Intraoperative protein sparing with glucose

Schricker, Thomas, Ralph Lattermann, and Franco Carli. Intraoperative protein sparing with glucose. J Appl Physiol 99: 898–901, 2005; doi:10.1152/japplphysiol.00172.2005.—We examined the hypothesis that glucose infusion inhibits amino acid oxidation during colorectal surgery. We randomly allocated 14 patients to receive intravenous glucose at 2 mg·kg⁻¹·min⁻¹ (glucose group) starting with the surgical incision or an equivalent amount of normal saline 0.9% (control group). These were the primary endpoints. Whole body leucine oxidation; secondary endpoints were leucine rate of appearance and nonoxidative leucine disposal as determined by a stable isotope tracer technique ([1-¹³C]leucine). Circulating concentrations of glucose, lactate, insulin, glucagon, and cortisol were measured before and after 2 h of surgery. Leucine rate of appearance, an estimate of protein breakdown, and nonoxidative leucine disposal, an estimate of protein synthesis, decreased in both groups during surgery (P < 0.05). Leucine oxidation intraoperatively decreased from 13 ± 3 to 4 ± 3 μmol·kg⁻¹·h⁻¹ in the glucose group (P < 0.05 vs. control group) whereas it remained unchanged in the control group. Hyperglycemia during surgery was more pronounced in patients receiving glucose (9.7 ± 0.5 mmol/l, P < 0.05 vs. control group) than in patients receiving normal saline (7.1 ± 1.0 mmol/l). The administration of glucose caused an increase in the circulating concentration of insulin (P < 0.05) resulting in a lower glucagon/insulin quotient than in the control group (P < 0.05). Intraoperative plasma cortisol concentrations increased in both groups (P < 0.05), whereas plasma concentrations of lactate and glucagon did not change. Plasma concentrations during cholecystectomy (8) and did not improve nitrogen balance in patients undergoing craniotomy (20). Part of this discrepancy may be due to the fact that the measurement of circulating amino acids and nitrogen balances does not allow insight into the kinetics of protein metabolism, i.e., protein breakdown, oxidation, and protein synthesis and, thus, represents only indirect parameters of protein catabolism.

The present study was designed to study the effects of intravenous glucose on amino acid release, oxidation, and incorporation into protein during abdominal surgery as assessed by a stable isotope tracer technique using [1-¹³C]leucine.

MATERIALS AND METHODS

The study was approved by the local Ethics Committee and conducted in accordance with the guidelines of Helsinki. Fourteen patients undergoing elective colorectal surgery were admitted to the study, and written, informed consent was obtained from all subjects. No patient was suffering from cardiac, hepatic, renal, or metabolic disorders or receiving any medication known to affect metabolism. None of the participants had developed more than 5% weight loss over the preceding 3 mo or had a hemoglobin <10 g/dl.

All patients were prepared for surgery in a standardized fashion. Solid food intake stopped after breakfast the day before surgery and only water were allowed until 2200. For bowel cleansing, 45 ml of Fleet Phospho Soda (Merck Frost Canada) diluted with 120 ml of cool water were given twice at 1400 and 2000 on the day before the operation.

Patients were randomly assigned to receive either intravenous glucose (10% beut dextrose anhydrous in normal saline, Avebe, Foxhol, Holland) at 2 mg·kg⁻¹·min⁻¹ starting with the surgical incision or 80 ml/h normal saline. The dextrose solution was prepared by the local pharmacy under sterile conditions and was tested for sterility, stability, and absence of pyrogens before intravenous infusion. Beet dextrose was chosen because of its low ¹³C content and therefore the lack of perturbation of ¹³CO₂ enrichment in expired air (5).

General anesthesia in all patients was induced with 5 mg/kg thiopentone and 5 μg/kg fentanyl. Endotracheal intubation was facilitated with 0.6 mg/kg rocuronium, and patients’ lungs were ventilated with 30% oxygen in air to maintain normocapnia. It has to be noted that no nitrous oxide was used because it has the same molecular weight as ¹³CO₂ and, thus, would interfere with the isotope ratio measurement of expired ¹³CO₂. General anesthesia was maintained by using desflurane at end-tidal concentrations as required to keep heart rate and blood pressure within 20% of preoperative values. Phenylephrine boluses (100 μg) were given to maintain a mean arterial pressure above 60 mmHg. Supplemental doses of rocuronium were administered for complete surgical muscle relaxation. Normal saline 0.9% was infused at a rate of 6 ml·kg⁻¹·h⁻¹ to replace insensible fluid losses. Blood losses were replaced with normal saline 0.9% at a rate of 3:1. All patients were covered with a warming blanket during surgery to maintain normothermia. Hemodynamic monitoring was performed using a three-lead electrocardiogram monitor and radial artery catheterization for continuous blood pressure measurement.

