EFFECT OF PEROPERATIVE NORMOTHERMIA ON POSTOPERATIVE PROTEIN METABOLISM IN ELDERLY PATIENTS UNDERGOING HIP ARTHROPLASTY

F. CARLI, P. W. EMERY AND C. A. J. FREEMANTLE

Surgical injury is followed by metabolic alterations secondary to tissue damage and neuroendocrine changes. Much of the protein loss occurs in skeletal muscle, as demonstrated by increased release of amino acids from muscle [1]. The accelerated release of amino acids is associated with increased loss of nitrogen and body cell mass.

In a previous study [2] protein breakdown was attenuated by conservation of body heat during major abdominal surgery, resulting in decreased urinary excretion of 3-methylhistidine (3-MeH) and urea nitrogen. However, the validity of 3-MeH excretion as an index of muscle breakdown has been questioned by Rennie and Millward [3], who suggested that large quantities of this amino acid come from breakdown of visceral protein in organs such as gut.

During abdominal surgery, the contribution of muscle breakdown to the excretion of 3-MeH might play a minor role compared with the gut, which is directly involved in such surgery. We decided, therefore, to study 3-MeH excretion using a surgical model associated with muscle damage but not gut involvement. In addition, we investigated the effect of peroperative normothermia on amino acid concentrations in plasma and muscle and on total body potassium, an index of body cell mass.

SUMMARY

We have examined in elderly patients the effect of maintenance of normothermia during hip surgery on postoperative protein metabolism. In one group of six patients (warmed group) heat loss was minimized during surgery and in the recovery period by warming fresh gases, i.v. fluids and wrapping the exposed parts of the body with a warming blanket. In a second group of six patients (cold group), routine care was provided. General anaesthesia consisted of thiopentone, tubocurarine and halothane in both groups. Urinary excretion of urea nitrogen and 3-methylhistidine (3-MeH) after surgery was significantly lower in the warmed group compared with the cold group ($P < 0.05$). There was little effect of normothermia on amino acid concentrations in plasma after surgery. Muscle glutamine concentration 4 days after surgery decreased by 50% in the cold group and 18% in the warmed group. Total body potassium (TBK), measured as an index of body cell mass, decreased significantly after surgery in both groups. However, 7 days after surgery the reduction in TBK in the cold group remained significantly lower than that of the warmed group ($P < 0.05$). Maintenance of normothermia during hip surgery appeared to attenuate, but not eliminate, protein breakdown and nitrogen loss after surgery.

F. CARLI, F.F.A.R.C.S., Department of Anaesthesia, Northwick Park Hospital and Clinical Research Centre, Watford Road, Harrow, Middlesex. P. W. EMERY, M.SC., PH.D., Department of Food and Nutritional Sciences, King's College, London. C. A. J. FREEMANTLE, M.SC., M.R.C. Cyclotron Unit, Royal Postgraduate Medical School, DuCane Road, London. Accepted for Publication: February 20, 1989.
Correspondence to F. C.

PATIENTS AND METHODS

Twelve elderly patients undergoing elective hip arthroplasty for osteoarthritis gave informed consent to participate in this study, which was approved by the local Ethics Committee. Patients who were grossly obese or malnourished or who had endocrine abnormalities were not included.

On the day before surgery, skinfold thicknesses and mid-arm circumference were measured to calculate the ratio of fat to body weight. The duration of preoperative starvation was recorded also.

Premedication comprised papaveretum 15–20 mg and hyoscine 0.2 mg i.m. 60 min before surgery. General anaesthesia was induced with thiopentone and neuromuscular block achieved with tubocurarine. The lungs were ventilated with a mixture of 70% nitrous oxide in oxygen supplemented by halothane. Normocapnia was ensured by monitoring the end-tidal concentration of carbon dioxide. The patients studied were allocated randomly to two groups (cold and warmed) of six patients each. The cold group served as a control, and no precautions were taken during anaesthesia and surgery to maintain normothermia. In contrast, in the warmed group, efforts were made to minimize heat loss during surgery. This was achieved by warming i.v. fluids to 37 °C using a blood warmer and delivering inspired gases by means of a cascade humidifier (Bennett) set to deliver gases at 36 °C at mouth level. All exposed parts of the body except the operated site were covered with heated blankets at 37 °C. At the end of surgery, a metallized plastic sheet was placed over the patient to minimize heat loss during recovery.

