Metabolic fate of non-esterified fatty acids in isolated hepatocytes from newborn and young pigs

Evidence for a limited capacity for oxidation and increased capacity for esterification

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In hepatocytes isolated from 48 h-old starved or suckling newborn pigs or from 15-day-old starved piglets, the rate of ketogenesis from oleate or from octanoate is very low. This is not due to an inappropriate fatty acid uptake by the isolated liver cells, but results from a limited capacity for fatty acid oxidation. Some 80–95% of oleate taken up is converted into esterified fats, whatever the age or the nutritional conditions. Three lines of indirect evidences suggest that fatty acid oxidation is not controlled primarily by malonyl-CoA concentration in newborn pig liver. Firstly, the addition of glucagon does not increase fatty acid oxidation or ketogenesis. Secondly, the rate of lipogenesis is very low in isolated hepatocytes from newborn pigs. Thirdly, the rates of oxidation and ketogenesis from octanoate are also decreased in isolated hepatocytes from newborn and young piglets. The huge rate of esterification of fatty acids in the liver of the newborn pigs probably represents a species-specific difference in intrahepatic fatty acid metabolism.

The newborn pig, which has an extremely small amount of body fat at birth (less than 2%; Manners & McCrea, 1963), receives a large supply of lipids from the milk (Salmon-Legagneur, 1965). This provides a source of fatty acids for the increase in fat storage, which is very rapid during the preweaning period (Manners & McCrea, 1963), but probably implies also an active hepatic fatty acid metabolism. In newborn animals of several species (rat, rabbit, guinea-pig, man), it has been shown that the absorption of a high-fat diet, the milk, leads to a physiological hyperketonaemia (for a review see Robinson & Williamson, 1980). In contrast, several studies have shown that newborn pigs do not show any hyperketonaemia, despite high concentrations of plasma non-esterified fatty acids (Bengtsson *et al.*, 1969; Gentz *et al.*, 1970; Pégorier *et al.*, 1981), suggesting that ketogenesis and/or β -oxidation are decreased. The reasons for the limitation of ketogenesis in the liver of the newborn pigs are unknown. It has been reported that the rate of oxidation of palmitate in homogenates of newborn-pig liver is very low at birth and increases 4-fold by day 7 after birth (Mersmann & Phinney, 1973; Wolfe *et al.*, 1978). This is probably the result of the low number

of mitochondria in liver at birth, which increases rapidly within the first 24–48 h after birth (Bischoff *et al.*, 1969; Mersmann *et al.*, 1972). However, the capacity of liver mitochondria of newborn pigs to oxidize fatty acids is only half that of 24-day-old piglets (Bieber *et al.*, 1973; Helmrath & Bieber, 1974). As the metabolic fate of fatty acids in the liver of newborn pigs has never been studied, it is not known whether the low rate of hepatic ketogenesis results from a limited capacity for fatty acid oxidation. The purpose of the present study was to determine the capacity for fatty acid oxidation, esterification and synthesis, in isolated hepatocytes from 48 h-old starved and suckling pigs. The results are compared with those determined in isolated hepatocytes from 15-day-old piglets, which are known to possess a well-developed adipose tissue (12–15% of body fat; Manners & McCrea, 1963) and an increased capacity for fatty acid oxidation (Mersmann & Phinney, 1973).

Experimental

Animals

Pigs of the Large White Strain, farrowed in the Institut National de la Recherche Agronomique

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(Jouy-en-Josas, France), were used. Pregnant sows were fed daily with 2.5 kg of cereals/soya-bean-meal diet. The lactating sows were fed *ad libitum* with the same diet. In this strain, normal delivery occurs during the night of day 114–115 of pregnancy. As precise timing of birth was desired, parturition was induced by injecting pregnant sows on day 113 of gestation with a prostaglandin analogue (cloprostenol, 10 µg/kg body wt; Bellon, Neuilly, France). With this technique, newborns were delivered on the morning of day 114, i.e. a few hours before the normal time of delivery. Starved newborn pigs were separated from the mother immediately after birth and maintained at 34°C for 48 h. Suckling newborn pigs were left with the mother under a heating lamp which maintained a local temperature of 32–33°C and were allowed to suckle at will for 48 h or 15 days after delivery. In some experiments 15-day-old suckling piglets were starved for 48 h at room temperature (22°C) with free access to water.

