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Brain substrates and the effects of nutrition

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The major role of the substrates utilized by the central nervous system is for oxidation to provide energy for maintenance of cerebral function. In addition, certain substrates can act as precursors for biosynthetic processes, for example, synthesis of myelin lipids during development (glucose and ketone bodies) or synthesis of proteins (amino acids). Apart from their role as respiratory fuels, cerebral substrates may also act as signals to control appetite or affect neurotransmission. The present paper deals with the short-term changes in substrate supply to the brain rather than the longer term, but equally important, effects of dietary restriction or imbalance.

Alternative substrates for the brain

For a long period it was accepted biochemical dogma that the only energy-yielding substrate utilized by the brain was glucose. This view was based on (a) the impairment of cerebral function in hypoglycaemic states, (b) the failure of the potential energy-yielding substrates to overcome the deleterious effects of hypoglycaemia and (c) measurements of arterio-venous difference across the head which indicated that glucose was the only circulatory substrate taken up by the brain. In the last two decades a number of studies have challenged the dogma and necessitated reappraisal of the capacity of nervous tissue to utilize alternative substrates. The present consensus is that although glucose is usually the major substrate for brain, and brain cells appear to have an obligatory requirement for a proportion of their oxidizable substrate to be supplied as glucose, when glucose availability is decreased the brain can utilize other substrates (Table 1) if they are

Table 1. *Potential substrates for brain*

Substrate	Reference
Glucose	Gibbs <i>et al.</i> (1942)
Lactate	Fernandes <i>et al.</i> (1984)
Pyruvate	Fernandes <i>et al.</i> (1984)
Glycerol	Sloviter <i>et al.</i> (1966)
Acetoacetate	Owen <i>et al.</i> (1967)
3-Hydroxybutyrate	Owen <i>et al.</i> (1967)
Acetate	Juhlin-Dannfelt (1977)
Amino acids	Eriksson <i>et al.</i> (1983)

available. It must be emphasized, however, that of the substrates listed in Table 1, only glucose is able to produce ATP (via glycolysis), albeit at a diminished rate, in cerebral anoxia. This may in part explain its apparent role as an obligatory substrate.

A key experiment in our understanding with regard to alternative substrates for the brain was the demonstration by Cahill and his colleagues (Owen *et al.* 1967; Cahill *et al.* 1968) that in prolonged starvation (5–6 weeks) the human brain extracted ketone bodies (acetoacetate and 3-hydroxybutyrate) and that these substrates could account for about 60% of the oxygen consumed by the brain. This study provided the explanation for how human subjects could survive prolonged starvation despite the fact that calculations suggested that the normal glucose requirement of human brain could not be maintained by endogenous glucose precursors. The question raised by this novel finding was whether this was an adaptation to prolonged starvation or whether ketone bodies and other oxidizable substrates could be utilized in the fed state if available in the circulation. The present experimental evidence indicates that the latter view is the correct one (see Robinson & Williamson, 1980).

Although it is now widely accepted that ketone bodies are the major alternative substrates to glucose for nervous tissue, in special situations it appears that lactate can replace ketone bodies. In normal situations lactate and pyruvate derived from glycolysis are released from brain (Table 2, Fig. 1) and the amount of pyruvate oxidized depends on the activation state of pyruvate dehydrogenase, an interconvertible enzyme (see Wieland, 1983). Patients with hepatic glycogen storage disease due to glucose-6-phosphatase deficiency sometimes have severe hypoglycaemia without an increase in circulatory ketone bodies, yet they do not exhibit any cerebral symptoms. However, these patients have raised blood lactate and measurements of arterio-venous differences across the head indicate that extraction of this substrate can account for 35% of the O₂ uptake (Table 2; Fernandes *et al.* 1984). Simultaneously, there is a suppression of ketone body utilization by the brain compared with normal neonates (Table 2; Kraus *et al.* 1974). This latter finding can be explained by the fact that the mitochondrial pathway for ketone body utilization is freely reversible (Fig. 1; see Robinson & Williamson, 1980) and therefore when lactate (pyruvate) is available at higher than

Table 2. Use of lactate by brain in glucose-6-phosphatase (EC 3.1.3.9) deficiency (from Kraus et al. 1974; Fernandes et al. 1984)

Metabolite	Normal neonates			Glucose-6-phosphatase deficiency		
	Arterial concentration	A-V difference	O ₂ uptake (% total)	Arterial concentration	A-V difference	O ₂ uptake (% total)
Glucose	4.56	0.57	88	4.21	0.19	65
Lactate	1.65	-0.28		8.83	0.21	35
Pyruvate	0.12	-0.02		0.45	0.02	
3-Hydroxybutyrate	0.16	0.04	6	0.68	0.03	
Acetoacetate	0.10	0.04	6	0.26	0.00	

A-V, arterio-venous.

normal concentrations (hyperlactataemia) the supply of acetyl-CoA derived from pyruvate oxidation (an irreversible step) prevents the utilization of acetoacetate until the availability of acetyl-CoA decreases. The high blood pyruvate concentration associated with glucose-6-phosphatase (EC 3.1.3.9) deficiency presumably activates pyruvate dehydrogenase (Wieland, 1983). An alternative explanation is that lactate and ketone bodies compete for entry into the brain via the monocarboxylic acid transport system (Oldendorf, 1973). Another situation in which lactate may be an important cerebral substrate is in the newborn infant with hypoglycaemia (Hellmann *et al.* 1982).

