Fatty acids as energy sources

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The notion that fatty acids (FA) can act as a significant source of energy for higher animals has been so much a part of our understanding of metabolism that just to express it seems platitudinous. We can however sharpen the notion a little by suggesting that this is the major function of FA. While protein and carbohydrates can and do act as energy sources they have other quantitatively important functions. The amounts of FA used for other purposes such as membrane synthesis or prostaglandin formation must be quite small relative to their use as an energy source. Moreover, whereas FA when mobilized need not be oxidized, they can be deposited in essentially the same form: the carbon cannot be used for the synthesis of carbohydrate or amino acids whereas these can be converted to FA.

Probably most tissues that can carry out aerobic oxidations have at least some capacity to use FA or their products as an energy source. The pregnant uterus appears to be largely carbohydrate-dependent, but it does appear to use small amounts of ketones (A. Domanski, B. P. Setchell & D. B. Lindsay, unpublished results) and this is also true of the foetus itself (Morriss, Boyd, Makowski, Meschia & Battaglia, 1974). The brain was also for many years supposed to use only carbohydrate, but there is evidence that ketones can be utilized in starving man (Owen, Morgan, Kemp, Sullivan, Herrera & Cahill, 1967) and rats (Hawkins, Williamson & Krebs, 1971). The sheep brain does not normally appear to use acetate or ketones (Lindsay & Setchell, 1972, 1974) but we have recently found that when acetoacetate is infused intravenously into sheep so that the concentration in blood is higher than is normally found even in ketotic animals, there does seem to be a small utilization of acetoacetate.

Even if we accept provisionally that most tissues have at least some capacity to use FA or their oxidation products as energy sources, it is clear that the extent to which this occurs varies considerably between tissues; moreover, for many tissues the amount used is likely to depend on the relative availability of substrates and the physiological state of the animal. I propose in this review to discuss how far we can estimate quantitatively the contribution of FA in meeting energy requirements. In principle this should be a fairly straightforward problem since ^{14}C-labelled FA have become available.

Starved sheep

The advantage of studying starved animals is that we already know the result we should obtain. For many years it has been established by standard nutritional

