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Effect of Ethanol on Fatty Acid Oxidation in the Perfused Livers of Starved, Fed, and Fat-fed Rats

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On the basis of measurements of oxygen uptake, ethanol and oleate removal, and the production of ketone-bodies and acetate by the perfused liver, the effect of ethanol on the flow through the citric acid cycle and the β-oxidation pathways was calculated. Studies were carried out with livers from fed, 48 h starved, and fat-fed animals. Ethanol depressed flow through the citric acid cycle by 40 to 60 % depending on the nutritional state of the rat. The addition of pyruvate partly overcame this inhibition. The pathway of β-oxidation was also blocked by the addition of ethanol, although except for the fat-fed rats, ketone-body production was unaltered. The results suggest that, if inhibition of the citric acid cycle by ethanol favours an accelerated rate of ketogenesis, this effect may be concealed by a block in β-oxidation which limits the supply of acetyl-CoA for ketone-body formation.

Leloir and Muñoz [1] have calculated that in appropriate circumstances as much as three-quarters of the oxygen consumed by the liver may be used for the oxidation of ethanol. In more recent works [2–8] it has been proposed that the primary effect of ethanol oxidation in influencing lipid metabolism in the liver, results from its effect on the [free NAD^+]/[free NADH] ratio. However, the detailed nature of the interaction is still under debate.[9] Conflicting results have also appeared regarding the quantitative effects of ethanol oxidation on the β-oxidation pathway and on ketone-body metabolism. Thus Williamson *et al.*[7] indirectly showed that ethanol inhibited the β-oxidation of olelate while other authors [10] suggested unaltered flow through this step. Perfusion experiments [4] and investigations *in vivo* [9,11] indicate a tendency towards increased rate of ketone-body formation although an opposite effect has also been reported.[12]

Consequently, the present study of the perfused liver was undertaken to examine the interaction and its nature between ethanol oxidation and the metabolic steps related to fatty acid oxidation.

MATERIAL AND METHODS

Animal treatment. Female Wistar rats (180 – 230 g) from the laboratory's stock were used. The animals were divided into three groups and pretreated as follows: (1) starved for 48 h, (2) fed *ad libitum* the standard small-animal diet (Astra-Ewos, Södertälje, Sweden), (3) fed a high-fat diet [13] for 10 days. The high-fat diet consisted of 90 parts margarine and 10 parts casein, and was supplemented with salts and vitamins. During the first two days on this diet, the animals ate little and lost weight (10 – 30 g). After 2 or 3 days, the rats accepted the diet and their weights remained constant.

Chemicals. Oleic acid (*puriss.*) was obtained from Fluka AG Chemische Fabrik, Buchs, Switzerland. Oleic acid of very high purity, as shown by gas chromatography, was a generous gift from Prof. E. Stenhagen of the Institute of Medical Biochemistry, Gothenburg, Sweden. There were no notable differences in the results obtained with the two oleic acid compounds. Other substrates, co-enzymes, and crystalline enzymes were supplied by Biochimica Boehringer, Mannheim, West Germany, Sigma Chemical Co, St Louis, USA and E. Merck AG, Darmstadt, West Germany. Oleic acid was added to the perfusate as a neutral solution bound to albumin.[14] All other substrates were neutralized before addition.

Liver perfusions. The method of liver perfusion was that described by Hems *et al.*[15] The perfusion medium was composed of physiological saline,[16] to which had been added 2.6 % bovine serum albumin, fraction V (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.), and aged washed human erythrocytes. The medium was gassed with CO_2:O_2 (6.5:93.5) during the perfusion. Unless otherwise stated, substrates were added to the medium 38 min after the start of the perfusion. At various time intervals, usually every 10 min, samples of perfusate were taken. These samples were used for the determination of different substances. Oxygenated samples (3 ml) were taken 5 min after the start of the perfusion. Venous samples (3 ml) were taken at 37, 53, and 68 min or more frequently. Oxygen was measured in the arterial and venous samples. In the presence of oleate (2 mM) the arterial oxygen content decreased 17.4 ± 2.5 S.E.M. %/h ($n = 7$), due to haemolysis. It was therefore necessary to estimate the arterial-oxygen content more frequently (at 15, 50, and 70 min) when a fatty acid had been added. The rates in the tables refer to maximum rates during 30 min after addition of substrate.

