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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the Clarendon Suites, Birmingham, on Wednesday and Thursday, 1/2 December 1993, when the following papers were presented.

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Correction of acidosis in chronic renal failure improves insulin-mediated glucose utilization but not insulin-mediated changes in protein degradation. By T.H.J. GOODHSIP[1], D. REAICH[1], C. SCRIMGEOUR[2]. [1]Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 4LP and [2]Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN.

The correction of acidosis in patients with chronic renal failure decreases (CRF) protein degradation and amino acid oxidation (Reaich *et al.* 1993). Insulin resistance is a well established feature of both CRF (DeFronzo *et al.* 1981) and acidosis (DeFronzo & Beckles, 1979), and insulin decreases whole body protein degradation (Fukagawa *et al.* 1985). In order to test the hypothesis that acidosis contributes to the insulin resistance of CRF and impairs the action of insulin to decrease protein degradation, eight CRF patients were studied using the combined L-[1-^{13}C]leucine-euglycaemic clamp technique before (pH 7.30) and after (pH 7.37) 4 weeks treatment with $NaHCO_3$. Protein degradation was estimated sequentially from the kinetics of a primed constant infusion of L-[^{13}C]leucine in the basal state and during a hyperinsulinaemic euglycaemic clamp. Insulin sensitivity was measured during the clamp.

The correction of acidosis significantly increased peripheral glucose uptake during hyperinsulinaemia (acid, 6.44 (SE 0.89) *v.* bicarbonate, 7.38 (SE 0.90) mg/kg per min, $P<0.01$) and significantly decreased protein degradation in the basal state (acid, 126.4 (SE 8.1) *v.* bicarbonate, 100.1 (SE 6.9) μmol/kg pr h, $P<0.001$). Hyperinsulinaemia decreased protein degradation in both studies (acid, basal 126.4 (SE 8.1) *v.* clamp 96.5 (SE 7.7), $P<0.001$; bicarbonate, basal 100.1 (SE 6.9) *v.* clamp 88.2 (SE 5.5) μmol/kg per h, $P=0.06$), its effect being unaltered by acidosis, with a reduction of 24 % before and 12 % after the correction of acidosis.

In conclusion: acidosis contributes to the insulin resistance of CRF but does not affect the action of insulin on protein degradation.

DeFronzo, R.A., Alvestrand, A., Smith, D., Hendler, R., Hendler, E. & Wahren, J. (1981). Journal of Clinical Investigation **67**, 563-568.

DeFronzo, R.A. & Beckles, A.D. (1979). American Journal of Physiology **236**, E328-E334.

Fukagawa, N.K., Minaker, K.L., Rowe, J.W., Goodman, M.N., Matthews, D.E., Bier, D.M. & Young, V.R. (1985). Journal of Clinical Investigation **76**, 2306-2311.

Reaich, D., Channon, S.M., Scrimgeour, C.M., Daley, S.E., Wilkinson, R. & Goodship, T.H.J. (1993). American Journal of Physiology **265**, E230-E235.

Nutrient-induced thermogenesis of a complete total parenteral nutrition mixture: relative contributions of glucose and amino acids. By G.L. CARLSON[1,2], P. GRAY[1], J. ARNOLD[2], R.A. LITTLE[2], and M.H. IRVING[1]. [1]Department of Surgery and [2]North Western Injury Research Centre, Hope Hospital, Salford M6 8HD.

The metabolic effects of intravenous glucose and amino acid administration are well documented, but the contributions of each to the metabolic effects of a complete total parental nutrition (TPN) mixture are less clear. The thermogenic, hormonal and metabolic effects of glucose alone (866 kJ/h, n=7), amino acid alone (0.91 gN/h, n-6) and a TPN big bag (904 kJ/h, 0.91 gN/h n=11) have been studied in healthy subjects receiving TPN for intestinal failure. Continuous indirect calorimetry was used to measure respiratory quotient (RQ) and resting energy expenditure (REE) for a 1h fasting baseline, and during 2h of nutrient infusion. Venous blood samples were taken at 15 min intervals for measurement of plasma glucose, lactate, free fatty acid, insulin, glucagon and catecholamine concentrations. Thermic effects were compared using the Mann-Whitney U (MWU) test, and metabolic effects examined with repeated measures ANOVA.

The thermic effect of glucose (% of energy infused) was smaller than that of the big bag, and amino acids alone (median 1.2% v 2.8% and 9.5%, $P<0.03$ and $P<0.05$ respectively, MWU). The thermic effect of the amino acids alone exceeded that of the big bag (9.5% vs 2.8%, $P<0.03$ MWU). The increment in REE associated with the big bag was almost identical to the sum of the increments associated with its components, indicating that glucose and amino acids made similar contributions to the total effect of the mixture.

RQ rose with infusion of glucose alone and with the big bag, but was not affected by amino acid infusion. Glucose and big bag administration resulted in similar increases in plasma glucose and lactate concentration, but no significant changes were observed when amino acids were infused alone. All nutrients led to a fall in free fatty acid concentrations, but the effect of glucose and the big bag was greater than that of amino acids ($P<0.002$, ANOVA).

While all three infusions led to a rise in plasma insulin, the rise in plasma glucagon associated with amino acid administration was negated by the inhibitory effect of glucose in the big bag. No significant changes in plasma catecholamine levels were observed.

The total effect of a TPN mixture is determined by the net effect of the stimulatory and inhibitory actions of its components. Although the thermic effect of glucose is smaller than that of amino acids, both nutrients contribute equally to the thermic effect of a big bag.

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Dual X-ray absorptiometry measurements of fat mass: comparison with direct analysis. By SUSAN A. JEBB, GAIL R. GOLDBERG, G. JENNINGS and M. ELIA. MRC Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2DH

Dual X-ray absorptiometry (DXA) is increasingly being used for the measurement of soft tissue composition, yet there has been little systematic evaluation of its absolute accuracy. We have compared measurements of fat mass, using a Hologic QDR-1000W, with those obtained by direct analysis in a sample of pork meat. In addition we have investigated the effect of changes in tissue thickness on the measured soft tissue composition.

Eighteen boneless shoulders of pork (approximately 3 kg each) were divided into three batches and a series of whole-body scans was performed. Each batch was scanned individually as a single layer (5 cm), then combinations of two layers stacked on top of each other (12 cm), followed by all three layers together (19 cm). The conformation of the meat was then rearranged to produce four (26 cm) and five (33 cm) layers of meat. Scans were performed in triplicate and analysed so that the entire sample was contained within the region corresponding to the trunk, using software package 5.5P. The meat was then minced and homogenized keeping the layers separate. Portions of each layer were removed for direct analysis of fat using the standard petroleum ether extraction following treatment with KOH.. The hydration of portions was also assessed by freeze drying and was found to range from 77.4 - 79.0% for the three layers.

At thicknesses of up to 25 cm there was a significant underestimate of the proportion of fat in the meat measured by DXA compared with the value from chemical analysis, ranging from 5 to 8% of fat as a proportion of weight (see Table).

Batch	Tissue thickness (cm)	% Fat (DXA)		% Fat (direct analysis)	
		Mean	SEM	Mean	SEM
1 †	5	17.4	0.10	22.6 *	0.64
1 + 2 †	12	13.2	0.27	21.1 *	0.69
1 + 2 + 3 ‡	19	13.0	0.11	20.7 *	0.85
1 + 2 + 3 ‡	26	14.7	0.77	20.7 **	0.85
1 + 2 + 3 ‡	33	22.8	0.72	20.7	0.85

† Other individual batches, or combinations, gave similar results.

‡ Arranged in different conformations to produce different tissue thicknesses. The highest thickness is unlikely to be encountered except in extremes of obesity.

Significantly different from the value obtained by DXA: *$P < 0.01$, **$P < 0.001$

Thus as the absolute amount of fat increased the absolute error increased. At thicknesses greater than 25 cm the measured fat increased markedly and at 33 cm the DXA-measured fat mass was greater (+2%) than that measured by direct analysis. This depth is beyond that see in human subjects where the tissue thickness, even across the abdomen, is unlikely to exceed 25 cm. The effect of changes in tissue thickness on the measured fat by DXA are comparable to those previously observed using an experimental tank containing mixtures of oil and water at different depths (Jebb et al. 1992).

At physiological tissue thicknesses this study suggests that there is a substantial underestimate of fat mass measured using the Hologic QDR-1000W. This study raises serious questions regarding the validity of the current algorithms for body composition analysis.

Jebb, S.A., Goldberg, G.R. & Elia, M. (1992). Clin. Nutr. 11, (Suppl.), P145.

Muscle wasting in multiple organ failure measured using ultrasound. By I.T. CAMPBELL[1,2], S. SUKUMAR[3], T. WATT[1,2], D. WITHERS[3], R. ENGLAND[3] and D. MARTIN[3]. [1]Intensive Care Unit, [2]University Dept of Anaesthesia and [3]Dept of Radiology, Withington Hospital, Manchester M20 8LR.

Patients suffering multiple organ failure waste away regardless of nutritional support (Streat et al. 1987, Green et al. 1990, Helliwell et al. 1991) but attempts to document or even identify this in individuals is confounded by abnormal fluid retention (Green et al. 1990). The oedema in MOF is mostly within the subcutaneous tissue and relatively little within muscle (Helliwell et al. 1991). We have previously demonstrated a significant relationship in normal individuals between fat free mass (measured using skinfold thickness) and muscle thickness measured using ultrasound at three sites, biceps, forearm and anterior thigh. The correlation was as good as arm muscle area and arm muscle circumference and better than mid upper arm circumference (MAC; Watt et al. 1993). Muscle thickness was measured at these three sites in patients suffering MOF to determine whether wasting, at these sites, could be identified in individual patients.

Six patients in MOF were studied. One patient with Guillain-Barré syndrome requiring only ventilatory support was also studied. Muscle thickness was measured at the three sites every 1-3 days, using ultrasound (ALOKA SSD 500 Portable Ultrasound Machine with 3.5 MHz linear array transducer) over periods of from 5 to 11 days. In five patients MAC was measured at the same time. Muscle thickness measurements were also made daily in eight normal volunteers for five days.

Rates of change in total muscle thickness were analysed using linear regression analysis. All six patients in MOF showed a steady and significant ($P<0.05$) decline in total muscle thickness over the time they were studied. The rate of decline ranged from 4.3 to 9.3 (median 7.4)% of the initial value per day. The patient with Guillaine-Barré syndrome showed a non significant decline in muscle thickness of 0.9% per day. No patient showed a statistically significant relationship between arm circumference and time. In the controls there was no change in muscle thickness over the five days. Coefficients of variation of repeat measurements in the volunteers were 2.9% for biceps, 2.7% forearm and 2.3% midthigh.

Serial measurements of muscle thickness over the biceps, forearm and anterior aspect of the thigh may represent a means of documenting rates of wasting in patients with MOF.

Green, C.J., Helliwell, T.R., McClelland, P., Gilbertson, A.A., Wilkes, R.G., Bone, J.M. & Campbell, I.T. (1990). Proceedings of the Nutrition Society **49**, 17A.

Helliwell T.R., Coakley J.H., Wagenmakers, A.J.M., Griffiths, R.D., Campbell, I.T., Green, C.J., McClelland, P. & Bone, J.M. (1991). Journal of Pathology **164**, 307-314.

Streat, S.J., Beddoe, A.H. & Hill G.L. (1987). Journal of Trauma **27**, 262-66.

Watt, T., Withers, D., England, R., Sukumar, S., Faragher, B., Martin, D.F. & Campbell, I.T. (1993) Proceedings of the Nutrition Society **52**, 345A.

Recovery of ^{13}C in breath from infused $NaH^{13}CO_3$ increases during euglycaemic hyperinsulinaemia due to a time-dependent effect. By T.H.J. GOODSHIP[1], D. REAICH[1], B. COOPER[1], K.A. GRAHAM[1] and C.M. SCRIMGEOUR[2]. [1]Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 4LP and [2]Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN

Measurement of the rate of production of $^{13}CO_2$ and $^{14}CO_2$ from ^{13}C- and ^{14}C-labelled substrates, especially L-[1-^{13}C]leucine, is widely used to estimate substrate oxidation rate. A correction factor, usually 0.81, is applied in this calculation to account for incomplete recovery in breath of the label (Allsop *et al.* 1978). Although it is known that both length of infusion (Elia *et al.* 1992) and metabolic state (Barstow *et al.* 1990) affect this factor, the same value is commonly used in both periods of the combined L-[1-^{13}C]leucine-euglycaemic clamp technique (Fukagawa *et al.* 1985).

