Disposition of Dietary Ethanol Carbons in Rats: Effects of Gender and Nutritional Status

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Dietary ethanol is an important contributor to total caloric intake and has been associated with gender-specific alterations in body weight and the risk for coronary heart disease. To understand the metabolic basis of these effects, it is important to first clarify the effects of gender and nutritional state on the metabolic fate of dietary ethanol. Tracer studies were therefore performed using ^{14}C-labeled ethanol in fasted or fed male and female Sprague-Dawley rats (N = 64) previously unexposed to ethanol. 1-^{14}C-ethanol (4.5 μCi) was mixed with unlabeled ethanol (for a total ethanol dose equal to 10% of total daily caloric intake) and a 3-kcal liquid meal and administered through gastric feeding tubes. $^{14}CO_2$ production was measured over the subsequent 8 hours. The ^{14}C content of skeletal muscle, liver, adipose tissue, gastrointestinal (GI) tract, brain, heart, kidney, and serum was determined at 4 time points following tracer administration (20 minutes and 3, 8, and 24 hours; $n = 4$ at each time point). Tracer content on a whole-body level was significantly greater in skeletal muscle compared with liver in all groups ($1.32 \pm 0.02 \times 10^6$ v $0.27 \pm 0.02 \times 10^6$ dpm, $P < .001$). Skeletal muscle tracer content decreased rapidly after 3 hours, whereas liver tracer content remained fairly constant throughout the study period. Fed female rats were the exception, with a significant increase in the tracer content of total liver and liver lipid at 8 hours. The tracer content was higher in the lipid extracts in liver from fed rats compared with fasted rats ($1.08 \pm 0.19 \times 10^5$ v $0.48 \pm 0.08 \times 10^5$ dpm, $P = .002$). While male rats exhibited a fairly constant tracer content in adipose tissue throughout the 24-hour period, female rats showed an increase in adipose tissue tracer content at 8 and 24 hours, with levels 3 to 4 times those of the male animals ($5.91 \pm 1.42 \times 10^4$ v $1.55 \pm 0.42 \times 10^4$ dpm, $P = .02$). These results demonstrate that (1) skeletal muscle plays an important role in the metabolism of dietary ethanol, (2) the fed state appears to favor the conversion of ethanol-derived carbons to lipid, and (3) female rats have a greater propensity to convert ethanol-derived carbons to lipid and to store these carbons in adipose tissue.

ETHANOL is responsible for over 5% of the energy consumed in the average American diet, and for those who drink alcohol on a regular basis, it accounts for approximately 10% of energy intake.[1] Ethanol is therefore an important macronutrient, yet its effects on energy and nutrient metabolism remain unclear. Epidemiological studies have also shown that body weight does not increase with ethanol consumption despite a greater total daily caloric intake. In fact, ethanol intake in women appears to reduce body weight.[2-5] Rat studies have also shown reductions in body weight gain when ethanol is added to the diet.[6-9]

Not only does ethanol play an important role in dietary intake and energy balance, there is increasing evidence that mortality rates are reduced in those who consume low to moderate amounts of ethanol compared with abstainers and heavy drinkers.[10-14] This health benefit has been primarily linked to a decreased risk of cardiovascular disease induced by multiple factors, including an increase in high-density lipoprotein cholesterol. However, the exact mechanisms by which the addition of ethanol to the diet alters lipid metabolism continue to be a matter of much debate.

To fully comprehend ethanol's effects on energy balance and lipid metabolism, the metabolism and processing of dietary ethanol must be better understood. It is generally accepted that the majority of ethanol is initially oxidized in the liver to acetaldehyde by one of 3 systems: alcohol dehydrogenase in the cytosol, the microsomal ethanol-oxidizing system (MEOS), and catalase in peroxisomes. The resulting acetaldehyde is then converted to acetate in the mitochondria associated with the conversion of NAD to NADH. However, the subsequent fate of ethanol-derived acetate is not known. A few studies have suggested that because of changes in the redox potential (high NADH/NAD ratio), ethanol-derived acetate is released from the liver for extrahepatic metabolism. Other studies have shown increased hepatic de novo lipogenesis in the fed state with ethanol and acetate treatment.[15-21] However, the relative importance of de novo lipogenesis versus extrahepatic metabolism is not known. And in contrast to the epidemiological studies, studies on whole-animal lipid oxidation and lipolysis suggest that acute ethanol intake results in a milieu favoring fat storage.[22-30]

