The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundredth Meeting of the Nutrition Society was held in the Medical and Biological Sciences Building, University of Southampton, Bassett Crescent East, Southampton, on Thursday and Friday, 13/14 *July* 1984, *when the following papers were presented:*

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Inhibition by indomethacin of the insulin stimulation of muscle protein synthesis in post-absorptive rats. By P. J. REEDS, S. M. HAY, R. T. GLENNIE and P. J. GARLICK, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent evidence has suggested that changes in prostaglandin metabolism are intimately involved in the short-term control of protein synthesis (Smith *et al.* 1983; Palmer *et al.* 1983) and degradation (Rodemann & Goldberg, 1982) in skeletal muscle. This work has been extended to include the control of muscle protein synthesis by insulin (Reeds & Palmer, 1983). However, these conclusions have been based on experiments with isolated muscles in vitro. Their relevance to regulation in vivo has been tested by studying the ability of an inhibitor of prostaglandin synthesis, indomethacin, to inhibit insulin-stimulated protein synthesis in the intact rat.

Young male rats (94 (SE 2) g) were fasted from 23.00 hours. The following morning, twelve rats were injected intravenously with indomethacin (0·25 mg in 8% ethanol) and twelve rats received vehicle. After 5 min an intravenous infusion of insulin (200 mU/h) was commenced in six rats from each group, the others receiving diluent. The infusion was continued for 30 min, during the last 10 of which muscle protein synthesis was measured with [^{3}H]phenylalanine (Garlick *et al.* 1980).

Insulin infusion lowered blood glucose. It also stimulated protein synthesis in gastrocnemius muscle (no insulin, no indomethacin, 9·48%/d; insulin, no indomethacin 12·6%/d; $P<0{\cdot}005$). The latter effect is similar in magnitude to earlier reports in another strain of rats (Garlick *et al.* 1983). Indomethacin reduced ($P<0{\cdot}005$) protein synthesis in insulin-treated animals to 10·2%/d, a value not significantly different from that of animals given neither insulin nor indomethacin. Indomethacin had no effect on muscle protein synthesis in animals that had not received insulin (indomethacin, no insulin, 9·50%/d).

The presence of indomethacin completely blocks the effect of insulin on protein synthesis in isolated muscles from fasted rabbits (Reeds & Palmer, 1983). Insulin also stimulates the release of $PGF_{2\alpha}$, an arachidonic acid metabolite which in itself stimulates muscle protein synthesis in vitro (Smith *et al.* 1983). We have concluded that activation of arachidonic acid metabolism to, specifically, $PGF_{2\alpha}$ is an important link between the binding of insulin at the plasma membrane and the activation of protein synthesis. The present results, obtained in intact animals of another species, support the conclusion that this mechanism is of general physiological relevance.

We are grateful to Mr I. Grant for insulin and Mr W. S. Mackie for prostaglandin assays.

Garlick, P. J., Fern, M. & Preedy, V. R. (1983). *Biochemical Journal* **210**, 669–675.
Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.
Palmer, R. M., Reeds, P. J., Atkinson, T. & Smith, R. H. (1983). *Biochemical Journal* **214**, 1011–1014.
Reeds, P. J. & Palmer, R. M. (1983). *Biochemical Biophysical Research Communications* **116**, 1084–1090.
Rodemann, H. P. & Goldberg, A. L. (1982). *Journal of Biological Chemistry* **257**, 1632–1638.
Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983). *Biochemical Journal* **214**, 153–161.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Membrane phospholipid fatty acids: relation with (Na^+–K^+)-ATPase in normal human erythrocytes. By MOUSA NUMAN AHMAD and ANTHONY RICHARD LEEDS, *Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill, London W8 7AH*

At the present time, there is general acceptance of the lipid bilayer model for the erythrocyte membrane with the phospholipid classes asymmetrically distributed within it. Fluidity of the phospholipid is dependent upon the nature of the fatty acid pool, which is in turn dependent on lipid nutrition.

The (Na^+-K^+)-ATPase is an important constituent of the cell membrane that provides the enzymic basis for the 'sodium pump' and uses a considerable part of cellular ATP energy to drive it. The active centre of this enzyme is localized at the cytoplasmic site of the erythrocyte membrane and phosphatidyl serine plays a specific role in its functioning. The general trend indicates that (Na^+-K^+)-ATPase activity is sensitive to the fluidity of the membrane phospholipid, but the available evidence is confined to animals (Shinitzky *et al.* 1980).

In an attempt to assess the relation between the status of the Na pump and membrane phospholipid fluidity, (Na^+-K^+)-ATPase activity and phospholipid fatty acid composition were determined in erythrocytes from forty-nine normal healthy individuals (eighteen men and thirty-one women), between 21 and 54 years of age, kept on their normal diets. (Na^+-K^+)-ATPase activity was significantly correlated with the ratio, double bond index: saturated fatty acid (DBI) and the ratio, unsaturated: saturated fatty acids (U/S) (r +0·63, $P<0·001$ and r +0·71, $P<0·001$ respectively), which are taken as indicative of the membrane phospholipid fluidity. Similar correlations emerged between the enzyme activity and the phospholipid content of linoleic acid (18:2ω6) and arachidonic acid (20:4ω6) (r +0·50, $P<0·001$ and (r +0·38, $P<0·01$ respectively). None of the other fatty acids or fatty acid families showed a correlation with (Na^+-K^+)-ATPase activity. In the case of sixteen individuals (eight men and eight women), significant correlations also emerged between (Na^+-K^+)-ATPase, mediated ^{86}Rb-uptake into erythrocytes and indices of phospholipid fluidity (r +0·76, $P<0·001$ for DBI and r +0·70, $P<0·001$ for U/S). In addition to illustrating that the fluidity of the fatty acid chains of the phospholipid may be involved in the control of (Na^+-K^+)-ATPase activity, the results also indicate that some fatty acids, or fatty acid families, may be involved in such control. Furthermore, the results shed light on the possible involvement of dietary lipid in the dynamics of the membrane phospholipid through modulating the membrane fluidity and fatty acid composition and in turn affecting function.

In view of the possibility that membrane fluidity may be abnormal in obesity (Beguinot *et al.* 1983), our findings suggest that the relation between membrane composition and (Na^+-K^+)-ATPase activity in obese subjects merits further study.

Beguinot, F., Mattioli, P., Duilio, C., Formisano, F. & Mancini, M. (1983). *Fourth International Congress on Obesity*, New York, October 1983. pp. 309 (Abstract).
Shinitzky, M., Borochov, H. & Wilbrandt, W. (1980). In *Membrane Transport in Erythrocytes*, pp. 91–102 [U. V. Lassen, H. J. Ussing and J. O. Wieth, editors]. Copenhagen: Munksgaard.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

The effect of food deprivation on adipocyte plasma membranes as detected by two fluorescent membrane probes. By ROBERT C. MUDD and J. I. DAVIES, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

One of the principal functions of adipose tissue is to respond to the cyclic changes that occur in food intake. Many of these responses are mediated by hormones, the actions of which are critically dependent upon the properties of the plasma membrane (Loh & Law, 1980).

It was shown previously that changes in the properties of adipocyte plasma membranes caused by food deprivation can be detected by measuring the fragillity of intact cells as induced by drugs such as propranolol (Mudd *et al.* 1981). The present study was designed to ascertain whether these changes in membrane properties could survive the processes of cell disruption and membrane isolation.

