assay of Tanabe *et al.* (1981). Supernatant (50 µl) was preincubated at 37 °C for 30 min in 100 µl of buffer (pH 7.4) containing 100 mM-Tris/HCl, 20 mM-MgSO₄, 10 mM-sodium citrate, 1 mM-dithiothreitol and 10 mg of fatty-acid-free bovine serum albumin/ml. After preincubation, the reaction was initiated by the addition of an assay buffer (pH 7.4) to produce final concentrations of 100 mM-Tris/HCl, 0.5 M-EDTA, 10 mM-MgSO₄, 15 mM-NaH¹⁴CO₃ (0.5 µCi/µmol), 20 mM-sodium citrate, 2.5 mM-ATP, 0.15 mM-acetyl-CoA, 1 mM-dithiothreitol and 10 mg of fatty-acid-free bovine serum albumin/ml in a total volume of 0.5 ml. The reaction was stopped after 2 min by the addition of 5 M-HCl. For control flasks, the assay was stopped at zero time. The reaction mixture was dried under N₂, 0.5 ml of distilled water was added, followed by 10 ml of scintillation fluid, and radioactivity was determined as described above. The assay was linear with respect to time and tissue protein concentration. Protein was determined by a modification (Schacterle & Pollack, 1973) of the method of Lowry *et al.* (1951).

Fatty acid synthetase. Fatty acid synthetase activity was measured by the method of Halestrap & Denton (1973). Assays were performed at 30 °C. Supernatant (50–200 µl) was added of 1 ml of 100 mM-potassium phosphate buffer (pH 6.5) containing 0.1 mM-NADPH and 25 µM-acetyl-CoA, and the reaction was started by the addition of 60 µM-malonyl-CoA. The disappearance of NADPH was measured spectrophotometrically at 340 nm in a Pye–Unicam spectrophotometer. A correction was made for the autoxidation of NADPH after performing the assay in the absence of malonyl-CoA. Reaction rates were linear with respect to time and tissue protein concentration.

Phosphatidate phosphohydrolase (EC 3.1.3.4) Phosphatidate phosphohydrolase activity was measured by the method of Bowley *et al.* (1977). Enzyme preparation (0.2 ml) was incubated at 37 °C for 20 min in a reaction mixture containing 20 mM-Tris/maleate buffer (pH 6.4), 1 mM-dithiothreitol, 2 mM-MgCl₂, 0.2 mM-EGTA and 3 mM-sodium phosphatidate (final reaction volume 1 ml). The reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid and placed on ice for 20 min. The precipitate was removed by sedimentation in a

Table 1. Influence of chronic ethanol feeding on the incorporation of D-[U-¹⁴C]glucose, [3-¹⁴C]pyruvate and [1-¹⁴C]acetate into fatty acids of rat adipose tissue

Adipose-tissue slices from six pairs of animals were incubated for 1 h at 37 °C in Krebs–Ringer bicarbonate buffer (5 ml) containing 5.6 mM-glucose and 4% fatty-acid-free bovine serum albumin. Saponifications were performed on chloroform/methanol extracts as described in the Methods section. Results are expressed as nmol of precursor incorporated/g of triacylglycerol.

	Ethanol-fed rats	Pair-fed controls	Significance
[U- ¹⁴ C]Glucose	197 ± 25	744 ± 144	<i>P</i> < 0.01
[3- ¹⁴ C]Pyruvate	372 ± 136	1134 ± 240	<i>P</i> < 0.025
[1- ¹⁴ C]Acetate	152 ± 48	738 ± 192	<i>P</i> < 0.01

bench centrifuge, and P_i was measured in the supernatant by the method of Bartlett (1959). The assay was linear with respect to time and protein concentration. The dispersion of sodium phosphatidate used in the reaction was obtained by sonication for 1 min of phosphatidic acid in 0.25 M-sucrose adjusted to pH 9–10 with NaOH. The pH was adjusted to neutrality before use.

Lipid determinations

Adipose-tissue lipids were extracted with chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957) and washed (Somer *et al.*, 1974). Triacylglycerol and total cholesterol contents were determined by Auto Analyzer (Technicon Instrument Corp., 1968). Total tissue phospholipids were measured by the method of Bartlett (1959).

Chemicals and reagents

All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radiolabelled compounds were obtained from New England Nuclear Corp., Boston, MA, U.S.A. General laboratory chemicals and solvents were of analytical-reagent grade.

Statistics

Results were expressed as means ± S.E.M. Statistical comparisons were made by Student's *t* test (Snedecor & Cochran, 1980).

