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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Seventy-ninth Meeting of the Nutrition Society (*One Hundred and Forty-eighth of the Scottish Group*) *was held in the Freedom Inn, Aviemore, on Wednesday and Thursday,* 3/4 *November* 1982, *when the following papers were read:*

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Flow of endogenous N from the rumen and abomasum of cattle given protein-free nutrients. By E. R. ØRSKOV and N. A. MACLEOD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Measurement of the flow of N at the abomasum or duodenum in ruminants is often used to estimate the amount of protein of microbial and dietary origin which is available for absorption in the small intestine. The endogenous contribution has been very difficult to measure and is often ignored. The technique of intragastric nutrition (Ørskov *et al.* 1979) has made it possible to give protein-free nutrients to ruminants, and recently some values for endogenous N outflow from the rumen have been determined (Ørskov & MacLeod, 1982*a*). These values can be related to the animal fed normally since the mitotic index of rumen epithelial tissue was similar in infused animals to that in normally fed ruminants (Dinsdale *et al.* 1980). The infusion technique has now been used to estimate the outflow of N from the abomasum. PEG 4000 was used as a liquid-phase marker to determine rumen volume. Chromium-EDTA and volatile fatty acid (VFA) solutions were infused into the rumen at a constant rate. The concentrations of Cr-EDTA in rumen and abomasal fluid were used to determine the addition of fluid to the abomasum so that the flow of N from the abomasum could be determined.

Three steers weighing from 240 to 275 kg live weight were used and the VFA solution infused at a rate of 450 kJ/kg live weight ($W^{0.75}$). The amount of fluid infused into the rumen was 25 l/d.

The outflows of non-ammonia-N from the rumen and abomasum are given in the Table together with the liquid flow rate. About two-thirds of the endogenous N in the abomasum are derived from the rumen, mainly in the form of abraded epithelial tissue from the rumen, mouth and oesophagus.

The outflow of liquid and endogenous non-ammonia-N from the rumen and abomasum in steers

	Rumen				Abomasum		
			Non-ammonia-N			Non-ammonia-N	
Steer	Volume (l)	Outflow (l/d)	(g/d)	(mg/kg $W^{0.75}$ per d)	Outflow (l/d)	(g/d)	(mg/kg $W^{0.75}$ per d)
1	22·9	34·6	6·6	98	49·0	9·6	142
2	20·5	30·0	4·9	74	43·2	7·6	116
3	18·1	29·9	5·3	86	38·6	8·1	131

Dinsdale, D., Cheng, K-J., Wallace, R. J. & Goodlad, R. A. (1980). *Appl. Environ. Microbiol.* **39**, 1059.
Ørskov, E. R., Grubb, D. A., Wenham, G. & Corrigall, W. (1979). *Br. J. Nutr.* **41**, 553.
Ørskov, E. R. & MacLeod, N. A. (1982*a*). *Proc. Nutr. Soc.* **41**, 76A.
Ørskov, E. R. & MacLeod, N. A. (1982*b*). *Br. J. Nutr.* **47**, 625.

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Effect of replacing soya-bean meal by fish meal on milk production by Friesian cows on commercial farms. By E. L. MILLER and N. W. GALWEY, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX* and I. H. PIKE, *IAFMM, Hoval House, Potters Bar, Herts EN6 3AR* and G. NEWMAN, *The Travellers Rest, Timberscombe, Taunton YA24 7UK*

Previously, supplementation of normal farm diets with fish meal, replacing barley or beet pulp, increased milk yield (Miller *et al.* 1981). The present experiment investigated the effect of replacing soya-bean meal by fish meal on 3 farms.

Farms 1 and 2 were under the same management, used the same concentrate diets at a flat rate together with different grass silages, and started the experiment in October 1980 shortly after housing. On both farms, one group of cows each received 0·83 kg selected fish meal/d plus 0·42 kg sugar beet pulp/d while a second group each received 1·25 kg soya-bean meal/d. On farm 3, cows were housed and given a diet containing fish meal. At the start of the trial in February 1981, one group of cows continued to receive 0·544 kg fish meal/d plus 0·363 kg barley/d while the second group received 0·907 kg soya-bean meal/d, as part of concentrates, given at a flat rate with grass silage *ad lib.* Milk yields were recorded for 24 h each week. Results from cows over 29 d into lactation at the start of the trials were used. Mean days of lactation at the start of the trials were 66, 57 and 78 for farms 1 to 3 respectively. Milk yields averaged over the first four and second 4 weeks of the trials and adjusted by covariance for milk yield immediately before the start of the trials are given in the Table.

(Mean values; no. of cows per group in parentheses)

Farm	Lactation (weeks)	Milk yield (l/d): Fish meal diet	Milk yield (l/d): Soya-bean meal diet	Fish–Soya effect	Standard error of difference	Significance of difference *P*
1	10–13	29·93 (20)	27·93 (22)	2·00	0·664	<0·01
	14–17	26·89 (20)	25·63 (22)	1·26	0·743	NS
2	9–12	29·95 (11)	27·95 (23)	2·00	0·708	<0·01
	13–16	27·59 (11)	26·65 (23)	0·94	0·825	NS
3	12–15	22·82 (25)	20·11 (18)	2·71	0·577	<0·001
	16–19	20·81 (25)	17·72 (18)	3·09	0·650	<0·001

NS, not significant.