Isolation of hepatocytes

Hepatocytes were isolated essentially by the method of Berry & Friend (1969). After anaesthesia (15 mg of sodium thiopental/kg body wt.; Specia, Paris, France) the liver was perfused *in situ* through the portal vein at a perfusion rate of 100 ml/min as described previously (Pégrier *et al.*, 1982). The viability and the choice of result expression has been discussed previously (Pégrier *et al.*, 1982).

Measurement of oleate and octanoate oxidation and esterification

Hepatocytes were incubated in Krebs–Henseleit (1932) bicarbonate medium, pH 7.4, at 37°C for 60 min in a final volume of 4 ml. All incubations were performed in duplicate in the absence of non-esterified fatty acids, or in the presence of oleate (1 mM) or octanoate (2 mM). In these experiments fatty acids were bound to dialysed albumin (fraction V, fatty acid-free; final concn. 3%, w/v). In some experiments, L-carnitine (1 mM) and glucagon (0.5 ng/ml) were added to the incubation medium. Incubations were ended by adding 0.5 ml of 40% (v/v) HClO₄ to the incubation flasks. The incubations with [1-¹⁴C]oleate and [1-¹⁴C]octanoate were performed as described by Whitelaw & Williamson (1977) for oleate and by McGarry & Foster (1971) for octanoate. The separation of acid-soluble labelled oxidation products from the traces of [¹⁴C]oleate or [¹⁴C]octanoate was performed as described respectively by Mannaerts *et al.* (1979) and McGarry & Foster (1971). Lipids were extracted as described by Folch *et al.* (1957). The radioactivity in the chloroform phase was used as a measure of the [1-¹⁴C]oleate or [1-¹⁴C]octanoate converted into esterified fats (Whitelaw & William-

son, 1977; McGarry & Foster, 1971). The uptake of [1-¹⁴C]oleate was determined and calculated as described by Whitelaw & Williamson (1977).

Measurement of lipogenic rates

As lipogenesis is very low in liver of adult pigs (O'Hea & Leveille, 1969) and of newborn pigs (Mersmann *et al.*, 1973a,b), 20–25 million hepatocytes were incubated in a final volume of 2 ml of Krebs–Henseleit buffer. The flasks were preincubated during 30 min at 37°C in the presence of oleate (1 mM) plus carnitine (1 mM) with or without 10 mM-lactate. In some experiments, glucagon (0.5 ng/ml) was added to the incubation medium. Measurements of lipogenic rates were initiated 30 min later by adding 250 µCi of ³H₂O. Incubations were ended 30 min later by centrifugation for 0.5 min at 5000 g. The lipids were extracted as described by Folch *et al.* (1957). Fatty acids were extracted as described by Windmueller & Spaeth (1966) and Harris (1975).

Measurements of metabolites

Glucose, lactate, pyruvate, acetoacetate and D-3-hydroxybutyrate were determined in the neutralized extracts by standard enzymic methods (Werner *et al.*, 1970; Bergmeyer, 1974; Williamson *et al.*, 1962). The results are given as means ± S.E.M., and significant differences were tested by variance analysis (Snedecor & Cochran, 1967).

Chemicals

All enzymes and coenzymes were obtained from Boehringer Corp. (Meylan, France). Fatty acid-free albumin, oleate and octanoate were purchased from Sigma (St. Louis, MO, U.S.A.). [1-¹⁴C]Oleate and ³H₂O were from The Radiochemical Centre, Amersham, Bucks., U.K. [1-¹⁴C]Octanoate was obtained from New England Nuclear, Dreieich, W. Germany. Glucagon was purchased from Novo Corp. (Paris, France).

