

Effect of riboflavin deficiency on lipid metabolism of liver and brown adipose tissue of sucking rat pups

By JULIA M. DUERDEN AND C. J. BATES*

Dunn Nutritional Laboratory, Downham's Lane, Milton Road, Cambridge CB4 1XJ

(Received 6 February 1984 – Accepted 27 July 1984)

1. An increase in liver:body-weight and in hepatic triacylglycerol content, together with changes in the fatty acid profiles of hepatic phospholipids, were observed as a result of moderate riboflavin deficiency in sucking rat pups. Oxygen consumption by hepatic mitochondria, with palmitoyl L-carnitine as substrate, was not significantly impaired.
2. Mitochondria from interscapular brown adipose tissue, however, showed a marked impairment of O₂ consumption, with palmitoyl L-carnitine as substrate, in the riboflavin-deficient pups. This impairment was also apparent after uncoupling with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, but was not consistently observed after the addition of GDP to suppress uncoupled oxidation. It was much less evident, and did not reach statistical significance, for the mitochondria of brown adipose tissue of the corresponding deficient dams.
3. Binding of ³H-labelled GDP by brown adipose tissue mitochondria was unaffected by riboflavin deficiency in the pups, suggesting that the effect on O₂ consumption is more likely to be due to impaired integrity of the mitochondrial respiratory chain, than to impairment of the specific capacity for uncoupling of respiration which is characteristic of brown adipose tissue mitochondria. Total cytochrome *c* oxidase (EC 1.9.3.1) activity of the brown adipose tissue of riboflavin-deficient pups was not significantly reduced.
4. A small but significant impairment was observed in the stimulation of whole-body O₂ consumption by injected noradrenaline in the riboflavin-deficient pups, suggesting that the impairment of brown adipose tissue mitochondrial function may be accompanied by impaired physiological capacity *in vivo*.

In weanling rats which are made riboflavin-deficient, the mitochondrial oxidation of fatty acyl substrates is reduced more rapidly and more severely than the oxidation of other mitochondrial substrates (Hoppel *et al.* 1979; Olpin & Bates, 1982*a, b*). This metabolic lesion may be of crucial importance during the preceding period of sucking, when dependency on lipid substrates as a source of energy is particularly high (Drahota *et al.* 1965-66; Hahn & Koldovsky, 1966; Yeh & Zee, 1979).

Whereas hepatic mitochondria release energy to support a wide range of metabolic processes, it is now becoming clear that brown adipose tissue mitochondria have a rather specific function in supporting heat production through non-shivering thermogenesis. This process is mediated through the uncoupled oxidation of fatty acids, which is linked to the control of body temperature (Nicholls, 1979; Cannon & Johansson, 1980; Sundin *et al.* 1981). No studies have yet been published on these processes in relation to riboflavin status in sucking animals.

Having established a model of marginal riboflavin deficiency compatible with successful reproduction in young female rats (Duerden & Bates, 1985), we have investigated its effects on some hepatic indices of lipid metabolism, and on the capacity for fatty acyl substrate oxidation by brown adipose tissue mitochondria. A possible physiological consequence of the mitochondrial lesion has also been investigated.

* For reprints.

MATERIALS AND METHODS

Animal and tissue preparation

Young female rats were made marginally riboflavin deficient as described in the accompanying paper (Duerden & Bates, 1985), were mated and the pups raised on the mother's milk alone, to day 15. Riboflavin was provided at a concentration of 0.12 or 0.25 mg/kg diet (deficient groups) or 15 mg/kg diet (pair-fed and *ad lib.*-fed controls) in addition to the 0.40 mg/kg from casein. It was usually maintained at this level until the time of death, with the exception of one experiment on liver mitochondrial function in which the riboflavin content was reduced from 0.12 mg/kg to no extra riboflavin between parturition and the time of death.

Dams and pups were killed by diethyl ether anaesthesia; livers were removed and weighed, the pups' livers being pooled within each litter. Riboflavin status was measured biochemically by the activation coefficient (stimulated:basal activity; AC) of erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2) as described by Duerden & Bates (1985). Samples of the pups' livers were used for the measurement of triacylglycerol content and for the analysis of fatty acid profiles of the phospholipid fraction by gas-liquid chromatography, as previously described (Olpin & Bates, 1982*a*). Further samples were used for the preparation of liver mitochondria and measurement of mitochondrial oxygen consumption (see p. 109). Interscapular brown adipose tissue from the dams, and from the pups pooled within litters, was removed and used for the measurement of total cytochrome *c* oxidase (EC 1.9.3.1) activity, as an index of total mitochondrial activity, and for the measurement of the specific activity of mitochondrial oxidation of palmitoyl L-carnitine.

