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Fatty acid uptake and metabolism to ketone bodies and triacylglycerol in rat and human hepatocyte cultures is dependent on chain-length and degree of saturation

Effects of carnitine and glucagon

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Rat and human hepatocyte cultures were incubated with 5 common plasma longchain fatty acids (C16-C18). Rates of fatty acid uptake were similar in rat and human hepatocytes and were of the order: 16:1 > 16:0; 18:2 > 18:1 > 18:0. Rates of ketogenesis were lower in human compared to rat hepatocytes. In rat hepatocytes glucagon stimulated ketogenesis only in the presence of exogenous carnitine and rates of ketogenesis were higher from unsaturated compared to corresponding saturated fatty acids. Glucagon decreased triacylglycerol secretion irrespective of the fatty acid substrate and it increased intracellular triacylglycerol accumulation. The latter effect of glucagon was more marked in the absence of carnitine supplementation.

Fatty acid; Ketone body; Triacylglycerol; Carnitine; Glucagon; Cepatocyte (Rat, Human)

1. INTRODUCTION

The most abundant non-esterified fatty acids in plasma are: oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2), stearic acid (18:0) and palmitoleic acid (16:1) [1,2]. These fatty acids are taken up by the liver and esterified to glycerolipid or degraded to acetyl-CoA which is converted to ketone bodies or oxidised in the citrate cycle. The effects of saturated and unsaturated fatty acids on glycerolipid formation in liver have been studied using the perfused rat liver [3]; rat liver slices [4]; freshly isolated rat and pig hepatocyte suspensions [5,6] and rat hepatocytes in primary culture [7–10]. There is little agreement whether rates of glycerolipid synthesis or secretion are lower [8], higher [3,7] or the same [6,10] with saturated compared to unsaturated fatty acids. Few studies have compared the rates of total fatty acid uptake and the relative partitioning of fatty acids between esterification and β-oxidation for different fatty acids [3]. It is commonly assumed that saturated and unsaturated fatty acids are taken up by the liver and oxidised at the same rate [3]. However, differential oxidation of saturated and unsaturated fatty acids has been demonstrated in vivo [11]. Hepatocytes in primary culture are currently the preferred technique for studying fatty acid metabolism in liver, because during the first few days in culture they express differentiated liver functions and their long-term viability permits studies of longer term duration than is possible with the perfused liver or hepatocyte suspensions.

L-Carnitine (3-hydroxy-4-*N*-trimethylaminobutyrate) is essential for the transfer of long-chain fatty acids into mitochondria [12]. Hepatocyte carnitine is lost during collagenase perfusion of the

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liver [13] and when the cells are maintained in carnitine-free medium [14]. As a result, hepatocytes cultured in serum-free medium are usually carnitine deficient. Studies on glycerolipid formation in hepatocyte cultures have been conducted in the absence of carnitine supplementation. Consequently, partitioning of fatty acids towards β-oxidation is very low [9,15]. Differing degrees of carnitine deficiency might explain some of the anomalies in the literature on the effects of fatty acid chain length and unsaturation on glycerolipid formation [3,6,8,10].

We examined rates of total fatty acid uptake and formation of ketone bodies and triacylglycerol in rat hepatocytes incubated with 5 plasma fatty acids in the presence or absence of exogenous carnitine or glucagon. In order to investigate the validity of extrapolating from rat hepatocytes to the human situation we compared the rates of fatty acid metabolism in rat hepatocytes with human hepatocyte cultures.

2. MATERIALS AND METHODS

2.1. *Materials*

Glucagon, lactic acid and fatty acids or their sodium salts were from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum albumin, fraction V, was from Boehringer (Mannheim, FRG); it was defatted [16] and dialyzed before use. Dimethyl sulphoxide (DMSO) was from BDH Ltd. (Poole, Dorset, England). Sources of other reagents and culture materials were as described [17].