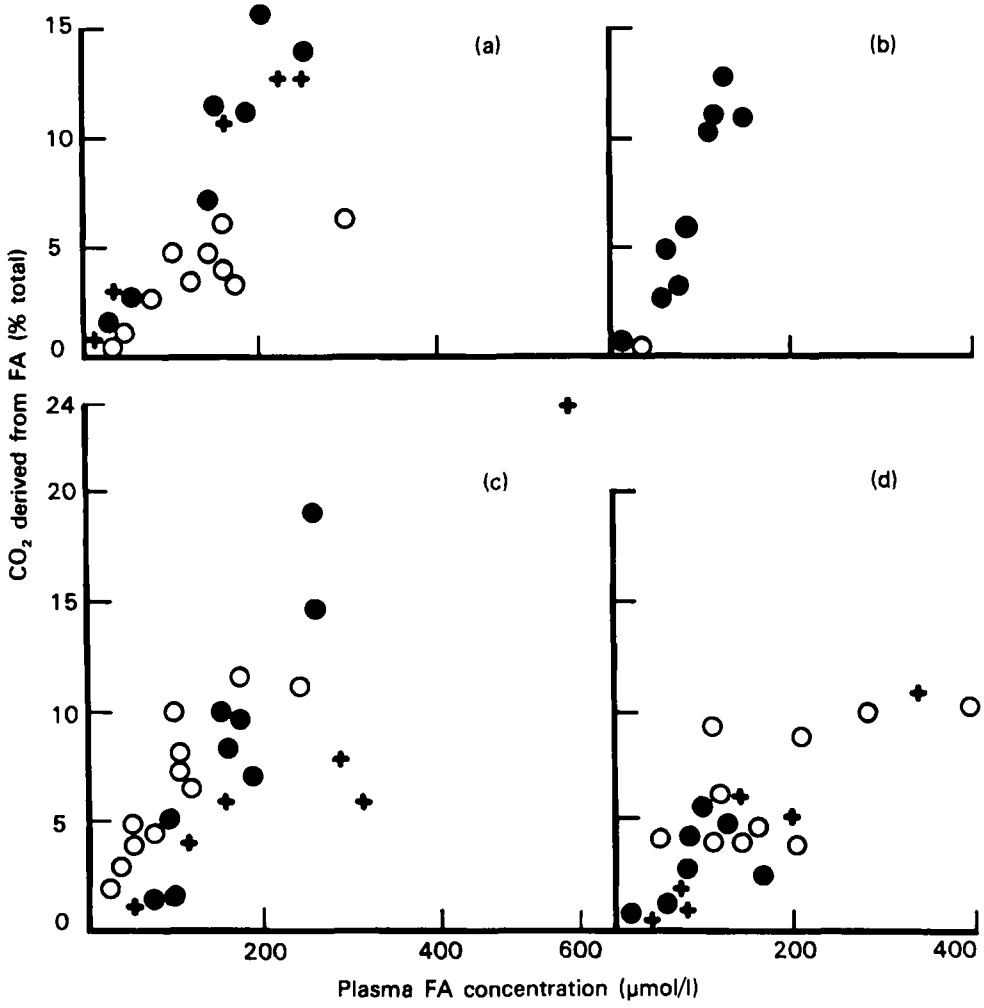

Fig. 1. Oxidation of ^{14}C-labelled fatty acids (FA) as a function of plasma concentration of the FA: (a) oleic acid, (b) linoleic acid, (c) palmitic acid, (d) stearic acid. Results for sheep (○) from Annison, Brown, Leng, Lindsay & West (1967), Leat & Ford (1966), Leat, Lindsay & Valerio (1975) and W. M. F. Leat & D. B. Lindsay (unpublished results), pigs (+) from Freeman, Noakes & Annison (1970) and chickens (●) from Infield & Annison (1973).

techniques that when animals rely on their tissues to supply their energy requirements, at least 80% of the energy is derived from fat, the remainder coming from protein (Blaxter, 1963). To meet the energy needs of a fasted sheep (50–60 kg), about 100 g fat/d is utilized. Even in sheep dependent only partly on body reserves for energy, fat is the predominant tissue used. Burton, Anderson & Reid (1974), in work which included a study of fed sheep which were losing body-weight, reported a loss of roughly 100 g fat/d (and only 10 g protein/d). This fat will be largely in adipose tissue, and the FA must be mobilized to be made available to other tissues such as muscle, heart, liver and kidneys. We know that predominantly, if not exclusively, the form of FA leaving adipose tissue is non-esterified or free FA (FFA) (Frederickson & Gordon, 1958). The flux (entry rate) of

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palmitic, stearic and oleic acids, as determined by constant infusion of the appropriate labelled acid and measurement of the 'plateau' specific activity, has been estimated in sheep starved for several d, and is in sum about 160 g/d. Since these three acids constitute about 80% of the total FFA in sheep plasma, it is entirely possible that the total flux is about 200 g/d. Thus even if the FA are significantly recycled, the flux is ample to account for the nutritional requirements.

When we examine the contribution of the FA to respiratory CO_2, by comparing the 'plateau' CO_2 specific activity with that of the FA, we find a rather different story. Fig. 1 shows how the percentage CO_2 derived from a plasma FFA is related to the plasma concentration of the FA. The amount of CO_2 derived from a FA increases as the plasma concentration increases, with no indication of a limiting value. Nevertheless, in sheep starved for 3–4 d for example, the maximal contribution to CO_2 from these acids (palmitic, stearic+oleic) is only about 30%. If the other FA contributed correspondingly we might expect 35–40% of CO_2 to be derived from the FFA. The results shown in Fig. 1, however, suggest that it is not really permissible to generalize from results obtained with one FA to the whole FFA fraction. They suggest that linoleic acid may be the most readily oxidized, that is, contributes most to respiratory CO_2 at a given plasma concentration. It is true that the results are only really valid for chickens, since the linoleate content of the FFA of sheep plasma is normally too low to draw any conclusions about the relation of concentration to extent of oxidation; nevertheless, there are indications that in man too, linoleic acid differs metabolically from the other FFA (Havel, Carlson, Ekelund & Holmgren, 1964). Stearic acid seems to be least readily oxidized in all species, while 'oleic' acid is perhaps less readily oxidized in sheep compared with other species, although this may simply reflect the significant content of *trans* acid, and possibly monoenes other than $\Delta 9$ in the 'oleic' acid fraction as usually isolated from the plasma FFA of ruminants. In a recent study by Thompson, Gardner & Bell (1975) of the uptake of palmitic, oleic and stearic acids by the liver of sheep fasted and subjected to cold exposure, the uptake of stearic acid, in contrast to the other acids, was largely unaffected by an increase in the plasma concentration of this FA. It is thus fairly certain that individual FA may be metabolized to a quantitatively different extent; moreover this feature is perhaps of especial importance in sheep, in which the stearate content of the plasma FFA is rather higher than that of most species. Despite this qualification, it seems highly unlikely that the 20% of the FFA fraction not studied would contribute up to 40% of the CO_2.