RESULTS

The rats appeared healthy and gained weight throughout the feeding period. Ethanol-fed animals gained less weight than controls (0.7 ± 0.1 versus 1.0 ± 0.1 g/day; *n* = 9 pairs), in accord with earlier observations by Saville & Lieber (1965). Ethanol-fed animals consumed 14.8 ± 0.9 g of ethanol/day per kg body wt. (*n* = 9), in accord with the experience of Lieber & DeCarli (1982).

Radiolabel incorporation studies

In ethanol-fed animals, labelled glucose incorporation was much less than control values (Table 1). When labelled pyruvate and acetate were used as precursors, similar differences were observed (Table 1), suggesting an ethanol-induced impairment of fatty acid synthesis at or distal to the acetyl-CoA carboxylase step.

Table 2. Effect of chronic ethanol feeding on rat adipose-tissue acetyl-CoA carboxylase, fatty acid synthetase and phosphatidate phosphohydrolase activities

Activities were assayed in 100000 g supernatants and, for phosphatidate phosphohydrolase, in the microsomal fraction as described in the Methods section. *n* = 6 pairs of animals. N.S., not significant.

	Ethanol-fed rats	Pair-fed controls	Significance
Acetyl-CoA carboxylase (nmol of $\text{H}^{14}\text{CO}_3^-$ incorporated/min per mg of protein)	3.5 ± 0.6	10.8 ± 1.5	$P < 0.005$
Fatty acid synthetase (nmol of NADPH oxidized/min per mg of protein)	11.5 ± 0.8	39.5 ± 2.3	$P < 0.0001$
Phosphatidate phosphohydrolase (nmol of P_i released/min per mg of protein)			
(a) Cytosolic	2.5 ± 0.6	2.6 ± 0.3	N.S.
(b) Microsomal	1.0 ± 0.2	1.0 ± 0.1	N.S.

Table 3. Triacylglycerol, total cholesterol and total phospholipid contents of adipose tissue from ethanol-fed and control rats

Lipids were extracted with chloroform/methanol. Triacylglycerol and total cholesterol contents were measured by Technicon Auto Analyzer. Total phospholipids were measured by the method of Bartlett (1959). Results are expressed as mg of lipid/mg of lipid-free dry weight. Results from at least 11 pairs of animals are presented. There was no significant difference between ethanol-fed animals and controls.

	Ethanol-fed rats	Pair-fed controls
Triacylglycerols	33.8 ± 5.4	29.7 ± 6.2
Total cholesterol	0.09 ± 0.02	0.08 ± 0.02
Total phospholipids	0.13 ± 0.02	0.15 ± 0.03

Enzyme assays

Activities of acetyl-CoA carboxylase and fatty acid synthetase in adipose tissue from ethanol-fed animals were approximately one-third of control values (Table 2). These decreased enzyme activities would explain, at least in part, the incorporation data presented in Table 1. In contrast, activity of phosphatidate phosphohydrolase, a rate-limiting enzyme for triacylglycerol synthesis (Lamb & Fallon, 1974), was similar in ethanol-fed animals and controls (Table 2).

As inclusion of ethanol in the diet necessarily involves a concomitant decrease in carbohydrate content, a third

group of animals was studied. These animals received an isoenergetic diet in which 36 % of carbohydrate energy was replaced by protein energy rather than ethanol energy. The energy intakes of these animals were individually matched to those animals receiving the ethanol and carbohydrate control diets. In these animals, activities of adipose-tissue acetyl-CoA carboxylase and fatty acid synthetase were not decreased as compared with carbohydrate control values (shown in Table 2), being 16.04 ± 1.11 nmol of $\text{H}^{14}\text{CO}_3^-$ incorporated/min per mg of protein and 37.49 ± 4.32 nmol of NADPH oxidized/min per mg of protein respectively (*n* = 8). In fact, values of acetyl-CoA carboxylase activities in animals fed on this low-carbohydrate control diet were significantly higher than those observed in animals on regular control diet ($P < 0.005$).

Tissue lipid contents

Adipose-tissue triacylglycerol, cholesterol and phospholipid contents were similar in ethanol-fed animals and their controls (Table 3).

DISCUSSION

This study has revealed that chronic consumption of ethanol results in decreased incorporation of labelled glucose, pyruvate and acetate into fatty acids of rat adipose tissue. In addition, it was found that activities of acetyl-CoA carboxylase and fatty acid synthetase were markedly lower in ethanol-fed animals when compared with control values. These findings suggest an ethanol-induced impairment of adipose-tissue fatty acid synthesis.