Plasma kinetics of leucine were determined before and during surgery by stable isotope tracer technique using primed continuous infusions of [1-¹³C]leucine (Cambridge Isotope Laboratories, Cambridge, MA). Sterile solutions of the isotopes were prepared as previously described and were kept at 4°C until administration (6).

All patients were studied on the day of surgery between 0700 and 0800. A superficial vein of the arm was cannulated to the major objective of the nutritional management of surgical patients is to prevent the loss of functional and structural proteins to accelerate wound healing and increase resistance to infection. Parenteral provision of nutrients represents a useful therapeutic modality to achieve this goal, particularly in patients undergoing abdominal surgery with temporary intolerance to oral food intake. The protein saving properties of glucose administration after surgery appear to be well established (24). Its use during the acute phase of surgical tissue trauma, however, has produced conflicting results. Glucose infused during upper abdominal surgery decreased plasma concentrations of amino acids indicating an inhibition of whole body protein breakdown (11). In contrast, the intravenous administration of glucose did not affect plasma amino acid concentrations during cholecystectomy (8) and did not improve nitrogen balance in patients undergoing craniotomy (20). Part of this discrepancy may be due to the fact that the measurement of circulating amino acids and nitrogen balances does not allow insight into the kinetics of protein metabolism, i.e., protein breakdown, oxidation, and protein synthesis and, thus, represents only indirect parameters of protein catabolism.
provide access for the infusion of L-\([1-\text{\textsuperscript{13}C}]\)leucine. A catheter was placed in the radial artery for hemodynamic monitoring and drawing blood samples. Blood and expired air samples were then taken to determine baseline enrichments. Thereafter, priming doses of \(\text{NaH}^{13}\text{CO}_3\) 1 \(\mu\text{mol/kg}\) and L-\([1-\text{\textsuperscript{13}C}]\)leucine 4 \(\mu\text{mol/kg}\) were administered followed immediately by continuous infusions of L-\([1-\text{\textsuperscript{13}C}]\)leucine 0.06 \(\mu\text{mol-kg}^{-1}\text{-min}^{-1}\). Isotope infusion was uninterrupted throughout the entire study period. Expired breath and arterial blood samples for the determination of isotopic enrichments as well as for the measurement of metabolic substrates (glucose, lactate) and hormones (insulin, glucagon, cortisol) were collected according to Fig. 1.

Expired air samples were collected through a mouthpiece in a 3-liter bag and transferred immediately to 10-ml Vacutainers to await 13CO2 isotope enrichment analysis. During controlled ventilation, expired gases were collected by means of a one-way valve into a 5-liter bag. Each blood sample was immediately transferred to a heparinized tube and centrifuged at 4°C (3,000 g, 15 min). The plasma obtained was stored at −70°C until analysis.

Whole body carbon dioxide production (V\(\text{CO}_2\)) was measured by indirect calorimetry before and during surgery by using the open-system indirect calorimetry device Deltatrac Metabolic Monitor (Deltatrat Instrumentarium, Helsinki, Finland). V\(\text{CO}_2\) was also measured during surgery (70 min after skin incision). The values of V\(\text{CO}_2\) represent an average of the data obtained over a period of 20 min on each occasion, with a coefficient of variation <10%.

Plasma \(\alpha-\text{[1-\text{\textsuperscript{13}C}]KIC}\) enrichment was determined by electron-impact selected-ion monitoring gas chromatography-mass spectrometry (19), except that \(t\)-butyldimethylsilyl rather than trimethylsilyl derivatives was used. Expired 13CO2 enrichment for the calculation of leucine oxidation was determined by isotope ratio mass spectrometry (Analytical Precision AP2, 003, Manchester, UK).

Plasma glucose concentrations were measured with a glucose analyzer 2 (Beckman Instruments, Fullerton, CA) based on a glucose oxidase method. The plasma lactate assay was based on lactate oxidase by using the synchron CX 7 system (Beckman Instruments, Fullerton, CA). Cortisol, insulin, and glucagon plasma concentrations were measured by a double antibody radioimmunoassay (Amersham International, Amersham, Bucks, UK).