2.2. *Hepatocyte isolation and culture*

Hepatocytes were isolated from livers of adult female Wistar rats (255–265 g body wt) by collagenase perfusion [18] and cultured in monolayer in 25 cm^2 flasks [17], 4 h was allowed for cell attachment. The medium was then replaced by serum-free MEM containing dexamethasone phosphate (10 nM) either without or with glucagon (100 nM). Human hepatocytes were isolated from liver sections from organ donors (M, 49 yr, F, 47 yr) [19] and cultured as for rat hepatocytes except that 12 h was allowed for cell attachment. The medium was replaced by serum-free MEM containing 10 nM dexamethasone phosphate. The flasks were incubated at 37°C equilibrated with 5% CO_2/air. The incubations with fatty acids were performed after 14 h for the rat cultures and a further 1 h for the human cultures.

2.3. *Incubations with fatty acids*

The fatty acid solutions were prepared by dissolving: 150 μmol of palmitoleic acid or oleic acid in 600 μl of DMSO or 150 μmol of the sodium salts of palmitic, stearic or linoleic acid in 7 ml distilled water (at 60°C). These solutions were added to 26 ml of defatted bovine serum albumin (10%, w/v). 7 ml of water or 600 μl of DMSO were then added to the solutions of free acids or sodium salts respectively. These solutions were diluted in MEM containing 5 mM L-lactate to a final fatty acid concentration of 1.0 mM. The fatty acid concentrations were assayed enzymically [20] and were usually within 2% of 1.0 mM and small differences were adjusted by appropriate additions. An albumin/MEM solution containing DMSO without fatty acid was also prepared. All solutions were clear.

The rat hepatocyte monolayers, precultured without or with glucagon, were incubated with 2 ml of MEM/albumin without or with 1.0 mM fatty acid. For each fatty acid or fatty acid-free solution parallel incubations (each in duplicate flasks) were set up either without added carnitine or with 0.5 mM L-carnitine. Glucagon (100 nM) was added to the monolayers that had been precultured with glucagon. Human hepatocytes were incubated with 0.5 mM carnitine. All incubations were at 37°C for 3 h. The medium was then decanted and an aliquot was deproteinized with $HClO_4$ (0.33 M) for immediate determination of acetoacetate and D-3-hydroxybutyrate [21]. Fatty acid [20] and triacylglycerol [22] were determined in the untreated medium using kits from Wako, Japan and Roche Diagnostics, Basle, Switzerland, respectively. The hepatocyte monolayers were rinsed three times with 150 mM NaCl and frozen for later extraction [21] and determination of triacylglycerol [22] and protein [23]. Preliminary time course experiments on fatty acid clearance from the medium and formation of ketone bodies (acetoacetate and D-3-hydroxybutyrate) established that rates were linear with time for at least 3 h. Rates of fatty acid uptake and ketogenesis were determined from the difference in substrate or product concentration at the beginning of the incubation and after 3 h and are expressed as nmol of fatty acid utilized/h per mg cell protein and nmol of acetoacetate + D-3-hydroxybutyrate formed/h per mg protein. The accumulation of triacylglycerol during the 3 h incubation was determined by subtracting the cellular triacylglycerol content of control flasks (precultured without or with glucagon and terminated at the start of the fatty acid incubations) from the triacylglycerol content in monolayers incubated with the fatty acids for 3 h. Values are means ± SE for duplicate flasks from 4 rat and 2 human hepatocyte cultures. Statistical analysis for rat hepatocyte data was by the Student's paired *t*-test.

3. RESULTS

3.1. *Effects of L-carnitine in rat hepatocytes*

Rat hepatocytes pre-cultured without or with glucagon (100 nM) were incubated in medium containing 1 mM fatty acids either in the absence or presence of L-carnitine (fig.1). The presence of carnitine did not affect the rate of fatty acid uptake but it increased ($P < 0.005$) the rates of ketogenesis from the fatty acids by 95–175%. In carnitine-free incubations the intracellular accumulation of triacylglycerol was greater ($P < 0.05$) than in the carnitine supplemented incubations. The increased accumulation of triacylglycerol in carnitine-free incubations was different for

the various fatty acids and was greatest for palmitate (61 and 78% increase, without and with glucagon respectively) and lowest for palmitoleate (13 and 19% increase, without or with glucagon respectively).

