Special Article

On the Maximal Possible Rate of Ketogenesis

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SUMMARY

The conversion of fatty acids to ketones is an energy-generating process. An evaluation of its quantitative significance on the energy metabolism of the human liver is presented. It is shown that in the decompensated diabetic state ketogenesis provides most of the energy used in this organ. Thus, under these conditions, ketogenesis appears to be limited by the rate of energy utilization in the liver. Since gluconeogenesis increases the energy expenditure in the liver, gluconeogenesis can be seen to have a permissive effect on ketogenesis. It is suggested that this interaction, at the level of the energy metabolism in the liver, explains why pathological rates of ketogenesis are found in metabolic situations where gluconeogenesis is very rapid. DIABETES *21*:50-53, January, 1972.

Under conditions of starvation, the production by the liver of ketones from fatty acids increases markedly and assumes an important role in supplying the peripheral tissues,[1] particularly the brain[2] and the kidney,[3] with a metabolic fuel capable of replacing glucose as a primary substrate for oxidative phosphorylation. In the uncontrolled diabetic state, the production of ketones exceeds their removal by the peripheral organs, leading to ketonuria and ketoacidosis. This latter type of pathological ketogenesis may be the result of massive free fatty acid mobilization and greatly increased levels of acyl-CoA and/or acetyl-CoA, and excessive oxaloacetate utilization for gluconeogenesis, possibly compounded by a shift toward malate in the mitochondrial equilibrium between the oxaloacetate-malate substrate pair.[1,4] As a consequence, the mitochondrial oxaloacetate concentration becomes insufficient to sustain the citrate synthetase reaction and hence the flow of intermediates through the citric acid cycle.[1,4] In turn, the formation of ketones from fatty acids increases as this process generates reducing-equivalents for energy production, thus making up for the deficient operation of the citric acid cycle.[1,4]

The extent to which the activity of the citric acid cycle can be reduced was recently illustrated by the finding that in livers from rats maintained on a high fat diet, when perfused with oleate, up to 90 per cent of the oxygen consumption could be attributed to fatty acid conversion to ketones.[5] Ketogenesis from fatty acid is thus tied into the liver cell's energy metabolism. Therefore, like any other energy-yielding process, ketogenesis has a theoretical upper limit which will be reached when ketogenesis provides enough reducing-equivalents to sustain the total energy expenditure of the liver. The maximal possible rate can be evaluated as follows:

In man, the consumption of oxygen by the splanchnic bed including liver, intestine, pancreas and spleen, is approximately one fifth of the total basal O_2 consumption, with the liver itself accounting for three fourths of the splanchnic O_2 consumption.[6] Since the total basal oxygen consumption by man is about 0.25 L./min., the daily consumption of O_2 by the liver can be estimated roughly at:

$$0.25 \text{ L./min.} \cdot 1/5 \cdot 3/4 \cdot 60 \text{ min./hr.} \cdot 24 \text{ hr./day} =$$

$$54 \text{ L./day, equivalent to } 54 \text{ L./day} \cdot \frac{1}{22.4 \text{ L/mole}} =$$

2.4 moles O_2/day.

Oleic acid closely resembles the average composition of free fatty acids mobilized from adipose tissue. If the ratio between the amounts of β-hydroxybutyrate and acetoacetate released by the liver is taken as 2,[3] the conversion of fatty acid to ketones is summarized by the following equation:

$$C_{18}H_{34}O_2 + 6O_2 \rightarrow 3C_4H_8O_3 + 1\ 1/2C_4H_6O_3 + 1/2\ H_2O.$$

It follows that a conversion of

$$\frac{2.4 \text{ moles } O_2/\text{day}}{6\ O_2/\text{mole}} = 0.4 \text{ mole/day of oleic acid}$$

to ketones is possible. This corresponds to a daily production of 0.4 x 3 moles of β hydroxybutyrate + 0.4 x

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1 1/2 moles of acetoacetate. The molecular weight of these acids being respectively 104 and 102, the highest possible rate of production of ketones would be approximately 185 gm./ day.

The oxygen consumption by perfused liver is increased by 30 per cent when free fatty acids are added to the perfusate. Because free fatty acid levels are markedly elevated in diabetes, the liver can be expected to consume more oxygen in the diabetic state.[4] Furthermore, reducing-equivalents are utilized in the synthesis of glucose from some of the gluconeogenic precursors. Together this allows for a corresponding increase beyond the rate of ketogenesis calculated above. However, this increase is partially offset by the inclusion of the oxygen consumed by the Kupffer cells in the figure for the O_2 consumption, although these cells presumably cannot meet their energy requirements through ketogenesis. Thus, on theoretical grounds, the maximal possible rate of ketogenesis may be evaluated at no more than 185 gm. + 30 per cent or some 240 gm./day.

