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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A meeting of the Nutrition Society (Clinical Metabolism and Nutritional Support Group) was held in the Armitage Centre, University of Manchester, on Thursday and Friday, 22/23 November 1990, when the following papers were read.

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Increased metabolic rate associated with cancer cachexia in mice bearing an adenocarcinoma (MAC 16). By J. C. DAY, I. D. MORRIS and N. J. ROTHWELL, *Department of Physiological Sciences, University of Manchester, Manchester M13 9PT* and M. J. TISDALE, *Pharmacological Sciences Institute, Aston University, Birmingham B4 7ET*

Severe and sustained weight loss is a common feature of many forms of malignant disease. This cachexia has been ascribed to both reductions in food intake and increases in metabolic rate, although data from animal and human studies are variable. Mice bearing a transplantable adenocarcinoma (MAC 16) exhibit weight loss without a reduction in food or water intake (Bibby *et al.* 1987). The objective of the present study was to assess food intake and metabolic rate in relation to weight loss and tumour growth in these mice.

Male and female NMRI mice (6–8 weeks) were injected subcutaneously with approximately 2×10^6 MAC 16 cells (tumour) or saline (9 g sodium chloride/l; control) and studied for 25–45 d. Development and growth rates of tumours were variable, but tumour mass was always less than 0·6 g at time of killing. Body-weight was similar for control and tumour-bearing mice for at least 18 d after implantation, thereafter the latter group lost weight at variable rates (1–15% over days 20–30). Food intake was similar for both groups of mice during the first part of the study, but over days 14–29 after implantation, intake was significantly greater in animals bearing tumours (5·0 (SE 0·1) g/mouse per d) than controls (4·5 (SE 0·1), $P<0·001$). Individual mice with the highest intakes failed to exhibit weight loss.

Resting oxygen consumption (26°), measured over periods of 2–4 h, was similar for all mice over days 1–15, but was significantly ($P<0·05$) elevated in tumour-bearing animals over days 16–20 (13% above controls) and days 21–26 (27%).

In vitro activity of brown adipose tissue (BAT), assessed from mitochondrial guanosine 5′-diphosphate binding, was increased in tumour-bearing mice in four separate experiments (15–90% above controls), but due to large variations, particularly in the MAC 16 group, the differences were not statistically significant. However, BAT activity correlated significantly ($P<0·01$) with percentage body-weight loss (r 0·75, n 10), whereas weight loss was less well correlated (r 0·6) with tumour size.

These results indicate that the primary cause of weight loss in mice bearing MAC 16 tumours is an increase in metabolic rate, possibly associated with activation of BAT. Increased food intake in tumour-bearing mice presumably represents an attempt to compensate for the hypermetabolism.

Bibby, M. C., Double, J. A., Ali, S. A., Fearon, K. C. H., Brennan, R. A. & Tisdale, M. J. (1987). *Journal of the National Cancer Institute* **78**, 539–546.

Evidence for the stimulation of human tumour growth by the amino acid L-arginine. By K. G. M. PARK[1,2], S. D. HEYS[1,2], K. BLESSING[3], M. A. MCNURLAN[2], O. EREMIN[1] and P. J. GARLICK[2], *Departments of [1]Surgery and [3]Pathology, Aberdeen University, Foresterhill, Aberdeen AB9 2ZD* and *[2]Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The amino acid L-arginine has been shown to reduce the growth and metastatic spread of a variety of tumours in rodents (Barbul, 1986). Despite the known low toxicity of arginine there have been few studies of the effect of arginine on human tumour growth. It is, however, difficult to measure accurately the growth of human tumours in vivo, with conventional static measurements of tumour bulk being insufficiently sensitive to detect the effects of relatively short courses of treatment. In this study rates of protein synthesis were measured as a dynamic assessment of the response of breast cancers to arginine. These were compared with conventional histological indices of tumour multiplication.

Twenty patients with localized breast cancer were randomized either to receive arginine supplements (30 g/d in four doses, n 10) or not (n 10), for 3 d before surgery. Rates of protein synthesis were determined, as previously described, using a 'flooding' dose technique with [1-^{13}C]leucine (Garlick *et al.* 1989). Histological sections of the tumours were labelled with the Ki67 monoclonal antibody (Dako, UK) which recognizes a nuclear antigen associated with actively dividing cells (Gerdes *et al.* 1984).

The rates of tumour protein synthesis were higher following arginine (median 25·6%/d, range 9·0–37·5%/d) than in the controls (median 9·7%/d, range 5·5–15·8%/d), ($P<0·01$, Wilcoxon rank sum). Furthermore, there was a positive correlation between Ki67 expression and the rate of tumour protein synthesis (r 0·8, $P<0·001$, Spearman's rank correlation test).

These results show that arginine stimulates synthesis in human breast cancers in vivo and that this is associated with a high number of cells in the proliferative phase of the cell cycle. This is the first evidence to indicate that L-arginine may stimulate the growth of a human tumour in vivo. This stimulation could possibly be used to sensitize tumours to the effects of cell cycle specific chemotherapeutic regimens.

KGMP was the recipient of the CMNSG Baxter research fellowship.

Barbul, A. (1986). *Journal of Enteral and Parenteral Nutrition* **10,** 227–238.

Garlick, P. J., Wernerman, J., McNurlan, M. A., Essen, P., Lobley, G. E., Milne, E., Calder, G. A. & Vinnars, E. (1989). *Clinical Science* **77,** 329–336.

Gerdes, J., Lemeke, H., Baish, H., Wacker, H. H., Schwab, U. & Stein, H. (1984). *Journal of Immunology* **133,** 1710–1716.

The effect of inhibiting gluconeogenesis on the synthesis of glycogen and lipid in cachectic tumour-bearing rats. By D. MATTHEWS, O. A. OBEID and P. W. EMERY, *Department of Food and Nutritional Sciences, King's College, London W8 7AH*

Hepatic glycogenesis following a meal is increased in tumour-bearing rats (Obeid & Emery, 1991). In order to determine whether this is associated with increased gluconeogenesis we have measured glycogen synthesis in the presence of 3-mercaptopicolinic acid (MPA), which suppresses gluconeogenesis by inhibiting the enzyme phosphoenolpyruvate carboxykinase. Fourteen male Fischer 344 rats bearing a transplantable Leydig cell tumour (TB) and 14 sham-injected controls were fasted overnight, and half the rats in each group were injected intraperitoneally with 45 mg MPA. One hour later all the rats were tube-fed a 16 kJ liquid meal and immediately injected intraperitoneally with ^3H_2O. The rats were killed 1 h later and the livers and epididymal fat pads were analysed for ^3H incorporation into glycogen and saponifiable lipid.

						Analysis of variance		
		C	TB	C+MPA	TB+MPA	Tumour	MPA	Interaction
Blood glucose (mg/l)	Mean	1450	1460	1180	1220	NS	$P<0.01$	NS
	SE	30	50	60	100			
Glycogen synthesis*	Mean	37·26	61·18	7·31	5·91	$P<0.01$	$P<0.01$	$P<0.01$
	SE	4·48	3·97	2·83	1·80			
Lipid synthesis†:								
Liver	Mean	10·62	9·03	16·55	11·35	$P<0.01$	$P<0.01$	NS
	SE	1·56	1·15	1·36	1·01			
Fat pad	Mean	2·87	3·75	2·70	1·81	$P<0.01$	$P<0.01$	$P<0.01$
	SE	0·39	0·32	0·48	0·31			

NS, not significant.
* μmol ^3H_2O incorporated into glycogen/h per g tissue.
† μmol ^3H_2O incorporated into saponifiable lipid/h per g tissue.

In control rats MPA treatment caused a massive decrease in glycogenesis, confirming that most of the glycogen synthesized after a meal is formed via gluconeogenesis. MPA treatment caused an even greater reduction in glycogenesis in TB rats, and abolished the differences between TB and controls. This indicates that increased glycogenesis in TB rats is entirely dependent on increased gluconeogenesis. The residual rate of glycogen synthesis in MPA-treated rats may be coming from the fructose moiety of the sucrose in the test meal, as well as by direct synthesis from glucose. Hepatic lipogenesis was increased by MPA treatment, presumably because of increased precursor availability. In contrast, MPA treatment decreased lipogenesis in adipose tissue, probably because of decreased blood glucose. However, in both tissues lipogenesis was lower in MPA-treated TB rats than in corresponding controls. It is thus unlikely that chronic inhibition of gluconeogenesis would increase body fat content.

Financial assistance from the Hariri Foundation is gratefully acknowledged. MPA was a gift from Smith Kline and French Ltd.

Obeid, O. A. & Emery, P. W. (1991). *Proceedings of the Nutrition Society* **50**, 141A.

Rates of synthesis of glycogen and lipid following a meal in cachectic tumour-bearing rats.

By O. A. OBEID and P. W. EMERY, *Department of Food and Nutritional Sciences, King's College, London W8 7AH*

We have previously suggested that decreased lipogenesis in tumour-bearing rats may be due to decreased food intake (Obeid & Emery, 1990). We have therefore measured lipogenesis and glycogenesis in response to a standard-sized meal. Male Fischer 344 rats bearing a transplantable Leydig cell tumour (TB) and sham-injected controls were fasted overnight and given a 16 kJ liquid meal by gavage. At defined time intervals after the meal, groups of five rats were injected intraperitoneally with ^3H_2O and killed 1 h later.

Period after tube feeding (h) . . .		0	1	2	3	6
Hepatic lipogenesis†:						
TB:	Median	3·55	8·14*	9·64	7·94	6·77*
	Range	2·7–4·7	6·5–10·1	7·3–11·8	7·0–9·6	5·2–7·0
C:	Median	3·43	12·28	10·18	8·49	4·74
	Range	3·2–3·8	9·5–24·6	8·9–12·6	7·2–10·8	3·9–5·8
Adipose tissue lipogenesis†:						
TB:	Median	1·35	1·15*	1·12	1·01	1·12*
	Range	0·8–1·8	0·9–1·8	0·8–1·7	0·8–1·6	1·1–1·3
C:	Median	1·20	2·08	1·41	1·13	0·85
	Range	0·8–1·7	1·5–2·2	0·8–3·2	0·8–1·7	0·6–1·0
Hepatic glycogenesis‡:						
TB:	Median	5·19*	76·47*	122·60**	112·70**	1·05
	Range	0·7–10·5	63·4–101·2	74·9–136·2	40·8–115·8	0·6–8·1
C:	Median	0·25	40·49	43·79	10·21	1·02
	Range	0·0–0·6	14·4–63·6	42·6–51·0	2·2–28·7	0·4–2·3

TB, tumour-bearing rats; C, control rats.
Significantly different from control value (Mann-Whitney test): $*P<0·05$, $**P<0·01$.
† μmol tritiated water incorporated into saponifiable lipid/g tissue per h.
‡ μmol tritiated water incorporated into glycogen/g tissue per h.

Hepatic lipogenesis rose after the meal by much less in TB rats than in controls, and in adipose tissue there was no post-prandial rise in TB rats. Glycogen synthesis rose to a much higher peak, and the increase was sustained for longer, in TB rats. We conclude that lipogenesis in TB rats does not respond to nutrient ingestion in the normal way. The reduced post-prandial rise in lipogenesis may contribute to loss of body fat. Increased glycogenesis is likely to be associated with increased gluconeogenesis, which may in turn contribute to increased energy expenditure. Moreover, the prolonged high rate of glycogenesis may act to inhibit the initiation of the next meal, thereby causing the increased interval between meals which is the main feature of the anorexia observed in these rats.

Obeid, O. A. & Emery, P. W. (1990). *Proceedings of the Nutrition Society* **49**, 40A.

Increased whole-body and skeletal muscle protein synthesis in weight-losing cancer patients. By T. PRESTON, *SURRC, East Kilbride G75 0QH*, D. C. MCMILLAN, *Department of Surgery, Royal Infirmary, Glasgow G4 0SF*, K. C. H. FEARON and O. J. GARDEN, *Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW*

We have reported previously that when rates of whole-body protein synthesis (WBPS) are estimated using a primed constant infusion of [^{15}N]glycine, weight-losing cancer patients have an increased rate compared with healthy controls (Fearon *et al.* 1988). In a subsequent study to determine the role of the liver in such increased tracer flux, we demonstrated that the non-export component of hepatic protein synthesis was reduced rather than increased (Fearon *et al.* 1990). The aim of the present study was to examine further the process of WBPS and to determine whether skeletal muscle might be the site of the increased rates of synthesis derived from the whole-body tracer kinetics.

