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Research Article

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Differential Effects of Insulin Deficiency on Albumin and Fibrinogen Synthesis in Humans

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Abstract

Insulin deficiency decreases tissue protein synthesis, albumin mRNA concentration, and albumin synthesis in rats. In contrast, insulin deficiency does not change, or, paradoxically, increases estimates of whole body protein synthesis in humans. To determine if such estimates of whole body protein synthesis could obscure potential differential effects of insulin on the synthetic rates of individual proteins, we determined whole body protein synthesis and albumin and fibrinogen fractional synthetic rates using 5-h simultaneous infusions of [14C]leucine and [13C]bicarbonate, in six type 1 diabetics during a continuous i.v. insulin infusion (to maintain euglycemia) and after short-term insulin withdrawal (12±2 h).

Insulin withdrawal increased ($P < 0.03$) whole body proteolysis by $\approx$ 35% and leucine oxidation by ~100%, but did not change $^{13}CO_2$ recovery from $NaH^{13}CO_3$ or estimates of whole body protein synthesis ($P = 0.21$). Insulin deficiency was associated with a 29% decrease ($P < 0.03$) in the albumin fractional synthetic rate but a 50% increase ($P < 0.03$) in that of fibrinogen.

These data provide strong evidence that albumin synthesis in humans is an insulin-sensitive process, a conclusion consistent with observations in rats. The increase in fibrinogen synthesis during insulin deficiency most likely reflects an acute phase protein response due to metabolic stress. These data suggest that the absence of changes in whole body protein synthesis after insulin withdrawal is the result of the summation of differential effects of insulin deficiency on the synthesis of specific body proteins. (*J. Clin. Invest.* 1991. 88:833–840.) Key words: leucine • KIC • amino acids • diabetes • protein metabolism

Introduction

Insulin exposure to rat tissues both in vitro and in vivo decreases in vitro estimates of proteolysis and stimulates in vitro estimates of protein synthesis (1–9). In humans, insulin infusion, in the absence of an exogenous source of amino acids, decreases the plasma concentrations of most essential amino acids (10) and whole body proteolysis (as assessed by decreased rates of leucine appearance) (11, 12), but does not increase whole-body protein synthesis (as reflected by decreased rates of

nonoxidative leucine disposal) (11, 12). During a combined insulin and amino acid infusion, measures of proteolysis are similarly suppressed, whereas the estimates of protein synthesis are sustained or slightly increased, depending on the experimental conditions and/or the isotope model employed (13–15). In addition, studies employing forearm perfusion (16) and/or muscle biopsy techniques (17, 18) in humans support the efficacy of insulin in suppressing rates of proteolysis, but have failed to demonstrate a stimulatory effect of insulin on muscle protein synthesis.

During insulin withdrawal in type 1 diabetic subjects, rates of whole-body proteolysis are increased (17, 19–22), an effect consistent with an inhibitory action of insulin on protein breakdown. However, in contrast to in vitro and in vivo results in rats (1–9), insulin withdrawal in humans results in either no change (20, 21) or, paradoxically, in an increase in the nonoxidative leucine disposal (17, 19, 22).

Taken together, these data support a consistent effect of insulin on protein breakdown but the effects of insulin on protein synthesis remain controversial. This lack of agreement on the effects of insulin on protein synthesis could be the result of problems inherent in the assumptions of the isotope model used to estimate protein synthesis (e.g., CO_2 fractional recovery is constant) and/or due to the fact that estimates of whole-body protein synthesis in humans reflect the net result of differential effects of insulin (some stimulatory and some inhibitory) on the synthesis of specific proteins. This latter hypothesis is supported by the results of a number of studies demonstrating a selective effect of insulin on the cell concentrations of specific mRNAs and, consequently, on the synthetic rates of individual proteins (23–25). In particular, albumin mRNA concentration and albumin synthesis are increased by insulin, both in vivo in rats (26, 27) and in liver cell cultures (25, 28), whereas no effect of insulin on fibrinogen synthesis has been observed (29, 30). Thus, the metabolic stress induced by insulin deficiency could result in an increase in the synthetic rates of acute phase reactant proteins that could obscure the concomitant decrease in the synthesis of other insulin-sensitive proteins.

Therefore, the present study was designed to determine the effects of insulin withdrawal on CO_2 fixation and on the estimated rates of protein synthesis using simultaneous infusions of $NaH^{13}CO_3$ and [14C]leucine infusion, respectively, and to determine the fractional protein synthetic rate of albumin and of the acute reactant phase protein fibrinogen.

Methods

Materials. Purity and sterility of L-[1-14C]leucine (> 55 mCi/mmol, Amersham Corp., Arlington Heights, IL), and sodium [13C]bicarbonate (Merck Sharp and Dohme, St. Louis, MO) were determined before use (31). Radio purity of [1-14C]leucine was determined by its elution pattern on amino acid analysis (32). Fraction V albumin,

thrombin, and $CaCl_2$ were obtained from Sigma Chemical Co., St. Louis, MO.