In a recent review, the capacity of the liver to produce ketones was taken to be 150 gm./kg. liver/day,[4] or 240 gm./day for an adult with a liver weighing 1.6 kg. Measurements made during ketoacidosis in two diabetic patients (weighing 43.4 and 67 kg.) gave ketone production rates of 3.1 and 4 mg./min./kg. body weight,[7] or 195 and 385 gm./day respectively, but the second of these values appears excessively high. In some extreme cases of ketoacidosis, the excretion of ketones in the urine was found to be 110,[8] 120,[9] 145[10] gm./day, even in emaciated patients (body weights, 55, 55, 49 kg.). If one considers all these data and the fact that ketone utilization by peripheral tissues is about 90 gm./ day during physiological starvation ketosis,[3] one is led to estimate the highest rates of ketone production encountered in man to be in the vicinity of 200 gm./day.

This figure is of the same order as the maximal possible rate calculated on the assumption that the oxygen consumed by the liver is utilized entirely for the reoxidation of the reducing-equivalents produced during ketogenesis. It is evident, then, that the generation of reducing-equivalents during the conversion of fatty acids to ketones can reach a magnitude such that the ability of the liver to utilize these reducing-equivalents could become a rate-limiting factor for ketogenesis. In fact, this limitation, at the level of the liver cell's energy balance, can provide an explanation for the upper limit observed in the rate of ketogenesis.

It has been noted that extreme rates of gluconeogenesis are generally accompanied by high rates of ketogenesis.[1,4,11,12] Renold and Cahill[12] have pointed out that intensive gluconeogenesis must entail rapid catabolism of fatty acids to ketones to provide the reducing-equivalents needed for the synthesis of glucose. If, as suggested by the above calculation, the balance between energy production and utilization should play a role in controlling ketogenesis, then gluconeogenesis could exert a controlling action on ketogenesis, not only by decreasing the supply of oxaloacetate available for the entry of acetyl-CoA into the citric acid cycle, but also by raising the energy expenditure, thus allowing for a greater flow through the energy-generating pathway of fatty acid conversion to ketones. Conversely, antiketogenic effects could conceivably be due in part to a sudden decrease in the expenditure of reducing-equivalents and ATP for gluconeogenesis which is one of the most significant energy-consuming processes in the diabetic liver. Another mechanism for antiketogenic effects could be exerted by substrates which feed into the citric acid cycle at a point beyond isocitrate, since this cycle appears to be most powerfully regulated at the citrate synthetase and isocitrate dehydrogenase steps.[13,14]

Recent investigation performed during prolonged starvation has shown that the liver primarily extracts alanine,[15] a glucogenic amino acid, from the circulation. The amount of acetyl-CoA produced by amino acid degradation in the liver is therefore limited, in accordance with the findings that ketones are primarily derived from fatty acids.[1,4] Thus the ketogenic moieties of amino acids are in fact used for energy production in peripheral tissues, rather than for ketone production in the liver. While gluconeogenesis from lactate and glycerol presumably proceed at comparable rates in decompensated diabetes and prolonged starvation, gluconeogenesis from protein is greatly enhanced in the former. The difference in the rates of gluconeogenesis from protein can be judged from the urinary losses of nitrogen, up to 25 gm./day in diabetes,[9,16] but only 4-5 gm./day in prolonged starvation.[2,3] Under conditions of high demand for gluconeogenesis, the excretion of 1 gm. of nitrogen in urine indicates the synthesis of 3.65 gm. of glucose from protein.[17] In decompensated diabetes, gluconeogenesis from protein thus yields some 20 gm. N/ day x 3.65 gm. glucose/gm. N = 75 gm. more glucose per day than in starvation.

The synthesis of 1 mole of glucose from 2 moles of alanine begins with the formation of pyruvate through transamination. Two moles of reducing equivalents are produced and 4 moles of ATP used when the two amino groups are subsequently metabolized to urea. Since the conversion of 2 moles of pyruvate to glucose requires two reducing-equivalents and 6 moles

of ATP, the net requirement for gluconeogenesis from alanine is 10 moles of ATP per 180 gm. of glucose synthesized.