Five patients with colonic cancer, who had lost an average of 10% of their body-weight, were entered into the study. WBPS and skeletal (abdominal) muscle protein synthesis (MPS) were measured simultaneously, using a primed constant infusion of [^{15}N]glycine. Values (g protein/d) obtained were compared with those from weight-stable non-cancer controls (*n* 6).

	WBPS		MPS		MPS/WBPS	WBPS–MPS	
Patients	Mean	SE	Mean	SE	(%)	Mean	SE
Control	217	31	65	10	30	152	49
Cancer	345*	35	161	22	47	184	54

*$P<0{\cdot}05$.

Protein synthesis in skeletal muscle was found to be significantly elevated in the cancer patients. When muscle mass was calculated from urinary creatinine excretion this increase in muscle protein synthesis accounted for most of the rise observed in the whole body. Clearly muscle contains proteins apart from actin and myosin and therefore the precise nature of the increased synthesis noted in the biopsy cannot be ascertained from these results. Moreover, because of the assumptions involved with flux measurements in mixed proteins using a single amino acid tracer, these findings will require confirmation by methods which rely on a different model.

Fearon, K. C. H., Hansell, D. T., Preston, T., Plumb, J. A., Davies, J., Shapiro, D., Shenkin, A., Calman, K. C. & Burns, H. G. J. (1988). *Cancer Research* **48**, 2590–2595.

Fearon, K. C. H., McMillan, D. C., Preston, T., Winstanley, P., Cruikshank, A. M. & Shenkin, A. (1990). *Proceedings of the Nutrition Society* **49**, 167A.

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Whole-body protein turnover and the acute-phase response in relation to the survival of colon cancer patients. By K. C. H. FEARON[1], D. C. MCMILLAN[2], T. PRESTON[3], D. T. HANSELL[2], A. SHENKIN[4] and O. J. GARDEN[1], [1]*Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW,* [2]*Department of Surgery, Royal Infirmary, Glasgow G4 0SF,* [3]*SURRC, East Kilbride G75 0QU* and [4]*Department of Chemical Pathology, Royal Liverpool Hospital, Liverpool L7 8XW*

The aim of this study was to determine if there is a relationship between duration of survival and the whole-body protein turnover (WBPT) or acute-phase protein response (APPR) of patients with colon cancer. Patients with a range of clinical diseases (*n* 32; Duke's B, *n* 11; C, *n* 8; D, *n* 13) underwent measurement of WBPT (primed constant 24 h infusion of [^{15}N]glycine) and assessment of their APPR (C-reactive protein (CRP), albumin (Alb) and CRP:Alb ratio) before resective surgery or diagnostic laparotomy. All patients were followed for a minimum of 5 years and survival noted. Survival analysis was performed using Cox's proportional hazards model. The results of the univariate analysis are shown in the Table.

Variable	χ^2	$P=$
Log (CRP):Alb	15·3	0·0001
Log (CRP)	13·3	0·0003
Alb	10·0	0·0016
Stage of disease (Duke's)	11·1	0·0039
WBPT	4·5	0·0348

In addition to the stage of disease, there was a significant relationship between each of the metabolic variables studied (log (CRP), Alb, WBPT) and the ultimate survival of the patients. In a bivariate analysis including stage of disease plus one other variable, log (CRP) and Alb were significant predictive factors ($P<0·02$) but WBPT was not. With a multivariate analysis including log (CRP), Alb and stage of disease, only Alb was an independent predictor of survival ($P<0·03$). Stage of disease and log (CRP) were sufficiently closely related that they were not independent predictors but contributed jointly to the power of the model.

These results suggest that the nutritional and metabolic consequences of continuing tumour growth as documented by an APPR and elevated WBPT are not only a reflection of tumour burden but, in the case of Alb, are also a reflection of the imbalance in the host–tumour relationship which, independent of stage of disease, can ultimately be related to the death of the patient.

Effect of vitamin E deficiency on the response of brain protein synthesis to anaesthesia with volatile anaesthetic agents. By K. FERGUSON[1,2], G. DUTHIE[1], A. G. S. BAILLIE[1], J. ARTHUR[1], S. D. HEYS[1,3], C. R. DUNDAS[1,2] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and *Departments of* [2]*Anaesthesia* and [3]*Surgery, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

Halothane is a potent producer of free radicals during its metabolism. Enflurane and isoflurane have less biotransformation and are not known to produce free radicals during their metabolism. We have demonstrated previously that all these volatile anaesthetic agents significantly depress brain protein synthesis in the rat in vivo (unpublished). The pathogenesis of this iatrogenic effect is unclear. Free radicals may produce disturbances in protein synthesis by means of lipid peroxidation and changes in membrane structure and function or by damaging enzymes associated with the endoplasmic reticulum. The aim of this study was therefore to determine the effect of volatile anaesthetic agents on tissue protein synthesis in vivo in a free-radical-susceptible animal model.

Groups of male rats were randomly assigned to receive either a vitamin E-deficient or sufficient diet. Vitamin E deficiency produces a free-radical-susceptible state. After 6 weeks on the vitamin E-deficient diet, groups of six animals were treated with halothane (1·4%), enflurane (1·5%), isoflurane (1·8%), or air (control). Exposure to anaesthesia lasted 1 h and the rate of brain protein synthesis was measured during the final 10 min of anaesthesia by incorporation of [^{3}H]phenylalanine into tissue protein as described by Garlick *et al.* (1980).

	Rate of protein synthesis (%/d)							
	Control		Halothane		Enflurane		Isoflurane	
Vitamin E	Mean	SE	Mean	SE	Mean	SE	Mean	SE
+	15·0	0·3	12·6	0·4	13·3	0·4	14·3	0·7
−	15·1	0·7	13·2	0·5	13·9	0·4	13·8	0·7

Plasma vitamin E levels showed that the animals on the deficient diet had significantly lower vitamin E levels than those on the sufficient diet (control +vitamin E 4·1 (SE 0·4) μg/ml and control −vitamin E 0·3 (SE 0·1) μg/ml). The free radical state was assessed by means of plasma malondialdehyde levels and the vitamin E-deficient groups had significantly raised levels compared with the sufficient groups. The results in the Table demonstrate that there is a depression in brain protein synthesis during exposure to the volatile anaesthetic agents, but this decrease is not influenced by vitamin E deficiency. This suggests therefore that the depression in brain protein synthesis is not related to the production of free radicals.

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.

Effects of dobutamine on energy expenditure and substrate mobilization. By CERI J. GREEN[1], S. FRAZER[1], S. UNDERHILL[1], PAULA MAYCOCK[2], JUDITH FAIRHURST[2], and I. T. CAMPBELL[1]*, [1]*University Department of Anaesthesia, Royal Liverpool Hospital, Liverpool L69 3BX* and [2]*North Western Injury Research Centre, Hope Hospital, Salford 6*

Dopamine and dobutamine are adrenergic drugs used to support myocardial function in critical illness. Adrenaline and noradrenaline are both known to have widespread metabolic effects with elevations in oxygen consumption ($\dot{V}_{O_2}$) and stimulation of lipolysis and glycogenolysis. It has recently been shown that dopamine has metabolic effects ($\dot{V}_{O_2}$ increased by 14% at 10 μg/kg per min) (Campbell *et al.* 1988) but there are no such data for dobutamine.

Eight healthy male volunteers aged 22–42 (median 32) years were infused with dobutamine in 50 g glucose/l at 0, 2, 5 and 10 μg/kg per min over three consecutive periods of 45 min each. $\dot{V}_{O_2}$ was measured throughout and blood taken at 30 and 45 min of each infusion period for measurement of free fatty acids (FFA), glycerol, glucose, insulin, adrenaline, noradrenaline, dopamine and dobutamine.

$\dot{V}_{O_2}$ increased in proportion to the rate of infusion and was significantly higher than during infusion of glucose alone ($P<0{\cdot}001$) (specifically) at 5 and 10 μg/kg per min ($P<0{\cdot}01$). At 10 μg/kg per min $\dot{V}_{O_2}$ was 33% higher than during the glucose control infusion. Insulin, glycerol and free fatty acids all increased significantly ($P<0{\cdot}01$), the last two by 150 and 225% respectively. Respiratory exchange ratio decreased significantly ($P<0{\cdot}01$) during dobutamine infusion compared with glucose alone. Adrenaline and noradrenaline were depressed by dobutamine infusion ($P<0{\cdot}05$) but recovered to pre-infusion levels on stopping the infusion. Dopamine remained below 0·1 nmol/l during both infusions. Dobutamine concentrations increased in proportion to the rate of infusion and both systolic and diastolic pressure increased ($P<0{\cdot}001$), the former by 30–40 mm Hg at 10 μg/kg per min.

It is concluded that like dopamine, dobutamine increases $\dot{V}_{O_2}$ and stimulates lipolysis but the thermogenic and lipolytic effects are more marked than with dopamine. Unlike dopamine, however, it promotes fat oxidation, decreases blood glucose levels and depresses endogenous catecholamine secretion.

Campbell, I. T., Regan, C., Duckworth, R., Fairhurst, J., Maycock, P. & Frayn, K. (1988). *Proceedings of the Nutrition Society* **47**, 60A.

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Changes in plasma catecholamines following surgery: the effect of body heat conservation or epidural anaesthesia. By F. CARLI and J. PEARSON, *Northwick Park Hospital, Harrow, Middlesex HA1 3UJ* and I. A. MACDONALD, *University of Nottingham Medical School, Nottingham NG7 2UH*

Surgery is associated with increased circulating concentrations of adrenaline and noradrenaline. This study determined the effect of perioperative epidural anaesthesia or warming on the sequential changes of these catecholamines during the first 24 h after surgery.

Twenty-one patients scheduled for major abdominal operations were randomly allocated into three equal groups. Group A (control) received routine anaesthetic care. In group B (normothermia), patients' body temperatures were maintained during surgery by active warming. At the end of surgery the group B patients were transferred to a thermostatically-controlled room (28–30°) for 24 h. Patients in group C received epidural blockade (T4-S5 with bupivicaine) which was established before surgery and maintained for 24 h. Otherwise general anaesthesia, surgery, fluid replacement and post-operative care were similar for all groups. Before the induction of anaesthesia a cannula was placed in a radial artery, and blood samples taken preoperatively, at the end of surgery and 1, 2, 3, 4, 8 and 24 h post-operatively for the measurement of plasma adrenaline and noradrenaline (Macdonald & Lake, 1985).

Both adrenaline and noradrenaline data were log transformed before statistical analysis.

In groups A and B, surgery was accompanied by increases in plasma noradrenaline (from baselines of 2·68 and 2·25 nmol/l to 6·26 and 3·78 in A and B respectively, $P<0·001$) and adrenaline (from 0·86 to 7·52 in A and 0·53 to 2·50 in B, $P<0·001$). By contrast, there were no significant changes in either catecholamines in group C. The changes during surgery for both adrenaline and noradrenaline were significantly different between groups ($P<0·001$).

During the post-operative period a significant quadratic trend was found for noradrenaline only in group A ($P = 0·017$). In this same group the peak was reached 3 h after surgery (9·75). In group B there was a rapid increase during the first hour and afterwards the level remained constant (peak value at 4 h = 5·33). There was a highly significant linear trend in adrenaline ($P<0·001$) which was different between groups ($P<0·001$). In groups A and B, adrenaline levels decreased to reach preoperative values by 24 h and 8 h respectively. There were no changes post-operatively in group C in either catecholamine.

Thus, preventing hypothermia markedly reduces, and epidural anaesthesia abolishes, the catecholamine responses to abdominal surgery. It remains to be determined whether this influences post-operative metabolism.

Macdonald, I. A. & Lake, D. M. (1985). *Journal of Neuroscience Methods* **13**, 239–248.

