The Independent Effect of Propofol Anesthesia on Whole Body Protein Metabolism in Humans

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Background: The purpose of this study was to examine the effect of general anesthesia with propofol in the absence of surgical stimulation on whole body protein metabolism.

Methods: Six unpremedicated patients were studied. General anesthesia included propofol (120 μg·kg⁻¹·min⁻¹), vecuronium bromide, and oxygen-enriched air. Changes in protein breakdown, protein oxidation, and synthesis were measured by an isotope dilution technique using a constant infusion of the stable isotope tracer 1-[¹³C]leucine (0.008 mg·kg⁻¹·min⁻¹) before and during 100 min of propofol anesthesia. The plasma concentrations of glucose, lactate, non-esterified fatty acids, and cortisol were measured before and during anesthesia.

Results: An isotopic steady state of plasma [¹³C]ketoisocaproate (taken to represent the intracellular leucine precursor pool enrichment for protein synthesis) and expired ¹³C-carbon dioxide were obtained before and during propofol infusion. Whole body protein breakdown decreased during propofol anesthesia by 6% (P < 0.05), whereas protein synthesis and oxidation did not change significantly. Plasma concentration of cortisol decreased after 90 min of propofol anesthesia (P < 0.05). No significant changes of plasma concentrations of glucose, lactate, and non-esterified fatty acids occurred during propofol administration.

Conclusions: Propofol anesthesia did not significantly affect whole body protein synthesis and oxidation but caused a small, although significant, decrease in whole body protein breakdown, possibly mediated through the suppression of plasma cortisol concentration. (Key words: Hormones; leucine; stable isotopes.)

Surgery initiates in patients a complex series of metabolic alterations such as increased circulating plasma concentrations of essential amino acids and negative nitrogen balance.¹ These changes in nitrogen economy reflect either an increased whole body protein breakdown or decreased protein synthesis, or both. Although the impact of nutritional intervention and intensity of surgical trauma on the metabolic endocrine response to surgery has been extensively studied,²⁻⁵ little is known about the effect of anesthesia alone. Modified neuroleptanalgesia using fentanyl and midazolam has been shown to have no influence on muscle protein synthesis in humans.⁶ An increase in whole body protein breakdown and oxidation and a decrease in protein synthesis has been reported using halothane⁷ and isoflurane⁸ in dogs. Halothane anesthesia in humans has been found to reduce leucine flux and protein synthesis,⁹ whereas 1 MAC of enflurane anesthesia showed only minor effects on whole body protein metabolism.⁸ It appears from the results of these studies that some of the changes in whole body protein and nitrogen kinetics known to occur with surgical trauma may be attributable to the anesthetic agents themselves.

Propofol, an intravenous anesthetic agent with a short duration of action, has proven effective as an induction agent and for continuous intravenous maintenance of anesthesia.¹⁰ Although a large amount of data has been accumulated on the clinical use of propofol in humans, few studies have addressed the metabolic consequences of propofol administration during surgical trauma. Propofol anesthesia supplemented with opioids has been found to attenuate the hypothalamic-pituitary–adrenal response, as reflected in lower plasma cortisol and catecholamine concentrations during and after surgery, but this suppressory influence was mainly ascribed to the action of opioids used in these studies.¹¹⁻¹⁴ To interpret correctly the results from studies performed during anesthesia and surgery it is necessary to identify the separate metabolic effect of the anesthetic agent. Thus, the aim of the current investigation was to dissect the acute influence of propofol anesthesia on whole body protein metabolism and intermediary metabolites in a group of unpremedicated patients.

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Patients and Methods
The study was approved by the Hospital Ethical Committee and informed consent was obtained from all patients. Six patients (four men, two women) of mean age of 43 yr (range, 25–64 yr), mean body weight of 70 kg (range, 51–84 kg), and mean height of 170 cm (range, 154–180 cm) with non-metastatic rectosigmoid carcinoma scheduled for elective colorectal surgery were studied. None of the patients was suffering from cardiac, hepatic, renal, or metabolic disorders or receiving medication. No subject had developed recent weight loss or had a plasma albumin concentration below 40 g/l.

Study Protocol
Anesthesia. Anesthesia was induced with 2 mg/kg propofol, the trachea was intubated after administration of vecuronium bromide, 0.1 mg/kg, and the lungs were ventilated to normocapnia with oxygen-enriched air (FiO₂ = 0.55). Nitrous oxide was not used in the study because it has the same molecular weight as carbon dioxide and thus interferes with the isotope ratio measurement of expired air ¹³C-carbon dioxide. Continuous propofol infusion was maintained for 100 min at a rate of 120 μg · kg⁻¹ · min⁻¹ as it provides plasma propofol concentrations between 2 and 6 μg/ml, adequate for anaesthesia without concomitant surgery. Normal saline solution, 0.9%, was infused intravenously during the whole study period at a rate of 2 ml · kg⁻¹ · h⁻¹. The degree of muscle relaxation was measured using the train-of-four-ratio, and supplemental doses of vecuronium bromide were given to achieve complete muscular relaxation. Electrocardiography (ECG), noninvasive systemic arterial pressure, end-tidal concentrations of carbon dioxide, inspired concentration of oxygen, and oxygen saturation were monitored and recorded during anesthesia.

