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Effect of Ethanol, Noradrenaline and 3′,5′-Cyclic AMP on Oxidation of Fatty Acids and Lipolysis in Isolated Rat Hepatocytes

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GRUNNET, N. AND J. KONDRUP. *Effect of ethanol, noradrenaline and 3′,5′-cyclic AMP on oxidation of fatty acids and lipolysis in isolated rat hepatocytes.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 245–250, 1983.—Improved methods for the determination of the rate of fatty acid oxidation and lipolysis are presented and evaluated. Hepatocytes from fed rats were incubated with 1.3 mM palmitate. The rate of oxidation of exogenous fatty acids was determined with [1-^{14}C]palmitate. The rate of oxidation of endogenous fatty acids was determined either as the difference between total fatty acid oxidation and oxidation of exogenous fatty acids, or as the oxidation of intracellular lipids, prelabelled with ^{14}C. The two methods agreed completely, indicating that the only endogenous source of acetylCoA was fatty acids. The rate of oxidation of exogenous and endogenous fatty acids was estimated to be 858 and 284 nmol C2-units/min 10^8 cells, respectively, and the rate of lipolysis to be 1640 nmol C2-units/min 10^8 cells. Ethanol caused a 17% and 70% inhibition of the oxidation of exogenous and endogenous fatty acids, respectively. Lipolysis was inhibited ~10% by ethanol. Noradrenaline was without effect on the oxidation of exogenous and endogenous fatty acids and on lipolysis. 3′,5′-Cyclic AMP enhanced the oxidation of exogenous and endogenous fatty acids by 25% and 31%, respectively, and the rate of lipolysis by 38%, suggesting the presence in hepatocytes of a cAMP sensitive lipase.

Ethanol Fatty acid oxidation Hepatic lipolysis Noradrenaline 3′,5′-Cyclic AMP Hepatocytes β-Oxidation Ketogenesis

STUDIES of triacylglycerol metabolism in the perfused rat liver have indicated a considerable rate of recycling of the fatty acid moiety of neutral lipids [12, 13, 14]. Ethanol enhanced the rate of recycling, and the results thus suggested a lipolytic activity of the hepatocyte, sensitive to ethanol or the metabolic changes caused by ethanol. The results of these studies also inferred that the rate of β-oxidation was unaffected by ethanol, despite a 45% inhibition of the oxidation of [1-^{14}C]palmitate. It was proposed that the diminished conversion of [1-^{14}C]palmitate to oxidation products during ethanol metabolism might be due to isotope dilution of the acylCoA pool, caused by the increased rate of recycling of the fatty acid moiety of neutral lipids. These results are in contrast to the general belief that ethanol causes an inhibition of the β-oxidation pathway.

Measurements of lipolysis and β-oxidation in hepatocytes have hitherto been done by indirect methods only. In this paper, improved methods for the determination of the rate of fatty acid oxidation and lipolysis in hepatocytes are presented and evaluated and the effect of ethanol, noradrenaline and 3′,5′-cyclic AMP (cAMP) on these processes are reported.

METHOD

Hepatocytes were prepared by the collagenase perfusion technique with 20 mM glucose added to all media as described previously [15]. Two hundred gram female Wistar rats with free access to food were used. More than 85% of the hepatocytes excluded trypan blue.

Incubations were carried out in a shaking water bath at 37°C in 20 ml conical flasks with a center cup containing a filter paper. Incubations in a total volume of 1.6 ml were initiated by addition of approximately 10^7 cells in Krebs-Henseleit buffer [16] to an equal volume of the same buffer containing 4% purified albumin [3], [1-^{14}C]palmitate (final concentrations 2% albumin and 1.3 mM palmitate) and other substrates as indicated. The gas phase was O_2/CO_2 (95/5, v/v). ^{14}C-Palmitate was purified by extraction with diethyl ether from an acidified solution and was mixed with unlabelled palmitate dissolved in KOH before being complexed to albumin.

Reactions were terminated by injecting 200 μl 65% citric acid into the main compartment of the flasks (final pH ~3) and 300 μl 2.5 N NaOH into the center cup. After 30 min, the filter paper was removed for determination of radioactivity in CO_2. Recovery of added $^{14}C\text{-}HCO_3^-$ was larger than 95%. An aliquot of the content in the main compartment was removed for neutralization and enzymatic determination of acetoacetate and 3-hydroxybutyrate [19,28]. A second aliquot (500 μl) was transferred to another incubation flask with 300 μl 2.5 N NaOH on a filter paper in the center cup.