Protein synthesis rate in human skeletal muscle is unaffected by anaesthesia but decreases after abdominal surgery irrespective of total parenteral nutrition. By PIA ESSÉN[1], MARGARET A. MCNURLAN[2], JAN WERNERMAN[1], GRAHAM A. CALDER[2] and PETER J. GARLICK[2], [1]*Department of Anaesthesiology and Intensive Care at Huddinge University Hospital, St Göran's Hospital, Stockholm, Sweden* and [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Several components contribute to the stress imposed by surgery. This study was undertaken to assess the separate impact of anaesthesia, surgery and post-operative nutritional state on protein metabolism in man. We have recently developed the 'flooding dose' technique for assessing protein synthesis in human muscle with [1-^{13}C]leucine (Garlick *et al.* 1989). The method is very appropriate for this type of study, since its ability to make rapid and sequential measurements allows acute responses to be systematically investigated.

Protein synthesis in muscle was studied in four groups of metabolically-healthy patients undergoing elective abdominal surgery. In all patients, the basal determination of muscle protein synthesis was performed in the post-absorptive state just before surgery. The second assessment was performed during anaesthesia (*n* 7), immediately after surgery (*n* 7), and 3 d post-operatively in patients receiving saline (9 g sodium chloride/l) only (*n* 8), or total parenteral nutrition (TPN) of 135 kJ/kg per 24 h and 0·2 g nitrogen/kg per 24 h (*n* 9). The direct effect of TPN on muscle synthesis was not evaluated as patients in the TPN group were studied after an overnight fast.

For each measurement of muscle protein synthesis rate, percutaneous biopsies were taken from the quadriceps femoris muscle before and 90 min after an intravenous injection of [1-^{13}C]leucine (0·05 g/kg, 20 atoms % excess). The rate of muscle protein synthesis was calculated from the increase in enrichment of leucine in protein during the 90 min period, using isotope ratio mass spectrometry. The enrichment of plasma α-ketoisocaproate was used to indicate the enrichment of the precursor for protein synthesis and analysed by gas chromatography mass–spectrometry.

Protein synthesis in quadriceps muscle (%/d)

	Anaesthesia		Surgery		Surgery+3 d fast		Surgery+3 d TPN	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal	1·97	0·18	2·17	0·07	2·34	0·13	1·98	0·07
Final	2·01	0·18	1·55**	0·15	1·31**	0·18	0·91***	0·14

**$P<0·01$, ***$P<0·001$.

Protein synthesis rate was unaffected by general anaesthesia alone. However, surgery, in the form of cholecystectomy, resulted in a rapid decrease in protein synthesis to about 70% of the basal value. When the effect of surgery was combined with 3 d post-operative fasting, the rate of protein synthesis declined further to 55% of the basal level. Maintenance of adequate nutrition in the 3 d post-operative period by TPN, however, did not alter the decline in protein synthesis. Thus, the immediate metabolic response to surgery was rapid and substantial. The decrease in muscle protein synthesis was still apparent after 3 d, irrespective of post-operative nutrition.

Garlick, P. J., Wernerman, J., McNurlan, M. A., Essén, P., Lobley, G. E., Milne, E., Calder, G. A. & Vinnars, E. (1989). *Clinical Science* **77**, 329–336.

Post-operative protein kinetics. The effect of nursing patients for 24 h in a thermoneutral environment. By J. WEBSTER[1], F. CARLI[1], M. PEARSON[2], M. READ[3], S. VENKATESAN[1] and D. HALLIDAY[3], [1]*Division of Anaesthesia*, [2]*Dietetics Department* and [3]*Nutrition Research Group, Clinical Research Centre, Harrow, Middlesex HA1 3UJ*

Surgery is associated with changes in plasma and muscle tissue amino acids resulting in increased losses of nitrogen. The maintenance of normothermia during and immediately after surgery has been shown to reduce protein loss from the body. In this study, stable isotopes were used to determine whole-body protein breakdown, oxidation and synthesis in sixteen patients undergoing surgery for adenocarcinoma of the colon or rectum. They were randomly allocated to two groups. The control ('cold') group were maintained in a room at 19–23° and received routine intraoperative and post-operative care. Patients in the 'warm' group were maintained at normothermia during anaesthesia and surgery and nursed after surgery in a warm room (ambient temperature 28–30°) for 24 h. A controlled diet of 0·1 g N and 84 kJ (20 kcal)/kg per d was provided orally for 7 d before surgery and intravenously for 4 d post-operatively. Urine collections (24 h) were made two consecutive days before surgery and for 4 d after surgery and analysed for N, creatinine, adrenaline and cortisol. Whole-body protein kinetics were determined before surgery and 2 and 4 d post-operatively using a primed continuous infusion of L-[l-^{13}C]leucine (0·5 mg/kg per h) for 4 h. Protein turnover was measured.

Cumulative N excretion was significantly greater after surgery in the cold group (5·5 (SE 1·0) mmol N/mmol creatine per kg per d) compared with the warm group (4·1 (SE 1·2) mmol) ($P<0·05$). Leucine flux increased significantly in both groups 2 and 4 d after surgery. The increase was significantly greater in the cold group (28%) than the warm group (18%) ($P<0·04$) 2 d post-operatively. Oxidation increased significantly in the cold group on day 2 (24%) ($P<0·001$) and remained elevated on day 4 ($P<0·001$). The warm group showed a significant elevation only on day 4 ($P<0·01$). Protein synthetic rate increased significantly in both groups on days 2 and 4 ($P<0·01$) but no differences were observed between the groups.

Adrenaline excretion increased significantly after surgery in both groups ($P<0·05$). The increase was greatest in the cold group, 1 and 4 d ($P<0·05$) after surgery. Urinary cortisol excretion increased in both groups but the increase was significantly less in the warm group on days 1, 2 and 3 ($P<0·05$).

It is concluded that maintenance of thermoneutrality diminishes the response of cortisol and adrenaline to surgery. The attenuated protein breakdown and amino acid oxidation observed in the warm group after surgery might partially be explained by the specific hormonal suppression achieved with thermoneutrality.

Mechanisms involved in the fever and hypermetabolism induced by turpentine injection in the rat. By A. L. COOPER[1], E. KIRKMAN[2], R. A. LITTLE[2], S. J. HOPKINS[3] and N. J. ROTHWELL[1], [1]*Department of Physiological Sciences*, [2]*North Western Injury Research Centre, University of Manchester M13 9PT* and [3]*Department of Rheumatology, Hope Hospital, Salford M30 8HD*

Intramuscular injection of turpentine in laboratory rodents is associated with a sustained fever and hypermetabolism (McWilliams *et al.* 1989). The aim of the present study was to assess the involvement of neural and non-neural (cytokine) mechanisms in both the development and the maintenance phase of the response to turpentine injection in the rat.

In all experiments, adult male Sprague-Dawley rats received an intramuscular injection (0·6 ml) of turpentine or sterile saline (9 g sodium chloride/l). Oxygen consumption ($\dot{V}_{O_2}$) and colonic temperature (Tc) measurements were made either 0–2 or 16–18 h later (at 24°). In one group of rats, peripheral neurones (C-fibre afferents) were depleted by intraperitoneal injection of capsaicin (50 mg/kg), 2 d after birth.

Turpentine injection alone, caused a 32 (SE 5)% rise in $\dot{V}_{O_2}$ and a 0·8 (SE 0·2)° rise in Tc by 2 h (n 8). These responses were significantly reduced ($P<0·001$, ANOVA) in capsaicin-treated rats (12 (SE 4)% rise in $\dot{V}_{O_2}$ and 0·3 (SE 0·1)° rise in Tc). Eighteen hours after turpentine injection, there was no difference in the $\dot{V}_{O_2}$ and Tc of capsaicin- (n 6) and control- (non-capsaicin)-treated (n 5) rats. Both groups exhibited an elevated colonic temperature (39·0°) and $\dot{V}_{O_2}$ (25%) compared with respective control groups ($P<0·001$, ANOVA).

The plasma concentration of interleukin-l (IL-l) did not change after turpentine injection. The plasma concentration of interleukin-6 (IL-6) was elevated 4 h after turpentine injection (856 (SE 223) pg/ml, range 402–1771 pg/ml), compared with saline-injected animals (21 (SE 7) pg/ml, range 0–36 pg/ml). A peak response ($P<0·001$, ANOVA), was observed 12 h after injection (12 526 (SE 3030) pg/ml, range 1976–20 973 pg/ml). By 24 h, the concentration of IL-6 was declining, although still elevated compared with saline-injected rats (2151 (SE 900) *v.* 50 (SE 19) pg/ml, range in turpentine group, 421–11 461 pg/ml, in saline group, 0–127 pg/ml). The peak plasma IL-6 concentration correlated with the fever observed in turpentine-treated rats.

These results indicate that different mechanisms are involved in the early and later responses to turpentine injection. The initial response (0–4 h) is dependent, at least in part, on peripheral neural afferents whilst the sustained response (18 h) appeared to have no peripheral neural component. This latter phase may be mediated by IL-6.

McWilliams, G., Burns, H. J. G., Fearon, K., Carter, K. & Shenkin, A. (1989). *Proceedings of the Nutrition Society* **48,** 70A.

Nutritional assessment using simple pulmonary function studies. By R. DAS-GUPTA[1], A. W. GOODE[1], A. WADE[2], J. POWELL-TUCK[3], J. TREASURE[4], P. WOODRUFF[4] and N. S. WILLIAMS[1], [1]*Surgical Unit,* [2]*Department of Physiology,* [3]*Department of Human Nutrition, London Hospital, Whitechapel, London E1* and [4]*Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London SE5*

In order to find simple methods for monitoring progress during nutrition repletion, six non-smoker female anorexic patients suffering no lung pathology were studied before and after supervised inpatient enteral support (12·55 MJ (3000 kcal)/d). Mean age 25·3 (SEM 3), mean body mass index 15·7 (SEM 0·9), mean weight 40·3 kg (SEM 1·45), and attained weight 51·5 kg (SEM 1·35). The increment in lean body mass during this treatment was measured using a ten-sensor whole body 40K detector which had previously been calibrated using 112 lean normal subjects, of which 56 were women. Patients increased their mean (SEM) total body potassium (TBK) from 1954 (115) mmol to 2294 (129) mmol, and thus approached after treatment, the mean 2446 (58) mmol TBK seen in the 56 control women, of similar mean age and height. This increment was compared with changes in other more simple measurements both of body composition and of muscle function.

Serum albumin, total protein and leucocyte values were normal in all cases. No significant improvement in hand-grip strength was recorded. Mid-arm muscle circumference (MAMC), body mass index (BMI, weight/height^2) and per cent body fat (% Fat) as measured by anthropometry improved, but were not correlated with improvement in TBK.

Lung function was measured using a computer-dependent microloop spirometer. Forced expiratory volume in 1 min (FEV_1), peak expiratory flow rate (PEF), vital capacity (VC), peak inspiratory flow rate (PIF), were measured. FEV_1 and PEF were found to be significantly correlated with restoration of LBM.

	Body-wt (kg)		Increase in:					
Subject	Initial	Final	TBK (mmol)	% Fat	BMI	FEV_1 (litres)	PEF (l/min)	MAMC (bar)
A	43·0	55·8	533	8·18	4·74	0·2	40	3·1
B	38·3	49·1	268	14·6	6·77	0·4	69	5·4
C	34·5	47·3	811	6·04	5·64	1·6	90	4·2
D	44·5	54·3	152	6·8	3·7	0·4	15	2·6
E	40·8	52·7	209	4·9	2·1	0·2	42	0·9
F	40·5	49·3	72	8·0	3·9	0·0	9	2·0
Correlation coefficient with TBK			1	0·15	0·46	0·83	0·78	0·46
Significance of correlation				NS	NS	P=0·04	P=0·05	NS

NS, not significant.

FEV_1 and PEF appear to be promising as sensitive measures of nutritional improvement, in the management of non-smoking malnourished patient with previously healthy lungs.

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The use of C-reactive protein, transferrin and prealbumin in monitoring the nutritional status of patients receiving total parenteral nutrition. By F. D. L. FINLAY[1], A. S. HUTCHISON[1] and J. C. FERGUSON[2], [1]*Department of Clinical Biochemistry* and [2]*Surgical Unit, Southern General Hospital, Glasgow*

Biochemical monitoring of patients receiving nutritional support employs the measurement of proteins such as albumin, transferrin and prealbumin. However, during periods of trauma or infection the concentration of these proteins will be affected by the acute-phase reaction (APR), as well as by nutritional factors, which limit their usefulness as monitors of nutritional status. The aims of the present study were to measure transferrin and prealbumin concentrations as markers of nutritional status in patients receiving total parenteral nutrition (TPN), and to try to improve the interpretation of these proteins as pure nutritional markers by use of C-reactive protein (CRP) as a measure of the APR.