Plasma membranes from the adipose tissues of fed and 48-h fasted rats were isolated by a method involving a self-forming Percoll density gradient centrifugation (Belsham *et al.* 1980). The polarization of diphenylhexatriene located in the plasma membranes was not appreciably influenced by food intake, although prior exposure of the tissue to insulin caused an increase which was greater in membranes derived from fed rats. Membranes obtained from fed and fasted animals were more directly distinguishable by determining the points at which discontinuities were observed in the relation between fluorescence polarization and temperature (22–23° and 18–19° respectively).

The addition of propranolol (500 μM) to membranes stained with anilino-naphthalene sulphonate resulted in an increase in membrane fluorescence. Starvation caused the temperature at which a propranolol-induced discontinuity in fluorescence intensity occurred to increase from 24–25° to 25–26°.

The nature of these changes in membrane properties is not known. They may, however, represent important adaptive responses to food deprivation.

Belsham, G. J., Denton, R. M. & Tanner, M. J. A. (1980). *Biochemical Journal* **192**, 457–467.
Loh, H. H. & Law, D. Y. (1980). *Annual Reviews of Pharmacology and Toxicology* **20**, 201–234.
Mudd, R. C., Grahn, M. F. & Davies, J. I. (1981). *Biochemical Society's Transactions* **9**, 561–562.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

The effect of acute cold exposure on mitochondrial GDP binding in brown adipose tissue of *ob/ob* mice before and after the development of insulin resistance.
By S. W. Mercer and P. Trayhurn, *MRC Dunn Nutrition Laboratory, Milton Road, Cambridge CB4 1XJ*

Acute cold exposure leads to a substantial increase in the thermogenic activity of brown adipose tissue (BAT) in normal mice and rats, due to an 'unmasking' of GDP binding sites on the mitochondrial uncoupling protein. Adult, genetically obese *(ob/ob)* mice have a reduced BAT thermogenesis and show little increase in GDP binding during acute cold exposure (Himms-Hagen & Desautels, 1978), despite a normal sympathetic stimulation (Zaror-Behrens & Himms-Hagen, 1983). However, preweanling *ob/ob* mice show a considerable increase in binding in response to cold stress (Goodbody & Trayhurn, 1982). Thus the ability of *ob/ob* mice to activate the proton conductance pathway of BAT during acute cold exposure would appear to be related to their developmental stage.

In the present study we have investigated the effect of acute cold exposure on BAT thermogenesis in lean and obese *(ob/ob)* mice of the Aston variety at 26 and 35 d of age, following weaning at 21 d on to a high-carbohydrate/low-fat diet. These two ages were chosen since in a previous study pronounced changes in the rates of lipogenesis in BAT of *ob/ob* mice were observed over this period, apparently reflecting the rapid and preferential development of insulin resistance in the tissue during the fifth week of life (Mercer & Trayhurn, 1983).

Male mice were kept at room temperature (22°) or exposed to cold (4°) for 1 h, following which GDP binding was measured in isolated BAT mitochondria (Goodbody & Trayhurn, 1982). At room temperature, both 26- and 35-d-old *ob/ob* mice had lower GDP binding values than lean littermates. Following acute cold exposure the GDP binding of the lean mice increased by 118 and 116 pmol/mg mitochondrial protein (49 and 53%) for 26- and 35-d-old animals respectively. In contrast, the binding in the 35-d-old *ob/ob* mice rose by only 49 pmol/mg mitochondrial protein (30%); thus, by the fifth week of life the defective response of BAT to cold, previously reported in older *ob/ob* mice (Himms-Hagen & Desautels, 1978), was already established. However, in the 26-d-old mutants there was a substantial activation of the proton conductance pathway in the cold, the GDP binding values increasing by 165 pmol/mg mitochondrial protein (115%). Consistent with the GDP binding results, the fall in rectal temperature of the 26-d-old *ob/ob* mice during exposure to 4° was less than in 35-d-old mutants (2·3 (SE 0·3)° *v.* 3·6 (SE 0·4)°; $P<0.05$), despite the smaller size of the younger group.

It is concluded that the ability of *ob/ob* mice to activate the proton conductance pathway of BAT mitochondria in response to acute cold is markedly decreased between days 26 and 35 of age. This coincides with the development of insulin resistance in the tissue (Mercer & Trayhurn, 1983).

Goodbody, A. E. & Trayhurn, P. (1982). *Biochimica et Biophysica Acta* **680**, 119–126.
Himms-Hagen, J. & Desautels, M. (1978). *Biochemical and Biophysical Research Communications* **83**, 628–634.
Mercer, S. W. & Trayhurn, P. (1983). *Biochemical Journal* **212**, 393–398.
Zaror-Behrens, G. & Himms-Hagen, J. (1983). *American Journal of Physiology* **244**, E361–E366.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Influence of dietary eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids on cell-mediated immunity in the mouse. By

T. A. B. SANDERS, MARLENE GRAHAME and M. MISTRY, *Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH*

Dietary linoleic acid (18:2ω6) is necessary for cell-mediated immunity (CMI) but high intakes cause immunosuppression in mice probably by influencing the production of prostaglandins (Mertin & Stackpoole, 1981). Dietary eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids can displace arachidonic acid (20:4ω6) from membrane lipids and can partially inhibit prostaglandin and leukotriene formation from 20:4ω6 (Sanders, 1983). We report the influence on CMI of both acute and chronic administration of 20:5ω3 and 22:6ω3 on CMI in CBA×CBA mice using the popliteal lymph node assay technique of Mertin & Stackpoole (1981).

To study the acute effects, mice (20 g, six per group) were dosed orally with 90 μl oil and after 3 h inoculated with allogeneic and syngeneic spleen cells (5×10^6) in the left and right foot pads respectively; F_1 CBA×B10 mice were used as a source of allogeneic cells. The difference in popliteal lymph node weight, which corresponds to the CMI response, was determined on day 3 following inoculation. Dosage with ethyl linoleate, ethyl eicosapentaenoate and MacEPA oil (180 g 20:5ω3 kg, 120 g 22:6ω3 kg; Seven Seas Health Care, Hull) inhibited the CMI response compared with ethyl oleate (0·1 (SE 0·07), 0·2 (SE 0·17), 0·2 (SE 0·16) mg respectively compared with 1·6 (SE 0·22) mg). To study the long-term effects, mice (eight per group) were fed on semi-synthetic diets containing, as percentage total energy, either 2% 18:2ω6 or 4% 18:2ω6 or 2% 18:2ω6+1·2% 20:5ω3+0·8% 22:6ω3 (provided as MaxEPA) for 4 weeks. Spleen lipids were markedly altered by the 20:5ω3 and 22:6ω3, in particular the proportion of 20:4ω6 was greatly diminished but the CMI response was not inhibited (table).

Dietary fatty acid ...	18:2ω6		18:2ω6		18:2ω6+ 20:5ω3 +22:6ω3	
Percentage energy intake ...	2		4		2 1·2 0·8	
Spleen fatty acid	Mean	SE	Mean	SE	Mean	SE
20:4ω6 (wt %)	17	1·0	19	0·2	6	0·6
20:5ω3 (wt %)	Trace		Trace		5	0·2
22:6ω3 (wt %)	3	0·2	2	0·1	9	0·4
CMI response:						
Change in lymph node weight (mg)	2·0	0·21	2·5	0·25	2·2	0·16

It is suggested that the mode of administration of fatty acids may influence the CMI response.

We are grateful to Dr J. Mertin and Dr A. Stackpoole for their help and advice and to Seven Seas Health Care for a grant.