TABLE 1

EFFECT OF ETHANOL, NORADRENALINE AND 3'5'-CYCLIC AMP ON FATTY ACID OXIDATION BY HEPATOCYTES FROM FED RATS

	No additions	Ethanol	Noradrenaline	3',5'-Cyclic AMP
1. [1-^{14}C]palmitate → Ketone bodies	638 ± 56	103 ± 4	83 ± 8	131
2. [1-^{14}C]palmitate → CO_2	220 ± 18	26 ± 2	141 ± 11	101
3. [1-^{14}C]palmitate oxidation	858 ± 62	83 ± 2	103 ± 6	125
4. Δ Ketone bodies	888 ± 78	110 ± 4	90 ± 7	138
5. AcetylCoA → CO_2	256 ± 24	28 ± 2	151 ± 20	103
6. AcetylCoA → CO_2 + ketone bodies	1142 ± 92	92 ± 3	107 ± 8	131
7. Endogenous acetylCoA formation	284 ± 42	118 ± 7	113 ± 16	162

Hepatocytes were incubated with 1.3 mM [1-^{14}C]palmitate with no further addition, 20 mM ethanol, 5 μM noradrenaline or 1 mM cAMP. Total acetylCoA formation (line 6) and acetylCoA formation from endogenous substrates (line 7) include acetylCoA formation from ethanol.

Figures with "No additions" are nmol C2-units/min 10^8 cells ±S.E.M. (n=10). Other figures are percent of "No additions" ±S.E.M. (n=9) for "Ethanol", n=4 for "Noradrenaline" and n=2 for cAMP.

The flask was closed and 200 μl aniline citrate [20] injected into the main compartment. After 2 hr shaking at 37°C, the filter paper was removed for determination of radioactivity in carbon-1 of acetoacetate. Determination of radioactivity in carbon 3 of acetoacetate was carried out by decarboxylation of acetoacetate by H_2SO_4 and collection of the CO_2 in NaOH and of the acetone in hydrazine lactate, pH 5 [17]. In some experiments a third aliquot (500 μl) was used for determination of radioactivity in water soluble products and triacylglycerols: The sample was shaken for 30 min with 6 ml chloroform:methanol (1:2, v/v) and for another 30 min after addition of 2 ml chloroform and 1.5 ml 2 M KCl. After centrifugation the volume of the two phases was measured. An aliquot of the upper phase was counted to obtain radioactivity in water soluble products. An aliquot of the lower phase was evaporated, dissolved in 2 ml heptane and fatty acids and phospholipids extracted twice with 2 ml 0.1 N KOH in 50% ethanol. The heptane was counted to obtain radioactivity in triacylglycerols [2]. ^{14}C-Hydroxybutyrate, ^{14}C-palmitate, ^{14}C-triacylglycerol and ^{14}C-phosphatidylcholine was recovered in the respective fractions by 90–100%.

For determination of radioactivity, sample plus 30 mM acetic acid in a total of 3 ml was mixed with 10 ml Insta-Fluor™: triton X-114 (3:2, v/v).

The rates given in Results are average rates for the first 30 min of incubation. During this period, the palmitate concentration fell to about half and the total rate of palmitate oxidation somewhat less. No change in the effects of ethanol, noradrenaline or cAMP was observed during the incubation. Results are expressed per 10^8 cells corresponding to approximately 1 g wet wt. [5]. The conversion of ethanol to ketone bodies and CO_2 was determined in incubations with unlabelled palmitate and [1-^{14}C]ethanol. The filter papers were dried twice over P_2O_5 before their radioactivity was determined.

[1-^{14}C]Palmitate was from The Radiochemical Center, Amersham, U.K., albumin (fraction V, bovine) from Armour Pharmaceutical Company, Ltd., Eastbourne, U.K., Insta-Fluor™ from Packard Instrument Company Inc., IL, collagenase from Worthington Biochemical Corporation, Freehold, NJ, and other enzymes from Boehringer Mannheim GmbH, Mannheim, West Germany. Noradrenaline and 3',5'-cyclic AMP was obtained from Sigma Chemical Company, St. Louis, MO.