All patients given TPN (providing 14 g N and 10·46 MJ (2500 kcal) in 3 l/d) in the general surgical wards of the hospital over an 18-month period were included in the study (thirty-five patients, mean age 62 years, mean duration of TPN 18 d (range 4–48 d)). Blood samples were taken before commencing TPN and then twice weekly. Transferrin and prealbumin were measured by immunoturbidimetry and CRP by a fluorescence polarization immunoassay. A CRP concentration of >50 mg/l was taken to indicate a significant APR.

Table. *Nutritional markers in plasma after 2 weeks of TPN*

	All patients (*n* 19)				Patients with CRP >50 mg/l excluded (*n* 9)			
	Day 1		Days 14–16		Day 1		Days 14–16	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Albumin (g/l)	25	4·7	26	4·9	25	5·1	28*	5·4
Transferrin (g/l)	1·40	0·30	1·52	0·33	1·40	0·38	1·70*	0·34
Prealbumin (mg/l)	83	41	111*	52	82	44	129	60

*$P<0·05$.

After 2 weeks of treatment with TPN, initial analysis showed a significant change only in prealbumin concentration, but excluding patients with elevated CRP levels from the analysis produced significant changes in all three proteins (Table). Prealbumin also showed a greater percentage increase (65%) than transferrin (25%) over 15 d in eight patients who had low CRP values throughout their treatment with TPN.

These results suggest that prealbumin is the most useful of the protein markers studied, and that a more specific and reliable use of transferrin and albumin as markers of nutritional status can be achieved if CRP concentrations are first used to identify the periods of the APR.

Six-month responses to growth hormone therapy for growth disorders are predicted by early changes in body composition and energy expenditure. By J. GREGORY[1], S. A. GREENE[1], R. T. JUNG[2], C. M. SCRIMGEOUR[3] and M. J. RENNIE[3], *Departments of* [1]*Child Health,* [2]*Medicine* and [3]*Anatomy and Physiology, University of Dundee, Dundee*

It is well recognized that growth hormone (GH) induces anabolic changes during therapy for short stature. The monitoring of its efficacy normally requires the measurement of height velocity at intervals of not less than 3 months, and classically of 1 year, because of the relative lack of precision in the measurement of standing height. We therefore attempted to determine whether early changes in body composition and energy expenditure, as indices of net anabolism, would be good predictors of increases in growth velocity only discernible over the longer term.

We studied fifteen children receiving GH (0·6 IU/kg per d) for a variety of clinical conditions (panhypopituitarism (*n* 2), isolated GH deficiency (*n* 4), Turner syndrome (*n* 4), and normal variant short stature (*n* 5)). All were studied before and at 6, 12 and 26 weeks of therapy. We measured body composition (by skinfold methods (Durnin & Rahaman (1967) for adolescents; Brook (1971) for younger children), resting energy expenditure (REE) (by ventilated-hood indirect calorimetry) and total daily energy expenditure (TEE) (by the doubly-labelled water method).

Height velocity changes at 6 weeks were not discernible but at 12 and 26 weeks the observed increases were significant (height velocities: pre-treatment, 49 (SEM 4); 12 weeks, 86 (SEM 6); 26 weeks, 86 (SEM 5) mm/year). Despite the lack of further increments in longitudinal growth after 12 weeks, fat-free mass continued to rise (% increases: 0–6 weeks, 5·9 (SEM 0·7); 0–12 weeks, 7·7 (SEM 1·0); 0–26 weeks, 12·4 (SEM 1·3)). These results indicate major changes in body composition which were early and sustained.

Correlation analysis indicated that changes at 6 weeks in body composition, and in both resting and total energy expenditure, accurately predicted the 26-week height velocity increments (Spearman Rank correlation coefficients as follows: % decrease in fat mass, −0·75 (P=0·005); increment in REE, 0·75 (P=0·007); TEE, 0·60 (P=0·02)).

The results suggest that early changes in body composition and energy expenditure are predictive of responses of height to GH therapy before such increases are themselves discernible. Furthermore, it is possible that monitoring such indices may enable prediction of long-term efficacy even earlier, perhaps in the first 2 weeks of therapy.

Supported by the Biomedical and Clinical Research Committee of the Scottish Home and Health Department, Child Growth Foundation, Novo Nordisk Gentofte and the University of Dundee.

Brook, C. G. D. (1971). *Archives of Disease in Childhood* **46,** 182–184.
Durnin, J. V. G. A. & Rahaman, M. M. (1967). *British Journal of Nutrition* **21,** 681–689.

Assessment of changes in total body water in patients undergoing renal dialysis using bioelectrical impedance analysis. By SUSAN A. JEBB and M. ELIA, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

The ability to measure body water in a simple, non-invasive manner at the bedside using bioelectrical impedance analysis has tremendous potential. Patients requiring dialysis represent a group in which there are rapid changes in body-weight, which are almost entirely attributable to water and which can be precisely quantified. Thus these patients provide a good model in which to establish the relationship between changes in body water and changes in the measured impedance. The present study aimed to assess this relationship and to examine whether changes in fluid can be accurately predicted from the change in whole-body impedance with an appropriate correction for the conductor length (i.e. height).

Eight studies were performed on six patients (five male and one female) with chronic renal failure and requiring dialysis. Two male subjects were studied on two separate occasions with a similar weight at the start of each study. Subjects had a mean (SD) age of 40·1 (12·1) years, height 1·73 (0·08) m and weight 57·4 (10·3) kg. Impedance (Holtain Ltd) was measured using a tetrapolar system with electrodes positioned on the wrists and ankles. The position of the electrodes was marked and care was taken to ensure that the subjects were in a supine position on each occasion with the limbs slightly abducted.

A baseline measurement of whole-body impedance was made immediately before the start of dialysis and between three and nine measurements per subject were performed during dialysis. On each occasion the amount of fluid removed was noted from the dialysis machine (specified accuracy 1%). The measured loss of fluid from the beginning to the end of dialysis agreed well with the change in body-weight. Subjects were dialysed for a mean (SD) of 3·75 (1) h and 1174 (706) ml of water were removed at a rate of 5·3 (2·9) ml/min.

In theory the change in the conducting volume (i.e. total body water$_1$ − total body water$_2$) is proportional to (height^2/impedance$_1$) − (height^2/impedance$_2$), where subscripts 1 and 2 describe the measurement before and after dialysis respectively and height is used as an index of conductor length. The regression lines of this plot for each subject had correlation coefficients ranging from 0·880 to 0·999. However, although there was a consistent change in the impedance with the loss of water in individuals, there was great variability between subjects, and indeed also for the two individuals who were studied on two separate occasions. The mean (SD) change in (height^2/impedance$_1$) − (height^2/impedance$_2$) for the removal of 0·5, 1 and 2 litres of water was 1·75 (0·96), 3·41 (1·55) and 6·72 (2·91) m^2/ohms respectively.

The cause of this variability has not been identified, but possible explanations include changes in electrolyte concentration which will alter the specific resistivity of the body and the disproportionate loss of water from body segments which have a variable impedance relative to their size or water content (Fuller & Elia, 1989).

The lack of a fixed relationship between changes in impedance and changes in body water precludes the use of the impedance technique as a means of accurately assessing small fluid changes in this patient group. This study highlights the potential for error when the impedance technique is used to assess body composition in subjects with abnormal or changeable water status. In such situations, which may be common in clinical practice, results from the method should be interpreted with great caution.

Fuller, N. J. & Elia, M. (1989). *European Journal of Clinical Nutrition* **43**, 779–791.

Percutaneous endoscopic gastrostomy for nutritional support: technique, indications and initial results. By BRENDAN J. MORAN and ROGER A. FROST, *Department of General Surgery, Salisbury General Infirmary*

The construction of a gastrostomy for access to the gastrointestinal tract has been used successfully for decades. However, this has involved a surgical procedure, usually under general anaesthesia. Furthermore, the many variations in the technique of surgical gastrostomy all have a significant morbidity and mortality. Percutaneous endoscopic gastrostomy (PEG) under local anaesthetic was described in 1980 by Gauderer *et al.* It is a relatively simple procedure which provides safe, reliable access to the stomach for either nutritional support or for long-term gastric decompression (Moran *et al.* 1990).

The abdominal wall is transilluminated with the gastroscope and a cannula is inserted percutaneously into the stomach. A string is passed through the cannula and grasped with a snare. The gastroscope, snare and string are drawn out of the mouth, the string is attached to the gastrostomy tube and the tube pulled into position.

We have attempted PEG for nutritional support in thirty-one patients with a mean age of 67 (range 22–84) years. All procedures were performed using sedation and local anaesthesia. Indications were, neurological disorders of swallowing (*n* 25), supplemental feeding (*n* 3), postlaryngectomy fistula (*n* 2) and oesphageal tumour (*n* 1). PEG was unsuccessful in one patient who had a previous partial gastrectomy. There was one insertion complication; a peristomal infection which was treated with antibiotics. In-use complications have included hub displacement in six cases, tube blockage in two and aspiration pneumonia in one patient.

Eight tubes were removed at the end of treatment lasting a mean of 122 (range 49–390) d. Ten tubes are still functioning 172 (range 8–585) d post-insertion. Eleven patients died 96 (range 12–320) d post-insertion of PEG.

The clinician, faced with a patient who requires prolonged tube feeding should consider PEG. PEG is safer, easier to perform, faster and cheaper than surgical gastrostomy (Moran *et al.* 1990) and avoids the complications and discomfort associated with prolonged or repeated nasogastric intubation.

Gauderer, M. W. L., Ponsky, J. L. & Izant, R. J. (1980). *Journal of Pediatric Surgery* **15**, 872–875.
Moran, B. J., Taylor, M. B. & Johnson, C. D. (1990). *British Journal of Surgery* **77**, 858–862.

A new cuffed polyurethane catheter for long-term central venous access: clinical evaluation confirms safety and efficacy. By BRENDAN J. MORAN[1], GRAHAM L. SUTTON[2], HUGH PARRY[1] and STEVEN J. KARRAN[2], [1]*Department of Surgery, Salisbury General Infirmary* and [2]*University Surgical Unit, Southampton General Hospital*

Patients receiving prolonged intravenous nutrition and some patients with malignancies require long-term venous access. The development and widespread use of 'Hickman–Broviac' catheters has been a major advance (Hickman *et al.* 1979). However, there are mechanical problems with these catheters, many of which are due to the silicone catheter material. Catheter fracture and blockage appear to be particularly serious (Pessa & Howard, 1985). Polyurethane (PU) may have advantages over silicone as PU is physically stronger and has a lower thrombogenicity. Its superior strength allows a reduction in external diameter for the same lumen size compared with silicone. This has two advantages: insertion by a percutaneous technique is easier, and the volume of intravascular foreign material is reduced.

We now have experience with the use of a PU catheter in more than 700 patients. However, the catheter requires suturing to the skin as there are difficulties in affixing a dacron cuff to polyurethane. A unique PU catheter (CUFF-CATH, VIGGO, Swindon), with a fixed dacron cuff, has recently been produced and we report the initial clinical experience.

We have inserted fifty catheters in forty-eight patients using a percutaneous technique under local anaesthesia. The indications for long-term catheterization were for chemotherapy (*n* 36), for long-term total parenteral nutrition (*n* 5) and a miscellaneous group of seven patients (two with systemic sclerosis and one each with AIDS, cystic fibrosis, chronic renal failure, infected aortic aneurysm graft and bacterial endocarditis).

There were three insertion complications; failure to cannulate at the first attempt, pneumothorax (resolved spontaneously) and malposition (repositioned with a guidewire). In-use problems included catheter sepsis (*n* 4), subclavian vein thrombosis (*n* 2), catheter falling out (*n* 1) and blockage (*n* 1) (unblocked with a guidewire). No catheter has fractured. The total catheter days has been 6607 d (mean 132 d, range 18–831 d).

