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Albumin Synthesis Rates Are Not Responsive to Hyperglycemic Hyperinsulinemia in Postoperative Patients

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Background: Insulin regulates albumin synthesis in vitro and in various experimental models. The current study was undertaken to determine the effects of a physiologic hyperinsulinemia on albumin synthesis in postoperative patients in whom plasma albumin concentrations are decreased. *Methods:* Studies were performed in postabsorptive patients after major abdominal operations. Mass spectrometry techniques were used to directly determine the incorporation rate of 1-[^{13}C]-leucine into albumin. Consecutive blood samples were taken during a continuous isotope (D-Glc) infusion (0.16 μmol/kg/min). Isotopic enrichments were determined at baseline (period I) and after a 4-hour D-glucose (D-Glc) infusion at currently recommended rates (170 mg/kg/h, n = 10) or after infusion of saline (control group, n = 8) (period II). *Results:* After D-Glc infusion, plasma insulin concentrations increased significantly (period I, 6.6 ± 1.8 μU/mL; period II, 21.4 ± 2.1 μU/mL; $P < .01$). In contrast, plasma insulin concentration remained constant in control patients (period I, 3.8 ± 0.9 μU/mL^{-1}; period II, 5.9 ± 1.1 μU/mL; not significant vs period I, but $P < .005$ vs the corresponding value at the end of period II in the control group). Hyperinsulinemia was without effect on fractional albumin synthesis (period I, 12.8% ± 1.9%/d; period II, 11.9% ± 1.9%/d; not significant), and synthesis rates corresponded to those measured in controls (period I, 13.0% ± 1.2%/d; period II, 12.1% ± 0.1%/d; not significant vs period I and vs D-Glc infusion). *Conclusions:* A standard D-Glc infusion is insufficient to increase albumin synthesis in postoperative patients. (*JPEN J Parenter Enteral Nutr*. 2011;35:405-411)

Keywords: albumin; protein synthesis; stable isotopes; glucose; operation

Clinical Relevancy Statement

The finding that a carbohydrate infusion at currently recommended rates (170 mg/kg/h) was unable to stimulate albumin synthesis in postoperative patients emphasizes the resistance of albumin metabolism toward parenteral nutrition measures. In healthy patients, albumin synthesis may be only stimulated by markedly elevated insulin concentrations (at least 3 to 4 times higher than the postabsorptive level), which, however, cannot be achieved by currently recommended carbohydrate infusion rates and may be still ineffective because of an altered insulin dose/response kinetics during conditions of postoperative stress.

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Introduction

Insulin rapidly stimulates protein synthesis by activating components of the translational machinery, factors including eukaryotic initiation factors and elongation factors. In the long term, insulin also increases the cellular

content of ribosomes to augment the capacity for protein synthesis.[1] Insulin regulates the expression of more than 150 genes, indicating that this is a major action of this hormone.[2] In primary cultures of rat hepatocytes, insulin was found to increase albumin gene expression, albumin messenger RNA (mRNA) content, and albumin synthesis in a dose-dependent manner.[3,4] Correspondingly, experimental insulin deficiency was associated with decreased albumin gene transcription, mRNA content, and synthesis, and these decreases could be prevented by maintaining animals on insulin therapy in these animals.[5-7] Comparable results could be obtained in diabetic humans in whom insulin withdrawal reduced albumin synthesis and insulin replacement normalized this rate again.[8]

In the present study, we examined the effect of mild hyperinsulinemia on albumin synthesis in postoperative patients in whom plasma albumin concentrations are decreased and in whom postabsorptive albumin synthetic rates (ASRs) are insufficient to rapidly correct the postoperative albumin deficit.[9] To produce physiologic hyperinsulinemia, patients received an experimental parenteral infusion of D-Glc, which was given at currently recommended rates. Studies were performed in patients who were recovering from major abdominal surgical procedures. During the postoperative period, the systemic catabolic response is known to peak. At this stage, these patients may serve as an ideal model to examine the effects of hepatic insulin during metabolic stress.

Methods

Patients

Two groups of postoperative patients (control n = 8; D-Glc infusion group, n = 10) with rectal carcinoma were carefully screened via medical history, physical examinations, and routine blood tests. All patients underwent a curative, low anterior resection. The groups were comparable (mean ± SEM) with respect to age (control, 64.8 ± 3.9 years; D-Glc 64.0 ± 2.9 years), body mass index (control, 24.9 ± 1.0 kg/ cm; 24.1 ± 0.6 kg/cm^2), and postoperative tumor stage (Dukes B). No patient (before operation) and no control patient had a history of metabolic diseases or other clinically important comorbidity. Malnutrition before operation was excluded by a stable body weight during the preceding 2 months, albumin concentrations in the normal range, and subjective global assessment.