The effect of euglycaemic hyperinsulinaemia (insulin infusion 50 mU/m^2 per min) on the recovery of ^{13}C in expired CO_2 has therefore been assessed in six normal subjects. Each was studied on three occasions; once with a 6 h primed constant infusion of $NaH^{13}CO_3$ combined with a euglycaemic hyperinsulinaemic clamp for the last 3 h (Study 1), once with a 6 h primed constant infusion of $NaH^{13}CO_3$ alone (Study 2) and once with a 6 h infusion of normal saline combined with a hyperinsulinaemic clamp for the last 3 h (Study 3). Measurements of ^{13}C enrichment of expired CO_2 and VCO_2 were made in the third and sixth hour of each infusion. There was no significant increase in enrichment during Study 3 (3 h, 0.00047 (SE 0.00016) *v.* 6 h, 0.00069 (SE 0.00028) atom percent excess) with potato-starch-derived dextrose used to maintain euglycaemia. ^{13}C recovery increased in the sixth hour of both studies 1 and 2 (Study 1: 3 h, 74.3 (SE 2.0) *v.* 6 h, 85.5 (SE 2.6) %, $P < 0.01$; Study 2: $NaH^{13}CO_3$ 3 h, 72.1 (SE 2.4) *v.* 6 h, 81.7 (SE 1.4) %, $P < 0.01$). There was no significant difference in recovery between Studies 1 and 2. These results suggest that increased recovery during a sequential euglycaemic clamp is time dependent. Studies which use this technique to examine the effect of insulin on substrate oxidation should take this into account.

Allsop, J.R., Wolfe, R.R. & Burke, J.F. (1978) Journal of Applied Physiology **45**, 137-139.

Barstow, T.J., Cooper, D.M., Sobel, E.M., Landaw, E.M. & Epstein, S. (1990). American Journal of Physiology **259**, R163-R171.

Elia, M., Fuller, N.J. & Murgatroyd, P.R. (1992). American Journal of Physiology **263**, E676-E687.

Fukagawa, N.K., Minaker, K.L., Rowe, J.W., Goodman, M.N., Matthews, D.E., Bier, D.M. & Young, V.R. (1985). Journal of Clinical Investigation **76**, 2306-2311.

A new tracer method (the labelled bicarbonate-urea method) for estimating energy expenditure in man. By M. ELIA, M.G. JONES, G. JENNINGS., S.D. POPPITT, N.J. FULLER, P.R. MURGATROYD and S.A. JEBB. Dunn Clinical Nutrition Centre, Hills Road, Cambridge, CB2 2DH.

The aim of this study was to validate a new tracer method for estimating energy expenditure. We have shown that a continuous infusion of ^{14}C- bicarbonate without a priming dose is almost entirely recovered as gaseous CO_2 between 12-36 hours of infusion (95.6 (SD 1.1)%, Elia *et al*, 1992). The extent of isotopic dilution of labelled CO_2, which is used to calculate energy expenditure, can be estimated from measurements of acid labile CO_2 present in urine but the technique has limitations because of the marked temporal variation in its urinary excretion, its rapid exchange across the human bladder, and the difficulties of collecting and storing urine under conditions that prevent exchange of label with atmospheric CO_2. In the present study the specific activity of urinary urea, which is formed from CO_2, was used to estimate total CO_2 production during a prolonged subcutaneous infusion of labelled bicarbonate.

Five healthy male subjects (34 (SD 10) years, 71 (SD 5) kg, 1.77 (SD 0.018)m) were studied for 5 days in a whole body indirect calorimeter whilst being infused with alkalinised bicarbonate solution ($\sim$1.5ml/day containing 12.5 μCi ^{14}C-bicarbonate) using a portable minipump which was attached to a belt. Total energy expenditure was manipulated over a range of 1.35-1.75 times basal metabolic rate simulating both sedentary and physically active days. Urine collections to estimate the specific activity of urea were obtained throughout the study. Changes in the size and specific activity of the urea pool were estimated from the change in blood urea concentration and specific activity of urinary urea (which reflects that of blood) in urine samples obtained over 2 hour periods at the end of each day. Calculations of total CO_2 production assumed that 95% of infused label is recovered as gaseous CO_2 and that the specific activity of urinary urea is 85% of that of CO_2 in breath and arterial blood (see Fuller and Elia, 1989).

Continuous recovery of labelled bicarbonate as gaseous CO_2 was 95.6 (SD 1.2)%, and was independent of physical activity. A further 1.4 $\pm$ 0.4% was recovered as urinary urea. CO_2 production, estimated from the urinary measurements and the small adjustments associated with the changes in the size and specific activity of the urea pool, was 100 (SD 5)% of the calorimeter estimate for 1 day periods (20.80 (SD 1.44) moles CO_2/day; calorimeter CO_2 - tracer estimate = 0.07 (SD 1.27) moles/day) for 1 day periods and 100 (SD 2)% for 4 day periods (calorimeter CO_2 - tracer estimate = 0.07 (SD 0.40) moles/day) (the first day was excluded from the calculations to allow equilibration of label with bicarbonate pool). It is concluded that the labelled ^{14}C-bicarbonate-urea method can provide reasonable estimates of total CO_2 production in normal subjects undertaking typical daily activities. It has the advantages over the doubly labelled water method in that it is considerably cheaper and provides estimates of energy expenditure over much shorter periods of time.

Elia, M., Fuller, N.J. & Murgatroyd, P.R. (1992). American Journal of Physiology **263**, E676-E687.
Fuller, N.J. & Elia, M. (1989). Clinical Physiology **9**, 345-352.

Early endogenous mediators of the response to trauma. By G.E. CURTIS, A. WALSH, L. FORMELA and A. SHENKIN. Department of Clinical Chemistry, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XW

Following trauma, various immunological, inflammatory and hormonal changes occur to promote repair and healing, including the acute-phase protein response (APPR). These changes are mediated by a number of endogenous factors, including the cytokines, with interleukin-1ß (IL-1ß) and tumour necrosis factor (TNFα), the early mediators in the cascade to interleukin-6 (IL-6), the major inducer of the APPR. In previous studies from our group neither IL-1ß nor TNFα have been detected in serum. Since cytokines have been reported to release soluble receptors, the aim of the present study was to determine whether the IL-1 and TNF systems had been activated by measuring the levels of soluble TNF receptors and IL-1 receptor antagonist in serum.

Nineteen patients undergoing elective surgery for gastrointestinal malignancies (six gastrectomies, five colectomies and eight pancreatectomies) were studied. Blood samples were taken pre-operatively, peri-operatively at time of incision (0), ½, 1, 2, 4, 6, and 8 h, and post-operatively on days 1, 2, 3, 4 and 5, for the measurement of C-reactive protein (CRP) as a marker of the APPR by immunoturbidimetry, cortisol by RIA (Diagnostic Products Corporation), IL-6 by in-house eluted stain bioassay, IL-1ß and TNFα by ELISA (Medgenix Diagnostics), and IL-6 soluble receptor (IL-6sR), IL-1 receptor antagonist (IL-1ra), and the soluble TNF receptors RI and RII (sTNFR-I and sTNFR-II) by ELISA (British Biotechnology).

	Pre-op level		Peak level		Peak time
	median	range	median	range	(h)
CRP (mg/l)	10	10 - 143	202*	90 - 366	48
Cortisol (nmol/l)	415	210 - 1144	1247*	613 - 2736	8
IL-6 (ng/ml)	0.04	0.01 - 12.60	2.48*	0.29 - 68.80	8
IL-1ß (pg/ml)	9.9	<5 - 20.5	NPD		-
TNFα (pg/ml)	22.5	10.4 - 54.5	NPD		-
IL-6sR (ng/ml)	38.7	17.9 - 51.2	38.8	18.3 - 66.9	-
IL-1ra (ng/ml)	0.45	0.19 - 1.43	23.23*	2.45 - 120.0	6
sTNFR-I (ng/ml)	2.01	0.81 - 3.77	3.60*	1.53 - 8.13	4
sTNFR-II (ng/ml)	3.58	1.69 - 12.96	6.31*	2.96 - 19.93	6-8

* significantly different from the pre-op levels (Mann-Whitney U Test): $p < 0.05$
NPD, no peak detected.

Significant increases were seen post-operatively in serum CRP, cortisol and IL-6, with the IL-6 peak preceding the CRP peak. No rise in IL-1ß or TNFα was observed throughout the time period of study. This may be explained by the local transient release and rapid proteolytic degradation of cytokines or the presence of circulating soluble receptors. However the IL-1ra and sTNFR-I and sTNFR-II did rise significantly within 6 to 8 h reflecting an earlier release of the corresponding cytokines. Hence the IL-1 and TNF systems were activated and preceded the IL-6 release.

Effect of protein malnutrition on food intake, protein distribution, and acute-phase protein response in the rat. By G. JENNINGS and M. ELIA. Dunn Nutrition Unit, Downham's Lane, Milton Road, Cambridge CB4 1XJ

Although catabolic states cause a negative N balance and loss of muscle mass, the gross changes that occur in other tissues are less well documented particularly in malnourished animals. The present study aimed to assess the effects of 'injury' in normal and protein-deficient animals on protein distribution between various tissues, and the associated changes in food intake and acute-phase protein response.

Normal animals fed on a 200g/kg protein diet (ad lib.) and protein-depleted animals (fed on a 30g/kg protein diet ad lib for 2 weeks) were injected subcutaneously with turpentine (5 ml/kg body weight which produces aseptic abscesses) at 42 d of age and killed 2 d later for measurement of weight and protein content of organs (N x 6.25; N measured using a Carlo Erba Nitrogen analyser 1500) and circulating α_2-macroglobulin (a major acute-phase protein in the rat measured by radial immunodiffusion). Animals pair-fed to maintain a similar intake as the animals with aseptic abscesses were injected with saline (9g NaCl/l; n 6 in each group).

In the normally-nourished rats injected with turpentine, the combined protein content per kg body weight of the liver, heart, kidneys and lungs showed a 33.3 % increase ($P<0.0001$) relative to the pair-fed animals (31.8 % liver, $P<0.0001$; 32.6 % kidneys, $P<0.001$; 49.8 % lungs, $P<0.02$; and 30.2 % heart, $P<0.05$). There were also significant increases compared with the animals fed ad lib. (19.2 % combined organs, $P<0.001$; 16.6 % liver, $P0.001$; and 23.3 % kidney, $P<0.005$). The changes in total tissue protein showed a similar pattern of change as did the changes in organ weight. The changes induced by turpentine in the protein-deficient animals were attenuated (only 11.9 % for the protein content of the combined organs, $P<0.001$ compared with the pair-fed animals, and no significant increase for the lungs and heart). Furthermore, in the protein-deficient rats there was only a 0.5 % reduction in food intake during the 48 h following turpentine injection (compared with a 41 % reduction in the normally-nourished animals) and the acute-phase protein response was attenuated (α_2-macroglobulin concentration 1.2 (SD 0.28) v. 4.85 (SD 0.55) g/l, $P<0.0001$).

Turpentine also caused a decrease in the weight and protein content of the small and large intestine of normally-nourished rats, but this effect could be reproduced by pair-feeding. Neither pair-feeding nor turpentine injection produced a significant change in protein content or weight of the small and large intestines of protein-deficient animals.

It is concluded that (a) the overall 'catabolic' response in the turpentine model of injury is associated with an 'anabolic' response in the major abdominal and thoracic organs, but not in the intestinal tract, and (b) protein deficiency abolishes or attenuates many of the acute-phase responses including anorexia, acute-phase protein response, and changes in protein distribution between various tissues.

The effect of dietary glutamine on the structure of the gastrointestinal mucosa of rats undergoing an acute-phase response to tissue injury. By M. WUSTEMAN[1], H. TATE[1], L. WEAVER[1], G NEALE[2] and M. ELIA[1]. [1]MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and [2]Department of Gastroenterology, Addenbrooke's Hospital, Cambridge CB2 2QQ

In a previous study we reported that the change in mucosal mass and N content that follows systemic aseptic injury in rats ingesting a diet with or without glutamine diet is due to reduce food intake (Wusteman et al, 1993a). The aim of the present study was to examine the possibility that, while changes in mucosal structure occur as a consequence of the systemic injury itself, they are either undetectable as changes in wet weight and N content or occur beyond the proximal tercile from where the initial samples were taken.

Forty rats (43-44 d old) were fed on either a glutamine-free diet (OG; n 20) or one in which 50% of the non-essential amino N was replaced by glutamine (GLN; n 20). Each diet was fed for 4 d after which half the rats on each diet underwent a series of three subcutaneous injections of turpentine (each 2 ml/kg body weight) to generate an acute-phase response (Wusteman et al, 1993b). The other ten rats were pair-fed with the injected group (PF). At 48 h after the last injection (6 d after the first) each rat was killed, the small intestine was removed and its length measured. A 10 mm section from the mid-point of each of the first (Q1), third (Q3) and fifth (Q5) quintiles was taken and stored in Clarke' s solution until microdissection for morphometry. The colon was also removed, its length and weight were measured and a 10 mm section was taken from the proximal end for histological examination.

Villus height (VH; μm) was unaffected by the presence of tissue injury in comparison with the PF group (ANOVA, $P>0.05$) but was significantly lower in the OG rats compared with the GLN group ($P<0.001$), (e.g. mean (SEM) in Q1 of PF rats, OG 736 (9.8), GLN 856 (14); $P<0.01$. Crypt depth (CD) was also unaffected by tissue injury compared with pair-feeding, but was significantly smaller in the OG rats than in the GLN group ($P<0.001$), (e.g. CD mean (SEM), Q3 of PF rats, OG 126 (3.71), GLN 145 (1.4). CD was unaffected by diet in Q5 ($P>0.05$) and VH was only increased by the GLN diet in Q5 in the rats that had been treated with turpentine ($P<0.001$). Finally, colon wet weight (mg/cm), mucosal thickness and gross histological appearance were unaffected by diet whilst its wet weight was only slightly affected by injury (inj) (GLN, Inj, 55.2 (SEM 1.9), PF 50.0 (SEM 1.0); $P<0.05$).