Analytical methods. The procedures for the determinations of β-hydroxybutyrate, acetoacetate, glucose, lactate, pyruvate, and ethanol were as previously described by Williamson *et al.*,[17] Ross *et al.*,[18,19] and Krebs *et al.*[20] Acetate was determined by a modifica-

Table 1. Rates of removal of ethanol and formation of acetate in perfused livers of starved, fed and fat-fed rats. After a preliminary perfusion period of 38 min, ethanol was added to a final concentration of 10 mM. Other substrates were also added at 38 min. The rats were starved for 48 h, fed a normal diet or fed a high-fat diet (see "Material and Methods"). In the experiment with starved rats, the ethanol which evaporated was caught in a trap containing cold destilled water (2°). The rate of evaporation was 11.5 ± 1.0 S.E.M. μmol/(h g wet wt), ($n = 7$). The rates, which have been corrected for evaporation, are given as means ± S.E.M. with the numbers of observations in parentheses.

	Metabolic changes (μmol/h per g wet wt)					
	Starved for 48 h		Normal diet		High-fat diet	
Substrate added	Ethanol	Acetate	Ethanol	Acetate	Ethanol	Acetate
None	118 ± 7.4 (7)	81 ± 4.5 (7)	137 ± 6.4 (6)	101 ± 6.3 (6)	89 ± 5.3 (4)	77 ± 4.3 (15)
Oleate (2 mM)	–	–	101 ± 6.3 (6)	95 ± 6.0 (6)	72 ± 3.0 (4)	62 ± 3.6 (4)
Pyruvate (10 mM)	169 ± 3.8 (7)	–	–	–	–	–

tion of the microdiffusion method fo Serlin and Cotzias [21] as described by Keane.[22] Fatty acids were estimated by the colorimetric method of Itaya and Ui.[23] Oxygen was measured manometrically according to Van Slyke and Neill.[24]

RESULTS

The effects of ethanol on approximate rates of β-oxidation of fatty acids and flow through the citric acid cycle are shown in Table 4. The data are based on measurements of ethanol uptake and acetate formation (Table 1), oxygen uptake and ketone-body metabolism (Table 2), and gluconeogenesis from pyruvate and lactate formation (Table 3). The flow through the different metabolic stages is expressed as flow of the two-carbon unit 'acetate'. For example the columns "changes of acetate calculated for total β-oxidation" and "changes of acetate calculated for citric acid cycle" equal the total production of acetyl-CoA in the β-oxidation step and the utilization of acetyl-CoA in the citric acid cycle, respectively. The calculations presuppose that oxygen-dependent reactions other than those listed in Table 4 do not occur. The assumption is also made that acetate derived from ethanol, which is not recovered in the medium (Table 1), is metabolized in the citric acid cycle. The figures in the column "β-oxidation resulting in acetate formation" (Table 4) have been calculated from the observed rates of acetate formation in the presence of oleate (2 mM). The rates are 27.5 ± 5.2 S.E.M. and 28.9 ± 2.7 S.E.M. μmol/(h g wet wt) ($n = 7$, resp. $n = 7$) for livers from rats on normal and high-fat diets, respectively. Endogenous rates of acetate production were very low (Fellenius and Kiessling, unpublished observations) and were therefore ignored.

The calculations used to obtain the data in Table 4 are illustrated by the following example in which ethanol (10 mM) and oleate (2 mM) were added to livers from normal-diet rats. In the first column "β-oxidation resulting in ketone-body formation" the rate of acetoacetate and β-hydroxybutyrate formation is expressed as rate of acetyl-CoA production according to the formula in Fig. 1 [$5.3 \times 9/4.5 + 32.7 \times 9/4.5 = 76$ μmol/(h g wet wt)]. The second column "β-oxidation not resulting in ketone-body formation" is obtained indirectly by substracting from the total oxygen uptake of 269 μmol/(h g wet wt) (Table 2) the sum of oxygen consumed in the following reactions: ethanol removal [Table 1, 101 μmol/(h g wet wt)], utilization of acetate in the citric acid cycle [the difference between ethanol removal and acetate formation shown in Table 1, 12 μmol oxygen/(h g wet wt)], and total ketone-body formation [Table 2, 48 μmol oxygen/(h g wet wt)]. The obtained rate of 108 ($269 - 161 = 108$) μmol oxygen/(h g wet wt) is equal to the rate of oxygen uptake due to complete oxidation of oleate. The proportions of this respiration associated with the β-oxidation is 32 [$(7.5/25.5) \times 108$] μmol oxygen/(h g wet wt) (Fig. 1). The remaining 76 μmol oxygen/(h g wet wt) is equivalent to the respiration in the citric acid cycle. If these two values are expressed as the production of acetyl-CoA by β-oxidation and the utilization of acetyl-CoA *via* the citric acid cycle (Fig. 1), the following rates are obtained: 38 ($32 \times 9/7.5$) and 38 ($76 \times 1/2$) μmol acetyl-CoA/(h g wet wt). The total rate of synthesis of acetyl-CoA (column 7, Table 4), is the sum of two-carbon units derived