The introduction of ethanol into the liquid diet necessarily results in a concomitant decrease in carbohydrate content. For this reason, another group of animals was given a diet in which 36 % of carbohydrate energy was replaced by protein energy, and the dietary intakes of these animals were individually matched to those of animals receiving the ethanol diet. Activities of acetyl-CoA carboxylase and fatty acid synthetase for this group were not decreased, suggesting that the decreased enzymic activities in ethanol-fed rats were due to ethanol itself rather than to a decrease in carbohydrate intake.

Our results contrast with those of previous studies. Alexander *et al.* (1966) reported that adipose-tissue lipogenesis was not influenced by chronic ethanol feeding. Furthermore, Cascales *et al.* (1983) reported that ethanol feeding enhanced adipose-tissue lipogenesis. Both these groups used male rats which were given ethanol for periods of 3 and 9 months respectively, much longer feeding periods than those used in this present study. However, the major difference between the present study and those of Cascales *et al.* (1983) and Alexander *et al.* (1966) was in the way ethanol was administered to the experimental animals. The earlier studies involved addition of ethanol to drinking water, a method which results in an ethanol intake which is insufficient to result in sustained appreciable concentrations of ethanol in the blood (Lieber & DeCarli, 1982). In contrast, when rats are given nothing to eat or drink but the nutritionally adequate ethanol-containing liquid diet formula used in the present study, their intake is sufficient to sustain a high daily ethanol intake of 12–18 g/kg, 2–3 times more than that achieved through the drinking-water technique (Lieber & DeCarli, 1982); furthermore, blood ethanol concentrations between 15 and 30 mM are frequently

observed (Lieber & DeCarli, 1982). A further point to note is that both Alexander *et al.* (1966) and Cascales *et al.* (1983) fed their animals on solid rat chow with a fat content of 3–10% of energy, whereas the liquid diets used in the present study have a fat content of 35% of energy, which more accurately represents Western dietary habits.

Ethanol oxidation results in elevated serum concentrations of acetate (Julkunen *et al.*, 1985) and presumably increased tissue acetate pools, and it might be argued that such an increase in acetate pool size would account, at least in part, for the incorporation data of this study (Table 1). However, the experimental animals were not given ethanol for 15 h before death and, according to the data of Julkunen *et al.* (1985), serum acetate concentrations (and presumably tissue acetate pools) would have normalized by the time of death. In addition, the effect of ethanol feeding on incorporation of labelled precursors was of the same magnitude as that obtained with the relevant enzyme assays (65–75% decrease), suggesting that the data for labelling *in vitro* are not due to alterations in acetate pool size.

Although this study provides evidence for a suppression of adipose-tissue fatty acid synthesis in animals chronically fed on ethanol, adipose-tissue contents of acylglycerol lipids were similar in ethanol-fed animals and their controls. There are several possible explanations for this apparent discrepancy. Endogenous synthesis of fatty acids is not the only source of adipose-tissue fatty acid content, as fatty acids in adipose tissue can also originate from exogenous sources. Therefore, decreased activity in this pathway may not be reflected in overall adipose-tissue lipid contents. Alternatively, similar lipid contents in ethanol-fed animals and their controls could reflect an effect of ethanol feeding on adipose-tissue lipolysis and the nutritional state of the animals at the time of death. Nilsson & Belfrage (1978) have reported that acetate (an oxidation product of ethanol) inhibits lipolysis in isolated rat adipocytes. Intact adipose-tissue function in control animals in the period of starvation before death may have allowed more rapid lipid mobilization, thus obscuring small differences in lipid content.

The mechanism of the ethanol-induced depression in adipose-tissue fatty acid synthesis reported here is not known. Insulin is known to be an important modulator of fatty acid synthesis via an enhancement of acetyl-CoA carboxylase activity (Halestrap & Denton, 1973). However, serum insulin concentrations are similar in ethanol-fed rats and their controls (Wilson *et al.*, 1986). Ethanol feeding in this animal model results in a post-prandial hypertriacylglycerolaemia (Baraona & Lieber, 1970; Wilson *et al.*, 1986). Consequently, the adipocytes of ethanol-fed animals may be faced with a greater post-prandial influx of fatty acids derived by the action of lipoprotein lipase, and these fatty acids, in turn, may result in a down-regulation of fatty acid synthesis similar to the down-regulation of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase by ingested cholesterol (Dempsey, 1974). However, such a mechanism must remain speculative.

Activities of acetyl-CoA carboxylase were higher in animals fed on the high-protein/low-carbohydrate diet than in animals fed on the regular (high-carbohydrate) control diet. This finding is consistent with the data of Herzberg & Rogerson (1981), who found that high-protein diets increased hepatic lipogenesis. An alternative

explanation for our data is that the higher carbohydrate content of the regular control diet may have resulted in relatively higher concentrations of circulating triacylglycerols, with subsequent down-regulation of adipose-tissue fatty acid synthesis. This explanation is supported by reports that a high carbohydrate intake results in elevated concentrations of serum triacylglycerols (Garton & Wahle, 1975).

