GLYCEROL-3-PHOSPHATE CONTENT AND TRIACYLGLYCEROL SYNTHESIS IN ISOLATED HEPATOCYTES FROM FED AND STARVED RATS

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Received 16 December 1980

1. Introduction

For a given load of fatty acids the perfused liver or isolated hepatocytes from fed rats esterify a greater proportion of the incoming fatty acids than do similar preparations from starved rats. In the latter oxidation is increased relative to esterification [1–3]. Since esterification and oxidation constitute a branch-point, the nutritional and hormonal control of esterification and oxidation can theoretically be exerted either on one arm of the branch-point or in reciprocal manner on both arms simultaneously. Recently, evidence has been offered that fatty acid oxidation is controlled by the hepatic levels of carnitine and especially of malonyl-CoA, a potent inhibitor of carnitine palmitoyltransferase I [4]. It is not clear, however, whether there exists a separate control on esterification. The moderate decrease in the activity of glycerol-3-phosphate acyltransferase that is observed in the starved state [5–7], has been proposed as a possible factor regulating esterification. The availability of glycerol-3-phosphate has been considered as another regulatory factor [1,7–11]. However, a wide range of overlapping glycerol-3-phosphate concentrations has been reported for fed and starved animals [2,10,12–15]. Moreover, interpretation of the data is complicated by the fact that the concentration range of glycerol-3-phosphate which might be regulatory in the intact hepatocyte, is not known. Here, the intracellular concentration range within which glycerol-3-phosphate exerts a regulatory function, was determined.

2. Materials and methods

Male Wistar rats (150–200 g) were maintained either on a standard laboratory diet or on a high-sucrose, fat-free diet as in [16]. Starved rats were deprived of food for 24 h. Hepatocytes were isolated and incubated in the presence of albumin and various concentrations of [1-^{14}C]palmitate as in [17]. Incubations were terminated with $HClO_4$ and glycerol-3-phosphate was determined on the neutralized extracts using a fluorimetric adaptation of the method in [18]. Radioactive CO_2, acid-soluble radioactivity (oxidation products) and incorporation of radioactivity in diglycerides and triglycerides were measured as in [17]. Incorporation of radioactivity in lysophosphatidate and phosphatidate was measured after extraction of hepatocytes with butanol. The extracts were evaporated, the residues dissolved in $CHCl_3$:CH_3OH (1:1) and the phospholipids separated on Silicagel thin-layer plates in a two-dimensional way with $CHCl_3$:CH_3OH:NH_4OH:H_2O (65:30:2.5:2.5) and acetone:$CHCl_3$:CH_3OH:acetic acid:H_2O (48:36:12:12:6) as the first and second solvent system, respectively.

3. Results and discussion

Fig.1 illustrates the oxidation and esterification to triglycerides of [1-^{14}C]palmitate by hepatocytes from fed and starved rats. Hepatocytes from starved rats oxidized palmitate at higher rates than hepatocytes from fed rats, especially at low palmitate concentrations (A). Rates of triglyceride synthesis were much lower in cells from starved rats than in those from fed rats at all palmitate concentrations used (B). To investigate the degree to which the decreased esterification in cells from starved animals was a consequence of the increased palmitate oxidation, rates of esterification were compared under conditions where fatty acid oxidation was suppressed by the addition of (+)-octa-

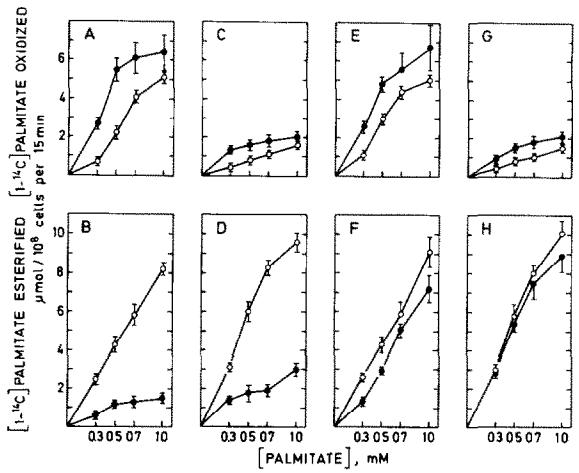

Fig.1. [1-^{14}C]Palmitate oxidation (upper) and esterification (lower) was measured in isolated hepatocytes from fed (○) and starved (●) rats, on a standard diet. Preincubation (10 min) and incubation (15 min) conditions were: (A, B) no additions (controls); (C, D) 2 mM (+)-octanoylcarnitine; (E, F) 1 mM glycerol; (G, H) 1 mM glycerol and 2 mM (+)-octanoylcarnitine. Incubations were started by the addition of palmitate. Oxidation represents the formation of CO_2 and acid-soluble products and is expressed as palmitate equivalents. Esterification is expressed as palmitate equivalents incorporated in triglycerides. Results are means ± SEM for 4 expt.