CALCULATIONS

The formation of acetylCoA from [1-^{14}C]palmitate was calculated from the oxidation of [1-^{14}C]palmitate. This was determined as the sum of [1-^{14}C]palmitate metabolized to CO_2 and to ketone bodies. Radioactivity in ketone bodies was calculated as (1 + C_3/C_1 radioactivity ratio) × specific radioactivity of carbon 1 of acetoacetate × content of acetoacetate plus 3-hydroxybutyrate. It agreed within 10% with radioactivity in water soluble products.

The total formation of acetyl-CoA was calculated as the sum of CO_2 formation and chemically determined ketone body formation. CO_2 formation from acetylCoA was calculated from the radioactivity in CO_2, assuming a specific radioactivity of acetylCoA equal to the specific radioactivity of carbon 1 of acetoacetate (see Discussion section).

Formation of acetyl-CoA from endogenous substrates was calculated as the difference between total acetyl-CoA formation and acetyl-CoA formation from [1-^{14}C]palmitate.

In the experiments of Table 3, the oxidation of ^{14}C-labelled lipids was calculated as radioactivity in CO_2 + ketone bodies divided by the specific radioactivity of the fatty acids of neutral lipids. The latter was calculated from the radioactivity in neutral lipids and the fatty acid content of the neutral lipid fraction, thus ignoring the compartmentation of triacylglycerols in hepatocytes (see Discussion).

RESULTS

β-Oxidation of Fatty Acids

The rate of oxidation of [1-^{14}C]palmitate of 860 nmol C2-units/min × 10^8 cells, with 26% being oxidized to CO_2 (Table 1), is similar to the oxidation measured in the perfused liver from fed rats [14]. Total acetyl-CoA formation was somewhat higher than formation from [1-^{14}C]palmitate, with acetylCoA formation from endogenous substrates amounting to 284 nmol C2-units/min × 10^8 cells (Table 1).

The endogenous source of this acetylCoA is probably the fatty acid moiety of endogenous lipids, as shown by the results of Table 2 and 3. The results of Table 2 show that addition of substrates, which would be expected to contribute to the formation of acetylCoA via pyruvate, had no effect on the specific radioactivity of carbon 1 of acetoacetate, indicating that flux through the reaction catalysed by pyruvate

dehydrogenase was negligible. This conclusion is in accordance with previous reports of pyruvate dehydrogenase flux in the presence of oleate [8]. NH_4Cl, which strongly inhibits proteolysis in isolated hepatocytes [24], should have caused an increase in the specific radioactivity of carbon 1 of acetoacetate if amino acids were a significant endogenous source of acetylCoA. This was, however, not the case (Table 2). Butyrate, as expected, lowered the specific radioactivity of acetoacetate carbon 1 (Table 2). The results of Table 3 show that the rate of oxidation of neutral lipids, prelabelled with ^{14}C in the fatty acid moiety, agreed with the rate of formation of acetylCoA from endogenous substrates, leaving no room for other endogenous sources than the fatty acids of lipids.

Ethanol had no effect on the formation of ketone bodies from [1-^{14}C]palmitate and caused, as expected, a 74% decrease in $^{14}CO_2$ formation, resulting in a 17% decrease in the oxidation of exogenous palmitate. Total ketogenesis, oxidation of acetylCoA to CO_2 and endogenous acetylCoA formation (line 4, 5 and 7, Table 1), includes conversion of ethanol to ketone bodies and CO_2. This was determined to 200±10 nmol C2-units/min × 10^8 cells (mean±S.E.M. n=5) which means that total acetylCoA formation from substrates other than ethanol was reduced by 26% during ethanol metabolism ((line 6: 1142×0.92 − 200)/1142=0.74). Endogenous acetylCoA formation was decreased from 284 to 344 − 200 = 144; i.e., by 50%, suggesting a more marked inhibition of oxidation of endogenous lipids compared to exogenous fatty acids (see below).

Noradrenaline enhanced the oxidation of [1-^{14}C]palmitate to CO_2 by 40%, in agreement with earlier reports [26]. Total oxidation of [1-^{14}C]palmitate as well as total acetylCoA formation from endogenous sources was unaffected by noradrenaline. Also, acetylCoA formation from endogenous sources was unaffected by noradrenaline suggesting the absence of a noradrenaline sensitive lipase in isolated hepatocytes (see also below).

3′,5′-Cyclic AMP, as has been reported earlier [9, 11, 29], caused a 30% increase in ketone body formation from [1-^{14}C]palmitate and a 25% increase in oxidation of endogenous fatty acids (Table 1). Total ketone body formation was increased by 38% and total CO_2 formation from acetylCoA was unchanged in the presence of cAMP (Table 1). AcetylCoA formation from endogenous substrates was increased by 60% (Table 1), suggesting a cAMP sensitive lipase activity in isolated hepatocytes.

