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THE GLUCOSE–FATTY ACID–KETONE BODY CYCLE

Role of ketone bodies as respiratory substrates and metabolic signals

J. C. STANLEY

In this paper, the glucose–fatty acid cycle is extended to include the ketone bodies acetoacetate and 3-hydroxybutyrate. The physiological roles of acetoacetate and 3-hydroxybutyrate as respiratory substrates and of 3-hydroxybutyrate as a metabolic signal are discussed. The paper includes descriptions of the regulation of ketogenesis in the liver, the use of ketone bodies as alternative fuels to glucose in the brain, the kidney cortex and the small intestine and the regulation of insulin secretion by the β-cells of the endocrine pancreas and of lipolysis in adipose tissue by 3-hydroxybutyrate. Emphasis will be placed upon the ability of ketone bodies to integrate the metabolism of different tissues and the contribution ketone bodies make towards glucose conservation in conditions of carbohydrate deprivation such as starvation.

Acetoacetate and 3-hydroxybutyrate, with acetone, were first discovered in the urine of diabetic patients. This association with a pathological condition led to the idea that these organic compounds were by-products of metabolism. Although it is unlikely that acetone has any physiological role, both acetoacetate and 3-hydroxybutyrate are of crucial physiological importance as described below. In an attempt to get away from the idea that ketone bodies are by-products of metabolism, some authors refer to acetoacetate and 3-hydroxybutyrate as the ketoacids. However, in this series of articles the term ketone bodies will be used to describe acetoacetate and 3-hydroxybutyrate. Nevertheless, it is possible to distinguish between the physiological ketosis of prolonged starvation when the plasma ketone body concentration increases to approximately 8 mmol litre^{-1} and the pathological ketosis of diabetes when the plasma ketone body concentration may reach 30 mmol litre^{-1}.

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Diabetes is not the only pathological condition in which the ketone body concentration in the blood increases. Some hypoglycaemic conditions of childhood are characterized by a deficiency in hepatic glycogen synthase activity and hence the liver is unable to synthesize glycogen. After an overnight fast the ketone body concentration in the blood of such children increases to about 8.5 mmol litre^{-1}. Since ketone bodies inhibit the release of the gluconeogenic precursor alanine from muscle (see later), then the rate of gluconeogenesis in such children is also depressed. Hence the low blood glucose concentration (1.5 mmol litre^{-1}) seen after an overnight fast is a consequence of both an inadequate liver glycogen store and a low rate of gluconeogenesis (Aynsley-Green, Williamson and Gitzelmann, 1977).

As can be seen from table II in the previous paper (Stanley, 1981), the ketone body concentration in the blood can vary over a 20-fold range during starvation. Such changes in concentration are not found in the case of other blood metabolites such as glucose and fatty acids. This emphasizes the potential of ketone bodies as metabolic signals for integrating the metabolism of different tissues.

Inhibition of glucose utilization by the oxidation of ketone bodies in the brain, kidney cortex and small intestine

In the postabsorptive state the brain uses approximately 120 g of glucose per day. This glucose is completely oxidized to carbon dioxide and water via the pathways of glycolysis, the tricarboxylic acid cycle and the respiratory chain. Hence glucose utilization by the brain represents loss of carbohydrate to the body which must be replaced either from dietary sources or, during starvation, from endogenous sources such as liver glycogen or gluconeogenesis. Since liver glycogen can only provide sufficient glucose for the brain for about

24 h during starvation, then either glucose must be provided by gluconeogenesis or the brain must oxidize some alternative fuel. Although gluconeogenesis does provide glucose for the brain during starvation this creates an additional problem since the amino acid alanine, the major precursor for gluconeogenesis in the liver, is derived from the degradation of muscle protein. Since muscle protein is essential for the survival of the animal it cannot be degraded indefinitely to satisfy the glucose requirement of the brain. Therefore, it is necessary for the brain to use an alternative fuel to glucose during starvation and hence allow the conservation of body protein.