The patients were covered with a warming blanket to maintain normothermia. Core body temperature was measured with a thermocouple probe (Mono-atherm, Mallinckrodt Medical Inc., St. Louis, MO) inserted in the aural canal and positioned adjacent to the tympanic membrane.

Measurement of Protein Kinetics. L-[¹³C]leucine (99% ¹³C) and sodium bicarbonate (99% ¹⁵C) obtained from Cambridge Isotope Laboratories (Cambridge, MA) were prepared under sterile conditions in the hospital pharmacy. An aliquot of tracer was dissolved in a known volume of sterile water. The solution was passed through a 0.22-μm filter into injection bottles. The bottles were sealed off, heat sterilized at 121°C for 15 min, and kept at 4°C until administration. Each set of solutions was confirmed to be free of pyrogens.

All patients were studied on the day of surgery at 8:00 am after fasting 12 h overnight. A superficial vein in the dorsum of the hand was cannulated, and the cannula was kept patent with heparinized saline solution. A second superficial vein in the contralateral arm was cannulated to provide access for the infusion of L-[¹³C]leucine. Blood and expired air samples were collected before the infusion to determine baseline carbon-13 enrichment. Primed doses of ¹⁵NaHCO₃, 0.08 mg/kg, and L-[¹³C]leucine, 0.5 mg/kg, were administered and followed immediately by a continuous infusion of L-[¹³C]leucine, 0.008 mg · kg⁻¹ · min⁻¹, which was maintained for 280 min. The tracer infusion rate was determined directly by weighing the tracer initially and at the end of the study. Five blood and expired air samples were collected after 3 h of isotope infusion and before the induction of anesthesia (baseline) and toward the end of propofol anesthesia, when the tracer was assumed to have reached an isotopic steady state. A schematic representation of the procedure is shown in figure 1. Each blood sample collected before and during the isotope infusion was transferred immediately to a heparinized tube and centrifuged at 4°C. The plasma obtained was stored at −70°C until [¹³C]α-ketoisocaproate (α-KIC) enrichment was measured. Expired air samples were collected through a mouthpiece in a 2 l latex bag and transferred immediately to 20 ml vacutainers to await ¹³C-carbon dioxide isotope enrichment analysis. During artificial ventilation, expired gases were collected by means of a one-way valve into a 5 l bag. Production of carbon dioxide (VCO₂) was measured by indirect calorimetry (Datex Deltatrac, Helsinki, Finland) over a 20-min period during plasma L-[¹³C]leucine steady state before and during anesthesia.

Analytical Methods
Protein Kinetics. Whole body leucine kinetics were calculated by conventional isotope dilution practice using a two-pool stochastic model during steady state conditions obtained at each phase of the studies. Plasma enrichment of α-KIC was used as the basis for calculating flux and oxidation of leucine. Thus, under steady state conditions, leucine flux (Q) is defined by the equation:

\[ Q = S + O = B + 1 \]  \hspace{1cm} (1)

where S is the rate at which leucine is incorporated into
L-[^13]C]leucine 0.5 mg kg⁻¹
[^13]C-NaHCO₃ 0.08 mg kg⁻¹

Continuous L-[^13]C]leucine 0.008 mg kg⁻¹ min⁻¹

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<th>Baseline</th>
<th>Propofol Anesthesia</th>
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<tr>
<td>0</td>
<td>140 160 180</td>
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<td>240 260 280</td>
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Fig. 1. Time course of the infusion of isotope and collection of plasma and expired air samples for isotope enrichment analysis (O), indirect calorimetry (open box), and collection of plasma for the determination of metabolic substrates and cortisol (closed box) before and during propofol anesthesia.

body protein. O is the rate of oxidation of leucine. B is the rate at which unlabeled 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 L-[^13]C]leucine (μmol · kg⁻¹ · h⁻¹), or both. Inspection of equation (1) indicates that when studies are conducted in the postabsorptive state, flux is equal to breakdown.

