Effects of Exogenous Fatty Acid Concentration on Glucagon-induced Changes in Hepatic Fatty Acid Metabolism

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SUMMARY

Studies were conducted to clarify the relationship between the external fatty acid concentration and glucagon in the regulation of hepatic fatty acid metabolism. Hepatocytes from fed rats were incubated with increasing concentrations of oleate (up to 1 mM) in the presence and absence of glucagon and the time sequence of changes in cellular malonyl-CoA levels, fatty acid synthesis, fatty acid oxidation, and ketogenesis were measured. At low concentrations of fatty acid the effect of glucagon was to abolish malonyl-CoA synthesis and lipogenesis and to produce a marked stimulation of fatty acid oxidation and ketogenesis. Similar effects were obtained with high concentrations of fatty acid in the absence of glucagon and, under these conditions, the additional presence of the hormone produced little further response. The results are consistent with the concept that the rate of fatty acid oxidation in liver is dictated largely by the relative concentrations of long-chain acyl-CoA (substrate for carnitine acyltransferase I) and malonyl-CoA (inhibitor of the transferase). They also indicate that the preemptive effect of fatty acids on glucagon-induced changes in fatty acid metabolism stems from their ability to reduce the tissue malonyl-CoA content, probably through long-chain acyl-CoA suppression of acetyl-CoA carboxylase. DIABETES 29:236–240, March 1980.

Evidence has been previously adduced that the ketogenic effect of glucagon on mammalian liver stems primarily from the ability of the hormone to switch the direction of fatty acid metabolism from synthesis to oxidation.[1–5] The key event in this metabolic transformation appears to be a cyclic AMP-mediated inactivation of key glycolytic enzymes (such as phosphofructokinase and pyruvate kinase) and acetyl-CoA carboxylase, which results in depletion of hepatic malonyl-CoA content and cessation of lipogenesis. Since malonyl-CoA is a potent inhibitor of carnitine acyltransferase I, the enzyme catalyzing the first step in fatty acid oxidation, a fall in its concentration serves to activate β-oxidation and thereby accelerate the production of acetoacetic and β-hydroxybutyric acids.

The above formulation was arrived at largely from studies in which the effects of glucagon on fatty acid metabolism were measured in hepatocytes from fed rats incubated with low concentrations of exogenous oleic acid (0.4 mM or less).[4,5] Under these conditions the hormone produced marked enhancement of fatty acid oxidation and ketogenesis. However, studies from other laboratories have demonstrated that the stimulatory effect of glucagon on fatty acid oxidation is much less pronounced when higher concentrations of fatty acid are employed.[6–8] Theoretically, this phenomenon might be explained as follows. Exposure of the liver to increasing concentrations of long-chain fatty acids produces concomitant elevation of intracellular levels of long-chain acyl-CoA.[9–11] The latter are potent inhibitors of acetyl-CoA carboxylase[12] and, as such, result in suppression of malonyl-CoA synthesis and lipogenesis.[6,11] Insofar as malonyl-CoA levels are reduced by this mechanism, the inhibitory effect of glucagon on fatty acid synthesis and its stimulatory effect on fatty acid oxidation should be preempted.

To test the validity of this thesis we felt it important to simultaneously examine rates of fatty acid synthesis, fatty acid oxidation, and ketogenesis together with changes in tissue malonyl-CoA levels in hepatocytes exposed to various concentrations of exogenous fatty acid, in the presence and absence of glucagon. The results obtained are entirely consistent with the predictions outlined above and emphasize the pivotal role of malonyl-CoA in regulating rates of hepatic fatty acid synthesis and oxidation.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing approximately 140 g at the time of experiments, were meal-fed a high sucrose, fat-free diet as previously described.[13] They were used for isolation of hepatocytes immediately after their last feeding.

Preparation of hepatocytes. Hepatocytes were prepared as described earlier[4] and were routinely adjusted to a cell density of 200 mg/ml in Krebs-Henseleit bicarbonate buffer (KHB).