In conclusion, using VH and CD as indices of changes in mucosal structure, we have confirmed our earlier conclusions, made on the basis of measurement of mucosal weight and N content, that mucosal structure along the length of the small intestine is unaffected by the systemic response to tissue injury. However, in contrast to the previous study, we have shown elements of mucosal structure that are responsive to the glutamine content of the diet. No evidence was found for gross changes in the structure of the colonic mucosa in response to either systemic injury or the supply of enteral glutamine.

Wusteman, M., Jordan, N., Weaver, L., Austin, S. & Elia, M. (1993a). Proceedings of the Nutrition Society (in the press).

Wusteman, M., Hayes, A., Stirling, D. & Elia, M. (1993b) Journal of Surgical Research (in press)

N-acetyl cysteine, glutamine and arginine may be essential to the ability of InmunoNutril[R] to enhance antioxidant defences in rats challenged with endotoxin.

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Malnutrition can impair immune function, deplete antioxidant defences and delay recovery after surgery. Parenteral and enteral nutritional preparations bring about clinical benefit in malnourished patients. The optimal composition for such preparations has been the subject of considerable research. Arginine, glutamine, sulphur amino acids and *n*-3 polyunsaturated fatty acids have been identified as potentially beneficial nutrients. InmunoNutril (g/kg: protein 145.6, arginine 41, glutamine 17.9, *n*-acetyl cysteine (NAC) 1.6, RNA 4.1 and 17.5 MJ/kg) is a compound enteral feed , based on milk proteins, containing these nutrients. In addition the feed contains NAC to act as an additional source of sulphur amino acids.

We examined the benefits of including glutamine, arginine and NAC in the mixture on the response of a protein-depleted rat model given an inflammatory challenge of Escherichin Coli endotoxin (400 ug/kg given intraperitoneally). After a period of 7 d consuming a diet containing 80 g casein/kg weanling rats were fed for a further 7 d on InmunoNutril (group E) or a modification in which glutamine, arginine and NAC had been replaced on an isonitrogenous basis by alanine, proline and aspartic acid (group D). Additional groups of rats were fed on a diet containing 200 g casein/kg for 7 (group B) and 14 d (group C) and an 80 g casein/kg diet for 7 d (group A). Half of each dietary group were given an endotoxin injection (endo) and half were given saline (sal). The latter group was pair-fed, for a 24 h post-injection period, the intake of their partners who had received endotoxin. Animals were killed 24 h after injection and serum albumin, and liver and lung reduced glutathione (GSH) levels were assayed.

Group		A	B	C	D	E	SEM
Liver GSH	sal	2.9^{a}	2.3^{a}	5.4^{b}	3.4^{a}	4.6^{b}	0.8
(μmol/g)	endo	2.9^{a}	4.9^{b*}	4.0^{a}	3.2^{a}	3.2^{a*}	0.8
Lung GSH	sal	1.2^{a}	1.1^{a}	1.0^{a}	1.2^{b}	1.0^{a}	0.3
(μmol/g)	endo	1.4^{a}	1.3^{ab}	2.0^{a*}	1.0^{b}	2.0^{ab*}	0.3
Serum Albumin	sal	27.3^{a}	30.4^{b}	33.6^{c}	32.7^{bc}	34.2^{c}	1.9
(mg/ml)	endo	24.1^{a*}	28.1^{b}	32.4^{c}	28.7^{b*}	27.5^{abc*}	1.9

Values are means with pooled SEM (5 rats/group). Values on the same line with different superscripts were significantly different (by ANOVA); $P<0.05$. Significantly different from endotoxin-injected rats (Wilcoxons rank mean square test); $*P<0.05$.

The 8% casein diet (group A) resulted in a lowering of serum albumin and liver GSH concentrations. These animals were unable to increase lung GSH concentrations when challenged with endotoxin. Either formulation of Inmunonutril permitted normalisation of serum albumin concentrations in animals given saline (group D & E). However, inclusion of NAC, glutamine and arginine in InmunoNutril permitted higher liver GSH concentrations in these animals and an enhancement of lung GSH concentration when they were injected with endotoxin (group E). Further studies are needed to identify which of the trio of amino acids is critical in the ability of InmunoNutril to enhance antioxidant defences in the presence of an inflammatory challenge.

The effects of tuberculosis and undernutrition on protein turnover and the response to nutrition.

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Chronic infections such as tuberculosis (TB) are associated with extensive protein wasting. In acute infections and in Human Immunodeficiency Virus (HIV) infection such protein loss is associated with accelerated rates of protein synthesis and degradation. However, nutritional state is also known to influence protein turnover. Although in the short term malnutrition may reduce turnover, recent reports of studies in chronic energy deficiency have shown increased protein turnover (Soares *et al.* 1991).

The present study set out to investigate whole body protein synthesis and degradation in patients with TB (before or within 2 days of starting treatment) and in normal control (C; body mass index (BMI) $\geq$ 20) and chronically undernourished (UN; BMI < 19) individuals in Bangalore, South India.

Whole-body protein metabolism was assessed by primed constant infusion of L-[1-^{13}C]leucine over an 8 h period, as described previously (Melville *et al.* 1989). For the first 4 h subjects remained fasting, and for the second 4 h they were fed. Results, expressed in μmol leucine/h per kg lean body mass (LBM; derived from skinfold measurement), are shown in the Table.

			BMI		Synthesis		Degradation		Balance	
			kg/m^2		μmol leucine /hr per kg LBM					
		n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	Fasted	7	22.5	1.52	105	13.1	125	10.7	-20.5	4.8
	Fed				118	17.3	83 §	12.7	+35.2	6.8
UN	Fasted	6	17.5	0.89	122 †	15.1	145 *	14.6	-23.1	3.6
	Fed				120	14.3	95 §	12.6	+25.6†	10.5
TB	Fasted	7	17.7	2.50	110	11.0	136 †	9.0	-25.5	8.8
	Fed				106	18.4	85 §	11.8	+20.8**	9.1

Significantly different from controls: * P< 0.05, ** P< 0.005, † 0.05<P<0.07.
Significantly different from fasted: § P<0.005 (not shown for balance).

In the fasting state whole-body protein degradation in the undernourished group was 16 % higher than in normal controls. There was also an elevation of degradation in tuberculosis (8 % above control; not significant at 0.05) which was considerably less than that seen in subjects with HIV infection (+25 %, Macallan *et al.* 1992). In response to feeding there was a reduction in protein degradation in all groups but no significant increase in synthesis. However, the change in protein balance on feeding was impaired in patients with tuberculosis and this phenomenon may contribute to protein wasting in chronic infection.

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The effect of parenteral nutrition on neutrophil adhesion molecule expression. By S. M. PLUSA, R. HORSMAN, J. N. PRIMROSE and N. WEBSTER. Nutrition Team, Academic Unit of Surgery, St James's University Hospital, Leeds LS9 7TF

Intravenous nutrition (IVN) has been shown to impair neutrophil oxidative activity and chemotaxis which may increase the susceptibility to bacterial infection. In order to permit transmigration of neutrophils out of the circulation into the site of infection it is essential for adhesion to occur between the neutrophils and the vascular endothelium. This adhesion is mediated by receptors on the neutrophils and endothelial cells, which as components of the cell membrane may be particularly sensitive to lipid emulsions.

We have therefore examined the expression of the adhesion molecules L-selectin and the β-2 integrin CD11b in ten patients (seven male; median age 56 years, range 27-84 years) before and after 24 h of IVN. Both adhesion molecules are sensitive to neutrophil activation, the level of CD11b is increased and that of L-selectin is decreased on stimulation. Patients received approximately 125 kJ/kg and 0.2 gN/kg per d. Lipid provided 55% of energy (Intralipid 20%; Kabi Pharmacia) and was infused as an all-in-one mixture over 24 h.

Whole blood samples were taken before and after 24 h of IVN and incubated with fluorescein-conjugated monoclonal antibodies to CD11b and L-selectin. The samples were then incubated with the bacterial wall peptide and neutrophil stimulant fMLP at 37° and portions were withdrawn at 5, 15 and 30 min and incubated with anti-CD11b. The expression of the adhesion molecules on the cell surface was then determined by flow cytometry and expressed as median channel fluorescence (MCF).

There was no change in the basal expression of L-selectin (pre-IVN MCF 49, (interquartile range 31-78), post-IVN 46,(interquartile range 21-73)), nor was there any significant alteration in the basal expression of CD11b or the response to stimulation. The results are tabulated below.

Sampling time (min)	CD11b Pre -IVN + fMLP		CD11b Post-IVN + fMLP	
	MCF	Inter-quartile range	MCF	Inter-quartile range
0	62	37-117	52	39-71
5	281	206-428	280	168-344
15	337	267-471	322	214-406
30	337	266-470	322	231-395

Thus, 24 h of TPN has no effect on the basal expression of L-selectin or CD11b nor on the expression of CD11b in response to neutrophil stimulation.

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Paediatric referrals for combined small bowel and liver transplantation: timing and indications; Room for Improvement?

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Small bowel transplantation has been attempted since the 1950s, but one-year survival rates of 70% have only recently been achieved (Tzakis et al. 1992). Suppressing rejection of the gut, essentially a lymphoid organ, remains a major problem associated with considerable morbidity, even with the availability of the new immunosuppressant FK506. Since the quality of life of adults and children on total parenteral nutrition is often acceptable, the timing of transplantation is difficult.

Thirteen children (seven male) were evaluated for combined small bowel and liver transplantation (CSBLTx) between August 1989 and August 1993. The indications for evaluation of CSBLTx were liver failure (*n* 11), or end-stage venous access (*n* 2). Nine had short gut syndrome secondary to either gastroschisis (*n* 5), necrotizing enterocolitis (*n* 2), multiple small bowel atresias (*n* 1) or intestinal volvulus (*n* 1). Four had intestinal failure secondary to either microvillus atrophy (*n* 1), hollow visceral myopathy (*n* 1), autoimmune enteropathy (*n* 1) or idiopathic diarrhoea of infancy (*n* 1).

Results of clinical evaluation at referral are shown in the Table.

Diagnosis	age (months) Median	Range	Wt (kg) Median	Range	Hepatic cirrhosis (%)	Bilirubin (μmol/L) Median	Range	Spleno-megaly (%)	Outcome
Short gut (*n* 9)	10	4-29	7.0	4-12	88	240	12-629	100	7 unfit (5 died) 1 awaiting CSBLTx 1 received CSBLTx
Intestinal failure (*n* 4)	30	6-46	11.3	6-14	75	301	10-760	100	3 unfit (all died) 1 awaiting CSBLTx

CSBLTx was contraindicated for ten out of thirteen children, who were unfit because of either small size (<6kg *n* 3), advanced liver disease (bilirubin >400 μmol/L; *n* 6) or fulminant fungal sepsis (*n* 1). The development of portal hypertension as indicated by splenomegaly, occurred in all of the children before the rise in bilirubin and other signs of hepatic decompensation (i.e. before referral). In view of the long waiting time for donors, early referral before hepatic decompensation and loss of adequate venous access is essential.

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Colonic secretion induced by enteral feeding in man is reversed by short-chain fatty acids.

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Short-chain fatty acids (SCFA) stimulate salt and water absorption in the colon. Previous in vivo perfusion studies in normal subjects have demonstrated a secretion of salt and water in the ascending colon in response to enteral feeding (Bowling et al. 1993a), which may be relevant to the pathogenesis of enteral feeding related diarrhoea. The aim of the present study was to investigate the influence of SCFA on this secretory effect.

Six healthy volunteers underwent a colonic perfusion study, using an established technique (Bowling et al. 1993b) which measures colonic in-flow volumes as well as water and electrolyte movement in the ascending and distal colon by sampling both at the hepatic flexure and from the rectal effluent. Once a steady state had been achieved after 2 h perfusion of an isotonic electrolyte solution directly into the caecum, the study consisted of three stages. Stage 1 established baseline fasting colonic water and electrolyte movement. In stage 2 samples were taken every 20 min for 3 h during the nasogastric infusion of a standard polymeric enteral diet (1.4 ml/min; 5.8 kJ/min; 8.75 mgN/min). In stage 3 similar samples were taken during diet infusion, but the electrolyte solution infused into the caecum, instead of containing mannitol to achieve isotonicity as in stages 1 and 2, contained SCFA in physiological concentrations (acetate 50 mmol/l, propionate 20 mmol/l, butyrate 20 mmol/l). The electrolyte concentrations and osmolality of these two solutions were identical.

	Water movement (ml/min) *		
	Stage 1	Stage 2	Stage 3
Ascending colon	$+1.1^{a}$	$-1.0^{a,b}$	$+1.6^{b}$
Distal colon	$+1.3^{c}$	$+3.7^{c}$	+2.8

^{a,b,c} Values with different superscript letters are significantly different: $p < 0.05$.