During surgery all patients received an i.v. infusion of normal saline 4 ml kg^{-1} h^{-1}. Dextran 70 in saline was administered when blood loss exceeded 20% of the patient's circulating volume. After operation, i.v. fluids consisted of a mixture of 4% dextrose in 0.18% normal saline at a rate of 40 ml kg^{-1} day^{-1} for 2 days, followed by oral diet.

Before induction of anaesthesia, a thermocouple probe was inserted under direct vision in the aural canal and secured with cotton wool to avoid draughts. Skin surface temperature probes were applied to the chest, mid-arm, mid-thigh and calf and mean skin temperature was calculated. All the probes and the thermometer had been calibrated previously and were accurate to 0.1 °C over the temperature range studied. The measurements were taken before induction of anaesthesia and at the end of surgery. Mean skin temperature was calculated using the four-point formula proposed by Ramanathan [4]: mean skin temperature (°C) = 0.3 ($t°$ chest + $t°$ arm) + 0.2 ($t°$ thigh + $t°$ calf). Ambient temperature and relative humidity in the operating theatre were kept at 21 °C and 55%, respectively, during the study.

All patients were maintained on a meat- and fish-free diet for a period of 4 days before surgery and for 4 days afterwards. Oral diet, based on 40–60 g of proteins and approximately 1000 calories per day was resumed on the 3rd day after operation. Surgery was performed by the same surgeon during the morning hours.

Venous blood samples were collected after a 12-h overnight fast on the day of surgery, and on the 2nd and 4th days after surgery for measurement of plasma concentrations of amino acids. Urine was collected for 24 h before and for 4 consecutive days after operation for measurements of urinary concentrations of urea nitrogen, creatinine and 3-MeH.

Percutaneous needle muscle biopsies were taken after a 12-h overnight fast from the lateral portion of the quadriceps femoris muscle, approximately 12–20 cm above the knee [5]. These were performed on the non-operated thigh after induction of general anaesthesia before surgery, and on the 4th day after operation under local anaesthesia. In addition, a small sample of the quadriceps femoris muscle on the operated side was taken by the surgeon immediately after skin incision. The wet biopsy material was dissected carefully to remove visible fat and connective tissue and frozen in liquid nitrogen. The samples were used for measurement of free amino acids.

Biochemical assay methods

Urea nitrogen and creatinine were determined by the SMA automated procedure.

Urinary 3-MeH was measured by high pressure liquid chromatography (HPLC) using a modification of the method of Jones, Shorley and Hitchcock [6]. Urine was diluted 1:10 with water, and 100 μl was mixed with 400 μl of borate buffer (0.1 mol litre^{-1}, pH 9) and 500 μl of fluorescamine (1.6 ng ml^{-1} in acetonitrile). After the mixture had stood for 5 min, 500 μl of HCl 2 mol litre^{-1} was added and the mixture heated to 80 °C for 1 h. After cooling, 20 μl was injected onto a reversed phase column (Ultrasphere ODS from Beckman, Bucks) and eluted isocratically with a mixture of 52% acetate buffer (sodium acetate 1 g and glacial acetic acid 2.5 ml litre^{-1}) and 48% methanol at a flow rate of 1 ml min^{-1}.

The detector was a Gilson Spectra Glo fluorimeter with excitation below 390 nm and emission above 460 nm. 3-MeH concentration was calculated from peak areas by comparison with external standards run with each batch of urine samples.

Plasma samples were deproteinized by the addition of an equal volume of 8% sulphosalicylic acid. After centrifugation the supernatants were analysed for amino acids by HPLC as described previously [7].