Using arteriovenous catheterization techniques it has been shown that, in obese patients undergoing a 5–6-week period of therapeutic starvation, the glucose requirement of the brain decreases to about 35 g per day (Owen et al., 1967). Hence glucose utilization by the brain during prolonged starvation decreases by about 70%. This study also demonstrated that, as the concentration of ketone bodies in the blood increased during starvation, so did the rate of ketone body utilization. The capacity of the brain for ketone body oxidation was demonstrated by the finding that all the enzymes necessary for ketone body oxidation were present in this tissue. Thus the brain has the capacity for ketone body oxidation which is used in conditions such as starvation when the plasma ketone body concentration increases. It should, however, be pointed out that even in prolonged starvation ketone bodies do not completely replace glucose as a metabolic fuel. Hence gluconeogenesis must still provide about 35 g of glucose per day to the brain in prolonged starvation. It is unclear whether this represents an absolute requirement for this amount of glucose by the brain or whether, at high concentrations of ketone bodies, the brain could use less glucose.

The mechanism whereby ketone body oxidation inhibits glucose utilization in the brain is likely to be similar to the mechanism whereby fatty acid oxidation inhibits glucose utilization in muscle. The oxidation of ketone bodies, like the oxidation of fatty acids, will increase the concentration of acetyl-CoA within the mitochondria. It is likely that the resultant increase in the concentration ratio acetyl-CoA : CoA inhibits pyruvate dehydrogenase activity. Furthermore, it has been shown that, in the rat brain, the oxidation of ketone bodies increases the concentration of citrate and inhibits the activity of phosphofructokinase. This will presumably lead to a decrease in the activity of hexokinase via an increase in the concentration of the inhibitor glucose-6-phosphate. However, more work needs to be done in this area; in particular the effect of ketone body oxidation on the transport of glucose into brain cells needs to be studied. Nevertheless, it is the author's view that the mechanism is likely to be broadly similar to that in muscle, but that details such as the mechanism of citrate accumulation may be different.

In the postabsorptive state the kidney cortex uses about 34 g of glucose per day. However, it can be calculated that glucose production via gluconeogenesis during starvation is only sufficient to meet the glucose requirement of the brain and the anaerobic tissues such as red blood cells which obtain their energy from the conversion of glucose to lactate. Hence it is necessary for the kidney cortex to oxidize alternative fuels to glucose during starvation. It has been shown that kidney cortex slices oxidize fatty acids and ketone bodies and that this leads to an increase in the concentration of citrate and a consequent inhibition of phosphofructokinase activity and glucose utilization. Recent work has demonstrated that the small intestine also oxidizes ketone bodies during starvation and that this inhibits glucose oxidation in this tissue. A reduction in the rate of glucose utilization by the small intestine during starvation has also been observed, but this effect is apparently not dependent upon the accumulation of citrate but rather upon a decrease in the concentration of the glycolytic enzymes hexokinase, phosphofructokinase and pyruvate kinase (Hanson and Parsons, 1978). Hence the ability of ketone bodies to act as an alternative fuel to glucose in tissues such as the brain, the kidney cortex and the small intestine will contribute towards the conservation of glucose in conditions such as starvation when the ketone body concentration in the blood increases.

The quantitative estimates of glucose production and utilization by various tissues referred to above are based on arterio–venous difference measurements. A fuller discussion of these estimates and their relation to starvation in man will be found in Cahill (1976).

The regulation of ketogenesis

The synthesis of the ketone bodies acetoacetate and 3-hydroxybutyrate from fatty acids involves

the co-operation of two tissues, namely adipose tissue and the liver. Fatty acids are first mobilized from adipose tissue then taken up by the liver and finally converted into ketone bodies. Regulation of ketogenesis, therefore, involves control of fatty acid mobilization in adipose tissue and of the conversion of fatty acids into ketone bodies in the liver. Furthermore, the metabolism of these two tissues must be integrated to ensure that the capacity for ketone body synthesis in the liver is matched by the rate of supply of fatty acids from adipose tissue. The control of fatty acid mobilization has already been discussed in a previous section and hence only factors which act at the level of the liver and which integrate the metabolism of the two tissues will be discussed in this section.