+, net absorption; -, net secretion.

* Median values

Na, Cl and K movement were similar to that of water throughout the study, but HCO_3 was secreted during the SCFA infusion.

Infusing SCFA directly into the caecum reversed the fluid secretion seen in the ascending colon during enteral feeding. This could have implications in the management of enteral feeding related diarrhoea.

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The effect of the first course of chemotherapy on dietary intake and urinary urea nitrogen in acute myeloid leukaemia. By H.V. FAWCETT[1], A OWENS[1], B COYLE[1], J DEEKS[2], A C NEWLAND[3] AND J POWELL-TUCK[1]. [1]Department of Human Nutrition. [2]Departments of Epidemiology and Statistics and [3]Haematology, The London Hospital Medical College E1 1BB.

Six newly-diagnosed patients with acute myeloid leukaemia and a normal Quetelet index have been studied for 21-24 d following their first course of chemotherapy with food diaries and continuing 24 h urine collections. There was a statistically significant ($P < 0.01$) mean weight loss of 6.0 (SD 4.5) kg during the admission. In all, dietary N intake was substantially reduced from the mean 13.6 (SEM 3.9) g/24 h on admission to 5.5 (SEM 1.0) g soon after the onset of chemotherapy ($P < 0.00001$). Urinary urea N excretion (urease) rose markedly from about 4-6 d post-chemotherapy to a mean over the first 10 d of 17.0 (SEM 3.8) g. Mean (SEM) N balance, taking urinary urea to be the only output, was -11.5 (3.6), -6.34 (2.4), -3.54 (1.6) g N in the 10 d chemotherapy period and the two following weeks respectively. The balance between N intake and urinary urea N was examined in relation to three predictors: temperature, N intake and time period. Temperature (a result of disease activity and complicating infections) had little effect. Increasing N intake increased balance ($P < 0.00001$) and balance was enhanced by 2.8 g N week following chemotherapy ($P < 0.00001$). In a simple analysis of variance of urinary urea N output compared with N intake in the three time periods, there was a significant ($P < 0.0001$) trend for N output to decrease in the 2 weeks following chemotherapy (by about 2.7 g N/week). The effect of N intake was not significant. In a more complicated analysis of variance we examined how N output was predicated by time trend after chemotherapy, body temperature and N intake. Time was again highly statistically significant ($P < 0.00001$). In conclusion, large N losses during the first admission for chemotherapy are associated with both poor nutritional intake and high N losses as urinary urea. Chemotherapy appears to be the likely principal cause.

A comparison of two types of peripheral intravenous feeding line and of the effect of heparin on line occlusion. By S. M. PLUSA, R. HORSMAN, S. KENDALL-SMITH, J. N. PRIMROSE and N. WEBSTER. Nutrition Team, Academic Unit of Surgery, St James's University Hospital, Leeds LS9 7TF

The use of the silicon 23 gauge, EpicutaneoCavaCath (ECCC; Vygon, Germany) for the administration of peripheral intravenous nutrition (IVN) is associated with a high occlusion rate in our unit (24 of 54 lines, 44 %), resulting in a median line survival of only 3 d (interquartile range 1-6 d).

We have therefore evaluated the use of a 22 gauge, polyurethane catheter (Hydrocath; Viggo-Spectramed, Swindon) in a controlled trial. The use of heparin in peripheral IVN solutions may be associated with flocculation of lipid and Ca so we also studied the effect of heparin on line occlusion.

Thirty-one adult patients referred for IVN were randomized on forty occasions to a Hydrocath or ECCC catheter, with or without 500 iu heparin/500 ml of IVN solution. Twenty catheters of each type were studied. All catheters were inserted under sterile conditions by members of the nutrition team and IVN was infused over 24 h by infusion pump. The patients received 2 - 2.5 l IVN containing 7531 kJ and 9 - 14 gN in 24 h. There was no significant difference in the fluid volume or amount of nitrogen administered between the groups. Hydrocortisone (25 mg) was added to all IVN solutions, a nitrate patch was applied to the arm and line care was the responsibility of the nutrition team.

The median survival of the Hydrocaths was 7 (interquartile range 3 - 10) d, compared with 4 (interquartile range 2 - 7) d for the ECCC, which was not significant. However the survival of the Hydrocath allowing for elective removal, was significantly different to the ECCC ($P<0.01$, Kolmogorov-Smirnov test).

The reasons for line removal are shown in the Table. The only significant difference was in the incidence of line occlusion ($P<0.02$).

Catheter type ...	ECCC				Hydrocath			
n ...	20				20			
Reason for removal	Heparin	%	No heparin	%	Heparin	%	No heparin	%
ELECTIVE	4	20	4	20	6	30	5	25
OCCLUSION	5	25	5	25	0	0	0	0
PHLEBITIS	1	5	1	5	4	20	2	10
INFECTION	0	0	0	0	0	0	2	10
CATHETER FAILURE	0	0	0	0	0	0	1	5

The median survival of Hydrocaths removed for complications was 9 d interquartile range 8 - 12), significantly greater than for the ECCC (4 d, interquartile range 2 -7, $P<0.004$). The presence of heparin had no effect on the incidence of line occlusion or of any other complication.

The Hydrocath is a better line for the administration of peripheral IVN.

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Gastric volumes and regurgitation of feed during enteral tube-feeding in severe head injury. By E. WEEKES[1] and M.ELIA[2], [1]Department of Nutrition and Dietetics, Addenbrooke's Hospital and [2]Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH

Aspiration pneumonia during enteral tube-feeding is more likely to develop in recumbent unconcious patients who have an impaired swallowing reflex in association with poor gastric emptying and regurgitation of feed. The aims of this study were to obtain an estimate of the gastric volume after introducing a feed into the stomach and to obtain evidence of possible regurgitation of feed from the stomach to the mouth in critically ill subjects who have suffered a severe head injuy.

Six male subjects aged 21-30 years were studied 3-5 days following head injury (Glasgow Coma Scale score 6.5 (SD 0.8)) whilst being artificially ventilated. Gastric emptying was assessed with the phenol red technique (Hurwitz, 1981). Feed (200 ml Fortison Standard: Cow and Gate) and 2 ml phenol red (1.25mg/ml) were introduced into an empty stomach that had been washed with water. Sequential additions of doubling quantities of phenol red and sequential sampling (2 ml) of stomach contents after mixing were made at intervals so that the gastric volume could be determined at 15, 30, 45, 60, 90 and 120 min. Four healthy, male controls underwent the same procedure. The energy intake of the patients during the previous day was $\leq$ 1500 KJ. Evidence of regurgitation of feed from the stomach to the mouth was obtained by measuring the glucose concentration in secretions of the mouth and/or pharynx (normally <0.2 mmol/l). Between days 3 and 5, thirty-one samples of saliva were aspirated during routine suction as clinical condition dictated. The presence of a glucose concentration >0.5 mmol/l was taken to imply that regurgitation of feed from the stomach into the mouth. had occurred For comparison, saliva samples were also obtained from eleven healthy subjects after an overnight fast.

The changes in gastric volume after placing 200 ml feed into the stomach were variable. In the patients the mean (SD) values were 167 (108), 135 (75), 104 (35) and 110 (67) ml at 30, 60, 90 and 120 min respectively. These were higher than in the control subjects (mean (SD) 97 (13), 54 (29), 13 (14) and 0 ml for the corresponding times). The area under the volume-concentration curve was two-fold greater in the patients than in the normal subjects ($P<0.001$). Sixteen of the thirty-one samples obtained from the patients had a glucose concentration greater than 0.5 mmol/l, and in eleven of these the concentration was greater than 1 mmol/l. (The glucose concentration of feeds was 5-10 mmol/l.) No patient had clinical evidence of pneumonia. In normal subjects the glucose concentration in saliva was always < 0.2 mmol/l. The results obtained from these subjects with severe head injury suggest the possibility of frequent regurgitation of feed from the stomach to the mouth, and disturbances in gastric emptying that result in high gastric volumes and poor feed intake.

Hurwitz, A. (1981). Gut **22**, 85-93

Incidence of infection related to insertion sites of long lines in intensive care patients. By E. RAINFORD,[1] S.T. TAN,[2] P.M. FORD,[1,2] and M. TAYLOR.[3] Intensive Care Unit,[1] Department of Anaesthetics,[2] and Aseptic Unit, Pharmacy.[3] Royal Albert Edward Infirmary, Wigan WN1 2NN

A 6-month study of insertion techniques, intercurrent care and outcome of the use of long lines was instituted following an increase in the number of pathogens from both the catheter tips and insertion sites. The study was limited to twelve long-stay patients (average duration of stay 17d.) The sex ratio was male:female 2:1. Nine admissions were surgical patients and three, medical. Eight patients survived (66 %).

The insertion sites were limited to the internal jugular, subclavian and basilic veins. The lines were either single or triple lumen inserted by catheter over guide wire method or single lumen drum catheters inserted by catheter through needle technique. Lines were inserted in theatre or in the Intensive Care Unit. Insertion was limited to three consultants, four registrars and three senior house officers, all anaesthetists. Surgical gowns were omitted, personnel wore plastic aprons, scrubbed and donned sterile gloves. The skin preparation was limited to povidone iodine (PI) (McLure & Gordon, 1992.) All cannulae except the drum catheters were sutured to the skin near the insertion sites. No line was tunnelled. The insertion site was covered with a transparent non-porous dressing. Parenteral nutrition was reserved for a single designated line. All other lines received multiple therapeutic infusions. Continuing care involved 24 h changes of dressings, giving sets and 3-way taps using aseptic technique. The site was cleaned with PI daily, as were all connections on the system. Culture swabs of the insertion site were taken on alternate days and results were available after 48 h. Following catheter removal, the terminal 6 cm of line was despatched for culture. The mean life of all catheters was 6.5d. Seven patients were ventilated via a tracheostomy tube.

No pathogens were isolated from the catheter samples from subclavian or basilic sited lines. In contrast, jugular sited catheter tips were contaminated with Staphylococcus epidermidis (S Ep) in 44 % cases. Swabs from the entry sites of both basilic and subclavian catheters had a 3 % incidence of enterococcal contamination, 3 % S Ep in the basilic group and 23 % S Ep in the subclavian entry point. In contrast, the jugular sites had an 18 % incidence of other pathogens, predominantly Gram negative with Candida albicans in one isolate.

Culture report	Jugular site	Jugular catheter	Subclavian site	Subclavian catheter	Basilic site	Basilic catheter
No growth (%)	39	56	73	100	93	100
S Ep (%)	42	44	23	0	3	0
Other organisms(%)	18	0	3	0	3	0

Interpretation of the results has linked the high infection rate of jugular lines to contamination from the beard area in male patients (nineteen infected sites and two catheter tips,) migration of gut organisms around the tracheostomy wound (seven tracheostomy patients with twentyfour infected sites and three tips,) difficulty in applying occlusive dressings with increased sweating in neck folds and the predominance of theatre (i.e. emergency) jugular lines with less meticulous skin hygiene. The lowest incidence of contamination is the drum catheter inserted in the basilic vein. It is not certain whether this is linked to the simplicity of insertion with packaging methods ensuring a 'no-touch technique' of the long line, to the fact that the basilic line is solely used for parenteral nutrition or to the cannula composition. Following the study of infection incidence linked with this unit, the jugular approach has been abandoned whenever possible and full aseptic technique with surgical gowns and complete towelling of the patient is employed during long line insertion.

McLure A.R. & Gordon J. (1992) Journal of Hospital Infection **21,** 291-299.

Catheter-related morbidity in patients on home parenteral nutrition. By N. WILLIAMS, G.L. CARLSON, S. WALES, J.L. SHAFFER and M.H. IRVING. Nutrition Unit, University of Manchester, Hope Hospital, Salford M6 8HD

Catheter-related complications (CRC) are the commonest cause of morbidity and hospital re-admission for patients receiving home parenteral nutrition (HPN; Burnes et al. 1992). We investigated this problem by reviewing the case notes of all current patients to obtain information regarding catheter insertion details and all subsequent episodes and admissions for CRC. Data were corroborated by patient interview.

Fifty patients (twenty-six females, twenty-four males) are currently receiving HPN from this unit, thirty because of Crohn's disease, sixteen for short bowel syndrome and four with miscellaneous other diagnoses. This cohort has received a total of 2667 patient-months of HPN (median 45 months, range 4-123 months). All catheters were inserted in the operating theatre by direct cutdown and screened into position. The majority of these ($\approx$69) were single-lumen silicone (Broviac) catheters and the rest were polyurethane single-lumen catheters. Eighty-eight catheters have been required in this cohort, an average of 1.8 per patient. Twenty-eight patients were still using their initial catheter (median age 29 months, range 4-123 months) whereas eleven patients required two, six required three and five patients required four catheters respectively. At removal, the median longevity for a first catheter was 36 (range 1-92; n 22) months, 30 (range 8-53; n 11) months for the second and 20 (range 10-24; n 5) months for the third catheter. Fifty-seven episodes of CRC were managed, of which the catheter was saved in nineteen cases (33%), the other thirty-eight cases requiring catheter replacement. Thrombotic occlusion (18), catheter sepsis (14) and exit site sepsis (10) were the main causes of morbidity. The median hospital stay (range) was 2 (2-8)d for exit site sepsis and 2 (1-7)d for catheter occlusion whereas that for catheter sepsis was 16 (4-45)d reflecting the use of an antibiotic-fibrinolytic lock technique. The overall incidence of catheter-related problems was one episode/3.8 years of treatment or 0.25 episodes/year.

