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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 Royal College of Physicians, Edinburgh, on Thursday and Friday, 16/17 November 1989, when the following papers were presented.

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Effect of protein deficiency on circulating protein concentrations during the acute phase response to injury. By G. JENNINGS and M. ELIA, *MRC Dunn Nutrition Unit, Downham's Lane, Milton Road, Cambridge CB4 1XJ*

Acute phase proteins have been used to assess the severity of injury and to monitor the activity of inflammatory disease processes. However, there is little information as to whether the circulating acute phase protein concentrations after trauma or sepsis are influenced by nutritional factors such as protein deficiency. The present study was undertaken to explore this possibility by measuring the plasma concentrations of α_2 macroglobulin (one of the most responsive acute phase proteins in the rat), albumin (a negative acute phase protein) and total protein during the development and resolution of abscesses induced by turpentine injection in male Dunn Hooded rats. The measurements were made before and at timed intervals of up to 3 weeks after administering a single dose of turpentine (5 ml/kg body-weight injected subcutaneously into two dorso-lumbar sites) into two groups of rats comprising normal animals (n 6) fed on a 200 g protein/kg diet based on sucrose, starch and casein, and animals made protein deficient by being fed on a 30 g protein/kg diet (n 6). The turpentine was injected into 28-d-old rats, 2 weeks after being given the above diets. These diets were continued during the course of the whole study. Control animals for each dietary group (n 6) were given saline instead of turpentine.

As expected the basal plasma albumin and total protein concentrations in the protein-deficient animals were reduced (albumin 27·6 (SE 2·1) v. 33·0 (SE 1·2) g/l, $P<0·001$; total protein 53·0 (SE 1·8) v. 60·7 (SE 1·0) g/l, $P<0·0001$). In contrast the basal α_2 macroglobulin concentration was higher in the protein-deficient animals compared with that in normal animals (0·201 (SE 0·011) v. 0·075 (SE 0·005) g/l, $P<0·001$).

Protein deficiency attenuated the α_2 macroglobulin response to turpentine injections by more than half, and delayed the time taken for the plasma α_2 macroglobulin concentrations to reach maximum concentrations. Thus, the peak α_2 macroglobulin concentrations in the animals fed on the 200 g protein/kg diet occurred 2 d after turpentine administration (7·5 (SE 1·1) g/l), whilst the peak concentration in the protein-deficient animals occurred on day 3 after turpentine administration (3·3 (SE 0·7) g/l).

The total plasma protein concentrations rose in both groups of animals but again the rise was delayed in the protein-deficient animals (peak concentration on day 3: 66·1 (SE 4) g/l) compared with normal rats (peak concentration on day 2: 79·7 (SE 0·5) g/l). The plasma albumin concentration decreased by a mean of 13–16 g/l in both groups of animals, reaching minimum concentrations on day 2 (protein-deficient animals) and day 3 (normally fed animals) after turpentine administration. There were no significant changes in the plasma protein concentrations in the groups of animals given saline instead of turpentine.

It is concluded that disturbances in the circulating protein concentrations during the acute phase response in the rat depend not only on the injury but also on the protein nutritional status.

Which cytokine modulates the acute phase response to elective surgery and accidental injury? By E. A. PULLICINO[1], F. CARLI[2], B. RAFFERTY[3], S. POOLE[3], S. T. A. MALIK[4] and M. ELIA[1], [1]*Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*, [2]*Department of Anaesthetics, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ*, [3]*National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG*, [4]*Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 3PX*

Interleukin 6 (IL-6) and tumour necrosis factor α (TNFα) have both been implicated in the acute phase response to sepsis. However, there is little information on the role of these cytokines in human injury especially after elective surgery and accidental injury. Therefore we have studied the temporal patterns of change in circulating concentrations of IL-6 and TNFα in the first 24 h after injury. These were then related to changes in the acute phase proteins (C-reactive protein (CRP), α1-antichymotrypsin (α1-ACT)), pyrexia, leucocytosis, duration of surgery and estimated blood loss.

Measurements were made in three patients suffering from accidental injury (subject nos. 4, 5, 6 with injury severity scores of 38, 20 and 8 respectively), and ten patients undergoing elective surgery as follows: insertion of Harrington's rod for correction of scolioses (subject nos. 1–3), cardiac bypass surgery (subject nos. 7 and 8), and surgery for colorectal cancer (subject nos. 9–13).

Circulating human IL-6 was measured by a specific radioimmunoassay employing a goat antiserum raised against human IL-6, with a limit of detection of 0·1 μg/l. Circulating TNFα was measured by bioassay using a WEHI 164 clone 13 murine fibrosarcoma cell line. The lower limit of detection of TNFα by this assay is 10 ng/l.

Subject no.	Peak IL-6 (μg/l)	Peak CRP (mg/l)	Peak α1-ACT (g/l)	Peak body temperature (°)	Peak leucocyte count ($\times 10^9$/l)
1	<0·10	91	1·06	39·0	13·3
2	0·28	30	0·71	37·5	9·7
3	<0·10	168	1·00	38·0	8·8
4	0·62	248	2·04	38·5	11·4
5	1·30	348	1·83	38·0	10·9
6	<0·10	140	1·06	37·7	13·1
7	1·50	152	1·03	37·8	16·0
8	1·20	160	1·29	38·3	17·0
9	1·50	140	1·22	37·2	7·5
10	0·28	180	1·58	37·2	6·9
11	1·59	188	1·59	38·2	9·9
12	<0·10	148	1·10	37·1	8·1
13	0·31	97	1·00	37·4	7·9

TNFα was not detected in any of the samples examined, while the changes in circulating IL-6 concentrations were variable both with respect to the pattern and extent of change. Although the highest measured concentrations of IL-6 typically occurred 4 to 8 h after injury (and usually before the rise in circulating acute phase proteins) these did not relate to peak levels of acute phase proteins or to other injury-related changes.

The results suggest: (a) serum TNFα is not associated with the acute phase response to elective surgery and accidental injury (although small changes below the limit of detection of the assay may have occurred), (b) immunologically measured serum IL-6 is variably involved in the acute phase response to injury. The lack of correlation between the serum concentration of IL-6 and the metabolic response to injury raises uncertainties about its precise role.

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Serum interleukin-6 and C-reactive protein response to elective surgery of varying magnitude. By ANNE M. CRUICKSHANK[1], W. D. FRASER[1], H. G. J. BURNS[2] and A. SHENKIN[1], *Departments of [1]Biochemistry and [2]Surgery, Royal Infirmary, Glasgow G4 0SF*

Recent studies have suggested that interleukin-6 (IL-6) is a major mediator of the acute phase protein response in man. The aim of the present study was to examine the relationship between serum IL-6, C-reactive protein (CRP) and the magnitude of tissue damage during elective surgery.

Five categories of surgical procedure were studied: minor surgery (varicose vein stripping, lumpectomy, thyroidectomy), cholecystectomy, vascular surgery (aortic aneurysm resection and bifurcation grafts), hip replacement, colorectal surgery. Blood samples collected pre-operatively and at timed intervals post-incision were separated and the sera stored at −20° prior to analysis for IL-6 (hybridoma growth stimulation assay, Coulie *et al.* 1987) and CRP (Abbott TDX).

Surgical category	*n*	Peak IL-6 (U*/ml) Median	Peak IL-6 (U*/ml) Range	Peak CRP (mg/l) Median	Peak CRP (mg/l) Range	Operation time (min) Median	Operation time (min) Range
Minor	6	36[o]	28–46	<10[e]	10–15	45	15–55
Cholecystectomy	6	156[o]	56–225	122[f]	57–203	67	45–135
Vascular	6	497[b]	192–1220	262[g]	124–335	122	75–240
Hip	6	208[c]	122–395	180[h]	121–285	120	60–190
Colorectal	5	552[d]	397–657	166[i]	85–259	105	75–140

a *v.* b, a *v.* d, c *v.* b, c *v.* d $P<0{\cdot}05$, Mann-Whitney U test.
o *v.* a, b, c, d $P<0{\cdot}01$; e *v.* f, g, h, i $P<0{\cdot}01$.
* 1 U is approximately 1 pg IL-6.

Serum IL-6 concentration rose in all groups, peaking 4–6 h post-incision in the minor and cholecystectomy patients, 6–8 h post-incision in the colorectal patients, about 9 h post-incision in the vascular patients, and at about 12 h post-incision in the hip replacement group. Although the minor group demonstrated an increase in serum IL-6 in response to surgery, no measurable CRP response was obtained in this group. Serum CRP rose in the remaining groups peaking between 24 and 48 h post-incision.

Peak serum IL-6 correlated better (r 0·80, $P<0{\cdot}001$) with length of operation time than did peak serum CRP (r 0·65, $P<0{\cdot}001$). Peak serum IL-6 and CRP also correlated significantly (r 0·76, $P<0{\cdot}001$).

These results are consistent with the hypothesis that changes in the serum IL-6 concentration are related to the duration of the operation, and that serum IL-6 is a more sensitive marker than serum CRP. Furthermore, intra-abdominal surgery may be a more potent stimulant of systemic IL-6 release than extra-abdominal surgery.

Coulie, P. G., Cayphas, S., Vink, A., Uyttenlove, C. & van Snick, J. (1987). *European Journal of Immunology* **17**, 1217–1220.

The effect of human interleukin-1β on rat liver protein synthesis compared with a systemic inflammatory insult induced by turpentine. By P. E. BALLMER, M. A. MCNURLAN, B. G. SOUTHORN, I. GRANT and P. J. GARLICK, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Interleukin-1 (IL-1) induces fever, contributes to the humoral and cellular host defence and leads to metabolic changes, including increased liver protein synthesis and peripheral protein wasting (Fong *et al.* 1989). However, little is known about the time-course of its metabolic effects and whether this relates to the changes observed in a classical acute-phase reaction. We therefore measured the time-course of metabolic changes and temperatures in fasted male Lister rats (95–110 g body-weight), given either IL-1β (6·25 μg subcutaneously) or turpentine (5 ml/kg subcutaneously) and compared with control animals injected with saline. The rate of synthesis of total liver protein was measured at intervals during the following 24 h by the method of Garlick *et al.* (1980).

Body temperature increased rapidly in the IL-1β group and peaked at 3 h, followed by a hitherto unreported hypothermic response which continued for 24 h. The turpentine group had a slower rise, reaching a plateau at 9 h and then decreasing to normal. The fever reaction did not parallel the response in liver, which is shown in the Table.

Effect of IL-1 or turpentine on the fractional rate (k_s) of protein synthesis in liver

	k_s (%/d)							
Period after injection (h) . . .	1		3		9		24	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
IL-1β	127	6	125	11	147**	8	131*	9
Turpentine	127	12	139	12	145**	21	187**	14
Control	125	11	131	11	119	8	117	9

Significantly different from control: * $P<0·05$, ** $P<0·01$.

The results indicate that IL-1β is a rapid modulator of liver protein synthesis, but that the reaction is not sustained following a single injection. The initial changes in liver protein synthesis following turpentine were similar to those induced by IL-1β, supporting the view that the onset of an acute-phase response is mediated by IL-1β. The study does not, however, show whether the further increase of liver protein synthesis in the turpentine group is mediated by an on-going production of IL-1β or by involvement of additional mechanisms.

The authors thank Ciba-Geigy, Basle, Switzerland for the gift of IL-1.

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

Adrenalectomy reverses the impaired pyrogenic responses to interleukin-1β in obese Zucker rats. By N. J. BUSBRIDGE[1], J. A. CARNIE[2], M. J. DASCOMBE[1], J. A. JOHNSTON[2] and N. J. ROTHWELL[1], [1]*Department of Physiological Sciences, University of Manchester, Manchester M13 9PT,* [2]*Department of Biochemistry, UMIST, Manchester M60 1QD*

Interleukin-1 (IL-1) is an endogenous pyrogen which stimulates fever by a central action involving activation of metabolic rate (Dascombe *et al.* 1989*b*). Genetically obese rats show impairments in diet-induced thermogenesis which are abolished by adrenalectomy and appear to be due to inhibitory effects of glucocorticoids (Marchington *et al.* 1983). We have recently demonstrated that responses to IL-1β are markedly attenuated in these mutants (Dascombe *et al.* 1989*a*). Therefore the object of the present study was to assess whether adrenalectomy influences the effects of IL-1β in obese mutants.

Genetically obese (*fa/fa*) and lean (+/?) Zucker rats were surgically adrenalectomized or sham-operated. The effects of central intracerebroventricular injections (via indwelling cannulae) of recombinant murine IL-1β on colonic temperature, oxygen consumption ($\dot{V}o_2$ by indirect calorimetry) and brown adipose tissue (BAT) activity (from mitochondrial GDP binding) were tested.

In lean, sham-operated rats, IL-1β elicited increases, which were maximal at a dose of 5 ng, in body temperature (1·3 (SEM 0·5)°, *n* 6–7, $\dot{V}o_2$ (27 (SEM 8)%) and BAT activity (24 (SEM 5)%). Peak responses occurred after 90–120 min. Injection of vehicle did not affect these parameters. The changes in $\dot{V}o_2$ and temperature were not significantly influenced by adrenalectomy although the BAT response was enhanced (60 (SEM 6)%).

In contrast, sham-operated obese rats showed no significant increases in temperature (0·5 (SEM 2)°), $\dot{V}o_2$ (2 (SEM 3)%) or BAT activity (0%) in response to injection of 5 ng IL-1β. Even very high doses (up to 100 ng) produced only small, transient responses. Adrenalectomy caused markedly enhanced responses to IL-1β in obese rats. Increases in body temperature in obese adrenalectomized rats (1·8 (SEM 0·2)°) and BAT activity (44 (SEM 4)%) were comparable with lean animals, but the rise in $\dot{V}o_2$ (49 (SEM 8)%) was significantly greater.

These results show that adrenalectomy completely restores the diminished responses to IL-1β in obese Zucker rats, indicating that the suppression may be due to glucocorticoids.

Dascombe, M. J., Hardwick, A. J., LeFeuvre, R. A. & Rothwell, N. J. (1989*a*). *International Journal of Obesity* **13**, 367–373.

Dascombe, M. J., Rothwell, N. J., Sagay, B. O. & Stock, M. J. (1989*b*). *American Journal of Physiology* **256**, E7–E11.

Marchington, D., Rothwell, N. J., Stock, M. J. & York, D. A. (1983). *Journal of Nutrition* **113**, 1395–1402.

Coconut oil feeding suppresses hypothalamic prostaglandin E_2 production in response to tumour necrosis factor α and endotoxin. By D. BIBBY and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Prostaglandins (PG) have been implicated in several metabolic effects of cytokines such as fever, shock and enhanced muscle proteolysis.

PG production can be modified by fats which alter the amount of the PG precursor, arachidonate (AA), in cell membrane phospholipids. One such fat is coconut oil which is poor in linoleic acid, the precursor of AA. We have shown that giving rats a coconut oil enriched diet blocked the loss of protein from muscle (Wan & Grimble, 1987) which follows exposure to endotoxin. The febrile response of these animals was inhibited by 50%. The present study examines whether PGE_2 production from the hypothalamus in response to tumour necrosis factor α (TNFα) and endotoxin can be modified by coconut oil.

Weanling male Wistar rats were fed for 56 d on diets containing either 200 g maize oil/kg or 190 g coconut oil and 10 g maize oil/kg. Rats attained 360 ± 5 g body-weight on both diets. After killing, the hypothalami were rapidly removed, finely chopped and pre-incubated in minimal essential medium (MEM, Gibco). After this wash, the tissue (15 mg) was resuspended in 1 ml MEM containing 0·25, 2·5 or 25 μg human recombinant TNFα (BASF/Knoll AG; endotoxin content <0·137 ng/mg) or 0·5, 5 or 50 μg of endotoxin (*Escherichia coli* serotype 0·127:B8; Sigma Chemical Co., Poole, Dorset) and incubated at 37° in a shaking water-bath for 50 min. Supernatant fractions were assayed for PGE_2 by radioimmunoassay.

PGE_2 (pg/mg hypothalamus per min)

TNFα dose (μg/ml)	Endotoxin dose (μg/ml)	Maize oil				Coconut oil			
		TNFα		Endotoxin		TNFα		Endotoxin	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	9·7	0·5	12·1	0·4	10·9	0·4	11·9	0·3
0·25	0·5	14·3*	0·9	15·4	0·2	12·7	1·0	13·1	0·9
2·5	5	13·2	1·5	17·4*	1·3	11·1	1·9	13·0	0·9
25	50	15·7*	1·7	18·7*	1·2	10·4	0·6	12·9	1·0

n 6 per measurement.
Significantly different from 0 dose (by ANOVA): * $P<0·05$.