Measurements of fatty acid oxidation, fatty acid synthesis, and cellular malonyl-CoA levels. Experiments were carried out at 37°C in 25-ml Erlenmeyer flasks. For studies of fatty acid oxidation the flasks initially contained 200 mg of hepatocytes in 2 ml of KHB with or without glucagon (10 μg).* After a preincubation period of 30 min, each flask received 0.5 ml of 14% defatted albumin in KHB containing sodium [1-^{14}C]-oleate (specific activity 0.4 μCi per μmol) in a concentration of 0, 1, 3, or 5 mM. This resulted in a final albumin concentration of 2.8% and final oleate concentrations of 0, 0.2, 0.6, or 1 mM in the incubation mixtures. The respective ν values (molarity fatty acid:molarity of albumin) were 0, 0.49, 1.48, and 2.46. When fatty acid synthesis and tissue malonyl-CoA levels were measured, radioactive oleate was replaced by nonradioactive fatty acid at the same concentrations. Fatty acid synthesis was assessed using ^3H_2O [4] and malonyl-CoA was assayed using a previously described radioisotopic method.[13] All other analytical procedures have been published.[4] In the text the term "fatty acid oxidation" will always refer to the conversion of labeled oleate into total acid-soluble products plus CO_2. The term "ketones" denotes the quantity of isotope from labeled oleate recovered in acetoacetate plus β-hydroxybutyrate.

Materials. The sources of all materials have been given previously.[3,4]

RESULTS

Figure 1 shows the effects of different oleate concentrations on the rate of fatty acid synthesis and the cellular malonyl-CoA content in hepatocytes from fed rats. As noted earlier,[4] in the absence of any additions the rate of fatty acid synthesis accelerated throughout the period of incubation (open circles, Figure 1A). This was accompanied by an initial rapid rise in the tissue malonyl-CoA level, which tended to plateau between the 30- and 60-min time points (Figure 1B). Both parameters were suppressed upon addition of oleate at 30 min, the degree of suppression increasing as the concentration of fatty acid was raised from 0.2 to 1 mM. Similarly, both began to revert toward control values as the added fatty acid was metabolized, though from the figure this is more obvious for malonyl-CoA concentrations than for the rate of fatty acid synthesis. In the presence of glucagon both the malonyl-CoA level and the rate of fatty acid synthesis were severely depressed during the initial 30 min of incubation. It is known that this action of the hormone results from inhibition of glycolysis and the acetyl-CoA carboxylase

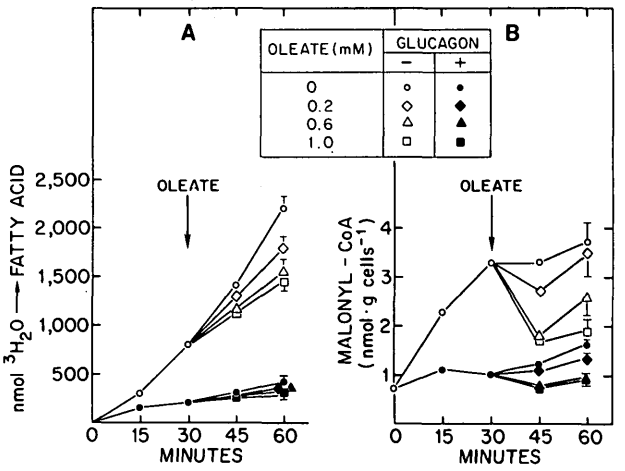

FIGURE 1. Effect of increasing concentrations of oleate on fatty acid synthesis and malonyl-CoA levels in hepatocytes from fed rats. Hepatocytes (200 mg) were initially incubated with 2 ml of Krebs-Henseleit bicarbonate buffer in the presence and absence of glucagon (10 μg) for the measurement of fatty acid synthesis (panel A) and cellular malonyl-CoA content (panel B), as described under MATERIALS AND METHODS. At 30 min 0.5 ml of 14% albumin in the same buffer, containing different concentrations of sodium oleate, was added to give the final concentrations of oleate indicated. Results are means ± SEM for 6–8 experiments.

step, probably through cyclic AMP-mediated enzyme phosphorylation.[4,5,14] As expected on the basis of previous studies,[4] the effects of the hormone began to wane during the latter period of the experiment. In this case also, the effect of increasing the exogenous oleate concentration was to cause a proportional further suppression of malonyl-CoA synthesis and lipogenesis.

