EFFECT OF GENERAL ANAESTHESIA ON WHOLE BODY PROTEIN TURNOVER IN PATIENTS UNDERGOING ELECTIVE SURGERY

F. CARLI, V. RAMACHANDRA, J. GANDY, H. MERRITT, G. C. FORD, M. READ AND D. HALLIDAY

SUMMARY
To determine if general anaesthesia alone or in conjunction with surgery alters body protein turnover, we studied six healthy, unpremedicated females undergoing elective total abdominal hysterectomy. Changes in protein metabolism, synthesis and breakdown were estimated by an isotope dilution technique using a continuous infusion of the stable isotope tracer, L-[1-13C]leucine, before anaesthesia (4 h), during anaesthesia alone (1 h), during anaesthesia and surgery (1 h) and in the recovery period (2 h). General anaesthesia comprised thiopentone, pancuronium, enflurane (1 MAC) and oxygen-enriched air. An isotopic steady state in plasma 13C-α-ketoisocaproate (13Cα-KIC) and expired 13C-carbon dioxide were obtained during the four periods. Collections of plasma and expired air were made during the steady state periods and plasma α-KIC enrichment measured to indicate precursor pool labelling from which leucine flux (equal to protein breakdown in the post-absorptive state) and oxidation were calculated, and whole body protein synthesis was derived. Whole body protein breakdown did not change with anaesthesia, but decreased with both surgery and during the acute recovery period (P < 0.05). Protein synthesis did not change with anaesthesia and surgery, but decreased significantly after surgery (P < 0.05).

KEY WORDS
Metabolism; protein.

Elective surgery is associated with postoperative negative nitrogen balance and increased plasma concentration of branched chain amino acids [1]. These changes must reflect an increase in whole body protein breakdown, a decrease in synthesis, or both [2]. Factors such as preoperative nutritional status, immobilization, intensity of trauma, substrate administration and anaesthetic techniques should be considered, but, whilst the contribution of some of these factors is well recognized, the effect of anaesthesia on protein metabolism has not received attention in surgical studies.

Anaesthesia alone by the in vitro administration of halothane has been reported to result in a small but reversible decrease in tissue protein synthesis [3]. More recently, an investigation on the effect of halothane and nitrous oxide anaesthesia in dogs showed that leucine oxidation and whole body protein breakdown increased, while protein synthesis was depressed [4]. We have examined the acute effect of general anaesthesia alone followed by anaesthesia and surgery on whole body protein turnover in patients undergoing elective surgery of moderate intensity.

PATIENTS AND METHODS
We studied six females (mean age 44 yr (range 38–50 yr), mean weight 65 (50–75) kg and mean body mass index 25.3 (21.4–26.7) kg m⁻²) undergoing elective total abdominal hysterectomy for menorrhagia. None of the patients was suffering from endocrine disease or receiving medication.

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The study was approved by the local Ethics Committee and informed consent was obtained from each patient.

Anaesthesia

None of the patients received any premedication. General anaesthesia was induced with thiopentone 5 mg kg\(^{-1}\), the trachea was intubated following administration of pancuronium bromide 0.1 mg kg\(^{-1}\) and the lungs were ventilated to normocapnia with oxygen-enriched air (\(F_{1\text{O}_2} = 35\%\)).

Nitrous oxide was not used in this study, as it has the same molecular weight as carbon dioxide and thus interferes with the isotope ratio measurement of expired air \(^{13}\text{C}\)-carbon dioxide. Anaesthesia was maintained with an end-expiratory enflurane concentration of 1.67 ± 0.13 % (1 MAC) throughout the study and a dose of papaveretum 10 mg was administered i.v. before skin incision. Normal saline 0.9 % was infused i.v. during anaesthesia, surgery and in the recovery period at a rate of 6 ml kg\(^{-1}\) h\(^{-1}\). Blood transfusion was not required during surgery or recovery.