Muscle samples were freeze-dried then homogenized in a small volume (30 µl mg⁻¹, dry weight) of 8% sulphosalicylic acid. After centrifugation, the protein-free supernatant was analysed for amino acids by HPLC as above. The method is based on that described first by Turnell and Cooper [8]; in our laboratory we have found its accuracy and precision to be similar to that reported by those authors. Thus for intracellular amino acids present at low concentrations (approximately 10 µmol litre⁻¹ in the final sample) the within assay coefficient of variation was 5–10%; for amino acids present at higher concentrations (up to 1000 µmol litre⁻¹ in the final sample) the within assay coefficient of variation was 1–5%.

The results were expressed as µmol g⁻¹, wet weight (instead of dry weight), for ease of comparison with previous data [9].

Total body potassium (TBK) was measured non-invasively by use of a whole body counter with a precision of 2% and an accuracy of 4%. The method relies on the measurement of gamma emission from naturally radioactive potassium (potassium-40) present in the body as 0.012% of the stable isotopes of potassium (potassium-38 and potassium-41). As 98% of potassium is located in the cells of the body cell mass, this measurement represents an index of the lean tissue mass. Age, sex, preoperative body weight and height of each patient were recorded, and a predicted value calculated according to formulae proposed by Boddy [10]. The degree of preoperative depletion was calculated from the ratio between the predicted and measured preoperative values, and the metabolic response to surgery was obtained from the difference between the preoperative and postoperative measured TBK concentrations. TBK was measured before surgery and 4 and 7 days after surgery.

Data are presented as mean and standard deviation. Comparison of data within a group was made by applying Student's paired *t* test. Comparisons between groups were performed by the two-sample *t* test for unpaired data.

RESULTS

The groups were comparable except for core and

TABLE I. *Physical characteristics of the two groups, clinical data and body temperature changes during surgery (mean (SD)).* ** *Statistically significant difference between values for cold and warmed groups* (P < 0.01)

	Cold (n = 6)		Warmed (n = 6)
Age (yr)	71 (6)		67 (8)
Sex M:F	4:2		3:3
Body weight (kg)	71.4 (13.8)		68.3 (19)
Body height (m)	1.68 (11.2)		1.59 (7.9)
Body fat (%)	33.4 (4.2)		32.9 (2.8)
Preoperative starvation time (h)	14 (2)		12 (2)
Duration of surgery (min)	168 (41)		157 (30)
Blood loss (ml)	1368 (400)		1346 (520)
Change in aural canal temperature (°C)	−1.3 (0.3)	**	+0.2 (0.2)
Change in mean skin temperature (°C)	−1.4 (0.2)	**	+0.4 (0.2)

mean skin temperatures, which decreased significantly in the cold group by 1.3 and 1.4 °C, respectively, at the end of surgery. In the warmed group, body temperature was maintained at preoperative values during surgery and in the immediate postoperative period. Patients in the cold group rewarmed to the preoperative body temperature during the first 4 h after operation (core of 36.6 °C), while the warmed group maintained core at 36.8 °C (table I).

Urea nitrogen and 3-MEH excretion

Preoperative values of urinary urea nitrogen were similar for both groups. Cumulative concentration of urea nitrogen over 4 days after operation was significantly greater in the cold group compared with that of the warmed group: 1240 + 558 mmol day⁻¹ for the cold group and 728 + 254 mmol day⁻¹ for the warmed group (*P* < 0.05). Urinary excretion of 3-MeH, expressed as the ratio of 3-MeH over creatinine, showed a significantly greater increase 4 days after surgery in the cold group compared with the warmed group (fig. 1).