These findings suggest a number of questions: Do ethanol-derived carbons undergo oxidation by skeletal muscle? Do these carbons follow lipogenic pathways ultimately to be stored in adipose tissue? Are the effects of ethanol on lipogenesis related to gender or nutritional state? And what is the relative contribution of lipogenesis and storage as compared with oxidation? The present studies were undertaken to answer these questions by tracing the movement of dietary ethanol through the body over time. It was hypothesized that skeletal muscle plays an important role in the metabolism of ethanol, the fed state favors lipogenesis, and males are more likely to store ethanol-derived carbons. These hypotheses were tested by administering 1-^{14}C-ethanol via surgically placed gastric feeding tubes in male and female Sprague-Dawley rats in the fed state and fasted state and following the fate of the labeled carbons over the subsequent 24 hours. By placing the tracer directly in the relevant pool, ie, the diet, a more physiological feeding paradigm was achieved.

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MATERIALS AND METHODS

Animals

Male and female Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and housed in a temperature-controlled room on a 12-hour light/dark cycle (8 PM to 8 AM) at the Animal Resource Center of the Surgical Research Facility of Denver Health Medical Center. Rats were fed chow (laboratory rodent diet: 23% protein, 10% fat, and 50% carbohydrate by calories) ad libitum except where noted. The protocols were approved by the Animal Use Committees of the University of Colorado Health Sciences Center and Denver Health Medical Center.

Gastric Feeding Tube Placement

To facilitate the delivery of tracers to previously fed rats, gastric feeding tubes were surgically placed with a method similar to that used by Sclafani and Nissenbaum[31] in the rats 7 days before the studies. Previously fasted rats were anesthetized with ketamine and xylazine. A midline incision was made through the abdominal skin and linea alba. The peritoneum was incised and the stomach was pulled through the abdominal wound. A purse-string suture was placed in the antrum of the stomach. An incision was made in the wall of the stomach, and a sterile silastic tube that was previously prepared with a small drop of silicone rubber on the end was placed through the wall into the lumen. The purse-string suture was tightened and tied, and a small piece of Marlex (Davol, Cranston, RI) mesh was glued to the outer stomach surface with methyl methacrylate. The muscular layer of the abdominal wall was then closed with interrupted sutures. The silastic tubing was tunneled subcutaneously from the abdominal incision to exit through the skin at the nape of the neck. The abdominal skin incision was closed with interrupted sutures. The silastic tubing exiting at the nape of the neck was then cut to the proper length, a Luer Loc hub (Becton Dickinson, Franklin Lakes, NJ) was secured in the exposed end of the tube with methyl methacrylate, and the skin incision was closed with interrupted sutures. The rat was then returned to its cage to recover. The tubes were kept clean by flushing with water.

Feeding Study

Following surgery, the rats were examined and weighed daily to ensure recovery. Rats that lost greater than 10% of their baseline weight and sustained the weight loss, were not used for the studies. The animals were fed rat chow and water ad libitum during the postoperative period until the studies were performed. To acclimate the rats to the use of feeding tubes, 3-mL liquid meals were introduced through the tubes over 15 minutes on a daily basis beginning on postoperative day 2. Studies were performed 7 days following insertion of the feeding tubes. Prior to the studies the daily chow consumption was measured to determine the daily caloric intake. For studies in the fasted state, rats were fasted for 15 hours (5 PM to 8 AM). For studies in the fed state, rats were allowed to eat ad libitum the night before the studies were performed. The rats had no prior exposure to ethanol. Meal tracer studies were performed in a manner similar to that previously described.[32] A 4.5-μCi volume of 1-^{14}C-labeled ethanol was mixed with an amount of unlabeled ethanol equivalent to 10% of the rat's predetermined daily caloric intake. This was mixed with 3 mL semisynthetic liquid diet (Ensure; Ross Products, North Chicago, IL: 21% protein, 24% fat, and 55% carbohydrate). This "meal" was then administered through the gastric feeding tube at a rate of 30 mL/min. After the meal plus tracer was administered, the rats were placed in a metabolic chamber for determination of $^{14}CO_2$ production. The air passing through the metabolic chamber was bubbled through methyl-benzethonium hydroxide to trap CO_2 and collected in 20-minute aliquots continuously for up to 8 hours. The rats were then removed from the chamber for tissue collection. For the 24-hour time point, rats from both fed and fasted groups were returned to their usual quarters and fed chow ad libitum until tissue collection. This was necessary to prevent the metabolic consequences of a prolonged period of continuous fasting.