Protocol. Six type 1 diabetic women without complications or other cardiovascular or renal disease were studied twice in the presence or in the absence of an insulin infusion. All subjects had a normal physical examination, chemistry group, urinalysis, and hematology group before being admitted to the study. Other clinical features of the patients are summarized in Table I. No subject had a C-peptide response > 0.04 nM after a 1-mg intravenous glucagon injection. Absence of endogenous insulin secretion was confirmed by lack of increase in the C-peptide concentration during the hyperglycemic component of this study (see Results).

For the 4 d before the first study, all subjects consumed a known caloric diet in the Clinical Research Center (CRC) of the Mayo Clinic. The diet provided 30–35 cal · kg^{-1} · d^{-1}, 50–60% carbohydrate, 10–15% protein, and the remainder as fat. At least 72 h before the first study all intermediate or long-acting insulin was stopped and the subjects were treated with four daily subcutaneous injections of regular insulin.

On the evening of the admission to the CRC, the patients received their last subcutaneous injection of regular insulin at 16:30 h and consumed their standard supper meal at 17:00 h. At 20:00 h, two intravenous catheters were placed in a vein of both forearms, one for the purpose of insulin and/or glucose infusion and the other for frequent plasma glucose determinations (20–30-min intervals).

The insulin infusion was adjusted to maintain the subjects at near euglycemia ($\approx$ 5.5 mM) and was continued until the end of study 1 (13:00 h). At 05:00 h on the day of study an intravenous catheter was positioned in retrograde fashion in a dorsal hand vein and the hand placed in a warming box to obtain arterialized venous blood (33). At 06:00 h, study 1 was started and the subjects received a 5-h primed infusion of [^{14}C]leucine (10 μCi, 0.33 μCi · min^{-1}) and of [^{13}C]bicarbonate (4.2 μmol/kg, 0.14 μmol · kg^{-1} · min^{-1}). 15 ml of blood were drawn for plasma hormone, substrate, amino acid, leucine, and KIC concentrations and leucine and KIC sp act at −15, 0, 60, 120, 180, 200, 220, 240, 260, 280, and 300 min. 10 ml of additional blood were withdrawn at 0 and 300 min for blood hematocrit and determination of the plasma concentrations of albumin, fibrinogen, and catecholamines. Arterialized venous blood gases were determined at 0, 180, and 300 min. Breath samples for measuring the rates of expired total CO_2, total $^{14}CO_2$, $^{14}CO_2$ sp act and $^{13}CO_2$ enrichments were collected at −15, 0, 180, 200, 220, 240, 260, 280, and 300 min.

Upon completion of study 1 (300 min) the isotope and insulin infusions were discontinued, and the subjects were permitted to ambulate freely in the CRC. They consumed their lunch meal but received no insulin. They were subsequently fasted but permitted to drink water *ad libitum* until the completion of study 2. The subjects were followed carefully; fluid balance, plasma glucose, and urinary ketone bodies were measured every 2–4 h. Upon appearance of urinary ketones, arterialized venous blood gases were determined and repeated every 3–4 h. When the plasma bicarbonate reached the concentration of $\approx$ 20 mM (12±2 h after insulin withdrawal), study 2 was initiated and

Table I. Clinical Features of the Diabetic Subjects

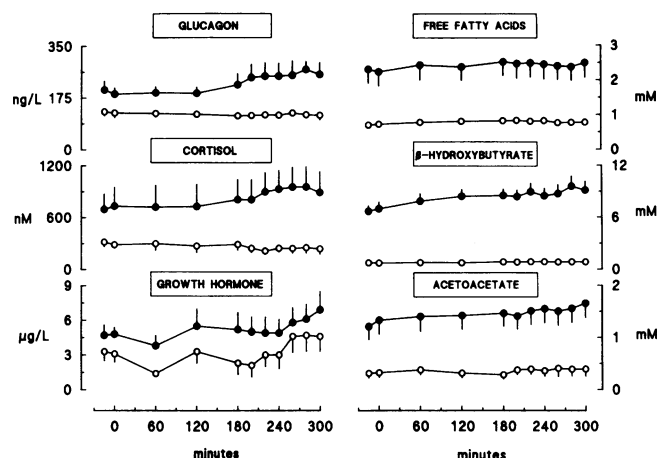| Subject	Age	Weight	Diabetes duration	Insulin dosage	HBA1
	yr	kg	yr	U/d	%
A	25	63	12	45	12.0
B	23	71	8	38	19.4
C	20	61	15	43	13.1
D	20	56	11	51	12.4
E	20	69	4	45	10.8
F	21	65	12	62	13.5
Mean±SEM	21±1	64±2	12±2	47±3	13.5±1.3

Figure 1. Plasma concentrations of glucagon, cortisol, growth hormone, FFA, and ketone bodies during insulin infusion (0.21±0.02 mU · kg^{-1} · min^{-1}; study 1, *open circles*) and after insulin withdrawal (study 2, *solid circles*) in six type 1 diabetic subjects.

carried out in a fashion identical to that of study 1 with the exception that no insulin was infused and patient hydration was maintained by infusing 0.45% NaCl at the rate of 4 ml · min^{-1}. After completion of the study plasma electrolyte concentrations were determined, insulin was infused intravenously and fluid and electrolyte infusions were continued until the mild ketoacidosis was corrected (3–5 h).

