Biochemical Pharmacology, Vol. 35, No. 12, pp. 2025–2028, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

DECREASE IN LIPOGENESIS AND GLUCOSE OXIDATION OF RAT ADIPOSE TISSUE AFTER CHRONIC ETHANOL FEEDING

JEREMY S. WILSON, MARK A. KORSTEN,* PETER W. COLLEY and ROMANO C. PIROLA

Department of Medicine, Prince Henry Hospital, NSW 2036, Australia; VA Medical Center, Bronx, NY 10468, U.S.A.; and Mt. Sinai School of Medicine (CUNY), NY 10029, U.S.A.

(*Received* 28 *May* 1985; *accepted* 29 *November* 1985)

Abstract—This investigation was performed to determine whether chronic ethanol feeding affects adipose tissue lipogenesis and glucose metabolism. Female Wistar rats were pair-fed nutritionally adequate liquid diets containing ethanol as 36% of energy or an isocaloric amount of carbohydrate for 3 weeks. Chronic ethanol feeding resulted in a depression of adipose tissue lipogenesis as assessed by labeled glucose incorporation into glyceride glycerol and glyceride fatty acids. Glucose oxidation was also impaired after chronic ethanol feeding. Such changes may contribute to the postprandial hypertriacyglyceridemia observed in alcoholics.

Chronic consumption of ethanol has been shown to promote hypertriacylglyceridemia [1, 2] and to alter lipid metabolism in a number of tissues including liver [3] and heart [4]. However, despite the central role of the adipocyte in lipoprotein kinetics and overall lipid homeostasis, the effects of chronic ethanol administration on adipocyte metabolism have received relatively little attention. Scheig and his colleagues [5, 6] have reported that lipogenesis is unaffected in adipose tissue of rats chronically fed ethanol in drinking water. In contrast, Cascales *et al.* [7] using a similar feeding regimen, have reported recently that chronic ethanol consumption stimulates rat adipose tissue lipogenesis. We now wish to report that chronic administration of ethanol, as part of a nutritionally adequate liquid diet, markedly impairs rat adipose tissue lipogenesis and glucose metabolism.

MATERIALS AND METHODS

Animals and experimental diets. Littermate female Wistar rats (100–260 g) were pair-fed nutritionally adequate liquid diets containing either ethanol as 36% of energy or an isocaloric amount of carbohydrate for 3 weeks [8]. Diets were purchased from Bioserv Inc. (Frenchtown, NJ). During the third week, tail vein blood was collected from animals in the fed state for serum glucose, insulin and lipid determinations. To ensure equal duration of fasting before sacrifice, the animals were given their liquid diets by gastric intubation (5 ml/100 g body wt) and then allowed water only overnight. The following morning, they were killed by cervical dislocation and exsanguination. Periovarian adipose tissue was then removed for lipid estimations and incorporation studies. In some experiments, livers were also removed for assessment of lipid content. Neck vessel blood was collected at the time of sacrifice for fasting serum lipid determinations.

Lipid incorporation studies. Adipose tissue slices were prepared at 4° and then incubated at 37° for 1 hr in 25-ml Erlenmeyer flasks containing 5 ml Krebs–Ringer bicarbonate buffer (pH 7.4) with 5.6 mM glucose, 4% fatty acid free bovine serum albumin, and 5 μCi D-[U-^{14}C]glucose (310 mCi/mmole; final specific activity 0.18 mCi/mmole). 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) [9] and washed once with one-fifth volume 0.73% NaCl containing 10% glucose and three times with 5 ml of pure solvent upper phase (chloroform–methanol–water, 3:43:47, by vol.) containing 1% glucose. No radioactivity was detected in the final wash. Saponifications were performed as previously described [10] by heating lipids at 100° in a 15% (w/v) solution of KOH in 90% ethanol. After 4 hr, the hydrolysates were cooled and nonsaponiable lipid was extracted with hexane (3 × 10 ml). The remaining hydrolysate was acidified with 6 N HCl, and fatty acids were extracted with diethyl ether (3 × 10 ml). Each 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 using the external standardization method.