Plasma and muscle amino acids

Changes in amino acid concentration in plasma and muscle after surgery are shown in figures 2 and 3. Plasma and muscle concentrations of branched chain amino acids increased in both groups. There was no difference in the magnitude of this response between the two groups. The concentration of glutamine in muscles decreased
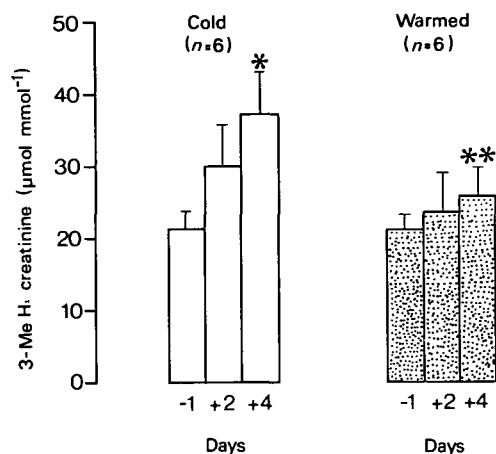
FIG. 1. Urinary excretion of 3-MeH before and 2 and 4 days after surgery in the cold and warmed groups. *Significant difference between values for preoperative and postoperative periods in the cold group ($P < 0.05$); **Significant difference between postoperative values for cold and warmed groups ($P < 0.01$).

in both groups after surgery; however, the decrease in the cold group was more than 50%, compared with 18% in the warmed group (fig. 3). The difference between the two groups was not significant because of the small number of patients from whom muscle biopsies were taken (four in each group). Before operation the concentration of glutamine in the muscle of the side to be operated on was significantly lower than that of the unaffected side (10.1 (2.2) v. 13.4 (2.3) µmol g^{-1} (wet weight) $n = 8$, $P < 0.05$ by paired sample t test). There were no other significant differences in muscle amino acid concentrations between the two legs before operation. The preoperative samples had a water content of approximately 71%, and postoperative samples one of 75%.

Total body potassium (TBK)

No preoperative depletion, calculated as a percentage ratio of measured over predicted value of TBK was observed in either group of patients
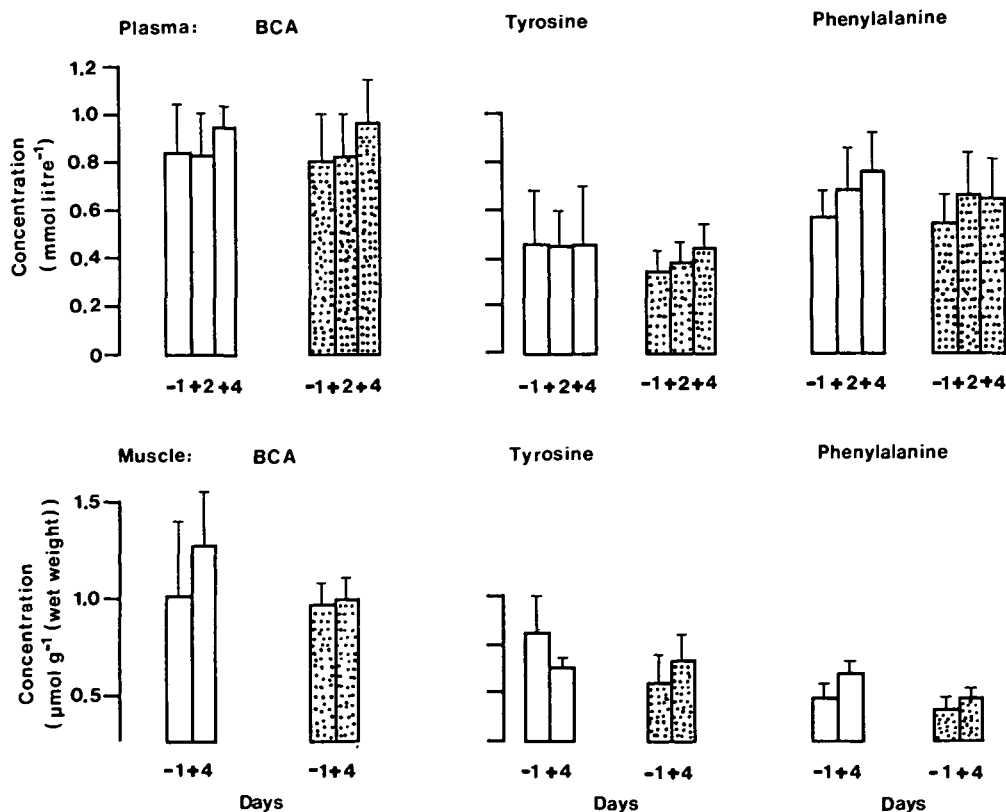