Tissue ^{14}C Content Determination

At 4 time points following administration of the tracer (20 minutes and 3, 8, and 24 hours; n = 4 at each time point), tissue samples were obtained. After induction of deep anesthesia by intraperitoneal Nembutal (Abbott Laboratories, North Chicago, IL), samples of skeletal muscle (soleus and medial gastrocnemius) were removed. Three milliliters of blood was collected from the inferior vena cava for determination of ^{14}C content and biochemical analysis. The rats were then euthanized by an intracardiac injection of Nembutal, and the adipose tissue (gonadal and omental), liver, gastrointestinal (GI) tract, heart, kidneys, and brain were removed. The ^{14}C content of all excrement was measured by swiping down the metabolic chamber. Tissues were individually weighed and homogenized in 3:1 mL/g normal saline. Duplicate 0.5-mL aliquots of each tissue and serum were transferred to scintillation vials and 1 mL tissue solubilizer (Solvable; Packard, Downers Grove, IL) was added and placed in a 50°C water bath for 3 hours. Then, 0.1 mL H_2O_2 (to bleach the sample) and 10 mL scintillation cocktail (Aquasol; Packard) were added to each sample. The ^{14}C content of the sample was then determined by scintillation counting. Adipose tissues were processed in a different manner. Each sample was individually weighed and homogenized in 12 mL 2:1 chloroform:methanol and then rotated for 4 hours. The organic and aqueous phases were separated by the addition of 2 mL H_2SO_4 and centrifuged at 2,000 rpm for 10 minutes. The procedure was repeated, the bottom layer (lipid class) was removed, and the ^{14}C content was determined by scintillation counting.

Separation of Lipids

Lipid extraction was performed on skeletal muscle, liver, and serum to determine the relative amount and nature of the lipid containing ethanol-derived ^{14}C. The tissues were individually weighed and homogenized in 3:1 mL/g normal saline. Six milliliters of 2:1 chloroform: methanol was added to the homogenate and rotated for 2 hours. Two milliliters of saline was added and centrifuged for 15 minutes. The top layer was removed, and this procedure was repeated. The bottom layer was allowed to dry and was then resuspended in a small volume of chloroform. The aqueous and lipid ^{14}C content of each sample was determined by scintillation counting.

Biochemical Serum Analysis

Biochemical analyses were performed on serum obtained from the groups at the 8-hour time point. Ethanol concentrations were measured by the enzymatic reduction of NAD to NADH (kit #333; Sigma, St Louis, MO). Glucose concentrations were measured using the YSI Sidekick model 1600 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Triglyceride concentrations were measured based on enzymatic measurement of glycerol (Sigma kit #320). Insulin and leptin concentrations were measured with radioimmunoassay kits (Linco Research, St. Charles, MO; #RI-13K and #RL-83K, respectively).

Calculations and Statistical Analysis

Each rat received the same tracer dose of ^{14}C-labeled ethanol mixed with an amount of unlabeled ethanol calculated to represent 10% of the total daily caloric intake. All values were therefore corrected for the specific activity (SA) of ^{14}C in the study meal. The SA was calculated as the ratio of dpm of ^{14}C-ethanol to the total dose of unlabeled ethanol administered to the rat. Tissue data are expressed as the total ^{14}C content in each tissue at each time point. This was calculated as the ^{14}C content per gram of tissue multiplied by the total weight of the tissue. The total adipose tissue mass and skeletal muscle mass were calculated by

Table 1. Rat Characteristics at the Time of Study

Characteristic	Female	Male
Body weight (g)	241.6 ± 2.2*	312.6 ± 6.1*
Fat-free mass (g)	222.8 ± 1.9*	296.4 ± 6.5*
Body fat (%)	8.0	7.1
Caloric intake (kcal/d)	76.1 ± 2.4*	102.8 ± 2.3*
Ethanol dose		
g	1.07 ± 0.03*	1.44 ± 0.03*
g/kg	4.44 ± 0.15	4.67 ± 0.13

NOTE. Values are the mean ± SEM (n = 32 for each gender).
*$P < .001$ between gender groups.

multiplying the total body weight times either the percent body fat (males, 7%; females, 8%) or percent skeletal muscle (males, 45%; females, 44%). The medial gastrocnemius muscle is a mixed-fiber muscle (78% fast-twitch, 17% fast-twitch oxidative, and 5% slow-twitch) and thus is a good representative of the muscle fiber type composition of the total body (76% fast-twitch, 19% fast-twitch oxidative, and 5% slow-twitch).[32] The ^{14}C activity per gram of this tissue was therefore used to calculate total skeletal muscle ^{14}C activity. Total serum ^{14}C content was calculated as the ^{14}C content per gram multiplied by the plasma mass (0.0385 times the body weight). The values for percent body fat, percent skeletal muscle, and plasma mass were derived from previous data on male rats.[33,34] Values for the females were derived by body composition analysis on a subset of rats (n = 10) by carcass lipid extraction. $^{14}CO_2$ production is expressed in dpm per kilogram of fat-free mass, correcting for the mass of tissue responsible for oxidation. Fat-free mass was calculated as the total body weight times the percent nonfat mass. The data are presented in graphical form as the mean ^{14}C activity at each time point (mean ± SEM). Statistical analysis was performed with SigmaStat statistical software (Jandel Scientific, San Rafael, CA). The figures were visually inspected for potential differences between groups at each time point within a tissue. A 2-way ANOVA was then performed between groups (fed/fasting and female/male) at these time points. For comparisons of $^{14}CO_2$ production between groups, a 2-way ANOVA of the integrated area under the curve was performed. For direct comparisons between two groups or two different tissues, Student's *t* test was used.