noylcarnitine, an inhibitor of carnitine palmitoyltransferase [19] (C, D). Esterification increased slightly but differences between both nutritional states remained equally large, suggesting that the activity of one or more regulatory enzymes involved in esterification was much smaller in the starved state or that the levels of glycerol-3-phosphate, the co-substrate for esterification, were not sufficiently high in cells from starved animals. To test these possibilities glycerol and a combination of glycerol plus (+)-octanoylcarnitine were added. Glycerol alone barely stimulated esterification in cells from fed rats but caused a dramatic increase in triglyceride synthesis in cells from starved animals suggesting that glycerol-3-phosphate deficiency was limiting esterification in these cells. Consistent with this idea were the findings that, at the end of the incubations, cells from fed animals contained 0.42 ± 0.05 µmol glycerol-3-phosphate/10^8 cells (n = 6) whereas cells from fasted animals contained only 0.16 ± 0.02 µmol/10^8 cells (n = 6). Addition of glycerol raised the glycerol-3-phosphate content to 4–5 µmol/10^8 cells. The stimulatory effect of glycerol was most marked at elevated palmitate concentrations so that rates of esterification in cells from fed and starved animals became almost equal. At low palmitate concentrations, where differences in oxidation were largest (E), a substantial difference in esterification persisted. All differences in esterification between both nutritional states disappeared completely when oxidation was suppressed by the addition of (+)-octanoylcarnitine to the cells incubated in the presence of glycerol (G, H). The latter experiments indicate that:

(i) In the starved state the increased oxidation succesfully competes for fatty acids with the esterification sequence;

(ii) In the presence of glycerol the esterification capacity is not deficient in cells from starved animals.

Similar results were obtained with rats on a fat-free, high-sucrose diet. In these cells the esterification capacity was even higher in the starved than in the fed state (not shown).

The strong stimulatory effect of glycerol on esterification and the low glycerol-3-phosphate content of cells from starved animals strongly suggested that glycerol-3-phosphate availability was limiting triglyceride synthesis and that such cells might provide a unique opportunity to study the concentration range in which glycerol-3-phosphate could exert a regulatory role in esterification. Therefore cells from starved animals were incubated in the presence of different precursors of glycerol-3-phosphate and its content as well as rates of triglyceride synthesis were measured (fig.2). In the absence of precursors, glycerol-3-phosphate was 0.1–0.2 µmol/10^8 cells and gradually rose to 0.3–0.4 µmol/10^8 cells in the presence of alanine or lactate. Triglyceride synthesis, which was very low in the absence of precursors, steeply increased with rising glycerol-3-phosphate content of the cells and reached a plateau at 0.3–0.4 µmol glycerol-3-phosphate/10^8 cells. Half-maximal rates of esterification were attained at ~0.2 µmol glycerol-3-phosphate/10^8 cells. Extrapolation of the curves shows an intersection with the x-axis 0.11–0.17 µmol glycerol-3-phosphate/10^8 cells. This suggests that this amount of glycerol-3-phosphate may not be available for esterification possibly as a result of binding or compartmentation. A sigmoidal shape of the curves is another possibility that cannot be excluded.

The concomitant increase of glycerol-3-phosphate levels and rates of triglyceride synthesis as depicted in fig.2 strongly suggest that glycerol-3-phosphate sti-