Results and discussion

Rate of ketogenesis in isolated hepatocytes from newborn and young pigs

The rate of ketogenesis from endogenous substrates and from added oleate or octanoate is very low in isolated hepatocytes from starved or suckling newborns (Table 1). This suggests that the low concentration of blood ketone bodies observed in the suckling newborn pig (Pégrier *et al.*, 1981) results from the low rate of hepatic ketogenesis rather than from a high rate of peripheral ketone-body utilization. Although the rate of ketone-body production is higher in isolated hepatocytes from 15-day-old animals than in those of the newborns (Table 1), the rate of ketogenesis remains 10–15-fold lower than

Table 1. Rate of ketone-body production in hepatocytes from 48 h-old and 15-day-old piglets

For details see the Experimental section. Final concns. of oleate, octanoate and carnitine were respectively 1, 2 and 1 mM. Glucagon was added to a final concn. of 0.5 ng/ml. Results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. Production of ketone bodies is expressed as nmol/h per 10^6 hepatocytes. Endogenous ketone-body production is not subtracted. * $P < 0.05$ compared with ketone-body production in hepatocytes from starved newborn pigs; † $P < 0.01$ compared with ketone-body production in hepatocytes from suckling pigs; ‡ $P < 0.01$ compared with endogenous ketone-body production.

Substrates added	Newborn pigs		15-day-old piglets	
	Suckling	Starved	Suckling	Starved
None	7.0 $\pm$ 1.4 (9)*	2.8 $\pm$ 0.6 (12)	13.6 $\pm$ 3.5 (4)*	18.3 $\pm$ 4.9 (9)*†
Oleate	9.7 $\pm$ 2.1 (5)*	4.5 $\pm$ 0.6 (8)‡	20.3 $\pm$ 8.8 (4)*	36.9 $\pm$ 5.3 (9)*†‡
Oleate + carnitine	12.7 $\pm$ 2.5 (5)*	6.6 $\pm$ 0.7 (8)‡	21.5 $\pm$ 5.3 (4)*	41.4 $\pm$ 9.3 (5)*†‡
Oleate + carnitine + glucagon	14.6 $\pm$ 1.7 (4)*	8.2 $\pm$ 0.9 (6)‡	—	—
Octanoate	14.0 $\pm$ 2.3 (9)*	8.0 $\pm$ 1.3 (10)‡	42.3 $\pm$ 9.2 (4)*†‡	41.4 $\pm$ 5.0 (9)*†‡

Table 2. Rates of [$1\text{-}^{14}\text{C}$]oleate uptake and percentage distribution in various products of [$1\text{-}^{14}\text{C}$]oleate in hepatocytes from 48 h-old and 15-day-old piglets: effect of glucagon

For details see the Experimental section. Final concn. of oleate was 1 mM and of glucagon 0.5 ng/ml. Results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. Rates of [$1\text{-}^{14}\text{C}$]oleate uptake are measured in the presence of 1 mM-carnitine and expressed as nmol/h per 10^6 hepatocytes. Metabolic distribution of [$1\text{-}^{14}\text{C}$]oleate is expressed as a percentage of [$1\text{-}^{14}\text{C}$]oleate uptake. * $P < 0.01$ compared with starved newborn pigs.

	Glucagon	Newborn pigs		15-day-old piglets
		Starved	Suckling	Starved 48 h
[^{14}C]Oleate uptake	—	80.5 $\pm$ 6.7 (6)	82.2 $\pm$ 6.7 (5)	108.1 $\pm$ 10.1 (4)
	+	77.6 $\pm$ 7.2 (4)	79.6 $\pm$ 10.4 (4)	—
Distribution of [^{14}C]oleate (% of oleate uptake)				
Metabolic CO_2	—	1.4 $\pm$ 0.2 (6)	4.9 $\pm$ 0.8 (5)*	4.4 $\pm$ 0.6 (4)*
	+	2.8 $\pm$ 0.2 (4)	6.2 $\pm$ 0.9 (4)*	—
Acid-soluble products	—	2.9 $\pm$ 0.5 (6)	12.8 $\pm$ 3.5 (5)*	16.7 $\pm$ 1.2 (4)*
	+	3.6 $\pm$ 0.7 (4)	16.2 $\pm$ 0.6 (4)*	—
Esterified fats	—	95 $\pm$ 0.8 (6)	82.2 $\pm$ 2.2 (5)*	77.2 $\pm$ 1.8 (4)*
	+	93.5 $\pm$ 0.7 (4)	77.5 $\pm$ 1.0 (4)*	—