Replacement of soya-bean meal by fish meal resulted in greater milk yields on all farms in the first 4 weeks. The effect decreased in the 5th to 8th week of feeding on two farms but not on the third.

Miller, E. L., Galwey, N. W., Pike, I. H. & Newman, G. (1981). *Anim. Prod.* **32**, 368.

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Some effects of fish lipids on rumen metabolism and digestion in sheep.

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Addition of free oils, including fish oils, to the rumen may depress fibre digestion, decrease acetate:propionate and, in lactating cows, depress fat content of milk (Storry, 1981). The reasons for these changes are not fully understood but effects on rumen fermentation have been implicated. It is not known whether smaller intakes of fish lipids given as part of fish meal would have similar effects on rumen fermentation.

Five rumen-cannulated wethers were given daily 500 g chopped hay plus 500 g of one of five concentrate mixes in four periods according to a balanced, incomplete block design (Type V; Cochran & Cox, 1957). The concentrates contained (g/kg; code): no fish products (0; control), pressed cake meal (93; FM), ethanol-extracted FM (86; EFM), EFM + ethanol-extractable fish lipids (86+7·5; EFM + RFL) or FM + pressed fish oil (93+7·5; FM + FO). All fish products were from the same batch of mackerel. Antioxidant was added during ethanol extraction of FM and to FO at the time of diet preparation. Neutral-detergent fibre (NDF) digestion was assessed by incubating hay within polyester bags in the rumen and overall digestion with reference to Cr_2O_3. Treatment means, adjusted for differences between sheep, are given in the Table.

Treatment	Rumen ammonia (mM)	Rumen acetate:propionate	Fractional NDF disappearance from bags 12 h	48 h	Apparent digestibility of NDF
Control	6·8	3·6	0·16	0·53	0·66
FM	8·2	3·6	0·22	0·55	0·69
EFM	8·3	3·7	0·16	0·55	0·70
EFM + RFL	8·2	3·6	0·19	0·55	0·72
FM + FO	8·9	3·6	0·22	0·55	0·68
SE of mean	0·35	0·14	0·024	0·043	0·027

Apart from an increase in rumen ammonia with added fish products, none of the treatments had a significant effect on the variables given in the Table. The lack of effect of fish lipids on rumen fermentation in this experiment may be due to the low levels of intake and frequent feeding regimen used or may indicate that any detrimental effects are minimized when the lipids are included with fish meal.

This work was supported financially by the International Association of Fish Meal Manufacturers.

Cochran, W. G. & Cox, G. M. (1957). *Experimental Designs*. New York: Wiley.
Storry, J. E. (1981). In *Recent Advances in Animal Nutrition—1981*, p. 3 [W. Haresign, editor]. London: Butterworths.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Some effects of dietary fats on rumen metabolism and digestion in sheep.

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The inclusion of additional fat in diets for dairy cows in early lactation has been suggested as a means of increasing energy intake and the fat content of milk but fat may have adverse effects on fibre digestion and rumen acetate:propionate; consequently, the response in milk fat is limited (Storry, 1981). Fat supplements designed to avoid these problems are being developed.

Four rumen-cannulated wethers were given four diets at maintenance level. The basal diet consisted of grass nuts (0·7 of diet digestible energy (DE)) and a loose supplement (0·3 of diet DE) based on flaked barley with casein. Fat supplements of (a) formaldehyde-treated casein–tallow, (b) spray-dried tallow–casein emulsion or (c) free fatty acids (FFA; prepared as prills) replaced barley to supply 0·15 of calculated DE, with casein equalized. Neutral-detergent fibre (NDF) digestion was assessed by incubating 5 g of each diet in polyester bags (pore size 43 μm) in the rumen of sheep given the corresponding diet and overall digestion was estimated with reference to Cr_2O_3.

Fat added	Rumen acetate:propionate	Fractional NDF disappearance from bags		Apparent digestibility	
		12 h	48 h	NDF	Fat
None (basal)	4·04	0·34	0·63	0·64	0·37
Formaldehyde-treated	3·73	0·29	0·68	0·61	0·83
Tallow–casein	3·72	0·23	0·64	0·66	0·80
Free fatty acids*	3·88	0·33	0·68	0·68	0·63
SE of mean	0·164	0·018	0·025	0·005	0·028

*Predominantly palmitic and stearic acids.

Supplemental fat had only minor effects on rumen fermentation, indicated by small, non-significant depressions in rumen acetate:propionate, a reduced NDF disappearance ($P<0·05$) after 12 h and no difference in NDF digestibility.