Overall, forty-one (82%) catheters have functioned satisfactorily; twenty-six have been removed at the end of treatment; six patients have died; and eleven catheters are still in use, a mean of 154 d post-insertion. Furthermore, this catheter allows insertion by a new technique which prevents catheter displacement (Moran & Sutton, 1990).

This new catheter combines the mechanical advantages of PU, together with the advantages of a dacron cuff. The results of this study show that this PU catheter may be a useful alternative to silicone catheters in providing safer, cheaper and more effective long-term venous access.

Hickman, R. O., Buckner, C. D. & Clift, R. A. (1979). *Surgery Gynecology and Obstetrics* **148,** 871–875.

Moran, B. J. & Sutton, G. L. (1990). *Journal of Parenteral and Enteral Nutrition* **14,** 546–547.

Pessa, M. E. & Howard, R. J. (1985). *Surgery Gynecology and Obstetrics* **161,** 257–260.

Superior vena cava thrombosis in patients on home parenteral nutrition; aetiology and mortality. By D. J. LEINHARDT, T. O'HANRAHAN, J. SHAFFER, M. M. MUGHAL and M. H. IRVING, *University of Manchester, Department of Surgery, Hope Hospital, Eccles Old Road, Salford M6 8HD*

The major complications for patients on long-term home parenteral nutrition (HPN) are catheter-related (Mughal & Irving, 1986). The HPN register includes a yearly update on virtually all UK patients.

Symptomatic superior vena cava (SVC) thrombosis was reported in twenty-one (6·1%) of 346 patients registered from January 1977 to January 1990. Seven of the twenty-one (33·3%) died, compared with an overall mortality for HPN of 11%. A retrospective analysis was performed to examine the relationship of previous catheter-related complications to the mortality of SVC thrombosis.

In those with SVC thrombosis there was a previous history of mechanical catheter complications (malposition, displacement, occlusion) in seven cases, and catheter-related sepsis (local or systemic) in nine others. The remaining five patients had no previous history of catheter-related complications.

	Catheter-related complications		
	Mechanical	Infective	None
SVC thrombosis	7	9	5
Deaths	0	7	0

Of the seven deaths, six were related to HPN; five of these were due to pulmonary embolism with septic emboli being proven in three cases.

Mortality of HPN-related SVC thrombosis was 77·7% in patients with a previous history of catheter-related sepsis. The finding that septic pulmonary emboli are a frequent cause of death in such patients has important implications for their management. Should we now consider treating this serious complication with antibiotics as well as thrombolytics and anticoagulation? Furthermore, should we now treat patients with catheter-related sepsis with long-term anticoagulation therapy?

Mughal, M. M. & Irving, M. H. (1986). *Lancet* **ii**, 383–386.

Resting energy expenditure in children with liver disease. By S. R. WILSON[1], P. AMOROSO[1], A. BAKER[2], J. C. PONTE[1] and C. BALL[2], *Departments of* [1]*Anaesthesia* and [2]*Child Health, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PU*

Failure to thrive is a common and difficult problem in the management of children with liver disease. The mechanism for this is unknown. There is evidence that increased resting energy expenditure may contribute to impaired growth seen in these children (Pierro, 1989). We aimed to demonstrate an increased basal metabolic rate in children with liver disease and failure to thrive.

Failure to thrive was defined as, weight or triceps skinfold thickness falling from a previously maintained growth centile by at least one standard deviation for 3 months or longer. All children were studied at least 2 h after the last meal. They were divided into three groups: liver disease with failure to thrive (n 10, mean age 0·82 years, mean weight 6·4 kg), liver disease but thriving (n 8, mean age 1·72 years, mean weight 11·4 kg) and control children (n 12, mean age 0·97 years, mean weight 9·1 kg). Resting energy expenditure (REE) was measured by indirect calorimetry using an open canopy technique and mass spectrometer. Oxygen consumption and carbon dioxide production were measured continuously at rest.

Mean (SD) resting O_2 consumption in children with liver disease and failure to thrive was 14·4 (3·4 ml/kg per min compared with controls of 9·7 (2·44) ml/kg per min ($P<0·001$); for those with liver disease and thriving it was 11·3 (4·1) ml/kg per min).

We conclude that this increase in REE may be a major determinant of malnutrition in chronic liver disease.

Pierro, A. (1989). *Journal of Paediatric Surgery* **24,** 534–538.

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Practical and nutritional aspects of an elemental diet in the management of Crohn's disease. By M. PEARSON, K. TEAHON, A. J. LEVI and I. BJARNASON (Introduced by P. BRERETON) *Northwick Park Hospital and MRC Clinical Research Centre, Harrow, Middlesex*

Despite initial scepticism, the use of an elemental diet has now gained acceptance as a primary therapy for Crohn's disease and is shown to be comparable in efficacy to corticosteroids. The treatment is not just symptomatic, it has clearly been shown to reduce intestinal inflammation. However, little attention has been given to the practical and nutritional aspects of such treatment.

To date we have treated seventy-seven patients suffering from active Crohn's disease using an elemental diet (Elemental 028; Scientific Hospital Supplies, Liverpool, UK) as the only nutritional intake for 4 weeks. Starter regimens are important not only for gut tolerance but also to gain the patients' confidence and in their adjustment to a high-volume, liquid diet. Nasogastric feeding was necessary in seventeen patients (22%) because of, refusal to try the diet (5), inability to cope with the volume (2), too ill to try (5), pregnancy (2) and necessity to use modular elemental diet (3). Twelve patients (16%) remained in hospital, the remainder were discharged and thirty patients (40%) returned to work during the treatment regimen. The problems encountered during treatment were postural hypotension, symptomatic hypoglycaemia, iron intolerance, caffeine withdrawal and vivid food dreams. 'Cheating' on normal food was surprisingly rare (*n* 1).

Detailed nutritional assessment was made pre-treatment, and at 2 and 4 weeks in twenty successfully treated patients. Lean body mass as measured by total body potassium and bioelectric impedance, was maintained throughout the treatment (42·7 (SEM 7·7) pre-treatment and 43·2 (SEM 7·1) kg post-treatment). There was evidence of improvement in functional nutrition with significant ($P<0·05$) increases in prealbumin (150 (SEM 59) pre-treatment to 199 (SEM 42) mg/l post-treatment), albumin (28 (SEM 4) pre-treatment to 30 (SEM 5) g/l post-treatment) and transferrin (2·1 (SEM 0·5) pre-treatment to 2·7 (SEM 0·1) g/l post-treatment). There were no significant changes in the trace elements measured (copper, magnesium and zinc).

Using a team approach, the practical aspects of an elemental diet can be overcome, few patients need to stay in hospital and many return to normal work. Such regimens are nutritionally adequate in the short term for patients with active disease.

Colonic preservation reduces the need for long-term intravenous nutrition, water and electrolyte therapy in the short bowel syndrome. By J. M. D. NIGHTINGALE, D. J. GERTNER, S. R. WOOD and J. E. LENNARD-JONES, *Department of Gastroenterology, St Mark's Hospital, City Road, London EC1V 2PS*

The case notes of eighty-two patients with less than 200 cm remaining jejunum and managed at this hospital over the last 10 years were reviewed. The patients had no overt disease in the remaining jejunum. The patients were divided into two groups; those with end jejunostomies (EJ) and those with jejuno-colic anastomoses (JC). Clinical details (Table 1) show that Crohn's disease was the commonest diagnosis; the treatment of small bowel volvulus and ischaemia usually resulted in colonic preservation.

Table 1. *Details of patients*

	End jejunostomy	Jejuno-colic anastomosis*
Sex	30♀, 13♂	26♀, 13♂
Mean age (range)	42 (16–63)	46 (7–70)
Diagnosis		
Crohn's	32	17
Irradiation	3	5
Ulcerative colitis	5	—
Volvulus	—	5
Ischaemia	1	6
Other	2	6

* Eight have ileo-caecal valve.

The nutritional/electrolyte supplements given were assessed 6 months and 2 years after the last small bowel resection. Table 2 shows that most EJ patients need long-term intravenous therapy if the jejunal length is less than 100 cm compared to about 50 cm in JC patients. The need for nutritional/water and electrolyte supplements did not appear to change from 6 months to 2 years in either group. Subsequently two JC patients with jejunal lengths 65 and 70 cm were able to stop the parenteral nutrition; whereas six in the EJ group (100–150 cm) needed to start taking oral electrolyte supplements after the 2 year period.

Table 2. *Patients receiving intravenous nutrition or electrolyte*

	End jejunostomy		Jejuno-colic anastomosis	
Jejunal length (cm)	6 months	2 years	6 months	2 years
0–50	3/3	2/2	6/9	5/6
51–100	9/13	8/10	1/12	2/11
101–150	3/20	4/17	0/11	0/10
151–200	0/7	0/5	0/7	0/7

No patient in the EJ group developed renal stones after their last small bowel resection. However, 9/39 (23%) of the JC patients did and these were analysed in three patients and were composed of calcium oxalate.

This study demonstrates that most patients with a residual jejunal length of 50–100 cm can be maintained on an oral regimen when the residual jejunum is in continuity with the colon, whereas most such patients with a terminal jejunostomy need intravenous supplements.

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Inter-observer variability in the measurement of body fat. By SUSAN A. JEBB, N. J. FULLER, GAIL R. GOLDBERG, T. J. COLE and M. ELIA, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

A variety of simple field techniques is currently available for the estimation of body composition. However, remarkably little attention has been given to assessing the observer error of the newer techniques relative to each other and to the more traditional body composition methods.

Measurements of weight (electronic digital scales), height, skinfold thicknesses (Durnin & Womersley, 1974), whole-body resistance (Valhalla Scientific Ltd.) and near infra-red interactance (Futrex-5000) were made on twelve healthy adult subjects (six male and six female) by six experienced observers. Each subject had the same measurement performed by each of the observers consecutively, over a period of less than 30 min. The ages, weights, heights and body mass indices of the subjects ranged from 20–52 years, 53·0–93·6 kg, 1·58–1·81 m and 19·6–32·1 kg/m^2, respectively. In instances where body composition was calculated from a combination of measurements, all the values obtained by that particular observer were used throughout. Two-way analysis of variance was performed on the basic measurements and on the estimates of body composition derived from them (including the body mass index (kg/m^2) obtained from weight and height and used to calculate body fat (Black *et al.* 1983)), using the Genstat 5 statistical programme for both the basic data and the natural log transformed data.

There was evidence of a systematic bias between observers in each of the basic measurements (except height) and in the estimates of body composition. The largest coefficients of variation (CV) for the basic measurements were found for skinfold thicknesses (11–18% for individual skinfold thicknesses and 9% for the sum of four skinfold thicknesses), and the lowest for weight (0·01%) and height (0·4%). CV for whole-body resistance (1·2%), and near infra-red interactance (optical density 1: 5·6% and optical density 2: 6·2%) measurements were found to have intermediate values. The variability in the estimate of body fat by skinfold thicknesses (CV = 4·6%) and near infra-red interactance methods (CV = 4·2%) was found to be greater than from the resistance method (CV = 2·6%) and the method based on weight and height alone (CV = 1·1%).

It is apparent that the extent of the residual error, after adjusting for observer differences, varies according to the type of measurement made. In general, use of the more empirical methods (e.g. those based on weight and height) are associated with less observer variability than those measurements which incorporate a degree of subjectivity (e.g. locating the correct anatomical site for the measurement of skinfold thickness). The present study demonstrates that the resistance technique provides more uniform results than those obtained by the measurement of skinfold thicknesses or near infra-red interactance.

In conclusion, this study provides data which may be useful in selecting field or bedside methods of body composition analysis involving measurements which are obtained by different observers. Observer error is a component of the precision of the measurement and is independent of its absolute accuracy. However, it is pertinent to note that in some cases the error on the estimate of body composition may be compromised more by the extent of observer variability than by the inaccuracy of the methodology.

Black, D., James, W. P. T. & Besser, G. M. (1983). *Journal of the Royal College of Physicians of London* **17**, 5–65.

Durnin, J. V. G. A. & Womersley, J. (1974). *British Journal of Nutrition* **32**, 77–97.