Mertin, J. & Stackpoole, A. (1981). *Nature* **294**, 456–458.
Sanders, T. A. B. (1983). *Clinical Science* **65**, 343–350.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Electroretinography of rodents reared on diets deficient in essential fatty acids and supplemented with either linoleic acid (18:2*n*6) or linolenic acid (18:3*n*3). By W. M. F. LEAT, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*, R. CURTIS and N. MILLICHAMP, *Unit of Comparative Ophthalmology, Animal Health Trust, Kennett, Newmarket, Suffolk CB8 7PN* and R. W. COX, *Institute of Ophthalmology, 17/25 Cayton Street, London EC1 V 9AT*

Long-chain polyunsaturated fatty acids derived from 18:3*n*3 are found in high concentrations in retinal lipids, and may be involved in the normal physiology of photoreceptors (see Fliesler & Anderson, 1983). To investigate this possibility we have examined the electroretinographs (ERGs) of rats and guinea-pigs reared to a number of generations on diets deficient in 18:3*n*3.

Expt 1. Rats. Rats were reared to three or four generations on an essential fatty acid (EFA)-deficient diet supplemented with either 18:2*n*6 or 18:3*n*3 (Leat & Northrop, 1981) and their ERGs recorded. There were large variations in the ERGs of all rats both within and between groups, but there were no statistical differences between rats fed on 18:2*n*6 (12) and those given 18:3*n*3 (6). In the total lipids of the retinas of rats fed on 18:2*n*6 the content of 22:6*n*3 fell from 17·5% to 8·5% in the first generation but remained relatively unchanged in further generations. The decline in the percentage 22:6*n*3 was compensated by an increase in that of 22:5*n*6. Histologically the retinas of some rats showed extensive atrophy which appeared to be related more to age and possibly exposure to artificial light than to dietary treatment.

Expt 2. Guinea-pigs. Guinea-pigs reared to a third generation on an EFA-deficient diet supplemented with 60 g 18:2*n*6 (as sunflower oil)/kg showed a reduction in the content of 22:6*n*3 in the retina from 7% to less than 1% (Leat *et al.* 1983). The ERGs of these animals (six eyes in three animals), compared with those from guinea-pigs fed on a commercial ration (six eyes from six animals), showed a similar b-wave response at all light intensities but the a-wave response appeared greater at high light intensities. It is concluded that if *n*3 fatty acids are involved in the visual process their role, in the guinea-pig at least, is of either minor importance or of a subtle nature not assessed by the techniques used here.

W. M. F. Leat thanks the Medical Research Council for a grant.

Fliesler, S. J. & Anderson, R. E. (1983). *Progress in Lipid Research* **22**, 79–131.
Leat, W. M. F. & Northrop, C. A. (1981). *Quarterly Journal of Experimental Physiology* **66**, 99–103.
Leat, W. M. F., Northrop, C. A. & Davidson, K. (1983). *Proceedings of the Nutrition Society* **42**, 98A.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Correlation of linoleic acid composition of erythrocyte membrane choline phosphoglyceride fraction with electrophoretic mobility in multiple sclerosis patients following a diet rich in polyunsaturates. By L. S. HARBIGE and M. A. CRAWFORD, *Nuffield Laboratories of Comparative Medicine, Department of Biochemistry and Nutrition, Zoological Society of London, Regent's Park, London NW1* and R. JONES, *Oncology Research Unit, Radiotherapy Centre, Bristol Royal Infirmary, Bristol.*

Multiple sclerosis (MS) subjects have been found to have reduced erythrocyte electrophoretic mobility (EPM) in the presence of linoleic acid (LA) (Jones *et al.* 1983), reduced linoleate levels in plasma and erythrocytes compared with matched controls, and certain abnormalities in the fatty acid (FA) composition of brain tissue (Thompson, 1966).

The aim of this pilot study was to investigate erythrocyte FA composition and EPM of two groups of MS subjects who attended the ARMS Research Unit (Central Middlesex Hospital) before and after dietary intervention (Crawford *et al.* 1979). These subjects were selected on the grounds that they had not changed their diet before joining the programme.

Venous blood samples were taken periodically before and after dietary intervention. The choline phosphoglycerides (CPG) were separated by thin-layer chromatography and the FA content determined by gas–liquid chromatography. Erythrocyte EPMs were determined using glutaraldehyde-fixed erythrocytes and laser cytopherometry (Jones *et al.* 1983).

	Pre-dietary intervention (n 8)		>6 months after dietary intervention (n 8)	
	Mean	SE	Mean	SE
mg LA/g CPG	155	9·6	208	7·0
Erythrocyte EPM	144	2·9	165	2·9

The difference in EPM before and after dietary intervention was significant ($P<0·001$). There was also a significant difference ($P<0·001$) in the percentage of LA in the CPG fraction of erythrocytes. Correlation analysis of selected subjects who showed a wide range of EPM changes and on whom we obtained both erythrocyte EPM and compositional data, showed a significant correlation (r 0·70, $P<0·01$, linear regression line $= -6·931 + 0·164x$) between percentage LA and EPM.

Field & Joyce (1976) have suggested that a reduced erythrocyte EPM is diagnostic of MS. The pilot study reported here suggests that dietary management, when it results in an increase in erythrocyte CPG LA content, corrects the abnormal mobility.

Crawford, M. A., Budowski, P. & Hassam, A. G. (1979). *Proceedings of the Nutrition Society* **38**, 373–389.
Field, E. J. & Joyce, G. (1976). *Lancet* **ii**, 367–368.
Jones, R., Preece, A. W., Luckman, N. P. & Forrester, J. A. (1983). *Physics in Medicine and Biology* **28**, 1145.
Thompson, R. H. S. (1966). *Royal Society of Medicine* **59**, 260–276.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Effect of alteration of dietary fat on erythrocyte electrophoretic mobility in people with multiple sclerosis. By D. C. HEWSON, *ARMS Research Unit, Central Middlesex Hospital, London, NW10*, and R. JONES, *Oncology Research Unit, Radiotherapy Centre, Bristol Royal Infirmary, Bristol*

It has been suggested that a diet low in saturated fat and high in essential fatty acids (EFA) would be beneficial to the management of multiple sclerosis (MS) (Crawford *et al.* 1979). Reports of low levels of EFA in blood of MS patients (Neu, 1983) and results of trials using EFA supplements support this view. Jones *et al.* (1983) have shown that erythrocytes from MS subjects show a reduced mobility in the presence of linoleic acid compared with controls.

The nutrient intake of fourteen subjects, who said they had not altered their diet as a result of MS, was assessed using the 7-d weighed intake method. These subjects were participating in the disease management study at the ARMS Research Unit (Central Middlesex Hospital). After 1 month, subjects were advised on a diet high in EFA and low in saturated fat (Harding & Crawford, 1981). Further food records were completed after 6 and 12 months and the nutrient intakes calculated. Fasted venous blood samples were taken from the subjects and healthy age-matched controls. At least one, and usually two, samples were taken, 4 weeks apart, before subjects were given dietary advice. Further samples were taken from subjects and controls at 3-month intervals over 1 year. All samples were stored and run together under the same conditions.

Changes in fat intake v. *erythrocyte electrophoretic mobility (EPM) over 1 year*

Decrease in saturated fat (g)		Increase in polyunsaturated fatty acids (g)		Increase in linoleic acid (g)		Increase in polyunsaturate: saturated fatty acids		Percentage change in erythrocyte EPM	
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
19·6	2·4	15·8	2·3	15·5	2·0	1·13	0·1	12·4	1·8

All subjects managed to effect and sustain changes in their diets following advice. They reduced their intake of saturated fat with simultaneous increase in EFA (mainly linoleic acid). Within 1 year on the diet, the erythrocyte EPM, for all the subjects, had moved into the normal range.