In summary, chronic ethanol feeding results in altered adipose-tissue lipogenesis in the rat, with decreased activities of acetyl-CoA carboxylase and fatty acid synthetase. These findings are not due to the lower carbohydrate content of the ethanol diet. Although the mechanism responsible for such alterations is not clear, these changes may represent an adaptive response to altered hepatic lipogenesis and increased hepatic excretion of lipoproteins, resulting in an increased influx of fatty acids into peripheral fat stores, with consequent down-regulation of adipose-tissue fatty acid synthesis.

This project was supported in part by the Clive and Vera Ramaciotti Foundations of Australia, the National Health and Medical Research Council of Australia, the Australian Associated Brewers and the National Heart Foundation of Australia.

REFERENCES

- Alexander, N. M., Scheig, R. & Klatskin, G. (1966) *J. Lipid Res.* **7**, 197–203
- Baraona, E. & Lieber, C. S. (1970) *J. Clin. Invest.* **49**, 769–778
- Baraona, E. & Lieber, C. S. (1979) *J. Lipid Res.* **20**, 289–315
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Bowley, M., Cooling, J., Burditt, S. L. & Brindley, D. N. (1977) *Biochem. J.* **165**, 447–454
- Brownsey, R. W., Hughes, W. A. & Denton, R. M. (1979) *Biochem. J.* **184**, 23–32
- Cascales, C., Benito, M., Cascales, M., Caldes, T. & Santos-Ruiz, A. (1983) *Br. J. Nutr.* **50**, 549–553
- Crouse, J. R. & Grundy, S. M. (1984) *J. Lipid Res.* **25**, 486–496
- Dempsey, M. E. (1974) *Annu. Rev. Biochem.* **43**, 967–990
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–504
- Garton, G. A. & Wahle, K. W. J. (1975) *Proc. Nutr. Soc.* **34**, 257–264
- Halestrap, A. P. & Denton, R. M. (1973) *Biochem. J.* **132**, 509–517
- Herzberg, G. R. & Rogerson, M. (1981) *Br. J. Nutr.* **45**, 529–538
- Julkunen, R. J. K., DiPadova, C. & Lieber, C. S. (1985) *Life Sci.* **37**, 567–573
- Lamb, R. G. & Fallon, H. J. (1974) *Biochim. Biophys. Acta* **348**, 166–178
- Lieber, C. S. (1982) in *Medical Disorders of Alcoholism* (Lieber, C. S., ed.), pp. 141–143. W. B. Saunders Co., Philadelphia
- Lieber, C. S. & DeCarli, L. M. (1982) *Alcohol.: Clin. Exp. Res.* **6**, 523–531
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Nilsson, N. O. & Belfrage, P. (1978) *J. Lipid Res.* **19**, 737–741
- Saville, P. D. & Lieber, C. S. (1965) *J. Nutr.* **87**, 477–484

- Savolainen, M. J. & Hassinen, I. E. (1978) *Biochem. J.* **176**, 885–892
- Schacterle, G. R. & Pollack, R. L. (1973) *Anal. Biochem.* **51**, 654–655
- Snedecor, G. W. & Cochran, W. G. (1980) *Statistical Methods*, 7th edn., p. 83, Iowa State University Press, Ames, IA
- Somer, J. B., Bell, F. P. & Schwartz, C. J. (1974) *Atherosclerosis* **20**, 11–21
- Somer, J. B., Colley, P. W., Pirola, R. C. & Wilson, J. S. (1981) *Alcohol.: Clin. Exp. Res.* **5**, 536–539
- Tanabe, T., Nakanishi, S., Hashimoto, T., Ogiwara, H., Nikawa, J.-I. & Numa, S. (1981) *Methods Enzymol.* **71**, 5–16
- Wilson, J. S., Colley, P. W., Sosula, L., Pirola, R. C., Chapman, B. A. & Somer, J. B. (1982) *Alcohol.: Clin. Exp. Res.* **6**, 117–121
- Wilson, J. S., Somer, J. B. & Pirola, R. C. (1984) *Exp. Mol. Pathol.* **41**, 289–297
- Wilson, J. S., Korsten, M. A., Colley, P. W. & Pirola, R. C. (1986) *Biochem. Pharmacol.* **35**, 2025–2028

Received 23 December 1987; accepted 10 February 1988