RESULTS

The rats did not lose a significant amount of weight in the postoperative period. Characteristics of the experimental groups at the time of the studies are shown in Table 1. There were statistically significant gender differences in body weight, fat-free mass, and caloric intake ($P < .001$). Because the total administered dose of ethanol was based on caloric intake, there was also a significant gender difference in this parameter. There were no significant gender differences in body composition, although the male animals were larger. Triglyceride (fasted *v* fed, 29.8 ± 2.3 *v* 58.0 ± 11.2 mg/dL, $P < .05$) and leptin (fasted *v* fed, 0.40 ± 0.04 *v* 1.02 ± 0.16 ng/mL, $P < .01$) concentrations were significantly higher in fed animals. The nutritional state did not significantly affect glucose (fasted *v* fed, 202.3 ± 7.5 *v* 191.1 ± 14.1 mg/dL) or insulin concentrations (fasted *v* fed, 1.55 ± 0.29 *v* 2.14 ± 0.40 ng/mL). Triglyceride, leptin, glucose, and insulin levels were not significantly affected by gender, although the number of measures was small.

Figure 1 shows the corrected $^{14}CO_2$ production adjusted for fat-free mass over 8 hours, depicting ^{14}C-ethanol oxidation. There was a quick increase in $^{14}CO_2$ production, which then appeared to reach a steady state by 2 hours. At 24 hours, the mean $^{14}CO_2$ production over 20 minutes was low but still measurable (1.19 ± 0.32 × 10^4 dpm/kg/20 min). There were no statistically significant differences in the total area under the curve across gender or nutritional state. However, there was a statistically significant gender difference during the first 3 hours (female *v* male, 1.19 ± 0.16 × 10^6 *v* 0.70 ± 0.07 × 10^6 dpm/kg, $P = .002$). Interestingly, there was a decline in $^{14}CO_2$ production in fed female rats between 180 and 200 minutes that coincided temporally with an increase in tracer accumulation in liver lipid (as will be shown later).

Figure 2 shows the total ^{14}C content in the total body skeletal muscle over time. The tracer content increased to very high levels over the first 20 minutes after tracer administration and remained high over the following 3 hours. Approximately 24%

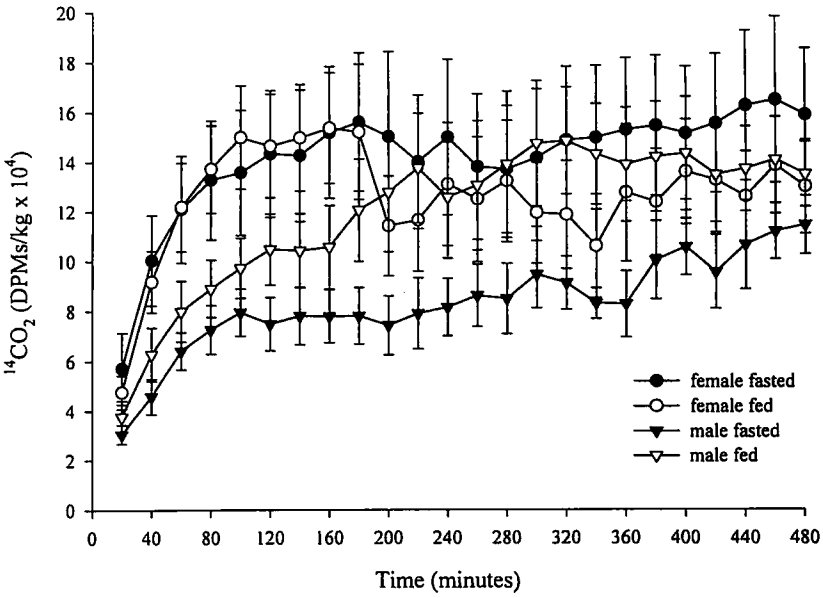

Fig 1. Production of $^{14}CO_2$ collected every 20 minutes over 8 hours following administration of a meal containing 1-^{14}C-ethanol at time 0. Over the first 3 hours, $^{14}CO_2$ production was greater in females *v* males ($P = .002$). Data are corrected for SA of tracer in the meal and expressed as the mean ± SEM dpm/kg fat-free mass (oxidizable tissue). n = 16 at 20 minutes, n = 12 at 3 hours, and n = 8 at 8 hours per group.