Supplemental fat increased markedly the apparent digestibility of dietary fat. By comparison with the basal diet, digestibilities of the added fats were calculated to be 0·96, 0·92 and 0·71 (±0·026) for formaldehyde-treated, tallow–casein and FFA respectively.

This work was supported by Volac Ltd, Royston, Hertfordshire.

Storry, J. E. (1981). In *Recent Advances in Animal Nutrition—1981*, p. 3 [W. Haresign, editor]. London: Butterworths.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Respiration of liver mitochondria from new-born lambs deficient in cobalamin. By J. E. HESKETH and CHARLOTTE A. MALTIN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* (Introduced by A. K. LOUGH)

Since cobalamin-deficiency in rats has been shown to cause changes in mitochondrial function (Matlib *et al.* 1979) we have studied the respiration of isolated liver mitochondria from new-born lambs of adequate and deficient cobalamin status. This forms part of a collaborative study into the effects of cobalt deficiency on sheep and their lambs (Duncan *et al.* 1981; Fell, 1981). Twelve lambs were studied; two from Blackface ewes given a cobalt-supplemented diet, seven from Blackface ewes given a cobalt-deficient diet and three from Finn Dorset × Suffolk ewes given a standard ration. Lambs born to ewes given the deficient diet showed low plasma cobalamin concentrations (<250 pg/ml) while the others had concentrations of more than 2000 pg/ml. It was only possible to obtain complete analytical data from six animals, as indicated in the Table. Results from the other animals supported these observations. Mitochondria were prepared by a modification of the method of Loewenstein *et al.* (1970). Oxygen consumption was measured polarographically using 20 mM-succinate or glutamate as substrate. The maximum rate of O_2 consumption during the response to ADP and the rate following the phosphorylation of ADP (state 4) were measured and their ratio (respiratory control index, RCI) calculated.

Mitochondrial respiration, plasma cobalamin and birth weight in new-born lambs

(Mean values with their standard deviations)

	Birth wt (kg)		Plasma cobalamin (pg/ml)		State 4 O_2 consumption (n atoms O_2/min per mg protein)				Respiratory control index			
					Glutamate		Succinate		Glutamate		Succinate	
Cobalamin	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adequate (*n* 3)	3·1	1·3	>2000		12·6	1·8	26·0	1·3	2·9	0·2	2·2	0·2
Deficient (*n* 3)	4·1	0·8	190	59	6·3	2·2	28·9	10·8	7·2	2·6	2·3	0·1

State 4 O_2 consumption in the presence of glutamate was lower, and the RCI elevated, in mitochondria from cobalamin-deficient lambs compared to lambs from ewes given a standard ration (see Table). ADP-stimulated O_2 consumption with glutamate as substrate was similar in all groups of animals. There was no difference in mitochondrial respiratory rates when succinate was used as substrate.

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Matlib, M. A., Frenkel, E. P., Mukherjee, A., Henslee, J. & Srere, P. A. (1979). *Arch. Biochem. Biophys.* **197**, 388.

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

The synthesis of cholesteryl esters in the developing chick embryo. By
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The development of the chick embryo is accompanied by a progressive accumulation of esterified cholesterol in the liver (Moore & Doran, 1962; Noble & Moore, 1966). The accumulation is such that in the 19-d-old embryo, cholesteryl esters account for 70–80% of the lipid present and for about 30% of the dry matter. A feature of the cholesteryl ester is its very high content of oleic acid, some 70–75% of the total fatty acids present (Noble & Moore, 1964). The source of these cholesteryl esters is unknown. However, during development, a progressive increase in the cholesteryl oleate content of the yolk has also been observed (Noble & Moore, 1967). The relative abilities of the three major possible sites, namely yolk, yolk-sac membrane and liver, to account for the accumulation of cholesteryl esters within the chick embryo has therefore been investigated at day 15 of development by techniques involving the incorporation of [^{14}C]-cholesterol in vitro.

Although an active acyl-CoA-cholesterol acyltransferase system was present in the liver, the fatty acids that became esterified to cholesterol during incubation were dissimilar from those of the cholesteryl esters present in the liver; for instance, only 45–50% of the fatty acids that became esterified to the cholesterol during incubation were oleic acid. Incubation of the yolk contents failed to demonstrate any significant cholesterol esterification. However, extensive esterification of cholesterol occurred within the yolk-sac membrane during incubation. Furthermore, the fatty acid distributions of the cholesteryl esters synthesized were not only identical to those found in the membrane, but also to those of the liver. Thus oleic acid comprised about 70% of the fatty acids that became esterified to the cholesterol during incubation compared to values of 66% and 77% respectively for the oleic acid contents of the cholesteryl esters that were present in the yolk-sac membrane and liver. Although an unequivocal reason for the synthesis of the cholesteryl oleate cannot be provided, a role in the assembly of a lipoprotein complex for the transfer of lipid from yolk to the embryo seems probable.