Analytical methods. The plasma concentration of glucose (glucose analyzer; Yellow Spring Instrument Co., Yellow Spring, OH), free insulin (34), C-peptide (35), growth hormone (36), cortisol (37), glucagon (38), free fatty acids (39), beta-hydroxybutyrate, and acetoacetate (40) were determined as previously described. Plasma catecholamines were measured through the Mayo Regional Laboratory by HPLC (41). Arterialized venous measurements of pH, P_{CO_2}, P_{O_2} and bicarbonate were determined by the Mayo Clinic Respiratory Laboratory using an IL-1310 gas analyzer (Instrumentation Laboratory, Inc., Lexington, MA). Blood hematocrit, plasma albumin, and fibrinogen concentrations were measured through the Mayo Clinic General Chemistry and Hematology Laboratories.

Plasma concentration and sp act of KIC and leucine were determined by HPLC as previously described (42). Plasma amino acid concentrations were measured by ion exchange chromatography (model 7300, amino acid analyzer; Beckman Instruments, Inc., Palo Alto, CA) (32). Expired rates of $^{14}CO_2$ were measured by aspirating 2-min collections of expired air through an ethanolamine solution (43). In addition, the sp act of breath $^{14}CO_2$ was determined at each breath sampling time by aspirating expired air through hydroxyde of hyamine (43). The ^{14}C radioactivity in KIC, leucine, and CO_2 was determined with a model LS5000 TD series liquid scintillation counter (Beckman Instruments, Inc.).

The sp act of leucine in plasma fibrinogen was determined as previously described (42) with the exception that peak areas were used. Plasma albumin was isolated using the following procedure. Plasma globulins were crudely removed from 2 ml of defibrinated plasma by adding 1.33 ml of a supersaturated ammonium sulfate solution. The supernatant was subsequently extracted with 2 ml of absolute ethanol (44). The ethanol fraction was dried and subsequently analyzed by SDS-PAGE using an overloaded gel and yielded a single large band which comigrated with fraction V albumin. The purified plasma albumin fraction was subsequently hydrolyzed (at 110°C for 72 h), and the sp act of leucine contained in the albumin fraction was analyzed utilizing procedures identical to those employed for the fibrinogen analysis.

$^{13}CO_2$ enrichments and $NaH^{13}CO_3$ infusion rates were determined using an automated isotope ratio mass spectrometer as previously described (45).

Calculations. The actual rates of $NaH^{13}CO_2$ infusion were determined as the product of the infusate stable isotope concentration, isotope enrichment, and the pump infusion rate (45). Total CO_2 production ($mmol \cdot kg^{-1} \cdot min^{-1}$) was calculated by dividing expired $^{14}CO_2$ ($dpm \cdot kg^{-1} \cdot min^{-1}$) by the $^{14}CO_2$ sp act ($dpm \cdot mmol^{-1}$) as previously described (43). The expired rate of $^{13}CO_2$ was calculated by multiplying the $^{13}CO_2$ enrichment by the total rate of expired CO_2 ($mmol \cdot kg^{-1} \cdot min^{-1}$). $^{13}CO_2$ recovery in breath was calculated by dividing the rate of $^{13}CO_2$ expired by the $NaH^{13}CO_2$ infusion rate. Rates of radiolabeled isotope administration were determined by multiplying the $dpm \cdot ml^{-1}$ of infusate by the infusion rate of the pump ($ml \cdot min^{-1}$). Estimates of whole body leucine metabolism were made at substrate and isotopic steady state between 200 and 300 min using the reciprocal pool model as previously described (31). The rate of leucine oxidation was calculated as previously described (31) using the $[^{14}C]KIC$ sp act and $^{14}CO_2$ expired rate after correcting for the $^{13}CO_2$ recovery determined simultaneously.