All patients had comparable perioperative management, including preoperative bowel preparation (orthograde flushing by oral colonoscopy fluids) and prophylactic intravenous antibiotics perioperatively. Anesthesia included epidural application of analgetic and anesthetic drugs continued until postoperative day (POD) 5. Duration of operation, amount of blood loss, fluid replacement, and extent of the procedure were comparable. All patients had an uneventful postoperative course and were free from signs of organ malfunction and local or systemic infection at the time of the study.

Patients were studied between PODs 8 and 10, when body protein loss and depression of muscle protein synthesis were found to be maximal.[10,11] According to clinical and biochemical examinations, symptoms of systemic inflammatory response syndrome (SIRS), including fever and leukocytos, but also perioperative volume shifts had abated at that time, and all patients were in a similar phase of postoperative recovery. Fluid balance was equilibrated and combined with a normal hydration state. A portion of the data (ASRs and amino acid concentrations of the 8 control patients) were presented in a previous publication.[12]

Informed consent was obtained after the experimental protocol had been explained in detail. The study was approved by the local institutional review board.

Experimental Protocol

All patients were inpatients of the general surgical service. Patients received approximately 15 kcal/kg/d on PODs 1–3 and then 25 kcal kg/d until the day of the study. If spontaneous oral intake was insufficient, missing amounts of calories or protein were given parenterally. During the days before the study, calorie and protein/amino acid supplies were comparable between groups. After 10 PM, all patients were fasted, except for the consumption of mineral water. A primed, constant infusion of 1-^{13}C-leucine (Tracer Technologies, Sommerville, MA, 99.3 atom percent enrichment) was started at 7 AM the next day and continued for 10 hours. The infusion rate was 0.16 μmol/kg min (prime 9.6 μmol/kg^{-1}). A blood sample was collected before isotope infusion to determine the background enrichment of albumin-bound leucine and plasma ketoisocaproic acid (KIC). In all patients, the first arterialized blood sample was taken after 180 minutes of isotope infusion, the second after 360 minutes, and the third after 600 minutes. Period I ranged from minutes 180 to 360, and period II from minutes 360 to 600. Arterialized samples were obtained from a heated dorsal hand vein. For heating, the patients placed their nondominant hand in a warm box containing air at 60°C–70°C for a 20-minute period.[13]

Between minutes 360 and 600 (after the second sample, period II), patients in the D-Glc infusion group received a continuous D-Glc infusion (170 mg/kg/h). Patients in the control group remained in a fasting state (saline infusion). Arterialized blood samples for measurement of glucose and insulin concentrations were taken at the same time as blood samples for measurement of albumin synthesis.

Procedures

The principles of the methods and the generation of the data have been presented and discussed in detail in

previous publications.[9,14,15] Plasma KIC and albumin-bound leucine in plasma samples were separated by precipitation of the proteins.

Albumin was isolated by a highly specific separation method (ethanol extraction with subsequent electrophoretic purification). For capillary gas chromatography (GC)/combustion isotope ratio mass spectrometry (IRMS) analysis, amino acids from albumin were then converted to the N-acetyl n-propyl (NAP) ester. For GC/quadrupole mass spectrometry (GC/MS) analysis, we prepared the quinoxalinol-trimethylsilyl (TMS) derivative from KIC. NAP-amino acid derivatives were analyzed in a capillary GC/combustion IRMS system that consisted of a Hewlett-Packard 5890 Series II gas chromatograph (Böblingen, Germany) that was interfaced to a mass spectrometer Delta S (Finnigan MAT, Bremen, Germany). Quinoxalinol TMS derivatives were analyzed by a GC/MS system (MSD 5971D; Hewlett-Packard). Isotopomer ratios of the sample were obtained by electron impact ionization and selected ion monitoring at mass-to-charge ratios (m/e) 259 and 260. Data were expressed as tracer/tracee ratios z (leucine and KIC enrichment).

Albumin concentrations were measured on samples taken before leucine administration with the bromocresol green method. Plasma amino acid levels were measured by an autoanalyzer (Beckman Instruments, Fulton, CA) in all controls and in 5 patients receiving infusions of D-Glc.