Fatty acids taken up from the blood by the liver are first esterified to CoA in an ATP-dependent reaction. The resultant cytoplasmic fatty acyl-CoA may either be esterified with glycerol-3-phosphate to form triacylglycerol or be esterified with carnitine and transported as fatty acyl-carnitine into the mitochondrion which is the site of ketone body synthesis. Once inside the mitochondrion, fatty acyl-carnitine is converted back to fatty acyl-CoA which undergoes β-oxidation to form acetyl-CoA. If ketogenesis is to occur, then this branch point of metabolism must be regulated so that fatty acyl-CoA is directed towards the mitochondrion. Regulation of this branch point could, therefore, in principle involve inhibition of triacylglycerol synthesis, stimulation of transport of fatty acid residues into the mitochondrion or a combination of both processes. Mitochondrial acetyl-CoA may be metabolized either via the tricarboxylic acid cycle to form carbon dioxide or via the HMG-CoA pathway to form ketone bodies. If ketogenesis is to occur then mitochondrial acetyl-CoA must be directed towards the HMG-CoA pathway. Hence, in principle, regulation of this branch point could involve inhibition of the tricarboxylic cycle, stimulation of the HMG-CoA pathway or a combination of both processes. The pathways described above and their relationship to one another are presented in figure 1.

The regulation of ketogenesis in the liver has been studied using *in vitro* preparations of this tissue, in particular the perfused rat liver and isolated rat hepatocytes. When livers from fed, starved and diabetic rats were perfused with the same concentration of oleic acid it was found that, despite similar rates of uptake of this long chain fatty acid by all three livers, the rates of ketogenesis in the livers from starved and diabetic animals were very much greater. This finding implies that the greater rates of fatty acid mobilization characteristic of the starved and diabetic states are not alone sufficient to ensure high rates of ketogenesis. Hence control mechanisms must exist in the liver to ensure that in the starved and diabetic states fatty acids are directed towards the ketogenic pathway.

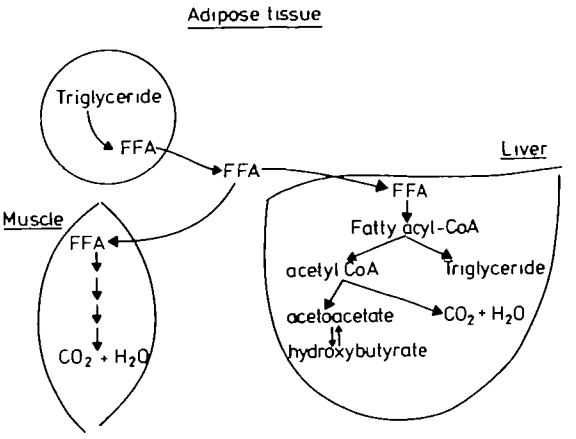

FIG. 1. Pathways of fatty acid oxidation and esterification in the liver.

Octanoate, a medium chain fatty acid, can cross the inner membrane without prior esterification to carnitine. The finding that octanoate is oxidized at similar rates by perfused liver from fed, starved and diabetic rats suggests that the transport of fatty acid residues across the inner mitochondrial membrane may regulate the distribution of fatty acyl-CoA between mitochondrion and cytoplasm. It is thought that the membrane-bound enzyme carnitine acyl transferase-I catalyses the rate-limiting step in the transport of fatty acid residues into the mitochondrion. Furthermore, the results also suggest that transport of fatty acid residues into the mitochondrion is less rapid in the livers from fed rats and more rapid in the livers from starved and diabetic rats thus allowing greater rates of ketogenesis in the starved and diabetic state.