The fractional synthetic rate of albumin and fibrinogen was calculated by dividing the rise of leucine sp act measured hourly ($dpm \cdot mmol^{-1} \cdot h^{-1}$) from 3–5 h by the mean plasma SA of KIC ($dpm \cdot mmol^{-1}$) during the same period of time (31). The daily fractional synthetic rate was extrapolated by multiplying the previous result by 24. The measurement of the fractional synthetic rate assumes that (*a*) labeled and unlabeled leucine are utilized identically in protein synthesis; (*b*) once synthesized the protein is released into the circulation without being degraded or stored (46, 47); (*c*) leucine SA in plasma protein increases linearly during the time course of the isotope infusion (see Fig. 3); (*d*) plasma $[^{14}C]KIC$ sp act accurately reflects the intrahepatic $[^{14}C]$leucine-tRNA sp act (48); (*e*) over the course of the isotope infusion no significant recycling of labeled nascent proteins occurs from the peripheral extravascular space to the intravascular space (49). By using radioiodinated albumin it has been estimated that 7–10 d are required for a complete equilibration of labeled albumin between the two pools (50). Therefore, we are able only to estimate the fractional rate of the intravascular albumin pool but not that of the total albumin pool. Consequently, valid estimates of the half-life of albumin cannot be obtained. In contrast, only $\approx$ 20% of total body fibrinogen is located in the extravascular bed (51). The half-life of plasma fibrinogen was calculated by dividing the natural log of 2 (ln2) by the fractional synthetic rate.

Statistics. Data are expressed as mean±SEM. Comparisons have been carried out using the Wilcoxon nonparametric test for paired observations (52). Values were considered to be significantly different with a $P < 0.05$. Linearity of label incorporation into plasma albumin and fibrinogen was tested according to the method suggested by Snedecor and Cochran (53). Model fitting was performed using the general linear procedure of the SAS-STAT package version 6.06 (54).

Results

Insulin, C-peptide, and glucose. Overnight insulin infusion preceding study 1 resulted in a mean plasma glucose concentration of 5.9±0.2 mM between midnight and 6 a.m. During study 1, insulin was infused at the rate of 0.21±0.02 $mU \cdot kg^{-1} \cdot min^{-1}$ and plasma free insulin levels were 37±1 pM, whereas during study 2 plasma free insulin was below limits of the assay (15 pM). Plasma C-peptide concentrations during two studies were superimposable and < 0.03 nM. Mean plasma glucose concentration during study 1 was 5.7±0.2 mM, whereas during study 2 it was 16.1±1 mM ($P < 0.03$).

Bicarbonate and pH. Arterialized venous bicarbonate concentrations decreased 36% by the end of study 2 in comparison to study 1 (300 min; study 1, 25±0.2 mM; study 2, 16±2 mM; $P < 0.03$); similarly, blood pH decreased from 7.40±0.01 (mean value of study 1) to 7.30±0.03 by the end of study 2 ($P < 0.03$).

Counterregulatory hormones (Fig. 1). Plasma concentrations of glucagon and cortisol were increased ($P < 0.03$) during the insulin withdrawal study when compared with the insulin infusion study ($P < 0.03$); plasma growth hormone concentration was higher during study 2 but the values were not statistically significant when compared with those of study 1. Plasma concentrations of catecholamines did not differ between the two studies (epinephrine study 1, 163±26 vs. 174±31 pM at 0 and 300 min, respectively; study 2, 184±33 vs. 201±43 pM at 0 and 300 min, $P = NS$; norepinephrine study 1, 1,246±193 vs. 1,198±188 pM at 0 and 300 min; study 2, 1426±224 vs. 1,476±196 pM, at 0 and 300 min, $P = NS$).

Free fatty acids (FFA) and ketone bodies (Fig. 1). During insulin withdrawal the plasma concentration of FFA increased ($P < 0.03$) by nearly threefold when compared with that of the insulin infusion study; similarly, plasma concentrations of β-hydroxybutyrate and acetoacetate were increased ($P < 0.03$) 10-fold and fivefold, respectively.

Amino acids (Table II). The total plasma concentration of the acid-neutral amino acids was similar in both studies. The total concentration of the essential amino acids was increased ($P < 0.03$) during study 2, whereas that of the nonessential amino acids was decreased ($P < 0.03$) during study 2 when compared with those of study 1. Most of the increase in the plasma concentration of the essential amino acids after insulin withdrawal was due to increases of the branched chain amino acids (BCAA), whose plasma concentration more than doubled ($P < 0.03$) between studies 1 and 2. Among the nonessential amino acids no significant difference between the two studies was observed for the plasma cysteine and tyrosine concentrations, whereas all the other amino acids measured showed a significant decrease after insulin withdrawal ($P < 0.03$).

Total CO_2 production and $^{13}CO_2$ recovery in breath (Table III). Total CO_2 production increased by 13% during study 2 in comparison with study 1; this difference, however, was not statistically significant. The recovery of $^{13}CO_2$ from infused $NaH^{13}CO_3$ in expired air during the last 100 min of study 1 was 85±3%, a value not statistically different from that observed during the same time interval of study 2 (84±3%).

Leucine kinetics (Tables II, III, and Fig. 2). Plasma leucine (Table II) and KIC (Table III) concentrations were stable throughout each of the two studies, although plasma leucine and KIC concentrations were 100 and 20% higher, respectively, during study 2 when compared with those of study 1 ($P < 0.03$).