Plasma α-KIC enrichment was determined by positive chemical ionization gas chromatography-mass spectrometry as previously described.¹⁶ Expired ¹³C-carbon dioxide enrichment was analyzed 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 ¹³C-carbon dioxide released by ¹³C-labeled leucine oxidation but retained within slow turnover rate pools of the body.¹³ Enrichment of plasma α-KIC during infusion of L-[^13]C]leucine has been used to determine whole body leucine kinetics. This steady state reciprocal pool model is considered to represent the intracellular precursor pool enrichment more precisely than leucine itself.¹⁷

**Plasma Metabolites and Cortisol.** Plasma concentration of glucose was measured by a glucose-oxidase method using a glucose analyzer 2 (Beckman Instruments, Fullerton, CA). Plasma lactate assay was based on lactate oxidase and was performed using the synchron CX 7 system (Beckman Instruments). The mean intraassay and interassay coefficients of variance were 3.0% and 4.5%, respectively.

Plasma concentration of non-esterified fatty acids (NEFA) was analyzed by using the Boehringer Mannheim enzymatic colorimetric kit (Boehringer Mannheim, Laval, Quebec, Canada). The mean intraassay and interassay coefficients of variance were 2.3% and 4.1%, respectively.

Circulating concentration of cortisol in plasma was measured using the Ciba Corning ACS 180 automated immunoassay (Ciba Corning Diagnostic Corp, East Walpole, MA). The mean intraassay and interassay coefficients of variance were 3.0% and 7.7%, respectively.

**Statistics**
Calculation of power and patient number was based on the results of a previous study investigating the effect of general anesthesia with enflurane and surgery on protein metabolism.⁸ Using Student paired t test and considering an expected mean change in protein flux: breakdown of 10% compared with baseline (power, 80%; α = 5.0), a total of six patients was calculated to be sufficient.

Data are presented as means (1 SD). Differences between whole body protein kinetics, i.e., protein flux: breakdown, protein oxidation and synthesis before and during anesthesia, were determined using the two-tailed Student paired t test. Analysis of variance with post hoc analysis by Student-Newman-Keuls test was used to examine within-group changes of hemodynamics and plasma concentrations of metabolic substrates and of cortisol. Statistical significance was accepted at P < 0.05.

**Results**

**Protein Kinetics**
Plateau enrichment for plasma [1-¹³C]α-KIC and expired ¹³C-carbon dioxide was achieved in all infusions
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Fig. 2. Plateau enrichment, expressed in atom percent excess (APE), of \[^{1-^{13}}\text{C}\alpha\text{-KIC}\] and \[^{13}\text{C}\text{-carbon dioxide}\] during the 280-min infusion in one patient.

before and during anesthesia, and the mean coefficient of variation was 2.74% (SD, 1.13) and 3.95% (0.94), respectively. Figure 2 illustrates the plateau enrichment of \[^{1-^{13}}\text{C}\alpha\text{-KIC}\] and \[^{13}\text{C}\text{-carbon dioxide}\] of one of the patients studied. Individual changes in protein metabolism are presented in table 1. Propofol anesthesia caused a significant decrease of whole body protein breakdown by 6% \((P < 0.05)\). In contrast, whole body leucine oxidation and protein synthesis showed no significant changes after 100 min of propofol administration.

**Hemodynamics, Metabolites, and Cortisol**

Hemodynamic parameters and plasma concentrations of intermediary substrates and cortisol are shown in table 2. No significant alterations of mean arterial pressure, heart rate, and oxygen saturation occurred during propofol infusion. Plasma concentrations of glucose and lactate also did not change significantly. The plasma concentrations of NEFA increased during propofol administration, but the changes were not significant. Plasma cortisol concentration significantly decreased during anesthesia, revealing statistical significance after 90 and 100 min of propofol infusion.

Baseline whole body carbon dioxide production at 159 ml/min (21) was not influenced by propofol (152 ml/min [28]). Mean baseline hematocrit was 39% (5) and 38% (4) during anesthesia. Baseline core temperature at 36.8°C (0.3) also remained unchanged after 100 min of propofol anesthesia (core temperature, 36.5°C [0.2]).

**Discussion**

The present study showed that propofol anesthesia in humans does not significantly affect whole body protein synthesis and leucine oxidation, although it decreases protein flux breakdown by a small but significant amount. Although the impact of anesthesia combined with surgery on perioperative protein metabolism has been widely studied, the influence of anesthetics alone has received little attention. The majority of published studies have been focused on the metabolic effects of inhalational anesthetic agents. In lung, liver, and lymphocyte preparations halothane has been shown to exhibit a dose-dependent and reversible depressive effect on fractional rate of protein synthesis. A progressive increase in protein breakdown and oxidation with increasing duration of anesthesia has been observed during isoflurane anesthesia in dogs. A report on the metabolic consequences of halothane in dogs demonstrated that leucine oxidation and flux significantly increased after 180 min of anesthesia. In contrast, 1 h of halothane anesthesia instituted after fentanyl premedication in patients undergoing cardiac surgery caused a decrease of leucine flux by 36%. More recently, 1 h of enflurane anesthesia produced no significant alterations of whole body breakdown and protein synthesis in subjects before abdominal hysterectomy.