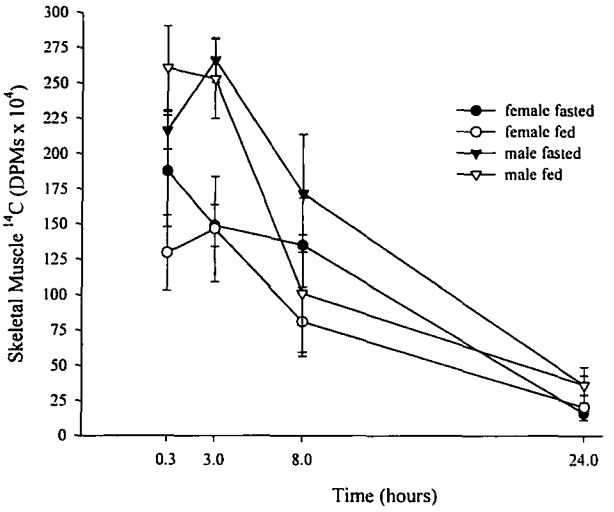

Fig 2. Total ^{14}C content of skeletal muscle over time. Data represent total body muscle ^{14}C content (tracer content per gram of tissue times percent total body muscle times body weight). Tracer content was greater in the males over the entire time course ($P < .001$). Data are corrected for SA of the tracer in the meal and expressed as the mean ± SEM dpm (n = 4 per group at each time point).

of the total administered ^{14}C dose was found in skeletal muscle at 20 minutes. Skeletal muscle tracer content then decreased rapidly after 3 hours. On a whole-body level, the mean tracer content over time was significantly greater in skeletal muscle compared with liver in all groups (muscle *v* liver, $1.32 \pm 0.02 \times 10^6$ *v* $0.27 \pm 0.02 \times 10^6$ dpm, $P < .001$). When expressed on a per-gram basis, ^{14}C content was greater in the liver versus the skeletal muscle (liver *v* muscle, $2.79 \pm 0.15 \times 10^4$ *v* $1.11 \pm 0.09 \times 10^4$ dpm/g, $P < .001$). There was a significantly greater skeletal muscle tracer content in the males compared with the females ($1.68 \pm 0.09 \times 10^6$ *v* $1.10 \pm 0.09 \times 10^6$ dpm, $P < .001$). This was likely due in part to the greater muscle mass of the males. When expressed on a per-gram basis, these gender differences disappeared. The nutritional state did not appear to affect skeletal muscle tracer content except at 8 hours, when fasted levels were significantly higher ($P = .029$). Separation of the skeletal muscle tissue into aqueous and lipid fractions demonstrated that over the first 3 hours, approximately 86% of the tracer was found in the aqueous fraction. By 8 to 24 hours, the lipid fraction increased to 50% of the total skeletal muscle ^{14}C content. There was also a trend for a greater increase in tracer content in the lipid fraction in the fed female group at 8 hours, consistent with the enhanced lipogenesis found in this group.

The total tracer content in the liver over time is depicted in Fig 3A. By 20 minutes, liver tracer content reached a plateau, which then remained fairly constant throughout the study period. Fed female rats were the exception, with an increase in liver tracer content at 8 hours ($4.02 \pm 1.24 \times 10^5$ *v* $1.98 \pm 0.24 \times 10^5$ dpm, $P = .02$). This difference was accentuated when specifically examining the ^{14}C content of the lipid fraction of liver tissue ($2.60 \pm 0.76 \times 10^5$ *v* $0.79 \pm 0.16 \times 10^5$ dpm, $P = .003$; Fig 3B). There was a significant increase in the average tracer content of liver lipid at 8 hours in the fed groups ($2.19 \pm 0.52 \times 10^5$ *v* $0.70 \pm 0.12 \times 10^5$ dpm, $P < .001$), and the liver had higher levels of tracer in the lipid extracts over the entire time course in fed rats compared with fasted rats ($1.08 \pm 0.19 \times 10^5$ *v* $0.48 \pm 0.08 \times 10^5$ dpm, $P = .002$), consistent with enhanced lipogenesis in the fed state.