Some FA will not be oxidized directly to CO_2, but will first be converted to ketones. Total uptake of FFA by the sheep liver as measured by Thompson *et al.* (1975) may be estimated to be about 50–60 g/d in conditions which are likely to reflect maximal mobilization of FFA. An appreciably greater estimate for the uptake of FFA in fasted sheep was obtained by Katz & Bergman (1969), but it is likely to be an overestimate since the method used for estimation of FFA is not sufficiently specific. Their estimation of ketone output, which should be realistic, may be calculated to be about 50 g/d. Thus about 70% of the FA taken up by the liver may be converted to ketones. If this amount were fully oxidized by peripheral

tissues, it could account for about 30% of the CO_2. We have no direct evidence of the amount of CO_2 derived from the oxidation of ketones in sheep starved at least 3 d. In sheep fasted for 24 h we found (Annison, Brown, Leng, Lindsay & West, 1967) that 2–8% of the CO_2 was derived from 3-hydroxybutyrate, while in undernourished, pregnant, ketotic sheep (Leng, 1965) the highest estimate obtained was 21%. It is likely therefore that in non-pregnant, starved sheep, ketones contribute 10–20% of CO_2. In man (Reichard, Owen, Haff, Paul & Bartz, 1974) after about 3 d starvation, total ketone-body production is about 150 g/d, of which about 130 g/d is apparently directly oxidized, which would account for about 33% of the energy requirement, equivalent to about 25% of the CO_2 production.

There is yet another product of incomplete oxidation of FA: acetate. Those of us who work with ruminants have long been familiar with the notion of acetate as an energy source, since it is a major product of rumen fermentation. It has rather been neglected as a possible energy source for non-ruminants, since except in some herbivores the blood acetate concentration is low. It was suggested some years ago (Annison & Lindsay, 1961; Annison & White, 1962) that in fasted sheep significant amounts of acetate are derived from endogenous sources, and this is now of some interest in relation to non-ruminant metabolism of long-chain FA (e.g. Söling, Graf & Seubert, 1974). The flux (entry rate) of acetate in sheep fasted for 3 d is quite substantial: according to Bergman & Wolff (1971) it is about 45 mmol/d. They found that about 80% of this was derived from endogenous sources. Thus about 50 g acetate/d would be produced in this way. We have again no direct evidence of the proportion oxidized, although in fasted, pregnant, ketonaemic sheep, Lindsay & Ford (1964) found nearly 10% of CO_2 was derived from acetate. Endogenous acetate probably is derived from a number of tissues. In the study of Bergman & Wolff (1971) about 20% was derived from the liver of starved sheep. Söling *et al.* (1974) found that the isolated rat liver perfused with long-chain FA released acetate at about one-third to one-tenth the rate of release of ketones. The mammary gland of lactating goats has also been shown to release as well as take up acetate from blood in both fed (Annison & Linzell, 1964) and fasted (Annison, Linzell & West, 1968) animals. Acetate production has also been shown to occur from rat and sheep heart slices (Knowles, Jarrett, Filsell & Ballard, 1974) and human forearm muscle (Hagenfeldt & Wahren, 1971). Annison & Armstrong (1970) refer to work of Annison and his colleagues which shows that acetate is produced by tissues of the head of the cow. Since B. P. Setchell and I have been unable to demonstrate the production of acetate by the sheep brain using a similar technique (fall in specific activity of acetate across a tissue when labelled acetate is infused), the tissues of the head producing acetate are probably muscular. In recent studies in pregnant sheep of tissue which is predominantly hind-limb muscle we have found in some undernourished animals that on occasion there is a net release of acetate. It is interesting that Annison, Scott & Waites (1963) found no significant production of acetate by the testis of sheep. Thus the two tissues for which there is no evidence for acetate production (brain and testis) are ones in which oxidative metabolism is substantially dependent on glucose.