Assuming that mobility of erythrocytes fixed in glutaraldehyde reflects aspects of membrane conformation and structure, it appears that a reduction in saturated fat with concomitant increase in EFA does have a membrane effect. We believe that EPM could be a useful method of monitoring membrane changes in an easily accessible cell.

Crawford, M. A., Budowski, P. & Hassam, A. G. (1979). *Proceedings of the Nutrition Society* **38**, 373–389.
Harding, J. & Crawford, M. A. (1981). In *Applied Nutrition*, vol. 1, pp. 20–23. [E. C. Bateman, editor]. London: John Libby.
Jones, R., Preece, A. W., Luckman, N. P. & Forrester, J. A. (1983). *Physics in Medicine and Biology* **83**, 1145–1151.
Neu, I. (1983). *Acta Neurologica Scandinavica* **67**, 151–163.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Changes in liver fatty acid composition and total triglycerides induced by ethanol in the Syrian golden hamster. By S. C. CUNNANE, M. S. MANKU and D. F. HORROBIN, *Efamol Research Institute, Kentville, Nova Scotia, Canada, B4N 4H8*

It has been widely reported that when ethanol is given to rats, significant changes in the essential fatty acid (EFA) composition of the liver lipids result. The major change induced by ethanol is an increase in the proportional composition of linoleic acid (18:2*n*6) and a decrease in arachidonic acid (20:4*n*6) which probably results from inhibition by ethanol of the Δ6- and Δ5-desaturases. Because rats exhibit a low preference for ethanol, force-feeding or liquid diets are required to increase ethanol intake to the point at which biochemical effects on fatty acid metabolism are seen. We have therefore sought to identify the effects of ethanol on liver fatty acid composition in the Syrian golden hamster, a species which voluntarily consumes ethanol (<20%) in preference to water alone. Male Syrian golden hamsters (100 g) were given rodent chow and 10% ethanol for 4 weeks followed by 15% ethanol for an additional 4 weeks. Controls were given rodent chow and tap water. Livers were removed and the lipids extracted with chloroform-methanol (2:1, v/v) and separated by thin-layer chromatography. Fatty acids were methylated and analysed by gas–liquid chromatography. Total energy intake was identical in both groups although ethanol was equivalent to 15% of total energy intake in the ethanol-fed group. Intake of essential nutrients was, nevertheless, adequate in the ethanol-fed group, according to published requirements. Liver weight was increased in relation to final body-weight in the ethanol-fed hamsters, a change partly attributable to increased liver triglyceride (15·8 (SD 2·0) *v.* 28·2 (SD 3·0) mg triglyceride/g liver). Fat accumulation in the liver was also evident histologically in the ethanol-fed hamsters. In the liver total phospholipids, levels of 16:1*n*7 and 18:1*n*9 were higher in the ethanol fed-group, as were the EFA desaturase substrates 20:3*n*6, 18:3*n*3 and 22:5*n*3. 18:0, 18:2*n*6 and 20:4*n*6 levels were decreased. In the liver triglycerides, 16:0 and the EFA were lower but 16:1*n*7 and 18:1*n*9 were higher in the ethanol-fed group. Our results are in partial agreement with those obtained by feeding rats ethanol in a liquid diet; 20:3*n*6/20:4*n*6 and 22:5*n*3/22:6*n*3 were increased suggesting an effect of ethanol on these desaturase-dependent conversions in the hamster as in the rat. 18:2*n*6/20:4*n*6 was not increased in liver phospholipids. This is contrary to results from the rat but similar to results from human alcoholics in which both 18:2*n*6 and 20:4*n*6 were decreased in the liver phospholipids from biopsy specimens (Cairns & Peters, 1983). Thus, the fatty acid compositions of the livers of the rat and hamster do not appear to respond equally to ethanol. This may be partly attributable to species differences and partly to the method of feeding the ethanol.

We are grateful for financial support from the National Research Council of Canada.

Cairns, S. R. & Peters, T. J. (1983). *Clinical Science* **65**, 645–652.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Effects of γ-linolenic acid and antioxidant administration on alcoholic fatty liver in the rat. By P. R. Ryle, J. Chakraborty, G. K. Shaw and A. D. Thomson (introduced by S. Cunnane), *Department of Gastroenterology, Greenwich District Hospital, London, and Bexley Hospital, Bexley, Kent*

Chronic ethanol feeding causes changes in the fatty acid composition of hepatic phospholipids with loss of more unsaturated acids, such as arachidonic acid, occurring. These changes may be the consequence of inhibition of microsomal Δ6-desaturase (the ennzyme that converts linoleic acid to γ-linolenic acid, the rate-limiting step in the production of dihomo-γ-linolenic acid, arachidonic acid and the prostaglandins derived from them) by ethanol, or of ethanol-induced lipid peroxidation. Both these effects of ethanol have been suggested as mechanisms by which ethanol causes liver injury. In order to elucidate which mechanism might be important in the pathogenesis of ethanol-induced liver injury (fatty liver), the effects of administering either γ-linolenic acid (as evening primrose oil (Efamol®), which will overcome inhibition of Δ6-desaturase activity) or a synthetic antioxidant (*N,N'*-diphenyl-*p*-phenylenediamine, DPPD) on the induction of fatty liver following chronic ethanol administration has been determined in the rat.

Male Wistar albino rats (220–240 g initially) were allocated to one of four groups (eight per group) representing an ethanol-only group (EC), ethanol with simultaneous administration of Efamol (EE) (1·0 ml/kg per d, intraperitoneally (i.p.)), ethanol with antioxidant administration (EA) (400 mg DPPD/kg per week, i.p.) and a control group (C) which was pair-fed with the EC group. Ethanol was administered as part of a nutritionally adequate liquid diet containing 65, 19 and 16% of the total energy as carbohydrate, fat and protein respectively over a period of 5 weeks. Ethanol was incorporated to a final level of 36% of total energy, replacing isoenergetic carbohydrate. Pair-fed controls (C) received a diet in which the energy from ethanol was replaced by glucose.

Ethanol consumption averaged 12·6 and 12·7 g/kg per d in the EC and EA groups respectively, whereas Efamol administration (EE) significantly lowered ethanol intake to 11·4 g/kg per d ($P<0·01$). Ethanol feeding alone (EC) caused a 102% increase in total hepatic lipid (90·9 *v.* 44·9 mg/g) and a 252% increase in hepatic triglyceride (41·9 *v.* 11·9 mg/g) when compared with the control group (C), antioxidant administration (EA) significantly reducing the ethanol-induced hepatic total lipid and triglyceride accumulation to 24 and 76% respectively ($P<0·001$). Efamol administration (EE) also reduced the total hepatic lipids in ethanol-treated rats (67·9 (EE) *v.* 90·9 (EC) mg/g, $P<0·01$), although this effect was less than that observed with DPPD and was probably due to the lower ethanol intake of the EE group.

In conclusion, it is suggested that impaired Δ6-desaturase activity does not appear to be an important factor in the pathogenesis of alcoholic fatty liver, whereas the antioxidant effect indicates that cellular injury arising from lipid peroxidation may be an important event in this condition.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

How people judge the healthiness of foods. By N. I. McNeil and R. McNeil,
Department of Medicine, The Rayne Institute, University College, London WC1E 6JJ

Nutritional advice for the public aims at the consumption of a healthy diet. The many factors that influence people's perception of what foods are healthy need to be established. A dichotomy may exist between what people consider healthy and what is of real nutritional value.

The perceived health value of new food products was scored on a scale 1–5 as part of a consumer assessment: simultaneously other aspects of the food including taste, texture, smell and appearance were independently scored 0–50. These tests were carried out by regional panels carefully selected to fully represent age and social class.