Figure 4 shows the ^{14}C content in adipose tissue over time. Male rats had constant levels of tracer in adipose tissue throughout the 24-hour period ($2.81 \pm 0.63 \times 10^4$ dpm). Female rats, on the other hand, showed a significant increase in adipose tissue tracer content at 8 and 24 hours, with levels 3 to 4 times the values in the male animals ($5.90 \pm 1.42 \times 10^4$ *v* $1.55 \pm 0.4 \times 10^4$ dpm, $P = .02$). However, adipose tissue levels at 24 hours represented only 0.36% to 0.8% of the total administered ^{14}C dose.

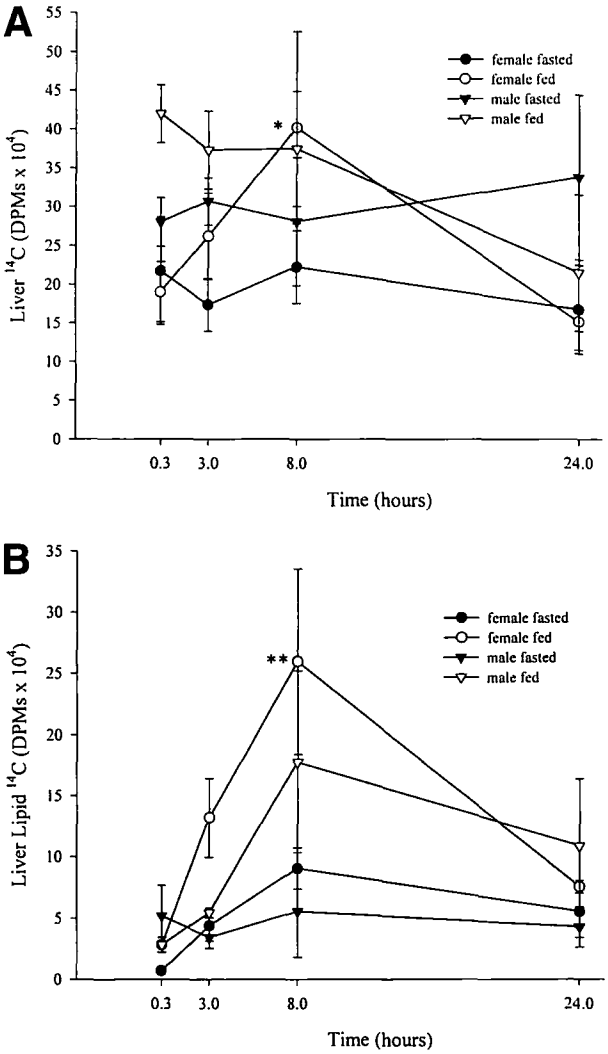

Fig 3. (A) Total ^{14}C content of liver over time. Data represent ^{14}C content of the entire liver (tracer content per gram times liver weight). Tracer content increased significantly at the 8-hour time point in the fed female group (*$P = .02$). (B) Total ^{14}C content of liver lipid over time. Data represent ^{14}C content of the lipid fraction of the entire liver. Tracer content increased at the 8-hour time point in the fed female group ($P = .003$). Mean values were significantly greater for fed groups *v* fasted groups over the time course ($P = .002$). Values are corrected for SA of the tracer in the meal and expressed as the mean ± SEM dpm (n = 4 per group at each time point).**

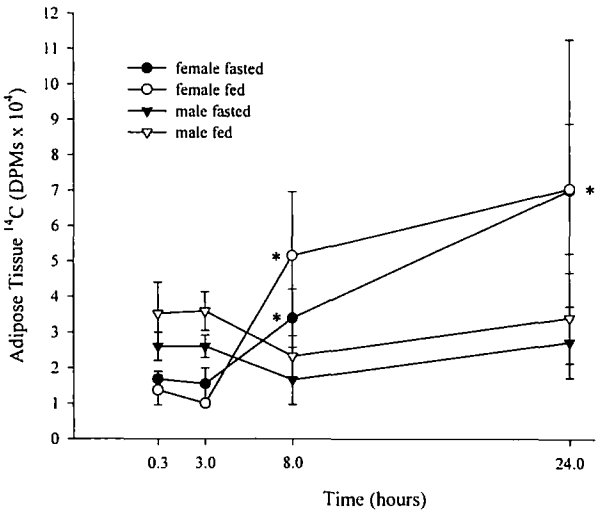

Fig 4. Total ^{14}C content of adipose tissue over time. Data represent total body fat ^{14}C content (tracer content per gram times percent body fat times body weight). Female rats showed a significant increase in adipose tissue tracer levels at 8 and 24 hours *v* males (*$P = .02$). Data are corrected for SA of the tracer in the meal and expressed as the mean ± SEM dpm (n = 4 per group at each time point).