Calculations

Albumin fractional synthetic rates (FSRs) in the metabolic steady state were calculated by dividing the increment in albumin-bound 1-[^{13}C]-leucine tracer/tracee ratio by the enrichment of the precursor pool enrichment.[16] As a substitute for the true precursor pool enrichment (transfer RNA–bound amino acid enrichment), we used the plasma [^{13}C]-KIC tracer/tracee ratio for albumin synthesis.[17] Delta increments of albumin-bound 1-[^{13}C]-leucine enrichment between samples were calculated from isotope ratios [^{13}C]/[^{12}C] using a correction factor that takes into account dilution of the label at the carboxyl position by the other carbon atoms in the derivatized NAP-leucine.[14] FSRs were then calculated as follows:

$$FSR=\frac{z_L(t_{i+1})-z_L(t_i)}{\{z_p(t_i)+z_p(t_{i+1})\}/2\Delta t}*\frac{1}{-}*60*100*24 \quad (\%/d)$$

where $z_L(t_i)$ and z_L (t_{i+1}) correspond to the tracer/tracee ratio of albumin-bound leucine in 2 subsequent samples (i and i+1), separated by the time interval Δt (minutes). z_P (t_i) and z_P (t_{i+1}) indicate the tracer/tracee ratios of plasma KIC in 2 subsequent samples. Average values between z_P (t_i) and z_P (t_{i+1}) were used as precursor enrichments for protein synthesis. The factors 60 (minutes), 100, and 24 are needed to express the FSR in %/d.

Table 1. Tracer/Tracee Ratios of 1-^{13}C-Ketoisocaproic Acid in Plasma

Group	Sample 1	Sample 2	Sample 3
Control	5.10 ± 0.40	5.62 ± 0.38	5.85 ± 0.29[a]
D-Glc infusion	5.75 ± 0.54	6.14 ± 0.50	5.88 ± 0.56

Values are given as % (mean ± standard error of the mean). Sample 1 was taken after 180 minutes, sample 2 after 360 minutes, and sample 3 after 600 minutes of isotope infusion. Patients in the D-Glc infusion group received 170 mg/kg/h of glucose between sample 2 and 3. Control patients continued to fast throughout the study.

[a]$P < .05$ vs sample 1.

Patients in the control group demonstrated a significant increase in the plasma KIC enrichment during the 10-hour isotope infusion (Table 1), possibly indicating a non–steady-state situation. However, Equation 1 can still be used, if piecewise constant functions between pairs of consecutive sampling points can be identified.[16] Since the plasma KIC enrichment did not differ significantly between samples taken at 180 and 360 minutes and at 360 and 600 minutes, respectively, one may assume a steady-state–like condition between sampling points 1 and 2 and between sampling points 2 and 3, justifying the use of Equation 1 to calculate FSR separately for those 2 study intervals.

The absolute ASRs, that is, the total amount synthesized per day (mg/kg/d), was measured by multiplying the FSR by the intravascular albumin mass and normalized for body weight. Intravascular albumin mass was assessed from the plasma albumin concentration and plasma volume, which was predicted from sex, age, and weight by using a nomogram.[18]

Statistics

Data are expressed as mean ± standard error of the mean. Albumin, glucose, and insulin concentrations and albumin protein synthetic rates from patients receiving parenteral D-Glc and from control patients were compared by the unpaired *t* test. The paired *t* test was used to compare albumin protein synthetic rates between periods I and II. A *P* value of ≤.05 was taken as indicating a significant difference.

Results

Plasma glucose and insulin concentrations rose significantly during D-Glc infusion (glucose, end of period II: 158.0 ± 3.8 mg/dL, $P < .05$ vs 102.2 ± 2.1 mg/dL at the end of period I; insulin, end of period II 21.4 ± 2.1 µU/mL, $P < .05$ vs 6.6 ± 1.8 µU/mL at the end of period I)