Both TNFα and endotoxin are able to stimulate PGE_2 production from rat hypothalamus in vitro. This phenomenon is prevented by chronic feeding of coconut oil. These results provide further evidence that fats which reduce PG production may be beneficial in modulating the inflammatory effects of cytokines such as TNFα.

The authors are grateful to BASF/Knoll AG for the gift of recombinant TNFα.

Stimulation of colorectal tumour protein synthesis, in vivo, by nutritional support. By S. D. HEYS[1,2], K. G. M. PARK[1,2], M. A. MCNURLAN[1], E. MILNE[1], R. A. KEENAN[2], J. D. B. MILLER[2], J. BROOM[2], O. EREMIN[2] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*, [2]*Department of Surgery, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

Malignant disease is frequently accompanied by weight loss and malnutrition, which are associated with an increase in patient mortality and morbidity. Supplemental nutrition has therefore been provided in an attempt to reverse this weight loss and improve nutritional status, but a major concern has been a possible stimulation of tumour growth. Animal experimental studies have shown that nutrition can stimulate tumour growth (Torosian & Daly, 1986), but to date there is no evidence for stimulation of human tumour growth in vivo. Growth of a tissue depends on an excess of protein synthesis over degradation. However, experimental studies have shown that in malignant cells in culture, protein synthesis is the primary determinant of cell growth (Baccino *et al.* 1980).

The measurement of human tumour protein synthesis in vivo using the 'flooding dose' technique has been previously described (Heys *et al.* 1989). This approach has now been applied to investigate the effect of intravenous nutrition on patients with colorectal cancer.

Patients with localized colorectal carcinoma with no evidence of metastatic disease were studied. They were randomly allocated to one of two groups: (1) to be fasted for 24 h before surgery (n 9), or (2) to receive intravenous nutritional support for the 24 h before surgery (n 9). Nutritional support comprised 1·25 g protein/kg body-weight (Vamin-9-glucose) and 105 kJ (25 kcal) energy/kg body-weight, with 40% of the total energy as glucose and 60% as lipid. The nutritional status of both groups before inclusion in the study was similar. Measurements of colorectal tumour protein synthesis were made at the end of this 24 h experimental period, by intravenous injection of L-[1-^{13}C]leucine, 4 g/70 kg body-weight, 19·6 atoms % in 200 ml saline (9 g sodium chloride/l). Biopsies from the tumour were then taken endoscopically, immediately after induction of anaesthesia, allowing 60–90 min for incorporation of the isotope into protein. The fractional rates of protein synthesis were calculated from the increase in enrichment of protein-bound leucine in the tumour and the average free leucine enrichment in the plasma, determined by isotope ratio and gas chromatography–mass spectrometry.

The mean rate of protein synthesis in colorectal tumour tissue was 22·6 (SEM 1·9)%/d in the fasted patients, but rose significantly to 43·9 (SEM 3·4)%/d ($P = 0·002$) in the group receiving nutritional support. This increase in tumour tissue protein synthesis provides evidence, in vivo, that nutritional support might lead to stimulation of human tumour growth.

Baccino, F. M., Tessitore, L. & Bonelli, G. (1980). *Toxicologic Pathology* **12**, 281–287.
Heys, S. D., Keenan, R. A., Wernerman, J., McNurlan, M. A., Milne, E., Calder, A. G., Buchan, V., Eremin, O. & Garlick, P. J. (1989). *Proceedings of the Nutrition Society* **48**, 101A.
Torosian, M. & Daly, J. M. (1986). *Cancer* **58**, 1915–1929.

The effect of feeding and fasting on human colonic tumours in vitro. By K. G. M. PARK[1,2], S. D. HEYS[1,2], O. EREMIN[2] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*, [2]*Department of Surgery, Aberdeen University, Foresterhill, Aberdeen AB9 2ZD*

Trials of nutritional supplementation before treatment for malignancy have yielded conflicting results (for review see Von Meyenfeldt *et al.* 1985). There is continuing concern that additional nutrition may stimulate the growth of the tumour. We have demonstrated that parenteral nutrition can increase the rate of tumour protein synthesis in vivo (Heys *et al.* 1990). This, however, represents an overall rate of synthesis for the tumour, which is composed of a variety of cell types including cancer cells, fibroblasts, macrophages and lymphocytes. The clinical effect of nutritional regimens will depend on the differential stimulation of each of these individual cell populations. To determine the effect of feeding on the malignant cells we have separated the tumour cells from colonic cancers and measured the effect of feeding on these cells in vitro.

Five patients with rectal cancers were given peripheral nutrition for 24 h (1·25 g protein/kg body-weight and 105 kJ (25 kcal)/kg body-weight). Plasma was taken in the fasting state and during feeding. The tumours were obtained fresh, at the time of surgery, and tumour cells obtained as previously described (Kuppner *et al.* 1987). The tumour cells were incubated at 37° for 7 d in complete medium (RPMI) + 20% fetal calf serum. After this time the medium was changed to RPMI + 25% enrichment with the patient's own serum, from either the fed or fasted state, and incubated for a further 12 h. The cells were labelled by the addition of tritiated phenylalanine at a concentration of 2·5 μCi and 2·5 μmol phenylalanine/ml. After incubation for 30 min the activity of cellular protein was measured (McNurlan & Clemens, 1986). The rates of protein synthesis (disintegrations/min per μg protein) in the cells treated with fasting and fed serum in each individual patient are shown in the Table.

Patient no.	Fasting	Fed	Difference
1	0·94	1·28	+0·34
2	0·80	1·2	+0·40
3	0·90	2·60	+1·70
4	2·0	3·0	+1·0
5	1·23	2·2	+1·07

In each case there was an increase when the added serum was taken in the fed rather than the fasted state. The difference between the two groups was significant at the $P<0·02$ level of probability (paired t test on log transformed data).

These results indicate that observations in vivo of increased rates of protein synthesis in patients with colorectal cancers, following feeding, are partially explained by increased rates of synthesis in the tumour cells. Furthermore these alterations in protein synthesis appear to be mediated by a factor in the patient's serum.

Heys, S. D., Park, K. G. M., McNurlan, M. A., Milne, E., Keenan, R. A., Miller, J. D. B., Broom, J., Eremin, O. & Garlick, P. J. (1990). *Proceedings of the Nutrition Society* **49**, 165A.

Kuppner, M., Wilkinson, S., Casson, E. & Eremin, O. (1987). *Cancer Immunology and Immunotherapy* **25**, 209–214.

McNurlan, M. A. & Clemens, M. J. (1986). *Biochemical Journal* **237**, 871–876.

Von Meyenfeldt, M. F., Fredrix, E. W. H. M., Haagh, W. A. J. J. M., Van der Aalst, A. C. M. J. & Soeters, P. B. (1985). *Bailliere's Gastroenterology* **2**, 869–885.

Reduced rates of hepatic protein synthesis, the acute phase protein response, and elevated circulating interleukin-6 and tumour necrosis factor in advanced malignancy. By K. C. H. FEARON[1], D. C. MCMILLAN[2], T. PRESTON[3], P. WINSTANLEY[2], A. M. CRUICKSHANK[2] and A. SHENKIN[2], [1]*Department of Surgery, Royal Infirmary, Edinburgh*, [2]*Departments of Surgery and Biochemistry, Royal Infirmary, Glasgow*, [3]*Department of Health Physics, SURRC, East Kilbride*

It has been suggested that the production of various cytokines (e.g. tumour necrosis factor (TNF) and interleukin-6 (IL-6)) and the ensuing acute phase protein response (APPR) may account for some of the changes in protein metabolism observed in patients with advanced malignancy. The aim of the present study was to compare circulating TNF and IL-6 concentrations, the APPR, and hepatic protein synthesis rates in a group of healthy controls (elective cholecystectomy: *n* 6, mean weight loss 0%) with those observed in a group of patients with hepatic metastases from colon cancer (*n* 7, mean weight loss 11 (SEM 3)%).

Direct measurement of hepatic protein synthesis was accomplished by following the incorporation of [^{15}N] glycine into liver protein during an 18 h intravenous infusion in the postabsorptive state (0·06 mg [^{15}N] glycine/kg per h). A wedge liver biopsy was taken within 20 min of the start of laparotomy. Following separation by high performance liquid chromatography, ^{15}N enrichment in hepatic free and protein bound glycine was measured by continuous flow, isotope ratio mass spectrometry (Preston & McMillan, 1988). Serum TNF was measured with a sensitive radioimmunoassay (IRE Medgenix: limit of detection approximately 5 pg/ml) and IL-6 was measured with a hybridoma growth stimulation assay (Coulie *et al.* 1987). The APPR was assessed by measuring the serum concentration of C-reactive protein (CRP; Abbot LLTDX). Blood samples were taken immediately before surgery. The results are shown in the Table.

		Hepatic protein synthesis (%/d)		CRP (mg/l)		TNF	IL-6 (U/ml)	
	n	Mean	SEM	Mean	SEM	No. positive	Mean	SEM
Control	6	12·6	0·8	9·5	0·9	0/6	12	1
Cancer	7	7·5**	0·8	64·3*	18·8	3/7†	121**	39

Mann-Whitney U test: * $P<0·05$, ** $P<0·01$.
† Range 15–22 pg/ml for the three positive subjects.

The patients with advanced cancer had rates of hepatic protein synthesis which were 30% lower than those observed in the controls ($P<0·01$). This reduced hepatic non-export protein synthesis was associated with a significant APPR (cf CRP) and with elevated circulating concentrations of TNF and IL-6. These results suggest that in patients with advanced malignancy, despite an APPR, non-export protein synthesis by the liver is markedly reduced. Whether the APPR, the net reduction in hepatic protein synthesis, or both, are mediated by the elevated circulating IL-6 or TNF remains to be determined.

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Preston, T. & McMillan, D. C. (1988). *Biomedical and Environmental Mass Spectrometry* **16**, 229–235.

The effect of arginine on the growth of experimental tumours. By K. G. M. PARK[1,2], S. D. HEYS[1,2], K. BLESSING[3], O. EREMIN[2], P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Departments of* [2]*Surgery and* [3]*Medicine, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

The amino acid L-arginine, when administered in large doses, inhibits the growth and spread of experimental animal tumours (review, Barbul, 1986). To date there are no data available regarding arginine and tumour growth in man, although one study has suggested that arginine inhibits the growth of a human breast cancer cell line in vitro (Cho-Chung *et al.* 1981). We have determined the effect of arginine on a human lung cancer cell line growing in vitro and as a xenograft in athymic nude mice.

A primary lung adenocarcinoma cell line (A549) was grown in monolayer in complete medium (RPMI) +10% fetal calf serum (FCS). After 24 h the medium was changed to include supplements of arginine at various concentrations. After 72 h the cells were counted and the cellular rate of protein synthesis measured by the incorporation of tritiated phenylalanine into the cells over a 30 min incubation period when given at a concentration of 2·5 μCi and 2·5 μmol phenylalanine/ml (McNurlan & Clemens, 1986). The Table shows the rates of synthesis under these different conditions.

Mean rate of incorporation of [^{3}H] phenylalanine into cellular protein (disintegrations/min per μg protein) with different concentrations of arginine

Arginine supplementation (mmol/l) . . .	0		1·5		3·0		6·0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Incorporation of phenylalanine	5·2	0·6	4·6	0·3	8·0*	1·2	1·9*	0·4

* $P<0·02$ (Student's t test).

The same cell type (A549) was injected subcutaneously (1×10^7 cells in 0·2 ml RPMI) into athymic nude mice. After tumour growth had been established the mice were randomized to receive a standard diet with or without 8 g arginine/l in drinking water. Subsequent tumour growth was measured using calipers and the volume calculated. After 30 d the mice were killed, plasma and tumour amino acid profiles measured and the tumour histology determined.

The plasma arginine was elevated in the supplemented group compared to the controls (2·52 (SD 0·43) *v.* 0·54 (SD 0·10) mmol/l). This was in the range at which arginine stimulated cell protein synthesis in vitro (Table). The in vivo rates of growth, as measured by increases in tumour volume, were higher in the supplemented animals (0·03 (SD 0·01) mm^3/d) compared with the control group (0·01 (SD 0·01) mm^3/d). The final tumour volume was also elevated in the arginine fed animals (0·76 (SD 0·21) mm^3 *v.* 0·28 (SD 0·16) mm^3) ($P<0·01$, Student's t test).

The results indicate that within the concentration range that can be obtained in vivo, arginine stimulates the growth of a human lung cancer cell line, both in vitro and in athymic nude mice. Although arginine stimulates the growth of this tumour, in the absence of a functional immune system, any effects of arginine in clinical practice are likely to represent a balance of the effects on the tumour and the host defence mechanisms.

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Effects of corticosteroid therapy on quadriceps protein synthesis and intramuscular prostaglandin concentration in patients with rheumatoid arthritis. By J. N. A. GIBSON[1], N. L. POYSER[2] and M. J. RENNIE[3], *Departments of [1]Orthopaedic Surgery and [2]Pharmacology, Edinburgh University, Edinburgh* and *[3]Department of Anatomy & Physiology, University of Dundee, Dundee DD1 4HN*

Although atrophy of skeletal muscle is associated with long-term corticosteroid administration, the mechanism of the wasting in terms of possible alterations in muscle protein turnover is obscure. We have investigated the effect of corticosteroid treatment (duration ranged from 2 to 14 years, mean 9 years) on muscle protein synthesis in six patients with rheumatoid arthritis (age 40–75 years, weight 56 (SD 12) kg) and related the results to intramuscular prostaglandin concentration. Values were compared with those from six other zero-positive patients who had not received steroids (age 62–83 years, weight 52 (SD 6) kg) and seven normal subjects (age 57–75 years, weight 62 (SD 7) kg).

L-[1-^{13}C]leucine was infused over 8 h to achieve steady state precursor pool labelling. Quadriceps muscle biopsies were then taken. Muscle protein synthesis was calculated by comparing the protein incorporation of ^{13}C (measured by preparative gas–liquid chromatography and isotope-ratio mass spectrometry; Smith *et al.* 1988) with the time-averaged enrichment of blood α-ketoisocaproate, used as an index of the intramuscular precursor for muscle protein synthesis, and measured by gas chromatography–mass spectrometry. Intramuscular prostaglandin (PGE_2 and $PGF_{2\alpha}$) concentrations were measured by radioimmunoassay.

Patients not receiving steroids had a normal rate of muscle protein synthesis (patients 0·056 (SE 0·005), normals 0·051 (SE 0·029) %/h) suggesting that atrophy was associated with an increased rate of muscle protein breakdown. These changes were associated with an elevation of PGE_2 (patients 0·12 (SE 0·06) ng PGE_2/mg protein, controls 0·06 (SE 0·03) ng PGE_2/mg protein, $P<0·05$). In contrast, corticosteroid treatment appeared to diminish muscle protein synthesis (treated 0·035 (SE 0·008)%/h, untreated 0·05 (SE 0·029)%/h, $P<0·05$) and this change was associated with a diminution in $PGF_{2\alpha}$/mg protein (untreated 0·016 (SE 0·008) ng $PGF_{2\alpha}$/mg protein, $P<0·01$).

The results suggest that major changes in muscle protein metabolism (specifically, an inhibition of muscle protein synthesis) are induced by corticosteroid administration, associated with alterations in the pattern of prostaglandin production or catabolism. Drug therapy directed at changing the concentration of specific intramuscular prostaglandins may be appropriate to inhibit muscle atrophy in patients with rheumatoid arthritis but should take into account the differences observed in those receiving or not receiving corticosteroids.

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Metabolic effects of lipid infusion in septic and non-septic humans. By J. ARNOLD[1,2], K. A. SHIPLEY[1], N. A. SCOTT[1], R. A. LITTLE[2] and M. H. IRVING[1], [1]*Department of Surgery and* [2]*North Western Injury Research Centre, Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT*

The rise in energy expenditure (heat production) occurring after the ingestion or infusion of nutrients is a well-known response. It has even been reported that carbohydrate and fat, given separately in isoenergetic amounts, elicit different degrees of nutrient-induced thermogenesis (Schwartz *et al.* 1985). By examining the mechanism behind the phenomenon, researchers have implicated the sympathetic nervous system (SNS).

Our group has recently investigated the thermic effect of a glucose-based parenteral nutrition regimen in septic patients whose SNS is particularly active (Arnold *et al.* 1989). The present study was a progression of our previous work in that the metabolic effects of lipid infusion were compared in septic and non-septic individuals.

Five septic, surgical patients (mean sepsis score of 14; Elebute & Stoner, 1983) and five non-septic (home parenteral nutrition) patients were recruited. Following an 8 h fast, a catheter was placed into an antecubital vein for blood sampling and the subjects were then familiarized with our ventilated canopy system for the measurement of respiratory gas exchange. Following the adaptation period subjects rested quietly for 60 min while resting values were determined. Thereafter, lipid (Intralipid; Kabivitrum Ltd, Uxbridge) was infused at 23 kJ (5·5 kcal)/min through the patient's central venous catheter, and indirect calorimetry was continued for a further 120 min.