It can be computed that 5 or 8 moles of ATP are gained respectively per mole of β-hydroxybutyrate or acetoacetate formed from oleic acid. Assuming that the liver releases these two acids in proportions of 2:1[3] the formation of 100 gm. of glucose from alanine uses the energy generated during the formation of

$$\frac{\text{100 gm. glucose}}{\text{180 gm. glucose/mole}} \bullet \frac{\text{10 moles ATP/mole glucose}}{\text{6 moles ATP/mole ketoacids}}$$

x 103 gm./mole ketoacid = 95 gm. ketoacids from oleic acid.

Assuming again that the utilization of energy generated during ketogenesis is rate-limiting for this process, one would expect that the rate of ketone production by the liver in prolonged starvation would be about 75 x 95/100 = 70 gm./day less than the 200 gm. formed during acute diabetic ketoacidosis. Since the production of ketones in man during prolonged starvation was found to approach 100 gm./day,[3] the difference in the rates of ketogenesis in this state and in acute diabetes could be attributed largely to the difference in energy utilization for gluconeogenesis from protein.

The studies on starvation in man reviewed by Cahill and Aoki[18] show that after twenty-four days of complete starvation the plasma concentrations of glucose, free fatty acids, β-hydroxybutyrate and acetoacetate reach levels which remain precisely constant for the continuation of the starvation period. Glucagon and growth hormone levels remain equally stable past day 24, while the insulin concentration decreases but slightly from 22 μU./ml. on day 24 to 17 μU./ml. on day 36. Because in such a steady-state situation the utilization of substrates by the various tissues can be expected to be constant, changes in rates of ketogenesis should be reflected by changes in urinary ketoacid excretion. Owing to the slight decrease in the levels of insulin, a powerful antiketogenic hormone, a small increase in ketoacid excretion would not be surprising. The opposite is the case, however. Relative to day 24, urinary ketoacids were found to be decreased by 13 and 15 mmoles,[18] or 1.4 and 1.6 gm., of β-hydroxybutyrate on days 31 and 36 respectively. In the absence of any other obvious explanation, it is of interest to try to explain this reduction through a decrease in hepatic gluconeogenesis from protein. The urinary nitrogen excretion decreases from day 24 to day 31 and day 36, by 0.9 and 1.2 gm. N/day respectively.[18] (N excretion on day 24 was calculated by averaging the N excretion on days 23, 24, and 25; N excretion for days 31 and 36 were obtained similarly.) Since under conditions of carbohydrate deprivation 1 gm. of urinary nitrogen allows maximally the synthesis of 3.65 gm. of glucose,[17] glucose synthesis from protein is 3.3 gm. less on day 31 and 4.4 gm. less on day 36 than on day 24. Liver and kidney have been found to contribute about equally to gluconeogenesis during prolonged starvation,[3] alanine being the major amino acid extracted by the liver.[15] Assuming that ketogenesis is limited by the liver's ability to dispose of the energy generated in the conversion of FFA to ketones, the production of ketones would be

$$3.3 \text{ glucose} \times 1/2 \times \frac{\text{10 moles ATP/180 gm. glucose}}{\text{6 moles ATP/103 gm. ketoacid}}$$

= 1.6 gm. less on day 31, and by a similar calculation 2.1 gm. less on day 36 than on day 24. These figures are surprisingly close to the observed decreases of 1.4 and 1.6 gm. respectively of urinary β-hydroxybutyrate excretion.

The discussion presented here shows that it may be possible to attribute the permissive role which gluconeogenesis appears to play in the development of ketosis to its effects on the energy expenditure in the liver. While the regulatory phenomena recognized at the enzymatic level achieve the indispensable reduction in the activity of the citric acid cycle, one may wonder whether they are deciding events or not for the degree of ketosis reached in a given metabolic situation.

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Enhancement of Liver Glucose-6-Phosphate Dehydrogenase by Dietary Carbohydrate and Insulin

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degradation is thirty-six hours in rats shifted from a 90 per cent glucose diet to one 90 per cent in protein. Hence, different rates of degradation as well as synthesis do appear to be involved in the regulation of levels of this enzyme. . . .

. . . The present work helps to focus on the rather spectacular effect of dietary carbohydrate on liver glucose-6-phosphate dehydrogenase and appears to clarify the indirect role of insulin acting through enhanced appetite. However, the exact mechanism by which carbohydrate triggers the changes in level of the enzyme is not yet clear. In this connection, it may be useful to recall parallel studies on the influence of diet on protein synthesis (see Nutrition Reviews 28:25, 1970). As a specific instance of such an investigation, J. S. Wittman III, K.-L. Lee, and O. N. Miller (Biochim. Biophys. Acta 174:536, 1969) showed that glucose, but not fat, reversed the disaggregation pattern of polysomes involved in protein (enzyme) synthesis. More investigations to delineate the primary inducer may be expected in the future.

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