ECG, non-invasive systemic arterial pressure, end-tidal concentrations of carbon dioxide (Hewlett-Packard) and enflurane (Emma, Engstrom), inspired concentration of oxygen, oxygen saturation, aural canal temperature and train-of-four were monitored and recorded during anaesthesia and surgery. In three of the subjects, a cannula was inserted into the radial artery after induction of anaesthesia and heparinized blood samples taken to measure pH and standard bicarbonate during anaesthesia, surgery and recovery. Attempts were made to keep the patients warm during the studies. Ambient temperature and relative humidity in the operating theatre were maintained at 23.5 ± 0.8 °C and 55 ± 5 %, respectively.

Following a period of 1 h of general anaesthesia, surgery was then started which lasted 1 h. The operations were performed by the same surgeon at the same time of the day on each occasion.

Following removal of the tracheal tube, the patients were transferred to the recovery room where they remained for 2 h; here, pain relief was achieved with papaveretum 5–10 mg administered i.v. as required.

Isotopes

\(L-[1-^{13}\text{C}]\)Leucine (99 % \(^{13}\text{C}\)) was obtained from Tracer Technologies (Newton, Ma, U.S.A.) and sodium bicarbonate (99 % \(^{13}\text{C}\)) from Cambridge Isotope Laboratories (Cambridge, Ma, U.S.A.).

All patients were studied on the day of surgery, at 08:00 after fasting approximately 12 h overnight (mean 11 h, range 10–13 h). A superficial vein in the dorsum of the hand was cannulated and the cannula kept patent with heparinized saline. A second superficial vein in the contralateral arm was cannulated to provide access for the infusion of \(L-[1-^{13}\text{C}]\)leucine. Blood and expired air samples were collected before the infusion to determine baseline carbon-13 enrichment. Primed doses of \(^{13}\text{C}\)-\(\text{NaHCO}_3\) 0.08 mg kg\(^{-1}\) and \(L-[1-^{13}\text{C}]\)leucine 0.5 mg kg\(^{-1}\), prepared in the hospital pharmacy, were administered and followed immediately by a continuous infusion of \(L-[1-^{13}\text{C}]\)leucine 0.5 mg kg\(^{-1}\) which was continued throughout anaesthesia, surgery and recovery for a total of 8 h. Two hours after the start of the infusion of isotope, when the tracer was assumed to have reached an isotopic steady state, venous blood and expired air samples were collected every 15 min for the remainder of the study. Each subject lay quietly on the bed for 4 h before anaesthesia and was then transferred to the operating theatre.

A schematic representation of the procedure is shown in figure 1. Each blood sample collected before and during the infusion was transferred immediately to a heparinized tube and centrifuged at 4 °C. The plasma obtained was stored at −70 °C until \(\alpha\)-ketoisocaproate (\(\alpha\)-KIC) enrichment was measured. Expired air samples were collected in a 2-litre latex bag and transferred immediately to 20-ml Vacutainers (Beckton, Dickinson) to await \(^{13}\text{C}\)-carbon dioxide isotope enrichment analysis.

During artificial ventilation, expired gases were

![Fig. 1. Time course of the infusion of isotope and collection of plasma and expired air samples (■) during the four periods.](image-url)
collected by means of a one-way valve into a 5-litre bag.

Production of carbon dioxide ($\dot{V}CO_2$) was measured by indirect calorimetry: expired air was collected for three periods of 3 min each and the expired volume ($\dot{V}E$) and the mixed expired concentration of carbon dioxide ($FE_{CO_2}$) were measured by a dry gas meter (Parkinson & Gowan) and an infra-red gas analyser (Hewlett-Packard). Both instruments had been calibrated previously. During anaesthesia and surgery, measurements of $\dot{V}CO_2$ were performed 30 min after tracheal intubation and 30 min after surgical incision. In the recovery period the measurements were obtained during the last 45 min of infusion of leucine, when patients were rousable and cooperative.

**Analytical methods**

Whole body leucine kinetics were calculated by conventional isotope dilution practice using a two-pool stochastic model [5] during steady state conditions obtained at each phase of the studies. Plasma enrichment of $\alpha$-KIC was used as the basis for calculating both flux and oxidation of leucine [6]. Thus, under steady state conditions, leucine flux ($Q$) is defined by the formula:

$$Q = S + O = B + I$$  \hspace{1cm} (1)

where $S$ is the rate at which leucine is incorporated into body protein, $O$ is the rate of oxidation of leucine, $B$ is the rate at which unlabelled leucine enters the free amino acid pool from endogenous protein breakdown, and $I$ is the rate of dietary intake or the rate of infusion of $^{13}$C-leucine ($\mu$mol kg$^{-1}$ h$^{-1}$) or both.