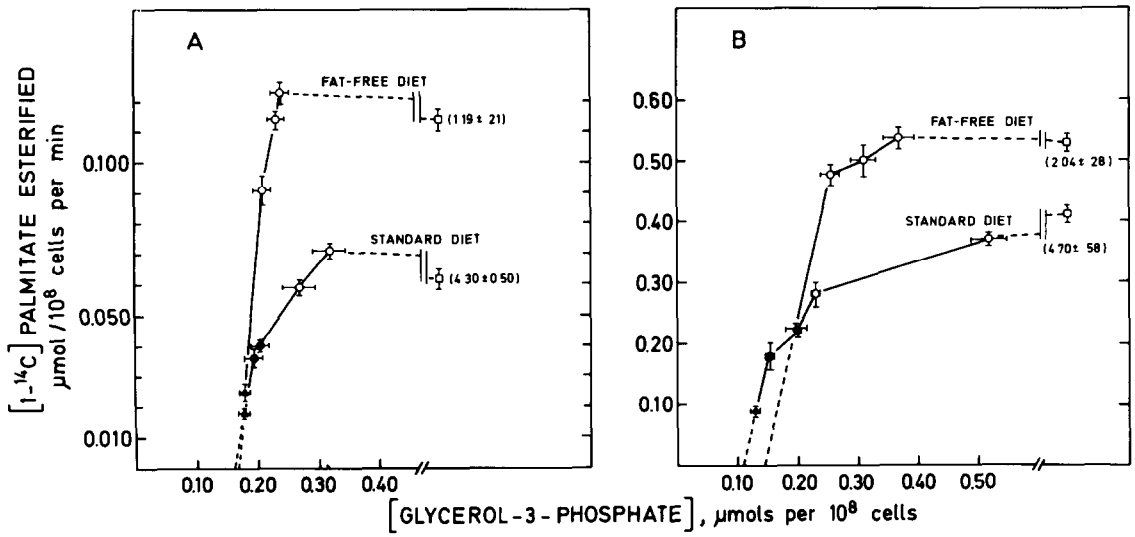

Fig.2. Hepatocytes from starved rats were incubated with 0.3 mM (A) or 1 mM (B) [1-^{14}C]palmitate. During preincubation (10 min) and incubation (10 min) the following glycerol-3-phosphate precursors were present: (▲) none (controls); (●) alanine, 0.5 or 5 mM; (○) lactate plus pyruvate, 0.5, 1 or 2.5 mM (ratio 10/1); (□) glycerol, 1 mM. [1-^{14}C]Palmitate incorporated into triglycerides and glycerol-3-phosphate content were measured at the end of the incubation. Results are means ± SEM for 4 expt.

mulated triglyceride synthesis by acting as the co-substrate for esterification. To exclude the possibility that glycerol-3-phosphate or perhaps another glycolytic/glyconeogenic intermediate that might rise concomitantly, would enhance triglyceride synthesis by allosterically activating one of the enzymes of the esterification pathway, we determined the incorporation of radioactivity from [1-^{14}C]palmitate into the intermediary products of esterification in cells from starved rats incubated in the absence and presence of glycerol. Fig.3 shows that glycerol increased the incorporation of radioactivity into all intermediates. These experiments indicate that glycerol acted by enhancing the flux through the first steps of esterification and that the increase in glycerol-3-phosphate levels was most probable the cause of the rise in esterification.

4. Conclusion

Our experiments demonstrate that:

(i) At saturating glycerol-3-phosphate levels the esterification capacity of cells from starved rats is not deficient;

(ii) Glycerol-3-phosphate deficiency is severely limiting esterification in isolated hepatocytes from starved rats incubated in the absence of precursors;

(iii) Variations in glycerol-3-phosphate from 0.1–0.3 µmol/10^8 cells strongly alter rates of esterification.

Knowledge of this regulatory concentration range in isolated hepatocytes should facilitate our understanding of the possible role of glycerol-3-phosphate in the regulation of esterification in various in vivo situations.

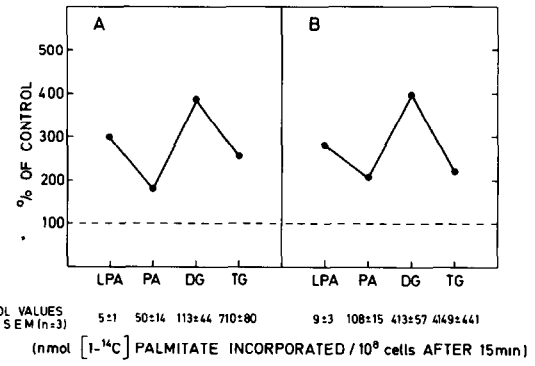

Fig.3. [1-^{14}C]Palmitate incorporation into intermediary products of triacylglycerol-biosynthesis and triacylglycerol was measured in hepatocytes from starved rats (standard diet) incubated with 0.3 mM (A) and 1 mM (B) [1-^{14}C]palmitate. During preincubation (10 min) and incubation (10 min) 1 mM glycerol was also present. The amount of [1-^{14}C]palmitate incorporated is expressed as % of control values (no glycerol added) shown on the figure. Results are means for 3 expt. *Abbreviations:* LPA, lysophosphatidic acid; PA, phosphatidic acid; DG, diacylglycerol, TG, triacylglycerol

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