Under steady-state conditions, the rate of appearance (Ra) of unlabeled substrate in plasma can be derived from the plasma enrichment (atom percent excess = APE) calculated by Ra = 1/APEinf/APEst - 1, where APEinf is the tracer enrichment in the infusate, APEst is the tracer enrichment in plasma at steady state, and I is the infusion rate of the labeled tracer. The APE values used in this calculation represent the mean of the APE values determined during each isotopic plateau. Steady-state conditions were assumed when the coefficient of variation of the APE values at isotopic plateau was <5%. In a steady state, leucine flux is defined by the formula Q = S + O = B + I, where S represents the rate of leucine uptake for protein synthesis, O is the rate of oxidation of leucine, B is the rate of leucine derived from endogenous protein breakdown, and I is the rate at which leucine is entering the free pool from dietary intake. When studies are conducted in the postabsorptive state, flux is equal to breakdown. The difference between the leucine Ra and oxidation represents nonoxidative leucine disposal, a measure of leucine incorporation into protein and, thus, whole body protein synthesis.

Plasma \(\alpha-\text{[1-\text{\textsuperscript{13}C}]KIC}\) enrichment was used for calculating both flux and oxidation of leucine. The steady-state reciprocal pool model represents the intracellular precursor pool enrichment more precisely than leucine itself (21). In the calculation of leucine oxidation, correction factors of 0.76 for the fasting state and 0.899 during glucose infusion were used to account for the fraction of 13CO2 released from 13C-labeled leucine oxidation but retained within slow turnover rate pools of the body (9, 22). The average baseline 13CO2 enrichment in the control group was used for the calculations in both the control and glucose group.

For metabolic substrates and hormones, the average of the two intraoperative measurements at 80 and 120 min after skin incision was calculated.

Statistics. Differences between and within groups were analyzed by two-way analysis of variance and post hoc test by Student-Newman-Keuls test. A probability of \(P < 0.05\) was considered to be significant. Data are presented as means ± SD.

RESULTS

There were no differences between the two groups regarding age, height, weight, and gender (Table 1). The estimated blood loss was minimal, and the amount of normal saline 0.9% administered during the study period was comparable in both groups.

In all patients, isotopic plateau of \(\alpha-\text{[1-\text{\textsuperscript{13}C}]KIC}\) and expired 13CO2 was achieved, allowing steady-state calculations (Fig.

Table 1. Biometric and clinical data of patients

<table>
<thead>
<tr>
<th>Control</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>60±17</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163±9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64±15</td>
</tr>
</tbody>
</table>
| Gender, male:female | 2:5    | 3:4 | 1
| Surgery, n | Hemicolectomy | 1 | 1 |
| | Anterior resection | 6 | 6 |
| Estimated blood loss, ml | 179±86 | 243±79 |

Values are means ± SD; n, number of patients.
2. Leucine Ra decreased to a similar extent during surgery in both groups (P < 0.05, Table 2). Leucine oxidation decreased during surgery in the glucose group (P < 0.05) resulting in a lower intraoperative leucine oxidation rate compared with the control group (P < 0.05, Table 2). Nonoxidative leucine disposal decreased in both groups during surgery (P < 0.05, Table 2). The leucine oxidation-to-leucine Ra ratio and the leucine oxidation-to-nonoxidative leucine disposal ratio intraoperatively decreased in the glucose group only (P < 0.05, Table 2). VCO₂ did not change intraoperatively in either group (Table 3). In the glucose group, the circulating insulin concentration increased during surgery (P < 0.05, Table 3). This increase was more pronounced during glucose administration (P < 0.05).

Circulating concentrations of lactate and glucagon remained unaltered (Table 3). In the glucose group the circulating insulin concentration increased during surgery (P < 0.05), resulting in a lower glucagon/insulin ratio than in the control group (P < 0.05). The intraoperative increase of the circulating cortisol concentration (P < 0.05) was comparable in both groups.

The results of this study demonstrate that the administration of glucose at 2 mg·kg⁻¹·min⁻¹ decreases the oxidative loss of protein during colorectal surgery as reflected by a 60% suppression of leucine oxidation.

Only few studies investigated the effect of glucose on intraoperative protein catabolism with conflicting results. Glucose administered at 3 mg·kg⁻¹·min⁻¹ during gastrectomy decreased the circulating concentrations of branched-chain amino acids without demonstrating any modifying influence during cholecystectomy (8, 11). In patients undergoing craniotomy the administration of glucose did not affect perioperative nitrogen balance (20). These discrepancies may be due to the fact that different types of surgical tissue trauma were studied. Methodological limitations, however, may also be responsible. The measurement of circulating concentrations of substrates provides no information about the underlying dynamic metabolic processes. Lower amino acid concentrations can be a result of decreased muscle proteinolysis and amino acid release, an increased uptake and incorporation into new proteins, or a combination of the two events. Urinary nitrogen measurements reflect only net gain or loss of protein from the body without elucidating the mechanisms how protein economy is maintained. For example, a negative nitrogen balance can occur if protein breakdown and amino acid oxidation increase and protein synthesis remains the same, or if protein breakdown and oxidation remain unchanged and the rate of protein synthesis decreases. These alterations in whole body protein breakdown and amino acid oxidation can be quantified and separated by using essential amino acids such as leucine that are labeled with stable isotopes such as ¹³C (9, 19).