TABLE 2

SPECIFIC RADIOACTIVITY OF CARBON 1 OF ACETOACETATE

Additions	% of "No additions"
Glucose, 12 mM	102 ± 1
Fructose, 11 mM	104 ± 8
Lactate, 12 mM	100 ± 5
NH_4Cl, 9 mM	95 ± 2
Alanine, 5 mM	104 ± 4
Glutamine, 9 mM + Asparagine, 9 mM	98 ± 5
Butyrate, 2 mM	52 ± 7

Hepatocytes from fed rats were incubated with 1.3 mM [1-^{14}C]palmitate for 30 min.

Figures are mean±S.E.M. (n=4). The specific radioactivity of carbon 1 of acetoacetate, relative to that of palmitate, was 0.78 ± 0.004 (S.E.M., n=10) with "No addition."

Lipolysis

Oxidation of the fatty acid moiety of neutral lipids amounted to 328 nmol C2-units/min × 10^8 cells, corresponding to 41 nmol C16-units (Table 3). In the experiments reported, 20% of the fatty acids taken up was oxidised and 80% esterified. Assuming a single pool of acetylCoA for oxidation and esterification, and a complete intracellular mixing of fatty acids taken up from the medium and those formed by lipolysis, this means that the rate of lipolysis was 41 × 100/20 = 205 nmol C16-units/min × 10^8 cells.

Ethanol caused a decrease in the oxidation of prelabelled lipids to 57% (Table 3). In the presence of ethanol, 13% of the fatty acids are oxidized (cf. Table 1 and 3) and the calculated

TABLE 3

EFFECT OF ETHANOL, NORADRENALINE OR cAMP ON HEPATIC TRIACYLGLYCEROL METABOLISM

	^{14}C-Lipid → CO_2 + Ketone Bodies		
Additions	% of "No additions"	% of AcetylCoA formation from endogenous substrates (excluding ethanol)	Esterification of [1-^{14}C]palmitate %
None	100*	102 ± 23	100†
Ethanol, 80 mM	57 ± 6	85 ± 30	128 ± 8
Noradrenaline, 5 μM	101 ± 23	78 ± 21	112 ± 8
cAMP, 1 mM	158 ± 23	92 ± 33	97 ± 2

Hepatocytes from fed rats were incubated for 1 hr with 1.3 mM [1-^{14}C]palmitate + 2% albumin to label hepatic lipids, washed once, and incubated for 20 to 40 min with 1.3 mM palmitate + 2% albumin to determine the oxidation of ^{14}C-labelled lipids to CO_2 and ketone bodies. AcetylCoA formation from endogenous substrates (and ethanol) and the esterification of [1-^{14}C]palmitate were determined in parallel incubations and as described in the Method Section with cells incubated for 1 hr with 1.3 mM palmitate + 2% albumin and washed once. Values are mean±S.E.M. of 5 experiments (no additions, ethanol) or 3 experiments (noradrenaline, cAMP).

*100%=328 ± 74 nmol C2-units/min × 10^8 cells.

†100%=3606 ± 182 nmol C2-units/min × 10^8 cells.

rate of lipolysis is 41 × 0.57 × 100/13 = 178 nmol C16-units/min × 10^8 cells or 88% of the control value. Most probably, ethanol had no or a slightly inhibitory effect on lipolysis in these experiments.

Noradrenaline had no effect on the fraction of [1-^{14}C]palmitate which is oxidized (cf. Table 1 and 3) or on the oxidation of prelabelled lipids (Table 3), allowing the conclusion that a noradrenaline-sensitive lipase does not exist in isolated hepatocytes.

In the presence of cAMP, the oxidation of prelabelled lipids was increased by about 60 (Table 3) and the fractional oxidation of fatty acids to 23% (cf. Table 1 and 3), resulting in a rate of lipolysis of 41 × 1.58 × 100/23 = 282 nmol C16-units/min × 10^8 cells. This value corresponds to a 38% increase in the rate of lipolysis by cAMP, and suggests the existence of a cAMP sensitive lipase in isolated hepatocytes.