FIG. 2. Preoperative and postoperative plasma and muscle concentrations of branched chain amino acids (BCA) and aromatic amino acids (phenylalanine and tyrosine) in cold group (open columns) and warmed groups (stippled columns).
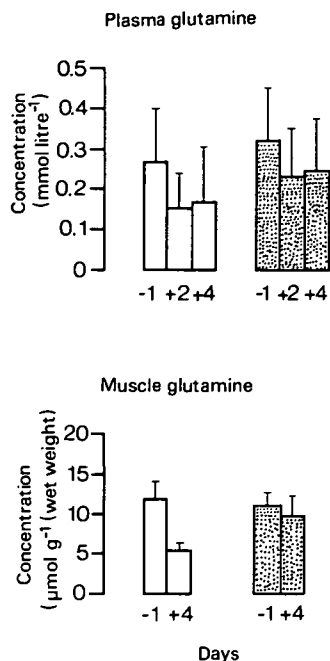
Plasma glutamine

FIG. 3. Preoperative and postoperative plasma and muscle concentrations of glutamine in the cold group (open columns) and warmed group (stippled columns).

FIG. 5. TBK concentration changes during the postoperative period represented as percentage changes from the pre-operative values in the cold group (○) and warmed groups (●). Significant differences $(P < 0.05)$: † between post-operative values for cold and warmed groups; * compared with day −1.
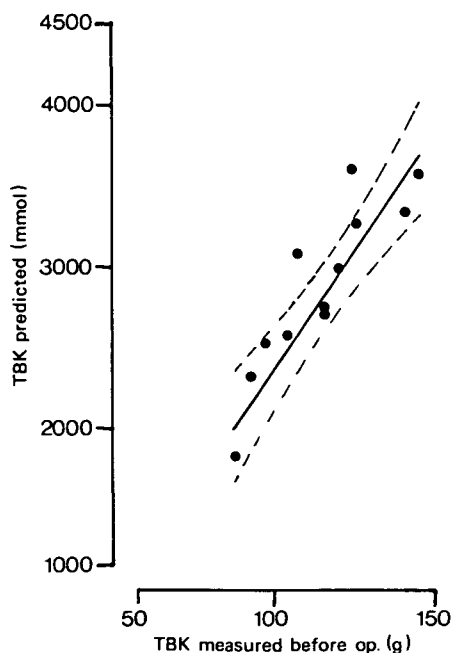

FIG. 4. Correlation between preoperative predicted and measured total body potassium concentration (TBK) in both groups studied, with 95% confidence limits.
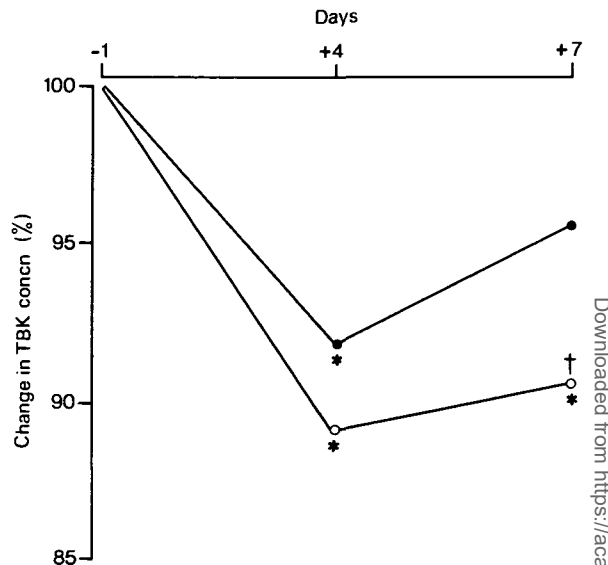
studied (fig. 4). The mean (SD) values for the cold and warmed groups were 0.97 (0.12) and 1.03 (0.15), respectively. The postoperative reduction in TBK, represented as percentage change from preoperative value, was similar in both groups 4 days after surgery. However, 7 days after surgery, the change in cell body mass in the warmed group was significantly smaller than that of the cold group $(P < 0.05)$ (fig. 5).