Data analysis from the HPN register for the United Kingdom suggests that catheter related problems remain the commonest source of morbidity, with an overall catheter complication rate of 1 episode per 0.98 years of treatment (O'Hanrahan & Irving, 1992). Our results suggest that substantial catheter longevity with low morbidity can be achieved for patients on HPN attending a specialist centre.

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O'Hanrahan, T. & Irving, M.H. (1992) Clinical Nutrition. **11**, 331-336.

Effect of undernutrition on muscle protein synthesis at local and distant sites following surgery in the rat. By P.W. EMERY and P. SANDERSON. Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH

Wound healing after surgery is often prolonged in patients who are chronically undernourished. Wound healing must involve the synthesis of a considerable amount of new protein, and we have recently found a major increase in the rate of muscle protein synthesis at the site of the wound 48 h after abdominal surgery in rats (Ghusain-Choueiri & Emery, 1992). We have now investigated whether this rise in protein synthetic rate is sensitive to undernutrition.

Sixteen mature female rats (body weight 150 g) were fed *ad lib.* on a high quality semisynthetic diet and a matched group of sixteen rats was restricted to 56% of the mean *ad lib.* intake of the same diet for 9 d. On day 7 half the rats in each group were anaesthetized with halothane, a 5cm midline incision was made through the peritoneum and the intestine was briefly mobilized. The muscle was then sutured and the skin closed with stainless steel clips. Control rats were anaesthetized but no surgery was performed. At 48 h after surgery the rate of protein synthesis was measured in abdominal muscle, both at the wound site and in undamaged tissue 2 cm away from the wound, and in the gastrocnemius muscle, using the large-dose phenylalanine method (Garlick *et al.* 1980).

	Protein synthetic rate (% per day)				Pooled
	Ad lib.		Restricted		
	Control	Surgery	Control	Surgery	SEM
Wound	8.2[b]	18.0[a]	5.4[c]	17.9[a]	0.5
Distant abdominal muscle	6.5[a]	6.2[a]	5.2[b]	5.3[b]	0.4
Gastrocnemius	7.2[a]	5.7[b]	5.5[b]	5.0[c]	0.3

a,b,c Values with different superscript letters were significantly different (two way analysis of variance); $P<0.05$

Food restriction caused a loss of 5 g body weight over the 9-d period, while the *ad lib.* group gained 19 g. Surgery caused a massive elevation in the rate of protein synthesis at the wound site, but this was completely unaffected by food restriction. In contrast, in the unoperated rats food restriction did cause a decrease in protein synthetic rate at the corresponding site. Similarly, at the sites away from the wound food restriction caused a decrease in muscle protein synthesis regardless of whether surgery also caused a decrease (gastrocnemius muscle) or not (distant abdominal muscle). Thus it appears that protein synthesis at the wound site is uniquely protected from the effects of poor nutritional status, so that inadequate protein synthetic activity during wound healing is unlikely to provide an explanation for the poor outcome of moderately depleted surgical patients.

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Ghusain-Choueiri, A. & Emery, P.W. (1992). Proceedings of the Nutrition Society **51**, 101A.

The effect of non-protein energy intake on protein metabolism kinetics in neonates receiving total parenteral nutrition. By M.O.* JONES[1], A. PIERRO[1], P.J. GARLICK[2], M.A. MCNURLAN[2] and D.A. LLOYD[1]. [1]The Royal Liverpool Children's Hospital, Alder Hey, Liverpool L12 2AP and [2]Rowett Research Institute, Aberdeen AB2 9SB.

The maintenance of adequate body protein is essential for normal health and growth. It remains unclear which glucose/fat ratio produces an optimal protein sparing effect in newborn infants (Pierro *et al* 1988; Bresson *et al* 1989). The aim of the present study was to determine the effect of different glucose/fat ratios on protein metabolism kinetics in newborn infants receiving total parenteral nutrition (TPN).

Fifteen studies were done on twelve infants (weight 3.09 (SE 0.22) kg, gestational age 37.2 (SE 0.9) weeks, postnatal age 14.5 (SE 3.7) d). Each infant was allocated to one of two groups, which were similar with regard to weight, gestation age, postnatal age and energy intake. Group A (six infants/eight studies) received TPN containing g/kg per d dextrose 10.0, fat 4.0 and amino-acids 2.5. Group B (six infants/seven studies) received TPN containing g/kg per d dextrose 19.0, fat 0.5 and amino-acids 2.5. Timed urinary N excretion was determined from 3-d urine collection. On the third day of the study, each infant received a 6h infusion of ^{13}C-leucine at a dose of 6 μmol/kg per h, preceded by a priming dose of 15 μmol/kg. Plasma and breath samples were taken at hourly intervals, and CO_2 production was measured by indirect calorimetry. Plateau levels of plasma ^{13}C-leucine enrichment and expired ^{13}C-CO_2 enrichment were determined by gas chromatograph mass spectrometry. Protein metabolism kinetics were then calculated using a simplified model of protein dynamics (San Pietro *et al* 1953).

There was no significant difference in any of the components of protein metabolism between the two groups (Table).

	Group A		Group B		P
	Mean	SE	Mean	SE	
nitrogen balance	2.40	0.15	2.35	0.15	0.39
protein flux	10.91	0.54	10.24	0.57	0.41
protein synthesis	9.99	0.56	9.26	0.51	0.36
protein breakdown	8.35	0.56	7.68	0.57	0.42
protein oxidation	0.92	0.05	0.97	0.08	0.61

All values expressed in g/kg per d.

This study demonstrates that carbohydrate and fat have an equivalent effect on the protein metabolism of newborn infants receiving TPN. This has important positive implications for the use of intravenous fat.

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Weight loss, interleukin 6 secretion and alpha 2-macroglobulin response in experimental colitis.

By P.J.D. NEILLY[1], G. JENNINGS[2], N.H. ANDERSON[3], M.D. McCAIGUE[1], K.R. GARDINER[1], S.J. KIRK[1], M. ELIA[2] and B.J. ROWLANDS[1]. [1]Departments of Surgery and [3]Pathology, Queen's University of Belfast BT12 6BJ, and [2]Dunn Nutrition Unit, Cambridge CB4 1XJ

Recently nutritional therapies have been advocated for use in inflammatory bowel disease (IBD). The aetiology and pathogenesis of IBD is uncertain but associated features of indefinite significance are weight loss, cytokine production and an acute-phase protein response. Using a model of hapten-induced chronic colitis (Morris *et al.* 1989) we have assessed the relationship between mucosal inflammation, weight loss, and interleukin 6 (IL6) and alpha 2-macroglobulin (α2M) production.

Colitis was induced in male Wistar rats (275-325 g) by intracolonic instillation of 30 mg trinitrobenzenesulphonic acid in 0.5 mL ethanol solution (500mL/L; TNBS/E). Controls received an equal volume of saline (9g NaCl/L). Animals were given food and water *ad lib.* and after 8 days the colon was excised and weighed and inflammation assessed using a colon macroscopic score (CMS). Plasma concentrations of IL6 and α2M were determined using a B9 cell bioassay and single radial immunodiffusion respectively. Change in body weight was recorded and expressed as a percentage of the initial weight (%CBW).

Data are expressed as means with their standard errors. Statistical significance was accepted if $P<0.05$.

		CMS		Colon wt (g)		%CBW		IL6 (pg/mL)		α2M (g/L)	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	6	0	0	0.65	0.02	13.87	1.64	0	0	<0.05	
TNBS/E	15	5.9*	0.5	2.56*	0.33	1.09#	2.65	174.2*	29.6	0.62*	0.12

Significantly different from controls (Mann Whitney U test): *P<0.0001, #P=0.006.

There was a negative correlation between %CBW and both CMS (P=0.0005, r=-0.8) and colon weight (P=0.0003, r=-0.82). CMS correlated positively with IL6 (P=0.0052, r=0.69) and α2M (P<0.0001, r=0.84). There was also a positive correlation between colon weight and both IL6 (P=0.0014, r=0.76) and α2M (P<0.0001, r=0.85)(Spearman's rank).

In this model of chronic inflammation and weight loss there is a cytokine and acute-phase protein response which correlates significantly with disease activity. This model could therefore be used to assess the systemic response to nutritional therapy.

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The effect of acute insulin administration on post-prandial glycogen and lipid synthesis in the rat. By J.W. POWLES, O.A. OBEID and P.W. EMERY. Department of Nutrition and Dietetics, King's College, London W8 7AH

When animals are refed after an overnight fast hepatic glycogen is synthesized predominantly by an indirect pathway which involves gluconeogenesis (McGarry *et al* 1987). Gluconeogenesis is known to be inhibited by insulin, yet it is commonly stated that insulin enhances hepatic glycogen synthesis. In order to clarify this paradox, normal male Sprague-Dawley rats were fasted overnight, then tube-fed a 16 KJ liquid meal and immediately injected subcutaneously with insulin (20 I.U./Kg body weight; group I) or saline (9 g NaCl/l; group C). At defined intervals after the meal groups of six to eight rats were injected intraperitoneally with 3 mCi of ^3H_2O and killed 1 h later, and the livers and epididymal fat pads (EFP) were analysed for ^3H_2O incorporation into glycogen and saponifiable lipid.

Time after meal (h)...		0		1		2		3	
	Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Hepatic glycogenesis†	C	6.7	0.2	50.7	8.1	25.0	11.0	2.1	0.5
	I	...	...	0.0**	0.0	0.0**	0.0	0.0**	0.0
Hepatic glycogen	C	8.8	1.0	22.9	0.8	29.3	2.0	32.4	3.8
content (mg/g)	I	...	...	3.3**	0.4	2.2**	0.2	1.5**	0.1
Hepatic lipogenesis‡	C	14.0	2.4	12.7	1.2	17.2	1.2	9.7	0.4
	I	...	...	14.9	1.4	14.8	1.0	13.1*	0.6
EFP lipogenesis‡	C	6.1	0.3	5.2	0.5	6.8	0.6	7.4	0.4
	I	...	...	19.0**	1.7	23.6**	2.2	17.4**	1.6

Significantly different from control group (*t* test); *$p<0.05$, **$p<0.001$.

† μmol ^3H_2O incorporated into glycogen/g tissue per h. ‡ μmol ^3H_2O incorporated into saponifiable lipid/g tissue per h.

In the insulin-treated rats there was no incorporation of ^3H into hepatic glycogen, and glycogen content did not increase throughout the 3 h post-prandial period. Clearly glycogen synthesis through both the direct and indirect pathways had been abolished by the acute administration of insulin. Hepatic lipogenesis was similar in the two groups except 3 h after the meal. However, adipose tissue lipogenesis was approximately three times higher in the insulin treated rats than in the controls throughout the 3 h post-prandial period. Thus, acute administration of insulin appears to divert glucose and its metabolites towards fatty acid synthesis in adipose tissue instead of hepatic glycogen synthesis. Whether chronic administration of insulin would have the same effect is not yet clear.

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Effect of intramuscular glutamine concentrations on muscle protein synthesis estimated after injury using a constant infusion of [^{3}H] phenylalanine. By M. WUSTEMAN, H. TATE and M. ELIA. MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ

We have previously reported experiments in which acute elevations in the intramuscular glutamine concentration ($[GLN]_m$) of rats injured with turpentine had no stimulatory effect on muscle protein synthetic rate (MPFSR; % per d) measured with a flooding dose of [^{3}H] phenylalanine (Wusteman & Elia, 1991). However, recent studies have indicated the possibility of a stimulatory effect of a flooding dose of an individual amino acid on MPFSR (e.g. Jahoor et al 1992; Smith et al 1992), an effect which may have interfered with the stimulation which would otherwise have occurred in response to the increases in $[GLN]_m$. Since this issue is of substantial biochemical and clinical importance, we have repeated the study using a constant infusion of [^{3}H] phenylalanine to measure MPFSR.

A systemic response to tissue injury was induced in thirty-two rats (age 38-40 d) by subcutaneous injections of turpentine (T): sixteen control animals were injected with saline 9 g NaCl/ls S). After 43 h all S rats and half the T rats were infused for 5 h via the lateral tail vein at a rate of 10 ml/kg body weight/h with saline (S/S and T/S) and the remaining T rats were infused for 5 h at the same rate with 0.22 M-glutamine (T/G). After 3 h the infusion solutions were supplemented with 1 μmol/ml of [^{3}H] phenylalanine (4.5 μCi/ml). After a further 2 h infusion the rats were all killed and the plasma and gastrocnemius muscles collected for estimation of glutamine concentration and phenylalanine specific activity.