DISCUSSION

Estimation of the Rate of β-Oxidation

Estimation of the rate of β-oxidation by using an exogenous, ^{14}C-labelled fatty acid requires the determination of radioactivity in all oxidation products (usually considered identical with water soluble products plus CO_2) and knowledge of the specific radioactivity of the mitochondrial and peroxisomal acylCoA-pool. Thus, estimates of the rate of β-oxidation in which only some of the oxidation products of fatty acid oxidation have been measured (e.g., CO_2 or carbon 1 of acetoacetate), or in which the isotope dilution of the ^{14}C-labelled fatty acid or of ^{14}C-labelled intermediates in the β-oxidation pathway are neglected, can be considered inaccurate. It can also be noted that erroneous estimates of ketone body radioactivity can result if isotopic equilibration is assumed between carbon 1 and 3 of acetoacetate, and total radioactivity of ketone bodies calculated from measurements of radioactivity in carbon 1 of acetoacetate. Especially in experiments with fed rats, this procedure gives rise to large errors. We find, in agreement with others [10,25], in hepatocytes from fed rats a radioactivity ratio between carbon 3 and 1 of 0.65±0.05 (mean±SE, n=7) and of 0.89±0.03 (mean±SE, n=4) in hepatocytes from starved rats. Furthermore, recent experiments [6] have shown that the liver can metabolize ketone bodies. Reincorporation of ketone bodies, or other oxidation products, into lipids may thus cause an underestimation of the rate of β-oxidation.

The method described in the present paper for the determination of the rate of β-oxidation, by using a [1-^{14}C]labelled fatty acid, involves the assumptions that (a) Oxidation products other than ketone bodies and CO_2 are negligible. This appears to be the case under the experimental conditions used, as radioactivity in ketone bodies almost equaled radioactivity in water soluble products (see Calculations section). (b) Carbon 1 of acetylCoA and carbon 1 of acetoacetate are in isotopic equilibrium. This would not be the case, if acetoacetate was formed by deacylation of acetoacetylCoA produced by amino acid metabolism. This pathway is, in all probability, negligible (see above). (c) AcetylCoA produced by oxidation of fatty acids constitutes a single pool regarding metabolism to CO_2 and ketone bodies [16a].

The rate of β-oxidation has also been estimated without the use of radioisotopes [7,30]. In such estimates, the oxygen uptake not accounted for by oxidation of added substrates to metabolic end products has been ascribed to the complete oxidation of fatty acids to CO_2. This may give rise to grossly erroneous estimates because the oxidation of endogenous substrates is neglected. Also fatty acid synthesis from added substrates will affect the calculations. As an example, the 25% inhibition of β-oxidation calculated by Williamson *et al.* [30] may be reduced to nil, if 50% instead of 100% of the unaccounted oxygen uptake is due to fatty acid oxidation to CO_2.

Estimation of the Rate of Lipolysis

Lipolysis, i.e., the formation of fatty acids by hydrolytic cleavage of neutral lipids, may be estimated by prelabelling of the fatty acid moiety of the lipids and determination of radioactivity in oxidation products. Some of the fatty acids formed by lipolysis will be reesterified, and the method therefore requires knowledge of the fraction of fatty acids, which is esterified and oxidized, respectively (see Results section). Estimates reported so far [4,22] of relative rates of lipolysis have not taken into account a possible variation in the fractional esterification of the fatty acids, and are therefore of little value. In the calculations it is also assumed that the partition between oxidation and esterification is the same for acylCoA formed from exogenous and endogenous fatty acids. This may not be the case, as ethanol was found to inhibit acetylCoA formation by 50% from endogenous substrates, but by 17% only from exogenous palmitate (see Results section). Furthermore, the existence of at least two intracellular pools of neutral lipids in hepatocytes [13] complicates the procedure. The probable precursor pool for lipolysis is neutral lipids in the membrane fraction [13], and the specific radioactivity of the fatty acids in this fraction should be known. In the experiments reported in this paper, neutral lipids have been considered a single pool with respect to lipolysis, which may cause an overestimation of the rate of lipolysis by ca. 15% (the specific radioactivity of membrane bound neutral lipids was about 1.2 times the average specific radioactivity of neutral lipids, unpublished observation). On the other hand, the extensive recycling of fatty acids results in a ca. 20% decline in the specific radioactivity of the fatty acid moiety of neutral lipids during the experiment. This fall will tend to underestimate the rate of lipolysis, and the rates reported in this paper are thus to be considered as rough estimates, which may be 10–20% inaccurate.