Integrating the areas under the response curves revealed the septic patients to have a resting oxygen consumption 17% higher than the control subjects (4·65 (SE 0·30) *v.* 3·98 (SE 0·24) ml/kg fat free mass per min, $P = 0{\cdot}122$). However, after accounting for the differences in resting metabolic rate, both groups were shown to respond identically to the lipid infusion; a 13% rise in O_2 uptake was noted during the 2 h infusion ($P = 0{\cdot}026$). The initial respiratory quotient (RQ) was nearly 7% higher in the septic patients compared with the controls (0·86 (SE 0·01) *v.* 0·80 (SE 0·01), $P = 0{\cdot}030$). During the infusion, RQ decreased similarly (≈6%) in both groups although the intergroup difference was not as marked at the end of the experiment. Plasma catecholamines were significantly elevated pre-infusion in the septic subjects and the levels did not change during the infusion. In contrast, noradrenaline increased in the non-septic controls during the 2 h infusion ($P = 0{\cdot}009$). Plasma cortisol levels, nearly 2·5 times higher in the septic patients, remained unaltered by the lipid in both groups. Glucagon followed a trend similar to that of cortisol although it increased slightly during the infusion in the two groups.

In summary, the significant rise in O_2 consumption occurring during lipid infusion appeared little influenced by sepsis. The results, considered in conjunction with previous observations (Arnold *et al.* 1989), suggest that the different macronutrients elicit different extents of nutrient-induced thermogenesis.

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Post-operative ketosis: relationship to serum non-esterified fatty acids, carnitine and metabolic hormone concentrations. By P. J. MOYNIHAN, CHRISTINE M. WILLIAMS and V. MARKS, *Division of Nutrition and Food Science, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

The ability to mount a ketotic response to accidental injury (Wedge *et al.* 1976) and surgery (Rich & Wright, 1979) has been suggested to be beneficial in preventing the excessive protein catabolism associated with these conditions. Factors regulating ketogenic potential in the stressed individual are, however, incompletely understood. Lower concentrations of insulin and glucagon have been observed in hyperketotic compared with normoketotic post-surgical patients (Rich & Wright, 1979). Availability of carnitine may also be a determinant of ketogenic capacity during stress (Cederblad *et al.* 1984). We have compared serum concentrations of carnitine, insulin and glucagon, as well as serum non-esterified fatty acids (NEFA), glycerol and glucose, in hyperketonaemic compared with normoketonaemic post-surgical patients.

Blood was collected from patients undergoing elective abdominal or thoracic surgery on days 1, 3 and 6 post-operatively. None of the patients received total parenteral nutrition or nasogastric enteral feeds during the first post-operative week but received some glucose (50 g/l) intravenously as part of their 24 h fluid requirements. Patients were divided into two groups on the basis of their combined day 1 and day 3 total ketone concentrations; hyperketonaemia was defined as a total ketone concentration greater than 0·2 mmol/l.

	Normoketonaemic (*n* 12)				Hyperketonaemic (*n* 9)			
Days after surgery . . .	1 + 3		6		1 + 3		6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total ketones (mmol/l)	0·14	0·04	0·08	0·04	0·38***	0·11	0·42**	0·28
NEFA (mmol/l)	0·65	0·24	0·50	0·24	0·95*	0·34	1·25***	0·24
Glycerol (mmol/l)	0·06	0·04	0·05	0·02	0·07	0·02	0·06	0·01
Glucose (mmol/l)	7·02	1·33	7·40	1·50	7·80	1·50	6·60	1·20
Total carnitine (μmol/l)	45·8	17·3	31·4	14·1	55·6	18·6	44·2	17·80
Acylcarnitine (μmol/l)	15·9	12·8	8·2	7·4	9·6	6·3	6·8	3·9
Insulin (pmol/l)	158	111	188	101	83·3	34	130	123
Glucagon (pmol/l)	20·8	17·1	14·6	21·5	9·7	9·0	20·1	27·0

Significantly different from normoketonaemic group on corresponding day: * $P<0·05$, ** $P<0·02$, *** $P<0·001$.

NEFA concentrations were significantly higher in the hyperketonaemic group in the early (days 1 and 3) and late (day 6) catabolic phase after surgery. Glycerol concentrations were similar in both groups and suggest that elevated NEFA and ketone concentrations were not due to higher rates of lipolysis in the hyperketonaemic group. Insulin and glucagon concentrations varied widely but did not differ significantly within the two groups. Mean concentrations of total carnitine were high in the hyperketonaemic group at both time periods but these differences were not statistically significant.

Elevated NEFA concentrations, in the absence of raised glycerol and lowered insulin concentrations, suggest that decreased peripheral tissue uptake and incomplete oxidation of NEFA may be a cause of the higher ketone concentrations in the hyperketonaemic group.

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Nutritional requirements in fulminant hepatic failure. By ALASTAIR FORBES[1], GEORGE K. GRIMBLE[2], CLAIRE WICKS[1], DAVID B. A. SILK[2] and ROGER WILLIAMS[1], [1]*Liver Unit, King's College Hospital, Denmark Hill, London SE5 9RS*, [2]*Department of Gastroenterology and Nutrition, Central Middlesex Hospital, London NW10 7NS*

Conventional management of fulminant hepatic failure (FHF)—severe acute liver failure associated with encephalopathy and coagulopathy—includes protein withdrawal and the provision of energy only to prevent hypoglycaemia. Little information exists on the nutritional requirements, but better intensive care leading to longer survival makes the development of nutritional deficiencies and their possible sequelae more likely. Requirements of macronutrients have therefore been studied in sixteen patients (four male) with FHF as the result of self-poisoning with paracetamol. On the 47 days during which the patients had marked (grade 3 or 4) encephalopathy, resting energy expenditure (REE) (indirect calorimetry; Nutrimon, Simonsen & Weel), and total nitrogen excretion (including losses in dialysis effluent) were measured.

Median energy expenditure on day 1 extrapolated from measurements made over 5 min was 6945 kJ (range 5397–8368)/24 h (1660 kcal (range 1290–2000)/24 h) rising to a median of 7447 kJ (range 5397–8368)/24 h (1780 kcal (range 1290–2000)/24 h) for the complete study period. Initial median N excretion was 3·9 g/24 h but this conceals the effects of therapy and in particular of haemodialysis. The median daily N excretion on 15 days of oliguria (<600 ml/24 h), untreated by dialysis on those days (nine patients), was 1·7 g (range 0·8–6·1); ultrafiltration in three of these patients removed approximately 1 g N/l ultrafiltrate. Haemodialysis achieved a median N excretion of 12·4 g (range 3·3–18·1) (14 d, seven patients). On 18 days when spontaneous urine output was >600 ml (seven patients), the median N loss was 8·6 g (range 3·6–24·0). In the ten patients who survived for 3 d or more, total energy provision (comprising glucose given in fluid management or treatment/prophylaxis of hypoglycaemia) reached a median of only 3096 kJ (range 1339–4393) at 72 h (740 kcal (range 320–1050) at 72 h). No N-containing fluids were given other than blood products (including human albumin solution for plasma expansion). In these ten patients the minimum median energy deficit at 72 h was 18·95 mJ (4530 kcal) with a median N deficit of 20·1 g. The true energy debt will have been substantially higher because of the increased energy expenditure during intervention (particularly dialysis) and is borne out by small but significant ($P<0·05$) anthropometric changes. Even in this short time-span, median mid-arm circumference fell from 255 to 250 mm and median triceps skin-fold thickness from 11 to 10 mm.

It is concluded that malnutrition develops rapidly in patients with fulminant hepatitis despite relatively modest REE. This may contribute to the high frequency of sepsis in these patients and suggests that nutritional support should be considered.

A controlled trial of sip-feed supplements in orthopaedic patients: post-discharge clinical outcome in relation to supplementation and compliance. By LYNN T. DRIVER[1], M. LUMBERS[1], J. OLDER[2] and CHRISTINE M. WILLIAMS[1], [1]*Division of Nutrition and Food Science, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*, [2]*Department of Surgery, Royal Surrey County Hospital, Guildford, Surrey*

In this study we have carried out a controlled trial of sip-feed supplements in elderly female orthopaedic patients. Patients admitted for surgery for proximal femoral fracture or total hip replacement were allocated into supplement and non-supplement groups alternately on admission. This method of allocation allowed each supplement patient to be 'paired' with a corresponding non-supplement patient. Supplement patients were asked to take sip-feed supplements (equivalent to 2090 kJ (500 kcal) energy and 20 g protein) daily during their stay in the acute ward and for 4 weeks after discharge. Patients in both groups were visited daily in hospital and at 2, 4 and 8 weeks post-discharge and later at 6 months post-discharge. Anthropometric, biochemical, muscle function and clinical assessments were made weekly in hospital and at the stated time periods post-discharge. Of the twenty-one patients who were offered supplements, eight refused to take them or continue with them after 2–3 d; these patients were classified as 'non-compliant'. The Table shows the weight changes from admission to 8 weeks post-discharge, and post-discharge clinical results in patients randomized to receive supplements or not. The supplement group has also been divided to show results for compliant and non-compliant patients; these values have been compared with the corresponding non-supplement admission 'pairs' of the compliant and non-compliant patients.

		Wt change (kg)		No. requiring convalescence		Duration of stay + convalescence (days)		Total no. of patient convalescence days †
Group	*n*	Mean	SD	*n*	%	Mean	SD	
Supplement	21	+0·67	1·89	8	38	30·1	17·5	217
Non-supplement	21	−2·43**	2·31	14	66	33·7	21·7	375
Supplement, compliant	13	+0·87	1·87	4	30	27·0	19·1	108
Non-supplement, pairs	13	−2·47**	2·80	11	85	36·2	23·4	246
Supplement, non-compliant	8	+0·43	2·20	4	50	33·0	18·0	109
Non-supplement, pairs	8	−1·90*	1·77	3	38	31·2	14·0	129

* $P<0·05$, ** $P<0·02$.

† Excluding patients who died.

Patients who did not receive supplements showed a significant loss of weight from admission to 8 weeks post-discharge. A greater number of these patients required convalescence so that although the average duration of stay was similar in all groups, the total number of patient convalescence days was greater in the non-supplement group. Compliance to the supplements was disappointing and poses problems in interpreting the significance of clinical outcome results in these patients.

Do 'novel' enteral substrates improve nitrogen economy following surgical stress in dogs?

By M. G. BROWN, G. R. CAMPBELL and B. J. ROWLANDS, *Department of Surgery, Royal Victoria Hospital, Queen's University of Belfast, Belfast BT12 6BJ*

Recently attention has been focused on the use of 'novel' substrates to support metabolism following surgical stress and trauma. Two such products are glutamine and ornithine α-ketoglutarate (OKGA). Following trauma, glutamine is metabolized by the jejunum to maintain mucosal integrity and its metabolites support hepatic protein synthesis and gluconeogenesis. Potential disadvantages are its instability in solution and its ability to enhance ammonia production. OKGA is a combination of ornithine, a urea cycle intermediate, and α-ketoglutarate, an end-product of glutamine deamination and a Krebs cycle intermediate. Ornithine has a direct anti-ureagenic effect and is a precursor of polyamine synthesis. α-Ketoglutarate acts as a nitrogen sink to trap N released from gluconeogenic amino acids and stimulates the Krebs cycle. The present study was designed to assess the efficacy of supplemental feeding with 'novel' substrates following surgical trauma, to evaluate the role of the jejunum in their metabolism and to measure ammonia production during administration.

Beagle dogs were stressed by laparotomy and either enterotomy (E) or 60% proximal enterectomy (T). Post-operatively, they received, via an enteral feeding catheter, a glutamine-free standard peptide diet (Peptisorbon (E. Merck Ltd.) equivalent to 2 g protein and 209 kJ energy/kg body-weight (BW) per d) to which were added equivalent amounts of glycine (C) (0·135 g/kg BW), glutamine (D) (0·2 g/kg BW) or OKGA (J) (0·143 g/kg BW) so that the diets were isonitrogenous and isoenergetic. Urine was collected for daily N balance and blood samples were obtained from catheters placed in the systemic and portal circulation to measure ammonia production over 72 h after surgery.

Cumulative N balance (g)

Period after surgery (h) . . .			24		48		72	
		n	Mean	SEM	Mean	SEM	Mean	SEM
Control	EC	7	0·53	0·31	−0·37	0·55	−3·70	1·27
	TC	5	1·25	0·55	0·68	1·04	−1·60	2·96
Glutamine	ED	6	2·16	0·49	3·09	0·68	4·61*	0·38
	TD	5	2·36	0·40	−0·09	1·80	−5·07	1·07
OKGA	EJ	6	1·36	0·48	1·71	0·91	−1·22	1·54
	TJ	5	1·35	0·66	1·46	0·34	−3·80	2·49

* $P<0·05$ (ANOVA).

Portal ammonia concentrations (normal range 150–250 mmol/l) were unchanged throughout the infusion period. Supplementation of enteral feeding regimen with glutamine improved N balance following enterotomy but not enterectomy, showing that the jejunum is an important site of glutamine metabolism. Ammonia production was not increased. OKGA, at the dosage used, improved N balance but did not reach a statistically significant difference compared with the standard regimen. It had no side-effects. Glutamine supplements improve post-traumatic metabolism in dogs indicating the need for assessment in clinical nutritional trials. OKGA requires evaluation using various doses and different stress states in both laboratory and clinical studies.

Effect of glutamine infusions on muscle glutamine concentration and protein synthetic rate in rat muscle. By MONICA WUSTEMAN and MARINOS ELIA, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

A reduction in muscle glutamine concentration is consistently observed after injury in both humans and experimental animals. It has been suggested that this is responsible for the reduction in muscle protein synthetic rate (Rennie *et al.* 1986) which is also observed after injury in experimental animals (Wusteman *et al.* 1989). In the present study we have (a) attempted to increase the intramuscular concentration of glutamine in both normal and injured rats using an infusion of glutamine and (b) assessed whether these changes relate to alterations in the fractional protein synthetic rate.

Forty-three hours after subcutaneous injections of turpentine or saline (9 g sodium chloride/l), male Dunn hooded rats were sedated with an intraperitoneal injection of Hypnorm (0·5 ml/kg) and infused for 5 h via the lateral tail vein with either saline or 0·22 M-glutamine: both infusions included 10 μl Hypnorm/ml to maintain sedation. Six turpentine-injected and six saline-injected rats received no infusion and were killed, together with the infused rats, 48 h after the injections. Plasma and muscle glutamine concentrations ($[GLN]_p$) were measured enzymically and intracellular muscle glutamine concentrations ($[GLN]_i$) calculated using the chloride technique. Gastrocnemius muscle protein synthetic rate (FSR) was measured using a phenylalanine flooding technique. Statistical comparisons were made using analysis of variance.

	$[GLN]_p$ (mmol/l)			$[GLN]_i$ (mmol/l)			Skeletal muscle FSR (%/d)			Plasma insulin (μUnits/ml)		
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
Saline injected												
No infusion	0·583	0·022	6	10·12	1·18	6	12·88	0·73	6	17·6	5·6	5
Saline infusion	0·490	0·032	8	7·96	0·94	8	8·97	0·81	8	15·0	3·2	6
Glutamine infusion	0·788	0·069	8	9·27	0·66	7	9·30	0·62	7	20·4	2·0	8
Turpentine injected												
No infusion	0·568	0·048	6	5·57	0·55	6	7·58	0·86	6	22·6	1·9	5
Saline infusion	0·574	0·038	9	4·39	0·59	8	7·94	0·81	9	21·4	3·1	10
Glutamine infusion	0·873	0·063	10	7·98	0·72	7	7·75	0·84	7	27·4	4·4	10

Turpentine injections significantly reduced both $[GLN]_i$ ($P<0·001$) and FSR ($P<0·001$) in muscle by about 42%. The saline/Hypnorm infusion produced a small but significant reduction in $[GLN]_p$ in the saline-injected rats ($P<0·05$): the $[GLN]_i$ was unaffected but FSR was reduced by 30% ($P<0·01$) in these animals. The saline/Hypnorm infusions had no significant effect on $[GLN]_p$, $[GLN]_i$ or FSR in the rats injected with turpentine.

Glutamine infusion elevated $[GLN]_p$ by 50–60% in both the saline-injected ($P<0·01$) and turpentine-injected ($P<0·001$) rats. It also increased $[GLN]_i$ in those injected with turpentine by 82% ($P<0·01$) but without changing FSR. Circulating insulin levels were low and similar in all groups of rats.