It is clear that in fasted animals the oxidation of FFA is incomplete; while some is oxidized to CO_2 directly, a significant amount is converted to ketones and some (and perhaps some of the ketones) is converted to acetate. It is even possible that some of the acetate is reconverted to long-chain FA, since Ingle, Bauman, Mellenberger & Johnson (1973) found significant (about 20% of the rate in fed animals) synthesis of FA from acetate in adipose tissue taken from sheep starved for 96 h. It is still doubtful, however, whether such findings will account for the apparently small proportion of CO_2 derived from FFA in fasted animals. It has previously been assumed (e.g. Annison *et al.* 1967) that in isotope experiments, when the proportion of CO_2 derived from a FA is estimated, this includes that occurring by indirect oxidation such as that through ketone formation. Leng & West (1969) found significant labelling of 3-hydroxybutyrate within 4–6 h of infusion of ^{14}C-labelled FA in sheep starved for 4 d, and Palmquist (1972) found significant labelling of acetate after infusion of palmitate for up to 10 h, in sheep fasted for 48 h. Studies of the labelling of CO_2 after infusion of FFA have usually been made over periods of 4–5 h, it being assumed that if the specific activity of CO_2 has not reached a plateau, this reflects incomplete equilibration with the CO_2 pool of the animal. The significance of indirect oxidation in accounting for the apparently small contribution of FA to the energy needs of starved sheep can probably be determined by experiments involving prolonged infusion of the ^{14}C-labelled FA.

Fed animals

In fed animals, particularly non-ruminants, we expect the major energy source to be glucose. About 31% of CO_2 was derived from glucose in fed pigs (Freeman, Noakes & Annison, 1970); in fed rats, the contribution can be even higher: about 44% in rats receiving a diet of nearly 700 g sucrose/kg, according to Shipley, Chudzik & Gibbons (1970). Depocas (1964), who used rats, and Issekutz, Paul & Miller (1967), who used dogs, infused glucose intravenously and found that the maximum proportion of CO_2 that could be derived from glucose was about 60%. It is uncertain what portion of the remaining CO_2 is derived from FA oxidation. The plasma FFA normally make only a small contribution, although in fed pregnant sheep the contribution of FA to CO_2 is appreciable (Leat & Ford, 1966). Infield & Annison (1973) found that in chickens on a high-carbohydrate diet, about 5–6% of CO_2 was derived from FFA, and a similar estimate was obtained by Freeman *et al.* (1970) with pigs. The latter authors, however, also found that about 12% of CO_2 was derived from acetate, while Annison, Shrimpton & West (1969) obtained a corresponding value for fed chickens of about 15%. In fed animals the origin of circulating acetate is not known: it is possible that a significant amount is derived from fermentation in the hind-gut of non-ruminants. Nevertheless, it is clear that FA provide at least 20% of the CO_2 in non-ruminants. In ruminants (at least in sheep) the volatile FA provide about 70% of CO_2, about 50% being derived from acetate and butyrate (Annison *et al.* 1967). The contribution in non-ruminants of FA to CO_2 may be even higher than is indicated, since not all the respiratory CO_2 is accounted for. Depocas (1964) suggested that intracellular stores (including FA) may also be oxidized. However, it should be noted that no account has been taken

of the oxidation of protein. There are indications that the direct oxidation of amino acids is appreciable in animals at nitrogen equilibrium (see e.g. Lindsay, 1975).

Endogenous FA

The possible role of intracellular FA in meeting energy requirements has been especially discussed in assessing the significance of FA oxidation in exercise. Glucose is only oxidized to a minor extent during exercise, supplying about 12–15% of CO_2. Although plasma FFA are the major energy source in both moderate and severe exercise in both men (Havel *et al.* 1964) and dogs (Issekutz, Miller, Paul & Rodahl, 1964), they account for only about 50% of the CO_2. Issekutz *et al.* (1964) showed that plasma esterified FA could not contribute appreciably to CO_2 production and obtained indirect evidence that intracellular FA were being oxidized. However, Masoro, Rowell, McDonald & Steiert (1966) were unable to demonstrate any decrease in the intracellular FA after several h electrical stimulation of hind-limb muscles of monkeys. Nor were they able to obtain any evidence of increased turnover of these FA. In contrast, Fröberg, Carlson & Ekelund (1971) have observed a significant decrease in the triglyceride content of biopsied samples of muscle taken from men before and after exhausting exercise. They also reported there was a decrease in the triglyceride content of rat muscle after electrical stimulation. There is no satisfactory explanation for the different findings. It is just possible that there may be errors due to the inclusion of intermuscular adipocytes as intramuscular triglyceride; indeed it has been suggested that FA could be in part available to muscle by diffusion from juxtaposed adipocytes as well as by passage in blood, although there is no direct evidence of this.