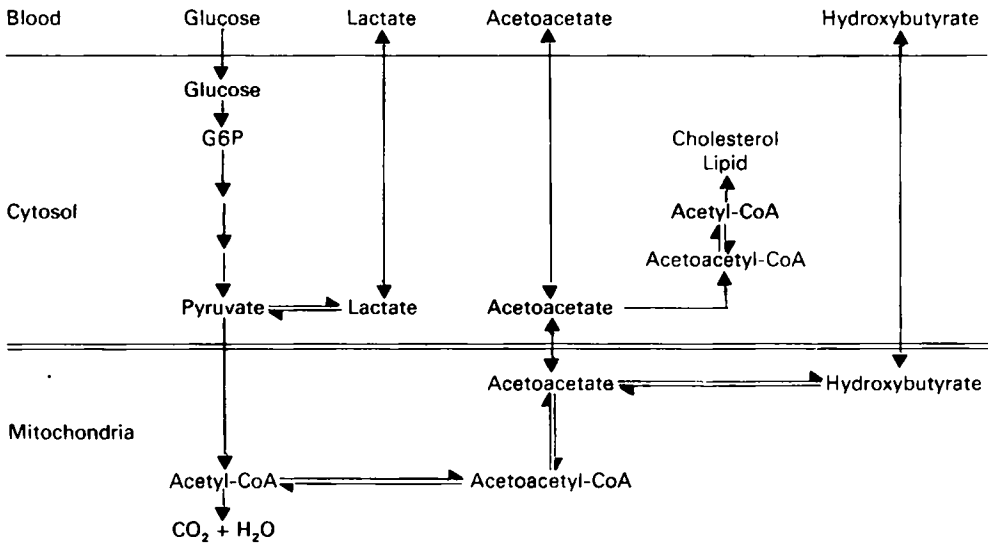


Fig. 1. Metabolism of brain substrates. G6P, glucose-6-phosphate.

In contrast to human and rat, ketone bodies do not appear to be important substrates for the adult brain of sheep (Jones *et al.* 1975; Lindsay & Setchell, 1976), dog (Wiener *et al.* 1971) or pig (Tildon & Sevdalian, 1972). The low ketone body utilization of these three species is associated with a small brain size in relation to total body-weight. Consequently, the relative demand for glucose is less and ketone bodies may not be required as an alternative fuel.

Factors regulating substrate utilization

The factors involved in regulation of substrate utilization by the brain and other mammalian tissues include: (a) the concentration of the substrate in the circulation, (b) the blood flow to the tissue, (c) the transport of the substrate into the tissue, (d) the concentration of the enzymes (initiating enzymes) which allow the entry of the substrate into the catabolic pathway, (e) the integration of the control of intracellular metabolism (for further discussion, see Williamson, 1984).

If one now considers ketone bodies as a particular case, their availability in the circulation is dependent on the rate of synthesis by the liver, which in turn is regulated at two levels: flux of long-chain fatty acids to the liver and their subsequent fate within the liver (see McGarry & Foster, 1980; Robinson & Williamson, 1980). A decrease in blood glucose will lower plasma insulin which in turn will lead to increased fatty acid release from adipose tissue. If the hepatic store of glycogen is already depleted then incoming fatty acids will be diverted to oxidation and formation of ketone bodies. Thus ketone body production increases when glucose must be spared, e.g. in starvation, or on a diet high in fat or protein and low in carbohydrate. An extreme example of this interrelation between the concentrations in the blood of the two key fuels for brain is to be seen in the large reciprocal changes of glucose and ketone bodies throughout the day in a case of hepatic glycogen synthase (*EC* 2.4.1.11) deficiency (Aynsley-Green *et al.* 1977). The concentration of ketone bodies in the blood increases in a number of situations (Table 3) and increased cerebral extraction of acetoacetate and 3-hydroxybutyrate has been confirmed in the majority of these.

There is a specific transporter for entry of ketone bodies into brain cells and in rats transport can be increased by starvation (Gjedde & Crone, 1975) or by

Table 3. *Range of blood ketone body concentrations in human and rat*

Situation	Ketone body concentration (mmol/l)	
	Human	Rat
Fed	About 0.1	Up to 0.3
Fasted 12–24 h	0.3	1–2
Fasted 48–72 h	2–3	2
Post-exercise	Up to 2	Up to 2
Late pregnancy	Up to 1	Up to 0.3
Late pregnancy, fasted 48 h	4–6	6–15
Neonatal	0.5–1	0.5–1.5
Untreated diabetes	Up to 25	Up to 10

fat-feeding (Moore *et al.* 1976). Transport of ketone bodies through the blood-brain barrier is considered to limit their utilization (Pardridge, 1983).