Blood ethanol concentrations and the total ^{14}C content of the serum over time showed no significant differences between groups. However, blood ethanol decreased much more rapidly than the ^{14}C content of the serum, especially in the first 8 hours (62% *v* 35%), indicating that the serum ^{14}C could represent acetate, HCO_3, CO_2, or lipid in addition to ethanol.

There were no differences in the total GI tract ^{14}C content (actual GI tract tissue plus its contents) between groups. The ^{14}C content of the excrement over 8 hours was $5.40 \pm 0.43 \times 10^4$ dpm and was not significantly different between groups. This suggested that differences in absorption between groups do not cause the observed changes. The total ^{14}C content of the other tissues analyzed (brain, kidney, and heart) also was not significantly different between groups and was low compared with the tissue content reported earlier (pooled mean, $1.82 \pm 0.16 \times 10^4$ dpm).

DISCUSSION

These studies were performed to examine the fate of ethanol-derived carbons over a 24-hour period after GI administration. The effects of gender and nutritional state were studied specifically. A number of conclusions can be drawn from these studies. First, skeletal muscle plays a vital role in the metabolism of ethanol-derived carbons. Second, ethanol-derived carbons are converted to lipid, especially in the fed state. And third, females store ethanol-derived carbons in adipose tissue. However, the magnitude of lipogenesis and storage is small compared with the oxidation of ethanol-derived carbons.

The present data demonstrate that skeletal muscle plays an important role in the metabolism of dietary ethanol. The level of ethanol-derived carbons found in skeletal muscle in the first 3 hours after administration was significantly higher versus all other tissues, including the liver. This is well depicted in Fig 5, which compares the mean tracer levels of different tissues pooled from all groups. The high skeletal muscle tracer content also coincided with a significant increase in $^{14}CO_2$ production, likely representing ethanol or acetate oxidation at the level of skeletal muscle. Because skeletal muscle contains little to no alcohol dehydrogenase, these findings likely represent acetate oxidation, although it is conceivable that ethanol metabolism by MEOS or catalase plays a role. Interestingly, as the skeletal muscle ^{14}C content decreased between 3 and 8 hours, $^{14}CO_2$ production remained constant, suggesting that either ethanol-derived carbons are oxidized by other tissues or enzyme kinetics are quickly saturated in skeletal muscle, resulting in slow, constant oxidation. Limb-balance studies have shown that as much as 25% to 50% of acetate is oxidized by skeletal muscle.[35-38] The present study shows the quantitative importance of skeletal muscle in the metabolism of ethanol on a whole-body level. In light of these findings, future studies on the effects of ethanol intake on energy balance and lipid metabolism should include investigations on the interaction of acetate and lipid fuels in skeletal muscle.

The present studies also suggest that a portion of dietary ethanol is converted to lipid. Hepatic levels of ethanol-derived carbons remained constant throughout the 24-hour period. This could represent persistent ethanol oxidation to acetate throughout the 24-hour period after administration, although this is unlikely since blood ethanol levels are essentially zero by 24 hours. The persistent liver tracer levels more likely represent carbons involved in direct or indirect hepatic de novo lipogenesis. This was most evident in the fed state, in which a significant increase in ethanol-derived carbons at 8 hours was observed in the liver lipid fraction of fed rats. In support of the present findings, prior studies have shown increased rates of hepatic de novo lipogenesis with ethanol and/or acetate.[15-21] However, these studies have not demonstrated that ethanol-derived carbons are directly involved in this process. The present findings also show a significant nutritional effect on the

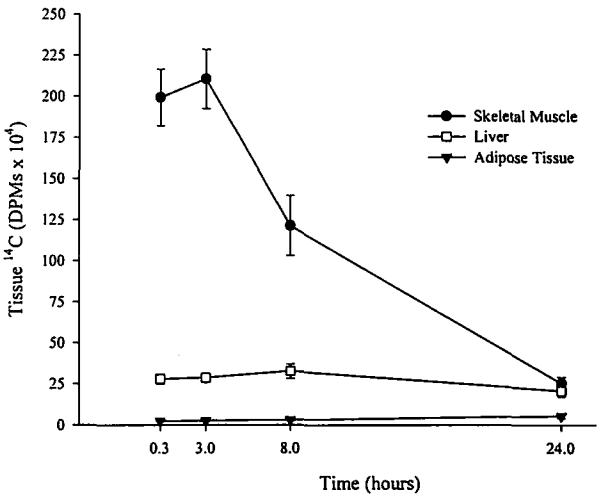

Fig 5. Mean ^{14}C content over time for total skeletal muscle, liver, and adipose tissue pooled from all groups, showing the importance of skeletal muscle relative to other tissues on a whole-body level in the metabolism of dietary ethanol. Data are corrected for SA of the tracer in the meal and expressed as the mean ± SEM dpm (n = 16 per group at each time point).