$^{14}CO_2$ evolution studies. Adipose tissue slices were prepared and incubated as described above in sealed center-well flasks according to the method of Somer *et al.* [11] in the presence of D-[U-^{14}C]glucose (5 μCi/flask, 310 mCi/mmole; final specific activity 0.18 mCi/mmole). After 1 hr, the medium was acidified with 6 N HCl, and the incubation was continued for an additional hour. The $^{14}CO_2$ evolved during this time was trapped by hyamine present in the center well. At the end of the incubation, the hyamine was transferred to a scintillation vial. Scintillation

* Address for correspondence: M. A. Korsten, M.D., Alcohol Research and Treatment Center, V.A. Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468.

fluid was added and radioactivity was measured as described above. To determine the contribution of the pentose phosphate pathway to glucose oxidation, D-[1-^{14}C]glucose (5 μCi/flask, 53.0 mCi/mmole) and D-[6-^{14}C]glucose (5 μCi/flask, 56.2 mCi/mmole) were utilized as radiolabeled precursors in addition to D-[U-^{14}C]glucose. $^{14}CO_2$ evolution studies were performed as above but, at the conclusion of the studies, tissue was removed from the flask and rinsed with ice-cold buffer. Tissue lipids were then extracted and saponified as described previously. The incorporation of ^{14}C into fatty acids, glycerol and CO_2 was then used to determine the per cent contribution of the pentose phosphate pathway to glucose oxidation using calculations C and D of Katz *et al.* based on their theoretical model of cellular glucose metabolism [12]. Values obtained were in close agreement with those reported previously [12].

Assessment of glucose uptake. Glucose uptake was assessed using the method of Livingston and Lockwood [13]. Adipose tissue slices were incubated under conditions described above in the presence of 1 μCi of 2-[^{14}C]deoxyglucose (57 mCi/mmole) for periods of either 15 sec or 3 min. Deoxyglucose is a glucose analogue which possesses little or no affinity for the enzymes involved in glucose utilization subsequent to hexokinase [14]. The rate of its uptake was calculated by subtracting tissue radioactivity at 15 sec from that present at 3 min. This calculation allows correction for the amount of sugar trapped extracellularly [13]. Preliminary experiments had established that uptake was linear for 5 min under these conditions. Reactions were terminated by immersing the tissue in ice-cold buffer. Following three 5-ml washes with buffer, the tissue was homogenized in 5 ml of buffer and the resulting homogenate was evaporated to dryness under N_2. The remaining residue was solubilized with 1 ml of hyamine and, after the addition of scintillation fluid, was measured for radioactivity as described above.

Lipid determinations. Adipose tissue and hepatic and serum lipids were extracted with chloroform–methanol (2:1) [9] and washed [10]. Hepatic free and esterified cholesterol were separated by thin-layer chromatography [10]. Triacylglycerol and cholesterol levels were determined by Auto Analyzer [15]. Total tissue phospholipid levels were measured using the method of Bartlett [16].

Glucose and insulin determinations. Blood glucose levels were determined using the glucose oxidase method of Raabo and Terkildsen [17]. Serum insulin levels were measured by radioimmunoassay [18] using rat insulin as the standard.

Reagents. All general chemicals were of analytical reagent grade. Reagents for the glucose assay and fatty acid free bovine serum albumin were purchased from the Sigma Chemical Co., St. Louis, MO. Hyamine was obtained from the Amersham/Searle Corp., U.S.A. Radiolabeled compounds were supplied by the New England Nuclear Corp., Boston, MA.

Statistics. Results were expressed as means ± S.E.M. Statistical comparisons were made using Student's *t*-test [19].

RESULTS

The animals appeared healthy and gained weight during the feeding period. The average weight gain for ethanol-fed animals was 1.5 ± 0.1 g/day and for controls was 1.6 ± 0.1 (N = 47 pairs). All serum and tissue parameters studied were independent of the weight of the animals.

Serum parameters. Random serum glucose and insulin levels as well as serum lipid levels are presented in Table 1. In the fed state, the ethanol-treated animals exhibited elevated serum triacylglycerol and cholesterol but serum glucose and insulin values were similar in both groups as were fasting serum triacylglycerol and cholesterol levels.

Tissue lipid levels. In keeping with the findings of DeCarli and Lieber [20], chronic ethanol feeding increased hepatic triacylglycerol content (68.3 ± 24.7 mg/g dry weight vs 37.6 ± 8.8 for controls; N = 9 pairs; $P < 0.01$). In addition, the cholesteryl ester content of the liver was also increased (4.9 ± 0.7 mg/g dry weight vs 2.5 ± 0.2 for controls; N = 8 pairs; $P < 0.01$). However, adipose tissue triacylglycerol, cholesterol and phospholipid levels were similar in both sets of animals (Table 2).

Lipid incorporation studies. In adipose tissue from ethanol-fed animals, labeled glucose incorporation into glyceride glycerol was reduced by approximately 50% (Table 3). Incorporation into glyceride fatty acids was also diminished but to a much greater extent (Table 3).