The values for MPFSR (% per d) achieved in the present study were in good agreement with those we have already reported with the flood of [^{3}H] phenylalanine (constant infusion: 12.82 (SD 0.44) % in saline-injected rats, 6.58 (SD 0.34) % in rats injected with turpentine; flood: 12.88 (SD 0.73) % in saline-injected rats, 7.04 (SD 0.67) % in rats injected with turpentine (Wusteman & Elia, 1991). There was, therefore, no evidence for a stimulating effect of a flood of phenylalanine on MPFSR, an effect which could have invalidated the conclusions drawn from our previous investigation. In the present study, the turpentine reduced MPFSR by 49 % (ANOVA, $P<0.001$) and this reduction was associated with a 39 % reduction in the $[GLN]_m$ ($P<0.001$). The reduction in $[GLN]_m$ was completely reversed by the 5 h intravenous infusions of 0.22 M-glutamine ($[GLN]_m$ (mmol/kg wet wt): S/S 6.27 (SD 0.28, n 13); T/S 3.80 (SD 0.24, n 15); T/G 6.64 (SD 48, n 14); S/S with T/G; $P>0.05$ ANOVA) but there was no accompanying increase in MPFSR (T/S 6.58 (SD 0.34) % per d; T/G: 7.12 (SD 0.5) % per d; $P>0.05$). The results of the present study, therefore, confirm those of our previous one and, since the group sizes were sufficient to give the study a 90 % power to detect a 30 % increase in MPFSR, they provide no evidence to support the proposal that changes in $[GLN]_m$ are a major factor in controlling MPFSR in skeletal muscle.

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Wusteman, M. & Elia, M. (1991). Journal of Parenteral and Enteral Nutrition **15**, 521-525.

The effect of acute starvation on tissue glutathione concentrations in the rat. By S.J. BEVAN, (Introduced by G. HARDY). Oxford Nutrition Ltd, Oxford OX4 3UH

The tripeptide glutathione (γ-glu-cys-gly) is a cytosolic antioxidant which protects cells from free radicals, particularly the reactive oxygen species which are produced as a consequence of normal aerobic metabolism. In addition to its redox properties, glutathione can also function as an amino acid carrier and the γ-glutamyl cycle may serve as an important route for the transport of amino acids in the gut (Meister, 1984).

Tissue glutathione levels are likely to be determined by the availability of its constituent amino acid precursors and cytosolic concentrations of glutamate, cysteine and glycine are likely to be influenced by nutritional status. Hence, the present study was undertaken to investigate the effect of acute food deprivation on the glutathione status of a number of tissues.

A group of male Wistar rats (n=6) was allowed food and water *ad lib.* and a second group (n=6) was deprived of food for 24 h before killing. Lung, liver, small intestine and blood were obtained from all animals and samples of the tissues were immediately analysed for total glutathione content (i.e. oxidized glutathione (GSSG) and reduced glutathione (GSH) forms) by an enzymic method. Frozen samples were later analysed for protein content. The results are shown below.

	Total glutathione (nmol/g tissue)				Total glutathione (nmol/mg protein)				Total glutathione (μmol/g Hb)				
	Control		Starved		Control		Starved		Control		Starved		Change
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	(%)
Lung	1536	118	1196*	68	11.68	0.96	7.62**	0.45					-35
Liver	3575	453	1447***	95	19.33	1.80	4.92***	1.00					-74
Intestine	2379	244	1322**	124	15.41	1.69	7.18***	0.51					-53
Blood									9.46	1.44	4.30**	0.58	-55

Significantly different from controls; *p<0.05, **p<0.01, ***p<0.001 (Students t test). Values are means with their standard errors for six observations.

As expected, all tissues that were studied exhibited a decrease in total glutathione content following nutritional deprivation, although differences in the magnitude of the responses were observed.

The liver appears to be most sensitive to nutritional status, with total glutathione levels decreasing to a quarter of that of control animals after 24 h starvation. This large decrease is considered to be due to the release of glutathione from the liver into the blood in order to meet the needs of extrahepatic tissues (Kaplowitz *et al.* 1985). However, in the present study blood glutathione homeostasis was not maintained and the total glutathione concentration decreased to a half of its original value.

The present study supports the hypothesis that there is selective uptake and maintenance of tissue glutathione by different extrahepatic tissues; this may relate to its functional importance in specific tissues. For example, the antioxidant activity of glutathione may be important in the lung (which shows the least dramatic decrease in concentration) because of its chronic exposure to O_2, whereas the gut may have a lower requirement for glutathione in starvation since in this tissue glutathione plays a role in amino acid transport. It remains to be investigated whether these decreases in tissue glutathione concentrations have pathological significance.

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Low muscle glutamine in the critically-ill patient is poorly repleted by intravenous glutamine of 5d duration. By T. E. A. PALMER, C. JONES and R. D. GRIFFITHS. Intensive Care Research Group, Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX.

Intramuscular glutamine (Gln) content falls rapidly in the critically-ill patient and it has been suggested that it remains low irrespective of the development of the disease (Gamrin *et al.* 1992). The addition of L-Gln to intravenous feeding has been shown to reduce the loss of intramuscular Gln following surgery. The ability to replete the very low intramuscular Gln content in the critically ill has been questioned. In these patients there is a variable expansion in muscle extracellular volume. This added complication in the measurement of muscle Gln has not been explored.

Patients (*n* 38, aged 19-77 (mean 55) years) in a general intensive care unit who were severely ill (APACHE II (Knaus, *et al.* 1985), 8-31, median 17) were recruited randomly to receive either a conventional total parenteral nutrition (TPN) or an isonitrogeneous, isoenergetic TPN supplemented with 25 g L-Gln (Hardy *et al.*1992). Consent was available in nineteen patients for two percutaneous muscle biopsies (day 2-4 and day 7-9). Ten patients received Gln (median APACHE 19) and nine patients (median APACHE 17) received the control feed. These patients are representative of the overall randomized group as judged by illness severity and outcome. Plasma and intramuscular Gln was measured following acid extraction by coupled enzymatic assay (Lund, 1983). Intracellular content was calculated using a correction for extracellular fluid (ECF) volume based upon muscle Na content (Jackson, *et al.*1985). Results before and after feeding are shown in the Table.

	Initial plasma Gln (mmol/l)			Initial muscle Gln (mmol/kg wet wt)			Final muscle Gln (mmol/kg wet wt)		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Gln feed	0.39	0.21	10	3.74	1.76	10	3.34	2.46	8
Control	0.20	0.10	9	2.51	1.65	9	2.73	1.32	8

Overall 5/18 patients given Gln (3/10 biopsied) and 11/20 patients given the control feed (6/9 biopsied) died. The initial plasma Gln was reduced in all but one of the patients biopsied (normal 0.64, CI 0.56-0.72 mmol/l). In line with previous reports, the ECF volume was high: mean ECF 39.7 (SD 13.6) % in Gln-fed and 38.7 (SD 7.0) % in control-fed. The average muscle Gln content before feeding was very low (reported normal range 12.66 (SD 2.37) mmol/kg wet wt). Between biopsies no consistent pattern of change was seen with or without exogenous Gln.

Intramuscular Gln in the first week of a severe illness appears unresponsive to the influence of additional Gln. However, many patients in both groups biopsied showed a 50 % or greater change in muscle Gln. An equal number of patients showed increases or decreases. This is illustrated by one septicaemic patient, Gln-supplemented, in whom muscle Gln fell from 7.17 to 1.82 mmol/l during the study and returned to normal, 12.79 mmol/l, within 2 months. Large changes in intramuscular Gln occur during a severe illness and very low levels are compatible with a good outcome. This suggests that while muscle is initially unresponsive to nutritional support there is a considerable nutritional debt to be repaid during convalescence.

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The use of bioimpedance to measure total body water in multiple organ failure. By K. FOLEY[1,2], M.A. KEEGAN[2], D. MILLICAN[1,2], B. MURBY[3], N. LATHAM[4] and I.T. CAMPBELL[1,2]. [1]Intensive Care Unit, [2]University Department of Anaesthesia and Departments of [3]Nuclear Medicine and [4]Biostatistics, Withington Hospital, Manchester M20 8LR.

One of the features of mutiple organ failure (MOF) is peripheral and pulmonary oedema resulting from abnormal retention of the fluid given for resuscitation. In adults this often amounts to 10-15 litres (Hall, Pollard & Campbell, 1992). There are difficulties in judging fluid status in these patients; fluid balance charts are notoriously inaccurate, the inaccuracy increasing the longer the period of use, and serial weighing is technically difficult and disturbing for the patient. Bioelectrical impedance is known to be a reasonable indicator of total body water (TBW) in subjects of normal body composition (Lukaski, 1985). In patients suffering MOF it would have obvious attractions as a non invasive indicator of TBW. In the present study TBW was measured in patients in MOF and the results compared with an estimate of TBW derived from impedance and height.

Twenty patients suffering MOF were studied (seven female, thirteen male; aged 32-75 years, median 57 years; height 143-188cm, median 172cm; admission weight 55.5-107kg, median 80.0kg). Total body water was measured using tritiated water; 3.7 MBq in saline (9g NaCl/l) were injected intravenously. Blood was taken hourly for 6h and the plasma assayed for tritium content by liquid scintillation counting. All urine passed and all abnormal losses were collected and assayed to correct for losses. TBW was calculated from the radioactivity and total body impedance (I) measured at 6 hours at 50 kHz using a Holtain body composition analyser (Holtain Ltd., Crymych). The relationship between TBW and total body conductance (C) derived from height and impedance (Ht^2/I) was analysed using linear regression analysis.

$$TBW = 0.29C+26.2$$
$$(SEE = 7.85 \text{ litres}; r=0.795; p<0.001)$$

It was deemed unethical to perform the study on a normal control population, but this equation is significantly different from the one derived by (Lukaski, 1985) for normal subjects

$$TBW = 0.68C+0.12$$
$$(SEE = 2.30\text{litres}; r=0.980; P<0.0001)$$

with an obviously larger standard error of estimate.

It is concluded that total body impedance can be used to measure total body water in MOF, but the relationship between conductance and TBW appears to be different from that seen in normal individuals and the prediction less precise. The technique may have a place in monitoring changes in TBW in individual patients suffering MOF.

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Supported by Holtain Ltd., Crymych, Dyffed.

Total energy expenditure and energy intake in Human Immunodeficiency Virus infected men.

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Wasting and weight loss are important clinical complications of infection with the Human Immunodeficiency Virus (HIV). Weight loss tends to occur in episodes during which energy balance must be negative (Macallan *et al.* 1993). Although hypermetabolism at rest has been well documented, it is unclear to what extent increased total energy expenditure (TEE), as opposed to reduced energy intake, contributes to weight loss episodes. The present study set out to test the hypothesis that episodes of weight change would be associated with changes in TEE and energy intake.

TEE was measured by the doubly-labelled water technique over a period of 14 d (Coward, 1988). Energy intake was ascertained contiguously from 7 d weighed food records and weight change was estimated from serial weight measurements with digital electronic scales. Twenty-seven measurements were made in twenty-two HIV-seropositive male subjects who were studied at different stages of their illness. Subjects were divided into groups according to their rate of weight change. Clinical and dietetic management were continued according to normal practices. Results of TEE and energy intake as mean energy /kg fat-free mass (derived from isotopic estimation of total body water assuming a hydration fraction of 0.73) are shown in the Table. Physical activity level (PAL) was calculated as TEE/resting energy expenditure from indirect calorimetry (ventilated hood).

Group	n	TEE (kJ/d per kg)		PAL		Energy Intake (kJ/d per kg)	
		Mean	SD	Mean	SD	Mean	SD
Weight-gaining	4	**242**	33	**1.50** *	0.23	**275** §	51
Weight-stable	8	**255**	27	**1.84**	0.23	**234**	16
Weight-losing, ≤3 kg/month	11	**207** **	23	**1.50** **	0.16	**187** **	37
Weight-losing, >3 kg/month	4	**168** **†	32	**1.19** **†	0.11	**86** **†	37

Significantly different from weight-stable subjects: * $P<0.05$, ** $P\leq0.001$, § $P=0.054$;
Significantly different from weight-losing ≤3 kg/month subjects: † $P<0.05$, by Student's *t* test.

Weight loss episodes were associated with a reduction in TEE. Thus hypermetabolism, although possibly contributing to overall negative energy balance, is not the primary cause of weight loss; indeed the increase in resting energy expenditure that we have observed during illness episodes is more than outweighed by the reduction in activity related energy expenditure. Energy intake showed very marked differences between groups. This demonstrates that the reduction in energy intake associated with illness episodes such as secondary infections is a far greater effect than any changes in eneergy expenditure.

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Changes in energy expenditure and body composition after severe head injury. By E. WEEKES[1] and M. ELIA[2] .[1]Department of Nutrition and Dietetics, Addenbrooke's Hospital and [2]Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH

Some studies of severe head injury have reported marked resting hypermetabolism exceeding 50% basal metabolic rate (BMR), whilst others have reported little or no hypermetabolism despite sequential measurements. Such studies have usually involved 'spot' measurements of energy expenditure (EE), which may not extrapolate to periods of 24h. The aims of the present study were (a) to measure EE continuously over periods of up to 24h, and to use the estimate of total EE in conjunction with measurement of nutrient intake and N excretion to calculate N and energy balances and (b) to relate the above to changes in N and energy balance calculated from changes in body composition over a more prolonged period of time.