Substrate	Overall reaction			
Oleate	$C_{18}H_{34}O_2 + 7.5\ O_2$	→	4.5	acetoacetate
	$C_{18}H_{34}O_2 + 5.25\ O_2$	→	4.5	β-hydroxybutyrate
	$C_{18}H_{34}O_2 + 7.5\ O_2$	→	9	acetate
	$C_{18}H_{54}O_2 + 25.5\ O_2$	→	18	cartbon dioxide
Ethanol	$C_2H_6O + O_2$	→		aceate
Acetate	$C_2H_4O_2 + 2\ O_2$	→	2	carbon dioxide
Pyruvate	$C_3H_4O_3 + 0.5\ O_2$	→		acetate + carbon dioxide
	$C_3H_4O_3 + 2.5\ O_2$	→	3	carbon dioxide

Fig. 1. Calculated quantitative relations between oxygen uptake and product formation from oleate, ethanol, acetate, and pyruvate.

from β-oxidation and ethanol oxidation, namely 120 (76 + 38 + 6) μmol acetyl-CoA/(h g wet wt). The total rate of utilization of acetyl-CoA by the citric acid cycle (column 8, Table 4) is 44 (38 + 6) μmol/(h g wet wt).

In the experiments with starved animals in the presence of ethanol the total sum of the oxygen-dependent reactions equals 237 (ethanol→acetate: 118, acetate→carbon dioxide: 74, oleate→ketone-body: 45) μmol oxygen/(h g wet wt), while the measured oxygen uptake is 200 μmol oxygen/(h g wet wt) (Table 2). To fulfil the condition that the measured oxygen uptake equals the calculated oxygen uptake it is, therefore, necessary to assume that only half of the acetate, *e.g.* 37 μmol/(h g wet wt), or 19 μmol 'acetate'/(h g wet wt) is completely oxidized in the citric acid cycle.

Ethanol, β-oxidation, and ketogenesis. In all experiments it was noted (Table 4) that ethanol depressed flow through the β-oxidation pathway. This is most directly shown in the experiments with livers from high-fat diet rats. Here the acetyl-CoA formed in the presence of oleate was exclusively used in the production of ketone-bodies. Thus, any effect of ethanol on β-oxidation will be reflected in ketone-body formation. As shown in Table 2 there was a significant ($p < 0.01$) decrease in ketone-body formation after the addition of ethanol to the perfusate. In livers from fed and starved animals ketogenesis was not depressed, but a lowered rate of β-oxidation could be demonstrated because of the decrease in "β-oxidation not resulting in ketone-body formation". As expected the [β-hydroxybutyrate]/[acetoacetate] ratio, an indicator of the redox state of the mitochondria, increased on the addition of ethanol or ethanol and oleate to the medium (Table 2).

Ethanol and the citric acid cycle. Inhibition of the citric acid cycle by ethanol varied with the nutritional state of the animals (Table 4). Inhibition was 64 % in livers from starved rats, but only 38 % when livers from normal fed rats were used. The decrease in flow through the citric acid cycle induced by ethanol in livers from fed rats was maintained in the presence of oleate. In livers from fat-fed rats, perfused with oleate, ethanol had no effect, since the citric acid cycle is already maximally inhibited by oleate oxidation.

It has been suggested [7] that the inhibition of the citric acid cycle by ethanol may be related to a decrease in oxaloacetate concentration. Pyruvate, one of the substrates for the synthesis of oxaloacetate in mitochondria *via*

Table 2. Effect of ethanol on oxygen uptake and ketone-body formation in the perfused livers of starved, fed and fat-fed rats. The experimental conditions were as described in Table 1. The initial concentration of ethanol was 10 mM. The term "total ketones" refers to the sum of β-hydroxybutyrate and acetoacetate. The rates are means ± S.E.M., with the numbers of observations in parentheses. The values for the [β-hydroxybutyrate]/[acetoacetate] ratio were determined from their concentrations in the medium 15 min after the addition of substrates. Metabolic changes are measured in μmol/(h g wet wt).