It is concluded, therefore, that acute elevations in intracellular glutamine concentrations can be brought about in rats with aseptic abscesses but these do not lead to any increase in protein synthetic rate in the muscle.

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Wusteman, M., Jennings, G. & Elia, M. (1989). *Proceedings of the Nutrition Society* **48**, 79A.

Are intracellular glutamine concentrations related to muscle protein synthesis during dietary restrictions? By K. KHAN, M. WUSTEMAN and M. ELIA, *Dunn Nutritional Laboratory, Downham's Lane, Milton Road, Cambridge CB4 1XJ*

Glutamine (GLN) is found in skeletal muscle at a higher concentration than any other amino acid. Recent work has suggested that it may have a specific protein sparing effect, particularly in muscle where it may stimulate protein synthesis (Rennie *et al.* 1986). Interestingly, trauma/sepsis which results in a decrease in muscle protein synthesis also produces a marked reduction in the intramuscular GLN concentration (Wusteman *et al.* 1989).

This study was concerned with assessing the relationship between the free GLN concentrations and protein synthetic rates in muscle during dietary restriction. The experiments were carried out using 40-d-old Dunn hooded rats which for the previous 2 d had been given one of three diets: (1) a diet based on casein, sucrose and starch containing 200 g protein/kg given *ad lib.* (*n* 9), (2) the same diet restricted to 25% of normal intake (*n* 9), and (3) glucose in amounts isoenergetic with diet (2) (*n* 9). The animals were killed at 10.00–12.00 hours on the day of the experiment. Protein turnover was assessed using a [^{3}H]phenylalanine flooding technique (Jepson *et al.* 1986). GLN was measured enzymically in plasma and also in the gastrocnemius/soleus muscle. The intracellular GLN concentrations were estimated using the chloride method. Before dietary intervention the weights of the animals were as follows: control group, 135·3 (SE 1·8) g; restricted mixed diet group, 136·7 (SE 2·7) g; restricted glucose group, 136·7 (SE 2·6) g. After 2 d of dietary manipulation the weights were 151·1 (SE 2·0) g for the control animals, 126·0 (SE 2·2) g for the animals given the restricted mixed diet, and 120·6 (SE 1·6) g for the animals given a restricted glucose diet.

	Control diet fed *ad lib.*		Restricted to 25% of control			
			Mixed diet		Glucose diet	
	Mean	SEM	Mean	SEM	Mean	SEM
Muscle protein synthetic rate (%/d)	17·68	0·61	10·88***	0·64	10·21***	0·54
Muscle glutamine:						
mmol/kg wet wt	5·57	0·16	6·48***	0·17	6·34*	0·19
mmol/kg intracellular water	8·68	0·29	10·01*	0·29	9·71	0·44

Significantly different from control animals (Wilcoxon rank sum test): * $P<0·05$, *** $P<0·001$.

Both groups of animals fed restricted diets showed a 40–45% decrease in fractional protein synthetic rate which was not associated with a decrease in the intracellular GLN concentration (Table).

It is concluded from this study that (a) severe dietary restriction alone does not reduce the intramuscular GLN concentration, although such a reduction does occur after trauma/sepsis which is associated with reduced dietary intake; (b) the reduction in skeletal muscle protein synthesis induced by dietary restriction is not related to a fall in the intramuscular GLN concentration.

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Comparative studies of the defunctioned and functioning colon in patients with a defunctioning colostomy confirm human colonic permeability to urea. By BRENDAN MORAN[1], STEVEN KARRAN[1] and ALAN A. JACKSON[2], *Departments of [1]Surgery and [2]Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

Previous investigators, using rapid large volume perfusion of the cleansed colon, have concluded that the human colon is impermeable to urea (Wolpert *et al.* 1971; Bown *et al.* 1975). We have disputed this and have previously presented evidence to the contrary using stable isotope labelled urea placed in the intact colon via the biopsy channel of the colonoscope (Moran *et al.* 1989). We concluded that the colon is permeable to urea, that intraluminal hydrolysis occurs and that a large proportion of urea nitrogen enters the metabolic pool of N. We reasoned that rapid handling of urea by colonic flora makes it difficult to assess reliably the movement of urea through the colon. A defunctioned colon, with reduced bacterial activity, should demonstrate more conclusively the permeability of the colon to urea.

Patients with a defunctioning colostomy provide a unique opportunity to compare a functioning and a defunctioned colon in the same individual.

In five subjects a dose of [$^{15}N^{15}N$] urea (1·5 mg/kg) was placed, on separate occasions, in either the right or left colon. Urine and stool were collected for the next 72 h and analysed for ^{15}N enrichment. The fate of [$^{15}N^{15}N$] urea as a median (range) percentage of the dose is shown in the Table.

	Urinary [$^{15}N^{15}N$] urea		Urinary [$^{15}N^{14}N$] urea		Stool		Retention	
	Median	Range	Median	Range	Median	Range	Median	Range
Right functioning colon	9	5–14	10	6–14	7	1–24	71	47–84
Left defunctioned colon	29	22–74	11	5–14	—	—	61	20–65

The recovery of unchanged [$^{15}N^{15}N$] urea in the urine represents intact urea that has crossed the colonic mucosa and was significantly greater ($P<0·01$ Wilcoxon rank sum) from the left defunctioned colon (29%) than from the right functioning colon (9%). Urinary [$^{15}N^{14}N$] urea represents urea that has been hydrolysed and absorbed as ammonia and reformed into urea in the liver.

In the defunctioned colon the activity of the intestinal flora is diminished due to a reduction in substrate. A reduction in urea hydrolysis and metabolism in the defunctioned colon allows contact between the mucosa and the intact urea molecules. It is likely that insufficient contact time in the perfusion studies, together with the rapid hydrolysis of available urea has led to difficulties in previous studies of colonic urea metabolism. The results of the present study confirm human colonic permeability to urea and illustrate some of the effects of the intestinal flora on the metabolism of urea N.

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Dietary reduction increases secretion in rat distal colon induced by secretagogues acting via calcium but not cyclic AMP. By HELEN C. NZEGWU and R. J. LEVIN, *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

Starvation for 72 h and acute undernutrition (33% of normal diet (CRM Labsure, Cambridge) for 9 d) create a hypersecretory state in the rat small and large intestine when stimulated by cholinergic secretagogues (Levin & Young, 1987; Levin *et al.* 1987) or bacterial toxins (Levin *et al.* 1988). Secretagogues activate colonic secretion via the mediation of the intracellular messengers calcium or cyclic AMP. We have examined their roles in the hypersecretion of starvation and acute undernutrition in rat distal colon.

Distal colons (30 mm segment, 130–160 mm from the caecum) were removed from rats (male Wistars (230–260 g) of the Sheffield strain) anaesthetized with sagatal (60 mg/kg body-weight, intraperitoneally), stripped of their external muscle and mounted as sheets between two chambers. The short-circuit current (I_{sc}) was measured by a standard technique and the electrogenic secretory function of the colon assessed by obtaining the maximal change in I_{sc} ($\triangle I_{sc}$) induced by application of the various stimuli. Electrogenic secretion was induced by serosal addition of 1 mM bethanechol (a Ca-mediated muscarinic agonist), 28 μM PGE_2 (a prostaglandin agonist mediated by cyclic AMP), 5 μM forskolin (an activator of adenylate cyclase to raise the intracellular cyclic AMP levels) or 1 mM dibutyryl cyclic AMP (DBcAMP, permeable derivative of cyclic AMP). The results are given in the Table. Statistical analysis was accomplished using the Kruskal-Wallis analysis of variance followed by Conover's multiple *t* test to identify significant differences.

	$\triangle I_{sc}$ (μA/cm² serosal area)											
	Bethanechol (1 mM)			Forskolin (5 μM)			PGE_2 (28 μM²)			DBcAMP (1 mM)		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Fed	31	5	13	43	5	8	19	2	30	41	4	8
72 h starved	46*	4	8	29†	3	8	7***	2	25	29*	4	7
Acute undernourished	67***	9	8	28	8	7	9*	5	8	17**	5	13

Significantly different from fed group: * $P<0{\cdot}05$, † $P<0{\cdot}02$, ** $P<0{\cdot}002$, *** $P<0{\cdot}001$.

Bethanechol induced an increased secretion in the starved (+48%, $P<0{\cdot}05$) and an even greater secretion in the acute undernourished animals (+116%, $P<0{\cdot}001$). Forskolin, PGE_2 and DBcAMP, however, gave reduced secretions in the starved and undernourished colons. The results indicate that the colonic hypersecretion is not activated by mechanisms involving cyclic AMP, whether by forskolin or by direct addition of cyclic AMP. This is in agreement with previous findings in acute undernourished rat jejunum and ileum (Pereira *et al.* 1989). Thus, the pathways involved in activating electrogenic secretion (in both small and large intestine) by Ca or cyclic AMP appear to behave as separate entities, suggesting that they do not share a final common route.

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[^{15}N]phenylalanine and L-[1-^{13}C]leucine leg exchange and plasma kinetics during amino acid infusion and euglycaemic hyperinsulinaemia: evidence for stimulation of muscle and whole-body protein synthesis in man by insulin. By WILLIAM M. BENNET[1,2], ALAN A. CONNACHER[1], CHARLES M. SCRIMGEOUR[2], ROLAND T. JUNG[1] and MICHAEL J. RENNIE[2], *Departments of* [1]*Medicine and* [2]*Anatomy & Physiology, University of Dundee, Dundee DD1 4HN*

Recent reports suggest insulin has little or no effect on whole-body (e.g. Castellino *et al.* 1987) or muscle (e.g. Gelfand & Barrett, 1987) protein synthesis in healthy man or in diabetic patients, despite the many reports of effects in animals (reviewed by Kimball & Jefferson, 1988). We recently confirmed the lack of any effect of insulin in diabetic patients (Bennet *et al.* 1990) in circumstances in which intramuscular concentrations of some amino acids (AA) essential to protein synthesis (e.g. tyrosine) fell. We hypothesized that if a sufficiency of AA could be maintained, an effect of insulin might be observed. Accordingly, leg exchange and plasma kinetics of [^{15}N]phenylalanine and [1-^{13}C]leucine were used to assess the effect of insulin during AA sufficiency. The methods used have been previously reported in detail (Bennet *et al.* 1990). Eight healthy subjects were studied first during AA infusion alone (84 mg/kg per h) and then during infusion of glucose (to maintain euglycaemia, i.e. 5·0 (SE 0·1) mM-glucose) and insulin (0·29 nmol/m^2 per min) with additional AA by infusion at four times the previous rate.

Insulin strongly stimulated the leg AA uptake seen with AA. Phenylalanine uptake into leg protein (protein synthesis) rose during insulin plus AA infusion (AA alone, 473 (SE 115) nmol/kg leg per min; insulin + AA, 731 (SE 73) nmol/kg leg per min, P=0·022) but with no reduction in leg phenylalanine release (protein breakdown) (AA alone, 447 (SE 81); insulin + AA, 400 (SE 79) nmol/kg leg per min). Leucine entry to leg protein increased slightly with insulin (AA alone, 1290 (SE 260); insulin + AA, 1460 (SE 210) nmol/kg leg per min) but this was possibly obscured by a fourfold elevation in leucine oxidation (P=0·012). Leg protein breakdown (leucine release) was reduced by insulin (1200 (SE 170) *v.* 840 (SE 100) nmol/kg leg per min, P=0·024). Whole-body protein synthesis (non-oxidative leucine-C disposal) increased with insulin (114 (SE 4) *v.* 126 (SE 4) μmol/kg per h, P=0·0048) and whole-body protein breakdown fell with insulin (i.e. leucine appearance, 118 (SE 5) to 99 (SE 6); phenylalanine appearance, 45 (SE 2) to 32 (SE 5) μmol/kg per h, both P<0·02).

The results provide strong evidence that insulin, given with sufficient AA, markedly stimulates leg and whole-body protein balance by mechanisms including stimulation of protein synthesis as well as inhibition of protein breakdown.

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Amino acids promote net phenylalanine balance and protein synthesis in legs of anaesthetized young pigs independently of insulin or glucagon. By P. W. WATT, Y. LINDSAY and M. J. RENNIE, *Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN*

The mechanisms of the beneficial effects of parenteral infusion of amino acid solutions have not been fully characterized, although recent evidence of an anabolic effect of amino acids alone has been obtained in studies of ^{13}C leucine leg exchange and incorporation into muscle protein in man (Bennet *et al.* 1989, 1990). Furthermore, infusion of large amounts of amino acids might be expected to influence hormones, e.g. insulin and glucagon, giving rise to a complicated situation in which any effects cannot be confidently attributed to one factor alone. We aimed to clarify matters by infusing six female anaesthetized pigs (27 (SE 4) kg) with somatostatin (Sandostatin) 8 μg/kg per h, replacement insulin and glucagon and tracer [^{15}N]phenylalanine (1 mg/kg per h) over 4 h. In a further seven pigs (25 (SE 2) kg), also infused with somatostatin, amino acids (3 g amino acid/kg per 12 h, as Synthamin 14) were also given. Blood samples were taken at intervals from the femoral vein and carotid artery. The measurements for the present results were taken between 210 and 240 min when isotopic plateau was achieved, with at least three samples for each animal. Plasma enrichments and phenylalanine concentrations were determined by gas chromatography–mass spectrometry after addition of phenylalanine [phenyl-D_5] as internal standard. Protein breakdown was calculated from the isotopic dilution of [^{15}N]phenylalanine across the leg and protein synthesis from the difference between balance and breakdown.

Administration of amino acids caused a 4·5-fold increase in the concentration of plasma phenylalanine and a significant increase in the rates of protein balance, protein breakdown and synthesis.

	Phenylalanine exchange (nmol/kg leg per min)					
	Balance		Breakdown		Synthesis	
	Mean	SEM	Mean	SEM	Mean	SEM
−Amino acid	−0·2	3·3	19·4	1·6	19·2	3·3
+Amino acid	31·6*	8·8	50·8*	7·4	82·4**	12·6

* $P<0·05$, ** $P<0·002$.

The results suggest: (1) amino acids alone, in the absence of changes in pancreatic hormone secretion, reverse net protein loss from muscle; (2) the mechanisms of the effect include a non-hormonally-induced increase in skeletal muscle protein synthesis (Bennet *et al.* 1989, 1990); (3) the unexpected effect of amino acids in apparently elevating muscle protein breakdown is either (a) an artefact of the model or (b) the result of trans-stimulation of the L-transport system by which phenylalanine leaves muscle; the stimulation of proteolysis by amino acids is difficult to envisage.

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Plasma leucine turnover and oxidation in critically ill patients: lack of a relationship to energy expenditure. By THERESE A. SAMUELS[2], J. CONOR DEVLIN[1], CERI J. GREEN[2], LESLIE J. HIPKIN[3], IAIN T. CAMPBELL[2] and MICHAEL J. RENNIE[4], [1]*Intensive Therapy Unit and* [2]*University Department of Anaesthesia, Royal Liverpool Hospital, Liverpool,* [3]*Sub-Department of Paediatric Endocrine Pathology, Alder Hey Hospital, Liverpool* and [4]*Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN*

Information about the relationship between the extent of the use of protein as a fuel and the hormonal and metabolic state of severely ill patients is sparse. We have therefore examined the kinetics of plasma leucine turnover using a tracer infusion of [^{13}C]leucine over 4 h and mass spectrometric methods in six critically ill patients (five men, one woman; age 41–68 (median 53) years; weight 63–118 (median 78·2) kg; APACHE II scores 11–31 (median 23); sepsis scores (Elebute & Stoner, 1983) 13–30 (median 18)) studied on nine occasions. The results were compared with those obtained on four healthy volunteers (one woman, three men; age 43–57 (median 55) years; weight 53·4–96·8 (median 73·7) kg). Four patients were in acute renal failure and one in chronic renal failure. Measurements of respiratory gas exchange were made during the period of leucine infusion and expired air collected for [^{13}C]CO_2 enrichment. Blood was taken hourly for enrichment of plasma 2-keto-isocaproate and once every 2 h for serum cortisol, growth hormone, glucagon and insulin.

All patients were studied 16 h after cessation of total parenteral nutrition and 6 h after cessation of glucose (50 g/l) infusion if this had been clinically indicated. The patients were ventilated and their cardiovascular systems were stable during the period of measurement.

Leucine flux (i.e. whole-body protein breakdown) was elevated in the patients compared with the healthy controls (90–213 (median 130) *v.* 30–95 (median 76) μmol leucine/kg body-weight per h respectively; $P<0·01$). Leucine oxidation was also elevated compared with controls (11–34 (median 15) *v.* 5–18 (median 11) μmol/kg per h; $P<0·02$). Both leucine oxidation and synthesis, expressed as a proportion of flux, were very similar in the two groups (% oxidation: patients 13·6 (SD 2·7), controls 15·8 (SD 2·1); % synthesis: patients 86·4 (SD 2·7), controls 84·2 (SD 2·1)).