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Noble, R. C. & Moore, J. H. (1964). *Can. J. Biochem.* **42**, 1729.
Noble, R. C. & Moore, J. H. (1966). In *Physiology of the Domestic Fowl*, p. 87 [C. Horton-Smith and E. C. Amoroso, editors]. Edinburgh: Oliver and Boyd.
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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Absorption and excretion of L(+) and D(−) lactic acid in pigs following intragastric injection of milk plus racemic lactic acid. By M. J. Sissons, P. D. Cranwell and A. W. Bell, *School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia*

That young pigs can utilize small quantities of D(−) lactic acid has been demonstrated by Christie & Cranwell (1976). To determine the absorption, utilization and excretion of both isomers of lactic acid, blood and urinary lactate concentrations were assayed following intragastric injections of milk plus racemic lactate at concentrations similar to those found in the stomach of suckling pigs reared under conventional conditions (Cranwell *et al.* 1976).

Five Large White, sow-reared pigs, aged 4 to 9 weeks, were prepared with chronic jugular catheters and gastric fistulas. Each animal received, at random, each of the following treatments at 5–10 d intervals: hourly intragastric injections (15 ml/kg $W^{0.75}$) of cows' milk (control) or 250 mM- or 500 mM-racemic lactic acid in cows' milk for 19 h. Blood was sampled at intervals and all urine collected for 28 h from the start of the experiment. Plasma and urine were assayed for L(+) lactate (Lundholm *et al.* 1963) and D(−) lactate (Brandt *et al.* 1980).

Mean plasma D(−) and L(+) lactate concentrations during control injections were 0.15 ± 0.01 mM and 1.47 ± 0.06 mM respectively. Following intragastric injections of 250 mM-lactic acid, plasma D(−) lactate reached a plateau 6-fold above baseline 250 min after the first injection and decreased to baseline levels 240 min after the last injection. Following intragastric injections of 500 mM-lactic acid, plasma D(−) lactate reached a plateau 7·5-fold above baseline 285 min after the first injection and decreased to baseline levels 480 min after the last injection. Plasma L(+) lactate was variable during all treatments and not significantly elevated by either dose of lactic acid. The mean plateau D(−) lactate concentration was 34% greater during 500 mM than during 250 mM injections ($P < 0.025$). Urinary excretion of total lactate, L(+) and D(−), as percentages of the total amounts given were 6.0 ± 0.6, 2.2 ± 0.3 and 10.5 ± 1.1 respectively. The proportions excreted were not significantly affected by dose.

The observations that (i) plasma D(−) lactic acid reaches a stable elevated concentration following repeated intragastric injection of lactate, (ii) the increase in plasma D(−) lactic acid was only 34% greater when the dose was doubled and (iii) only a small proportion of the lactic acid is excreted in the urine suggests that D(−) lactic acid is readily metabolized by tissues of the young pigs but at a slower rate than L(+) lactate; the proportion absorbed may be lower at the higher dose rate.

Brandt, R. B., Siegel, S. A., Waters, M. G. & Bloch, M. H. (1980). *Anal. Biochem.* **102**, 39.
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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

The use of an 'elasticity' measure in regression analysis of the results of metabolism studies with [^{14}C]-labelled D(–) and L(+) lactate in young pigs. By J. J. QUILKEY, P. D. CRANWELL and ANNE CHRISTIE, *School of Agriculture, La Trobe University, Bundoora, Victoria* 3083, *Australia*

Results from experiments in which [^{14}C]-labelled sodium D(–) or L(+) lactate were given to young pigs by intraduodenal injection indicated that the L(+) isomer is more readily utilized than the D(–) isomer (Christie & Cranwell, 1976). It therefore appears that the two isomers may be metabolized by enzyme systems which operate at different rates (Giesecke & Stangassinger, 1980). If this is so and, since carbon dioxide is the major end-product of lactate metabolism, it is likely that the maximum activity in blood CO_2 will occur earlier when [^{14}C]-L(+) lactate is injected than when the D(–) isomer is given.

The results from the experiments of Christie & Cranwell (1976) have been analysed by regression analysis of a log polynomial function (Allen, 1979). The analysis in this form provided estimates of the 'elasticity' of blood CO_2 radioactivity with respect to time. The elasticity measure (E) of the change in blood CO_2 radioactivity with respect to time (t) is defined as:

$$E = \frac{d(\log CO_2)}{d(\log t)}$$

and is not affected by the scale of the variables, thus enabling comparisons both within and between experiments. Estimates of elasticities for the D(–) and L(+) isomers varied with time as shown in the Table.

	Elasticity						
Time after injection (min)...	5	10	20	40	70	100	130
D(–) isomer	1·167	0·816	0·463	0·110	–0·175	–0·357	–0·490
L(+) isomer	0·707	0·376	0·045	–0·286	–0·553	–0·723	–0·848

The results are consistent with the hypothesis that maximum blood CO_2 radioactivity, indicated by a zero value, occurs with the L(+) isomer than with the D(–) isomer. In addition, the subsequent decline in activity is seen to be greater when L(+) lactate is injected.

