Acetoacetate: a major substrate for the synthesis of cholesterol and fatty acids by isolated rat hepatocytes

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Received 19 September 1983

Evidence is presented that isolated, intact rat hepatocytes can synthesize fatty acids and cholesterol from acetoacetate. The quantitative importance of these processes is evaluated by measuring total rates of fatty acid and cholesterol synthesis by incorporation of ^3H from ^3H_2O. The contribution of acetoacetate varies from 14–54% and from 21–75% for de novo synthesized fatty acids and cholesterol, respectively, depending on the physiological condition of the donor rat. The relative contribution of acetoacetate to cholesterol synthesis is 1.4–2.3-times greater than to fatty acid synthesis.

Isolated hepatocyte *Acetoacetate utilization* *Lipogenesis* *Cholesterogenesis* *Fatty acid synthesis* *Ketone bodies*

1. INTRODUCTION

Ketone bodies are generally considered hepatic export products: they are produced by the liver and utilized only by extra-hepatic tissues. This notion was based on the finding that enzymes which activate acetoacetate in liver are too low in amount to permit substantial hepatic utilization of ketone bodies. Low activities of the mitochondrial acetoacetate-activating enzyme, 3-oxoacid CoA-transferase (EC 2.8.3.5), and of the cytosolic enzyme acetoacetyl-CoA synthetase (EC 6.2.1.x) were reported [2,3].

Recently, this concept has been questioned. 3-Oxoacid CoA-transferase was measured in liver mitochondria and it was proposed that this enzyme plays a role in the control of hepatic ketogenesis [4]. Furthermore, authors in [5] reported much higher activities of acetoacetyl-CoA synthetase than were previously observed in liver. Finally, the data in [6] show that ketone bodies can be produced and utilized simultaneously by the perfused liver, leaving open the possibility that these processes proceed in different cell types. The study in [6] also indicates that ketone bodies are good precursors for lipid synthesis in adult rat liver.

From a physiological viewpoint it seems unlikely that in one cell compartment, the mitochondrion, fatty acids are degraded to yield ketone bodies which in another cell compartment, the cytosol, are utilized for fatty acid synthesis. It is possible, however, that ketone bodies function as intermediates in the formation of long-chain fatty acids from fatty acids of short and medium-chain length. Preferential use of ketone bodies for hepatic cholesterogenesis is another possibility. Arguments in favour of the latter hypothesis are:

(i) Acetoacetyl-CoA synthetase [5] and HMG-CoA reductase [7] have a similar diurnal rhythm, suggesting that acetoacetate can serve to carry C_4 units from mitochondria to the cytosol where the acetoacetyl moiety can be incorporated directly into cytosolic HMG-CoA and then into cholesterol;

(ii) Ketone-body utilization has been associated with cholesterogenesis in extra-hepatic tissues [8,9] and in isolated perfused rat liver [6].

The aim of this work was to answer the following questions:

(i) Are ketone bodies used for lipid synthesis by liver parenchymal cells or are other hepatic cell types involved?

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

(ii) Is acetoacetate used preferentially for the synthesis of cholesterol as has been shown in other tissues [8,9]?

Therefore, parenchymal cells isolated from rat liver were incubated with tritiated water and [3-^{14}C]acetoacetate alone or in combination with other lipogenic substrates and the amount of ^{3}H and ^{14}C label incorporated into fatty acids and cholesterol was measured. Hepatocytes from normal, starved and lactating female rats were compared. The possible importance of acetoacetate for hepatic lipid synthesis in different physiological states is discussed.