DISCUSSION

Maintenance of normothermia during hip surgery resulted in a significant decrease in postoperative urinary excretion of urea nitrogen and 3-MeH. These data are in agreement with two previous studies on protein metabolism in patients undergoing major abdominal surgery [2, 11].

The changes in amino acid concentrations in plasma and muscle were not significantly different between the two groups. The pattern of amino acids is known to be influenced by numerous factors, including age [12], sex distribution [13], dietary intake and physical activity [14]. As far as physical activity is concerned, patients on a waiting list for hip arthroplasty become inactive

and some muscle wasting may occur. This may explain the significantly different amino acid concentrations observed in the quadriceps of the operated side compared with the contralateral side. Although physical inactivity does not seem to change the composition of amino acids in muscle in the short term, disuse of specific muscles does lead to atrophy which is characterized by changes in intracellular amino acid patterns, including decreased glutamine concentration [14], as observed here. This finding is of interest because of possible therapeutic implications. Maintaining mobility before surgery may be expected to improve the rate of recovery after the operation.

No attempt was made to control dietary intake before operation, except for a meat- and fish-free diet. However, judging from the anthropometric characteristics of the subjects in both groups (height, weight, body fat %) and preoperative TBK concentration and urea nitrogen excretion, there was no difference between the two groups. Hypocaloric intake was ensured for the first 2 days after operation, followed on days 3 and 4 by a vegetarian diet which included a moderate amount of proteins. The alterations in plasma and muscle amino acids reported here are in good agreement with findings in patients who received different types of nutrition during the postoperative period [15]. This suggests that the metabolic milieu observed in the injury state causes changes in muscles which nutrition can affect only in minor ways. Concentrations of branched chain amino acids increased after surgery, presumably because of the net breakdown of muscle protein.

A greater decrease (50%) in muscle glutamine concentration was observed in the cold group than in the warmed group. This difference was not significant; however, the change observed was of some interest because it was consistent in the small number of patients studied. It has been suggested that the size of the intracellular free pool of glutamine may be important in determining acute changes in protein mass by controlling the rate of protein synthesis in muscle [16, 17]. Thus the attenuation of the loss of glutamine from muscle in the warmed group is further evidence that peroperative warming may ameliorate postoperative loss of muscle protein.

TBK was measured in these patients before and after surgery and used as an absolute index of body cell mass [18]. A reduction in the amount of

potassium in the total cell mass is likely to indicate a loss of protein [19]. The whole body counter method for calculating changes in body nitrogen depends on the assumption that nitrogen and potassium are lost in the same ratio as they exist in protoplasm [20]. The extent of loss of lean body mass observed with surgery appears to be related to severity of trauma [21]. In the present study the maximal decrease in TBK occurred 4 days after surgery in both groups. On the 7th day after operation the loss of cell mass remained significantly greater in the cold group than in the warmed group. This suggests that tissue breakdown began to be reversed more quickly in the warmed group.

It may be concluded from the present study that, when attempts are made to maintain patients normothermic during surgery, muscle tissue breakdown can be attenuated but not eliminated. The mechanism by which protein metabolism is affected has not yet been elucidated. Provision of thermoneutrality is known to decrease metabolic rate during the postoperative period [22]. In addition, it is not known how the rates of synthesis and breakdown of protein in muscle and other tissues are affected. Measurements of these processes should lead to a greater understanding of the physiopathology of nitrogen loss after injury and contribute to the development of more rational methods of modifying this loss in critically ill patients.