Allen, R. G. D. (1979). *Mathematical Analysis for Economists,* p. 352. London: MacMillan.
Christie, A. & Cranwell, P. D. (1976). *Proc. Nutr. Soc.* **35**, 27A.
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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Comparison of fatty acid intakes in contrasting socio-economic groups during pregnancy. By WENDY DOYLE, W. R. HARE and M. A. CRAWFORD, *Nuffield Laboratories of Comparative Medicine, Zoological Society of London, Regent's Park, London NW1 4RY*

The purpose of our study was to examine maternal food and fatty acid intakes during pregnancy in the UK with special reference to a poor community. Of 100 women studied, seventy-six attended the principal maternity hospital (The Salvation Army Mothers Hospital; SAMH) in Hackney in the East End of London and were from social groups III, IV and V, and twenty-four were from social groups I and II attending the Royal Free Hospital (RFH) in Hampstead, North-west London: most were non- and none were heavy smokers (>10/d).

Food intakes were measured by weight for 1 week in each trimester. Fatty acid intakes were calculated using information from Paul *et al.* (1980), the Agricultural Research Service (1976) and previous analyses of foods, particularly fats and oils, carried out by our own laboratory.

The fatty acid contents of the different brands of margarine, oils and other compound fats used by the mothers were determined, as were those of gravies from different meat sources, mayonnaises, soups and chips from different fish and chip shops. Forty-eight take-away food shops were visited to ascertain the brand of oil used. The fatty acid contents of made-up dishes to which fat was added (e.g. fried foods, roast, chipped or mashed potatoes, pastries, sauces, casserole dishes) were calculated from information on the raw ingredients and the specific fat used. The fatty acid and energy intakes of the mothers attending the two hospitals and the mean birth weights of their babies are presented in the Table.

Comparison of birth weights (g), daily energy and fatty acid intakes (g) of SAMH and RFH mothers

	SAMH (*n* 71)		RFH (*n* 23)		Significance of difference
	Mean	SD	Mean	SD	*P*
Birth weight	3026	645	3313	344	0·05
MJ	7·1	1·4	8·6	1·6	0·001
Kcal	1689	323	2044	376	0·001
Total fat intake	70·9	14·2	96·1	21·4	0·001
Total saturates	29·6	6·8	39·8	9·4	0·001
Total monoenes	26·8	5·4	36·0	10·1	0·001
Total ω6 fatty acids	7·94	3·45	10·74	3·86	0·002
Linoleic acid (18:2ω6)	7·83	3·35	10·52	3·82	0·002
Arachidonic acid (20:4ω6)	0·101	0·046	0·143	0·048	0·001
Alpha-linolenic acid (18:3ω3)	1·05	0·36	1·35	0·62	0·01
Long chain ω3 fatty acids (20:4ω3; 20:5ω3; 22:5ω3; 22:6ω3)	0·230	0·189	0·262	0·248	>0·1

Agricultural Research Service (1976). *Composition of Foods, Agriculture Handbook no.* 8 (1–7). Washington, DC: United States Department of Agriculture.

Paul, A. A., Southgate, D. A. T. & Russell, J. (1980). First Supplement to *McCance and Widdowson's The Composition of Foods.* London: HMSO.

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Phosphoglyceride levels of linoleic and arachidonic acids in pregnancy.
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Essential fatty acids (EFA) are required for structural lipids and hence for cell membrane development. They are likely to be of special relevance to early development and for the biosynthesis of prostaglandins which are regulators of pregnancy and blood flow.

We have reported the food intakes of poor families in Hackney where there is a high incidence of low birth weight (Doyle *et al.* 1982). Blood was collected from a random sample of these mothers at mid-term and birth; the phosphoglycerides were separated by thin layer chromatography and the fatty acid contents of the phosphoglyceride fractions were determined by gas–liquid chromatography.

The mean birth weight of our sample was 3025 g with 11·8% below 2500 g and 50% of the birth weights at or below 3000 g. The mean daily energy intake of all mothers, over a three week period, was 7·1 MJ (1689 kcal) with only 6% of the mothers meeting the DHSS recommended daily allowance (RDA) in any one trimester. The mean protein intake was 66·9 g/d and was above the RDA in each trimester.

Studies on the maternal and foetal bloods at term have shown that maternal EFA concentrations were significantly lower in those whose babies were born below 2500 g (see Table).

Birth weights (g) and maternal plasma CPG fatty acids (mg/100 mg)

Range in birth weight	*n*	Essential fatty acid (linoleic acid)		Derived prostaglandin precursor (arachidonic acid)	
		Mean	SE	Mean	SE
<2500	7	14·2	1·7	6·87	1·01
2501–2850	11	16·3	2·58	8·97	0·96
2851–3000	10	18·3	1·82	7·42	0·62
3001–3500	13	20·8	0·82	8·73	0·33
3501–5000	9	20·0	0·65	9·23	0·48

CPG, cholinephosphoglyceride, a principal source of EFA in plasma.

We have analysed foetal (cord) blood in different mammals and found that the placentae of different species consistently concentrate the long chain derivatives of the parent EFA in favour of the foetus.