Twenty-four h energy expenditure of the patients was 98·7–135·4 (median 112·9) % of basal energy expenditure predicted from the Harris Benedict equation and percentage contribution of protein oxidation to 24 h energy expenditure was 14·5–23 (median 15·7). There was no correlation between leucine flux and energy expenditure, percentage energy derived from protein oxidation, sepsis or APACHE II scores. Concentrations of glucagon and cortisol were higher in the patients than in the controls ($P<0·05$ and $P<0·01$ respectively) and there was a significant correlation between leucine flux and both glucagon levels ($P<0·01$, Kendal Rank correlation) and cortisol ($P<0·01$). There was no difference between the groups in insulin or growth hormone levels. In the three patients in whom flux was measured on more than one occasion the two measurements differed by between 105 and 138%.

It is concluded (1) that in critical illness there is no relationship between leucine flux and severity of illness using existing techniques of assessing severity; (2) protein breakdown is elevated but protein is not increased in importance as a source of fuel relative to fat and carbohydrate; (3) leucine oxidation may not accurately reflect whole-body amino acid catabolism in severe sepsis; (4) serum cortisol and glucagon concentrations are directly related to leucine flux.

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Elebute, E. A. & Stoner, H. B. (1983). *British Journal of Surgery* **70**, 29–31.

Effect of volatile anaesthetic agents on liver protein synthesis. By K. FERGUSON[1,2], S. D. HEYS[1,3], A. C. NORTON[2], C. R. DUNDAS[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*

A reduction in liver protein synthesis following exposure to the volatile anaesthetic agent halothane, has been demonstrated in cell culture and perfused organ preparations. Recently, a significant decrease in liver and lung protein synthesis, in vivo, in young fed rats following exposure to halothane has been shown (Heys *et al.* 1989). The aim of the present study was to confirm this effect in fasted rats and also to determine the effect of other commonly used volatile anaesthetic agents on liver protein synthesis, in vivo.

Groups of young male rats were fasted for 12 h before exposure to the anaesthetic agents. Treatments were halothane 1·4%, enflurane 1·5% and isoflurane 0·9% and 1·8%, and all were vaporized in oxygen-enriched air (FiO_2 0·33 Bar). A control group breathed atmospheric air. Exposure time was 1 h. Inspired concentrations of anaesthetic gases were monitored continually with a Datex Capnomac monitor and adjusted accordingly. O_2 saturation was monitored by intermittent pulse oximetry and the animal's core temperature was kept within 1° of starting value.

Protein synthesis was measured during the last 10 min of anaesthesia by the incorporation of [^{3}H]phenylalanine into protein as described by Garlick *et al.* (1980). The fractional rate of liver protein synthesis for the control group was (mean (SEM)) 84·6 (2·1)%/d. Treatment with halothane and enflurane reduced this rate to 74·9 (4·4)%/d ($P<0·03$) and 73·6 (3·3)%/d ($P<0·01$) respectively. Isoflurane, however, did not significantly decrease liver protein synthesis rates at either concentration used (79·6 (1·6)%/d and 78·9 (1·8)%/d for isoflurane at 0·9% and 1·8% respectively).

These results show that both halothane and enflurane in clinically comparable concentrations significantly reduce liver protein synthesis. In contrast, isoflurane caused a minor but non-significant depression of protein synthesis rate. Although the pathogenesis of these differing effects is unclear, they may have clinical implications with respect to liver functions, such as the production of acute-phase reactants following surgery and anaesthesia.

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Protein synthesis in human term placenta, umbilical cord and uterus. By P. F. W. CHIEN[1], P. W. WATT[2], D. J. TAYLOR[1] and M. J. RENNIE[2], [1]*Department of Obstetrics and Gynaecology, Ninewells Hospital and Medical School, Dundee DD1 9SY* and [2]*Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN*

Despite the importance of the placenta as an organ regulating fetal nutrition, relatively little information is presently available on its metabolism and protein turnover. We have therefore begun to study protein metabolism in human placenta, umbilical cord and maternal uterine muscle to provide baseline information against which to measure possible changes in placental insufficiency.

A primed, constant intravenous infusion of L-[1-^{13}C]leucine (2 mg/kg per h) was administered, over 3–5 h, to six mothers with no antenatal complications undergoing elective caesarian section at term. Plateau labelling was achieved by 0·5 h after infusion commenced. At delivery, samples of the tissue of interest were immediately frozen. The ^{13}C enrichment of leucine in proteins was measured by isotope ratio mass spectrometry after separation by preparative gas chromatography (Smith *et al.* 1988). Leucyl-tRNA enrichments were measured by gas chromatography–mass spectrometry (GCMS) following the isolation of aminoacyl-tRNA from placental samples by phenolic extraction. Enrichment of free intracellular leucine was also measured by GCMS.

Labelling of leucine in tissues, and protein synthesis rates

	^{13}C labelling (atoms % excess)						Protein synthesis rate (%/h) Assumed precursor:			
	Free intracellular leucine		Leucyl-tRNA		Protein leucine		Free intracellular leucine		Leucyl-tRNA	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Placenta	6·45	0·80	6·67	0·63	0·20	0·02	0·80	0·08	0·73	0·10
Umbilical cord	6·67	0·57	nd	—	0·045	0·003	0·17	0·03	nd	—
Uterine muscle	7·89	0·00	nd	—	0·011	0·001	0·05	0·001	nd	—
Rectus abdominus muscle	8·06	1·07	nd	—	0·025	0·004	0·06	0·004	nd	—

nd, not determined, insufficient sample.

Mean (SEM) maternal plasma leucine labelling (atoms % excess) was 12·44 (0·63) and that of plasma α-keto-isocaproate was 10·77 (1·21) at plateau, but fetal arterial leucine enrichment was 6·29 (1·74). This suggests that either placental protein breakdown contributes unlabelled amino acid at a high enough rate to cause a large dilution of the leucyl-tRNA, or that as in the gut the route of supply of amino acid to the precursor pool is via the basolateral membrane, i.e. from the fetal side. As expected the synthetic rate of placenta was higher than that of the umbilical cord (chiefly connective tissue and smooth muscle) or uterus (all smooth muscle). These values, which we believe are the first to be obtained for such tissues in women, extend our knowledge of lean tissue turnover rates and provide a basis for further pathophysiological studies.

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C-reactive protein, sickness scores and energy expenditure in critical illness. By A. E. MURRAY, M. BELL, CERI J. GREEN, P. MCCLELLAND, S. M. MOSTAFA and I. T. CAMPBELL, *Department of Medical Microbiology, Intensive Therapy Unit, University Department of Anaesthesia and Department of Renal Medicine, Royal Liverpool Hospital, Liverpool L69 3BX*

C-reactive protein (CRP) is one of a family of proteins whose plasma levels increase with infection, trauma, widespread malignancy and inflammatory disease. An increased CRP level represents a non-specific response to a pathological insult, but the extent of the elevation in specific conditions such as those outlined above is said to reflect closely the extent and activity of the disease (Pepys, 1987). The CRP response to multiple organ failure, however, has not been assessed.

The relationships have been investigated between a number of markers, scores and manifestations of severity of illness (including CRP) in a group of nineteen critically ill intensive care patients (twelve male, seven female; age 18–76 (median 57) years; mean APACHE II scores 11–28 (median 23); mean sepsis scores (Elebute & Stoner, 1983) 8–18 (median 16)) who were studied for between 5 and 51 (median 17) d.

Daily measurements were made of each of the following: serum albumin; serum CRP; APACHE II score; sepsis score; 24 h energy expediture (EE), measured using an Engstrom metabolic computer (Gambro Engstrom Bromma, Sweden) (Campbell & Snowdon, 1985); and the proportion of 24 h EE derived from oxidation of protein (a measure of protein catabolism, calculated from urinary nitrogen and changes in blood urea). Correlation coefficients were calculated between each pair of the above variables.

Significant correlations were found between CRP and sepsis score ($P<0{\cdot}001$), APACHE II score ($P<0{\cdot}001$) and 24 h EE ($P<0{\cdot}001$), between EE and both sepsis ($P<0{\cdot}001$) and APACHE II scores ($P<0{\cdot}001$), and a significant negative correlation between serum albumin and both EE ($P<0{\cdot}005$) and APACHE II score ($P<0{\cdot}001$). There was also, however, a significant correlation between body-weight and APACHE II score (r 0·539, $P=0{\cdot}017$) and a correlation between body-weight and sepsis score which just failed to achieve significance (r 0·404, $P=0{\cdot}086$), implying that the heavier individuals were sicker. When EE was expressed as a proportion of basal 24 h EE, calculated from the Harris Benedict equation, none of the above correlations involving EE were significant.

Within individuals there was a significant correlation between CRP and sepsis score in three patients, CRP and APACHE II in two, CRP and 24 h EE in two, EE and sepsis score in three, EE and APACHE II score in two, albumin and EE in one and albumin and APACHE II in none. Each of the other correlations were significant in only between one and four of the nineteen patients.

It is concluded that in critical illness serum CRP concentrations, for the population, correlate with clinical measures of severity of illness, i.e. sepsis score and APACHE II score. There also appears to be a relationship between severity of illness and body size, but no relationship between sepsis score, APACHE II score or CRP and either EE or percentage EE derived from the oxidation of protein.

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For how long and how often should oxygen consumption be measured in the ventilated patient to assess 24 h energy expenditure? By CERI J. GREEN, BRENDA PHILLIPS, P. MCCLELLAND and I. T. CAMPBELL, *Intensive Therapy Unit, University Department of Anaesthesia, Department of Renal Medicine, Royal Liverpool Hospital, Liverpool L69 3BX*

It is becoming increasingly common in hospital practice to assess 24 h energy expenditure (EE) by measuring oxygen consumption and respiratory quotient for a limited period and then extrapolating the results to 24 h. There is no way of knowing, for the individual patient, the accuracy of this technique. In normal, free living subjects, over a period of 6–12 months, it was shown to be accurate for the population, but individual errors were large and random (Acheson *et al.* 1980). The potential errors that might ensue from adopting such a technique in ventilated patients have been investigated.

The 24 h EE records obtained using an Engstrom metabolic computer (Campbell & Snowdon, 1985) of twenty-seven patients (nineteen male, eight female; age 18–76 (median 57) years) ventilated for between 5 and 31 (median 12) d, were studied. Mean APACHE II scores ranged from 3 to 34 (median 24) and sepsis scores (Elebute & Stoner, 1983) from 8 to 23 (median 17). A comparison was made between actual 24 h EE as measured and 24 h EE obtained by extrapolating to 24 h the highest and lowest hourly EE figures obtained during each 24 h period.

Twenty-four h EE extrapolated from the highest hourly figure overestimated true mean 24 h EE of each patient by between 8·7 and 23·4 (median 14·7)%. The worst overestimate for a single day was 43·7%. Extrapolation from the lowest hourly figure underestimated EE by between 3·1 and 13·5 (median 7·0)%. The worst underestimate for a single day was 34·3%. True day-to-day variation in EE as denoted by the coefficient of variation of 24 h EE as measured ranged from 3·5 to 14·0 (median 6·3)%. For a 24 h EE of 8·4 MJ this represents a day-to-day variation in EE of 1·2–4·7 (median 2·1) MJ/d.

In nine patients a significant change in 24 h EE occurred over the study period, either suddenly or as a steady trend. In six of the patients the change in EE was associated with a significant change in sepsis or APACHE II scores. In three patients there was a significant difference in time between the maximum and minimum EE. Mean maximum EE in these three patients was at 00.12 hours (SE 60 min) and minimum EE at 05.48 hours (SE 30 min) ($P<0·05$).

It is concluded that measuring the EE of ventilated patients for only 1 h will give an estimate generally within 10–20% of 'true' 24 h EE, but EE should be measured every few days. The time of measurement in most patients appears to be of little importance.

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Energy expenditure in the severely traumatized patient: early experience. By P. BLAIR[1], G. G. LAVERY[3], J. D. SMILLIE[3], H. N. MCLEOD[3], R. S. J. CLARKE[2] and B. J. ROWLANDS[1], [1]*Department of Surgery, Queen's University of Belfast,* [2]*Department of Anaesthetics, Whitla Medical Building, Queen's University of Belfast* and [3]*Regional Intensive Care Unit, Royal Victoria Hospital, Belfast*

An accurate means of assessing energy expenditure in trauma patients is vital to optimal nutrition. This study compared measured energy expenditure (MEE) with resting energy expenditure (REE) derived from the following equations: Harris Benedict, 'Dallas' (Ireton-Jones & Turner, 1989), weight (kg) × 22 (Hunter *et al.* 1988).

Twenty-three post-trauma patients (seventeen male, six female) were enrolled in the study. Their mean age was 32·1 (SD 11·7) years and mean weight 72 (SD 12·5) kg. All patients were mechanically ventilated. Standard exclusion criteria were applied (Van Lanschot *et al.* 1986). APACHE II and sepsis scores (Elebute & Stoner, 1983) were calculated on admission and 3 and 5 d after admission to the intensive care unit. Injury severity score was also recorded. On days 1, 3, and 5, over a 45 min period, MEE was obtained using a metabolic cart (Sensormedics 2900Z).

On admission, the mean APACHE II score was 14·4 (range 6–31) and the mean sepsis score 5·5 (range 1–9). REE derived from the Harris Benedict equation underestimated MEE on day 1 (MEE_1) in nineteen of the twenty-three subjects. The mean bias for the whole group was –1226 kJ (–293 kcal). Similarly, REE predicted by the equation weight (kg) × 22 underestimated MEE_1 in twenty of the twenty-three patients with a group mean bias of –1226 kJ (–293 kcal). In contrast, REE from the 'Dallas' equation exceeded MEE_1 in twenty-one out of twenty-three subjects the group mean bias being 2054 kJ (491 kcal). Seven patients remained ventilator dependent throughout the 5 d of the study. In these patients the mean values (kJ (kcal)/d) of MEE_1, MEE_3 and MEE_5 were 8678 (2074), 8895 (2126) and 11 397 (2724). In all seven subjects MEE increased between days 1 and 5.

These preliminary data show that in the post trauma period MEE on day 1 varies significantly from REE derived from formulae.

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The effect of underfeeding for 7 d on thermogenesis and glucose metabolism during infusions of glucose and insulin in healthy women. By I. W. GALLEN[1], M. DONALDSON[2] and I. A. MACDONALD[1], [1]*Department of Physiology and Pharmacology, University of Nottingham Medical School* and [2]*Dietetic Department, University Hospital, Clifton Boulevard, Nottingham NG7 2UH*

Underfeeding in man reduces both resting metabolic rate and the thermic response to food ingestion (Alban-Davies *et al.* 1989; Gallen *et al.* 1989). After a 48 h fast the thermic effect of glucose during glucose and insulin infusions is absent, but glucose storage is unaltered (Mansell, 1988). The present study investigates the effect of underfeeding on thermogenesis and glucose metabolism during glucose and insulin infusions in healthy women.

Six subjects (all 22 years old; mean body mass index 23·7, range 21·1–28·4) were each studied on two occasions after an overnight fast either, in random order, whilst on their normal diet (C) or after 7 d underfeeding at 60 kJ/kg ideal body-weight per day (U). Studies took place 1 month apart in a temperature-controlled room (30°) with the subjects resting supine, wearing a T-shirt and shorts only. Baseline measurements were made for 30 min. This was followed by a 90 min continuous insulin infusion (100 mU/m^2 per min), during which the arterialized venous blood glucose was maintained at 4·5 mmol/l by infusing glucose as required (De Fronzo *et al.* 1979). Measurements were made of glucose infusion rate and respiratory gas exchange ratio (RER), from which glucose storage rate (GSR), glucose oxidation rate (GOR) and metabolic rate (MR) were calculated, and of forearm blood flow (by venous occlusion plethysmography) and arterialized venous and forearm muscle effluent venous blood glucose concentrations from which forearm glucose extraction (FGE) was calculated. Results are given as means (and SEM) and were statistically analysed by Student's paired *t* test.

Underfeeding caused weight loss of 2·4 (0·4) kg, a reduction in RER (C 0·86 (0·02), U 0·75 (0·01), $P<0·01$), but no change in resting MR (C 4·25 (0·3), U 4·08 (0·3) kJ/min) or forearm glucose uptake (C 7·9 (2), U 8·0 (2) μmol/l per min). During the final 30 min of the glucose and insulin infusions, MR rose in C by 0·43 (0·01) but only by 0·1 (0·01) kJ/min in U ($P<0·01$). GOR (C 24·2 (1·2), U 20·1 (0·7) μmol/l per min, $P<0·05$) and RER (C 0·97 (0·02), U 0·80 (0·01), $P<0·01$) were lower after underfeeding compared with the controls. By contrast GSR (C 23·7 (2·6), U 27·1 (3·5) μmol/kg per min) and FGE (C 62·0 (10), U 79·0 (12) μmol/l per min) during glucose and insulin infusions were unaffected by underfeeding. Thus, the net energy cost of glucose storage was reduced by underfeeding (C 10·9 (1·3), U 2·9 (1·0)% stored energy, $P<0·01$).