Preparation of hepatic mitochondria

Liver (1 g) was homogenized in 10 ml buffer, pH 7.4, containing (mol/l): mannitol 0.22, sucrose 0.07, morpholinopropane sulphonic acid 5×10^{-3} and ethylene diaminetetraacetic acid 2.17×10^{-3} , using a Potter-Elvehjem homogenizer with a loose-fitting pestle. The homogenate was centrifuged for 10 min at 700 g to remove unbroken cells, nuclei and debris. The supernatant fraction was centrifuged for 15 min at 6000 g; the mitochondria were washed by resuspension in a further 10 ml buffer, collected by centrifugation and resuspended at 20 mg protein/ml.

Preparation of brown adipose tissue mitochondria

Brown adipose tissue mitochondria were prepared, essentially as described by Cannon & Lindberg (1979), as follows. After removal of contaminating tissue, the brown adipose tissue was homogenized in 5 ml buffer, pH 7.2, containing (mol/l): sucrose 0.25, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid 10^{-3} and ethylene diaminetetraacetic acid 2×10^{-3} , using a Potter-Elvehjem homogenizer with a loose-fitting pestle. The homogenate was centrifuged for 15 min at 12000 g and the pellet resuspended and centrifuged at 700 g to remove nuclei and debris. The supernatant fraction containing the mitochondria was filtered through nylon material and the mitochondria were washed twice more with buffer before resuspension at 5 mg protein/ml.

O₂ consumption by mitochondria

Liver mitochondrial O₂ consumption, with palmitoyl L-carnitine as the substrate, was measured as described previously (Olpin & Bates, 1982*b*) except that the O₂ monitor was a model 53, Yellow Springs instrument.

For brown adipose tissue mitochondria the incubation medium, volume 3.0 ml, pH 7.0, contained (mol/l): potassium chloride 0.08, morpholinopropane sulphonic acid 0.05, ethylene glycol tetraacetic acid 10^{-3} , sodium phosphate 5×10^{-3} , bovine serum albumin

2.5 g/l. The operating temperature was 20° for brown adipose tissue mitochondria and 30° for hepatic mitochondria; the concentration of mitochondrial protein in the incubation mixture was 0.5 mg/ml for mitochondria from brown adipose tissue and 1–2 mg/ml for mitochondria from liver. Substrate (palmitoyl L-carnitine) concentration was 4×10^{-5} mol/l and malate, 2×10^{-3} mol/l, was added to prime the Krebs cycle. An excess of ADP (1 μ mol) was added to ensure state-3 respiration (Chance & Williams, 1955). In the brown adipose tissue studies, after measurement of the initial rate, an excess of GDP was added to achieve complete coupling (final concentration 10^{-3} mol/l) and, after measurement of the coupled rate, complete uncoupling was obtained by the addition of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP; Sigma Chemical Co., Poole, Dorset) at 5×10^{-6} mol/l.

Mitochondrial protein concentration was determined by a modification (Bensadoun & Weinstein, 1976) of the Lowry *et al.* (1951) method, in which protein was precipitated with trichloroacetic acid before the assay.

[³H]GDP binding

Binding of ³H-labelled GDP to brown adipose tissue mitochondria was measured as follows (Goodbody & Trayhurn, 1981). The mitochondria were suspended at a concentration of 0.4 mg/ml, total volume 1.0 ml, in a buffer, pH 7.1, containing (mol/l): sucrose 0.1, choline chloride 10^{-2} , EDTA 10^{-3} . They were mixed with [U-¹⁴C]sucrose (0.1 μ Ci/ml; Amersham International, Amersham, Bucks) and with [8-³H]GDP (10^{-5} M, 1.25 μ Ci/ml; Amersham International). After 5 min incubation at 25° they were collected by centrifugation (3 min at 25000 g) and were dissolved in 0.1 ml sodium hydroxide (0.75 mol/l) at 55° before addition of scintillation fluid containing NCS tissue solubilizer (25 g/l), 2,5-diphenyloxazole (5 g/l) and 1,4-di-(2-(5-phenyloxazolyl)) benzene (0.1 g/l). Double-label scintillation counting was used to measure the radioactivity present in ³H and ¹⁴C, and the amount of GDP bound was calculated after correction for the amount present in extra-mitochondrial fluid, estimated from the [¹⁴C]sucrose content.