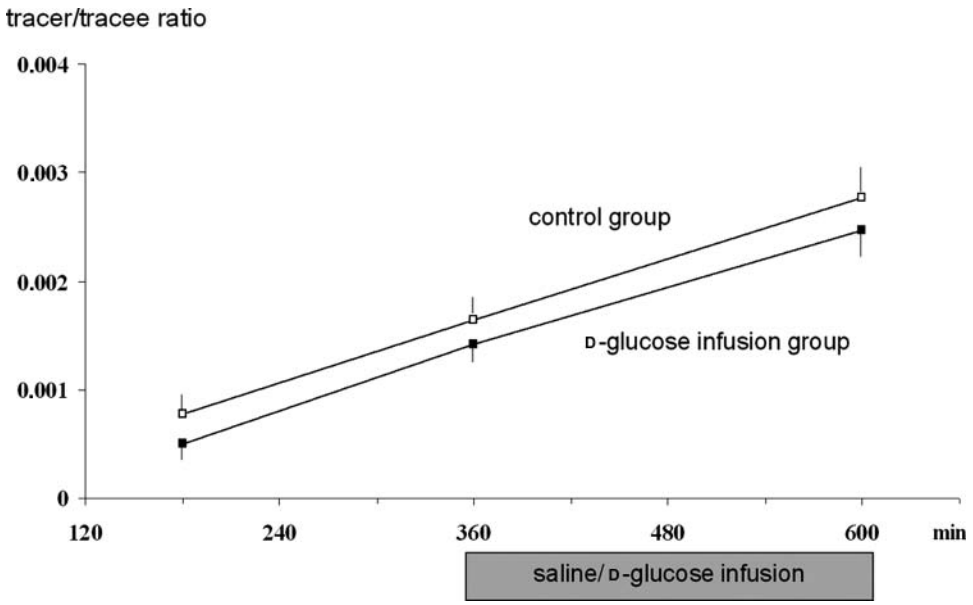

Figure 1. Time curves of tracer incorporation into serum albumin (tracer/trace ratio) in control patients and patients receiving D-Glc infusion. Patients in the D-Glc infusion group (solid squares) received 170 mg/kg/h of glucose between minutes 360 and 600 of tracer infusion. Control patients (open squares) fasted throughout the study. Values are given as mean ± standard error of the mean.

whereas plasma glucose and insulin concentrations remained unchanged in control patients (glucose, end of period II: 96.3 ± 2.0 mg/dL, not significant vs 103.5 ± 3.8 mg/dL at the end of period I; insulin, end of period II 5.9 ± 1.1 μU/mL, not significant vs 3.8 ± 0.9 μU/mL at the end of period I) resulting in a significant difference of serum insulin and plasma glucose concentrations between controls and patients receiving infusion of D-Glc at the end of period II ($P < .05$).

Total amino acid concentration remained unchanged in control patients (end of period II: 2,921 ± 86 μmol/L, not significant vs 2,871 ± 83 μmol/L at the end of period I) and in those patients in whom amino acid concentrations were measured before and at the end of the carbohydrate–glucose infusion (end of period II: 2,770 ± 181 μmol/L, not significant vs 2,700 ± 216 μmol/L at the end of period I).

Baseline serum albumin concentrations were comparable between control patients and patients receiving D-Glc (3.7 ± 0.2 g/dL, not significant vs 4.6 ± 0.4 g/dl in controls). Correspondingly, patients in both groups demonstrated baseline albumin FSRs that were comparable (13.0% ± 1.2%/d and 12.8 ± 1.9%/d, not significant).

Analysis of tracer data showed that only in the D-Glc infusion group did the tracer/tracee ratio in the plasma KIC pool (= precursor pool for albumin synthesis) not change significantly over time (Table 1). In control patients, the precursor pool enrichment rose significantly over time. Thus, the enrichment in sample 3 (600 minutes of isotope infusion) was significantly higher than the corresponding enrichment in sample 1 (180 minutes of isotope infusion).

Figure 1 shows the values of albumin-bound leucine enrichment and the time curve of tracer incorporation into serum albumin in the D-Glc infusion and control patients studied. In both groups, tracer incorporation over time was comparable and linear. Corresponding to the linear tracer incorporation, also FSR of albumin remained stable during the 10-hour study. Thus, in patients in D-Glc infusion group, albumin synthesis was 12.8% ± 1.9%/d in period I and 11.9% ± 1.9%/d in period II (not significant vs period I). Also in control patients, we observed comparable FSR of albumin in period I (13.0% ± 1.2%/d) and in period II (12.1% ± 0.1%/d, not significant vs period I). Similar results were obtained when absolute ASRs were calculated in both groups (Figure 2).

Discussion

This study shows that in patients who underwent a major abdominal surgical procedure, mild hyperinsulinemia/hyperglycemia had no effect on albumin synthesis. Thus, after an increase of insulin concentrations from about 7 to 21 μU/mL, FSRs and ASRs remained unchanged.