that measured in isolated hepatocytes from newborn rats or rabbits (Ferré *et al.*, 1981; El Manoubi *et al.*, 1981). The low capacity of the liver to synthesize ketone bodies seems to persist in the adult pig. Indeed a low rate of ketogenesis has been shown in the starved adult miniature pig despite an increased hepatic uptake of fatty acid (Müller *et al.*, 1982). The differences in the rate of ketogenesis between newborn and older piglets are also observed when the results are expressed per mg dry wt. or per mg fresh wt. (results not shown). As carnitine is lost during the preparation of isolated hepatocytes (Christiansen & Bremer, 1976), and as carnitine is required for the entry of oleate into the mitochondria (for a review see McGarry & Foster, 1980), we have investigated whether the low rate of ketogenesis from oleate was not due to carnitine depletion of isolated hepatocytes. The addition of 1 mM-carnitine does not enhance the rate of ketogenesis from oleate in hepatocytes isolated under various conditions of age or nutrition (Table 1). At

least three mechanisms can theoretically be involved in the limitation of ketogenesis in isolated hepatocytes from the piglets: (1) an inappropriate fatty acid uptake by pig liver cells; (2) a low rate of hepatic fatty acid oxidation; (3) a channelling of fatty acyl-CoA toward esterification rather than into β -oxidation.

Rate of fatty acid uptake by isolated hepatocytes from newborn and young piglets

Oleate uptake is similar in isolated hepatocytes from newborn or young piglets whatever the nutritional conditions (Table 2) and is similar to the rate of oleate uptake measured in isolated hepatocytes from fed or starved adult rats (Whitelaw & Williamson, 1977) or in hepatocytes from newborn rats (Satabin *et al.*, 1982). About 98–99% of the [$1\text{-}^{14}\text{C}$]oleate taken up by the hepatocytes during a 60 min incubation could be accounted for by the radioactivity found in three fractions: metabolic CO_2 , acid-soluble products, esterified fats (Table 2).

This indicates that changes in the rate of formation of oleoyl-CoA are not responsible for the variations in the distribution of [$1-^{14}\text{C}$]oleate (Table 2).

Metabolic fate of fatty acids in hepatocytes isolated from newborn and young piglets