Inspection of equation (1) indicates that, when studies are conducted after absorption, flux is equal to breakdown. Both plasma $\alpha$-KIC enrichment and concentration were determined by chemical ionization selected-ion monitoring gas chromatography–mass spectrometry using ketovaleric acid as internal standard. Expired breath $^{13}$C-carbon dioxide enrichment was determined on the day of the study by means of isotope ratio mass spectrometry and used to calculate leucine oxidation. A factor of 0.81 was applied to account for the fraction of $^{13}$C-carbon dioxide released by $^{13}$C-labelled leucine oxidation but retained within slow turnover rate pools of the body [5].

Enrichment of plasma $\alpha$-KIC during infusion of $^{13}$C-leucine has been used to determine whole body leucine kinetics. This steady state reciprocal pool model is considered to provide a more precise representation of intracellular precursor pool enrichment than leucine itself [7, 8].

Plateau enrichment for both plasma $\alpha$-KIC and expired carbon dioxide during the four periods (control, anaesthesia, surgery and recovery) exhibited a coefficient of variation of less than 4% in all cases. The control mean value (range) was 1.82 (1.06–3.04)%; that during anaesthesia 2.8 (1.22–4.1)%; during surgery 2.0 (1.2–3.3)% and during recovery 3.1 (1.0–8.2)%.

**Statistics**

Data are presented as means (SD). Differences between whole body protein kinetics during anaesthesia, surgery and recovery were deter-

**Fig. 2.** Plateau enrichment, expressed in atom percent excess (ape), of $^{13}$C-$\alpha$KIC ($\bullet$) and $^{14}$C-carbon dioxide ($\diamond$), and concentration of total $\alpha$KIC (□) during the 8-h infusion of isotope in one patient.
TABLE I. Mean (SD) measurements of cardiovascular status, body temperature, production of carbon dioxide and concentration of \( \alpha \)-ketoisocaproate (\( \alpha \)-KIC) during the four phases (\( n = 6 \)). *\( P < 0.05 \) compared with control

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Anaesthesia</th>
<th>Anaesthesia and surgery</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat min(^{-1}))</td>
<td>88 (9)</td>
<td>96 (12)</td>
<td>103* (13)</td>
<td>98 (15)</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>125 (12)</td>
<td>111 (10)</td>
<td>128 (8)</td>
<td>121 (17)</td>
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<td>Aural canal temp. (°C)</td>
<td>36.8 (0.7)</td>
<td>36.6 (0.4)</td>
<td>36.5 (0.5)</td>
<td>36.9 (0.4)</td>
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<tr>
<td>( \dot{V} )( \text{CO}_2 ) (ml min(^{-1}))</td>
<td>168 (26)</td>
<td>153 (24)</td>
<td>156 (23)</td>
<td>176 (20)</td>
</tr>
<tr>
<td>([\alpha \text{-KIC}]) (( \mu \text{mol litre}^{-1}))</td>
<td>20.7 (6.1)</td>
<td>18.4 (4.3)</td>
<td>22.7 (7.9)</td>
<td>21.4 (6.3)</td>
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Fig. 3. Changes in leucine oxidation, flux and synthesis during anaesthesia, surgery and recovery. Compared with baseline: *\( P < 0.05 \); **\( P < 0.01 \).

RESULTS

Mean duration of surgery was 58 min (56–63 min); mean operative blood loss was 235 ml (210–262 ml). Mean values of systemic arterial pressure, heart rate, core temperature recorded, \( \dot{V} \)\( \text{CO}_2 \) and concentration of \( \alpha \)-KIC recorded during the steady states, before and during 1 h of general anaesthesia, 1 h of surgery and 2 h of recovery are presented in table I. There was a transient increase in heart rate during anaesthesia, but a significant increase occurred only during surgery. No significant changes in systemic arterial pressure occurred throughout the study. \( \dot{V} \)\( \text{CO}_2 \) decreased during anaesthesia and surgery by 8% compared with the preoperative values, but increased during the recovery. No significant changes in concentration of \( \alpha \)-KIC occurred.