In our study of the Hackney mothers, analysis of the foetal blood at term demonstrated that the maternal deficit of linoleic acid in relation to weight at birth appeared as a deficit of arachidonic acid in foetal blood. In addition, our results show that there was a progressive reduction in the proportion of EFA in the maternal circulation throughout pregnancy and that the reduction was greatest in those mothers who produced babies of low weight at birth from whatever cause.

Doyle, W., Crawford, M. A., Laurance, B. M. & Drury, P. (1982). *Human Nutr.: Appl. Nutr.* **36A**, 95.

https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Fatty acids and sterols in the British diet. By R. Burt, D. H. Buss and R. S. Kirk, *Ministry of Agriculture, Fisheries and Food, Horseferry Road, London SW1P 2AE* and *Laboratory of the Government Chemist, Stamford Street, London SE1 9NQ*

Mortality from coronary heart disease has been related to numerous components of the diet including the amount and type of fat (Department of Health and Social Security, 1974). In Britain, the total amounts of saturated, monounsaturated and polyunsaturated fatty acids and of cholesterol consumed have previously been calculated from the Ministry of Agriculture's national food supply and household purchase statistics. To supplement and amplify this, we have now investigated the contributions of the major food groups to each fatty acid in the diet by analysing total diet samples (Buss & Lindsay, 1978) obtained from eight towns in Britain. Total lipid was extracted and its fatty acid and sterol components analysed by packed and capillary column gas–liquid chromatography. The results are summarized in the Table. Intakes of campesterol, beta-sitosterol, stigmasterol and other plant sterols were 24, 56, 7 and 4 mg/person per d respectively.

Source of fat and its major components as derived from 'total diet' analyses (/person per d)

	Meat, meat products and eggs	Fish	Milk	Fats and dairy products other than milk	Cereals and cereal products	Total
Total fat (g)	21·9	1·5	13·6	46·9	12·2	96·1
Fatty acids (g)						
Saturated	9·4	0·4	8·7	25·5	5·1	49·1
cis-Monounsaturated	8·6	0·5	3·1	11·6	3·3	27·1
trans-Monounsaturated	0·5	—	0·6	1·6	0·5	3·2
All-*cis*-polyunsaturated, ω6 series	1·8	0·3	0·3	4·3	2·6	9·3
All-*cis*-polyunsaturated, ω3 series	0·3	0·2	0·1	0·6	0·2	1·4
Other polyunsaturated (including *cis, trans* isomers)	0·1	—	—	0·5	0·1	0·7
Cholesterol (mg)	174	13	40	101	9	337

Full details of the individual fatty acids and sterols in a wide range of foods will be published separately.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Leucocyte essential fatty acid metabolism in zinc-deficient pregnant rats.

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Zinc-deficient rats have previously been shown to have a defect in both linoleic acid (18:2, *n* 6) desaturation (Cunnane & Wahle, 1981; Huang *et al.* 1982) and in triglyceride turnover of dihomo-gamma-linolenic acid (20:3, *n* 6) to phospholipids (Cunnane & Huang, 1982). We have studied this defect using intact leucocytes from pregnant Zn-deficient rats.

The rats were maintained on a diet containing 10 mg Zn/kg from days 1–15 of pregnancy and then to term on one containing 0·5 mg Zn/kg. Control rats were given a diet containing 40 mg Zn/kg throughout pregnancy. Peripheral blood leucocytes were isolated by dextran sedimentation and centrifugation. The leucocyte pellet was resuspended in TC 199 medium with 20 mM-HEPES buffer and incubated with [1-^{14}C]linoleic acid and [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid for 1 h at 37°. The lipids were then extracted with $CHCl_3$:MeOH and the distribution of radioactivity quantified by neutral and phospholipid thin layer chromatography, and liquid scintillation counting.

Linoleic acid was found mainly in the free fatty acid (FFA) fraction (81%) with lesser amounts in triglycerides (12%) and phospholipids (3%). However, in Zn-deficient rats, 71% of the linoleic acid remained as the FFA (12% less than control, $P<0·05$) and 23% was incorporated into triglycerides (92% more than control, $P<0·01$). Arachidonic acid distribution in control leucocytes was FFAs 27%, triglycerides 66% and phospholipids <3%, but in leucocytes from Zn-deficient rats it was FFAs 17% (37% less than control, $P<0·01$), triglycerides 75% (14% more than control, $P<0·05$), with no changes in the incorporation of arachidonic acid into the phospholipids or other minor fractions.

Thus, intact rat leucocytes accumulated significantly more arachidonic acid than linoleic acid into the triglyceride fraction. This difference was further increased in Zn deficiency along with a significant decrease in the level of both linoleic and arachidonic acids in the FFA fraction. This suggests that, as in in vivo studies (Cunnane & Huang, 1982), the triglyceride and FFA fractions are of importance in the regulation of essential fatty acid metabolism. The relevance of the change in arachidonic acid metabolism in the leucocytes to changes in the synthesis of '2 series' prostaglandins in Zn deficiency remains to be elucidated.