Over 8 months, 402 manufactured foods were studied with a perceived health value score of 2·84 (SEM 0·02), range 1·67–4·36. A detailed comparison of the lower 10% (forty foods scoring under 2·27) and top 10·7% (forty-three foods scoring 3·5 and over) was made. Those foods considered to be less healthy rated 23·3 (SEM 1·2) on the criteria of taste, smell, etc. whereas the top-scoring group had a mean assessment of 37·0 (SEM 0·9) (t = 9·26, P<0·001). Apparently identical foods were considered healthier if they were more generally acceptable.

The top-scoring group contained many foods heavily promoted by the media as 'healthy', or whose names contained words associated with health. Included were many foods containing sugar, e.g. certain yoghurts, bran and muesli preparations and prepared salads. The low-scoring group contained many cakes and desserts as well as some ethnic foods.

In conclusion, the public perceives the healthiness of foods in a manner strongly modified by the palatability and presentation of the food and poorly related to real nutritional value. Media promotion of generic foods is another major influence. Nutritionists need to be aware how their patients perceive healthiness in foods so that they can then counteract misconceptions when restructuring their patient's diet.

We thank John Cooke of Cambridge Market Research, Haslingfield, Cambridge, for access to documentation.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Contrasting patterns of seasonal weight change within a rural community in the Sahel. By K. HILDERBRAND, A. THIAM and C. FOWLER, *Centre International pour l'Elevage on Afrique, Bamako, Mali* and E. DOWLER and A. TOMKINS, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Analyses of seasonal weight change in West Africa have usually emphasized the weight loss among women and growth faltering among children that occurs in rice- and millet-farming communities during the rainy season. However, an important proportion of rural populations are pastoralists; the impact of seasonality on their nutrition is unknown.

A group of adults and children in Dossou, a semi-arid area of Mali (rainfall less than 200 mm per year) comprising both pastoralists (Fulbe) and millet farmers (Rimaibe) were measured repeatedly throughout 1981 and 1982 (see table) in order to assess the weight change during three major seasons: the rains (May–August), cool dry (September–December) and hot dry (January–April).

Apart from a mild weight loss in the hot dry season there was insignificant seasonal weight change among women, adolescents or older children. This contrasted with the marked seasonal weight changes in men; the Fulbe lost weight during the hot dry season while the Rimaibe lost weight during the rains. Growth of younger children was slowest in the hot dry season with 'catch up' during the rains.

These findings are considerably different from elsewhere in rural West Africa and are discussed in the light of differences in patterns of energy expenditure, dietary habits, food availability and morbidity.

Seasonal changes in nutritional status of pastoralists and millet farmers in Dossou, Mali

Season ...	Cool dry	Hot dry	Rains
Change in body-weight (kg) during season			
Rimaibe men (*n* 21)	+1·7***	0·0NS	−1·7***
Fulbe men (*n* 24)	+2·3***	−2·2***	−0·1NS
Rimaibe women (*n* 15)	+0·4NS	−1·1*	+0·7NS
Fulbe women (*n* 14)	+0·7NS	−1·5**	+0·8NS
Change in body mass index (weight/height^2) during season			
Children 6+ years†			
Rimaibe (*n* 21)	+0·4NS	+0·5NS	+0·9NS
Fulbe (*n* 16)	+0·4NS	+0·2NS	−0·6NS
Weight gain (g/month) during season			
Children age 12–60 months‡	+160*	+104*	+228*

NS, not significant. *P<0·05, **P<0·01, ***P<0·001.
†Adolescents and sexes combined.
‡Sexes and groups combined.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Energy balance and growth of malnourished children with systemic infection. By A. TOMKINS, *Medical Research Council Laboratories, Fajara, nr Banjul, The Gambia* and *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

The energy cost of growth of malnourished children during recovery has been calculated by various methods involving measurement of dietary intake, faecal losses and rates of increase in body-weight. Weight gain may be rapid during intensive rehabilitation and slows during acute infection but the quantitative energy cost of infection is not known (Spady *et al.* 1976).

Children with severe protein–energy malnutrition (marasmus or marasmic–kwashiorkor) who were admitted to a paediatric unit received rehydration, antibiotics and graded introduction of a high-energy formula with solid food during the first week. During the next 7 d all dietary intake was weighed and faecal samples collected for 3–5 d; samples were freeze-dried prior to bomb calorimetry. Body-weight was measured, to within 50 g, daily.

A group (*n* 10) of malnourished children grew rapidly during the second week (mean weight gain 12·6 g/kg body-weight per d). Using the intercept of the regression line to calculate a maintenance value for requirement (410 kJ (98 kcal)/kg per d) the energy cost of growth was calculated as 32·6 kJ (7·8 kcal)/g body-weight gain. A group of malnourished children (*n* 6) was observed in whom intermittent fluctuations in temperature, abnormal chest radiographs and a history of content with tuberculosis was accompanied by poor rates of weight gain (4·6 g/kg body-weight per d). A similar calculation to estimate their energy costs of growth gave a figure of 157 kJ (37·6 kcal)/g body-weight gain, which is at variance with customary values. These children were started on anti-tuberculous chemotherapy and after 7 d the energy balance was repeated. In all cases the rate of growth had increased as pyrexia settled but the calculated energy cost of weight gain was still high at 50 kJ (11·9 kcal)/g body-weight gain.

These results will be discussed with reference to the factors which increase energy requirements especially in undernourished children.

Spady, D. W., Payne, P. R., Picou, D. & Waterlow, J. C. (1976). *American Journal of Clinical Nutrition* **29**, 1073–1078.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Carotene dioxygenase (*EC* 1.13.11.21) activity in vitamin A deficiency in pregnant rats. By LAURENCE VILLARD and C. J. BATES, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

It is not known whether variation in vitamin A status affects the efficiency with which carotene is utilized for vitamin A synthesis. One potential site of metabolic control might be the intestinal enzyme carotene dioxygenase responsible for conversion of dietary carotene to vitamin A. This enzyme was measured by a modification of the method of Goodman *et al.* (1967) in control and vitamin-A-deficient rats both in the non-pregnant and the pregnant state.

Non-pregnant rats, and rats at 7 and 20 d of pregnancy, were killed after 10 weeks on a vitamin-A-restricted diet from weaning. Scrapings of intestinal mucosa were incubated with [^{3}H]β-carotene. Retinol formed by carotene dioxygenase activity was separated and quantitated by scintillation counting. The figures in the table represent the percentage of the total added carotene (13×10^{-5} µmol) which was converted into retinol by 5 mg mucosal protein in 20 min.

	Controls			Deficient		
	Mean	*n*	SEM	Mean	*n*	SEM
Non-pregnant	15·9	7	0·9	19·4	5	3·1
Pregnant 7 d	16·1	4	1·9	23·1	8	2·2
Pregnant 20 d	18·7	10	1·5	25·0*	12	1·8
All animals combined	17·3	21	0·9	23·3***	25	1·3

**P*<0·02, ****P*<0·001.

Thus there was strong evidence of increased enzymic activity in the deficient group as compared with the controls. There appeared to be a greater difference in activity between the non-pregnant and pregnant state in the deficient group. Both groups showed a trend towards higher values as pregnancy progressed.

These observations suggest a possible adaptation to low-carotene intake by increased efficiency of carotene utilization, especially in pregnant animals. Further studies are needed to determine whether the increased enzyme activity in vitro is reflected in increased carotene conversion in vivo.