Insulin is commonly believed to be an important determinant of human albumin production, since in diabetic patients, insulin withdrawal and replacement directly correlate with albumin synthesis.[8] In contrast, it

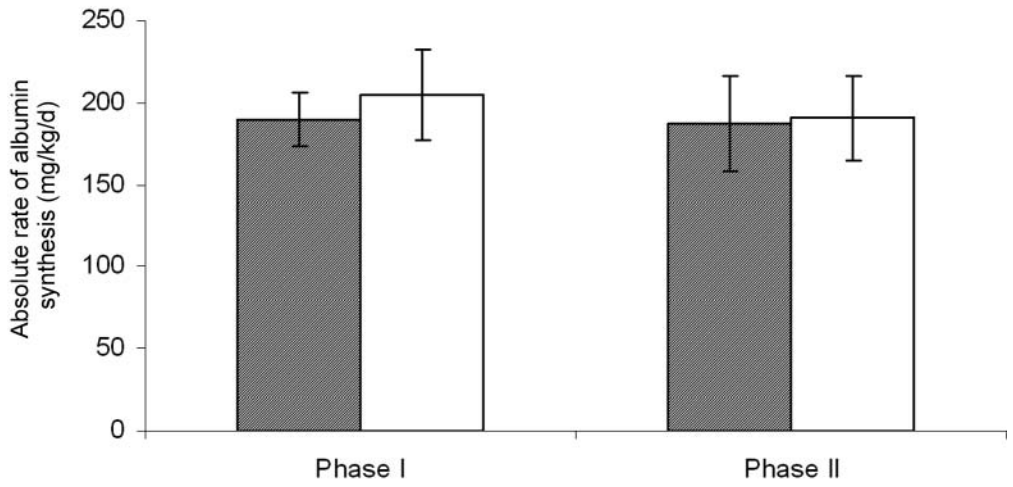

Figure 2. Absolute rates of serum albumin synthesis in control patients and patients receiving D-Glc infusion. Period I ranged from minutes 180 to minutes 360 and period II from minutes 360 to minutes 600 of tracer infusion. Patients in the D-Glc infusion group (hatched bars) received 170 mg/kg/h of D-Glc between minutes 360 and 600 (period II). Control patients (open bars) fasted throughout the study. Values are given as mean ± standard error of the mean.

is controversial whether supranormal insulin concentrations stimulate synthetic rates of splanchnic or global hepatic protein or of albumin. This controversy largely results from methodological restrictions in specific experiments and from the ignorance of an in vivo dose response effect of insulin.

Experiments in which exogenous insulin was infused to test allover anabolic effects at the site of the liver are compromised by 2 confounding factors. First, high insulin concentrations are known to inhibit peripheral amino acid release, with a subsequent reduction of visceral amino acid supply. Therefore, the associated hypoaminoacidemia may explain why in dogs and humans, various degrees of hyperinsulinemia had no significant effect on splanchnic amino acid balances.[19-21] Furthermore, when regional catheter techniques were combined with isotopic tracer infusion, studies in healthy humans revealed that profound hyperinsulinemia was associated even with a decrease in splanchnic protein synthesis.[22]

The second drawback relates to the study design. Many studies examined only global splanchnic protein synthesis during insulin infusion. However, interpretation of those global rates is difficult, since insulin might have differential and opposite effects on the synthesis of individual proteins. In fact, estimates of splanchnic protein synthesis reflect the mean synthetic rate of a mixture of proteins with different turnover rates. Thus, it is possible that the stimulatory effect of insulin on the synthesis of some proteins could be obscured by the concomitant inhibition of the synthesis of others with faster turnover rates and/or greater pool sizes. Such a hypothesis is supported by in vitro data indicating that insulin differentially regulates the production of specific mRNAs[23-25] and by data obtained in humans demonstrating that short-term variations in insulin availability have opposite effects on the synthesis of several hepatic proteins such as albumin, fibrinogen, or antithrombin.[8,26-28] Consequently, the fact that hyperinsulinemia did not increase splanchnic protein synthesis in healthy patients even when plasma amino acid concentrations were simultaneously maintained[29] would not exclude stimulatory effects on specific proteins such as albumin.