Isolation of sinusoidal membrane vesicles from human liver for studies of amino acid transport: a preliminary report. By A. AHMED[1], P. M. TAYLOR[1], J. M. BARUA[2], F. C. CAMPBELL[2] and M. J. RENNIE[1], [1]*Department of Anatomy and Physiology* and [2]*Department of Surgery, Ninewells Hospital, University of Dundee, Dundee DD1 4HN*

Membrane transport is recognized to be an important regulatory step in hepatic amino acid metabolism. Amino acid transport across the blood-facing (sinusoidal) membranes of rat liver has been investigated using isolated cells or membrane vesicles (Kilberg *et al.* 1980; Jacob *et al.* 1986). The lack of comparable information on amino acid transport in human liver led us to initiate the present study using techniques adapted from those used for rat liver.

With the consent of relatives, liver tissue (approximately 80 g) was obtained from an organ-transplant donor (male, aged 55 years, with no known liver disorder) during full liver autoperfusion. The tissue was homogenized in 0·25 M-sucrose buffer using a Ystral Ultraturrax (5 s at setting 6). Crude liver membranes were isolated from the homogenate by centrifugation (Jacob *et al.* 1986), and membranes were separated by discontinuous density-gradient centrifugation (0·25 M, 26, 34 and 51% sucrose) into three interfacial fractions. The membrane fraction obtained at the 26–34% sucrose interface had the highest (15-fold) enrichment of a sinusoidal membrane marker enzyme (potassium-stimulated phosphatase), and was depleted in activity of marker enzymes for bile canalicular and reticular membranes (γ-glutamyltransferase and glucose-6-phosphatase respectively) relative to the other membrane fractions. This fraction was used for transport studies.

L-[^{3}H]glutamine uptake into sinusoidal membrane vesicles (measured using a rapid gel-filtration method; Ahmed *et al.* 1990) was linear for up to 1 min. The initial (30 s) rate of uptake was six times higher with an inward gradient (100 mM) of sodium chloride than with choline chloride (1·8 (SE 0·45) *v.* 0·3 (SE 0·09) pmol/mg protein per min respectively with 1 μM-glutamine). NaCl-stimulated glutamine uptake showed an overshoot typical of ion-coupled transport processes in vesicles. Intravesicular volume (estimated from equilibrium L-glutamine uptake) was 0·7 (SE 0·13) μl/mg protein.

The results demonstrate that sinusoidal membrane vesicles prepared from human liver include a sodium-dependent component of L-glutamine transport. We are currently trying to establish the extent to which this Na-dependent glutamine transporter resembles the System N characterized in rat liver (Kilberg *et al.* 1980).

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Ahmed, A., Taylor, P. M. & Rennie, M. J. (1990). *American Journal of Physiology* **259,** E284–E291.
Jacob, R., Rosenthal, N. & Barrett, E. J. (1986). *American Journal of Physiology* **251,** E509–E514.
Kilberg, M. S., Handlogten, M. E. & Christensen, H. N. (1980). *Journal of Biological Chemistry* **225,** 4011–4019.

Are values of leucyl-tRNA labelling from muscle biopsies reliable? Some supporting evidence. By P. W. WATT[1], J. N. A. GIBSON[2], Y. LINDSAY[1], S. DOWNIE[1] and M. J. RENNIE[1], [1]*Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN* and [2]*Department of Orthopaedic Surgery, Princess Margaret Rose Hospital, Edinburgh EH10 7ED*

The amino-acyl tRNA pool used for protein synthesis is less than 1% of the free amino acid pool and its turnover time is probably of the order of seconds. When using isotope-labelled amino acid tracers, the time taken for tissue freezing and isolation of tRNA could lead to artefacts in the labelling of amino-acyl tRNA if the transferase remained active. The aims of the present work were to examine the effects of (1) delays in freezing on the labelling of skeletal muscle leucyl-tRNA and (2) the isolation procedure on labelling of leucyl-tRNA.

Six female Wistar rats (250 g) were anaesthetized with an intraperitoneal injection of Sagatal (50 mg/kg, May & Baker, Dagenham), and given a primed (6 mg/kg) constant infusion of L-[1-^{13}C,^{15}N]leucine (6·5 mg/kg per h) for 1 h. After this, gastrocnemius muscle samples were taken; one being immediately clamp-frozen and the other kept at room temperature for 2 or 5 min before freezing. Leucine enrichment was determined in plasma, the tissue homogenate-free amino acids and leucyl-tRNA, isolated according to Allen *et al.* (1969). Amino acids were prepared as *t*-butyl dimethylsilyl derivatives. To one portion of clamped muscle, 200 μg L-[2,5,4-methyl^{13}C]leucine (99 atoms %) were added before homogenization. Labelling of leucine was measured with either a Finnigan 1020B or Hewlett Packard 5971 GC-MS, using a selected ion monitoring; protein [1-^{13}C]leucine enrichment was measured on a Finnigan Delta D IRMS.

Results showed that 2 or 5 min at room temperature had no significant effect on labelling of leucyl-tRNA by ^{13}C or ^{15}N. Also tissue-free leucine labelling by ^{13}C was not significantly affected by such delays but ^{15}N labelling did show a small reduction (from 3·4 (SE 0·35) to 2·51 (SE 0·29) atoms % excess (APE)). Incorporation of ^{13}C label into protein-leucine was not significantly affected by a 5 min delay (0·0168 (SE 0·0042) *v.* 0·0186 (SE 0·0022) APE). There was no significant incorporation of L-[2,5,4-methyl^{13}C]leucine into leucyl-tRNA despite mixed homogenate-free leucine labelling at 30 APE. These results indicate that muscle leucyl-tRNA labelling is stable for several minutes after removal from the body, possibly reflecting (1) the size and stability of the intra- and extracellular free amino acid pools for charging tRNA and (2) a reduction in the activities of leucyl-tRNA transferase, amino acid transport and protein turnover. Short delays in sample freezing and the procedures for preparation of labelled tRNA are unlikely to introduce artefacts into the measurement of tRNA labelling, and thus subsequent calculations of protein synthesis based on it are probably robust.

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Allen, R. E., Raines, P. L. & Regen, D. M. (1969). *Biochimica et Biophysica Acta* **190**, 323–336.

Urinary nitrogen, urinary urea and urinary amino acids during intravenous feeding in multiple organ failure. By CERI J. GREEN[1], C. M. SCRIMEGOUR[2], M. BOSOMWORTH[2], KATH SHIPLEY[2], P. MCCLELLAND[1] and I. T. CAMPBELL[3], [1]*University Department of Anaesthesia and Department of Chemical Pathology, Royal Liverpool Hospital, Liverpool L69 3BX*, [2]*Department of Anatomy and Physiology, University of Dundee, Dundee DD1 9SY* and [3]*Department of Surgery, Hope Hospital, Salford M6 8HD*

There is some evidence that capillary permeability increases in trauma, sepsis and shock (Fleck *et al.* 1985) and that protein is lost into the urine (Gosling & Sutcliffe *et al.* 1986). Long *et al.* (1976) also presented some evidence that amino acids given intravenously to patients might be lost in the urine. If this were true, it would mean that the assumptions usually made about the interrelationships between urinary nitrogen and urinary urea in calculating protein oxidation in these patients, might not hold. It would also mean that a proportion of any amino acids infused would be wasted as a means of nutritional support.

Urinary urea and urinary N excretion (24 h) were measured in eight critically-ill patients (mean sepsis score 10, range 7–18; mean APACHE II score 19, range 10–27) for between 1 and 12 (median 4·5) d totalling forty-two patient-days in all. Urinary amino acid concentrations were measured using automatic ion-exchange analysis on urine from one healthy subject and on 24 h urine samples from three critically-ill patients (mean sepsis score 13, range 13–14; mean APACHE II score 11, range 9–25) on 1, 2 and 3 d respectively, while being given Vamin 14 (Kabi Vitrum UK, Uxbridge, UK) intravenously at a rate of 17·9 ml/kg fat-free mass per 24 h.

Urinary urea N as a percentage of urinary N was 78·3 (SD 10)%. Urinary amino acid N ranged from 139 to 492 (median 365) mg/24 h (normal range 267–431 mg/24 h) (Diem, 1962). Correlation coefficients of urinary amino acid concentrations on the six patient-days *v.* the amino acid concentrations in the healthy subject ranged from 0·557 (P=0·011) to 0·864 (P<0·001) (median 0·768; P<0·001). Correlation coefficients for the patient's urinary amino acid concentration *v.* amino acid concentration of Vamin 14 ranged from 0·068 to 0·217 (median 0·120, not significant).

It is concluded that in these critically-ill patients, the normal proportion of N was present in the urine as urea. The pattern of urinary amino acid excretion showed a better correlation with the amino acid excretion pattern in the normal subject than it did with the concentration in the feeding solution, and there was no excessive loss of infused amino acid in the urine.

Diem, K. (editor) (1962). *Documenta Geigy Scientific Tables*, p. 528. Manchester: Geigy Pharmaceutical Company Ltd.

Fleck, A., Raines, G. & Hawker, F. (1985). *Lancet* **i**, 781–784.

Gosling, P. & Sutcliffe, A. J. (1986). *Annals of Clinical Biochemistry* **23**, 681–685.

Long, C. L., Crosby, F., Geiger, J. W. & Kinney, J. M. (1976). *American Journal of Clinical Nutrition* **29**, 380–391.

Regulation of hepatic phosphofructokinase-1 activity in septic surgical patients. By J. ARNOLD[1,3], M. J. HAMER[2] and M. H. IRVING[1], *Departments of* [1]*Surgery* and [2]*Biochemistry and Molecular Biology* and [3]*The North Western Injury Research Centre, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT*

Carbohydrate metabolism is severely disrupted in sepsis and trauma. The ability of tissues to utilize glucose appears suppressed yet hepatic gluconeogenesis is seen to increase (Long *et al.* 1976; Black *et al.* 1982). Phosphofructokinase-1 (PFK-1, *EC* 2.7.1.11) plays an important role in the regulation of hepatic glycolysis. The amount of PFK-1 and its activity are controlled by the regulation of its synthesis and degradation, and by allosteric effectors (fructose-2,6-bisphosphate (F26BP) being the most potent) (Dunaway & Weber, 1974; Van Schaftingen *et al.* 1980). In the present study, PFK-1 activity, along with F26BP levels and phosphofructokinase-2 (PFK-2, *EC* 2.7.1.105) activity were determined in hepatic biopsies obtained at laparotomy from patients with and without abdominal septic foci.

A 60% reduction ($P<0{\cdot}05$) in hepatic PFK-1 activity (see Table) was observed in the septic patients along with significantly raised plasma levels of counter-regulatory hormones (glucagon, cortisol and adrenaline) and lactate. However, hepatic F26BP levels and PFK-2 activity along with plasma insulin and glucose levels were similar in the non-septic and septic patients. Hepatic glycogen and protein contents were very similar between the two groups of patients.

		F26BP (nmol/g)		PFK-1 (units/g)		PFK-2 (munits/g)		Glycogen (mg/g)	
Patients	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Non-septic	6	2·00	0·26	1·13	0·18	0·10	0·01	20·03	1·75
Septic	7	2·31	0·30	0·49*	0·11	0·10	0·02	23·38	1·23

*$P<0{\cdot}05$.

All values are per gram liver wet weight; enzyme activity units are μmol substrate consumed or product produced/min at 30°.

The results suggest that a decrease in hepatic PFK-1 activity may be responsible for the depressed glycolytic flux seen in sepsis. The decrease in PFK-1 activity does not appear linked to F26BP content or the capacity to synthesize F26BP. The elevated levels of counter-regulatory hormones may influence transcription/translation of the PFK-1 gene and hence alter the amount of PFK-1.

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Black, P. R., Brooks, D. C., Bessey, P. Q., Wolfe, R. R. & Wilmore, D. W. (1982). *Annals of Surgery* **196,** 420–435.

Dunaway, G. A. & Weber, G. (1974). *Archives of Biochemistry and Biophysics* **162,** 629–637.

Long, C. L., Kinney, J. M. & Geiger, J. W. (1976). *Metabolism* **25,** 193–201.

Van Schaftingen, E., Hue, L. & Hers, H. G. (1980). *Biochemical Journal* **192,** 897–901.