S.C.C. acknowledges a post-doctoral fellowship and grant from the Lalor Foundation, Wilmington, Delaware, USA.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Comparison of leucocyte linoleic acid metabolism in humans and rats.

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Previous studies of essential fatty acid metabolism have indirectly shown that major species differences in the rate of metabolism of linoleic acid may exist (Rivers *et al.* 1975; Stone *et al.* 1979). We have, therefore, used leucocytes to directly assess possible differences in delta 6 desaturation and lipid incorporation of linoleic acid (18:2, *n* 6) in the rat and man.

Peripheral blood leucocytes were isolated from healthy male volunteers and from male Sprague–Dawley rats weighing approximately 250 g. The method employs dextran sedimentation and centrifugation as previously described (Jones *et al.* 1980). The pellet of mixed leucocytes (polymorphonuclear cells and monocytes) was resuspended in TC 199 medium with 20 mM-HEPES buffer and incubated with [1-^{14}C]linoleic acid for 1 h at 37°. The lipids were extracted with $CHCl_3$:MeOH and the fractions separated by thin layer chromatography (TLC). Liquid scintillation counting was used to quantify linoleic acid desaturation. The desaturation studies involved saponification and methylation of the fatty acids which were separated according to the number of double bonds by silver nitrate TLC. The results are given in the Table.

Desaturation and distribution of ^{14}C from linoleate in rat and man

	Rat		Man		Significance of effect
	Mean	SEM	Mean	SEM	
Delta 5 and 6 desaturation products (pmol/h per 10^7 cells)	186	41	28	9	$P<0.01$
Percentage distribution of ^{14}C in					
Free fatty acids	56		4		$P<0.01$
Triglycerides	6		52		$P<0.01$
Phosphoglycerides	25		29		NS

Total desaturase products (delta 6 and 5) accounted for 2·0±0·3% from human males (*n* 4) and 2·6±0·4% from rats (*n* 4) of recovered radioactivity in leucocytes. However, when expressed as activity per cell it appeared that the rat desaturated linoleic acid at a rate which was approximately six times faster.

The high proportion of linoleic acid remaining as the free acid in the leucocyte lipids of rats was associated with the much faster rate of linoleic acid desaturation. This suggests that a key regulatory step in the metabolism of linoleic acid to arachidonic acid in intact cells may be its availability as the free acid. Thus the relatively low desaturation rate in human cells may be determined by the faster rate at which free linoleic acid is removed by incorporation into triglycerides and subsequently into phospholipids.

S.C.C. acknowledges a post-doctoral fellowship and grant from the Lalor Foundation, Wilmington, Delaware, USA.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Prevention of respiratory distress syndrome of premature babies by intra-amnial application of dipalmitoyl-phosphocholine. By L. VON KLITZING and F. KLINK, *Departments of Clinical Research and of Obstetrics and Gynaecology, Medical School of Luebeck, D-2400 Luebeck, West Germany* (Introduced by J. A. MILNE)

The respiratory distress syndrome (RDS) is one of the major problems associated with premature babies. It has been suggested that one reason may be the loss of 'surfactant factor' which reduces surface tension of the alveoli (Quirk *et al.* 1980). It is known that the main component of this 'surfactant factor' is dipalmitoyl-phosphocholine (DPPC) (Marino & Rooney, 1981). In amniotic fluid taken by amnioscentesis a low level of lecithin is considered to indicate a possible RDS (Diedrich *et al.* 1979).

In 'mini-pig' experiments we found labelled DPPC in the lungs of the foetus when this substance was administered into the amniotic fluid 12 h before delivery. The rate of DPPC incorporation into lung tissue was dependent on gestation age as well as on the DPPC level in the amniotic fluid. Intravenous administration of DPPC to the mother had no influence on the foetus because there is a placental barrier to this substance. For all applications DPPC was emulsified in soya-bean oil and mixed with Intralipid (KabiVitrum).

In forty-three imminent deliveries, before the 35th week of gestation, we administered the mixture of phospholipids with a total content of 500 mg DPPC. The lecithin level in amniotic fluid increased from the critical level (<50 µg/ml) to more than 120 µg/ml. The incidence of RDS was reduced drastically and no moderate or severe RDS was found.

In animal experiments with eight female Wistar rats the incorporation of labelled palmitic acid into lecithin in lung and liver was found 24 h after oral dosing. These qualitative results led to further experiments with a special diet of crisp bread enriched with 100–130 mg palmitic acid/24 h, given to a group of fourteen rats. Another group (seventeen rats) received DPPC (60–80 mg/24 h) instead of palmitic acid. After 1 week the concentrations of DPPC in maternal lung and liver increased significantly. In comparison to the control group (nine animals) the increase of DPPC in lung was 170–290% and in liver 30–110% 24 h after dosing. In a corresponding experiment with pregnant rabbits we found an increase of DPPC in amniotic fluid as well as in lung and liver tissues.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Insulin responsiveness of adipocytes of the Zucker rat. By R. L. Hood and D. J. Best, *CSIRO, Division of Food Research, North Ryde, NSW, Australia* and M. L. Trankina and D. C. Beitz, *Department of Animal Science, Iowa State University, Ames, Iowa, USA*

Sensitivity of rat adipocytes to insulin is dependent on cell size (Czech, 1976; Stevens *et al.* 1981). Most studies reporting insulin insensitivity of large adipocytes, however, have compared large cells from old, obese rats with small cells from young, lean rats. We have studied the insulin responsiveness of adipocytes of different sizes, isolated from the epididymal fat pads of either Fa/Fa (lean) or fa/fa (obese) Zucker rats.