$^{14}CO_2$ evolution studies. In adipose tissue, glucose can be oxidized via the tricarboxylic acid cycle or via the pentose phosphate shunt [21]. The latter pathway is important in supplying NADPH for fatty acid synthesis [21]. The results of the glucose oxidation experiments are presented in Table 4. Chronic ethanol feeding was associated with a reduced $^{14}CO_2$ production by adipose tissue. The contribution of the pentose phosphate pathway to glucose oxidation was similar in both groups.

Deoxyglucose uptake. Glucose uptake studies

Table 1. Serum lipids, glucose and insulin levels in ethanol-fed and control rats

	Postprandial serum triacylglycerols (mg/dl)	Postprandial serum cholesterol (mg/dl)	Fasting serum triacylglycerols (mg/dl)	Fasting serum cholesterol (mg/dl)	Random serum glucose (mg/dl)	Random serum insulin (mg/ml)
Control	62.3 ± 9.7 (8)	103.0 ± 3.6 (12)	31.3 ± 4.6 (9)	91.6 ± 2.2 (9)	162.0 ± 10.4 (6)	5.4 ± 0.1 (6)
Ethanol-fed	97.8 ± 4.0 (8)	129.0 ± 8.4 (12)	36.8 ± 4.7 (9)	87.2 ± 4.9 (9)	162.3 ± 7.0 (6)	5.3 ± 0.1 (6)
Significance	$P < 0.025$	$P < 0.025$	NS*	NS	NS	NS

Values are means ±S.E.M.; the number of pairs of rats is indicated in parentheses.
* Not significant.

Table 2. Triacylglycerol, total cholesterol and total phospholipid levels in adipose tissue from ethanol-fed and control rats

	Triacylglycerols	Total cholesterol (mg/mg lipid free dry weight)	Total phospholipid
Control	30.18 ± 9.35	0.08 ± 0.03	0.15 ± 0.03
Ethanol-fed	28.72 ± 4.11	0.10 ± 0.02	0.13 ± 0.02

Values are means ±S.E.M.; six pairs of rats were used for each experiment.

were performed using a radiolabeled glucose analogue, 2-[1-^{14}C]deoxyglucose. Ethanol feeding had no effect on the uptake of this compound by adipose tissue (338.9 ± 78.9 pmoles/g adipose tissue/3 min in ethanol-fed animals vs 378.8 ± 138.8 for controls; N = 6 pairs).

DISCUSSION

In the present investigation, we found that chronic administration of ethanol to rats decreased adipose tissue lipogenesis as indicated by decreased [^{14}C]glucose incorporation into both glyceride glycerol and glyceride fatty acids. Furthermore, chronic ethanol feeding appeared to impair adipose tissue glucose oxidation and to decrease both pentose phosphate shunt and tricarboxylic acid cycle activity.

It is unlikely that these findings resulted from a decrease in the availability of glucose. Random serum glucose and insulin levels were similar in both groups. In addition, ethanol feeding did not affect the uptake of the glucose analogue, 2-deoxyglucose, by adipose tissue.

Our results contrast with those of Scheig *et al.* [5, 6] who failed to demonstrate an effect of chronic alcohol consumption on rat adipose tissue lipogenesis. These investigators administered ethanol in drinking water—a method which results in negligible blood alcohol levels [22]. In contrast, the method of Lieber and DeCarli [8], which was employed in the present study, results in a greater alcohol intake and blood alcohol levels that are significantly higher (of the order of 65 mg/dl [22]). Cascales and her colleagues [7] have reported recently that chronic ethanol administration stimulates rat adipose tissue lipogenesis. These investigators used incorporation of ^3H_2O into lipids as an index of lipogenesis. However, it is difficult to exclude the possibility that the increased accumulation of label in adipose tissue lipids of ethanol-fed rats resulted from increased hepatic lipogenesis with subsequent transport of labeled triacylglycerol to peripheral fat stores.

Both Scheig *et al.* [5, 6] and Cascales *et al.* [7] administered experimental diets for longer periods than in the present study (up to 260 days) and did not withdraw food from their animals prior to sacrifice. Furthermore, the solid diets employed by these investigators contained less fat (3–10% of energy) than the liquid diets of the present study which contained fat as 35% of energy-lipid levels which more accurately reflect U.S. dietary composition [8]. However, it it not possible, at this time, to determine whether these dietary differences contributed to the different results obtained.