Cytochrome *c* oxidase

Cytochrome *c* oxidase activity in whole homogenates of brown adipose tissue was measured essentially by the procedure of Yonetani & Ray (1965). After preparation of homogenates as described previously, they were mixed with the non-ionic detergent Lubrol (Sigma Chemical Co.), final concentration 10 g/l. A portion (15 μ l) of the homogenate was added to 1.4 ml incubation medium, pH 7.0, containing potassium phosphate (10^{-2} mol/l) and ferrocytochrome *c* (5×10^{-5} mol/l). The rate of oxidation to the ferric form was measured at 550 nm, and complete oxidation at the end of each incubation was obtained by adding an excess of potassium ferricyanide. The total amount of cytochrome *c* oxidase activity present in the brown adipose tissue recovered and pooled from each litter of pups was calculated on the basis of the extinction coefficient: 0.92/mm per mm for ferricytochrome *c*.

Whole-body O₂ consumption

This index was measured as follows (Stock, 1975). Four 15-d-old pups from each litter (two male and two female) of equal weights were mildly sedated with pentobarbitone, 8 μ g/g body-weight, and were transferred to the chamber of a closed-circuit O₂ consumption apparatus, maintained at 33°. Carbon dioxide was absorbed with soda lime and water with silica gel. Consumption of O₂ was measured by recording the volume of air needed, per unit time, to maintain the unit at a constant pressure. After measurement of the basal O₂ consumption for 1 h, the pups received an intraperitoneal injection of noradrenaline, 0.8 μ g/g body-weight, and the noradrenaline-stimulated rate was then measured for a further 1 h.

Table 1. *Liver: body-weight, hepatic triacylglycerol and fatty acid profiles of hepatic phospholipids of riboflavin-deficient and pair-fed control pups*

(Mean values with their standard errors; no. of pooled litters in parentheses. The deficient group received the diet containing 0.12 mg added riboflavin/kg and had a mean activation coefficient of erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2) of 1.78 (SE 0.07; *n* 4) and the control group received the diet containing 15 mg added riboflavin/kg and had a mean activation coefficient of 1.21 (SE 0.03; *n* 5))

	Riboflavin-deficient pups (3)		Pair-fed control pups (5)	
	Mean	SE	Mean	SE
Liver: body-wt	0.051**	0.037	0.035	0.0019
Hepatic triacylglycerol (mg/g wet liver)	72.1***	2.3	31.0	4.3
Phospholipid fatty acid composition†:				
16:0/1‡	24.5	0.29	25.5	0.55
18:0	20.7	0.61	20.0	0.76
18:1	11.9***	0.26	8.6	0.36
18:2	9.3*	0.99	7.0	0.27
20:3	1.5	0.43	0.7	0.03
20:3/4§	22.3**	1.05	26.4	0.25
22:4/5	7.5	0.35	7.3	0.28
22:6	2.3***	0.20	5.1	0.18

Values were significantly different from the control values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Proportion by weight of total fatty acids recovered by gas-liquid chromatographic analysis.

‡ Mainly 16:0.

§ Mainly 20:4 ω 6.

|| Consists of 22:4 ω 6 and 22:5 ω 3.

Statistical analysis

Student's *t* test was used for the comparisons between groups, having first ascertained that the values approximated to a normal distribution and that multiple groups contained significant differences by analysis of variance.

RESULTS

Hepatic lipids and mitochondrial respiration

Liver: body-weight values and liver triacylglycerol concentrations were significantly higher in riboflavin-deficient compared with pair-fed pups (Table 1). These differences were accompanied by differences in the fatty acid profiles of the hepatic phospholipids (Table 1), oleic and linoleic acids being present as a significantly higher proportion of the total fatty acids of the deficient animals, whereas arachidonic and docosahexanoic acids were present as a significantly lower proportion.