Figure 2 shows the relationship between the average cellular content of malonyl-CoA between 45 and 60 min and the rate of fatty acid synthesis during this period. The expected linear plot was obtained. As routinely observed in this laboratory[4,5] such plots generally intersect the abscissa at a point equivalent to a tissue malonyl-CoA content between 0.5 and 0.7 nmol/g wet wt. We assume that this quantity of malonyl-CoA is bound to cellular macromolecules and is therefore unavailable for metabolic purposes.

Figure 3 shows the effects of glucagon on the rate of oxidation of [1-^{14}C]-oleate when the labeled fatty acid was added at the 30-min time point in the same concentrations as those employed in the experiments of Figure 1. From panel A it is evident that the degree of stimulation of overall fatty acid oxidation by glucagon decreased with increasing concentration of substrate. Qualitatively similar effects were seen as regards the rate of conversion of the labeled fatty acid into ketones (panel B). When the percent stimulation of oleate conversion into products over the 45–60-min interval was examined as a function of the concentration of fatty acid added at 30 min, the following relationships emerged. With 0.2, 0.6, and 1 mM oleate the stimulation of overall oxidation caused by glucagon was 167%, 93%, and 31%, respectively. Under the same conditions the hormone stimulated the conversion of oleate into ketones by 432%, 240%, and 115%, respectively. As pointed out earlier,[5] under these experimental conditions the stimulatory effect of glucagon on ketogenesis will always exceed the degree of enhancement of β-oxidation. The reason stems from the fact that by block-

* This pharmacologic concentration of glucagon was employed in order to obtain maximal effects of the hormone. As noted previously,[4] while much lower concentrations produce similar metabolic responses the effects are transient, probably as a result of hormone degradation by the hepatocytes.

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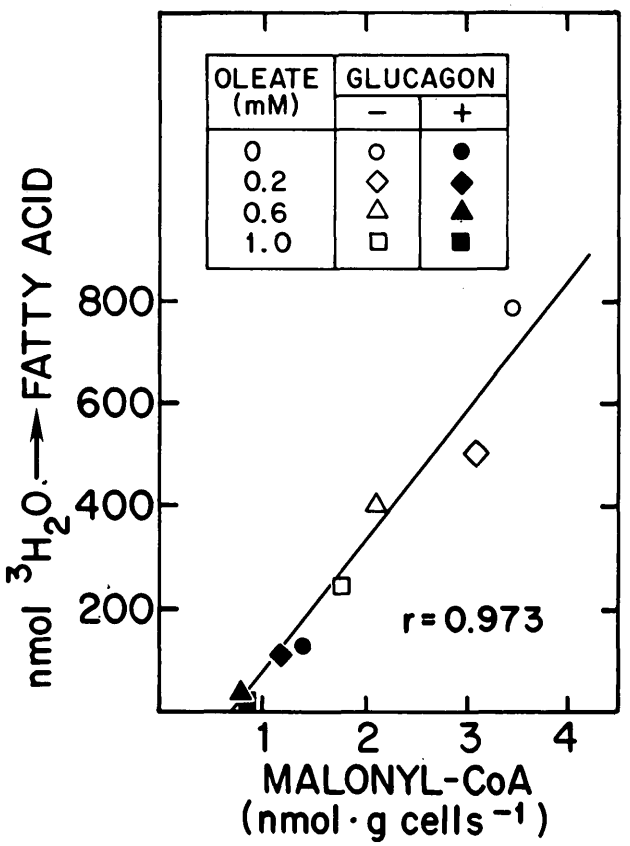


FIGURE 2. Relationship between the rate of fatty acid synthesis and cellular content of malonyl-CoA in hepatocytes from fed rats exposed to different concentrations of oleate. Taking the data from Figure 1, the rate of fatty acid synthesis has been plotted against the average tissue malonyl-CoA level between 45 and 60 min.