Underfeeding reduces the thermic effect of glucose and insulin infusion, despite preserved glucose storage and forearm glucose extraction and reduces the net energy cost of glucose storage.

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Glucose-induced thermogenesis is reduced in myotonia dystrophica. By I. W. GALLEN and I. A. MACDONALD, *Department of Physiology and Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH*

Myotonia dystrophica is an inherited multi-system disorder, characterized by weakness of, and reduced glucose uptake by, skeletal muscle. As glucose-induced thermogenesis in man is mediated in part by skeletal muscle (Astrup *et al.* 1987), the present study was designed to see if glucose-induced thermogenesis is abnormal in this disorder of skeletal muscle.

Six myotonic subjects (three male, three female; age 24–53 years; mean body mass index 23·3, range 19·5–27·4) and ten healthy subjects (six male, four female; age 21–30 years; mean body mass index 22·1, range 19·8–24·1) were each studied after an overnight fast. Studies took place in a temperature-controlled room (30°) with the subjects resting supine, wearing a T-shirt and shorts only. Baseline measurements were made for 30 min. This was followed by a 90 min continuous insulin infusion (100 mU/m^2/min), during which the arterialized venous blood glucose was maintained at 4·5 mmol/l by infusing glucose as required (De Fronzo *et al.* 1979). Measurements were made of glucose infusion rate and respiratory gas exchange from which glucose disposal rate (GDR), glucose storage rate (GSR), metabolic rate (MR) and respiratory gas exchange ratio (RER) were calculated. Results are given as means (and SEM) and were statistically analysed by Student's *t* test.

Resting MR was similar in myotonic (M) and healthy subjects (H) (M 3·75 (0·2), H 4·5 (0·7) kJ/min). During the final 30 min of the glucose and insulin infusions, MR rose in H by 0·44 (0·05) but only by 0·21 (0·05) kJ/min in M ($P<0·05$), and RER (H 0·91 (0·01), M 0·98 (0·01), $P<0·01$) was lower in healthy subjects. Both GDR (H 53·5 (4·1), M 39·9 (4·0) μmol/kg per min, $P<0·01$) and GSR (H 33·2 (4·0), M 16·9 (1·0) μmol/kg per min $P<0·01$) were lower in myotonic subjects during glucose and insulin infusions. The net energy cost of glucose storage was lower in myotonic subjects than in healthy subjects (M 5·1 (1·3), H 7·3 (2·0)% stored energy) although this did not reach statistical significance.

Although the myotonic subjects were older and heavier than the healthy subjects, it seems probable that the thermic effect of glucose infusion is reduced in this disorder. Furthermore, these differences cannot be explained by the observed reduction in glucose disposal alone as the net energy cost of glucose storage seen in myotonic subjects is also reduced and is similar to the theoretical cost of glycogen synthesis (Flatt, 1978). This suggests that facultative thermogenesis is absent during glucose infusion in this muscle disorder.

The study was approved by the University Hospital Ethical Committee and supported by a project grant from the Wellcome Trust.

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Brown adipose tissue activity in children in relation to disease state. By J. BRUCE[1], C. C. CHILDS[2], A. C. COOPER[3] and N. J. ROTHWELL[3], [1]*Royal Manchester Children's Hospital,* [2]*Booth Hall Hospital* and [3]*Department of Physiological Sciences, University of Manchester, Manchester M13 9PT*

Brown adipose tissue (BAT) is the major effector of non-shivering and diet-induced thermogenesis in small mammals, but can also be stimulated by pyrogens, injury or cancer (Rothwell & Stock, 1986). Functional BAT has been identified in man and its activity is high in patients with phaeochromocytoma (Ricquier *et al.* 1982). We are currently investigating the activity of perirenal adipose tissue (the major BAT depot in humans) in children and the preliminary results indicate that this may be increased in certain disease states.

Small samples of adipose tissue were removed from the perirenal depot of children (up to 13 years of age) during renal surgery or at postmortem. Tissue protein content was determined, and mitochondria isolated for assessment of the activity of the thermogenic proton conductance pathway from the specific binding of [^{3}H]guanosine diphosphate (GDP; binding was determined at a concentration of 2 μM, and non-specific binding at 200 μM). In tissue from 'controls' (children undergoing exploratory renal surgery with no metabolic disorders) (age 3 months–13 years), protein content was 2·1 (SE 0·3)% (n 14), of which 6·9 (SE 1·5)% was isolated as mitochondrial protein (recovery approximately 12%). Specific GDP binding was 22 (SE 3) pmol/mg protein (range 6–44) which was lower than values obtained for young adult control rats (40–80 pmol/mg protein). Higher values (83 (SE 14) pmol/mg protein) were obtained in three neonatal children. Scatchard analysis of a small number of samples revealed the presence of two separate binding sites with binding characteristics very similar to that of rat BAT. No significant binding was detectable in white adipose tissue.

Perirenal tissue was taken during surgery from eleven children with malignant disease (neuroblastoma, Wilm's tumours or lymphomas) who had shown recent weight loss. The protein content of the tissue was slightly greater (3·1 (SE 0·5)%) and GDP binding was significantly ($P<0·05$) greater (51 (SE 8) pmol/mg protein) than 'controls'. High levels of GDP binding (48 (SE 6)) were also observed in tissue taken at postmortem from three children who had suffered serious burn injury and had exhibited severe fevers.

These results demonstrate the presence of active BAT in 'normal' children and suggest that BAT may be stimulated in disease states associated with cachexia.

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Modification by clenbuterol of muscle atrophy induced by unilateral femur fracture plus protein–energy deficiency in the rat. By J. J. CHOO[1], M. A. HORAN[2], R. A. LITTLE[3] and N. J. ROTHWELL[1], *Departments of* [1]*Physiological Sciences and* [2]*Geriatric Medicine* and [3]*North Western Injury Research Centre, University of Manchester, Manchester M13 9PT*

Certain β_2-adrenoceptor agonists promote growth and increase muscle protein content in many mammalian species. These effects have stimulated increasing interest in the clinical application of such agents in counteracting muscle wasting as well as producing leaner meat in farm animals. Physical injury is a condition which induces a reduction of voluntary food intake and is usually associated with muscle wasting which is greater than would be expected from immobilization alone. We have therefore investigated the effects of the β_2-adrenoceptor agonist, clenbuterol, on muscle atrophy induced by a combination of unilateral femur fracture and food restriction in the rat.

Four groups of seven male Wistar rats weighing 150 g were studied. Two groups of animals were sham-operated and one of these groups was given a powdered diet containing 175 g protein/kg *ad lib.* (control) whereas the other was fed at about 40% of *ad lib.* intake (FR). The remaining two groups were anaesthetized and subjected to unilateral femur fracture and their food intake was restricted (FRF) as for the sham-operated, food-restricted animals. One of these groups received clenbuterol mixed into the diet at a concentration of 8 mg/kg (FRFC). Three days later animals were killed and gastrocnemius muscles from the intact and fractured leg were dissected and weighed.

	Mass of gastrocnemius muscle (g)			
	Intact		Fractured	
	Mean	SEM	Mean	SEM
Control	0·73[a]	0·01	0·72[a]	0·01
FR	0·66[b]	0·01	0·66[b]	0·01
FRF	0·62[c]	0·01	0·57[c]	0·01
FRFC	0·71[d]	0·01	0·63[d]	0·01

[a–d] Mean values with different superscript letters were significantly different: $P<0·05$.

Food-restriction caused reductions in muscle mass in both legs (Table). Femur fracture plus food restriction induced an extra 5% decrease in muscle from the intact leg whereas muscle from the fractured leg suffered a further 12% reduction. The administration of clenbuterol significantly increased the mass of the intact leg muscle and of the fractured leg muscle by 15% and 11% respectively compared with the femur fracture plus food-restricted group. These results imply that clenbuterol could be useful in modification of muscle wasting associated with physical injury.

Relationship of post-surgical ketosis and nitrogen loss to anthropometric and biochemical indices of nutritional status on admission to hospital. By CHRISTINE M. WILLIAMS and P. J. MOYNIHAN, *Division of Nutrition and Food Science, Department of Biochemistry, University of Surrey, Guildford GU2 5XH* and D. TEALE, *Department of Clinical Biochemistry and Nutrition, St Luke's Hospital, Guildford*

Patients who are able to maintain heightened ketone concentrations following accidental injury and surgery are reported to show lower urinary nitrogen losses than normoketonaemic individuals (Wedge *et al.* 1976; Rich & Wright, 1979). These observations have led to the development of feeding regimens which may promote ketogenesis and elevate circulating ketone concentrations in catabolic patients.

Blood was collected on days 1, 3 and 6 post-operatively for the determination of total serum ketone concentrations, and 24 h urine collections were made on the same days for determination of N losses. Albumin and IGF-1 concentrations were determined on days 1 and 6 post-operatively; anthropometric measurements were made weekly and on the day of discharge. Patients were divided into normo- and hyperketonaemic groups on the basis of their combined day 1 and day 3 total serum ketone concentrations, and admission and post-surgical measurements were compared between the two groups. None of the patients studied received total parenteral nutrition or nasogastric enteral feeding during hospitalization but received some glucose (50 g/l) intravenously as part of their 24 h fluid requirements.

Table 1.

Admission measurements		BMI	TSF (mm)	MUAC (mm)	Albumin (g/l)	IGF-1 (U/ml)
Normoketonaemic	Mean	22·2	22·7	269	34·6	0·49
(*n* 11)	SD	2·1	4·2	25	6·5	0·19
Hyperketonaemic	Mean	20·1*	18·2*	260	34·8	0·47
(*n* 9)	SD	1·3	4·6	25	5·5	0·14

BMI, body mass index; TSF, triceps skinfold; MUAC, mid arm circumference; IGF-1, insulin-like growth factor-1. * $P<0·05$ compared with normoketonaemic group.

Table 2.

		Change from day 1 to day 6		Average N balance (g/24 h)	Change from admission to discharge		
		Albumin (g/l)	IGF-1 (U/ml)		Body-wt (kg)	TSF (mm)	MUAC (mm)
Normoketonaemic	Mean	−11·6	−0·21	−9·50	−3·16	−3·29	−11·3
(*n* 11)	SD	4·9	0·16	4·80	2·00	2·50	8·4
Hyperketonaemic	Mean	−6·8	−0·17	−6·00*	−3·07	−3·52	−12·5
(*n* 9)	SD	4·3	0·12	2·00	1·50	2·28	11·2

* $P<0·05$ compared with normoketonaemic group.

Patients in the hyperketonaemic group had lower values for body-mass index (BMI) and TSF on admission. Patients in the normoketonaemic group lost significantly more N in the post-operative period and the mean fall in serum albumin was also higher (but not significantly so), in this group. Changes in IGF-1 and in anthropometric measurements from admission to discharge, were not significantly different between the two groups. The greater capacity for ketogenesis in the hyperketonaemic group may reflect a greater degree of fat loss, and adaptation to malnutrition in this group, before admission.

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Ketone body metabolism during starvation. By S. WOOD, K. KHAN, E. PULLICINO and M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 2QL*

The present study aimed to (a) assess the role of ketone bodies in the forearm muscle metabolism of lean subjects undergoing short-term starvation, and (b) assess possible differences in ketone body metabolism between lean and obese subjects (using the present data and results previously acquired by ourselves and other workers).

Thirty-three arterio-venous forearm catheterization studies were carried out on healthy, lean individuals undergoing starvation for 12–14 h (*n* 13), 30–36 h (*n* 7) and 60–66 h (*n* 13). Acetoacetate (AcAc) was found to be consistently taken up by muscle, but there was a net release of 3-hydroxybutyrate (BOH) (Table). The ratio of BOH:AcAc in the deep venous blood draining forearm muscle was greater than that in arterial or arterialized blood, by 39% at 30–36 h of starvation ($P<0{\cdot}005$) and 33% at 60–66 h of starvation ($P<0{\cdot}025$). The lactate:pyruvate ratio was also greater in deep venous blood than arterial blood by about 20% both at 30–36 h of starvation ($P<0{\cdot}05$) and 60–66 h of starvation ($P<0{\cdot}001$).

The concentration and flux of metabolites across forearm muscle at 12–14, 30–36 and 60–66 h of starvation

Period of starvation (h) . . .	Arterial concentration (μmol/l)			Flux (μmol/l muscle per min)		
	12–14	30–36	60–66	12–14	30–36	60–66
Glucose	5154	3915***	3439***	3·80	3·38	2·10
Lactate	698	719	706	−1·02	−4·41	−3·00
AcAc	78	460***	825***	0·40	3·72***	7·51*
BOH	65	1256***	2654***	0·46	−1·89***	−3·57***
BOH:AcAc	0·81	2·76***	3·2***	—	—	—
NEFA	429	838	809***	0·77	1·61	1·20
O_2	8245	8584	8280	61·60	60·40	72·00

Positive values for flux indicate net uptake and negative values net release.
Values significantly different from the 12–14 h values (*t* test): * $P<0{\cdot}05$, *** $P<0{\cdot}001$.

In contrast to these observations the forearm muscle of obese individuals has been reported (Owen & Reichard, 1971) to take up both AcAc and BOH at a rate which would account for 51% of the oxygen consumption at 84 h of starvation.

Using oxidative stoichiometries it can be calculated that in lean individuals the O_2 equivalents of AcAc plus BOH correspond to about 5, 10 and 20% of the O_2 consumption of forearm muscle at 12–14 h, 30–36 h and 60–66 h of starvation respectively. The O_2 equivalents of circulating glucose taken up by muscle corrected for the release of lactate diminished as starvation progressed. The O_2 equivalents of circulating non-esterified fatty acids (NEFA) taken up by muscle were higher than those of ketone bodies and glucose at all times tested. Another difference between lean and obese individuals is suggested by the present study, i.e. a significantly greater rate of rise of the circulating ketone body concentration and ratio of BOH:AcAc (~ 50%) in lean individuals undergoing short-term starvation.

The results suggest that during short-term starvation: (a) the mitochondrial redox state assessed by the circulating BOH:AcAc ratio changes more rapidly in lean subjects than obese subjects; (b) lean muscle becomes a particularly reduced tissue; (c) in lean subjects fat is a more important energy substrate for muscle than ketone bodies, although the reverse appears to be true of obese individuals (Owen & Reichard, 1971).

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Lipoprotein lipase activity in tissues of tumour-bearing rats. By O. OBEID and P. W. EMERY, *Department of Food and Nutritional Sciences, King's College, London W8 7AH*

Cancer cachexia is characterized by considerable loss of body fat. Rates of lipolysis may be increased (Thompson *et al.* 1981); lipogenesis is decreased, but only in proportion to the decrease in food intake (Obeid & Emery, 1990). Lipoprotein lipase (LPL, *EC* 3.1.1.34) activity in white adipose tissue is known to decrease, but the activity of this enzyme has not been studied systematically in other tissues. We have therefore used the method of Peterson *et al.* (1985) to measure LPL activity in heart, skeletal muscle (gastrocnemius) and liver as well as epididymal fat pads of rats bearing a transplantable Leydig cell tumour.

Groups of four rats were studied on days 1, 4, 7 and 10 of palpable tumour growth, after either *ad lib.* feeding or a 24 h fast. Measurements were also made on groups of eight sham-injected control rats.

Lipoprotein lipase activity (nmol fatty acid released/min per g tissue at 37°)

Tissue			Control (*n* 8)	Tumour bearing			
				Day 1 (*n* 4)	Day 4 (*n* 4)	Day 7 (*n* 4)	Day 10 (*n* 4)
Adipose	Fed	Mean	3880	2610**	1833**	1690**	1887**
		SE	364	326	103	90	207
	Fasted	Mean	1992††	1975	1519	1781	2598
		SE	132	265	303	184	479
Heart	Fed	Mean	6155	—	6602	7463	7705
		SE	661		593	561	155
	Fasted	Mean	4964	—	4869	5764**	6282**
		SE	203		130	24	517
Muscle	Fed	Mean	403	—	—	420	604**
		SE	47			55	20
	Fasted	Mean	331	—	—	431	429††
		SE	32			59	38
Liver	Fed	Mean	227·5	220·2	130·0**	146·4**	125·2**
		SE	10·0	42·0	20·0	18·0	3·6
	Fasted	Mean	189·9	161·1	127·5**	123·8**	83·8**
		SE	19·0	6·5	16·0	19·0	21·0

Significantly different from control rats (ANOVA): ** $P<0.01$.
Significantly different from fed rats (unpaired *t* test): †† $P<0.01$.