Goodman, D. S., Huang, H. S., Kanai, M. & Shiratori, T. (1967). *Journal of Biological Chemistry* **242**, 3543–3554.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

The Do-Do pill: potentiation of the thermogenic effects of ephedrine by methylxanthines. By A. G. DULLOO and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, University of London, London W8 7AH*

The ability of the sympathetic stimulant ephedrine to increase metabolic rate and to cause loss of body fat in various animal models of obesity is now well established (Massoudi *et al.* 1983; Dulloo, 1984; Dulloo & Miller, 1984). In this study we have investigated the thermogenic effect of the Do-Do pill; the latter contains a mixture of ephedrine, caffeine and theophylline, and is currently used for the treatment of asthma.

A 4-week energy balance study was conducted on 5–6-month-old female CFLP mice made obese using monosodium glutamate (MSG). All groups (*n* 6) were fed *ad lib* on a powdered stock diet, and the drug(s) were incorporated in the diet in the following dosages (g/kg diet): ephedrine, E (1·0); caffeine, C (1·36) and theophylline, T (2·27). Metabolizable energy intake was monitored continuously and at the end of the experiment all animals were killed and analysed for energy, fat and protein. Heat production over the entire experiment was estimated by the comparative carcass method. The effects of each treatment have been expressed in ratios relative to a no-drug control group, and such ratios are presented in the table.

Treatment	Body-weight	Body fat	Body protein	Food intake	Heat production
C+T	0·98	0·95	1·04	0·99	1·00
E	0·78	0·58	0·94	0·99	1·18
E+C+T	0·67	0·19	0·90	1·11	1·54

The C+T mixture had no effect on energy balance and body composition. In contrast, treatment with E alone caused losses of 22 and 44% in body-weight and body fat respectively, effects brought about mainly by an 18% rise in total heat production. These changes were accentuated when E was administered together with C+T: a 54% rise in heat production caused body-weight and body fat to be reduced by 32 and 80% respectively, while body protein and food intake were not significantly different from those of the no-drug control group. The body composition of the E+C+T (i.e. the Do-Do)-treated group was similar to that of the lean control mice.

These results indicate that when administered in the same dose ratios as in the Do-Do pill, the methylxanthines (caffeine and theophylline) potentiate the thermogenic effects of ephedrine and completely cures the obesity of the MSG-mice.

Dulloo, A. G. (1984). *Journal of Physiology* **349**, 66P.
Dulloo, A. G. & Miller, D. S. (1984). *British Journal of Nutrition* **52**, 179–197.
Massoudi, M., Evans, L. & Miller, D. S. (1983). *Annals of Nutrition and Metabolism* **27**, 26–37.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Effect of guar gum intake on chylomicron size and composition in the rat.

By M. SCHNELL, J. F. PACY and P. A. JUDD, *Department of Nutrition, Queen Elizabeth College, Campden Hill, London W8 7AH*

Guar gum (GG) has been shown to increase the viscosity of small-intestinal contents and give rise to an increase of the unstirred water layer in the rat (Blackburn & Johnson, 1981), thus possibly enhancing the barrier to absorption in the proximal small intestine. It has also been suggested that if fat absorption takes place in the distal small intestine, chylomicron size and composition change (Wu *et al.* 1980) with possible consequences to later fat metabolism. The effect of acute and chronic GG feeding on plasma chylomicron size and composition was therefore compared in rats.

Littermates of the Sprague-Dawley strain (108 rats) were randomly assigned to two groups (mean weight 174 g) and pair-fed for 14 d on isoenergetic diets with (G) or without (C) GG (50 g GG/kg diet). On day 15, after an overnight fast, the rats were tube-fed a test meal with (G_1) or without (C_1) GG. Plasma samples were obtained at 0, 60, 120 and 180 min and the size and composition of chylomicrons measured.

The size distribution of chylomicrons 1 h after the test meal suggested that in animals given GG with the test meal (G_1), chylomicrons were being synthesized in both proximal and distal small intestine compared with proximal only, in animals receiving a control test meal (C_1). There were no significant differences at other time periods.

Chylomicron triglyceride content (mmol/l) is shown in the table.

	Period after tests meal (min)					
	60		120		180	
Group	Mean	SE	Mean	SE	Mean	SE
CC_1	0·37	0·07	0·75	0·12	0·53	0·06
CG_1	0·38	0·04	0·50*	0·14	0·32*	0·06
GC_1	0·68*	0·17	0·91	0·12	0·82*	0·17
GG_1	0·47	0·10	0·67	0·07	0·82*	0·10

*$P<0·05$.

Triglyceride absorption would therefore seem to be delayed by meals containing GG (CG_1 *v.* CC, CC_1 *v.* GG_1) but after chronic feeding of GG, triglyceride absorption was apparently increased (GC_1 *v.* CC_1). The results suggest that acute feeding with GG delays fat absorption in the rat but chronic feeding creates an adaptative response overcoming the effect of any gel in the small intestine.

Blackburn, N. A. & Johnson, I. T. (1981). *British Journal of Nutrition* **46**, 239–245.
Wu, A-I., Bennett-Clarke, S. & Holt, P. R. (1980). *American Journal of Clinical Nutrition* **33**, 582–589.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Whole-body protein turnover in chronic renal failure and the effect of continuous ambulatory peritoneal dialysis. By T. H. J. GOODSHIP, S. LLOYD, M. B. CLAGUE, M. K. WARD and D. N. S. KERR, *Departments of Medicine and Surgery, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne*

In vitro and in vivo studies in uraemic animals suggest a decreased rate of protein turnover. Little work has been performed on whole-body protein turnover in uraemic man.

We have measured whole-body protein turnover in patients with chronic renal failure, before and after starting continuous ambulatory peritoneal dialysis (CAPD), using the method described by Clague *et al.* (1983) which involves a primed constant infusion of [^{14}C]leucine with measurement of plasma [^{14}C]leucine, [^{14}C]ketoleucine, [^{14}C]bicarbonate and total ^{14}C-labelled products. Approval for these studies was obtained from both the local ethical committee and the Isotope Advisory Panel of the DHSS.

Initial experiments used a priming dose of 0·59 that of the constant rate of infusion (CRI). This dose had previously been found to give a satisfactory plateau in surgical patients. However, in our initial experiments we were unable to achieve a satisfactory plateau for the [^{14}C]leucine specific activity. We therefore determined the appropriate priming dose using basic pharmacokinetic principles. Assuming a two-pool model the priming dose that should bring one 'instantly' to plateau is derived from the formula:

$$\text{Priming dose} = (t_{0\cdot5} \times \text{infusion rate})/0\cdot693$$

where $t_{0\cdot5}$ is the half time.

Five CAPD patients and four controls were given an intravenous bolus of approximately 0·3 μmol (15 μCi) [^{14}C]leucine under the same experimental conditions used in the constant infusion experiments. The bolus was given over 2 min and plasma was taken for [^{14}C]leucine estimation at 2, 5, 10, 15, 30, 60, 90, 120, 180 and 240 min. Two exponentials were fitted to the log decay curve for these results and the $t_{0\cdot5}$ for the second exponent derived. For controls this was 55·6 (SEM 2·4) min and for the CAPD patients 76·8 (SEM 3·2) min ($P<0\cdot01$). This gives calculated ratios of priming dose:CRI of 1·34 and 1·85 respectively.

Using these priming doses satisfactory plateaux have been obtained. The results (g/kg per d) for protein turnover studies in nine end-stage renal failure (ESRF) and thirteen CAPD patients are given in the table.