The infusion rate of radioactive leucine was 9.2±0.7 during study 1, and 11.4±0.7 $\cdot 10^3$ $dpm \cdot Kg^{-1} \cdot min^{-1}$ during study 2. Both plasma concentration and plasma $[^{14}C]KIC$ sp act were at near steady over the last 100 min of both studies 1 and 2 (Table III).

Leucine rate of appearance was increased ($P < 0.03$) by $\approx$ 35% (1.85±0.12 vs. 1.37±0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) during insulin withdrawal when compared with the insulin infusion study (Fig. 2). Concomitantly the rate of leucine oxidation was increased ($P < 0.03$) 100% during insulin withdrawal (0.54±0.08 vs. 0.24±0.03 $\mu mol \cdot kg^{-1} \cdot min^{-1}$); whereas the rate of nonoxidative leucine disposal was not significantly higher ($P = 0.21$), during insulin withdrawal (study 1, 1.12±0.11 vs. study 2, 1.31±0.08 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Plasma concentrations and fractional synthetic rates of albumin and fibrinogen (Table IV, Fig. 3). Plasma albumin concentration during the two studies did not change (4.1±0.2 vs.

Table II. Plasma Concentration of Acid-Neutral Amino Acids during Insulin Infusion (0.21 ± 0.02 mU $\cdot$ kg^{-1} $\cdot$ min^{-1}, Study 1) and after Insulin Withdrawal (Study 2) in Six Type 1 Diabetic Subjects

Hours of study	Insulin replacement			Insulin withdrawal		
	0	3	5	0	3	5
	μm	μm	μm	μm	μm	μm
Aspartate	5±1	5±1	4±1	4±1	4±1	4±1
Threonine	90±8	87±10	82±11	77±10	68±8	62±8
Serine	102±4	103±6	98±6	82±11	71±9	70±10
Glutamate	51±6	55±4	54±4	28±4	23±3	37±7
Proline	135±16	130±18	134±21	112±19	107±19	97±17
Glycine	192±9	196±8	196±8	140±20	115±11	126±19
Alanine	212±25	192±26	199±29	172±14	180±17	187±22
Valine	188±6	180±5	172±7	320±14	327±19	319±15
Cysteine	12±3	12±3	11±3	12±2	13±3	14±3
Methionine	14±2	13±1	13±1	14±2	11±1	13±1
Isoleucine	55±3	51±3	47±4	127±9	124±11	119±8
Leucine	105±4	100±4	91±6	222±13	219±17	211±13
Tyrosine	36±3	31±2	28±4	43±3	37±3	35±3
Phenylalanine	33±2	37±2	33±2	45±4	40±3	26±3
Essential AA	485±15	468±19	437±27	801±30	787±42	760±30
Nonessential AA	743±58	722±57	721±62	518±69	458±63	477±65
Total AA	1228±62	1190±71	1158±84	1320±54	1244±89	1237±59

4.1±0.2 g/dl at 0 and 300 min of study 1, respectively; 4.2±0.1 vs. 4.3±0.2 g/dl at 0 and 300 min of study 2, P = NS). Similarly no change in the plasma fibrinogen concentration (199±29 vs. 208±26 mg/dl at 0 and 300 min of study 1, respectively; 205±24 vs. 201±26 mg/dl at 0 and 300 min of study 2, P = NS)

or in blood hematocrit (study 1, 45±1 vs. 44±1% at 0 and 300 min, respectively; study 2, 44±1 vs. 43±2% at 0 and 300 min, respectively) was observed during or between the two studies.

Leucine sp act in plasma albumin and fibrinogen increased linearly from 60 to 300 min (P < 0.01). A straight line fit was

Table III. $^{14}CO_2$ Excretion, Specific Activity, Production and Recovery in Breath, Plasma KIC Concentration and Specific Activity during Study 1 (Insulin Infusion) and Study 2 (Insulin Withdrawal) in Six Type 1 Diabetic Subjects

| Minutes	200	220	240	260	280	300
$^{14}CO_2$ excretion (10^2 dpm $\cdot$ kg^{-1} $\cdot$ min^{-1})						
Study 1	12.3±1.4	12.8±1.9	13.6±1.7	13.8±1.7	14.4±1.6	13.9±1.6
Study 2	26.0±3.5*	26.6±3.6*	28.4±4.0*	28.9±3.0*	28.6±3.2*	29.0±3.0*
$^{14}CO_2$ SA (10^3 dpm $\cdot$ nmol^{-1})						
Study 1	9.8±0.9	10.3±0.9	10.2±0.8	10.3±0.9	10.9±0.9	10.8±0.8
Study 2	16.2±1.6*	16.5±1.7*	17.0±1.6*	17.9±1.7*	18.3±1.9*	17.9±2.2*
CO_2 production (μmol $\cdot$ kg^{-1} $\cdot$ min^{-1})						
Study 1	125±5	124±8	133±8	133±9	132±8	129±9
Study 2	145±11	146±11	152±14	146±13	143±15	147±12
$^{13}CO_2$ recovery (%)						
Study 1	80±2	82±3	84±4	88±3	88±4	87±5
Study 2	80±3	81±3	84±5	83±3	87±5	88±4
Plasma KIC (μM)						
Study 1	26±3	26±3	27±3	29±3	29±4	28±4
Study 2	32±5	34±5	31±4	35±4	35±6	32±5
Plasma KIC SA (dpm $\cdot$ nmol^{-1})						
Study 1	6.8±0.4	6.5±0.4	6.7±0.4	6.8±0.4	7.0±0.5	6.9±0.5
Study 2	6.6±0.5	6.1±0.6	6.0±0.5	6.5±0.5	6.2±0.5	6.0±0.4