2. MATERIALS AND METHODS

Hepatocytes were isolated from Wistar rats which had free access to water and were fed a stock, pelleted diet. In some experiments animals were starved for 24 h or were meal-fed between 4 and 7 a.m. by an automatic feeding machine. All animals were killed between 9 and 10 a.m. Liver cells were isolated as in [10], with modifications described in [11]. Cell viability (Trypan blue exclusion) was routinely >95%. Isolated hepatocytes (3–6 mg protein/ml) were suspended in Krebs–Henseleit bicarbonate buffer, supplemented with 3.5% bovine serum albumin (charcoal-treated and dialyzed). Incubations (final volume, 3 ml) were carried out for 60 min at 37°C in a metabolic shaker (90 strokes/min) in 25-ml Erlenmeyer flasks under an atmosphere of 95% oxygen and 5% carbon dioxide.

To monitor total rates of lipid synthesis $^{3}H_2O$ (2.3 mCi/ml) was added to the cell suspensions. In parallel incubations, the contribution of different lipogenic substrates to lipogenesis was assessed by adding [3-^{14}C]acetoacetate, [1-^{14}C]acetate or [U-^{14}C]lactate, each with a specific radioactivity of 0.07 Ci/mol, to 3 ml cell suspension.

The rates of incorporation of ^{3}H or ^{14}C into fatty acids and digitonin-precipitable sterols were measured as in [12] and [13] and expressed in terms of acetyl units · mg protein^{-1} · h^{-1} [14,15].

Absolute rates of lipid synthesis vary from experiment to experiment. However, percentages of contribution were very reproducible between experiments. Therefore, in the figure and the tables representative experiments are presented which were reproduced with at least two other preparations of hepatocytes.

Protein was determined as in [16]. Radioactive compounds were obtained from the Radiochemical Centre, Amersham; collagenase type I and bovine serum albumin, fraction V, were purchased from Sigma; biochemicals were from Boehringer; other chemicals were from Baker.

3. RESULTS

Rates of fatty acid and of cholesterol synthesis were determined in hepatocytes isolated from rats in different physiological conditions and incubated with 10 mM glucose and 5 mM acetoacetate (table 1). The total rates of fatty acid synthesis measured by the incorporation of ^{3}H from $^{3}H_2O$, varied over a wide range. A 70-fold difference was found between the highest and the lowest rates in lactating and starved female rats, respectively. The total rates of cholesterol synthesis varied less; only a 6-fold difference occurred between the highest and lowest rates.

Acetoacetate contributed 14–54% of the total rates of fatty acid synthesis and 21–75% of the total rates of cholesterol synthesis (table 1). The relative contribution of acetoacetate to cholesterol synthesis was 1.4–2.3-times greater than to fatty acid synthesis.

The total rate of fatty acid synthesis was independent of the concentration of acetoacetate (fig.1). The contribution of acetoacetate to fatty acid synthesis was concentration-dependent and increased from 21% at 0.9 mM to 60% at 8.5 mM acetoacetate. The contribution of acetoacetate to cholesterogenesis increased also with the concentration of acetoacetate, but less: from 45% at 0.9 mM to 77% at 8.5 mM acetoacetate.

Addition of acetoacetate and acetate or lactate to the medium stimulated fatty acid synthesis. Cholesterogenesis, on the other hand, was suppressed by addition of these substrates (table 2). The contribution of acetoacetate to lipid synthesis is also affected by the presence of acetate or lactate, which are both good lipogenic substrates [17,18]. Acetoacetate contributed more to fatty acid synthesis in the presence of lactate than in the presence of acetate. Also a greater proportion of the newly synthesized cholesterol in the hepatocytes is derived from acetoacetate in the

Table 1

Effect of the physiological condition of the donor rat on lipogenesis from acetoacetate by isolated hepatocytes

Donor rat	Incorporation into fatty acids		Incorporation into cholesterol		(Incorporation into cholesterol)/(Incorporation into fatty acids)	
	^3H_2O	[3-^{14}C]Acac	^3H_2O	[3-^{14}C]Acac	^3H_2O	[3-^{14}C]Acac
♂ Meal-fed	49	7.1 (14)	3.7	0.8 (21)	0.08	0.11
♀ Fed	141	50.3 (36)	10.3	6.1 (60)	0.07	0.12
♀ Starved	3	0.4 (15)	1.8	0.6 (34)	0.60	1.50
♀ Lactating	204	73.3 (36)	10.7	7.7 (72)	0.04	0.11
♀ Lactating, starved	4	2.2 (54)	5.7	4.3 (75)	1.08	1.95