These observations focused attention on the regulation of carnitine acyl transferase-I activity as a means of activating the pathway for the oxidation of fatty acyl-CoA in starvation and diabetes. A large number of intermediates of glucose and fatty

acid metabolism were tested for their ability to activate or inhibit this enzyme. As a result it was discovered that physiological concentrations of malonyl-CoA inhibited carnitine acyl transferase-I activity. Malonyl-CoA is the first intermediate unique to the pathway of fatty acid synthesis. Hence, when the rate of fatty acid synthesis in the liver is high and the malonyl-CoA concentration is high, carnitine acyl transferase-I activity will be inhibited and consequently the rate of fatty acid oxidation and ketogenesis will be low. Hence the role of malonyl-CoA as an inhibitor of carnitine acyl transferase-I and as an intermediate of the pathway of fatty acid synthesis allows reciprocal control of fatty acid oxidation and synthesis in the liver. It has been shown that rates of fatty acid synthesis and oxidation, when measured simultaneously in isolated rat hepatocytes are related in a linear and reciprocal fashion.

Glucagon stimulates the rate of ketogenesis in the liver. This effect may be partly a result of the ability of glucagon to decrease the malonyl-CoA concentration. The activity of the enzyme acetyl-CoA carboxylase controls the rate of malonyl-CoA synthesis in the liver, while the activity of the fatty acid synthetase enzyme complex controls the rate of its utilization. Hence it might be expected that glucagon decreases the malonyl-CoA concentration by changing the activities of one or both of these enzymes. It has recently been shown that impure preparations of acetyl-CoA carboxylase are phosphorylated by cAMP-dependent protein kinase—a process that is accompanied by inhibition of the enzyme (Kim, 1979). Hence glucagon can inhibit acetyl-CoA carboxylase since it increases cAMP concentration and consequently activates cAMP-dependent protein kinase. Inhibition of acetyl-CoA carboxylase by glucagon will reduce the rate of malonyl-CoA synthesis and hence decrease the malonyl-CoA concentration. As a result carnitine acyl transferase-I will be deinhibited and the rate of ketogenesis will increase.

It is not clear at the present time how far inhibition of the rate of fatty acid esterification during starvation and diabetes contributes to increased rates of ketogenesis in these situations. Nevertheless, it is likely that some control mechanism ensures that the rate of fatty acid esterification is inhibited in ketotic states otherwise deinhibition of fatty acid oxidation and ketogenesis when the malonyl-CoA concentration decreases would not necessarily direct fatty acyl-CoA into the oxidative pathway but merely allow competition between the oxidative and esterification pathways for the available fatty acyl-CoA.

A second problem concerning the regulation of ketogenesis is the nature of the factors which control the distribution of mitochondrial acetyl-CoA between the tricarboxylic acid cycle and the HMG-CoA pathway. No increase in the capacity of the HMG-CoA pathway in ketotic states has been detected. It has been shown that the capacity of the tricarboxylic acid cycle decreases during ketogenesis, but this effect is not essential for enhanced ketogenesis. The mechanism of regulation of this branch point in metabolism remains to be established. Factors which integrate the mobilization of fatty acids from adipose tissue and the synthesis of ketone bodies from fatty acids in the liver include the concentration of circulating insulin, glucagon and ketone bodies. During starvation the concentration of insulin in the blood decreases while that of glucagon increases. Enhanced ketogenesis during starvation is caused by a simultaneous increase in the rate of fatty acid mobilization from adipose tissue and an increased capacity for ketone body synthesis from fatty acids in the liver. The former is probably mediated by a decline in the concentration of the antilipolytic hormone insulin, while the latter is probably mediated by an increase in the concentration of the ketogenic hormone glucagon. However, the author considers that this kind of hormonal regulation alone may not be sufficiently precise to ensure that the rate of supply of fatty acids from adipose tissue is precisely matched to the rate of their utilization by the liver. Hence a further control mechanism which involves ketone bodies is necessary.