Intravenous anesthetics have been found to initiate negligible metabolic changes in humans. Modified neuroleptanesthesia using fentanyl and midazolam in the absence of surgery did not alter the flux of free amino
acids across one leg\textsuperscript{21} and muscle protein synthesis rate in patients undergoing cholecystectomy.\textsuperscript{4}

The results of these investigations using inhalation and intravenous anesthetics indicate that the effect of anesthesia on protein metabolism is variable and depends on the anesthetic agent used and the species studied. The measurement of whole body protein turnover, protein synthesis, and oxidation gives 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 the essential amino acid leucine for protein synthesis and oxidation is that derived from the breakdown of endogenous proteins. To obtain an \textit{in vitro} estimation of whole body protein metabolism, an isotope dilution technique has been applied infusing labeled L-[\textsuperscript{13}C]-leucine.\textsuperscript{14} The flux of L-[\textsuperscript{13}C]-leucine represents the total movement of leucine into and from the plasma pool. Oxidation of leucine results in its conversion to \textsuperscript{13}C-carbon dioxide. Therefore, leucine flux minus oxidation provides, indirectly, a measure of the rate of protein synthesis.

To achieve isotopic plateau conditions during anesthesia, we infused propofol for 100 min. It has been suggested that during the execution of tracer studies, at least 2 h should elapse before any observed establishment of a new isotopic plateau is interpreted as representing a real metabolic change. Studies reporting the effects of feeding or insulin therapy on leucine kinetics,\textsuperscript{22,23} however, indicate that major metabolic events can be observed within 1 h, as reflected by changes in the tracer enrichment, although the final isotopic plateau was not obtained for approximately 2 h after the intervention. We are aware that the present results might not provide absolute proof that they truly reflect metabolic events occurring during this study. However, confidence that the results are valid is strengthened as the mean coefficient of variation of plateau values for expired \textsuperscript{13}C-carbon dioxide and plasma α-KIC during propofol anesthesia was less than 4%.

Propofol infusion caused a minimal, although significant, decrease in whole body protein breakdown by 6%. This change appears to be of no clinical significance when compared with the 30-40% changes of protein breakdown observed in patients after colorectal surgery.\textsuperscript{24,25} Whole body leucine oxidation decreased by 14% during propofol administration. Because of the large variability of data and the relatively few patients studied, this decrease in leucine oxidation did not reach stati-

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<th>Patient Number</th>
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\textsuperscript{*} P < 0.05 versus baseline.
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Table 2. Measurements of Cardiovascular Status, Oxygen Saturation (SaO₂), Plasma Concentrations of Glucose, Lactate, Nonesterified Fatty Acids (NEFA), and Cortisol in Patients before (baseline) and during Propofol Anesthesia

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<th>Baseline</th>
<th>Anesthesia 60 min</th>
<th>Anesthesia 70 min</th>
<th>Anesthesia 80 min</th>
<th>Anesthesia 90 min</th>
<th>Anesthesia 100 min</th>
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<tr>
<td>MAP (mmHg)</td>
<td>88 (11)</td>
<td>83 (8)</td>
<td>85 (12)</td>
<td>82 (12)</td>
<td>87 (10)</td>
<td>91 (6)</td>
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<td>Heart rate (beats/min)</td>
<td>86 (14)</td>
<td>84 (13)</td>
<td>87 (15)</td>
<td>85 (15)</td>
<td>82 (11)</td>
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<td>SaO₂ (%)</td>
<td>98 (1)</td>
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<td>Glucose (mm)</td>
<td>5.2 (0.7)</td>
<td>4.9 (0.7)</td>
<td>4.9 (0.6)</td>
<td>5.1 (0.6)</td>
<td>5.3 (0.8)</td>
<td>5.2 (0.6)</td>
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<tr>
<td>Lactate (mm)</td>
<td>1.2 (0.3)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>0.9 (0.2)</td>
<td>1.0 (0.2)</td>
<td>0.9 (0.3)</td>
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<tr>
<td>NEFA (μM)</td>
<td>583 (318)</td>
<td>790 (220)</td>
<td>862 (196)</td>
<td>938 (214)</td>
<td>841 (319)</td>
<td>910 (189)</td>
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<td>Cortisol (nm)</td>
<td>423 (234)</td>
<td>298 (60)</td>
<td>261 (52)</td>
<td>231 (227)</td>
<td>227 (69)*</td>
<td>189 (44)*</td>
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Data are mean (SD).

* P < 0.05 versus baseline.

The authors thank T. Nordolillo for excellent technical assistance.

References