	Turnover		Breakdown		Catabolism		Synthesis		Balance	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ESRF	2·36	0·15	1·61	0·14	0·41	0·04	1·95	0·15	0·34	0·04
CAPD	2·87	0·18	2·12	0·18	0·34	0·02	2·53	0·17	0·41	0·02
	NS		NS		NS		$P<0\cdot05$		NS	

NS, not significant

In conclusion, these results suggest that protein synthesis is increased in patients on CAPD when compared with patients prior to the start of dialysis.

Clague, M. B., Kier, M. J., Wright, P. D. & Johnston, I. D. A. (1983). *Clinical Science* **65**, 165–175.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

The metabolic response to protein deficiency in relation to the concentration of dietary protein. By M. M. Jepson and D. J. Millward, *Nutrition Research Unit, Department of Human Nutrition, London School of Hygiene & Tropical Medicine, 4 St Pancras Way, London NW1 2PE*

Low-protein diets invariably reduce lean tissue growth, but have been reported to either increase or decrease metabolic rate and thyroid hormone status. We have investigated, therefore, the extent to which the response to protein deficiency is variable according to the degree of protein deficiency.

In one experiment, male albino rats (75 g) were fed *ad lib.* on diets containing 200 (control), 90, 50 or 0 g protein/kg for 16 d. Measurements of food intake and oxygen consumption were made throughout, and hormone status (thyroid, insulin and corticosterone) and muscle protein turnover were measured at the beginning and end of the treatment period. Food intakes fell by 12, 45, and 50% in the 90, 50 and 0 g protein/kg diet groups respectively, with a similar pattern of response for oxygen consumption (depressed at 50 and 0 but little changed at 90 g protein/kg diet). Growth rates were reduced on the protein-deficient diets so that after 16 d rats weighed on average 115, 80 and 56 g on the 90, 50 and 0 g protein/kg diet regimens respectively compared with 188 g on the control diet. In the second experiment slightly heavier rats (85 g) were given the 200, 90 and 50 g protein/kg diet and in this case, although there was a similar response to the 50 g protein/kg diet, there was a 25% increase in food intake on the 90 g protein/kg diet and near normal body-weights were achieved (173 g cf 193 g). Oxygen consumption was depressed in the 50 g protein/kg diet group but unchanged in the 90 g protein/kg diet group. In both experiments the reduced muscle growth was caused by reduced protein synthesis which varied with the extent of the elevation of urinary corticosterone and the reduction in plasma insulin which in turn varied with the severity of the protein deficiency and with the food intakes. In the first experiment, protein degradation was reduced to the same extent in all three protein-deficient groups, while in the second experiment the increased intake of the 90 g protein/kg diet resulted in no change in degradation. As previously reported (Cox *et al.* 1981), differential changes in free and total 3,5,3′-triiodothyronine (T_3) occurred since total T_3 was highest in the 0 g protein/kg diet group, but free T_3 levels were reduced in all three protein-deficient groups (i.e. 69, 48 and 54% of control values in the 90, 50 and 0 g protein/kg diet groups in Expt 1 and to 83 and 41% in the 90 and 50 g protein/kg diet groups in Expt 2 respectively). This means that the response to protein deficiency does vary with the severity, very low-protein diets resulting in anorexia and a hypothyroid, hypometabolic state, while marginally protein deficient diets maintain or even increase appetite and food intake so that in the latter case near normal growth can be achieved. However, in this case the relation between energy balance and thyroid status remains problematical since whereas energy expenditure was not reduced and could have been increased, free T_3 was reduced.

Cox, M. D., Dalal, S. S. & Heard, C. R. C. (1981). *Proceedings of the Nutrition Society* **40**, 39A.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

The effects of the synthetic androgen Stanozolol on muscle growth and protein turnover in male and female rats. By P. C. BATES, P. BROADBENT, L. F. CHEW and D. J. MILLWARD, *Nutrition Research Unit, Department of Human Nutrition, London School of Hygiene & Tropical Medicine, 4 St Pancras Way, London NW1 2PE*

Stanozolol, a 17-α-alkyl-testosterone analogue with a high anabolic:androgenic value, has been shown to successfully reverse muscle wasting in the elderly (Gribben & Flavell Matts, 1976) and reduce the catabolic response to surgery (Garden *et al.* 1984).

To investigate its mechanism of action, we have assessed the response of well-fed male and female rats to treatment at varying ages and dose levels, measuring growth and protein synthesis in muscle in vivo. We have also examined the extent to which it is anticatabolic by examining the response to treatment in normal and adrenalectomized fasted rats, and in rats treated with catabolic doses of corticosterone.

In adult male rats treated for 10 or 20 d with 1 mg/kg per d there was no effect on body or muscle growth and protein synthesis, and in a second experiment at this dose with male rats at three ages no response was observed. However, female rats treated at three ages responded with increased body- and muscle weights which reflected higher rates of protein synthesis, at least in the two oldest groups. Treatment of males with higher doses (1, 5 and 10 mg/kg per d) for 11 d at an age which was the most responsive for females (170 g) was also without effect. These results indicate that although the hormone can stimulate muscle growth and protein synthesis in the female rat, healthy male rats do not respond, presumably because their androgenic stimulus is fully expressed.

To determine whether the hormone has an anticatabolic effect such as blocking the inhibitory effect of corticosterone on muscle protein synthesis, the effect of 3-d pretreatment at 5 mg/kg per d on the response of muscle protein synthesis to a 24 h fast in normal and adrenalectomized rats was examined. No effect of the pretreatment was observed in either group. Pretreatment at a higher dose (50 mg/kg in intact rats) also had no effect on the 24 h fast-induced fall in protein synthesis. However, in rats treated with 50 mg/kg corticosterone for 4 d (a dose which stops growth), simultaneous treatment with stanozolol at 50 mg/kg did slightly reduce the catabolic response to the corticosterone. In contrast to the complete cessation of growth in the corticosterone-treated group, there was some growth in the rats given stanozolol, as a result of a smaller reduction in muscle protein synthesis (53% of the control rates) compared with the other group (44% of control group). These results do provide some evidence of a limited anticatabolic effect of stanozolol possibly through an interaction with corticosteroid hormones, although the dose levels required to achieve this are much higher than those which are apparently clinically effective in man (single doses of about 0·7 mg/kg).

Garden, O. J., Blamey, S. L., Shenkin, A. & Carter, D. C. (1984). *Proceedings of the Nutrition Society* **43**, 81A.

Gribbin, H. R. & Flavell Matts, S. G. (1976). *British Journal of Clinical Practice* **30**, 3–9.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Growth stimulation in female rats by inhibition of glucocorticoid production.

By M. N. Sillence, R. L. H. Reford, C. Tyrer, A. P. Wimbush and R. G. Rodway, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

We have previously demonstrated that a reduction in plasma glucocorticoid concentrations and a reduced adrenal response to ACTH are seen in female rats and sheep treated with the anabolic agent trenbolone acetate (Thomas & Rodway, 1982). It has also been shown that high levels of plasma glucocorticoids have catabolic effects on muscle and cause decreased growth rate in young animals (McGrath *et al.* 1981). We have therefore examined the effects of a 3β-hydroxysteroid dehydrogenase blocker, trilostane, which has been shown to reduce glucocorticoid production, on growth rate in young female rats.

Twenty female rats (4–5 weeks old) were fed *ad lib.* and kept on a 12 h light–12 h dark cycle with lights on at 07.00 hours. Animals were paired for growth rate before the start of treatment. Rats were housed individually and one from each matched pair was injected at 15.00 hours with trilostane (65 mg/kg, subcutaneously) suspended in saline (9 g sodium chloride/l). The other received saline only. Rats were weighed daily and on day 10 were killed at 17.00 hours. Plasma corticosterone was assayed using a competitive protein-binding assay, after removal of trilostane using a solid-phase separation method.