Despite significant differences in the rate of oleate oxidation in various experimental conditions, the most striking feature of hepatic fatty acid metabolism in newborn or in young piglets is the huge proportion of oleoyl-CoA converted into esterified fats, whatever the age or the nutritional conditions (Table 2). In newborn or young piglets, liver fatty acid oxidation does not seem to be limited by an inappropriate development of the carnitine acyltransferase system. Indeed Bieber *et al.* (1973) have shown that the activity of carnitine palmitoyltransferase is well developed in the liver of starved or suckling newborn or young piglets and that the activity of this enzyme is higher than in the liver of newborn rats (Augenfeld & Fritz, 1970), which are known to possess an active fatty acid oxidation and ketogenesis (Satabin *et al.*, 1982). Nevertheless, these results do not exclude a possible limitation of fatty acid oxidation and ketogenesis via an inhibition of the carnitine acyltransferase system by some intracellular metabolite and/or by an abnormal development of the other enzymes involved in β -oxidation or ketogenesis. Indeed, in adult rat liver, it has been shown that malonyl-CoA, a metabolic intermediate in the lipogenic pathway, is a potent inhibitor of carnitine acyltransferase I (for a review see McGarry & Foster, 1980). Although the measurements of hepatocyte malonyl-CoA content and of the effect of malonyl-CoA on carnitine acyltransferase have not been performed in the present study, several indirect pieces of evidence suggest that fatty acid oxidation and ketogenesis do not seem to be controlled primarily by malonyl-CoA concentration in newborn-pig liver. Firstly, the addition of glucagon, which has been shown to stimulate fatty acid oxidation mainly through a decrease in [malonyl-CoA] in the adult rat (McGarry *et al.*, 1978), has no effect on fatty acid oxidation (Table 2) and on ketogenesis in pig hepatocytes (Table 1). The absence of a stimulatory effect of glucagon on fatty acid oxidation and ketogenesis is not due to a loss of glucagon receptors during the preparation of isolated hepatocytes or to the low [glucagon] used, since under the same experimental conditions glucagon markedly decreases production of lactate + pyruvate measured from 5 mM-dihydroxyacetone (48 ± 5 versus 94 ± 6 nmol/h per 10^6 hepatocytes, $n = 6$) and stimulates significantly ($P < 0.05$) the rate of glucose production measured from 10 mM-lactate in isolated hepatocytes from starved newborn pigs (63 ± 2 versus 45 ± 4 nmol/h per 10^6 hepatocytes, $n = 6$).

Table 3. Rate of lipogenesis in isolated hepatocytes from 48 h-old starved and suckling piglets

For details, see the Experimental section. Final concns. of oleate, carnitine, lactate and glucagon were respectively 1, 1 and 10 mM and 0.5 ng/ml. Results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. The rates of lipogenesis are expressed as nmol of $^3\text{H}_2\text{O}$ incorporated into lipids/h per 10^6 hepatocytes.

Substrates added	Starved	Suckling
None	Undetectable	Undetectable
Oleate + carnitine	3.7 ± 0.3 (5)	4.2 ± 0.5 (4)
Oleate + carnitine + lactate	4.8 ± 0.3 (5)	5.3 ± 0.7 (4)
Oleate + carnitine + lactate + glucagon	3.7 ± 0.6 (5)	3.9 ± 0.7 (4)

Secondly, lipogenesis is very low in the hepatocytes isolated from newborn pigs (Table 3), even in the presence of 10 mM-lactate (Table 3). These results are in agreement with those of Mersmann *et al.* (1973a,b) showing that lipogenesis from glucose or acetate, as well as lipogenic enzyme activities, are very low in the liver from foetal and newborn pigs. Thirdly, the rates of ketogenesis from 2 mM-octanoate, which provides the same amount of carbon atoms as 1 mM-oleate, and which by-passes the carnitine acyltransferase step (McGarry & Foster, 1974), are very low in isolated hepatocytes from newborn and young piglets (Table 1). This results also from a limited capacity of isolated hepatocytes from starved and suckling newborn pigs to oxidize octanoate. Indeed, the rates of incorporation of [$1-^{14}\text{C}$]octanoate into metabolic CO_2 and into acid-soluble products are respectively 0.82 ± 0.23 and 1.63 ± 0.36 nmol/h per 10^6 hepatocytes ($n = 6$) in hepatocytes from starved newborn pigs and 0.90 ± 0.16 and 3.10 ± 0.41 nmol/h per 10^6 hepatocytes ($n = 5$) in hepatocytes from suckling newborn pigs. Thus it seems unlikely that the low rates of fatty acid oxidation and ketogenesis in isolated hepatocytes from newborn or young piglets could be only explained by changes in hepatic malonyl-CoA concentration.

Although further investigations are warranted in this area, it seems that the huge rate of esterification of fatty acids in the liver of the newborn pigs could contribute to enhancing the fat-depot stores, which have been shown to increase 7-fold during the suckling period (Manners & McCrea, 1963). This probably represents a species-specific difference in intrahepatic fatty acid metabolism.

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