3.2. *Uptake and metabolism of different fatty acids by rat hepatocytes*

Irrespective of whether cells were cultured with glucagon or incubated with carnitine, the rate of fatty acid uptake was higher for palmitoleate than for palmitate ($P < 0.05$) which in turn was higher ($P < 0.005$) than for stearate and oleate. In cells cultured with glucagon and incubated with carnitine the rates of ketogenesis were similar for the three unsaturated fatty acids but higher than for the corresponding saturated fatty acids (fig.1). Since the uptake of palmitoleate was higher than for oleate and linoleate, the relative partitioning of fatty acids towards ketogenesis was higher for the latter two fatty acids (46–52%) relative to palmitate, palmitoleate and stearate (32–35%). The accumulation of cellular triacylglycerol was higher for palmitate and palmitoleate than for the other fatty acids ($P < 0.05$) in the absence of carnitine supplementation but in the presence of carnitine it was higher only for palmitoleate ($P < 0.05$).

3.3. *Effects of glucagon in rat hepatocytes*

In the presence of carnitine, the glucagon-cultured cells had higher ($P < 0.05$) rates of ketogenesis from palmitate (22%), palmitoleate (37%), oleate (34%) and linoleate (38%), but in the absence of carnitine supplementation there was no increase in ketogenesis with glucagon. Hepatocytes cultured with glucagon secreted less triacylglycerol, irrespective of the substrate, than cells cultured without the hormone, and in the absence of carnitine the intracellular accumulation of triacylglycerol was higher in the glucagon cultures than in the controls (fig.1).

3.4. *Fatty acid metabolism in human hepatocytes*

The rates of fatty acid uptake in human hepatocytes were similar to the values in rat hepatocytes and were of the order (16:1 > 16:0 and 18:2 > 18:1 > 18:0). In contrast, the rates of ketogenesis in human hepatocytes were lower than in rat (fig.1). Consequently when the rates of ketogenesis are expressed as a percentage of the total rate of fatty acid uptake, the values for human hepatocytes were between 12 and 18% whereas the corresponding values for rat hepatocytes were 30–50%. Similar rates of palmitate uptake and ketogenesis from palmitate were observed in four other human hepatocyte preparations (Tosh, D. and Agius, L. unpublished). The rates of triacylglycerol secretion were between 10 and 30% of the rates of triacylglycerol accumulation in the cells and showed little variation with the different substrates. Glucagon (1–100 nM) decreased triacylglycerol secretion during incubation of human hepatocyte cultures with palmitate in 3 out of 6 preparations. The latter had a higher endogenous triacylglycerol content (507 ± 214 vs 120 ± 17 nmol/mg protein, means ± SE, $n = 3.3$).

4. DISCUSSION

This study shows that the rate of fatty acid uptake and metabolism by rat and human hepatocyte cultures is dependent on chain-length and degree of saturation. The general pattern for the different fatty acids was similar for human and rat hepatocytes. Rates of fatty acid uptake were higher for unsaturated than for corresponding saturated fatty acids. These findings agree with a study on fatty acid uptake in perfused rat liver [24] but disagree with other studies using the perfused rat liver and hepatocyte cultures [3,9] which concluded that rates of uptake of fatty acids of different chain-length and saturation are the same. In a study on glycerolipid formation in pig hepatocytes, although fatty acid uptake was not determined, triacylglycerol formation from palmitate was higher than from stearate, suggesting that even in this species stearate is metabolised at a lower rate than palmitate [5]. Since the liver plays a major role in the removal of fatty acids from the circulation, differences in the uptake of the common plasma fatty acids may account for differences in fatty acid composition between adipose tissue and plasma in the fasting state [2].