Hepatocytes were incubated for 60 min with 5 mM acetoacetate and 10 mM D-glucose in the presence of ^3H_2O. In parallel incubations tritiated water was omitted and acetoacetate was added as ^{14}C-labelled compound (Acac). Data are expressed in acetyl equivalents · mg protein^{-1} · h^{-1}. Values in parentheses are percentages of total synthesis measured by incorporation of ^{3}H from ^3H_2O

presence of lactate than in the presence of acetate. In combination with lactate and especially with acetate the contribution of acetoacetate to fatty acid synthesis is markedly diminished. The contribution of acetoacetate to cholesterol formation, however, is much less affected under these conditions (table 2). Results similar to those presented in table 2 for lactating rats were obtained for starved and fed female rats (not shown). The ratio of label incorporated into cholesterol over label incorporated into fatty acids was higher with [3-^{14}C]acetoacetate as substrate compared to label incorporation from other radioactive precursors (table 1,2). These results indicate that carbon from acetoacetate is preferentially incorporated into cholesterol compared to carbon from lactate and acetate. Glucose which is probably the endogenous carbon source for lipid synthesis in the absence of added substrates also appears to be less cholesterogenic than acetoacetate.

Table 2

Lipogenesis in isolated hepatocytes from various substrate mixtures

Substrate combination	Incorporation into fatty acids				Incorporation into cholesterol				(Incorporation into cholesterol)/(Incorporation into fatty acids)			
	^3H_2O	[3-^{14}C]-Acac	[1-^{14}C]-Ac	[U-^{14}C]-Lact	^3H_2O	[3-^{14}C]-Acac	[1-^{14}C]-Ac	[U-^{14}C]-Lact	^3H_2O	[3-^{14}C]-Acac	[1-^{14}C]-Ac	[U-^{14}C]-Lact
None	225	–	–	–	20.2	–	–	–	0.09	–	–	–
Acac	229	98 (43)	–	–	21.0	12.0 (57)	–	–	0.09	0.12	–	–
Acac + Ac	272	53 (20)	139 (51)	–	15.0	6.7 (45)	8.8 (59)	–	0.06	0.12	0.06	–
Acac + Lact	314	112 (36)	–	137 (44)	17.3	11.3 (65)	–	7.4 (43)	0.06	0.10	–	0.05

Hepatocytes from lactating rats were incubated with 10 mM glucose in the presence of ^3H_2O and one of the following substrate combinations: 5 mM acetoacetate (Acac), or 5 mM acetoacetate plus 5 mM acetate (Ac), or 5 M acetoacetate plus 5 mM lactate (Lact). In parallel incubations tritiated water was omitted and acetoacetate, acetate or lactate were added as ^{14}C-labelled compounds. Data are expressed in acetyl equivalents · mg protein^{-1} · h^{-1}. Values in parenthese represent percentages of total synthesis as measured by the tritiated water method

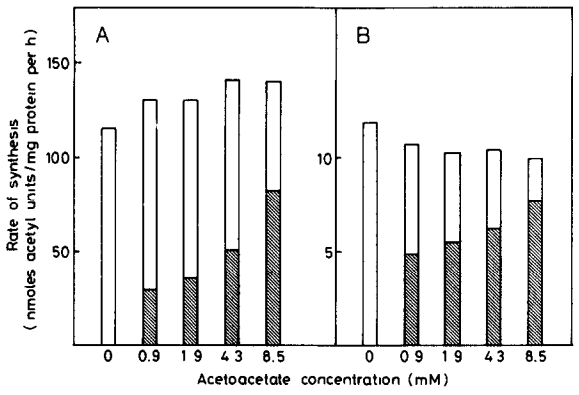

Fig.1. Lipogenesis in isolated hepatocytes as a function of the concentration of acetoacetate. Hepatocytes from fed female rats were incubated in the presence of 10 mM glucose, increasing concentrations of acetoacetate and ^3H_2O to assess the total rate of lipogenesis. In parallel incubations tritiated water was omitted and [3-^{14}C]acetoacetate was added to determine the contribution of acetoacetate to lipogenesis (shaded sections of the bars). Panel A: fatty acid synthesis; panel B: cholesterol synthesis.