Six adult males aged 23.2(SD 5.0)years, weight 73.4 (SD 7.4)kg, height 1.74 (SD 0.07)m, were studied 3-5 d after sustaining head injury (Glasgow Coma Scale score (GCS) 6.5 (SD 0.8). Four were studied again 12-19d post injury (15.5 (SD 3.5)d; GCS 14.5 (SD 0.6)). Apart from the head injury, three subjects had other injuries (ruptured diaphragm and fractured pubic ramus (1); chest injury plus laparotomy for ruptured spleen (1); and pneumothorax (1)). The first measurement of EE (up to 24h) was made when the patients were artificially ventilated, and the second (30 min) was made after an overnight fast while the patients were self-ventilating. A 24h urine sample was collected for N analysis by the Kjeldahl technique. Food intake was recorded daily and body composition was assessed at the beginning and end of the study by skinfold thickness, bioelectrical impedance (Holtain equation) and near infra-red interactance techniques.

The subjects lost a mean of 9.8 (SD 4.5)kg, of which 2.3kg was estimated to be due to fat and 7.5kg due to fat-free mass (mean of three body composition techniques). Such changes imply mean negative energy and N balances of about 7.1MJ/d and 14gN/d, which is similar to that observed on days 3-5 when indirect calorimetry was first carried out (-7.1MJ/d and -19gN/d; mean intake 2.7MJ and 4.6gN). Energy intake from the beginning to the end of the study averaged 3.76 (SD 0.8)MJ/d and 4.7 (SD 1.3)gN/d. Total 24h EE on days 3-5 was $\geq$ 30% predicted BMR by the Harris Benedict equation (this being almost entirely due to resting hypermetabolism since five out of six patients were paralysed throughout the day and one for most of the day). At the end of the study resting EE was 105 (SD 11)% of predicted.

Estimates of N and energy balance (days 3-5) as well as measurements of body composition (0 to 15.5 (SD 3.5) d) suggest that lean tissue accounted for about 70-80 % of the weight loss, which is considerably more than that which occurs during dietary restriction in healthy individuals with a similar degree of adiposity (Elia, 1992). The study also suggests that the negative energy balance in the head-injured subjects studied is largely due to reduced energy intake, while the negative N balance is probably largely due to the injury plus immobilization since even early total starvation is associated with a negative N balance of only about 10g/d (Elia, 1992).

Elia M (1992) in Protein energy interactions ed: N S Scrimshaw and B. Schurch, Nestle Foundation, Lausanne, Switzerland. pp 249-284

Validation of an activity questionnaire to assess total energy expenditure in Human Immunodeficiency Virus infection . BY C. BALDWIN[1], C. NOBLE[2], D.C. MACALLAN[2], S.A. JEBB[3] and A.M. PRENTICE[3]. [1]Department of Genitourinary Medicine, King's Healthcare, London, [2]Division of Communicable Diseases, St. George's Hospital, London and [3]MRC Dunn Nutrition Unit, Hills Road, Cambridge, CB2 2DH.

The main components of total energy expenditure (TEE) are basal metabolic rate (BMR) and activity. BMR can be measured relatively easily using indirect calorimetry, whilst measurements of TEE require access to more sophisticated facilities such as doubly-labelled water (DLW). In this way the energy cost of activity may be calculated by difference. A number of questionnaires are available which purport to assess the physical activity of individual subjects. These are widely used in epidemiological work to examine patterns of activity and sometimes to quantify energy expenditure. Few have been rigorously validated against independent measures of energy expenditure.

We have measured aspects of energy expenditure in 20 HIV positive patients (CDC classes II, III and IV), at varying stages of their disease ranging from good health to acute, severe illness. BMR was measured using a portable indirect calorimeter and total energy expenditure using DLW. Activity was assessed using a simplified form of the Allied Dunbar activity questionnaire. The questionnaire was administered by one of two observers, who then calculated the energy expenditure on activity from BMR and tabulated physical activity ratios for each activity (Dept. of Health 1991).

Linear regression analysis showed a good correlation between the estimates of TEE made by DLW of the activity analysis ($r = 0.74$, SEE= 1.73 MJ/day). However analysis of the difference between methods showed that the activity analysis underestimated TEE for DLW by 1.16 ± 2.01 (SD) MJ/day ($p<0.01$).

The activity questionnaire gave a reasonable estimate of total energy expenditure for the group as a whole. However, there were unacceptably large errors for individual subjects. It is possible that accuracy could be improved by modifications to the questionnaire, but it seems unlikely that a questionnaire-based approach could be a useful tool for studying clinical correlates of acute changes in activity. The results of this study emphasis the importance of performing objective tests of external validity when searching for new methods which can be readily applied in the clinical setting.

Department of Health (1991) Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Reports on Public Health and Medical Subjects, No. 41. London: HMSO.

Use of a three-component model for evaluation of density and hydration of fat-free mass and validation of body composition predicted from the same whole-body bio-electrical impedance measurement, in lean and obese women. By N.J. FULLER, M.B. SAWYER and M. ELIA. MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH

The aims of the present study were twofold: (1) to evaluate fat-free mass (FFM) density and hydration (D_{ffm} and H_{ffm} respectively) using a three-component reference model (Fuller *et al.* 1992) in discrete groups of twelve lean (body mass index 20.9 (SD 2.1) kg/m^2; body fat 24.4 (SD 3.1) %) and fifteen obese (body mass index 42.8 (SD 8.8) kg/m^2; body fat 48.0 (SD 6.8) %) women; and (2) to establish the extent of agreement between body composition estimates obtained with this model and eight different predictions of the same whole-body bio-electrical impedance (BI) measurement (see Fuller, 1993).

The extent of agreement between estimates of FFM (kg) and body fat (%) by the three-component model and BI predictions using bias (three-component model minus BI prediction) and 95% limits of agreement (95% LA) is shown in the Table. D_{ffm} was found to be 1.097 (SD 0.006) kg/l and 1.104 (SD 0.006) kg/l, and H_{ffm} was 73.0 (SD 1.6) % and 71.2 (SD 1.6) % for lean and obese women respectively. No material difference was found between either value for D_{ffm} and its traditionally-applied value (1.1 kg/l), despite significant differences between the lean and obese women for both D_{ffm} ($P < 0.01$) and H_{ffm} ($P < 0.01$).

	Fat-free mass (kg)				Body fat (%)			
	Lean women (*n* 12)		Obese women (*n* 15)		Lean women (*n* 12)		Obese women (*n* 15)	
	Bias	95% LA	Bias	95% LA	Bias	95% LA	Bias	95% LA
Predicted by :								
Bodystat-500 instrument	-0.51	4.38	1.61	7.20	0.85	7.74	-1.00	6.66
E-Z Comp 1500 instrument	1.44	4.52	5.67	10.22	-2.51	8.06	-4.34	9.50
Maltron BT-905 instrument	1.50	4.56	6.71	11.10	-2.67	8.16	-5.20	10.04
Valhalla 1990b instrument	-0.96	4.50	10.53	34.94	1.57	7.34	-6.34	24.60
Holtain equation	3.61	4.46	8.20	10.52	-6.39	8.02	-6.73	9.04
Lohman equation	-0.83	4.52	-8.69	9.68	1.33	7.56	7.79	7.08
Lukaski equation	0.86	4.52	5.38	10.46	-1.53	8.02	-4.03	10.00
RJL equation	-1.24	4.64	-4.07	7.20	2.10	7.80	4.03	8.02

Mean values for D_{ffm} and H_{ffm} obtained on a relatively small number of subjects indicate no radical need to alter those traditionally applied to FFM (although the accuracy of the three-component model is limited by the assumption of constant ratio of protein to mineral; Fuller *et al.* 1992). In general, agreement between methods was better for lean than for obese women (with few exceptions for each, in the obese women the bias was more positive for FFM and more negative for fat, and the 95% LA were considerably larger). The extent of variability from different predictions incorporating BI highlights a potential source of major error and confusion, especially in the obese. Thus, a coherent approach is recommended to ensure that appropriate predictions (derived against valid reference methods) can be applied with confidence to relevant populations.

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Accuracy and precision of techniques used for measuring height in critical illness. By T. WATT, F. DODD, M. KEEGAN and I.T. CAMPBELL. University Department of Anaesthesia, Withington Hospital, Manchester M20 8LR.

Height is often required to normalise measurements of, for example, cardiac output or metabolic rate to body size. This may present problems in critically ill patients. It is usually obtained either from the relatives or measured using a domestic 60 inch tape measure. How accurate any of these options are is unknown. We have compared five different techniques of obtaining height, three of them applicable to patients lying in bed.

Five normal individuals had their height measured by ten volunteers using five different techniques: (1) empirical estimation (Est) with the subject supine (2) conventional stadiometer (Stad; Avery, Liverpool) with the subject standing, (3) portable stadiometer (PStad; CMS Weighing, London) with the subjects standing (4) 60" domestic tape measure with the subject supine (Tape) and (5) surveyors measure (SM) modified for measuring the height of patients lying in bed (CMS Weighing, London) again with the subject supine. Each volunteer estimated then measured the height of the five subjects using each of the four measurement techniques. Techniques and subjects were randomised so that no two successive measurements were made on any subject by any one volunteer. Each measurement was made in triplicate and the mean of the three used in the analysis.

There was no significant difference in the figure obtained for mean height of the five subjects between the conventional stadiometer (167.3 (SD9.8)cm), the portable stadiometer (167.5 (SD8.8)cm), and empirical estimation (167.4 (SD10.1)cm) but the figures obtained with the tape measure (168.1 (SD10.0)cm) and the surveyors measure (168.8 (SD10.0)cm) were significantly higher than those obtained using the conventional stadiometer($P=0.011$ and $P<0.001$ respectively).

The individual values obtained for the five subjects by the 10 observers, using the different five techniques ranged (median) as follows: (1)Est: 7.4-15.3(10.2)cm (2)Stad: 0.5-7.6(1.2)cm (3)PStad:1.0-2.8 (1.3)cm (4)Tape: 5.6-16.3(6.9)cm (5)SM. 1.4-5.3(3.1)cm.

An index of the interobserver variation was obtained by calculating the interquartile range of each type of measurement and expressing it as a percentage of the median measurement obtained on that subject using that technique. The results (mean (SD)) in order of precision are as follows; Stad: 0.26 (0.07)%, PStad: 0.38 (0.09)%, SM: 0.74 (0.15), Est 1.5 (0.55)%, Tape: 2.46 (1.16)%.

All three of the techniques used to assess the height of supine patients were significantly less precise than the conventional stadiometer ($P<0.05$), but the surveyors measure was significantly better than either a tape measure or empirical estimation ($P<0.05$).

It appears that of the three techniques available to obtain the height of a supine patient, the tape measure and surveyors measure overestimate "true" height (obtained using a conventional stadiometer with the subject standing) but the random error with the surveyors measure is less than the tape or empirical estimation.

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Inhibition of adipose tissue lipolysis *in vivo* in man by ketone body infusion. By J. WEBBER and I. A. MACDONALD. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

During starvation the rate of lipolysis rises, but there is little further increase after 36 to 48 hrs of fasting (as reflected by stable plasma levels of glycerol over this time period; Webber & Macdonald 1993), despite insulin levels continuing to fall. It has been suggested that the rise in ketone bodies which occurs during starvation may play a role in the inhibition of lipolysis (Balasse & Fery 1989), but the site of this action has not been demonstrated. We therefore sampled arterialized venous blood (AV), deep forearm venous blood (DV) and blood draining the subcutaneous adipose tissue of the abdomen (ATV; Frayn *et al.* 1989) during ketone infusion.

Eight healthy subjects were studied (mean body mass index 21.0 (SE 1.3) kg/m^2, mean age 27.2 (SE 1.8) years) on two occasions in random order. After an overnight fast basal measurements of blood glycerol and plasma non-esterified fatty acids (NEFA) were made. Then on one occasion an incremental infusion of sodium β-hydroxybutyrate was commenced at 6 mg/kg per min for 20 min and then increased to 10 mg/kg per min for a further 20 min, whilst on the other occasion an equivalent volume of saline (9 g NaCl/l) was infused. ATV was obtained during six of the β-hydroxybutyrate infusions and five of the saline infusions and therefore the data are unpaired.

AV β-hydroxybutyrate levels were 0.07 (SE 0.04) mmol/l basally and 2.52 (SE 0.20) mmol/l at the end of the second increment during the ketone infusion visit, whilst they were 0.08 (SE 0.02) and 0.11 (SE 0.02) mmol/l respectively during the saline infusion visit. Glycerol and NEFA levels are shown in the Table.