	Substrate added	Oxygen uptake		β-Hydroxybutyrate	
		Without ethanol	With ethanol	Without ethanol	With ethanol
Starved (48 h)	None	188 ±4.5 (5)	200 ±6.3 (4)	−1.1±0.9 (5)	11.5±1.8 (4)
	Pyruvate (10 mM)	271 ±6.8 (5)	287 ±6.1 (7)	−0.5±1.0 (5)	7.6±0.7 (7)
Normal diet	None	192 ±9.9 (5)	228 ±7.2 (6)	0.6±0.2 (7)	2.7±0.7 (6)
	Oleate (2 mM)	254 ±7.8 (7)	269 ±9.0 (6)	28.5±2.2 (7)	32.7±3.8 (6)
High-fat diet	None	203 ±8.5 (8)	196 ±8.2 (12)	−1.9±2.3 (8)	19.4±2.9 (15)
	Oleate (2 mM)	310 ±7.2 (7)	284 ±7.7 (8)	165 ±7.3 (7)	147 ±8.4 (8)

		Acetoacetate		Total ketones		[β-Hydroxybutyrate]/[Acetoacetate] ratio	
		Without ethanol	With ethanol	Without ethanol	With ethanol	Without ethanol	With ethanol
Starved (48 h)	None	24.5±2.4 (5)	18.3±2.1 (4)	23.4±1.6 (5)	29.8±2.5 (4)	0.15±0.01	0.48±0.07
	Pyruvate (10 mM)	2.8±2.0 (5)	−5.8±1.3 (7)	2.3±1.6 (5)	1.8±1.1 (7)	0.39±0.05	0.46±0.09
Normal diet	None	1.7±0.4 (7)	1.0±0.3 (6)	2.3±0.5 (7)	3.7±0.8 (6)	0.62±0.07	1.32±0.21
	Oleate (2 mM)	13.8±1.4 (7)	5.3±1.9 (6)	42.3±3.3 (7)	37.9±3.6 (6)	2.23±0.38	4.84±0.37
High-fat diet	None	52.7±2.1 (8)	24.5±2.7 (15)	50.8±2.4 (8)	43.9±2.7 (15)	0.27±0.04	0.57±0.13
	Oleate (2 mM)	17.9±2.4 (7)	−2.5±3.6 (8)	183 ±7.4 (7)	145 ±6.1 (8)	2.69±0.11	3.65±0.28

Table 3. Effect of ethanol on lactate formation and gluconeogenesis from pyruvate. The experimental conditions were as described in Table 1. The rats were starved for 48 h. The initial concentration of pyruvate and ethanol was 10 mM. The rates have been calculated from the linear part of the progress curve during 30 min. The results are given as means ± S.E.M., with the number of observations in parentheses.

Removal of pyruvate μmol/(h g wet wt)		Production of lactate μmol/(h g wet wt)		Rate of gluconeogenesis μmol/(h g wet wt)	
Without ethanol	With ethanol	Without ethanol	With ethanol	Without ethanol	With ethanol
320 ± 15 (5)	343 ± 8.4 (7)	115 ± 5.6 (5)	157 ± 8.1 (7)	50.1 ± 4.3 (5)	55.1 ± 5.6 (7)

Table 4. Effect of ethanol on the flow *via* the β-oxidation and the citric acid cycle in livers from rats fed various diets. The calculation of flow, which are expressed as changes of the two-carbon unit acetate, have been made from the results given in Tables 1, 2, and 3 and formulas in Fig. 1. The concentration of the added oleate was 2 mM and of pyruvate and ethanol 10 mM. The details of the calculations are given in the text. Figures are given in μmol/(h g wet wt).