The activities of the initiating enzymes for acetoacetate (3-oxoacid-CoA transferase; *EC* 2.8.3.5) and 3-hydroxybutyrate (3-hydroxybutyrate dehydrogenase; *EC* 1.1.1.30) metabolism remain relatively constant in human brain throughout life (Page & Williamson, 1971; Patel *et al.* 1975). In contrast, their activities in rat brain increase dramatically (300%) in the preweaning period when the neonatal rat receives a high-fat diet in the form of the maternal milk, and then decrease again on weaning (Page *et al.* 1971). Surprisingly, the activities of these enzymes continue to rise in the period before weaning when the pups are beginning to eat solid chow (high in carbohydrate) and no satisfactory explanation for this finding has been put forward. The activities of the enzymes were increased two- to threefold in brains of fetal and newborn rats whose mothers were subjected to starvation (Thaler, 1972) or high-fat feeding (Dierks-Ventling & Cone, 1971; Sherman & Wilson, 1978). However, higher activities of the enzymes of ketone body metabolism are not observed in brains of adult rats fed on a high-fat diet or starved for a prolonged period. Experiments designed to elucidate the mechanism whereby the enzyme activities are induced in rat brain preweaning have shown the following: (a) increased synthesis of enzyme protein is involved (Haney & Patel, 1985), (b) the physiological hyperketonaemia of the preweaning period is not obligatory for the induction (Haney & Patel, 1985), (c) neonatal hypothyroidism retards the postnatal development of the enzymes (Patel, 1979).

Brain substrates as lipid precursors

The acetyl-CoA formed by the metabolism of glucose or alternative substrates (ketone bodies, lactate, pyruvate, etc.) can either be oxidized or transported from the mitochondrial matrix to the cytosol for the synthesis of cholesterol and lipid, particularly during the period of myelination. In the case of acetoacetate an alternative pathway (Fig. 1) has been described in rat brain involving a cytosolic acetoacetate-CoA ligase (acetoacetyl-CoA synthetase, *EC* 6.2.1.16) (Buckley & Williamson, 1973) and acetyl-CoA acetyltransferase (acetoacetyl-CoA thiolase, *EC* 2.3.1.9) (Middleton, 1973). This pathway appears to be particularly active in oligodendrocytes isolated from calf brain (Pleasure *et al.* 1979). Although the acetoacetate-CoA ligase has low activity it has a high affinity for acetoacetate. Experiments *in vivo* and *in vitro* have shown that ¹⁴C-labelled acetoacetate and 3-hydroxybutyrate are incorporated into brain lipids and cholesterol during development and that ketone bodies may be more effective precursors than glucose (Edmond, 1974; Yeh *et al.* 1977; Pleasure *et al.* 1979; Webber & Edmond, 1979; Koper *et al.* 1984).

Brain heterogeneity and substrate utilization

There is considerable interest in the question whether certain areas of the brain can preferentially utilize alternative substrates. Measurements of enzyme activities in dissected areas of human brain indicate that the capacity to utilize ketone bodies

is present in the major anatomical regions (Page & Williamson, 1971). With the use of the more sophisticated technique of injection of [$3\text{-}^{14}\text{C}$]hydroxybutyrate and autoradiography, it was concluded that certain areas of rat brain had the capacity to use a high proportion of ketone bodies and that permeability was a key factor (Hawkins & Biebuyck, 1979). This technique has also been used to examine possible differences in regional ketone body utilization between brains of fed, fasted and diabetic rats (Hawkins *et al.* 1986). The main conclusions from this study were that starvation increased plasma clearance of ketone bodies by the brain by about 50–60%, and that although there were regional differences in ketone body utilization, these were unrelated to the energy requirement as estimated from glucose utilization measured by the same technique (Lu *et al.* 1983).

Brain substrates as signals

An emerging area of interest is whether any of the circulatory substrates act as signals to the brain to regulate whole-body metabolism, dietary intake or behaviour. Prime candidates would be ketone bodies whose concentration in blood varies over a wide range (Table 2) and increases in response to decreased glucose availability. Subcutaneous injection of 3-hydroxybutyrate (10 mmol/kg; Langhans *et al.* 1983) decreased food intake whereas continuous infusion of small amounts into the third ventricle of the brain (36 $\mu\text{mol/d}$; Davis *et al.* 1981) decreased body-weight but had no significant effect on food intake. Intracerebroventricular infusion of a similar amount of glucose or glycerol decreased both body-weight and food intake (Davis *et al.* 1981). Elevation of plasma glycerol has been shown to cause weight reduction in rats (Wirtshafter & Davis, 1977; Carpenter & Grossman, 1983). Thus there is evidence to indicate that direct monitoring of metabolites by the brain, particularly those that increase in concentration in starvation, may play a role in the control of food intake and body-weight. However, with the notable exception of the intracerebroventricular infusion experiments (Wirtshafter & Davis, 1977) the plasma concentrations employed to observe the effects are at least an order of magnitude higher than physiological levels.

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