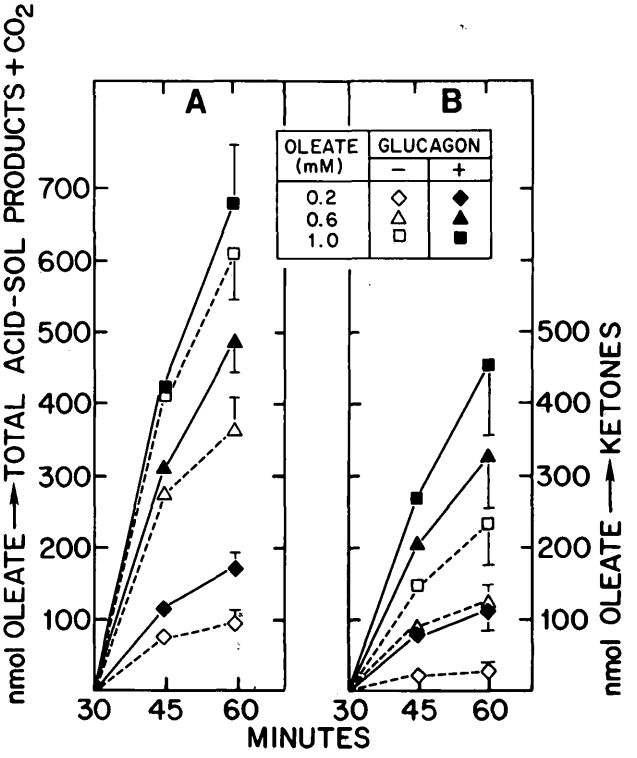


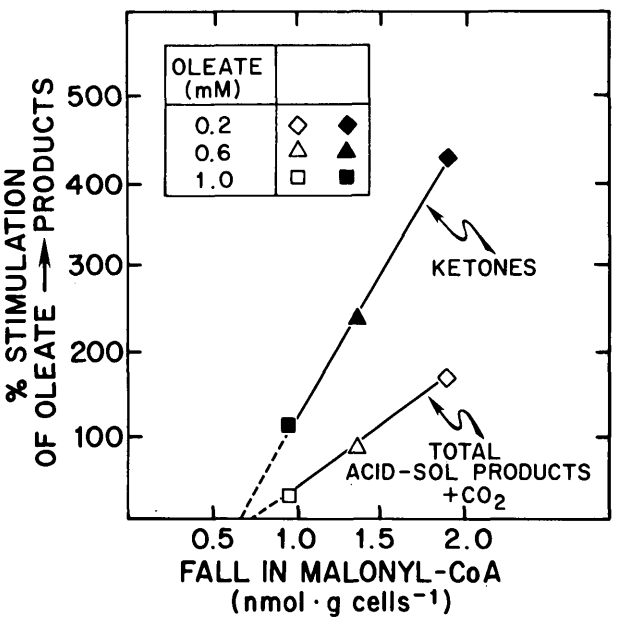
FIGURE 3. Effect of glucagon on the oxidation of different concentrations of oleate in hepatocytes from fed rats. Incubation conditions were identical with those described for Figure 1 except that radiolabeled oleate (1-^{14}C) was added at the 30-min time point. Oxidation products were measured as described under MATERIALS AND METHODS. Results are means ± SEM for four experiments.

ing endogenous pyruvate production, glucagon not only prevents the synthesis of malonyl-CoA but presumably also renders the cells deficient in oxaloacetate. As a result, in the presence of the hormone a much greater fraction of the acetyl-CoA generated through the β-oxidation process enters the ketogenic pathway rather than the tricarboxylic acid cycle.†