	Substrate added	β-Oxidation resulting in ketone-body formation	β-Oxidation not resulting in ketone-body formation	β-Oxidation resulting in acetate formation	Total β-oxidation	Complete oxidation of acetate to CO_2 and H_2O	Oxidation of pyruvate to acetyl-CoA	Total synthesis of acetyl-CoA	Citric acid cycle
Starved (48 h)	None	47	52		99			99	52
	Ethanol	60	0		60	37		97	19
	Pyruvate	4	40		44		106	150	146
	Ethanol+ pyruvate	3	0		3	52	76	131	108
Normal diet	None	5	66		71			71	66
	Ethanol	7	5		12	36		48	41
	Oleate	84	89	28	174			174	61
	Ethanol+ oleate	76	38		114	6		120	44
High-fat diet	None	102	41		143			143	41
	Ethanol	88	7		95	12		107	19
	Oleate	366	50	30	416			416	20
	Ethanol+ oleate	290	7		297	10		307	17

Table 5. Quantitative relations between the removal and the oxidation of oleate in livers from fed and fat-fed rats. Ethanol (10 mM) and oleate (2 mM) were added 38 min after the start of the perfusions. The calculations of fatty acid oxidized have been made from the numbers shown in Table 4 ("total β-oxidation"), and the formulas in Fig. 1. The rates of oleate removed are means ± S.E.M., with the numbers of observations in parentheses. The calculations presuppose that the added oleate is oxidized in preference to endogenous fatty acids. A: Without ethanol. B: With ethanol.

	Oleate removed [μmol/(h g wet wt)]		Oleate oxidized [μmol/(h g wet wt)]		Oleate removed / Oleate oxidized	
	A	B	A	B	A	B
Normal diet	45.4 ± 1.8 (7)	44.5 ± 1.4 (6)	19	13	2.3	3.5
High-fat diet	55.8 ± 5.9 (5)	46.5 ± 3.8 (6)	46	33	1.2	1.4

pyruvate carboxylase [25] is known to decrease in the presence of ethanol [20] and can thereby decrease the rate of the pyruvate carboxylase reaction. The inhibition by ethanol of the flow in the citric acid cycle was therefore examined in the presence of pyruvate (Table 4). Pyruvate alone increases the cycle activity almost threefold. After the addition of ethanol, the inhibition is 26 %. The results also indicate that the pyruvate dehydrogenase step is inhibited by ethanol, since it was calculated that the oxidation of pyruvate to acetyl-CoA decreases about 25 %.

Ethanol and triglyceride synthesis. Knowing the amount of oleate oxidized (Table 4) and the observed rate of oleate removal (Table 5) it is possible to estimate approximately the amount of added oleate which is transferred to triglyceride. The results are shown in Table 5. Added oleate being oxidized is about 60 % less in livers from fed rats than in livers from fat-fed rats. Since the rates of oleate removal in the two types of livers are approximately the same it follows that oleate is incorporated into triglycerides. This reaction is accelerated by the addition of ethanol.

DISCUSSION

The interaction and its nature between ethanol and fatty acid oxidation was examined. With a knowledge of the over-all metabolic balance of the perfused liver it was possible to calculate approximate flow rates of the β-oxidation pathway, the citric acid cycle and triglyceride synthesis. The manner of expressing flow in the form of the two-carbon unit,[6] acetate' is to be preferred rather than rates of oxygen utilization,[7] because not all reactions giving rise to NADH are coupled to the electron transport chain and oxygen utilization. Thus, in the presence of pyruvate and ethanol it can be calculated from the rates in Tables 1 and 3 that about 80 % of the NADH formed when ethanol is oxidized to acetate is required for the synthesis of lactate and glucose. The

calculations presuppose that oxygen-dependent reactions other than those listed in Fig. 1 do not occur. Fritz [26] calculated that in livers from fed rats glucose and amino acid oxidation contributed to the fuel of respiration to about 20 %. It follows that the 80 % inhibition of the β-oxidation by ethanol in normal livers (Table 4) might be overestimated. However, the 40 % inhibition of the citric acid cycle in the same experiment is probably not overestimated, since the inflow of carbon-units derived from glucose [27] or amino acids [20,28] is strongly suppressed during ethanol oxidation.

In spite of a strong suppression of the activity of the citric acid cycle by ethanol (Table 4), which according to current views [29–32] should be related to an increased ketone-body formation, ethanol was shown to be antiketogenetic or without effect on ketogenesis (Table 2). The most likely explanation is that ethanol inhibits the β-oxidation pathway directly. This has been suggested by others [7,9] and is directly shown in the present investigation. Advantage was taken of the observation that livers from rats fed a high-fat diet almost quantitatively converted added oleate to ketone-bodies. Thus any effect of ethanol on the ketone-body formation of these livers will reflect the changes in the β-oxidation step. As shown in Table 2, the addition of ethanol significantly inhibits ketone-body formation in these livers. It is of interest that ethanol has been found to have a depressing effect on ketone-body production in diabetic patients.[33] In these patients, the turnover rate of fatty acid to ketone-body is very high and presumably the citric acid cycle is already inhibited.