* P < 0.03 vs. study 1.
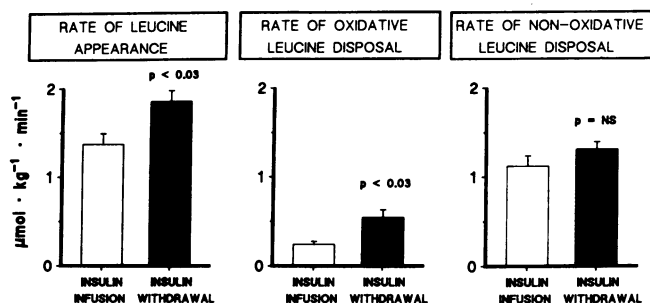
Figure 2. Rates of leucine appearance, of oxidative and of nonoxidative leucine disposal during insulin infusion (0.21±0.02 mU·kg⁻¹·min⁻¹; study 1, *open bars*) and after insulin withdrawal (study 2, *solid bars*) in six type 1 diabetic subjects.

adequate to describe the time course of label incorporation into the two proteins for each subject's data during both studies. Leucine sp act in plasma albumin and fibrinogen at the end of study 1 was 134 ± 14 and $256\pm20\cdot10^3$ dpm·nmol⁻¹, respectively. Basal leucine sp act of study 2 in plasma albumin and fibrinogen was 139 ± 18 and $301\pm29\cdot10^3$ dpm·nmol⁻¹, respectively. The slope of increase of albumin and fibrinogen sp act after the label infusion of study 1 was 0.0077 ± 0.001 and $0.0623\pm0.006\cdot10^3$ dpm·nmol⁻¹·min⁻¹. Assuming that this slope continued over the subsequent 5 h of study 2, it is evident that when compared with the actual slopes observed during study 2 (albumin 0.319 ± 0.06, fibrinogen $1.454\pm0.2\cdot10^3$ dpm·nmol⁻¹·min⁻¹) the baseline contribution to these results would be negligible, indicating that the albumin and fibrinogen fractional synthetic rates of the two studies can be compared in a valid fashion.

The estimated daily fractional albumin synthetic rate during the insulin infusion study was $9.9\pm1.2\%$ and decreased ($P<0.03$) by 29% during the insulin withdrawal study ($7.0\pm2.6\%$).

The estimated daily fractional fibrinogen synthetic rate during the insulin infusion study was $22\pm2\%$ and increased ($P<0.03$) to $33\pm5\%$ during the insulin withdrawal study, an increase of nearly 50%. The calculated half-life of plasma fibrinogen during the insulin infusion study was 3.1 ± 0.4 d and decreased ($P<0.03$) to 2.1 ± 0.3 d after insulin withdrawal.
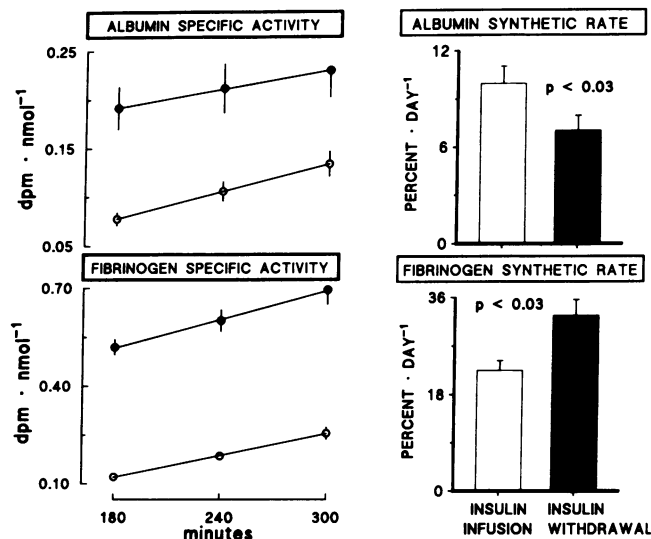

Figure 3. Albumin and fibrinogen sp act during insulin infusion (0.21 ± 0.02 mU·kg⁻¹·min⁻¹; study 1, *open circles*) and after insulin withdrawal (study 2, *solid circles*) in six type 1 diabetic subjects and calculated daily albumin and fibrinogen fractional synthetic rates during study 1 (*open bars*) and study 2 (*solid bars*).