content of ethanol-derived carbons in liver lipids possibly representing increased lipogenesis in the fed state. It has been shown previously that hepatic lipogenesis from ^{14}C-acetate is inhibited with fasting.[19] Although important, the overall contribution of ethanol-derived carbons to lipogenesis was small on a total-body level as compared with skeletal muscle oxidation. This may help to explain the epidemiological finding that ethanol intake does not cause weight gain.

A significant gender effect on the partitioning of dietary ethanol-derived carbons is evident in the present studies. The most striking difference is the accumulation of ethanol-derived carbons in the adipose tissue of females over 24 hours, which was not found in the males. Again, in comparison to skeletal muscle, adipose tissue ^{14}C content was relatively small. In contrast to the epidemiological data on chronic ethanol consumption that show reduced weight in women who are drinkers, the present findings indicate that acute ethanol intake appears to favor fat storage in females. A number of other metabolic studies have also found that acute ethanol intake may enhance lipogenesis and inhibit both lipolysis and fat oxidation, all promoting fat storage. These conflicting results cannot be reconciled by the results of the present study. In the fed state, the increase in female adipose tissue tracer levels coincided with the significant increase in liver tracer levels, suggesting hepatic lipogenesis as the source. However, the ethanol-derived carbons found in adipose tissue also could be explained by direct adipose tissue de novo lipogenesis. Prior studies have found conflicting results for the effects of ethanol/acetate on adipose tissue de novo lipogenesis, with some showing a decrease while others show an increase.[15,39-41] No conclusions about the site of lipogenesis can be made from the present studies. These findings also do not explain the more pronounced weight loss found in female drinkers.[2-5]

In these studies, the acute metabolism of ethanol-derived carbons has been examined and clarified. However, there are several limitations that must be discussed. First, these studies have been performed in rats previously unexposed to ethanol. As already mentioned, the epidemiological data on chronic ethanol consumption are in contrast to the metabolic data on acute ethanol consumption. However, it was believed that the "acute" metabolism of dietary ethanol must be determined before adaptation to chronic ethanol consumption can be studied. Second, the present studies have only examined the metabolism of a single dose of ethanol. The dose, 10% of the total caloric intake, was chosen to represent an average moderate dose in humans and the dose found to have maximal health benefits. Although this is proportionally a much larger dose for rats than for humans (when expressed as grams of ethanol per kilogram of body weight), this dose is significantly lower than most doses used in other published studies.[6-9] This is an important point, as different levels of daily ethanol intake have profound clinical implications. Third, in the present studies, the chronically consumed diet was low in fat content. Studies have shown that the fat content of the baseline diet may alter ethanol's metabolic effects.[7] Fourth, only a maximum of 47% of the administered tracer dose could be accounted for in the CO_2 and tissue analyses. This is likely due to incomplete tracer recovery in CO_2 or the analyzed tissues or to tracer in unanalyzed tissues. Fifth, the rats were anesthetized with Nembutal to obtain fresh, viable muscle samples. Nembutal has been shown to cause mild insulin resistance within 3 to 10 minutes after administration.[42,43] In this study, tissues were collected within 2 to 3 minutes after Nembutal injection, and in addition, all groups received the same treatment and were compared with each other and not with unanesthetized animals. Finally, using lipid separation techniques, we have been able to determine whether the ethanol-derived carbons are in the form of lipids or water-soluble substances. However, it is unclear whether the aqueous fractions of ethanol-derived carbons are in the form of ethanol, acetate, or other compounds such as protein or carbohydrate.

In conclusion, high levels of ethanol-derived carbons are found in skeletal muscle, signifying that skeletal muscle plays a vital role in the metabolism and possible ultimate oxidation of dietary ethanol. Based on these findings, future studies on the effects of ethanol consumption on energy balance and lipid metabolism should give particular attention to the effects at a skeletal muscle level. In addition, dietary ethanol is converted to lipid, albeit in small amounts. This likely represents increased hepatic de novo lipogenesis, which appears to be increased in the fed state and in females. Females are also more likely to store ethanol-derived carbons in adipose tissue than males. The mechanisms for these gender and nutritional effects are unclear. It is also unclear as to how ethanol itself may impact the metabolic milieu, such as by affecting metabolically active enzymes, further influencing its own disposal. Future studies will be necessary to examine these issues.

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