Mean (1 SD) arterial pH was 7.38 (0.02) during anaesthesia, 7.39 (0.02) during surgery and 7.37 (0.03) during the recovery period. Mean (1 SD) standard bicarbonate was 26 (1.8) mmol litre\(^{-1}\) during anaesthesia, 25 (1.2) mmol litre\(^{-1}\) during surgery and 24.8 (1.5) mmol litre\(^{-1}\) in the recovery period. Oxygen saturation exceeded 95% throughout the study.

Leucine flux/breakdown during anaesthesia alone did not change in the patients studied, but decreased during surgery and during the first 2 h of recovery by approximately 7 and 10%, respectively (\( P < 0.01 \)) (fig. 3). When the mean change in leucine flux occurring during surgery was compared with that observed with anaesthesia alone, no significant difference was found.

Oxidation of leucine decreased significantly during anaesthesia and surgery (\( P < 0.05 \)), but returned during recovery to preanaesthetic values. Whole body leucine rate of disappearance, an indicator of whole body protein synthesis plus oxidation, did not change with anaesthesia alone. During surgery there was a non-significant decrease in protein synthesis compared with the preoperative values, and this decreased by another 7% during the recovery period (\( P < 0.05 \)).
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DISCUSSION

Stable, non-radioactive isotopes have become more widely used in clinical research in recent times, especially for quantitative assessment of aspects of protein metabolism. This renewed interest has been stimulated both by the increased availability and diversity of stable isotope-labelled compounds and by improvements in mass spectrometric methods for quantitative analysis [9].

The present study showed that, during general anaesthesia supplemented with 1 MAC enflurane, leucine kinetics in unpremedicated patients undergoing elective surgery did not change. However, whole body protein breakdown decreased significantly during surgery ($P < 0.01$) and recovery ($P < 0.01$), while a significant reduction in protein synthesis occurred only during the recovery phase ($P < 0.05$).

Measurements of whole body protein turnover and assessment of protein synthesis and breakdown give a dynamic picture of the movement of proteins in the body compared with more conventional indicators of protein metabolism such as nitrogen balance or specific muscle degradation indicators such as urinary 3-methylhistidine or creatinine.

In the fasting state, the sole source of leucine for protein synthesis and oxidation is that derived from the breakdown of endogenous proteins. To obtain an in vivo estimation of whole body protein metabolism, an isotope dilution technique has been used which measures labelled [1-13C]leucine [5]. The flux of L-[1-13C]leucine represents the total movement of leucine into and from the plasma pool. Oxidation of leucine results in its conversion to 13C-carbon dioxide and leucine flux minus oxidation provides, indirectly, the rate of protein synthesis—a method used recently to measure protein kinetics associated with elective surgery, burns, malignancy and parenteral nutrition.

Ideally, in the execution of tracer studies, a period of at least 2 h should elapse before any observed establishment of a "new isotopic plateau" is interpreted as representing a real metabolic change. In the present study, ethical constraints limited the period of anaesthesia before surgery to little more than 1 h. Also, this surgery was chosen to represent surgical trauma of moderate intensity in which the surgical procedure, when performed by the same surgeon, was completed in 1 h. Thus anaesthesia was of 2 h total duration with surgery superimposed during the second 1 h. If highly significant metabolic changes had occurred as a result of either anaesthesia or surgery, they must have occurred to quantitatively the same extent, but in opposing directions, to produce the results obtained during the periods of anaesthesia and anaesthesia and surgery. Studies reporting the effect of feeding or insulin therapy on leucine kinetics [10, 11] indicate that major metabolic events can be observed within 1 h, as reflected by changes in the tracer enrichment, although the "final" isotopic plateaux were not obtained for approximately 2 h after the intervention. It may be argued, therefore, that the current results do not provide absolute proof that they truly reflect metabolic events occurring during the studies. However, confidence that the results are valid is strengthened in that the coefficient of variation of plateau values for both expired 13C-carbon dioxide and plasma α-KIC was less than 4% for each arm of the study.

