Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in the human forearm

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Hillier, Teresa A., David A. Fryburg, Linda A. Jahn, and Eugene J. Barrett. Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in vitro. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E1067–E1074, 1998.—Insulin clearly stimulates skeletal muscle protein synthesis in vitro. Surprisingly, this effect has been difficult to reproduce in vivo. As in vitro studies have typically used much higher insulin concentrations than in vivo studies, we examined whether these concentration differences could explain the discrepancy between in vitro and in vivo observations. In 14 healthy volunteers, we raised forearm insulin concentrations 1,000-fold above basal levels while maintaining euglycemia for 4 h. Amino acids (AA) were given to either maintain basal arterial (n = 4) or venous plasma (n = 4) or increment arterial plasma AA by 100% (n = 4) in the forearm. We measured forearm muscle glucose, lactate, oxygen, phenylalanine balance, and [3H]phenylalanine kinetics at baseline and at 4 h of insulin infusion. Extreme hyperinsulinemia strongly reversed postabsorptive muscle's phenylalanine balance from a net release to an accretion (P < 0.001). This marked anabolic effect resulted from a dramatic stimulation of protein synthesis (P < 0.01) and a modest decline in protein degradation. Furthermore, this effect was seen even when basal arterial or venous aminoacidemia was maintained. With marked hyperinsulinemia, protein synthesis increased further when plasma AA concentrations were also increased (P < 0.05). Forearm blood flow rose at least twofold with the combined insulin and AA infusion (P < 0.01), and this was consistent in all groups. These results demonstrate an effect of high concentrations of insulin to markedly stimulate muscle protein synthesis in vivo in adults, even when AA concentrations are not increased. This is similar to prior in vitro reports but distinct from physiological hyperinsulinemia in vivo where stimulation of protein synthesis does not occur. Therefore, the current findings suggest that the differences in insulin concentrations used in prior studies may largely explain the previously reported discrepancy between insulin action on protein synthesis in adult muscle in vivo vs. in vitro.

phenylalanine; insulin; proteolysis; amino acids; insulin-like growth factor I; blood flow

OVER SEVERAL DECADES, IN VITRO STUDIES CONVINCINGLY DEMONSTRATED THAT INSULIN STIMULATES PROTEIN SYNTHESIS IN ISOLATED SKELETAL AND CARDIAC MUSCLE (32, 35, 44). INSULIN INCREASES TRANSLATIONAL EFFICIENCY VIA INCREASED POLYSOME FORMATION (35), AN EFFECT MEDIATED IN PART VIA REGULATION OF THE PHOSPHORYLATION OF ONE OR MORE INITIATION FACTORS (34, 35, 37). LIKewise, IN ISOLATED MUSCLE PREPARATIONS, INSULIN INHIBITS THE DEGRADATION OF MUSCLE PROTEINS, ALTHOUGH THE CELLULAR MECHANISMS RESPONSIBLE REMAIN UNEDEFINED (24, 33). ON THE BASIS OF THESE OBSERVATIONS, INSULIN'S COMBINED EFFECTS TO STIMULATE PROTEIN SYNTHESIS AND RETARD PROTEOLYSIS WERE THOUGHT RESPONSIBLE FOR THE PROTEIN ANABOLIC ACTION OF INSULIN SEEN IN VIVO (51).

OVER THE PAST DECADE, A CONUNDRUM AROSE AS INVESTIGATORS BEGAN TO EXAMINE THE ACTION OF PHYSIOLOGICAL CONCENTRATIONS OF INSULIN ON WHOLE BODY AND MUSCLE PROTEIN METABOLISM IN VIVO. USING TRACER METHODS, SEVERAL LABORATORIES REPORTED THAT PHYSIOLOGICAL DOSES OF INSULIN INHIBIT WHOLE BODY PROTEIN DEGRADATION (22, 53). LIKewise, COMBINING TRACER AND REGIONAL CATHETERIZATION METHODS, MOST (27, 30, 38, 40, 41) BUT NOT ALL (9) LABORATORIES HAVE DEMONSTRATED THAT INSULIN SPECIFICALLY RETARDS MUSCLE PROTEOLYSIS IN HUMANS, FINDINGS IN ACCORD WITH INSULIN'S EFFECT ON PROTEOLYSIS SEEN IN VITRO. STRIKINGLY, IN THESE STUDIES, INSULIN DID NOT STIMULATE PROTEIN SYNTHESIS EITHER IN THE WHOLE BODY OR WITH ONE EXCEPTION (9) IN SKELETAL MUSCLE. Thus INSULIN'S PROTEIN ANABOLIC ACTION IN VIVO APPEARED TO ONLY PARTIALLY PARALLEL THAT WHICH WAS SEEN IN ISOLATED MUSCLE PREPARATIONS.