Discussion

The present study demonstrates that short-term insulin deficiency in type 1 diabetic subjects leads to an increase in the rates of whole body proteolysis and of leucine oxidation, but has no effect on the estimated rate of whole body protein synthesis. This latter result would suggest that insulin does not regulate protein synthesis in humans. However, during insulin deficiency, we detected a significant decrease in the fractional synthetic rate of albumin and a concomitant significant increase in that of fibrinogen. These data indicate a differential effect of insulin deficiency on the fractional synthetic rate of two hepatically synthesized plasma proteins. Thus, the observed absence of changes in the estimated whole body protein synthetic rate during insulin deficiency was most likely due to a concomitant increase in the synthesis of some body proteins

Table IV. Slopes of Plasma Albumin and Fibrinogen Specific Activities, Mean Plasma KIC Specific Activities (180–300 min) and Albumin and Fibrinogen Fractional Synthetic Rates during Study 1 (Insulin Infusion) and Study 2 (Insulin Withdrawal) in Six Type 1 Diabetic Subjects

| | SA increase (180–300 min) | | | | | | Fractional synthetic rate | | | |
| | Albumin | | Fibrinogen | | Plasma KIC SA | | Albumin | | Fibrinogen | |
Subject	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
	10^3 dpm·nmol⁻¹·min⁻¹				dpm·nmol⁻¹		%·d⁻¹			
A	0.496	0.259	1.043	1.119	8.26	7.30	8.6	5.1	18	22
B	0.469	0.410	0.694	0.902	5.72	5.85	11.8	10.1	17	22
C	0.475	0.239	1.422	2.117	7.03	6.15	9.7	5.6	29	50
D	0.700	0.569	1.279	2.296	7.05	7.74	14.3	10.6	26	43
E	0.477	0.290	0.822	1.358	7.31	7.38	9.4	5.7	16	26
F	0.211	0.146	1.011	0.931	5.25	4.10	5.8	5.1	28	33

All slopes of plasma albumin and fibrinogen sp act are statistically significant ($P<0.01$).

and to a concomitant and offsetting decrease in the synthetic rate of others.

In the present study, as has already been observed by others (18, 20, 21) insulin deficiency resulted in an increase in the plasma concentration of the essential amino acids, primarily the BCAA, attributable to the increased rate of whole body proteolysis observed after insulin withdrawal ($\approx 35\%$), because estimates of whole body protein synthesis did not change. In contrast to the essential amino acids, the plasma concentration of the nonessential amino acids significantly decreased after insulin withdrawal. Because proteolysis was increased, this reduction in the plasma concentration of the nonessential amino acids was most likely due to decreased *de novo* synthesis and/or increased rates of utilization. The increase in the rate of utilization of the nonessential amino acids is consistent with increased gluconeogenis, known to occur with insulin deficiency (55, 56).

During insulin deficiency, the rate of leucine oxidation was increased more than 100%. This observation is consistent with other reports both in vitro (57) and in vivo (58) that increased concentration of leucine and KIC are associated with an activation of the branched chain α-ketoacid dehydrogenase and with increased rates of oxidation of the carbon skeleton of leucine. Extrapolating the increase in leucine oxidation to other amino acids, these data are consistent with the nitrogen wasting known to occur in poorly controlled diabetics (59) and the increased use of amino acids as an energy source as has been shown in iatrogenically-induced protein catabolism associated with the administration of glucocorticosteroids in humans (60).

Leucine oxidation is calculated by dividing the rate of labeled CO_2 expired in breath by the precursor pool sp act (31). Because part of the labeled CO_2 generated from the oxidation of leucine is retained in the body during the course of the study, the rate of expired labeled CO_2 must be corrected for such fixation and has been estimated in normal subjects to be 19% (61). In previous studies in which the effects of insulin withdrawal were compared with those of insulin replacement (17, 19–22) the fractional CO_2 recovery was not determined and was assumed to be constant. During an infusion of [^{13}C]bicarbonate, recovery of tracer in breath increased linearly with CO_2 production (62; Horber, F., and M. Haymond, unpublished data), suggesting that increased CO_2 production, as might occur in ketoacidosis after insulin withdrawal, could result in an increase in the fractional CO_2 recovery. Failure to account for this could lead to overestimate leucine oxidation and to underestimate protein synthesis. However, because CO_2 production was slightly but not significantly increased in the present study this cannot be a factor. Conversely, CO_2 recovery might have been decreased during insulin deficiency as a result of a greater fractional fixation of labeled CO_2 due to increased gluconeogenesis during insulin deficiency (55, 56). This possibility is indirectly supported by the observation that factors known to suppress gluconeogenesis, such as feeding (62), decrease CO_2 recovery, whereas gluconeogenic stimuli, such as fasting (62) or steroid administration (Horber, F., B. Beaufrere, and M. Haymond, unpublished data), increase it.