The interpretation of the effect of ethanol on ketogenesis is thus complicated by an inhibition of both the β-oxidation and the citric acid cycle. Any inhibition of the citrate-synthetase reaction, leading to an accelerated ketogenesis, may be concealed by a block of the β-oxidation and, consequently, of the supply of acetyl-CoA for ketone-body formation. It is therefore not surprising that many authors have reported [11,34,35] almost unchanged ketone-body formation under the influence of ethanol.

The mechanism behind this effect of ethanol on the β-oxidation remains to be explored. Williamson *et al.*[7] suggest that, since the addition of ethanol in perfused liver supplemented with oleate causes a reduction of flavoproteins, the supply of reducing equivalents exceeds the rate at which they can be transferred from the flavin to the cytochrome system. This suggestion is probably also valid in the present investigation, when ethanol and oleate have been added, since the [β-hydroxybutyrate]/[acetoacetate] ratio increases above the normal range of 2 – 3. In addition, it is probable that ethanol oxidation competitively inhibits the β-oxidation of fatty acids by the formation of an excess of NADH. This is supported by the finding that ethanol oxidation is inhibited by oleate (Table 1), demonstrating the competitive nature of the interaction.

The redox state of the mitochondrial nicotinamide dinucleotides may be of importance in the regulation of ketone-body formation.[32] However, no correlation was found between the [β-hydroxybutyrate]/[acetoacetate] ratio and the ketone-body formation in liver supplemented with only oleate (Table 2). The ratio is about the same 15 min after the addition of oleate, despite a pronounced variation in ketone-body formation in all the livers tested. More-

over, in spite of an increase in the [β-hydroxybutyrate]/[acetoacetate] ratio, ethanol is antiketogenic when oleate is added to fat-fed rats.

The citrate-synthetase activity can be controlled by the mitochondrial concentration of oxaloacetate through its transformation to malate.[31,36,37] The production of oxaloacetate *via* the pyruvate-carboxylase reaction and the rate by which it is removed by gluconeogenesis are additional factors. Pyruvate concentration is one of the factors limiting the rate of the pyruvate-carboxylase reaction.[20] Addition of pyruvate (Table 4) partly overcomes the inhibition by ethanol of the flow through the citric acid cycle, probably by increasing the level of oxaloacetate. In addition to the concentration of oxaloacetate, the concentrations of adenine nucleotides may also play a key role in the regulation of the citrate-synthetase reaction.[38,39] Garland [40] pointed out that it is impossible to separate the significance of the intramitochondrial concentration of oxaloacetate from that of adenine nucleotides, since the K_m value of oxaloacetate in the citrate-synthetase reaction is dependent upon the concentrations of adenine nucleotides. The total inflow of two-carbon units to the citric acid cycle should be equivalent to the outflow of two-carbon units. This metabolic balance is not achieved in the experiment with the livers from starved animals, supplemented with ethanol (Table 4). The total synthesis of acetyl-CoA is 97 μmol/(h g wet wt) and the utilization of acetyl-CoA *via* the citric acid cycle and ketone-body formation is 79 μmol/(h g wet wt). This indicates that the flow through the cycle is decreased by inhibition of both citrate-synthetase and after the citrate-synthetase step, confirming the results of Williamson *et al.*[7] The latter proposed an inhibition at the isocitrate dehydrogenase step.

Ethanol is known to interfere with lipid metabolism in such a way that a single dose of ethanol causes a prompt increase in the triglyceride content of the liver.[41,42] The accumulation of α-glycerophosphate has been suggested to be one important mechanism responsible for the hyperlipemia.[43,44] It can be argued that the inhibition of the β-oxidation pathway might be an additional factor. However, as shown in Table 5 ethanol did not stimulate triglyceride synthesis in livers from animals on a high-fat diet, while the β-oxidation pathway was greatly inhibited (Table 4). As discussed elsewhere [45] the fraction of fatty acid undergoing esterification depends primarily on the level of α-glycerophosphate.

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