Treatment	Growth rate (g/d)		Adrenal (Percentage of body-weight)		Liver (Percentage of body-weight)		Kidney (Percentage of body-weight)		Food conversion efficiency*		Plasma corticosterone (nmol/l)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	2·81	0·13	0·023	0·002	4·85	0·06	0·88	0·01	6·04	0·16	483	97
Trilostane	3·44	0·16	0·036	0·002	5·24	0·10	0·80	0·02	5·13	0·18	384	91
P<	0·01		0·001		0·01		0·01		0·01		NS	

NS, not significant.
*g food intake/g weight gain.

Growth rate was increased by 22% and food conversion efficiency increased by 15%. Plasma corticosterone concentrations were reduced by trilostane, although this did not reach significance. Earlier dose-response trials had indicated that the dose used caused a 72% reduction. Presumably the rats achieved some degree of tolerance after 10 d of treatment.

These results indicated that growth may be held back by physiological concentrations of corticosterone and that inhibition of its production is of potential use in stimulating growth.

Trilostane was kindly donated by Stirling Winthrope Ltd.

McGrath, J. A., Kelley, F. I. & Goldspink, P. F. (1981). *Advances in Physiological Science* **24**, 179–184.
Thomas, K. M. & Rodway, R. G. (1982). *Proceedings of the Nutrition Society* **41**, 138A.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Brown adipose tissue cell sizing—a simple, histological method. By PAULINE M. JONES[1], A. J. NEWTON[2] and MARGARET ASHWELL[1], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and [2]*Department of Histology, Addenbrooke's Hospital, Cambridge CB2 2QQ*

Various methods have been used to study the size of fat cells (Gurr & Kirtland, 1978). Cell diameters can be measured in frozen tissue slices but the method is laborious and no permanent record is obtained without photography. Another common method involves the direct measurement of fat cell diameter of cells isolated by collagenase (*EC* 3.4.24.3) incubation. This is time consuming, not suitable for very large or small cells and again provides no permanent record. A quick, simple method for sizing white adipocytes by comparing the diameters of cells in histological sections with those of isolated cells, and deriving a correction factor to account for factors such as shrinkage in fixed sections has been suggested (Ashwell *et al.* 1976). The purpose of the present study was to devise a similar simple histological method for estimating the size of brown adipocytes.

Interscapular brown adipose tissue (IBAT) was removed from rats (four per group) of various ages and divided into two portions. Method I: adipocyte suspensions were made using the collagenase method of Nedergaard & Lindberg (1982). The diameter of 100 cells was measured using a calibrated occular microscope. Method II: tissue was fixed in Bouin's fixative before being sectioned and stained for reticulin fibres using Gordon & Sweet's (1936) method. Sections were studied using light microscopy and the number of cells in a defined area counted. Cells were only included if more than half their area fell within the defined area. The apparent mean cell diameter was then calculated from the apparent mean cell area (see table).

Age of rats (d)	Body-weight (g)		IBAT weight (mg)		Cell diameter (μm): Method I (collagenase-isolated cells)		Cell diameter (μm): Method II (fixed tissue)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	10·4	0·8	45·6	4·6	24·3	4·8	19·0	4·1
8	11·2	1·3	63·4	8·8	25·6	5·0	20·2	4·9
13	25·2	1·6	74·1	3·8	26·9	5·3	20·8	4·7
34	110·8	11·9	131·5	23·4	29·9	5·2	23·9	5·0
37	113·9	13·0	134·3	10·2	32·4	5·5	25·5	7·0

The agreement between the two methods is excellent (r 0·99, $P<0·001$) over the range of cells studied. The mean cell diameter determined by Method II is slightly smaller than that obtained by Method I. A correction factor of 1·27 is proposed when sizing cells by Method II so that real cell diameter = 1·27 × apparent cell diameter.

Ashwell, M., Priest, P., Bondoux, M., Sowter, C. & McPherson, C. K. (1976). *Journal of Lipid Research* **17**, 190–192.
Gordon, H. & Sweet, H. M. (1936). *American Journal of Pathology* **12**, 545–000.
Gurr, M. I. & Kirtland, J. (1978). *International Journal of Obesity* **2**, 401–427.
Nedergaard, J. & Lindberg, O. (1982). *International Review of Cytology* **74**, 188–210.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press

Fluorescence histochemical demonstration of catecholamines in brown adipose tissue from obese and lean mice acclimated at different temperatures. By MARGARET ASHWELL, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and S. B. DUNNETT, *Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB*

Sympathetic nervous system (SNS) activity in brown adipose tissue (BAT) from genetically obese mice has previously been evaluated by measuring noradrenaline (NA) turnover and has been reported to be lower than the SNS activity of lean mice, at 25° (Knehans & Romsos, 1982; Zaror-Behrens & Himms-Hagen, 1983). NA turnover increases in both types of mice if they are cold-acclimated and decreases in both genotypes if they are warm-acclimated.

Using fluorescent histochemical techniques to demonstrate the presence of catecholamines in nerve fibres, BAT has been shown to have two sympathetic nerve supplies—one directly to the parenchymal cells and another to the arterial blood vessels (Derry *et al.* 1969). The purpose of the present study was to use these techniques to compare SNS activity in BAT from lean and obese mice which had been acclimated at different temperatures.

Genetically obese male mice of the Aston strain *(ob/ob)* and lean counterparts (*ob*/+ or +/+) (aged 4 weeks) were kept at either 4° (lean only), 13°, 23° or 33° for 10 d (*n* 3 in each group). Before killing, the mice were perfused via the ascending aorta following the technique of Lorén *et al.* (1977). Interscapular BAT was then removed and frozen sections were treated according to the method of de la Torré & Surgeon (1976) so that catecholamine-containing nerve fibres could be visualized using fluorescence microscopy.

BAT taken from lean mice acclimated at either 4°, 13° or 23° showed the typical dual pattern of catecholamine-dependent fluorescence around both arterioles and parenchymal cells. However, BAT taken from warm-acclimated (33°) lean mice showed no catecholamine-dependent fluorescence around the cells. On the other hand, BAT taken from obese mice acclimated at 23° or 33° showed virtually no catecholamine-dependent fluorescence around the cells and the dual pattern of catecholamine-dependent fluorescence around arterioles and cells was only seen in obese mice which had been cold-acclimated at 13°.

These results are in accordance with the results from the previously mentioned NA turnover studies since they suggest that the difference in SNS activity between obese and lean mice is best demonstrated at normal environmental temperatures. The histochemical observations allow us to conclude that the reduced SNS activity of the obese mice is probably at the level of parenchymal cell innervation. The reduced SNS activity in BAT of obese mice is likely to be a major factor in their reduced nonshivering thermogenesis and resultant high efficiency of energy storage as previously suggested by other workers.

de la Torré, J. C. & Surgeon, J. (1976). *Histochemistry* **49**, 81–93.

Derry, D. M., Schönbaum, E. & Steiner, G. (1969). *Canadian Journal of Physiology and Pharmacology* **47**, 57–63.

Knehans, A. W. & Romsos, D. K. (1982). *American Journal of Physiology* **242**, E253–E261.

Lorén, I., Björklund, A. & Lindvall, O. (1977). *Histochemistry* **52**, 223–239.

Zaror-Behrens, G. & Himms-Hagen, J. (1983). *American Journal of Physiology* **244**, E361–E366.

https://doi.org/10.1079/PNS19850027 Published online by Cambridge University Press