Hepatic mitochondrial O₂ consumption, with palmitoyl L-carnitine as the substrate, was not significantly affected by riboflavin deficiency in either the dams or the pups, even when the deficiency was relatively severe. This is best illustrated by the response obtained from a group of animals given the diet containing 0.12 mg added riboflavin/kg during gestation, followed by a diet containing no added riboflavin until they were killed at 15 d post partum. The dams in the present experiment had a mean activation coefficient (stimulated: basal activity) of erythrocyte glutathione reductase (NAD(P)H) (EGRAC) of 1.90, and the

Table 2. Mitochondrial respiration of brown adipose tissue of riboflavin-deficient and pair-fed control dams and their pups
(Mean values with their standard errors; no. of animals (dams) or pooled litters (pups) in parentheses)

Diet and measurement	Pups						Dams					
	Riboflavin-deficient			Pair-fed controls			Riboflavin-deficient			Pair-fed controls		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0.12 mg added riboflavin/kg EGRAC	1.78***	0.07 (4)		1.21	0.03 (5)		2.04***	0.03 (4)		1.28	0.03 (4)	
Mitochondrial respiration†:												
Initial rate (A)	23.2***	0.9 (4)		57.4	4.7 (5)		107.1	16.7 (4)		82.2	14.2 (4)	
After addition of GDP‡ (B)	21.0	3.2 (4)		28.4	4.0 (5)		89.7	24.0 (4)		50.8	14.9 (4)	
After addition of uncouplers§ A/B	48.1 1.13***	9.0 (4) 0.13 (4)		120.5 2.27	13.2 (5) 0.11 (5)		122.8 1.18	24.0 (4) 0.10 (4)		155.0 1.85	52.1 (4) 0.46 (4)	
0.25 mg added riboflavin/kg EGRAC	1.59***	0.03 (9)		1.20	0.05 (6)		2.01***	0.08 (8)		1.31	0.03 (5)	
Mitochondrial respiration†:												
Initial rate (A)	27.4***	4.5 (9)		70.1	4.0 (6)		80.9	7.1 (8)		101.3	11.8 (5)	
After addition of GDP‡ (B)	20.3**	3.6 (9)		36.2	0.8 (6)		51.6	6.2 (8)		59.2	7.7 (5)	
After addition of uncouplers§ A/B	51.2 1.37***	9.2 (9) 0.05 (9)		130.3 1.98	20.9 (6) 0.12 (6)		158.5 1.63	22.9 (8) 0.11 (8)		175.1 1.78	28.8 (5) 0.22 (5)	

EGRAC, activation coefficient of erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2).

Values were significantly different from the control values: ** $P < 0.01$, *** $P < 0.001$.

† Expressed as atoms oxygen consumed/mg mitochondrial protein per min at 24°.

‡ GDP added at 10^{-3} mol/l final concentration.

§ Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazine added at 5×10^{-6} mol/l final concentration.

Table 3. [^3H]GDP binding by brown adipose tissue mitochondria, and cytochrome *c* oxidase (EC 1.9.3.1) activity of brown adipose tissue homogenates of the riboflavin-deficient, pair-fed control and *ad lib.*-fed control pups

(Mean values with their standard errors; no. of pooled litters in parentheses. The riboflavin-deficient group received the diet containing 0.12 mg added riboflavin/kg and had a mean activation coefficient of erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2) of 1.81, the pair-fed control group had a mean activation coefficient of 1.29, and the *ad lib.*-fed control group had a mean activation coefficient of 1.30)

	Riboflavin-deficient pups		Pair-fed control pups		<i>Ad lib.</i> -fed control pups	
	Mean	SE	Mean	SE	Mean	SE
[^3H]GDP bound to brown adipose tissue mitochondria†	809	53 (8)	730	40 (9)	871	115 (5)
Cytochrome <i>c</i> oxidase activity of brown adipose tissue‡	25.3	2.4 (6)	38.6	7.7 (6)	31.3	6.8 (5)

No significant differences were observed between groups.

† pmol GDP bound/mg mitochondrial protein.