Thin slices of epididymal fat pads of 14·5-week-old Zucker rats of the two genotypes were incubated with either 0·5 mM-glucose or 5 mM-palmitate. One-half of the incubations for each substrate contained insulin (0·1 i.u./ml) and the other half contained no added insulin. After 2 h, the reactions were terminated by the addition of osmium tetroxide and the fixed adipocytes were isolated and separated by screening on the basis of diameter (Hood & Thornton, 1980). Rates of substrate uptake were measured in adipocytes of each diameter category. The results are shown in the Table.

Glucose incorporation (fmol/(2 h × adipocyte))

Adipocyte diameter ranges (µm)	Fa/Fa rats				fa/fa rats			
	- insulin		+ insulin		- insulin		+ insulin	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
80–102	17	1	23	2	18	2	23	5
102–125	29	1	36	5	49	11	42	6
125–153	41	3	52	7	58	17	55	6
153–183	50	11	83	10	59	15	80	9
183–202	48	7	162	85	106	15	109	10
202–223	105	28	202	39	143	25	132	13

Incorporation rates of both glucose and palmitate increased with adipocyte size. Insulin had a highly significant ($P<0·01$) stimulatory effect on glucose incorporation/adipocyte of Fa/Fa rats but had no effect on that of fa/fa rats. Larger adipocytes were more responsive to insulin than smaller ones. When insulin was not added to the incubation media, lower rates of glucose uptake were observed in Fa/Fa rats.

Palmitate uptake was not influenced by insulin. Large adipocytes from fa/fa rats, however, incorporated more palmitate than did those from Fa/Fa rats, but the reverse was true for adipocytes that were less than 125 µm in diameter.

In this study of rat adipocytes isolated from a single site, we conclude that responsiveness to insulin is related to cell size and genotype.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press

Fatty acid synthesis in brown adipose tissue of genetically obese (ob/ob) mice. By STEWART W. MERCER and PAUL TRAYHURN, *MRC Dunn Nutrition Laboratory, Milton Road, Cambridge CB4 1XJ*

Recent studies have established that brown adipose tissue (BAT) is an important site of fatty acid synthesis (FAS) in rats and mice. On a high-carbohydrate/low-fat diet the rate of FAS in BAT is dependent on the temperature to which the animals are acclimated, high rates of synthesis being associated with high rates of thermogenesis (Trayhurn, 1979, 1981). In view of the reduced thermogenic activity in BAT of obese (ob/ob) mice (Himms-Hagen & Desautels, 1978; Thurlby & Trayhurn, 1980) we have measured the rate of FAS in interscapular BAT from normal and obese mutant animals and compared the rates with those in other tissues.

FAS was measured in vivo with ^3H_2O, as described previously (Trayhurn, 1981). The mice were maintained at $22 \pm 1°$ and studied before and after the development of hyperphagia, at 26 d and 8 weeks of age respectively. The synthesis rates obtained in BAT are shown in the Table.

Fatty acid synthesis (μg *atoms H incorporated/h*)

(Mean values with their standard errors for seven mice)

Age . . .	26 d				8 weeks			
	Mean (/g)	SE	Mean (/tissue)	SE	Mean (/g)	SE	Mean (/tissue)	SE
Lean	113·1	19·6	7·7	1·1	102·5	18·8	10·2	2·4
Obese	252·8	30·1*	49·0	8·1**	42·2	6·9*	14·8	2·4

Student's *t* test: **P*<0·01, ***P*<0·001.

At 26 d FAS in interscapular BAT was much higher in the obese than the lean mice, both /g and /tissue; this was also true for liver, epididymal white adipose tissue and the carcass. In contrast, at 8 weeks the synthesis rate/g BAT was lower in the obese than the lean mice and there was no significant difference ($P > 0·05$) /tissue, although synthesis in the other tissues of the obese mice remained greatly elevated. Subsequent measurements showed that the increased synthesis rate in BAT of 26-d-old ob/ob mice was not apparent at either 14 d or 35 d of age. It is concluded that there is a transitory hyperlipogenesis in BAT of ob/ob mice, but that after the 5th week of life BAT is the only major lipogenic tissue in the obese mutant which does not exhibit elevated rates of FAS.

S.W.M. holds a research studentship funded by Unilever.

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https://doi.org/10.1079/PNS19830014 Published online by Cambridge University Press