Figure 4 represents a combination of the data from Figure 1B and Figure 3 and shows the relationship between the ability of glucagon to lower the tissue malonyl-CoA level and the hormone's effect on fatty acid oxidation at the three concentrations of oleate used. Clearly, the higher the level of exogenous oleate, the less effective was the hormone in causing additional reduction of the malonyl-CoA concentration and further stimulation of the processes of β-oxidation and ketogenesis. Also evident from the figure is the extrapolation that had we used initial concentrations of oleate in excess of 1 mM, a point would presumably have been reached where glucagon would have produced no significant stimulation of fatty acid oxidation, while still causing a reduction in the tissue malonyl-CoA content of about 0.6–0.7 nmol/g. We assume that under such conditions the intracellular concentration of oleyl-CoA reaches a sufficiently high level to drive fatty acid oxidation maximally

† Carnitine was absent from all of the incubations reported here. However, had it been included together with glucagon, rates of fatty acid oxidation and ketogenesis would have reached values considerably higher than those seen with the hormone alone.[4,5]

FIGURE 4. Relationship between the stimulation of oleate oxidation and the fall in malonyl-CoA content caused by glucagon in hepatocytes from fed rats exposed to different concentrations of oleate. The data are taken from the 45–60-min intervals of Figures 1B and 3. Note that it is the fall in the average malonyl-CoA content that is plotted.



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such that this additional small fall in malonyl-CoA concentration has no discernible effect.

DISCUSSION

The purpose of the present investigation was to clarify the biochemical basis underlying the observation that the stimulatory effect of glucagon on fatty acid oxidation in livers from fed animals diminishes with exposure of the tissue to increasing concentrations of long-chain fatty acids. As noted in the introduction, a plausible explanation for this phenomenon could be advanced on the basis of isolated reports, each of which dealt with different aspects of hepatic fatty acid metabolism. However, validation of the scheme proposed required that four key metabolic parameters, namely fatty acid oxidation, ketogenesis, fatty acid synthesis, and tissue malonyl-CoA levels, be measured simultaneously and shown to correlate in the predicted fashion in cells exposed to glucagon and various concentrations of fatty acids. This was accomplished in the current studies.

Of the many biochemical factors that could potentially influence the overall rate of fatty acid oxidation in liver, it seems reasonable to conclude that four will be of major importance. These are the extracellular concentration of fatty acid and the intracellular levels of long-chain acyl-CoA, carnitine, and malonyl-CoA. Considering first the situation in vivo, two extremes can be clearly distinguished. In the carbohydrate fed state (low [glucagon]:[insulin]) plasma free fatty levels are low,[15] as are the intrahepatic concentrations of long-chain acyl-CoA[9,10] and carnitine,[16] while the tissue content of malonyl-CoA is elevated.[10,13] Under these conditions fatty acid synthesis is brisk and the physiologic role of malonyl-CoA can be viewed as a mechanism to ensure unidirectional flow of carbon from glucose → fatty acids → triglycerides → very low density lipoproteins by inhibiting carnitine acyltransferase I, and thereby preventing the futile reoxidation of newly synthesized fatty acids. At the same time, the oxidation of exogenous fatty acids and the production of acetoacetic and β-hydroxybutyric acids is restricted. By contrast, in ketotic states (high [glucagon]:[insulin]) the liver carnitine concentration rises,[16,17] malonyl-CoA levels fall,[10,13] fatty acid synthesis is greatly depressed,[5,10] plasma free fatty acids are elevated,[15] intrahepatic concentrations of long-chain acyl-CoA rise,[9,10] and the processes of fatty acid oxidation and ketogenesis occur at high (uninhibited) rates.[4,5,15]