Twenty-four-hour energy and substrate balance in patients receiving total parenteral nutrition. By EDGAR PULLICINO, GAIL R. GOLDBERG and MARINOS ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Many studies of energy expenditure and fuel selection (i.e. source of energy expended) in patients receiving total parenteral nutrition (TPN) have been carried out over short periods of time and mainly in patients at rest. We have investigated patterns of fuel selection and nutrient balance in twelve (four ♂, eight ♀) metabolically-stable patients, over 24 h periods (15.00–15.00 hours) by continuous whole-body indirect calorimetry. The study protocol included 4 h of resting energy expenditure measurements, 6 h asleep and the remainder of the time spent in voluntary activities. The subjects studied were patients in remission from Crohn's disease. They were all malnourished, underweight (mean (SD) weight 51·9 (9·5) kg, body mass index 19·2 (1·9) kg/m^2) and receiving high-energy intravenous feeding. The feeds, which contained all nutrients in a single bag (Intralipid®), were administered by a volumetric pump either continuously (four subjects) or cyclically between 22.00 and 10.00 hours (eight subjects).

In continuous feeders, the respiratory quotient (RQ) showed little variation at different times of the day and the mean value at rest was very similar to the 24 h value. In cyclic feeders the mean RQ during feeding was significantly greater than during the off-fed period (0·973 *v.* 0·889, $P<0·03$).

Feeding regimen			Intake (MJ)				Balance (MJ)			
			Energy	AA	Fat*	CHO	Energy	Nitrogen†	Fat	CHO
Continuous	4	Mean	9·97	1·43	3·90	4·52	3·27	0·43	2·83	0·01
		SD	0·6	0·08	0·44	0·27	0·76	0·05	1·30	1·62
Cyclic	8	Mean	10·15	1·42	3·34	5·39	3·26	0·21	2·54	0·51
		SD	2·00	0·09	0·94	1·49	1·76	0·24	1·38	0·80
All subjects	12	Mean	10·09	1·43	3·56	5·11	3·26	0·28	2·64	0·35
		SD	1·63	0·08	0·83	1·27	1·42	0·22	1·30	1·09

* Includes free glycerol present in Intralipid®.

† Assumes an energy content of 116 kJ for each g metabolized and excreted.

The mean (SD) energy expended over 24 h was 6·83 (1·10) MJ and comprised 68·1 (12·5)% from carbohydrate (CHO) oxidation, 12·4 (11·7)% from fat, 16·6 (3·2)% from amino acids (AA) and 2·9 (0·5)% from free glycerol, which was present in the fat emulsion. All nutrient balances, most notably fat, were positive in all subjects. In cyclic feeders, the contribution of fat oxidation to energy expenditure was greatest shortly before the onset of feeding. On-feed non-protein, non-glycerol RQ (npngRQ) exceeded 1·00 in these subjects, which is indicative of net fat synthesis from CHO, and persisted above 1·00 for 2·3 (SD 2·0) h after the cessation of feeding.

It is concluded that in these metabolically-stable patients, (1) high-energy feeding resulted in a CHO balance close to zero in all subjects which is consistent with the limited capacity to increase body glycogen stores; (2) in patients receiving continuous TPN, there was no evidence to suggest a circadian pattern in fuel selection or differences between rest and periods of light activity; (3) in the patients feeding cyclically there was a circadian pattern in fuel selection associated with intermittent net fat synthesis from CHO. When this persisted for prolonged periods after cessation of feeding, glycogen rather than glucose was the source of CHO for net lipogenesis.

Effect of local injury on protein synthesis in skeletal muscle in the rat. By A. GHUSAIN-CHOUEIRI and P. W. EMERY *Nutrition Department, King's College, London W8 7AH*

We have preliminary evidence suggesting that a needle biopsy may cause a local increase in muscle protein synthesis over the following 6 h in normal men. We therefore investigated the time course of changes in muscle protein synthesis in rats following a small injury. Rats were anaesthetized with halothane, an incision was made into the gastrocnemius muscle and about 5 mg tissue was removed from the interior of the muscle. The wound was closed with stainless steel clips and the animals were allowed to recover. Protein synthesis was measured using the flooding dose technique (Garlick *et al.* 1980) in both gastrocnemius muscles of the injured rats, and in control rats which had been anaesthetized but not injured.

		Protein synthesis (%/d)		RNA content (mg/g protein)	
Time after injury (h)	*n*	Mean	SE	Mean	SE
2 Injured	20	15·3	0·5	8·1	0·4
2 Uninjured	20	14·8	0·6	8·1	0·4
2 Control	5	16·4	0·7	9·0	0·5
6 Injured	10	12·4	1·2	7·9	0·6
6 Uninjured	10	12·1	1·0	8·6	0·5
6 Control	4	15·7*	0·9	9·3	0·5
24 Injured	17	12·6	0·6	7·7	0·5
24 Uninjured	17	13·3	0·9	7·1	0·5
24 Control	7	14·1	1·7	6·5	0·9
48 Injured	9	16·8†	2·3	6·7†	0·3
48 Uninjured	9	13·9	1·9	5·9	0·2
48 Control	5	14·5	1·2	5·6	0·3

* Significantly different from both legs of injured rats, $P<0·05$, unpaired t test.
† Significantly different from uninjured leg, $P<0·05$, paired t test.

There was no significant difference in the rate of protein synthesis between injured and uninjured muscles at any time during the first 24 h after injury, suggesting that the injury did not have a local effect on protein synthetic activity. However, in both legs the rate of muscle protein synthesis was lower than that in uninjured control rats. The decrease amounted to 22% 6 h after injury, but by 24 h the difference was not statistically significant. Thus the systemic response to this relatively minor injury appears to involve a rapid but brief decrease in the rate of protein synthesis in skeletal muscle. Forty-eight hours after injury the rate of protein synthesis was 20% higher in the injured muscle than in the uninjured leg. This was associated with an increase in RNA content, which had remained unaltered up to that time, and may reflect repair and regrowth of the damaged muscle.

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Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.

Time course of the effect of volatile anaesthetic agents on tissue protein synthesis. By K. FERGUSON[1,2], A. G. S. BAILLIE[1], A. C. NORTON[1,2], C. R. DUNDAS[2] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*Department of Anaesthesia, University of Aberdeen, Aberdeen AB9 2ZD*

We have demonstrated that the volatile anaesthetic agents halothane and enflurane significantly depressed liver protein synthesis in young rats during 1 h of administration, whilst isoflurane produced a non-significant fall (Heys *et al.* 1989; Ferguson *et al.* 1990). Smaller changes in protein synthesis also occur in lung and brain (unpublished). It is important to know whether changes in protein synthesis induced by anaesthesia contribute to the negative nitrogen balance after surgery. The aim of this study therefore, was to determine whether the effect of these volatile anaesthetic agents on protein synthesis in rat tissues persists after the termination of exposure.

Groups of young male rats were fasted for 12 h before exposure to the anaesthetic agents halothane and enflurane at concentrations of 1·4 and 1·5% respectively. Control groups breathed atmospheric air. Treatments lasted 1 h and then the animals were killed at time points of 1, 4 and 12 h after the termination of anaesthesia. The rate of protein synthesis was measured in each rat during a 10 min period immediately before death, by incorporation of [^{3}H]phenylalanine into protein as described by Garlick *et al.* (1980).

	Fractional rate of liver protein synthesis (%/d)					
	Control		Halothane		Enflurane	
Time (h)	Mean	SEM	Mean	SEM	Mean	SEM
1	75·9	6·7	79·1	4·5	63·1	4·7
4	78·8	4·1	86·8	4·5	85·6	4·9
12	69·7	2·6	74·6	2·8	79·5	4·6

The results show that the depression in tissue protein synthesis in vivo seen during a period of anaesthesia with these volatile agents does not persist after the termination of exposure (except possibly after 1 h in the enflurane group). There is no evidence of a significant depression at 1, 4 or 12 h post-anaesthesia, suggesting that these volatile anaesthetic agents do not contribute to the negative N balance observed after surgery.

Ferguson, K., Heys, S. D., Norton, A. C., Dundas, C. R. & Garlick, P. J. (1990). *Proceedings of the Nutrition Society* **49**, 182A.

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.

Heys, S. D., Norton, A. C., Dundas, C. R., Eremin, O., Ferguson, K. & Garlick, P. J. (1989). *Clinical Science* **77**, 651–655.

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The effects of dietary lipids on metabolic responses to tumour necrosis factor α. By HILDA MULROONEY and R. F. GRIMBLE, *Human Nutrition Department, Southampton Medical School, Southampton SO9 3TU*

Tumour necrosis factor α (TNFα) has been implicated as a mediator in various responses to infection, including fever, changes in acute-phase proteins and skeletal muscle wasting. Many of the effects of cytokines are mediated by alterations in prostaglandin synthesis. Changes in the fatty acid profile of the diet can alter the host response to inflammatory stimuli (Grimble, 1990). We examined the effects of fats with differing patterns of *n*-6 and *n*-3 polyunsaturated fatty acids, on responses to TNFα.

Forty-eight male weanling Wistar rats (mean weight 60 g), were fed *ad lib.* for 2 months on four diets similar in all respects but their lipid source (g/kg: casein 180, methionine 3, fat 100, vitamin/mineral mix 50, cellulose 100, sugar 280, starch 287). The fat in each diet was maize oil (high in linoleic acid), coconut oil (high in short-chain saturates, and low in linoleic acid), fish oil (high in eicosapentaenoic acid), or butter (high in medium-chain saturates and oleic acid, and low in linoleic acid). Diets were supplemented with 10 g maize oil/kg, to prevent essential fatty acid deficiency.

Dietary oil . . .	Maize		Coconut		Fish		Butter		Pooled SEM
	Saline	TNFα	Saline	TNFα	Saline	TNFα	Saline	TNFα	
FSR (%/d)									
Liver	57***	106	52***	78*	81**	98	62*	51***	3·3
Lung	41*	54	24***	74**	39*	21***	26***	27***	2·7
Kidney	38**	52	40*	52	76***	42*	65*	53	2·0
Muscle	12	11	12	20***	16*	13	12	12	0·5
Kidney Zn (μg/g)	39*	35	37	37	32*	31**	35	32	0·5
Liver Zn (μg/g)	35**	43	33***	41	37*	36**	33***	36**	0·7

Significantly different from maize oil-fed TNFα group: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

Half of each group were injected i.p. with 100 μg TNFα/kg body-weight (endotoxin content <2·9 pg/mg protein); the other half with sterile, non-pyrogenic saline. The latter group were pair-fed with the TNFα group. After 24 h, fractional rates of protein synthesis (FSR) were measured in the lungs, livers, kidneys and tibialis muscles (Garlick *et al.* 1980) together with tissue protein and zinc contents. TNFα increased protein FSR in the lungs, liver and kidney in the maize oil-fed animals. The increased rate of turnover was not matched by an equivalent increase in protein content of the liver or lung, possibly due to an increased rate of protein breakdown in these tissues, or the synthesis of acute-phase reactants in liver. Butter, fish and coconut oils suppressed the effects of TNFα. In the butter-fed animals, the response was totally abolished. The coconut and fish groups showed varying degrees of suppression. Maize oil-fed animals also showed an increase in Zn concentration in liver, and a decrease in kidney. Again, varying levels of suppression were seen in the other dietary groups.

This indicates that, not only may the presence of *n*-3, or low levels of *n*-6 polyunsaturates be important in the reduction of metabolic responses to cytokines, but that the fatty acid profile of the saturated fat may produce subtle modifications of the effects of low levels of linoleic acid.

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.
Grimble, R. F. (1990). *Nutrition Research Reviews* **3**, 193–210.