It has been shown that high concentrations of the ketone body 3-hydroxybutyrate stimulate the secretion of the antilipolytic hormone insulin. Furthermore, it has also been shown that 3-hydroxybutyrate directly inhibits the rate of mobilization of fatty acids from adipose tissue and increases the sensitivity of adipose tissue to the effects of insulin (Green and Newsholme, 1979). These are examples of the role of ketone bodies as a metabolic signal. These effects could form the basis of a sensitive feedback control mechanism for regulating the rate of release of fatty acids from adipose tissue in relation to the rate of ketone body synthesis in the liver. Indirect evidence for this control mechanism is provided by the pheno-

menon of post-exercise ketosis. Immediately after exercise has finished the rate of utilization of fatty acids by muscle will rapidly decrease while the rate of fatty acid mobilization from adipose tissue will remain unchanged. Consequently, the concentration of fatty acids in the blood will increase. This in turn will stimulate the rate of ketogenesis in the liver and hence the ketone body concentration will increase. The antilipolytic action of ketone bodies will ensure that fatty acid mobilization is inhibited so that it now exactly matches the new low rate of fatty acid utilization by muscle. Hence a new metabolic steady state is achieved.

A more detailed discussion of the regulation of ketone body synthesis will be found in McGarry and Foster (1980).

The glucose–fatty acid–ketone body cycle

The glucose–fatty acid cycle can now be extended to include the role of ketone bodies as respiratory substrates and the role of 3–hydroxybutyrate as a metabolic signal. The glucose–fatty acid–ketone body cycle is presented in figure 2. This extended cycle provides a more complete picture of the whole animal's response to conditions of carbohydrate deprivation such as starvation. The role of ketone bodies as respiratory substrates provides an explanation for the inhibition of glucose oxidation and utilization via the glycolytic pathway in the brain and kidney cortex and of glucose oxidation in the small intestine during starvation. Hence, the ability of these tissues to oxidize ketone bodies in preference to glucose in response to an increased ketone body concentration in the blood contributes towards the conservation of glucose during conditions of carbohydrate deprivation. In the case of the brain, inhibition of glucose utilization also allows vital muscle protein to be conserved since this protein is the ultimate source of the glucose used by the brain. The ability of 3-hydroxybutyrate to inhibit lipolysis in adipose tissue forms the basis of a sensitive feedback control mechanism for fatty acid mobilization. Thus the rate of fatty acid mobilization can be regulated, through the 3-hydroxybutyrate concentration in the blood, in relation to the plasma fatty acid concentration and hence to the rate of fatty acid utilization. Furthermore, the inhibitory effect of 3-hydroxybutyrate on lipolysis provides a mechanism for integrating the rate of supply of fatty acids from adipose tissue and the rate of their conversion into ketone bodies in the liver. Further details of the glucose–fatty acid–ketone body cycle are provided by Newsholme (1976).

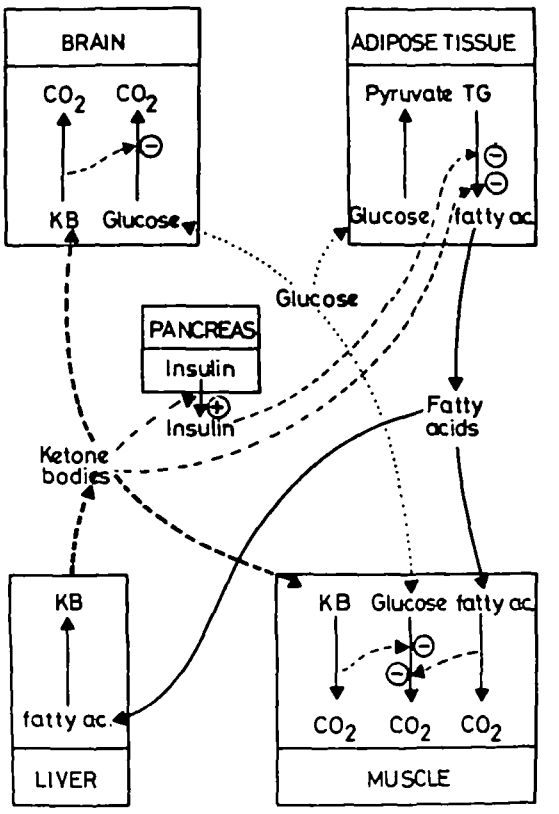

FIG. 2. The glucose–fatty acid–ketone body cycle.

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