Studies in vitro, such as those presented here and by others,[6–8] allow one to examine situations that are analogous to or intermediate between the two extremes noted above. For example, incubation of hepatocytes from fed rats with low concentrations of oleate simulated the in vivo environment of the liver in fed animals. Although not measured directly, it is reasonable to assume that under these conditions the tissue level of long-chain acyl-CoA (substrate for carnitine acyltransferase I) was also low.‡ The prevailing high concentration of malonyl-CoA (inhibitor of the transferase) served to drive lipogenesis and to suppress fatty acid oxidation. The effect of glucagon was to abolish malonyl-CoA synthesis and lipogenesis and to cause a marked stimulation of fatty acid oxidation and ketogenesis.† The key event in this switch of hepatic fat metablism from synthesis to oxidation presumably was the glucagon-induced lowering of the malonyl-CoA level without significant change in the concentration of long-chain acyl-CoA (because fatty acid levels were held low experimentally). On the other hand, exposure of fed hepatocytes to high concentrations of oleate (which ordinarily would occur only after fasting or in uncontrolled diabetes) revealed an additional level of metabolic control. In keeping with reports from other groups[6–8] the stimulatory effect of glucagon on fatty acid oxidation and ketogenesis was now far less pronounced. In confirmation of the findings of Cook et al.[6] we observed a fall in the cellular content of malonyl-CoA as the concentration of oleate in the medium was raised. This was accompanied by a proportional fall in the rate of endogenous fatty acid synthesis. There can be little doubt that the operative mechanism was inhibition of acetyl-CoA carboxylase due to an increase in the intracellular concentration of long-chain acyl-CoA.[12,‡] The net effect was that the high extracellular concentration of fatty acid indirectly enhanced its own oxidation by feedback inhibition of malonyl-CoA synthesis and lipogenesis. Under these circumstances it might be expected that glucagon, which exerts its effects by blocking synthesis of malonyl-CoA, would have progressively less impact on rates of fatty acid synthesis and oxidation as the external concentration of fatty acid is raised. This is in fact what was observed. In the latter situation the driving force for fatty acid oxidation was presumably also an elevation of the [long-chain acyl-CoA]:[malonyl-CoA] ratio, induced not by glucagon but by a rise in fatty acyl-CoA levels as the initial event.

It is noteworthy that over the time period during which metabolic events were studied, the tissue malonyl-CoA level swung from about 0.7 to 3.5 nmol/g wet wt. with the various manipulations employed. This is a similar range to that observed in our earlier studies[4,5] and, taken together with the fact that the Ki for malonyl-CoA toward carnitine acyltransferase I is in the region of 1.5 μM,[2,3] is entirely compatible with the postulated regulatory role of this compound in hepatic fatty acid oxidation.

Finally, the question has been raised as to whether the glucagon-induced removal of the malonyl-CoA inhibition of hepatic fatty acid oxidation has any relevance for the enhancement of ketogenesis in ketotic states; the argument here was that the high plasma fatty acid level itself should be sufficient to suppress acetyl-CoA carboxylase activity and thus allow rapid rates of ketone body production.[6] While conceivable in principle, evidence from a number of in vivo studies is against such a possibility. For example, in both dogs and humans[18,19] suppression of insulin and glucagon secretion by somatostatin produced a marked curtailment in hepatic ketone production as compared with rates observed when the glucagon deficit was corrected by exogenous administration of the hormone. In both situations plasma free fatty acid levels remained elevated because of insulin deficiency. Along the same lines, we infused a triglyceride emulsion plus heparin into saline- and glucagon-treated fed rats and found that ketonemia developed far more rapidly in the latter group, despite the fact that equally high plasma free fatty acid concentrations were achieved in both.[20] Similar studies were carried out by Schade and Eaton[21] in diabetic humans receiving a saline or glucagon infusion. Again, the ketogenic response to acute elevation of circulat-

‡ Hepatic levels of long-chain acyl-CoA are known to fluctuate in parallel with the plasma fatty acid concentration.[9–11]

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ing fatty acid levels was more pronounced in the individuals who were made hyperglucagonemic compared with the control group. Taken together, these observations suggest that in the intact animal the transformation of the liver from nonketogenic to ketogenic mode is initiated by elevation of the circulating [glucagon]:[insulin] ratio, independent of changes in the plasma free fatty acid concentration. As indicated by our studies with hepatocytes, the biochemical consequence of this hormonal perturbation at the level of the liver is glycogen depletion, reduction of the tissue malonyl-CoA content, and activation of the fatty acid oxidation machinery. High levels of plasma fatty acids, as would eventually be reached in prolonged fasting or uncontrolled diabetes, doubtlessly would contribute to the further suppression of lipogenesis and acceleration of the ketogenic process. However, we interpret this to be a secondary rather than the primary controlling mechanism.

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