‡ μmol cytochrome *c* oxidized/min for the entire interscapular brown adipose tissue recovered from each litter.

pups had a mean EGRAC of 2.67 compared with mean control values of 1.28 and 1.21 for dams and pups respectively. Mitochondrial O_2 consumption (natoms/mg mitochondrial protein per min) for the deficient dams was 39.1 (SE 6.8; *n* 7) compared with 29.0 (SE 3.6; *n* 21) for controls, and for the deficient pups it was 44.2 (SE 5.5; *n* 7) compared with 40.1 (SE 3.3; *n* 16) for controls. In each case, the deficient group did not differ significantly from the controls. The same conclusion was reached for comparisons involving somewhat less-severely-deficient groups of animals.

Brown adipose tissue mitochondrial respiration

In contrast to the picture obtained with hepatic mitochondria, respiration by brown adipose tissue mitochondria, with palmitoyl *L*-carnitine as the substrate, differed markedly between riboflavin-deficient pups (dams given diets containing 0.12 or 0.25 mg added riboflavin/kg) and pair-fed controls, for whom the riboflavin addition was 15 mg/kg diet (Table 2). For both groups of deficient pups, there was a highly-significant difference in their initial rates of O_2 consumption when compared with the respective control groups. Addition of GDP to promote coupling of oxidative phosphorylation caused a marked decrease in O_2 consumption by the control groups, but a much smaller effect on the deficient groups, so that the respiratory activity in the coupled mitochondria did not always differ significantly between deficient and control groups, but the ratio, coupled: uncoupled activity was always significantly different between control and deficient groups. Addition of an excess of the uncoupling agent FCCP caused a large increase in rate both in the deficient and the control groups, and the final uncoupled rate was considerably greater in the control groups than in the deficient groups.

In the mitochondria from the dams of these pups (Table 2), differences between deficient and control groups were much less pronounced than in the pups, and did not reach statistical significance.

In a separate experiment it was shown that very similar values for brown adipose tissue

Table 4. Whole-body oxygen consumption by the riboflavin-deficient, pair-fed control and ad lib.-fed control pups

(Mean values with their standard errors; no. of pooled litters in parentheses. The deficient group received the diet containing 0.12 mg added riboflavin/kg and had a mean activation coefficient of erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2) of 1.81; the pair-fed control group had a mean activation coefficient of 1.29, and the *ad lib.*-fed control group had a mean activation coefficient of 1.30)

Oxygen consumption†	Riboflavin-deficient pups (5)		Pair-fed control pups (6)		<i>Ad lib.</i> fed control pups (5)	
	Mean	SE	Mean	SE	Mean	SE
Before addition of noradrenaline	331	13	348	13	335	14
After addition of noradrenaline	467*	19	536	19	529	18

All groups showed a significant increase after the addition of noradrenaline (controls $P < 0.001$, deficient $P < 0.01$).

* Significantly different from either the pair-fed or the *ad lib.*-fed control group: $P < 0.05$.

† ml O₂ consumed/h per kg body-weight^{0.75}.

mitochondrial respiration in the pups were obtained for both pair-fed and *ad lib.*-fed control groups receiving the diets with 15 mg added riboflavin/kg. Thus the effect of riboflavin deficiency on the pups' brown adipose tissue mitochondria is not, apparently, attributable to an inanition effect.

Table 3 shows the extent of binding of labelled GDP to the brown adipose tissue mitochondria of control and deficient groups of pups. The deficient group gave a value which was intermediate between, and similar to, those of the two control groups, and there was no significant difference in binding between any of the three groups.

Also in Table 3 is shown the total activity of cytochrome *c* oxidase in the brown adipose tissue recovered from each litter of pups. Although the mean value was lower for the deficient pups than for either of the control groups, the differences between the groups were not statistically significant.

Whole body O₂ consumption and the effect of noradrenaline

Table 4 shows the effect of riboflavin deficiency on whole-body O₂ consumption by the pups, both before and after the injection of a standard dose of noradrenaline. Although the basal level of O₂ consumption was virtually identical in all three groups of animals, the rate after noradrenaline treatment was significantly higher in the control groups than in the riboflavin-deficient groups. Thus, in the deficient group the increase over the basal level was only 135 ml O₂/h per kg body-weight^{0.75}, compared with 192 and 193 ml O₂/h per kg body-weight^{0.75} in the two control groups, comprising a 43% difference between the responses of deficient and control animals.