SUBSEQUENT STUDIES HAVE PROBED THE ORIGIN OF THIS DISCREPANCY. As INSULIN IN VIVO LOWERS THE PLASMA (AND CELLULAR) CONCENTRATION OF MANY AMINO ACIDS, REPLACEMENT OF AMINO ACIDS DURING HYPERINSULINEMIA MIGHT BE REQUIRED FOR EXPRESSION OF INSULIN ACTION ON PROTEIN SYNTHESIS. However, NEITHER REPLACING BASAL AMINO ACID CONCENTRATIONS (LEUCINE-CLAMP METHOD; SEE REF. 16) NOR REGIONAL INSULIN INFUSIONS (WHICH DO NOT AFFECT PLASMA AMINO ACIDS CONCENTRATIONS; SEE REF. 38) UNMASKED ANY EFFECT OF INSULIN ON PROTEIN SYNTHESIS. COMBINED HYPERINSULINEMIA AND HYPERAMINOACIDEMIA DID INCREASE MUSCLE AMINO ACID UPTAKE AND PROTEIN SYNTHESIS (7, 8, 46). However, AS HYPERAMINOACIDEMIA ALONE INCREASES AMINO ACID UPTAKE AND PROTEIN SYNTHESIS IN BOTH WHOLE BODY AND LIMB BALANCE STUDIES (7, 10, 21, 39, 43, 57), ANY INDEPENDENT EFFECT OF INSULIN IN THESE STUDIES COULD NOT BE ASCERTAINED, SINCE AMINO ACID CONCENTRATIONS WERE NOT STRICTLY COMPARABLE (7, 46). A SUBSEQUENT STUDY CONTROLLED FOR AMINO ACID CONCENTRATIONS BY Raising SYSTEMIC AMINO ACID CONCENTRATIONS TWOFOLD AND SELECTIVELY INCREASING THE INSULIN CONCENTRATION IN ONE FOREARM. THIS DID NOT ENHANCE MUSCLE PROTEIN SYNTHESIS MORE THAN AMINO ACIDS ALONE IN THE CONTRAULER ARM (21).

TWO OTHER POTENTIAL MAJOR DIFFERENCES BETWEEN IN VITRO AND IN VIVO STUDIES ARE AGE AND INSULIN CONCENTRATIONS. Several recent studies have demonstrated that physiological doses of insulin in vivo stimulate skeletal muscle protein synthesis in young (25) but not mature or aged rats (3, 1). Likewise, in vitro studies demonstrating an effect of physiological doses of insulin on muscle protein synthesis have used muscle from young rats (50). A recent study has indicated that this effect is blunted or lost in muscle from older animals (17).
virtually all human studies have been performed in adults, the age of the subjects may contribute to the generally reported lack of effect of physiological insulin to stimulate protein synthesis.

A second potential major difference between in vitro and in vivo studies is the insulin concentrations used. Most in vitro studies demonstrating stimulation of protein synthesis in adult animals have used insulin concentrations of 2 mU/ml or above (32–34, 42). In contrast, much lower concentrations (e.g., 10–200 mU/ml) have generally been used in vivo (27, 30, 38, 40, 41). To further resolve this conundrum, we examined whether insulin at concentrations previously shown to stimulate protein synthesis in adult muscle in vitro exerted similar effects in vivo.

In the current study, we infused insulin (5 mU·min⁻¹·kg⁻¹) into the brachial artery to raise local forearm insulin concentrations by ~1,000-fold. This insulin infusion lowers circulating amino acids (23), an effect not seen in vitro in perfused tissues. We prevented hypoaminoacidemia by simultaneous systemic infusion of a balanced amino acid mixture at one of three rates selected to either replace or augment plasma amino acids and then examined the separate effect of amino acid supply on the response to marked hyperinsulinemia.

**METHODS**

Subjects. Fourteen healthy (9 females, 5 males), normal-weight (68 ± 3 kg), young adult (24 ± 2 yr) volunteers were admitted to the University of Virginia General Clinical Research Center the evening before study. No subject was taking any medication, and all female participants had a negative serum pregnancy test 1–2 days before study. The study protocol was approved by the University of Virginia Human Investigation Committee, and each subject gave written consent.

Experimental protocol. Figure 1 schematically depicts the experimental protocol. After an overnight 12-h fast, a brachial artery catheter and an ipsilateral, retrograde, median cubital (deep) vein catheter were placed percutaneously. In the contralateral arm, a second, antegrade, median cubital vein catheter was placed for infusion of glucose, amino acids, and tracer. Each subject received a primed (~33 mCi), continuous (0.43 mCi/min) infusion of L-[ring-2,6-³H]phenylalanine. After a 90-min tracer equilibration period, quadruplicate paired arterial and deep venous samples were taken over 30 min. After baseline samples were obtained, a balanced amino acid mixture was continuously infused at a rate of either 0.004 (n = 4), 0.007 (n = 6), or 0.015 (n = 4) ml·min⁻¹·kg⁻¹ for 4 h. Insulin (5 mU·min⁻¹·kg⁻¹ body wt⁻¹) was continuously infused intra-arterially. Twenty percent dextrose was infused into the contralateral arm at a variable rate to maintain euglycemia. Quadruplicate paired blood samples were again taken from each of the sampling catheters between 210 and 240 min of insulin infusion.