Adipose tissue plays a central role in the clearance and storage of circulating triacylglycerols. However, it cannot resynthesize triacylglycerols directly from glycerol because it lacks glycerol kinase [21, 23]. Therefore, in order to assemble triacylglycerols from fatty acids made available through the action of lipoprotein lipase, it must derive glyceride glycerol from dihydroxyacetone phosphate—an intermediate of the glycolytic pathway [21, 23]. As chronic ethanol feeding decreased synthesis of glyceride glycerol in adipose tissue (Table 3), this defect may contribute to the postprandial hypertriacylglyceridemia observed in rats (Table 1; [24]) and humans [25] following prolonged ethanol consumption and could explain the elevation of serum free fatty acids observed in alcoholics [25]. It is of interest that Fan *et al.* [26] (in a preliminary report) have also demonstrated that adipocytes obtained from patients with alcoholic hyperlipemia have a reduced capacity to synthesize glyceride glycerol.

Adipose tissue fatty acid synthesis was affected to

Table 3. Effect of chronic ethanol feeding on the incorporation of D-[U-^{14}C]glucose into rat adipose tissue glyceride glycerol and fatty acids

	[^{14}C]Glucose incorporated (nmoles/g triacylglycerol)	
	Glyceride glycerol	Fatty acids
Control	2702.0 ± 578.2	2633.8 ± 896.2
Ethanol-fed	1137.5 ± 115.9	60.1 ± 9.1
Significance	$P < 0.01$	$P < 0.001$

Values are means ±S.E.M.; eleven pairs of rats were used in each experiment.

Table 4. Effect of chronic ethanol feeding on the oxidation of D-[U-^{14}C]glucose by rat adipose tissue

	$^{14}CO_2$ evolution*	% Contribution of pentose phosphate pathway
Control	301.9 ± 100.8 (9)	6.3 ± 2.7 (3)
Ethanol-fed	79.0 ± 31.0 (9)	8.1 ± 8.7 (3)
Significance	$P < 0.01$	NS†

Values are means ±S.E.M.; the number of pairs of rats is indicated in parentheses.

* Results are expressed as nmoles [^{14}C]glucose oxidized/g adipose tissue/hr.

† Not significant.

a greater degree than glyceride-glycerol synthesis and was almost totally abolished by chronic ethanol feeding (Table 3). Although we suggest that chronic ethanol feeding may impair the ability of adipose tissue to resynthesize triacylglycerols and therefore contribute to ethanol-induced, postprandial hypertriacylglyceridemia, the adipocytes of ethanol-fed animals may still be faced with a larger postprandial influx of fatty acids derived from lipoproteins secreted by the liver. We speculate that these, in turn, may result in a down-regulation in fatty acid synthesis similar to the down-regulation of HMG-CoA reductase by ingested cholesterol [27].

This study also revealed that chronic ethanol intake impaired glucose oxidation by adipose tissue. Although the exact site of this defect has not been demonstrated, both the tricarboxylic acid cycle and the pentose phosphate shunt appeared equally depressed. Such an alteration in the adipocyte may impair a number of its physiologic functions such as mobilization of fatty acids and could, in part, be responsible for defective lipogenesis.

Despite the decreased lipogenesis demonstrated with labeled glucose as a precursor, adipose tissue triacylglycerol and phospholipid levels were not reduced in ethanol-fed animals. This lack of difference may reflect the relatively short period of ethanol administration—which was sufficient to alter lipogenesis (as indicated by sensitive radiolabeling techniques) but insufficient to lower total lipid content of the tissue. Alternatively, unchanged tissue lipid levels could reflect the nutritional state of the animals at the time of sacrifice. Intact adipocyte function in control rats may have allowed more rapid lipid mobilization during the pre-sacrifice period, thus obscuring small differences in lipid content. Another possible explanation for the observed similarity in glyceride lipid levels between ethanol-fed animals and controls is that chronic consumption of ethanol may also impair adipocyte triacylglycerol breakdown. This idea is consistent with the data of Nilsson and Belfrage [28] who have reported that acetate (a product of ethanol metabolism) impairs lipolysis in isolated rat adipocytes.

In summary, chronic ethanol feeding markedly altered adipose tissue lipogenesis and glucose oxidation. Thus, in addition to the documented effects of chronic ethanol administration on hepatic synthesis of lipoproteins [1], the present findings suggest an additional mechanism for the postprandial hypertriacylglyceridemia of alcoholics. More comprehensive studies using tracer isotopes will be necessary to determine the relative contributions of lipid metabolism in the liver and adipose tissue to alcoholic hyperlipemia.

Acknowledgements—This study was supported by the Ramaciotti Foundation of Australia, the National Health and Medical Research Council of Australia, the National Heart Foundation of Australia and the Australian Associated Brewers. J. S. W. was the recipient of an N.I.H. Fogarty International Fellowship and a C.R.B. Blackburn Travelling Fellowship of The Royal Australasian College of Physicians. The authors are grateful to Patrice M. Ardies for the expert typing of this manuscript.

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