REFERENCES

1. Dale G, Young G, Latner AL, Goode A, Tweedle D, Johnston IDA. The effect of surgical operation on venous plasma free amino acids. *Surgery* 1977; **81**: 295–301.
2. Carli F, Itiaba K. Effect of heat conservation during and after major abdominal surgery on muscle protein breakdown in elderly patients. *British Journal of Anaesthesia* 1986; **58**: 502–507.
3. Rennie MJ, Millward DJ. 3-Methylhistidine excretion and urinary 3-Methylhistidine/creatinine ratio are poor indicators of skeletal muscle protein breakdown. *Clinical Science* 1983; **65**: 217–225.
4. Ramanathan NL. A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology* 1964; **19**: 531–533.
5. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged 16 to 72 years. *British Journal of Nutrition* 1974; **32**: 77–97.
6. Jones D, Shorley D, Hitchcock C. The fluorometric determination of 3-methylhistidine in meat and meat products. *Journal of the Science of Food and Agriculture* 1982; **33**: 677–685.
7. Mann GE, Smith SA, Norman PSR, Emery PW. Fasting,

refeeding and diabetes modulate free amino acid concentration in the rat exocrine pancreas: role of transtimulation in amino acid efflux. *Pancreas* 1987; 3: 67–76.

8. Turnell DC, Cooper J. Rapid assay for aminoacids in serum or urine by precolumn derivatisation and reverse phase liquid chromatography. *Clinical Chemistry* 1982; 28: 527–531.

9. Askanazi J, Furst P, Michelsen CB, Elwyn DH, Vinnars E, Gump FE, Stinchfield FE, Kinney JM. Muscle and plasma amino acids after injury. *Annals of Surgery* 1980; 191: 465–472.

10. Boddy K, King PC, Hume R, Wyers E. The relation of total body potassium to height, weight and age in normal adults. *Journal of Clinical Pathology* 1972; 25: 512–517.

11. Carli F, Clark MM, Woollen JW. Investigation of the relationship between heat loss and nitrogen excretion in elderly patients undergoing major abdominal surgery under general anaesthetic. *British Journal of Anaesthesia* 1982; 54: 1023–1029.

12. Armstrong MD, Stave U. A study of plasma free amino acid levels. III. Variations during growth and aging. *Metabolism* 1973; 22: 571–578.

13. Moller P, Bergstrom J, Eriksson S, Furst S, Hellstrom K. Effect of aging on free amino acids and electrolytes in leg skeletal muscle. *Clinical Science* 1979; 56: 427–432.

14. Askanazi J, Elwyn DH, Kinney JM, Gump FE, Michelsen CB, Stinchfield FE, Furst P, Vinnars E, Bergstrom J. Muscle and plasma amino acids after injury: the role of inactivity. *Annals of Surgery* 1978; 188: 797–803.

15. Furst P, Elwyn D, Askanazi J, Kinney JM. Effects of

nutrition and catabolic stress on intra-cellular branched-chain amino acids. In Wesdarp R, Socters P, eds. *Clinical Nutrition*. Edinburgh: Churchill Livingstone, 1981; 10–17.

16. Rennie MJ, Babij P, Taylor PM, Hundal HS, MacLennan P, Watt PW, Jepson MM, Millward DJ. Characteristics of a glutamine carrier in skeletal muscle have important consequences for nitrogen loss in injury, infection and chronic disease. *Lancet* 1986; 1: 1008–1012.

17. MacLennan PA, Brown RA, Rennie MJ. A positive relationship between protein synthetic rate and intracellular glutamine concentration in perfused rat skeletal muscle. *FEBS Letters* 1987; 215: 187–191.

18. Moore FD, Olsen KO, McMurray JD, Parker HV, Ball MR, Boyden LM. *The Body Cell Mass and Its Supporting Environments*. Philadelphia: Saunders, 1963.

19. Shizgal HM, Milne CA, Spanier AH. The effect of nitrogen sparing, intravenously administered fluids on postoperative body composition. *Surgery* 1979; 85: 496–503.

20. Baker JP, Detsky AS, Whitwell J, Langer B, Jeejeebhoy KN. A comparison of the predictive value of nutritional assessment techniques. *Human Nutrition: Clinical Nutrition* 1982; 36C: 233–241.

21. Carli F, Freemantle C. Body cell mass following major elective surgery. *Clinical Science* 1988; 75 (Suppl. 19): 37P.

22. Carli F. Peroperative normothermia attenuates increase in postoperative metabolic rate in elderly patients. *British Journal of Anaesthesia* 1988; 61: 511–512P.