An alternative method for measuring the rate of lipolysis is to use the specific radioactivity of carbon 1 of acetoacetate relative to that of the exogenous [1-^{14}C]fatty acid (see Calculations section). This approach requires that the only source of ketone bodies is fatty acids. This appears to be the case when hepatocytes are incubated with 1.3 mM palmitate as the only substrate (cf., the value of 284 and 328 nmol C2-units/min × 10^8 cells of Table 1 and 3, respectively), but may not be the case under other experimental conditions.

The Effect of Ethanol on β-Oxidation

Literature reports on the rate of oxidation of exogenous fatty acids in the presence of ethanol show large variations (Table 4). Several explanations are likely to account for these variations: (a) The above-mentioned methodological problems may lead to erroneous measurements. (b) The concentration of fatty acids may be of importance for the effect of ethanol (cf., line 2 and 3 of Table 4). (c) The nutritional state of the animals could influence the results, as a larger fraction of acetylCoA is oxidized to CO_2, and a correspondingly smaller fraction converted to ketone bodies, in the fed compared to the starved animal and as a much larger

TABLE 4

EFFECT OF ETHANOL ON FATTY ACID OXIDATION

Liver Preparation	Nutritional State	Fatty acid concentration mM	Effect of ethanol on ^{14}C-Fatty Acid Oxidation to CO_2 + Ketone Bodies*	^{14}C-Fatty Acid Oxidation to CO_2*	^{14}C-Fatty Acid Oxidation to Ketone Bodies*	Reference
Liver Slices	Fed	1	34–40	26–46	29–52	[1]
Isolated	Fed	1.3	83	29	103	This study
Hepatocytes	Fed	0.3	100	18	246	Unpublished results
	Fed	0.7–1	94	40	109	[27]
	Starved	1.3	79	38	81	Unpublished results
	Starved	0.4	77	59	79	[21]
Perfused	Fed	1	54	28	61	[14]
Liver	Fed	0.2	40	33	64	[14]
	Fed	0.9	56	40	107	[23]
	Fed	4.5	86	62	126	[23]
	Fed	2	66†	62†	90†	[7]
	Starved	0.2–0.5	43	73	40	[18]
	Starved + 10 mM lactate	0.2–0.5	53	43	58	[18]
	Starved + 5 mM alanine	1	75†	13†	145†	[30]

*Percent of ^{14}C-fatty acid oxidation in the absence of ethanol. Fatty acid oxidation represents ^{14}C-fatty acids converted to CO_2 and ketone bodies or water soluble products, except for those in [7] and [30]. See footnote†.

†Experiments performed without ^{14}C-labelled fatty acids. Fatty acid oxidation calculated stoichiometrically.

fraction of the fatty acids are oxidized in starved animals. (d) The various liver preparations may respond differently to ethanol. There may be a tendency toward a smaller inhibition by ethanol of the oxidation of ^{14}C-fatty acids in isolated hepatocytes as compared to the perfused liver (Table 4). (e) The oxidation rate of [1-^{14}C]fatty acids does not necessarily reflect the rate of β-oxidation (see above), and dilution of radioactivity may occur to a different extent and at different intermediates in the metabolic pathways, again depending on the actual experimental conditions.

In conclusion, ethanol consistently appears to depress the formation of CO_2, probably by inhibition of the tricarboxylic acid cycle. The inhibitory effect of ethanol on the β-oxidation of fatty acids is less thoroughly documented, and may range from 0 to 50%, dependent on the experimental conditions. Thus, in some cases, the decrease in CO_2 formation seems to be accompanied by an increase in ketogenesis (Table 4). To what extent a decrease in the β-oxidation of fatty acids can be of importance for the induction of the ethanol-induced fatty liver is therefore uncertain.

Comparison with the Perfused Liver

The rates of oxidation of [1-^{14}C]palmitate to ketone bodies and to CO_2 are very similar in isolated hepatocytes from fed rats (this study) and in once-through perfused liver from fed rats [14]. In the latter work it was shown that ethanol increased the turn-over of fatty acids of triacylglycerols without increased accumulation of triacylglycerol. It was therefore suggested that the decreased oxidation of [1-^{14}C]palmitate was due to a decreased specific radioactivity of acylCoA, caused by increased flux of fatty acids from lipolysis to the β-oxidation pathway. The present experiments do not support this suggestion. It should be pointed out, however, that more thorough studies, e.g., studies which take into account the intracellular compartmentation of neutral lipids in hepatocytes, are required to evaluate these problems in detail.

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