The cellular uptake of fatty acids was thought to be a passive diffusional process but recent evidence suggests that it is, at least in part, mediated by a specific membrane binding protein [25–27]. The different rates of uptake of saturated and un-

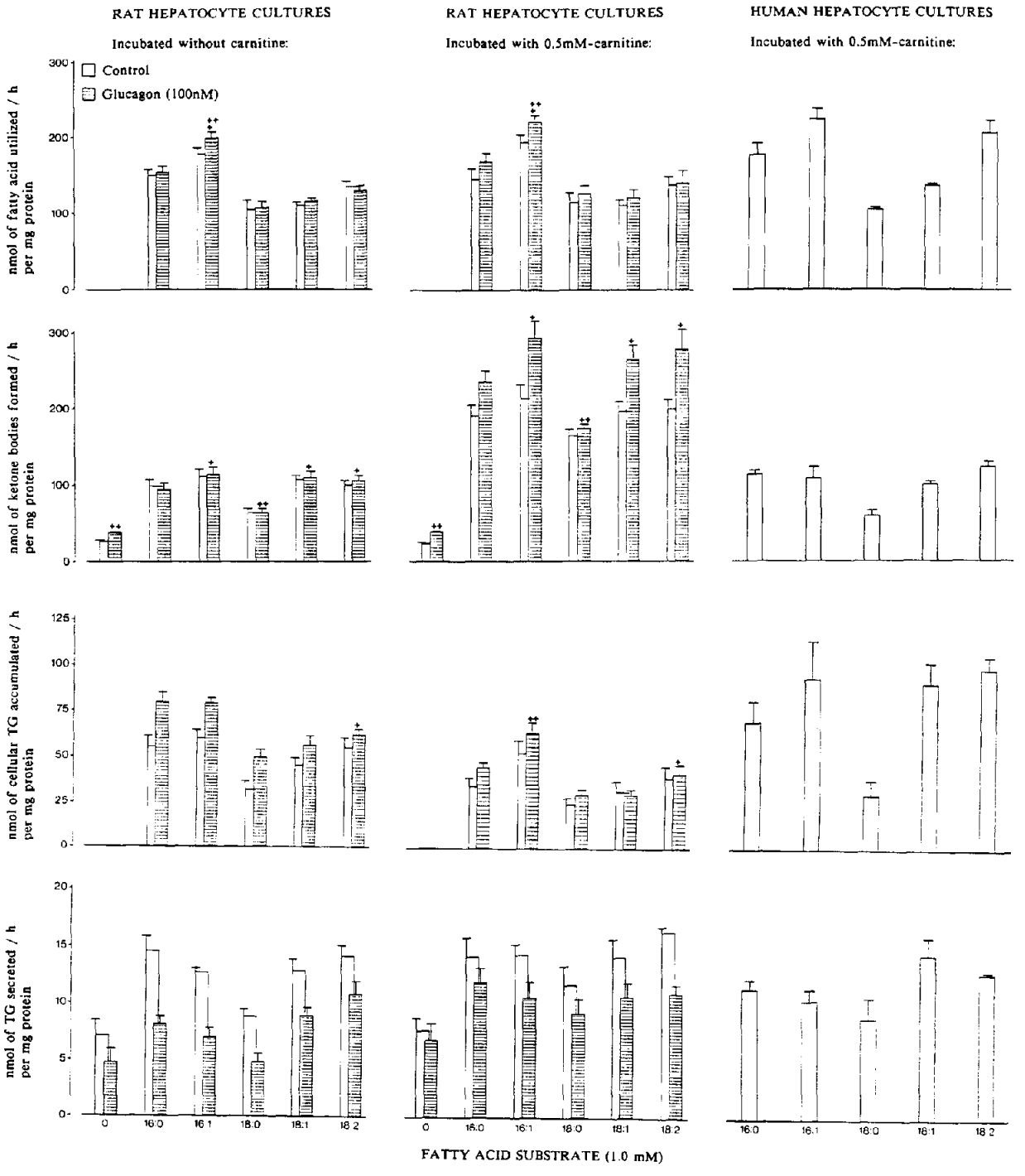

Fig.1. Fatty acid metabolism in rat and human hepatocyte cultures incubated with no fatty acid (0); palmitate (16:0); palmitoleate (16:1); stearate (18:0); oleate (18:1) or linoleate (18:2). Statistical analysis for rat hepatocytes (paired t-test): +, $P < 0.05$ for differences between unsaturated and corresponding saturated fatty acid; + +, $P < 0.05$ relative to all other fatty acids.