DISCUSSION

An increase in liver:body-weight values, accumulation of triacylglycerol in the liver, and a change in the fatty acid profile of hepatic phospholipids in sucking pups in the present study is qualitatively very similar to the changes previously observed in severely-riboflavin-deficient weanling animals (Olpin & Bates, 1982*a*), and for several of the indices the changes

were nearly as large as in the weanling animals. At the sucking stage, therefore, hepatic abnormalities may become manifest even when the extent of riboflavin deficiency is not sufficient to cause a marked reduction in growth rate of the pups. It is possible that they may be accompanied by the early stages of important structural changes in other tissues; for instance, those which lead eventually to peripheral neuropathy and nerve degeneration in animals with severe riboflavin deficiency (Phillips & Engel, 1938; Shaw & Phillips, 1941; Street *et al.* 1941; Norton *et al.* 1976).

Surprisingly, in view of these changes, it was not possible to detect an effect of riboflavin deficiency on hepatic mitochondrial respiration, using palmitoyl L-carnitine as the substrate. In weanling animals (Olpin & Bates, 1982*b*) there was generally a close association between compositional changes in the liver and changes in the mitochondrial capacity for fatty acid oxidation, but this does not appear to be true for sucking pups.

In contrast to the lack of an effect on hepatic mitochondrial respiration, the respiration of brown adipose tissue mitochondria with palmitoyl L-carnitine in the sucking pups was clearly sensitive to a moderate extent of riboflavin deficiency, as demonstrated by the significant reduction in O₂ consumption at two levels of riboflavin (0.12 and 0.25 mg added kg). The difference between deficient and control groups was clear-cut, both in the partially-uncoupled state as initially isolated, and in the fully-uncoupled state, but it became much smaller, and was statistically significant in only one of the two groups, after the addition of GDP to obtain complete coupling.

From the available evidence it seems unlikely that there was any alteration in the efficiency of coupling in the riboflavin-deficient animals. The reduction in response to GDP by the riboflavin-deficient animals' mitochondria was not accompanied by any significant reduction in labelled-GDP binding, which suggests that they have not lost their capacity to interact with this effector. Since the impairment in electron transport was most pronounced in the completely uncoupled mitochondria, it is unlikely that the defect in the mitochondria from the deficient animals was attributable to an altered extent of coupling by external effectors. Probably the simplest explanation for the reduced rate of respiration is that there is a partial loss of flavin cofactors from the electron transport chain, and that this becomes evident as a reduced rate of O₂ utilization when the mitochondria are partly or fully uncoupled, but not when they are tightly coupled, and thus less-severely stressed in terms of electron throughput.

Further studies are needed to investigate the possible occurrence and significance in brown adipose tissue of abnormal structures such as megamitochondria, which have been observed among the hepatic mitochondria of severely-deficient weanling animals (Tandler & Hoppel, 1972, 1980) and which are probably related to their functional impairment.

Whatever the explanation of the impairment of electron transport at the molecular and ultrastructural levels, it is highly probable that an impairment also occurs at the physiological level in the whole animal. This is the simplest explanation for the reduced capacity for whole-body O₂ consumption which is observed after noradrenaline treatment. It appears that a certain level of stress is required, imposed, for instance, by partial or complete uncoupling of the mitochondria *in vitro*, or by a noradrenaline stress *in vivo*, in order to permit the fundamental lesion to be manifested as an impaired response.

It is significant that the impairment in brown adipose tissue function in the riboflavin-deficient pups was more pronounced than the corresponding effect in the riboflavin-deficient dams, which confirms and extends the conclusion (Duerden & Bates, 1985) that the severity of deficiency at 15 d post partum was at least as great in the pups as in the dams, under conditions of marginal intakes of riboflavin, where the amounts given are just sufficient to support pregnancy.

Clearly, the observations of the present study may have implications for human subjects

in developing countries, where riboflavin deficiency at birth and during the sucking period is evident, at least in biochemical terms (Bates *et al.* 1982), but its functional significance is not yet understood. If the impairment were sufficient to affect the activity of the brown adipose tissue mitochondria, this lesion might have significant consequences for the maintenance of thermal equilibrium under conditions of stress.

The authors are indebted to Dr P. Trayhurn for helpful discussion. J.M.D. was supported by a Medical Research Council Studentship.

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