Modulatory effects of glycine and cysteine on the response of glutathione and acute-phase proteins to tumour necrosis factor α in rats. By R. F. GRIMBLE, A. A. JACKSON, C. PERSAUD and M. A. WRIDE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU* and F. DELERS and R. ENGLER, *Laboratoire des proteines de la reaction inflammatoire, 75270 Paris Cedex 06, France*

Tumour necrosis factor α (TNFα) brings about profound changes in hepatic metabolism. Protein synthesis is increased as the production of acute-phase proteins is enhanced. Glutathione (GSH) concentrations also increase in response to TNFα. Since many acute-phase proteins are rich in the interrelated amino acids glycine (Gly) and cysteine (Cys), and GSH is synthesized from glycine, cysteine and glutamine, the ability of the liver to respond to TNFα may be influenced by the availability of Gly and Cys. This hypothesis was tested by administering TNFα to male Wistar rats (148 (SE 1) g) fed on a low-protein (80 g casein/kg) diet supplemented with isonitrogenous amounts (g/kg) of alanine (Ala) (6), Gly (5), Cys (8), a mix of Gly + Cys (2·5+4) or Cys (4). A further group received a diet of normal protein content (200 g casein/kg) supplemented with Cys (8). Rats received the respective diets for 1 week. Growth during this period was 10, 19, 31, 27 and 35 g for the low-protein diets containing Ala, Gly, Cys (4), Cys (8) and Gly + Cys respectively. Those on the normal protein diet grew 45 g. Rats from each dietary group received either sterile non-pyrogenic saline or recombinant human TNFα (50 μg/kg, endotoxin content <2·7 pg/mg protein) injected i.p. Animals were killed 24 h after injection. Saline-injected rats were pair-fed on the intakes of the respective TNFα group during this period.

		Serum		Liver			
Group	Dietary protein (g/kg)	AGP (μg/ml)	AM (units)	Wt (g)	GSH (mg/g)	Zn (mg/g)	Protein (mg/g)
Ala	TNFα 80	403^{a}	9·7^{a}	7·2^{a}	8·8^{a}	29^{a}	196^{a}
	Saline 80	88^{d}	3·5^{c}	6·9^{a}	5·4^{d}	31^{a}	167^{b}
Gly	TNFα 80	546^{b}	10^{a}	8·1^{b}	7·5^{a}	19^{b}	173^{b}
	Saline 80	74^{d}	4·3^{c}	6·8^{d}	3·2^{d}	13^{e}	166^{a}
Cys (4)	TNFα 80	284^{c}	11·3^{a}	9·5^{c}	25·8^{b}	41^{c}	192^{a}
	Saline 80	94^{d}	3·7^{c}	8·8^{e}	19·8^{e}	31^{d}	155^{b}
Gly+Cys	TNFα 80	390^{a}	8·2^{a}	9·9^{c}	21·3^{c}	24^{ab}	202^{a}
	Saline 80	76^{d}	2·4^{c}	9·6^{f}	17·9^{e}	20^{e}	161^{b}
Cys (8)	TNFα 80	302^{c}	7·8^{a}	9·5^{c}	29·8^{b}	31^{a}	211^{a}
	Saline 80	100^{d}	3·0^{c}	8·6^{eg}	22·6^{f}	22^{e}	149^{b}
	TNFα 200	426^{a}	16·7^{b}	9·6^{c}	29·0^{b}	42^{c}	215^{a}
	Saline 200	85^{d}	1·9^{c}	9·0^{f}	23·9^{e}	43^{cd}	158^{b}

$^{a-g}$ Values with different superscript letters are significantly different (ANOVA): $P<0·05$.
AGP, α-1-acid glycoprotein; AM, α-2-macroglobulin.

In response to TNFα, no increase in liver mass occurred in the low-protein group supplemented with Ala. However, an increase occurred when diets were supplemented with Gly or Cys or a mixture of both, thereby restoring the stimulatory effect of TNFα on total protein, GSH and Zn. The rise in AM was suppressed in rats fed on the low-protein Ala diet while that of AGP was unaffected. However, differential effects were produced by Gly alone and Cys alone on AGP. Gly enhanced and Cys suppressed the response to TNFα. Gly and Cys have subtle modulating effects beyond that of directly providing substrate for the production of acute-phase proteins and GSH.

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The influence of total parenteral nutrition on rat liver protein synthesis in an acute-phase reaction induced by interleukin-1β or turpentine. By P. E. BALLMER, M. A. MCNURLAN, I. GRANT and P. J. GARLICK, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

We have recently demonstrated that recombinant human interleukin-1β (IL-1β) and turpentine injection in the rat produces a rapid increase of total liver protein synthesis (Ballmer *et al.* 1990). By 9 h after injection of either IL-1β or turpentine, liver protein synthesis rates rose by 15%. The elevated synthesis of liver protein may reflect the increased production of acute-phase proteins, and thus represents an early beneficial adaptation in the host defence system. It is therefore of interest whether nutritional support in an acute-phase reaction can positively influence liver protein synthesis. Consequently, we have measured fractional synthesis rates (FSR) of total liver protein in rats injected with IL-1β or turpentine and given previously total parenteral nutrition (TPN) or intravenous saline (9 g sodium chloride/l) for 2 h. Liver protein synthesis rates were assessed at intervals by a flooding dose of [^{3}H]phenylalanine. The Table summarizes the FSR (%/d) of total liver protein.

Period after injection (h)	Control				IL-1β				Turpentine			
	NaCl		TPN		NaCl		TPN		NaCl		TPN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
9	78	4	89	4	91	3	100***	3	95	5	97	5
24	72	5	90***	6	95	2	97	5	113	6	127*	8

Significantly different from NaCl infusion: *$P<0{\cdot}05$, ***$P<0{\cdot}001$.

Compared with IL-1β, turpentine-injected animals showed a continuing increase in FSR. Controls and turpentine-injected animals showed a significant positive response to TPN by 24 h, whereas IL-1β-treated rats exhibited higher FSR at 9 h with TPN. We conclude that TPN does increase synthesis rates of total liver protein in rats exposed to IL-1β and turpentine. This suggests that TPN is a potential means of enforcing the host defence, even in the early stages of an inflammatory insult.

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Ballmer, P. E., McNurlan, M. A., Southorn, B. G., Grant, I. & Garlick, P. J. (1990). *Proceedings of the Nutrition Society* **49**, 162A.

Unsaturated fatty acids inhibit interleukin-2 production by concanavalin A-stimulated lymphocytes. By P. C. CALDER, J. A. BOND, S. J. BEVAN and E. A. NEWSHOLME, *Cellular Nutrition Research Group, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Activated T-lymphocytes are characteristic of autoimmune and inflammatory disorders, and increases in soluble interleukin-2 (IL-2) and IL-2 receptors have been reported in rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus. This over-production of IL-2 leads to aberrant immune responses and so down-regulation of IL-2 production may be a useful therapeutic approach to such disorders. We have shown that a number of fatty acids inhibit proliferation of concanavalin A (Con A)-stimulated rat lymphocytes (Calder *et al.* 1991). Since T-lymphocyte proliferation is IL-2 dependent it was of interest to investigate whether fatty acids modulate IL-2 production in vitro.

Rat lymph node cells were prepared and cultured as described elsewhere (Calder *et al.* 1991). The cell culture medium was supplemented with 5 μg Con A/ml and 100 μM-fatty acid. After 48 h the medium was removed and assayed for prostaglandin E_2 (PGE_2) using a commercial radioimmunoassay kit or IL-2 using a bioassay involving the IL-2-dependent CTLL-2 cell line. Lymphocyte proliferation was measured as incorporation of [^{3}H]thymidine into DNA during the final 18 h of a 66 h culture period (Calder *et al.* 1991).

	Lymphocyte proliferation (thymidine incorporation, disintegrations/min)		PGE_2 (nM)		IL-2 (Units/well)	
No. of determinations . . .	8		6		3	
Fatty acid added	Mean	SEM	Mean	SEM	Mean	SEM
None	80180	3570	0·34	0·03	1·41	0·08
Myristic (14:0)	68955*	2405	0·52*	0·06	1·30	0·09
Palmitic (16:0)	48110***	3200	0·53**	0·03	1·18	0·12
Stearic (18:0)	31270***	1605	0·38	0·03	1·12	0·08
Oleic (18:1 *n*-9)	32875***	4010	0·49†	0·03	1·10*	0·07
Linoleic (18:2 *n*-6)	25650***	1650	0·46*	0·03	1·02†	0·06
Linolenic (18:3 *n*-3)	40090***	1720	0·49†	0·03	0·82†	0·11
Arachidonic (20:4 *n*-6)	22450***	1590	>1·42***		0·99*	0·09
Eicosapentaenoic (20:5 *n*-3)	16840***	1490	0·83***	0·03	0·90†	0·08
Docosahexaenoic (22:6 *n*-3)	36080***	1820	0·50**	0·02	0·91†	0·09

Statistical significance *v.* control: *$P<0·05$, †$P<0·02$, **$P<0·01$, ***$P<0·001$.

As previously reported (Calder *et al.* 1991), all nine fatty acids tested had the ability to inhibit T-lymphocyte proliferation, though the extent of inhibition depended on the particular fatty acid. Inhibition of proliferation was not correlated with PGE_2 concentration in the medium, suggesting that the inhibitory effect of fatty acids is not mediated via eicosanoids. Significant inhibition of IL-2 production was observed during culture with the polyunsaturated fatty acids and with oleic acid; IL-2 concentration and lymphocyte proliferation correlated well (r 0·803, $P<0·01$).

The mechanism by which fatty acids exert their inhibitory effect on cell proliferation is not known, but it is possible that their effect is mediated via changes in plasma membrane fatty acid composition and hence fluidity. Whatever the mechanism, these results suggest that T-cell activation and hence immune responses may be modulated by dietary lipid intake.

Calder, P. C., Bond, J. A., Bevan, S. J., Hunt, S. V. & Newsholme, E. A. (1991). *International Journal of Biochemistry* (In the Press).

L-Arginine stimulates human lymphocyte natural cytotoxicity. By K. G. M. PARK[1,2], P. H. HAYES[1], P. J. GARLICK[2] and O. EREMIN[1], [1]*Department of Surgery, Aberdeen University, Foresterhill, Aberdeen AB9 2ZD* and [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Human natural cytotoxicity is important in the primary defence against many potential pathogens and may be important in the immune surveillance against cancer (Trichieri, 1989). Many disease states, including HIV infection and malignancy, may depress lymphocyte natural killer activity and this is frequently of prognostic significance. In the present study we present the first evidence that human natural cytotoxicity can be enhanced by the amino acid L-arginine.

Lymphocytes obtained from a healthy donor were incubated in L-arginine (1 mg/ml) for 24 h; these were then washed and used as the effector cells in a standard 4 h ^{51}Cr release assay against K562 cells (Eremin *et al.* 1981). Killing of K562 cells is mediated by natural killer (NK) cells. Lymphocytes were incubated in interleukin-2 (IL-2) (1000 units/ml) in a medium enriched with arginine (0·5 or 1 mg/ml) or a standard medium. After 72 h, killing assays were determined against the NK-resistant Daudi cell line. This is an indicator of lymphokine-activated killer (LAK) cell activity.

There was no significant increase in NK activity following arginine. However, arginine appeared to sensitize the lymphocytes to the effects of IL-2 as there was a significant increase in LAK cell activity in the cells incubated in arginine. When the lymphocytes were depleted of the cells expressing the CD56 antigen (a marker of NK and LAK cells), there was no cytotoxicity directed against either the Daudi or K562 cells.

Arginine was given orally to thirteen volunteers (30 g/d in divided doses) for 3 d. NK and LAK cell activity and CD56 expression on peripheral blood lymphocytes were determined on each volunteer before and after 3 d of arginine. Following arginine there was a significant enhancement in NK and LAK cytotoxicity compared with the pre-arginine levels. The median increase in NK cell activity was 91% (range −33 to +347%, $P<0·01$, Wilcoxon signed rank test) and in LAK cell activity 51% (range −8 to +128%, $P<0·01$, Wilcoxon signed rank sum test). Furthermore these changes in NK and LAK cell activity were associated with a significant increase in the number of cells expressing the CD56 antigen in the peripheral blood, median increase 32% (range 7–123%, $P<0·01$).

In this study it has been demonstrated that arginine has a direct effect on peripheral blood lymphocytes in vitro, increasing their response to IL-2. The effects in vitro were mediated by $CD56^+$ cells. In vivo, arginine increased the expression of CD56 on peripheral blood lymphocytes and also resulted in an increased NK and LAK cell activity. It is postulated that arginine may be used to benefit in disease states, such as HIV infection and malignancy where NK and LAK cell activities are frequently depressed.

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Eremin, O., Coombs, R. R. & Ashby, J. (1981). *British Journal of Cancer* **44**, 166–176.
Trichieri, G. (1989). *Advances in Immunology* **47**, 187–376.