4. DISCUSSION

Liver cells isolated and purified by the procedure of Seglen are almost exclusively parenchymal cells. This procedure removes over 95% of the non-parenchymal liver cells [10]. It has been shown that ketogenesis in isolated parenchymal cells proceeds at rates fully comparable with rates observed in the intact liver [19] and thus the parenchymal liver cells are responsible for the production of ketone bodies. Evidence that the liver can also utilize ketone bodies has been reported [3–6]. In isolated hepatocytes, we also measured net utilization of ketone bodies (not shown) and incorporation of label from [3-^{14}C]acetoacetate into lipids. Therefore, this study strongly suggests that parenchymal liver cells can also synthesize fatty acids and cholesterol from ketone bodies and that production and utilization of ketone bodies can occur in the same cell type; i.e., the parenchymal liver cell.

Acetoacetate has been considered as a carrier for acetyl units in extra-hepatic tissues [8,20]. Our data, which corroborate the work in [6] with perfused liver, indicate that acetoacetate can fulfill a similar carrier function in rat liver. The preferential incorporation of acetoacetate into cholesterol over fatty acids observed both in brain [8,9] and in liver ([6], here) suggests that acetoacetate has a specific function in cholesterogenesis.

The data in table 1 confirm the wide range of lipogenic rates observed previously in rat liver under different metabolic conditions [6,21–23]. It was the aim of this study to evaluate the contribution of ketone bodies to lipid synthesis under various metabolic conditions. Authors in [6] reported on liver-perfusion experiments in which ketone bodies contributed 22 and 80% of the total carbon incorporated into fatty acids and sterols, respectively, in the presence of (−)-hydroxycitrate. This study indicates that acetoacetate contributes substantially to hepatic lipid synthesis also under more physiological conditions: up to 54 and 75% of the carbon of newly synthesized fatty acids and cholesterol, respectively, originated from acetoacetate (table 1).

The preferential incorporation of acetoacetate into cholesterol in the presence of other lipogenic substrates (table 2) suggests:

(i) that acetoacetyl-CoA does not equilibrate with cytosolic thiolase;
(ii) hence, that the 4-carbon unit of acetoacetate can be incorporated directly into HMG-CoA and then into cholesterol (cf. [6]).

It has been recently reported [24] that ^{14}C from [1-^{14}C]oleate is actively incorporated into sterols by isolated rat hepatocytes. We propose that acetoacetate might well be an intermediate in this pathway. The observation by these authors that the incorporation rate gradually increases supports our hypothesis because the ketone body concentration in the medium will also increase gradually after addition of oleate to hepatocytes (cf. the concentration dependence shown in fig.1).

In conclusion, our results confirm the observations in [6] that the liver can utilize ketone bodies for the synthesis of lipids, especially of cholesterol. Furthermore, our data extend their results:

(i) Ketone-body production and utilization can proceed in the same cell type, i.e., the parenchymal liver cell;
(ii) The contribution of acetoacetate to lipid synthesis in hepatocytes is influenced by the contribution of other lipogenic substrates and by the physiological state of the donor animal;

(iii) Ketone bodies are preferred to other lipogenic substrates for the de novo synthesis of cholesterol.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the expert technical assistance of Mrs T.A. Wisserhof and thank Professor S.G. van den Bergh for his critical reading of the manuscript. These investigations were supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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