LPL activity in adipose tissue was markedly decreased from day 1 of tumour growth, before any decline in food intake was apparent. Values in 24 h fasted rats were not significantly different from those in fed rats. LPL activity in adipose tissue normally rises in response to feeding, an effect which is believed to be mediated by insulin. Apparently this effect had been abolished by the presence of the tumour. The decreased LPL activity in adipose tissue and increased LPL activity in heart correspond to the known effects of the cytokine tumour necrosis factor α (TNFα) (Semb *et al.* 1987). Skeletal muscle appears to be affected in a similar manner to heart muscle. However, LPL activity in the liver was markedly reduced, in contrast to the increase which has been reported in response to TNFα.

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Whole-body protein homeostasis and protein requirements in man: validation studies of a new metabolic model. By GILLIAN PRICE and D. J. MILLWARD, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE* and P. J. H. PACY and D. HALLIDAY, *Nutrition Research Group, Clinical Research Centre, Harrow, Middlesex HA1 3UJ*

Millward & Rivers (1988) have proposed a new model of whole-body protein homeostasis within which protein requirements can be better understood. One feature of this model is that protein requirements are variable, and determined in part by the extent of oxidative losses of amino acids. These losses vary according to dietary protein content which conditions the activity of the amino acid oxidative pathways. The model predicts that postabsorptive nitrogen losses will increase as dietary protein intake increases, with the need for enhanced N gain during feeding to balance such losses. This is in contrast to an alternative model of fixed protein requirements with invariant postabsorptive losses, with balance achieved by a constant postprandial gain, and the excess intake oxidized during feeding.

We have commenced a study of diurnal changes in N balance and whole-body N turnover in normal adults after a 2 week period of adaptation to 0·35, 0·75 and 1·5 g protein/kg per d and isoenergetic, weight-maintaining intakes. Preliminary results reported here are from subjects studied over 48 h during alternating 12 h periods of feeding and fasting. N loss over 12 h was assessed from N excretion corrected for changes in the body urea pool. Whole-body protein turnover was estimated from urinary N loss and excretion of $^{13}CO_2$ measured over the last 4 h of the fasting period and the first 4 h of feeding during a primed constant intravenous infusion of [1-^{13}C]leucine. The preliminary results are shown in the Table.

N intake (mg/kg per d for 14 d) . . .	58		120		247	
	Mean	SD	Mean	SD	Mean	SD
N balance (mg/kg, *n* 6):						
Fasting (12 h)	−41·7	8·1	−54·8	9·0	−100·7	30·4
Feeding (12 h)	13·0	4·9	37·7	10·5	103·9	23·8
Balance (24 h)	28·6	10·4	−17·1	14·0	3·1	37·1
Whole-body N turnover (g/kg per d)	0·74	0·18	0·75	0·10	1·02	0·10

The 12 h N balances confirm the model predictions that with increasing protein intake, the amplitude of the diurnal changes in body N balance increases. Thus there were increasing postprandial gains and postabsorptive losses. Furthermore, these changes were accompanied by increasing rates of whole-body N turnover. These preliminary results appear to confirm the model of Millward & Rivers (1988) and indicate that protein requirements are in part conditioned by protein intakes.

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Assessment of body composition by bioelectrical impedance compared with anthropometry, total body potassium and tritiated water dilution. By R. A. RICHARDSON[1], K. C. H. FEARON[2], W. WATSON[3], R. HUME[3] and A. SHENKIN[1], [1]*Institute of Biochemistry, Royal Infirmary, Glasgow,* [2]*Department of Surgery, Royal Infirmary, Edinburgh* and [3]*Department of Nuclear Medicine, Southern General Hospital, Glasgow*

The aim of the present study was to evaluate whole-body bioelectrical impedance analysis in the assessment of fat mass (FM) and lean body mass (LBM). A group of twelve males and twelve females, representing a clinical spectrum from frankly malnourished to moderately obese (range of percentage ideal body-weight: male 67–118, female 65–103) underwent estimation of FM and LBM using a whole-body impedance analyser (RJL, Detroit). FM (and by subtraction fat free mass; FFM) was also assessed by skinfold measurements at four different sites (Durnin & Womersley, 1974). In addition, LBM (and by subtraction FM) was derived from measurement of total body potassium (TBK; measured via ^{40}K whole-body counting) using the predictive formulae of Boddy *et al.* (1972) and also from total body water using tritiated water dilution (assuming a constant hydration of LBM 730 g/kg; Pace & Rathburn, 1945). The results are shown in the Table.

		Impedance		Anthropometry		TBK		^3H_2O	
		♂	♀	♂	♀	♂	♀	♂	♀
	n	12	9	12	9	12	9	12	9
FM	Mean	11·1	11·3	10·4	9·2	15·6	11·3	12·2	11·3
	SEM	1·4	1·5	1·4	1·3	2·9	2·2	1·9	1·8
LBM*	Mean	51·8	36·7	52·8	38·9	47·7	36·8	51·1	36·9
	SEM	2·4	1·5	2·3	2·2	1·0	0·9	2·4	2·0
R^2†				0·771	0·844	0·359	0·590	0·666	0·830
Coefficient of variation (%)		1·51	0·85	5·0	3·4	3·5	4·4	ND	ND

ND, not determined (literature value 4%).
No significant difference between groups by Kruskal–Wallis test.
* FFM assumed to be equivalent to LBM for anthropometry.
† Correlation of LBM (impedance) against LBM (anthropometry, TBK, ^3H_2O).

In both males and females impedance provided an estimation of LBM which correlated well with values obtained by anthropometry and tritiated water dilution. There was a poorer correlation for males between LBM measured by impedance analysis and that derived from measurements of TBK. This may reflect the limitation of deriving LBM (rather than body cell mass) from TBK. The errors may be compounded further due to the possible variation in the concentration of K in lean tissues of a heterogenous patient population with varying severity of illness. The results suggest that impedance analysis compares favourably with other methods of body composition analysis which may be used in the clinical setting. Moreover, impedance analysis is precise, non-invasive and not observer-dependent.

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Evaluation of simple predictive methods for assessing body composition: comparison with deuterated water technique. By E. A. PULLICINO, W. A. COWARD and M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

There is as yet no simple method for assessing body composition in populations or hospital patients. Therefore the present study was undertaken to compare the results of simple predictive methods with those obtained by the deuterated water dilution technique.

Body composition was assessed by a single observer in fifty-five healthy subjects (thirty-eight males and seventeen females, body mass index 17–30 kg/m^2) using the following methods: (1) dilution of deuterated water assuming (a) that deuterium dilution space is 4% greater than total body water and (b) that fat free mass contains 719·4 g water/kg (Siri, 1961) (total body water was estimated from the isotopic enrichment of saliva samples obtained at 4, 5 and 6 h after dosing); (2) skinfold thickness at four standard sites (Durnin & Womersley, 1974); (3) whole-body resistance (Valhalla 1990B analyser plus equation package); (4) whole-body impedance (Holtain prototype analyser using equations supplied by the manufacturer; (5) body mass index formula (Black *et al.* 1983); (6) weight and height formula of Hume & Weyers (1971) (assuming that fat free mass contains 719·4 g water/kg).

The fat free mass (kg) obtained by deuterium dilution was 77·8 (SD 7·2)% of body-weight. The correlation coefficients *r* (and standard error of the estimate (SEE)) of fat free mass obtained by the deuterium dilution technique and alternative methods are indicated in the Table. Estimates of fat free mass (Table) and fat mass (values not shown) obtained by the deuterium technique correlated better with estimates obtained by the skinfold and impedance/resistance methods than with those obtained by the weight and height formula. A similar ranking order was obtained with respect to their limits of agreement. The skinfold method provided the lowest value for bias and the impedance formulae the highest bias (see Table).

The correlation coefficient r *(and SEE), bias, and limits of agreement of estimates of fat free mass by the deuterium dilution technique and by alternative methods*

Method	*r*	SEE (kg)	Bias* (kg)	Limits of agreement (± 2 SD) (kg)
Skinfold thicknesses	0·96	2·23	−1·29	4·60 (−5·89 to +3·31)
Resistance (Valhalla)	0·97	2·06	−2·36	4·20 (−6·56 to +1·84)
Impendance (Holtain)	0·95	2·48	+4·12	4·90 (−0·7 to +9·02)
Body mass index	0·93	2·93	−1·45	5·76 (−7·21 to +4·31)
Weight/height	0·93	2·99	−2·08	5·96 (−8·04 to +3·88)

* Negative values for bias indicate that the estimate is higher than that obtained by deuterium dilution.

It is concluded that in the population studied, skinfold thickness, measured by a single observer, and resistance (Valhalla package equation) are the best methods for predicting body composition as measured by deuterium dilution. However, in large populations or multicentre studies the use of multiple operators may lead to a greater variability in results obtained by the skinfold method than by the resistance method (Valhalla package).

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Assessment of body composition by near infra-red interactance. By M. ELIA, S. A. PARKINSON and E. DIAZ, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

The value of near infra-red interactance (NIRI) in predicting body composition measured by densitometry was assessed in twenty-nine healthy subjects (fifteen male, fourteen female) aged 18–40 years with a body mass index of 18·3–28·5 kg/m^2. The optical density measurements were made with the Futrex 5000 body composition analyser (Self-care Products Ltd, Amersham, Bucks) in the biceps area according to the procedure described in the Futrex manual. The equations for calculating body composition take into account not only the optical measurements at 940 nm (od_1) and 950 nm (od_2), but also weight, height, sex and estimated level of activity. Five other methods were used to predict body composition: (1) skinfold thicknesses (Durnin & Womersley, 1974); (2) whole-body resistance (Valhalla 1990B analyser plus equation package); (3) whole-body impedance (Holtain prototype analyser using equations supplied by the manufacturer); (4) body mass index (Black *et al.* 1983); (5) weight and height formula of Hume & Weyers (1971), assuming that fat free mass contains 719·4 g water/kg (Siri, 1961). Statistical analysis was carried out using correlation analysis and the method of Bland & Altman (1986).

The correlations *r* between fat (g/kg) estimated by densitometry and other variables were: od_1, *r* 0·71; od_2, *r* 0·75; height, *r* −0·53; activity, *r* −0·38; skinfold thicknesses, *r* 0·75. With mass of fat (kg), the following relationships were found: weight, *r* 0·45; body mass index, *r* 0·84; od_1, *r* 0·51; od_2, *r* 0·58; skinfold thicknesses, *r* 0·73.

The correlation coefficient r *(and SEE), bias, and limits of agreement between densitometry and alternative methods which are used to predict body fat (kg)*

	r	SEE (kg)	Bias (kg)	Limit of agreement (± 2 SD) (kg)
NIRI	0·90	2·47	2·49	5·39
Skinfold thicknesses	0·90	2·40	1·18	4·81
Resistance (Valhalla)	0·91	2·31	3·55	4·70
Impedance (Holtain)	0·92	2·12	−3·11	4·64
Body mass index	0·90	2·38	2·26	5·33
Weight/height	0·90	2·44	2·51	4·91

The correlation coefficients (and standard error of the estimate (SEE)) for estimates of body fat obtained by densitometry and alternative methods were remarkably similar to each other (Table). The bias (densitometry–alternative method) and the 95% confidence interval between estimates of body fat obtained by densitometry and NIRI were found to be greater than those obtained between densitometry and other methods and tended to increase as the degree of adiposity increased. It is concluded that NIRI has little or no advantage over other simple methods in predicting body composition.

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The recovery of labelled carbon dioxide during a 36 h continuous infusion of bicarbonate in man. By N. J. FULLER, P. R. MURGATROYD and M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

The recovery of labelled carbon dioxide during an infusion of bicarbonate is not only of relevance to studies involved with the assessment of rates of oxidation of carbon-labelled substrates, but also to the assessment of energy expenditure and CO_2 production by isotopic dilution (Elia *et al.* 1988). The present study aimed to assess the inter-individual variation in the recovery of labelled CO_2 during a 36 h infusion of labelled bicarbonate. In addition it aimed to assess the possible use of urea-specific activity as an indicator of CO_2 production (urea is formed from bicarbonate; see Elia *et al.* 1988 for the principles of isotopic dilution of CO_2), and to investigate the possible existence of mitochondrial compartmentation of CO_2 in the intact human liver. Such compartmentation has been suggested by hepatocyte studies, which have found the specific activity of the C-labelled bicarbonate incorporated into urea to be several-fold different compared with that of bicarbonate in the medium (Hems & Saez, 1983).

Unlike previous human studies involving bicarbonate kinetics, this one was conducted in a whole-body calorimeter, where both continuous and intermittent (spot breath) samples were obtained. The measurements were made in six healthy male adults who were given a constant unprimed infusion of $NaH^{14}CO_3$ over a 36 h period (*500* μCi). Urine was collected once every 3 h during the study, except during sleep.

Period of experiment (h)	Recovery (%)						Distribution of urinary label (%)					
	Gaseous CO_2		Urinary		Gaseous Co_2 + urinary		Urea		Acid-labile CO_2		Other	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0–3	49·91	2·45	0·35	0·21	50·26	2·63	46·48	14·53	47·97	14·77	5·55	3·10
3–6	85·99	4·01	0·81	0·50	86·80	4·29	72·08	10·11	20·72	10·66	7·18	2·14
6–12	87·38	4·12	1·44	0·57	88·81	3·94	77·82	7·45	18·22	7·03	3·97	1·45
12–36	95·57	1·07	1·85	0·43	97·42	0·84	84·22	3·28	10·00	2·34	5·80	1·98

The recovery of label in gaseous CO_2 rapidly rose to reach an apparent steady state between 4 and 12 h. The recovery of label in gaseous CO_2 from 12 to 36 h was found to be remarkably consistent (95·6 (SD 1·07)%) by continuous collection. Only a small proportion of the label was recovered in urine (Table). Virtually all (93–96%) of the label in urine could be accounted for by urea (46–84%) and acid-labile CO_2 (10–48%). The specific activity of urinary urea was found to increase more slowly than that of CO_2 in breath and urine. However, during the last 12 h of the study, the specific activity of urea was stable but slightly lower (84%) than that of CO_2 in expired air and urine. This difference can be largely explained by isotopic dilution of CO_2 in splanchnic tissues without having to invoke the existence of CO_2 compartmentation in the liver.

The results suggest that (1) a nearly complete recovery of $^{14}CO_2$ was achieved in all subjects, with little variation between them; (2) there is little or no evidence of hepatic mitochondrial compartmentation of CO_2 in vivo.

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Total body water measurement in children with growth disorders. By J. W. GREGORY and S. A. GREENE, *Department of Child Health, Ninewells Hospital and Medical School, Dundee DD1 9SY* and C. M. SCRIMGEOUR and M. J. RENNIE, *Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HN*

Changes in body composition measured by total body water (TBW) estimations may be a useful indicator of response to therapy in many conditions, including growth disorders. Prior to treatment we measured TBW in fourteen patients: mean age 12·1 (range 7·8 to 15·7) years; mean height, SD score −2·57 (range 1·06 to −4·60); mean body mass index 16·3 (range 15·2 to 18·8). Five patients had constitutionally delayed puberty and the remaining nine were undergoing investigation before growth hormone therapy.

TBW was measured by $H_2{}^{18}O$ dilution from the third voided urine specimen 5 h after the tracer was administered. Bioelectrical impedance was estimated by the Holtain body composition analyser. TBW was also derived from percentage body fat estimations by the sum of four skinfold thicknesses (triceps, biceps, subscapular and suprailiac sites) measured by a single observer, assuming fat free mass contains 730 g water/kg and using the regression equations of Brook (1971) for prepubertal children and those of Durnin & Rahaman (1967) for adolescents.

The regression equation for height squared (H^2) divided by impedance (I) with TBW measured by $H_2{}^{18}O$ is: TBW (litres) = $-0{\cdot}17 + 0{\cdot}59(H^2/I)$. Values for TBW by the three methods are shown in the Table.

	TBW (l)	
	Mean	SD
$H_2{}^{18}O$ dilution	17·42	4·32
Impedance	17·42	4·23
Skinfolds	17·35	4·49

The correlation coefficients between $H_2{}^{18}O$ values for TBW and H^2/I (r 0·979) and $H_2{}^{18}O$ and skinfold thickness (r 0·986) methods for calculating TBW were similar.

The results validate published data for the use of bioelectrical impedance (Davies *et al.* 1988) in children, which produced a similar regression equation (TBW = $0{\cdot}5 + 0{\cdot}6H^2/I$). Although in our hands the precision and likely accuracy of TBW measurements by skinfolds and impedance were similar, we believe that the impedance method has more value in the measurement of body composition in children, as it is better tolerated in young children and is independent of inter-observer error.