It has generally been assumed that during exercise the FA utilized are fully oxidized. Hagenfeldt & Wahren (1968), in studying forearm metabolism during exercise, found that although the arterial concentration of 3-hydroxybutyrate was increased, utilization (arteriovenous difference) was if anything less than in resting muscle; indeed one of their subjects consistently had a small output of 3-hydroxybutyrate. There is, however, also evidence (Johnson, Walton, Krebs & Williamson, 1969), that in non-athletes blood ketones increase after exercise, which has been attributed (Johnson & Walton, 1972) to more efficient utilization of ketones by athletically trained subjects. Hagenfeldt & Wahren (1971) have also found that at high work intensities human forearm muscle releases a significant amount of acetate, which was derived from infused ^{14}C-labelled FFA; the amount relative to that converted to CO_2 seems to vary markedly between individuals.

Oxidation in muscle

Several studies have been made of the uptake of metabolites by muscle. On the assumption that net uptake represented oxidation of a metabolite, the results shown in Table 1 have been obtained. Glucose uptake, although corrected for lactate and pyruvate output, has not been corrected for possible conversion to non-essential amino acids, which certainly occurs to some extent. Nevertheless, the results do suggest that even in fed ruminants there is substantial dependence on

glucose as an energy source. This, however, may be misleading; in dogs, Spitzer & Hori (1969), by infusing ^{14}C-labelled FFA, showed there was both uptake and output of FFA, and 34% of the CO_2 was derived from the oxidation of FFA. In our recent studies of sheep muscle, using [^{14}C]glucose, we find a variable fraction of CO_2 derived from glucose, about 1–12%, but it is in all instances substantially less than is suggested by Table 1. Only 2–4% of CO_2, not 8% as suggested by Table 1, appears to be derived from 3-hydroxybutyrate and 14%, not 20%, from acetate. Unfortunately, results of isotope experiments too are not unequivocal, since it is for example possible that glucose is converted to glycogen and that this is the direct source of carbohydrate-derived energy. Nevertheless, in an earlier study (Annison, Lindsay & White, 1963) little labelling of glycogen was found in sheep muscle following infusion of [^{14}C]glucose.

Table 1. *Maximum percentage contribution to total oxygen consumption by some metabolites taken up by hind-limb muscle in vivo*

	Fed sheep*	Fed sheep†	Fed steers‡	Dogs§
Glucose‖	38	17	47	103
Acetate	20	30	62	—
3-Hydroxybutyrate	8	17	—	—
Acetoacetate	0	2	—	—
Free fatty acids	—	33	Net output	Net output

*From Domanski, Lindsay & Setchell (1974).

†From Jarrett, Filsell & Ballard (1974); oxygen consumption was not measured, so values are percentages of total oxygen that could be used by the metabolites; lactate output communicated by authors.

‡From Bell, Gardner & Thompson (1974) and Bell, Gardner, Manson & Thompson (1975).

§From Spitzer & Hori (1969); dogs were anaesthetized and fasted for 18 h; results were calculated from CO_2 output.

‖Glucose uptake corrected for output of lactate and pyruvate.

Control of FA oxidation

Only brief comment is possible on the control of FA oxidation. We saw earlier that oxidation is linearly related to plasma concentration and there is no indication that this limited at any concentration. Nevertheless, at high rates of oxidation there is probably increased formation of ketones by the liver and of acetate by most tissues. These probably feed back to control the rate of FA mobilization. Raised concentrations of FFA (Crespin, Greenough & Steinberg, 1972) and of ketones (Madison, Mebane, Ungar & Lochner, 1964) stimulate insulin secretion, at least in the dog. In addition a raised concentration of acetate appears to limit adipose tissue lipolysis, both in sheep (Lindsay, 1959) and in man (Crouse, Gerson, DeCarli & Lieber, 1968) and 3-hydroxybutyrate has a similar effect in sheep (E. F. Annison, personal communication) although in this species it does not appear to stimulate insulin secretion (Horino, Machlin, Hertelendy & Kipnis, 1968).

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