In our study, hepatic albumin synthesis did not change significantly during mild hyperinsulinemia. It can be excluded that the duration of D-Glc infusion (4 hours) was insufficient to allow detection of a stimulatory effect. In humans, albumin synthesis was found to rise as soon as 30 minutes after an appropriate stimulatory trigger (elevated hepatic amino acid exposure through the portal vein).[30] It is also unlikely that an increased ASR that was already present before the D-Glc infusion prevented a subsequent rise. We have recently reported that postabsorptive albumin synthesis is accelerated in the postoperative state.[9] However, even with such elevated basal rates, it could be shown that an additional parenteral substrate supply may further stimulate albumin production, for example, during hemodialysis.[31] Finally, it can be excluded that a simultaneous fall in arterial amino acid concentration counteracted a possible stimulatory effect of insulin on albumin synthesis. The degree of hyperinsulinemia in our study was low enough not to interfere with plasma amino acid concentrations. Also, the accompanying hyperglycemia can be excluded as a potential

reason for the unaltered albumin synthesis. Hyperglycemia in combination with constant insulin concentrations does not interfere with human protein metabolism, leaving plasma leucine concentration or appearance rate unaltered,[32,33] and variations in carbohydrate concentration are usually not regarded as effectors of changes in protein metabolism observed during hyperinsulinemic hyperglycemia.[34] Consequently, other reasons must exist to explain the absent effect of insulin on albumin synthesis. A thorough analysis of the available literature reveals a possible insulin dose/response relationship that may, in addition, depend on the species under investigation.

It appears that the physiologic elevations of plasma insulin concentration observed during standard enteral or parenteral carbohydrate supply do not significantly alter albumin production in humans. Thus, a carbohydrate-based oral diet was found to increase plasma insulin concentrations 2 to 3 times above basal values but was without effect on FSRs or ASRs compared with a fat-based diet.[35] A similar rise in plasma insulin concentration was reported by Ballmer et al,[36] who administered parenteral carbohydrates at a rate of about 170 mg/kg/h in healthy humans. Using the flooding dose technique, these authors were also unable to identify a relevant change in albumin synthesis. Although the flooding dose technique may intrinsically interfere with the measurement of albumin synthesis,[37] the results by Ballmer et al[36] are well compatible with our negative results, which were obtained by a different tracer technique (continuous tracer infusion).

Only insulin concentrations that were 3 to 4 times higher than basal and that could be only produced by exogenous insulin infusion (0.4 mU/kg/min) increased albumin synthesis significantly. However, with those infusion rates and insulin concentrations, arterial amino acid levels may already start to decline.[26] Therefore, the insulin effect on albumin synthesis is not linear. Pacy et al[38] infused almost twice as much insulin (0.7 mU/kg/min) but did not observe significant changes of albumin synthesis. The most likely explanation for these negative results can be found in the associated decrease of plasma amino acid concentrations, which was more pronounced than with lower insulin infusion rates, thereby simultaneously blunting the stimulatory effect of insulin on albumin synthesis.[38] However, with simultaneous amino acid replacement, albumin synthesis can be accelerated in humans, if sufficient insulin (1.0–2.0 mU/kh/min) or carbohydrates (250 mg/kg/h) are being infused.[27,28,31] Interestingly, the insulin effect appears to be species-specific. Thus, insulin infusion rates between 0.7 and 0.8 mU/kg/min did not increase ASRs in pigs, even in the presence of supranormal amino acid concentrations.[17,39]

It is also currently unknown whether the insulin dose/response kinetics in healthy patients correspond to those found during catabolic stress. Thus, with respect to the stimulation of albumin synthesis, the half maximal insulin concentration might be significantly higher in surgical patients than in controls, and maximum insulin effects on albumin synthesis may depend on the underlying disease. It is therefore uncertain whether the stimulatory effects of large amounts of insulin together with sufficient amino acids[27,28,31] could be reproduced in postoperative patients.

Nevertheless, the available evidence clearly points to a concentration-dependent effect of insulin on human albumin synthesis. At least in healthy patients, a stimulatory effect will only become evident when peripheral insulin concentrations rise at least 3-fold to 4-fold above the postabsorptive level and if euaminoacidemia is simultaneously maintained. However, such markedly elevated insulin concentrations cannot be achieved by currently recommended carbohydrate infusion rates (200–250 g/d)[40] but would require the additional infusion of exogenous insulin.

In conclusion, our results demonstrate that a standard D-Glc infusion is insufficient to increase albumin synthesis in postoperative patients.

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