In agreement with studies showing depressed whole body protein metabolism during colorectal (18), cardiac (16), and pelvic procedures (4), the leucine Ra and nonoxidative leucine disposal decreased although patients received glucose or not. In contrast to the recent observation of a 20% reduction of amino acid oxidation during abdominal surgery in the absence of energy supply, leucine oxidation in the control group did not significantly change (18). This discrepancy may be explained by the fact that patients in the former study received a remifentanil-based anesthetic. Intravenous anesthesia using continuous infusions of opioids such as remifentanil or sufentanil has been shown to inhibit the catabolic responses to surgery to a greater extent than inhaled anesthesia as used in the present protocol (2, 17, 18).

Table 2. Leucine kinetics

<table>
<thead>
<tr>
<th>Control</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before surgery</td>
</tr>
<tr>
<td>Leucine Ra, μmol·kg⁻¹·h⁻¹</td>
<td>100±16</td>
</tr>
<tr>
<td>Leucine oxidation, μmol·kg⁻¹·h⁻¹</td>
<td>13±3</td>
</tr>
<tr>
<td>Nonoxidative leucine disposal, μmol·kg⁻¹·h⁻¹</td>
<td>87±13</td>
</tr>
<tr>
<td>Leucine oxidation/Leucine Ra, %</td>
<td>13±2</td>
</tr>
<tr>
<td>Leucine oxidation/Nonoxidative leucine disposal, %</td>
<td>15±2</td>
</tr>
</tbody>
</table>

Values are means ± SD.Ra, rate of appearance. *P < 0.05 vs. before surgery; †P < 0.05 vs. control group.

Table 3. Circulating concentrations of metabolic substrates and hormones

<table>
<thead>
<tr>
<th></th>
<th>Control Before surgery</th>
<th>During surgery</th>
<th>Glucose Before surgery</th>
<th>During surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>4.8±0.8</td>
<td>7.1±1.0*</td>
<td>4.7±0.8</td>
<td>9.7±0.5†</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.9±0.2</td>
<td>0.7±0.3</td>
<td>1.0±0.3</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>65±25</td>
<td>71±18</td>
<td>54±11</td>
<td>94±43*</td>
</tr>
<tr>
<td>Glucagon, pmol/l</td>
<td>16±3</td>
<td>16±8</td>
<td>16±3</td>
<td>14±6</td>
</tr>
<tr>
<td>Glucagon/insulin, %</td>
<td>27±8</td>
<td>24±7</td>
<td>28±9</td>
<td>16±7*†</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>299±142</td>
<td>864±122*</td>
<td>345±267</td>
<td>890±160*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. before surgery; †P < 0.05 vs. control group.
Although glucose was infused at a low dose, plasma glucose concentrations increased in the glucose group to almost 10 mmol/l, the concentration threshold for glucose excretion. Considering the clinical consequences of acute hyperglycemia, including immunosuppression (7, 13), electrolyte imbalances (3), and increased mortality (15, 23), this hyperglycemic response might gain clinical importance.

Although the present study design does not permit identification of specific mechanisms responsible for the inhibition of protein oxidation by glucose, it seems plausible that the increase in the circulating insulin concentration was a contributing factor (12). It has to be noted, however, that inhibitory effects of insulin on protein metabolism have been reported in nonsurgical patients with plasma levels greater than those observed in the present protocol (14). Low-dose glucose administration causing a doubling of insulin concentration (similar to the insulin response in the present study) failed to exert any anticatabolic effects in patients who were studied several days after surgery (6).

In summary, the intravenous provision of small amounts of glucose was associated with a decrease in amino acid oxidation during colorectal surgery. Because glucose infused during the acute period of surgical stress caused severe hyperglycemia, it seems questionable whether patients will benefit clinically from the protein-sparing action of intraoperative energy supply.

ACKNOWLEDGMENTS

We gratefully acknowledge Drs. Paul Beliveau and Judith Trudel for permission to study their patients.

R. Latterrmann is now working at the Department of Anesthesia, Regensburg University, Germany.

GRANTS

The study was supported by Funds provided by the Fonds de la recherche en sante du Quebec (to T. Schricker), and the Research Institute of the McGill University Health Center, Montreal, Canada (to T. Schricker).

REFERENCES