Some concern has been expressed in the past about the possible changes in the size of the body bicarbonate pool causing excess production or retention of label in breath carbon dioxide. In the present study, arterial pH and standard bicarbonate did not change significantly at any time. Increased oxidation calculated during the recovery phase resulted exclusively from an increase in the total production of carbon dioxide rather than from any change in the bicarbonate pool size or carbon dioxide enrichment. This latter observation indicates that neither changes in the bicarbonate pool size nor differential retention of 13C-carbon dioxide in slowly turning over components of that pool were responsible for the enhanced oxidation. Indeed, we have found that during 4-8 h infusion studies of this nature there was no significant increase in bicarbonate recovery, which is thus independent of the duration of its infusion over this time interval [12; Wenham and colleagues, unpublished data].

A gradual increase in α-KIC enrichment occurring during the study period, as shown in figure 2, could indicate either a reduction in flux or recycling of label. We do not have any evidence that the latter occurred during the time course of infusion, as shown by previous studies in which leucine was infused for a similar period [10, 11, 13]. The present results are in disagreement with the findings reported by Rennie and McLennan [14], who showed 45% and 36% de-
creases in protein synthesis and leucine flux, respectively, after halothane anaesthesia. These authors studied patients undergoing cardiac surgery who received halothane anaesthesia. However, no details relevant to preoperative nutritional status, drug history, concentration of anaesthetic agent used or duration of anaesthesia were reported and it is therefore difficult to make direct comparisons with the present study. It is of interest that these authors observed a change in α-KIC enrichment soon after the administration of an opioid premedication. In contrast, the administration of benzodiazepine before surgery does not seem to disturb isotopic equilibrium [15]. Because of these findings, it was decided to study unpremedicated patients in the first instance and look solely at the effect of general anaesthesia on protein kinetics.

The role of anaesthetic agents in the metabolic abnormalities of surgery and their effect on protein metabolism are not clear. The relatively few studies undertaken have looked at the volatile agent halothane and have been carried out in cell culture or isolated organ preparations. These studies have reported conflicting results: halothane inhibited protein synthesis in human lymphocytes [16] and rabbit pulmonary macrophages [17], but had no effect on protein synthesis in neuroblastoma cells [18]. In isolated perfused lung [19] and liver [20] preparations, halothane reversibly inhibited protein synthesis in a dosedependent manner. One study in the rat showed a depressant effect of two exposures of halothane, at an interval of 24 h, on albumin and transferrin synthesis [21], while another investigation also in rats exposed to different concentrations of halothane showed a reduction in lung and liver fractional rates of protein synthesis [22].

A report [4] on the effect of halothane anaesthesia on whole body leucine metabolism in dogs indicated results that differed from those reported in the current study; thus oxidation and flux of leucine increased significantly after 180 min of nitrous oxide–halothane anaesthesia, while non-oxidative disappearance of leucine (an indicator of whole body protein synthesis) was decreased by approximately 10%. The authors also reported significant changes in plasma concentrations of amino acids 30 min after induction of anaesthesia and speculated on possible causes for the increased oxidation of leucine such as an alteration in regulatory enzymatic system or a liberation of catabolic hormones. However, these changes reported with anaesthesia appeared to be insignificant when compared with the response to surgical stimulation.

Another difference between the present study and that of Horber and colleagues [4] was the duration of anaesthesia. Their sampling and the derived calculations were started 120 min after the beginning of anaesthesia and continued subsequently for 2 h. This methodology might present some limitations when applied to human studies, and it is not clear if the duration of anaesthesia is related to the changes in oxidation of leucine. Furthermore, because the isotope and anaesthetic agents were administered at the same time, the authors could not determine the time course of the onset of this response to the administration of the anaesthetic agents in these dogs. The significant changes in whole body protein flux and synthesis observed in this study during surgery and in the immediate postoperative period were not of great magnitude. A decrease in either synthesis or flux might be the result of depressed body metabolism, as demonstrated during the ebb phase following experimental trauma in rats [23]. It is not yet known if this occurs in humans.

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