saturated fatty acids may be due to: (i) differences in affinity of the membrane carrier towards the fatty acid; (ii) differences in intracellular binding to the Z-protein or activation to the CoA ester; (iii) differences in intracellular metabolism via β-oxidation or glycerolipid formation. The latter, however, seems unlikely to be the main factor because carnitine supplementation in the rat cultures caused a large increase in ketogenesis (and presumably β-oxidation [17]) without affecting the overall rate of fatty acid uptake.

To date, the majority of studies on fatty acid metabolism in rat hepatocyte cultures have been performed in the absence of exogenous carnitine, under conditions where β-oxidation is very low [9,15]. This study shows that in the absence of exogenous carnitine the net rates of intracellular triacylglycerol deposition are significantly higher than in the presence of carnitine and the patterns of triacylglycerol deposition for the various substrates were also different. Carnitine supplementation of the cultures is more likely to mimic the physiological situation than carnitine lack, because the endogenous carnitine content of the cultures is lower than in freshly isolated hepatocytes [17].

Although rat and human hepatocytes had similar rates of fatty acid uptake, the rate of production of ketone bodies was lower in human hepatocytes. In rat hepatocytes ketone bodies are the major products of fatty acid β-oxidation. Whether the low rate of ketogenesis in human hepatocytes is due to a low rate of β-oxidation or to diversion of mitochondrial acetyl-CoA to oxidation in the citrate cycle or to conversion to acetylcarnitine because of the high activity of carnitine acetyltransferase in human liver is not clear.

The role of hormones in regulating hepatic fatty acid metabolism and triacylglycerol secretion is of major interest in understanding changes in lipid metabolism in endocrine disease. The finding that glucagon increased cellular triacylglycerol in rat hepatocytes agrees with previous studies showing increased glycerolipid synthesis in rat hepatocyte cultures after long-term exposure to glucagon [28,29]. The finding that glucagon inhibited triacylglycerol secretion in rat hepatocyte cultures despite increasing intracellular triacylglycerol accumulation is of interest because it is frequently assumed that inhibition of triacylglycerol secretion by glucagon reflects inhibition of synthesis. Although studies on the perfused rat liver have clearly established that glucagon suppresses the secretion rate of triacylglycerol [30,31], at high perfusate fatty acid concentrations the secretion rate of triacylglycerol is low in comparison with the intracellular accumulation and consequently represents only a small proportion (20%) of total synthesis. The accumulation of cellular triacylglycerol does not appear to be affected by glucagon [30]. It therefore cannot be unequivocally assumed that suppression of secretion by glucagon reflects inhibition of synthesis. An inhibition of radiolabelled fatty acid incorporation into triacylglycerol during acute exposure to glucagon has been demonstrated in freshly isolated hepatocyte suspensions and monolayer cultures [32,33]. Incorporation of radiolabelled glycerol into triacylglycerol was, however, unaffected [33]. Whether these differences reflect changes in glycerolipid synthesis or changes in the specific activity of intermediates is not clear. In human hepatocyte cultures a decrease in triacylglycerol secretion in response to glucagon during incubation with palmitate was observed only in some experiments. Cultures in which glucagon did not decrease triacylglycerol secretion had a very high endogenous triacylglycerol content. Studies on rat hepatocyte cultures have suggested that accumulation of cellular triacylglycerol is a function of medium [fatty acid] whereas the secretion rate is related to the endogenous content [34]. The relation between hormonal control of triacylglycerol secretion and endogenous cell content warrants further study.

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