In the present study, the fractional CO_2 recovery did not significantly change under the two experimental conditions. The absence of significant changes in the fraction of CO_2 fixed between the insulin replacement and withdrawal protocols suggest that errors in CO_2 fixation could not be used to explain the failure of previous studies (17, 19–22) to observe a decrease in whole body protein synthesis during insulin deficiency or withdrawal.

Insulin deficiency induced both in vitro and in vivo significantly decreases in vitro measures of protein synthesis and, conversely, insulin administration stimulates it (1, 2, 4–9, 63). Insulin regulates protein synthesis at both the translational and transcriptional levels. At the translational level insulin stimulates peptide chain initiation factors (1, 9) and increases the number of ribosomes as evidenced by increased ribosomal RNA (4, 6). At the transcriptional level insulin acts by either increasing (23, 25) or decreasing (23, 24) the cell content of specific mRNAs. These latter observations indicate that insulin can selectively stimulate the synthesis of some proteins and inhibit that of others (23). In humans, measurements of whole body protein synthesis reflect the sum of the effects of insulin on the synthetic rates of all body proteins; therefore, during insulin deficiency a reduction in the synthesis of some proteins which anabolically respond to insulin could be obscured by a concomitant increase in the synthesis of other proteins whose production may be inhibited by insulin or stimulated by other factors.

The stimulatory effects of insulin on albumin synthesis have been clearly demonstrated both in liver cell cultures (25, 28) and in vivo in rats (26, 27). In diabetic rats, insulin deficiency significantly decreased the intracellular concentration of albumin mRNA with a resultant decrease of albumin synthesis (27). Subsequent insulin administration increased both albumin mRNA concentration and albumin synthesis, which were completely normalized after 3 d of treatment (27). In contrast, fibrinogen synthesis in vitro is not affected by insulin administration (29, 30), whereas it is increased by other hormones such as corticosteroids and triiodothyronine (29) and by interleukin-6 (64), a monokine that appears to play a key role in determining the response to stress of acute phase reactant proteins (64).

In our diabetic subjects during insulin replacement the calculated daily fractional synthetic rate of albumin ($10\pm1\%$) and that of fibrinogen ($22\pm2\%$) are comparable with those recently reported in normal subjects during rest (65) and to those recently obtained in normal subjects in our laboratory (unpublished data). Also the half-life of fibrinogen (3.1 ± 0.4 d) in the insulin-treated diabetic subjects was not different from that previously calculated in normal subjects (3–4 d) using other techniques (66–69). Short-term insulin withdrawal resulted in a 29% decrease in the albumin synthetic rate and in a concomitant 50% increase in the fibrinogen synthetic rate. Since between the two studies and in the time frame of the same study no change occurred in the hematocrit or in the concentration of either plasma albumin or fibrinogen, these changes in the calculated fractional synthetic rates are indicative of the relative changes in their synthetic rates.

In the present study we demonstrate, for the first time, that in humans reciprocal changes in the synthetic rate of these two hepatic proteins occurred under conditions of insulin deficiency. However, we cannot exclude that factors other than insulin deficiency *per se* might have been responsible for the results observed. Insulin withdrawal was associated with a significant increase in the plasma concentration of cortisol, glucagon, FFA, ketone bodies, and essential amino acids which have been reported to affect leucine and protein metabolism (13, 15, 56, 58, 70–75). However, in absence of other hormonal inter-

actions in vitro, insulin deficiency decreased the rate of albumin gene transcription and, conversely, insulin replacement increased it (28). In contrast, no effect of insulin on fibrinogen synthesis has been reported in vitro (29, 30), suggesting that the increase in fibrinogen synthetic rate observed in the present study was due to factors other than insulin deficiency. Other hormones such as cortisol and triiodothyronine are potent in vitro stimulators of fibrinogen synthesis (29), as well as interleukin-6 (64). Thus, the increase in the fractional synthetic rate of fibrinogen is consistent with the higher plasma cortisol concentration observed during insulin deficiency.

If our finding for albumin and fibrinogen synthesis can be extrapolated, populations of proteins throughout the body may be stimulated by insulin, whereas others may be unaffected or inhibited by insulin. This alone could account for the failure to demonstrate significant changes in whole body estimates of protein synthesis during insulin deficiency. Other than in rat liver (23), a selective effect of insulin on protein synthesis has also been reported in rat cardiac muscle, where insulin deficiency was associated with a decreased transcription of 11 mRNA species and with a concomitant increased transcription of eight other specific mRNAs (76). Studies in humans using organ balance (16) and muscle biopsies (17, 18) have failed to demonstrate an effect of insulin on whole muscle tissue protein synthesis. Therefore, future studies in humans must address the effect of insulin on the synthesis of specific muscle proteins.

Acknowledgments

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