This work was supported by the Scottish Home and Health Department, Novo-Nordisk and Organon Laboratories.

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The bromide space in endotoxaemia: a poor reflection of extracellular volume? By R. J. HANNON[1,2] and V. E. BOSTON[1,2], [1]*Royal Belfast Hospital for Sick Children* and [2]*Department of Surgery, Queen's University of Belfast, Royal Victoria Hospital, Belfast*

Fluid space measurements such as total body water (TBW) and extracellular space are useful in trying to follow changes in body composition with growth, illness or treatment. The 'gold standard' for TBW is the deuterium space but a variety of measurements are used to estimate extracellular space. All have disadvantages. In paediatric practice the best method of estimating extracellular space is regarded as the bromide dilution technique because it is well-established that bromide space is almost identical to chloride space and the chloride ion has a wide extracellular distribution. Bromide can be administered in non-radioactive form and easily measured using spectrophotometry, making it ideal for clinical use.

Some authors have attempted to use the bromide space to monitor response to total parenteral nutrition during childhood illness and the effect of resuscitation in paediatric sepsis. The use of bromide space in these circumstances may be flawed because severe illness states are associated with altered membrane potential. This directly alters chloride distribution in many tissues as the partition of chloride ions across most cell membranes is largely passive and related directly to membrane potential. The nature of the monovalent anion channels governing chloride and bromide distribution is such that the boundaries of the bromide space are probably also changed.

To observe the effect of altered membrane potential on tissue extracellular water distribution, TBW, bromide space and packed cell volume (PCV), rabbits were each given a single intravenous injection of endotoxin (100 μg/kg). The changes in TBW, bromide space, extracellular water and PCV were measured serially for 5 h after endotoxin administration and the trends correlated.

Period after injection (h) . . .	0	1	2	3	4	5	Statistical significance (ANOVA)
Membrane potential difference (mV)	−90·1	−79·2	−71·4	−67·1	−59·3	−54·5	$P<0·001$
Intracellular water (g/kg dry wt)	640	660	670	680	700	710	$P<0·001$
Extracellular water (g/kg dry wt)	90	80	50	40	40	30	$P<0·001$
Bromide space (g/kg body-wt)	264	267	270	280	281	284	NS
TBW (g/kg body-wt)	723	752	702	731	722	682	NS
PCV	0·318	0·332	0·340	0·353	0·378	0·400	$P<0·001$

NS, not significant.

The results confirm the widespread alteration of membrane function described previously in shock and sepsis which is associated with cellular swelling. TBW and bromide space showed no significant change during the course of the experiment. Tissue extracellular water declined and intracellular water increased. There was a significant negative correlation between tissue extracellular water and PCV (r −0·41, $P<0·01$) which suggests migration of fluid from extracellular to intracellular compartments. There was no correlation between total extracellular volume (i.e. bromide space) and tissue extracellular volume (r −0·07, $P = 0·63$).

We conclude that bromide space is an inadequate reflection of underlying changes in extracellular fluid volume in endotoxaemia. Its validity in children ill with sepsis must be seriously questioned.

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The time dependence of nitrous oxide interference in ^{13}C and ^{18}O isotope ratio measurements in breath and plasma after inhalation by mask. By J. M. BARUA[1,2], C. M. SCRIMGEOUR[2], I. M. MACKAY[3] and M. J. RENNIE[2], [1]*Departments of Surgery,* [2]*Anatomy and Physiology and* [3]*Plastic Surgery, University of Dundee, Dundee DD1 4HN*

The use of stable isotopes to study protein metabolism and energy expenditure is well established and is now being applied to post-operative patients and those in intensive care. The use of nitrous oxide (mass 44) for anaesthesia or pain relief either at or after operation (e.g. during physiotherapy) may cause isobaric interference with isotope ratio measurements of ^{13}C or ^{18}O in CO_2. We have therefore investigated the time course and magnitude of possible interference in expired breath CO_2 and in plasma equilibrated with CO_2 for ^{18}O measurements.

Four healthy male volunteers (mean age 34·25, range 30–41 years) inhaled a 50:50 mixture of nitrous oxide and oxygen by mask for 10 min. Breath and plasma samples were taken before inhalation and at intervals up to 24 h afterwards. Breath was analysed immediately for $^{13}CO_2$ and $C^{18}O_2$ with a Finnigan MAT Delta D breath gas analysis system (Scrimgeour & Rennie, 1988) and plasma was equilibrated with 5% CO_2 for 48 h before analysis for ^{18}O derived from $H_2{}^{18}O$ on the same instrument.

Breath CO_2 showed large isobaric interference by N_2O immediately post-inhalation (80–190 delta/ml for ^{13}C and 100–200 delta/ml for ^{18}O). The subsequent decay was fitted to a two compartment exponential decay curve with first order rate constants of 0·071 (SD 0·035) and 0·008 (SD 0·01)/min, probably reflecting exit from the water and lipid compartments of the body respectively.

Plasma ^{18}O showed no significant change in ratio within the precision of the measurement (0·25 delta/ml) between the pre-inhalation value and all samples taken 5 min or more after the inhalation ceased. The samples taken immediately post-inhalation were not more than 3 SD from the mean of the remaining samples.

N_2O contamination is not a significant factor in plasma ^{18}O measurements. Expired breath, however, may show significant contamination for up to 10 h after inhalation and studies using breath should be designed to avoid measurements during this period, or the CO_2 purified after by absorption into potassium hydroxide. Further studies are required with post-operative patients (who have had full NO_2 inhalation anaesthesia) to determine if the time course of N_2O interference clearance is different from that in healthy active subjects.

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Use of a hypoallergenic casein hydrolysate diet in the rehabilitation of severely malnourished Gambian children with chronic diarrhoea. By P. G. LUNN, C. A. NORTHROP-CLEWES and P. B. SULLIVAN, *Dunn Nutrition Laboratory, Milton Road, Cambridge CB4 1XJ*

According to the literature, children with chronic diarrhoea can develop intolerance to food antigens such as cow's milk protein and cereal prolamines. However, cow's milk and cereal flour are widely used in rehabilitation foods for malnourished infants even when there is a history of diarrhoea. It would therefore seem probable that the use of a non-allergenic, lactose-free food such as a casein hydrolysate formula should improve recovery rates in such cases. We have assessed the value of one of these foods, 'Pregestimil' (Bristol-Myers UK Ltd), by comparing it with a standard, locally-made rehabilitation formula, SSO, which contains sugar, skimmed-milk and maize oil. Severely malnourished Gambian children (mean age 18·4 months and mean weight/age 57·6% of National Center for Health Statistics standard, see Hamill (1977)) with chronic or intermittent diarrhoea for more than 3 weeks were admitted to hospital for study. Subjects (n 28) were allocated alternately to receive either Pregestimil or SSO and remained on their respective food throughout their hospital stay. The diet was given as discrete meals and, when necessary, administered by nasogastric tube. Other foods provided by their mothers, however, were given during their last week in hospital.

In addition to oral rehydration fluid, SSO-fed children took on average 111 ml/kg per d of formula which provided 464 kJ (111 kcal) and 3·1 g protein/kg per d. The Pregestimil-fed group consumed 122 ml/kg per d of the preparation at the recommended dilution but because it is less nutrient-dense, this provided only 347 kJ (83 kcal) and 2·3 g protein/kg per d. Attempts to increase the intake of Pregestimil were not successful because of poor patient compliance—it has an unpleasant taste and at times caused vomiting.

No differences were observed in terms of weight gain, resolution of symptoms or mucosal morphology between infants fed on the two formulae. However, some biochemical findings in the Pregestimil group were disturbing. After 1 week of treatment, children fed on SSO showed a significant rise in plasma albumin concentration from 35·0 to 40·1 g/l ($P<0·05$) but in those given Pregestimil, values were virtually unchanged at 34·5 and 35·6. Essential amino acid concentrations in the plasma (after an overnight fast) reflected this result: SSO children showed an increase from 708 to 1197 nmol/ml ($P<0·001$) but the corresponding Pregestimil group values were 679 and 666 nmol/ml ($P>0·1$). In the latter group, plasma levels of threonine, valine, isoleucine, leucine, tyrosine, lysine and histidine remained well below the near normal values observed in the SSO group. Two to 3 weeks later these low values had resolved but by that time subjects were receiving a mixed diet. The combination of low plasma albumin and low essential amino acid concentrations are classical features of dietary protein inadequacy and strongly suggest that the amount of protein provided by Pregestimil, given at the recommended dilution, is less than optimal for the rapid rates of catch-up growth which occurs in severely malnourished children.

We conclude that in the rehabilitation of infants from severe malnutrition and chronic diarrhoea, the casein hydrolysate preparation offered no advantages over the locally-made skimmed-milk-based formula and the biochemical evidence suggests it may have delayed recovery of protein nutritional status.

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Urea production rates with two different amino acid solutions. By G. A. PONTING, *Wexham Park Hospital, Slough, Berks* and A. J. W. SIM, *University Department of Surgery, Faculty of Medicine and Health Sciences, PO Box 15551, UAE University, Al Ain, United Arab Emirates*

The ideal amino acid profile of solutions used for intravenous nutrition has yet to be established. The World Health Organization recommendation of egg protein as the ideal has been used as the basis for one solution, Vamin (Kabi-Vitrum). A profile based on pharmacokinetic principles (transfer adapted), Eloamin (Leopold), has been reported to improve nitrogen balance and hepatic protein levels in multi-traumatized patients without development of high blood urea concentrations (Semsroth *et al.* 1982).

Urea production rates were compared in stable patients receiving parenteral nutrition, either Vamin or Eloamin, in a double blind, randomized 4 d crossover study. All intravenous nutrients were infused as a complete nutritional mixture containing fat, glucose, amino acids, vitamins and minerals through a central venous catheter to provide 14 g N and 8368 kJ (2000 kcal) non-protein/d. Plasma urea, urinary urea and urinary N were measured on a daily basis. Urea production rate was calculated from the urinary urea excretion and the change in whole-body urea (0·75 × change in plasma urea × body-weight). Eight patients were studied for eleven pairs of study periods (4 d).

	Urea production rate (mmol/d)		N balance (g/d)	
Infusion	Mean	SE	Mean	SE
Vamin	379	115	1·3	2·7
Eloamin	396	93	1·5	3·8

There were no significant differences (Wilcoxon matched pairs analysis) in either urea production or N balance between the two amino acid solutions. This study has demonstrated that, at these N and energy intakes, there is no difference in urea production rates between infusions of solutions based on egg protein or transfer-adapted amino acid profiles.

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Prevention and management of venous thrombosis complicating central parenteral nutrition, a clinical audit. By G. BARCLAY, K. BRADY, J. R. RICHARDS and C. R. PENNINGTON, *Departments of Medicine and Pharmacy, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Venous thrombosis (VT) is a potentially fatal complication of prolonged central parenteral nutrition. The present study reports an audit of clinical practice which aimed to analyse factors associated with VT, to monitor change in incidence following consequent modifications in management, and to describe treatment and outcome.

One hundred and twelve courses of central parenteral nutrition were administered to eighty-seven patients via tunnelled central venous catheters, using standard nutrient solutions compounded in 3-litre bags and cyclical infusion. The majority of patients suffered from Crohn's disease and most were at increased risk of thrombosis. All were treated in one medical unit with specially trained nurses using strict catheter-care protocols.

Initially two standard nutrient regimens were adopted, each providing 14 g nitrogen (Synthamin 14) and 8368 kJ (2000 kcal) non-protein energy in 2 litres. The energy source in solution A was glucose alone whereas 50% of the energy in solution B was in the form of lipid (Intralipid). Subsequently solution A was modified to provide 11·5 g N, 7530 kJ (1800 kcal) and 2500 units of heparin in 2·5 litres (solution C). To meet individual requirements some patients received solution B or C with additional fluid or electrolytes (miscellaneous solution, M).

Overall sixteen episodes of VT occurred during 112 courses of treatment after a mean time of 35 d. Thirteen episodes of VT occurred during forty-five courses of solution A, but only one episode during twenty-four courses of solution B ($P<0·05$). One episode occurred during seventeen courses of solution C and one episode during twenty-six courses of solution M. The incidence of VT with solution A (13/45) compared with the combined incidence with the other three solutions, B, C and M, (3/67) was significantly different ($P<0·001$).

When the catheter tip was identifiable in the superior vena cava (SVC), fourteen episodes of VT occurred during forty-eight courses of treatment but when the tip was in the right atrium no episodes occurred during twenty-eight courses ($P<0·005$). This was independent of the solution used as with solution A there were twelve episodes of VT during twenty-six courses when the tip was in the SVC but no episodes in sixteen cases when the tip was in the right atrium ($P<0·005$).

Early clinical and radiological resolution occurred in two of seven who received heparin, five of eight treated with streptokinase (three had inadequate treatment) and all three with tissue-plasminogen activator. There were no fatalities; two patients developed pulmonary emboli. Investigation for catheter sepsis was negative in all patients with thrombosis.

In conclusion, VT may be related to the nature of the nutrient solution and position of the catheter, the incidence may be reduced by changes in management, and when it occurs it can be effectively treated. However, the problem persists and basic research is required to investigate the role of individual factors in thrombus formation and thus methods of prevention.

The oxygen content of total parenteral nutrition mixtures stored in different container systems. By G. HARDY and R. HEMS, *Oxford Nutrition, Oxford OX4 3UH* and G. MARI, *Mirandola, Italy*

Total parenteral nutrition (TPN) mixtures, susceptible to air oxidation, are stored at refrigeration temperatures to maintain or extend stability. Unfortunately, oxygen has greater solubility at lower temperatures so that, in addition to air trapped in solutions during mixing, more O_2 will permeate traditional plastic containers to exacerbate nutrient oxidation.

We have determined free O_2 content in three 2 litre TPN mixtures: (1) Synthamin 14–glucose (with bisulphite), (2) Eloamin 16–glucose (without bisulphite) and (3) glucose at 200 g/l as a control, in four different plastic containers (bags): MixiEVA (M), PVC (T), Trilaminate (U) and Multilaminate (K). Bags were aseptically filled with the solutions and stored at 4°. The solutions were analysed periodically during 15 d. Free O_2 was determined with a Clarke O_2 electrode, connected to a chart recorder, set to zero with sodium dithionite and to maximum deflection at O_2–CO_2 of 95:5 (v/v) in water at 37°.

Initial O_2 content of the control solution (3) was similar in all bags, (0·36 μmol/ml) and during 2 weeks there was essentially no O_2 penetration into bags U (−5%) or K (−3%). In contrast, bags M (+ 8%) and T (+ 17%) showed an increase. Free O_2 levels in mixtures (1) and (2) were initially lower than in mixture (3) (0·31 μmol/ml in M and T, 0·23 μmol/ml in U and K), reflecting the low O_2 content of the unmixed amino acid solutions sealed under nitrogen or vacuum. However, during storage, mixtures (1) and (2) showed appreciable increases in O_2 uptake in bags M (+ 23%) and T (+ 33%) and final concentrations equalled that of controls (0·41 μmol/ml). Conversely, free O_2 for mixture (1) decreased slightly in bags U (−4%) and K (−7%), whilst the decrease for mixture (2) in both containers U and K was −43%.

These results confirm that traditional bags M and T are permeable to air at low temperatures (in this study T was more permeable than M) and laminated bags U and K appeared to be equally impermeable. The results further demonstrate that O_2 (i.e. air) trapped in solutions during mixing is taken up over time by the constituents of the mixture which may be oxidized. In an impermeable container this is a finite process, therefore minimal, since no additional O_2 can enter the mixture. However, in permeable bags O_2 uptake and hence oxidation continues throughout storage as more air continues to pass into the mixture to re-establish equilibrium. Bisulphite decreases O_2 uptake but our earlier studies with mixtures similar to (1) (Hardy & Mari, 1988) showed that the amino acids tyrosine, tryptophan and threonine are still susceptible to oxidation in plastic bags.

These mixtures contain approximately 400 000 μmol amino acids/l. Assuming 100 μmol O_2/l is absorbed during 2 weeks, oxidation losses could be 0·02–0·05% of the total. However, it is unlikely that all amino acids are equally oxidized so that tryptophan, for example, with an initial concentration of 3500 μmol/l could exhibit losses of 3–5%. Furthermore, losses will be approximately 50% more in 500 ml paediatric bags because of the greater surface area:volume ratio.

Losses of amino acids and other O_2-sensitive nutrients in plastic containers can have clinical implications for both adult and paediatric TPN therapy. The use of permeable bags can prevent O_2 penetration and hence minimize the degree of nutrient oxidation.

Hardy, G. & Mari, G. (1988). *Clinical Nutrition* Spec. Suppl. **7**, 75.