	Saline visit				Ketone visit			
	Basal		2nd Increment		Basal		2nd Increment	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AV glycerol (μmol/l)	33.9	6.8	38.2	10.7	32.3	4.5	14.5**	1.6
ATV glycerol (μmol/l)	171.6	18.3	167.7	10.6	117.1	26.0	37.8**	10.3
DV glycerol (μmol/l)	34.8	9.0	37.9	9.4	32.0	6.4	10.7**	3.4
AV NEFA (mmol/l)	0.62	0.07	0.74	0.07	0.45	0.11	0.16**	0.03
ATV NEFA (mmol/l)	1.48	0.09	1.69	0.08	1.19	0.28	0.24*	0.09
DV NEFA (mmol/l)	0.56	0.08	0.63	0.07	0.35	0.10	0.12**	0.03

Significantly greater fall in metabolite level from basal to 2nd increment for ketone visit v. saline visit by unpaired *t* test: $*P<0.05$; $**P<0.01$.

Hyperketonaemia appears to inhibit adipose tissue lipolysis strongly and thus may be a major factor preventing excess fat mobilization during starvation.

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Differential effects of high-fat diets on adipose tissue deposition in the rat. By E. J. SHERRINGTON, P. YAQOOB and P. C. CALDER. Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

There is evidence that obesity is linked to the development of disorders such as cardiovascular disease and diabetes mellitus. Although consumption of unsaturated fatty acid-containing oils (especially fish oil) has been proposed for the prevention and therapy of these diseases, there have been few studies investigating the link between the type of dietary fat consumed and the development of obesity. In the present study the effects of a variety of dietary lipids on adipose tissue deposition were investigated in the rat.

Male weanling Lewis rats were fed for 10 weeks on a low-fat diet (20 g/kg; LF) or on high-fat (200 g/kg) diets containing hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden oil (MO). After killing, seven distinct adipose depots (epididymal fat pad (EPI), dorsal wall of abdomen (DWA), mesenteric (MES), omental (OME), side groin (SGR), ventral groin (VGR) and intrascapular (INT)) were carefully dissected out and weighed. Data are expressed as g adipose tissue/kg body weight.

			Adipose tissue weight (g/kg body weight)													
	Body weight (g)		EPI		DWA		MES		OME		SGR		VGR		INT	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	301.6	7.1	13.5	1.5	15.6	1.6	12.5	1.5	4.5	0.3	14.0	1.3	4.7	0.6	5.3	0.5
HCO	398.7*	8.7	28.5*	2.3	38.5*	2.9	16.4*	0.7	6.7*	0.3	21.3*	1.9	8.7*	1.0	11.1*	1.7
OO	391.6*	13.6	20.8*	1.4	35.3*	1.6	14.9	0.5	5.8*	0.4	18.8	2.3	9.3*	1.4	9.9*	1.2
SO	407.7*	12.1	20.6*	1.5	37.5*	1.5	16.8	1.4	6.3	0.8	22.0*	1.6	10.0*	1.5	12.6*	1.1
EPO	374.0*	8.1	16.4	0.7	29.0*	1.6	13.4	0.7	6.0*	0.3	18.0	2.0	8.8*	1.3	9.0*	1.5
MO	386.4*	10.2	16.2	0.6	25.8*	1.8	14.1	1.2	5.2	0.2	21.3*	1.5	9.6*	1.0	11.0*	1.1

* Significantly different from LF (Student's *t* test); $P<0.05$ at least; n 7 for each diet.

LF-fed animals had lower body weights than animals fed on each of the high fat diets; there were no differences between the body weights of the animals fed on high-fat diets. The total adiposity (i.e. the sum of the weights of the seven depots in g/kg body weight) of LF-fed animals (69.7 (SEM 2.5)) was significantly less ($P<0.001$) than that of animals fed on HCO (134.7 (SEM 23.0)), OO (117.1 (SEM 6.2)), SO (125.5 (SEM 6.6)), EPO (102.3 (SEM 4.6)) or MO (104.8 (SEM 4.4)). The total adiposity of animals fed on HCO was greater than that of those fed on OO ($P<0.05$), EPO ($P<0.001$) or MO ($P<0.001$) while that of animals fed on SO was greater than that of those fed on EPO ($P<0.01$) or MO ($P<0.05$). Compared with the LF diet, the HCO diet increased the relative weights (g/kg body weight) of all seven adipose depots. The increases in relative weight were not uniform across all adipose depots; for EPI, DWA and INT the relative weight more than doubled but for MES the relative weight increased by only 25%. The OO and SO diets caused an increase in the relative weights of five depots, while the EPO and MO diets increased the relative weights of four depots. The relative weights of three depots (SGR, VGR, INT) were not different ($P>0.05$) between animals fed on the high fat diets. The relative weight of OME was lower in MO-fed animals than in those fed on HCO ($P<0.01$) or EPO ($P<0.05$) while the relative weight of MES was lower in animals fed on EPO than those fed on HCO ($P<0.01$). The greatest differences between the effects of the high-fat diets were observed in EPI and DWA: the relative weights of both DWA and EPI were lower in animals fed on either EPO or MO than in those fed on HCO, OO or SO ($P<0.05$ at least). The relative weight of EPI was lower in animals fed on OO or SO than in those fed on HCO ($P<0.02$). These results confirm that high-fat diets result in increased adipose deposition. However, this study also shows that the type of fat consumed is important in determining the extent of adiposity and that there are significant differences in deposition between sites.

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Dietary lipid manipulation alters lymphocyte phospholipid fatty acid composition but not membrane fluidity. By P. YAQOOB, D. J. HARVEY, E. A. NEWSHOLME and P. C. CALDER. Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

In vitro studies have shown that fatty acids, particularly the *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA), have profound effects on lymphocyte composition and functions (Yaqoob & Calder, 1993). It has also been shown that dietary lipid manipulation can affect lymphocyte proliferation (Yaqoob *et al.*, 1993*a*) and natural killer cell activity (Yaqoob *et al.*, 1993*b*). One mechanism by which fatty acids affect these functions may be through changes in lymphocyte membrane composition and/or fluidity. The present study investigates the effects of a variety of dietary lipids on the fatty acid composition of the phospholipids of spleen lymphocytes and on the fluidity of their membranes.

Weanling Lewis rats were fed for 10 weeks on a low-fat (LF; 20 g/kg) diet or on high-fat diets containing 200 g/kg of either hydrogenated coconut oil (HCO; rich in saturated fatty acids), olive oil (OO; rich in oleic acid, 18:1*n*-9), safflower oil (SO; rich in linoleic acid, 18:2*n*-6), evening primrose oil (EPO; containing the *n*-6 PUFA, γ-linolenic acid, 18:3*n*-6) or menhaden oil (MO; rich in *n*-3 PUFA). The membrane fluidity of spleen lymphocytes was determined by electron spin resonance spectroscopy at 37°*C*, using 5-DOXYL-stearic acid as the 'spin' label; membrane fluidity is expressed as the order parameter, *'S'*. The fatty acid composition of spleen lymphocyte phospholipids was determined by gas chromatography.

	Fatty acid (mol %)															
	14:0		16:1*n*-9		16:0		18:2*n*-6		18:1*n*-9		18:0		20:4*n*-6		*S*	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	1.9	0.5	2.5	0.6	31.8	2.3	8.1	0.1	9.4	0.5	24.6	1.4	9.8	1.0	0.52	0.01
HCO	8.0*	0.1	1.8	0.0	37.3	1.1	1.9*	0.5	7.7	1.0	29.3	0.7	6.3	0.2	0.53	0.00
OO	1.2	0.2	1.4	0.2	33.2	0.9	4.2*	0.3	20.9*	0.6	26.2	0.8	4.9*	0.6	0.53	0.01
SO	1.0	0.2	0.9	0.0	27.4	1.4	15.4*	0.8	8.3	0.8	24.7	1.5	11.0	0.9	0.54	0.00
EPO	0.9	0.4	1.4	0.4	34.7	1.1	12.3	1.7	5.7	1.0	29.7	3.1	8.3	0.5	0.53	0.00
MO	2.7	0.2	3.8	0.4	37.4	1.6	3.9*	0.7	10.3	0.6	26.8	2.4	3.5*	1.0	0.54	0.00

Data are mean (SEM); *n* 2 or 3 for each diet; *significantly different from LF (Student's *t*-test); ($P<0.05$ at least). Only the major fatty acids are shown; *n*-3 PUFA are not included.

Dietary lipid manipulation resulted in significant changes in the fatty acid composition of the phospholipid fraction of spleen lymphocytes. Lymphocyte phospholipids from HCO-fed animals contained a higher proportion of myristic acid (14:0) and a lower proportion of linoleic acid (both $P<0.01$) than those from the LF-fed animals. The OO-fed rats also contained a lower proportion of linoleic acid ($P<0.01$) in their lymphocyte phospholipids than the LF-fed rats. In addition, they contained a smaller proportion of arachidonic acid (20:4*n*-6; $P<0.05$), but a greatly increased proportion of oleic acid ($P<0.001$) compared with the LF-fed animals. Phospholipids from the spleen lymphocytes of the SO-fed animals contained a higher proportion of linoleic acid ($P<0.05$) than those from rats fed the LF diet. Only lymphocytes from EPO-fed rats contained γ-linolenic acid (0.7 (SEM 0.2) mol %). Phospholipids from lymphocytes of MO-fed rats incorporated only small amounts of *n*-3 PUFA (data not shown). However, they contained significantly lower proportions of linoleic ($P<0.02$) and arachidonic ($P<0.05$) acids than those of animals fed on the LF diet. These changes reflect the fatty acid composition of the diets. There was no difference in the order parameter, *S*, of spleen lymphocytes from animals fed different diets. Thus, despite significant changes in the fatty acid composition of phospholipids from spleen lymphocytes, dietary lipid manipulation does not alter their membrane fluidity.

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A 12-week programme of brisk walking and postprandial lipaemia in previously-sedentary women aged 40-55 years. By H.E. ALDRED[1], A.E. HARDMAN[1] and S. TAYLOR[2]. [1]Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Leics LE11 3TU, and [2]Department of Anaesthetics, Glenfield Hospital, Leicester LE3 9QP

Attenuation of postprandial lipaemia has been shown during recovery from a single bout of brisk walking (Aldred & Hardman, 1993). The purpose of the present study was to investigate the influence of a training programme of brisk walking on postprandial lipaemia. Twenty-six normolipidaemic previously sedentary women aged 41-55 years were randomly assigned to either a walking group (*n* 13) or control group (*n* 13). The controls continued their habitual lifestyle for the next 12 weeks while the walkers undertook a partially-supervised programme of brisk walking. Changes in endurance fitness were assessed by measuring the O_2 uptake at a reference blood lactate concentration during submaximal treadmill walking. Subjects performed an oral fat tolerance test (OFTT) pre- and post-training, refraining from exercise for 2 d before each trial. Subjects reported to the laboratory after an overnight fast and a cannula was introduced to a forearm vein. A blood sample was taken and the test meal was then ingested. This consisted of cereal, fruit, nuts, chocolate and cream; the weight of meal given provided 1.8 g dietary fat/kg subject's fat-free mass. Further blood samples were obtained 1, 2, 3, 4, 5 and 6 h after the meal. Food intake was weighed and recorded for the 2 d before the first test. The same food intake was consumed for the 2 d before the second test. Serum was analysed for triacylglycerol (TAG), total cholesterol (TC) and high-density-lipoprotein cholesterol (HDL-C). Two indices of postprandial lipaemia were adopted: (1) peak TAG concentration and (2) total lipaemic response, calculated by the area under the TAG *v.* time curve, normalized to the zero hour level. The responses of the walkers and controls were compared using the Mann Whitney U test on the pre- and post-training difference scores, adopting a 5% level of significance.

Eleven of each group completed the study, walkers completing a mean of 21(*SEM* 1) min/d of brisk walking at a speed of 1.79 (*SEM* 0.04) m/s. O_2 uptake at a reference blood lactate concentration of 3 mmol/l was increased by 2.4 ml/kg per min (12.9 (*SEM* 4.0) %) in the walkers. Changes in body mass differed in walkers (-1.1 (*SEM* 0.7) kg) and controls (+1.0 (*SEM* 0.5) kg). Of the fasting lipid concentrations, only low-density-lipoprotein cholesterol (LDL-C) and TC:HDL-C ratio differed between groups (Table). There was no change in either index of postprandial lipaemia (Table).

	Pre/Post -training	Fasting TAG (mmol/l)		TAG Area (mmol/l.h)		TAG Peak (mmol/l)		LDL-C (mmol/l)		TC:HDL-C	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Walkers	Pre	0.87	0.12	2.4	0.5	1.6	0.2	3.25	0.25	3.49	0.34
(n 11)	Post	0.80	0.11	2.6	0.4	1.6	0.2	3.07	0.26	3.32	0.29
									*		*
Controls	Pre	1.03	0.13	3.5	0.6	1.9	0.3	3.40	0.13	3.52	0.19
(n 11)	Post	1.09	0.15	3.6	0.6	2.1	0.3	3.49	0.14	3.73	0.20

* Changes significantly different between groups; $P<0.05$

These results suggest that, in women aged 40-55 years, training by brisk walking does not modify postprandial lipaemia when this is determined 48 h after the last training session.

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Aldred H.E. & Hardman A.E. (1993) Proceedings of the Nutrition Society **52**, 128A.