Analytic methods. Plasma glucose was measured using the glucose oxidase method. Lactate concentrations were measured by a combined lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Blood oxygen content was measured spectrophotometrically using an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark). Plasma insulin was measured by radioimmunoassay. Phenylalanine concentrations and specific activity were measured as previously described (6).

Calculations of forearm phenylalanine kinetics. The net forearm balance for amino acids was calculated from the Fick principle

\[
\text{Net balance} = (\{A\} - \{V\}) \times F
\]

where \(A\) and \(V\) are arterial and venous substrate concentrations, respectively, and \(F\) is forearm blood flow in milliliters per minute per 100 ml forearm volume. Measurement of the absolute rates of synthesis and breakdown of muscle protein requires knowing the phenylalanine specific activity in the phenylalanyl-tRNA pool being used for protein synthesis. This is not experimentally accessible in the forearm. The rates of protein synthesis and degradation can be estimated from the kinetic of exchange of labeled phenylalanine across the forearm as previously described (6, 27). Equations have been presented for estimating forearm protein synthesis and degradation that were based on use of either arterial or deep venous specific activity of [³H]phenylalanine to approximate
phenylalanyl-tRNA specific activity. Because recent data suggest that the specific activity of phenylalanine in venous plasma more closely approximates that of the TRNA pool (56, 59), we used venous specific activity to estimate rates of protein synthesis and degradation. Qualitatively comparable results were obtained using the arterial phenylalanine specific activity as the precursor pool (see below). With the use of the venous phenylalanine specific activity to reflect the precursor pool for protein synthesis, synthesis (S) is given by

$$S = \frac{(DPM_{\text{art}} - DPM_{\text{vein}}) \times F}{SA_{\text{vein}}}$$

(2)

where DPM_{art} and DPM_{vein} are disintegrations per minute in arterial and venous concentrations, respectively, and SA_{vein} is the specific activity in vein. Muscle protein breakdown (B) is calculated as

$$B = S - \text{net balance}$$

(3)

Data presentation and statistical analysis. All data are presented as means ± SE. Data on hormone and substrate concentrations, forearm substrate and oxygen balances, and forearm amino acid kinetics are presented for each of the sample periods, i.e., basal and 4 h. Stochastic comparisons were made using analysis of variance with repeated measures and post hoc comparisons with Duncan’s test (True Epistat; Epistat Services, Richardson, TX).

RESULTS

Forearm glucose, lactate, phenylalanine, and insulin concentrations. In the three study groups, the arterial concentration of glucose averaged 4.8 ± 0.2 mM in the basal period and remained at or slightly above basal levels throughout the 4-h experimental period (mean = 5.3 ± 0.2 mM over last 30 min). The arterial lactate concentration (measured in subjects given 0.007 ml of amino acid·min^{-1}·kg AA^{-1}) rose modestly during the 4-h insulin infusion (0.6 ± 0.1 mM basal vs. 1.0 ± 0.2 mM with insulin infusion). Infusion of amino acids at 0.004 ml amino acid·min^{-1}·kg AA^{-1} maintained arterial phenylalanine at basal values; however, the venous concentration declined ~20% (Table 1). The 0.007 ml amino acid·min^{-1}·kg AA^{-1} infusion raised the arterial concentrations ~35% above basal level, while venous concentrations were not different from basal (Table 1). The highest amino acid dose (0.015 ml·min^{-1}·kg AA^{-1}) significantly raised both arterial and venous phenylalanine (Table 1).

In the basal period, the venous plasma insulin concentration averaged between 7 and 9 µU/ml (Table 1). During the experimental period, forearm concentrations rose nearly 1,000-fold (average 7,100–11,300 µU/ml) and remained elevated for 4 h (Table 1). Euglycemia was maintained throughout and during the last 60 min of the insulin clamp. Glucose was infused at an average rate of 10.7 ± 0.9 mg·min^{-1}·kg^{-1}. There was no significant difference in the rate of glucose infusion in the three groups (9.6 ± 0.6, 12.2 ± 2.0, and 9.6 ± 1.4 mg·min^{-1}·kg^{-1} for the 0.004, 0.007, and 0.015 ml·min^{-1}·kg AA^{-1} amino acid groups, respectively).

Table 1. Concentration of insulin in the forearm vein and of phenylalanine in the forearm artery and vein basally (~30 to 0 min) and at the end of the insulin infusion (210–240 min)

<table>
<thead>
<tr>
<th>Insulin, µU/ml</th>
<th>Basal</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004 ml·min^{-1}·kg AA^{-1}</td>
<td>9 ± 2</td>
<td>8,600 ± 1,700†</td>
</tr>
<tr>
<td>0.007 ml·min^{-1}·kg AA^{-1}</td>
<td>7 ± 2</td>
<td>7,100 ± 1,600†</td>
</tr>
<tr>
<td>0.015 ml·min^{-1}·kg AA^{-1}</td>
<td>9 ± 1</td>
<td>11,300 ± 5,500†</td>
</tr>
</tbody>
</table>

Arterial phenylalanine, mM

| 0.004 ml·min^{-1}·kg AA^{-1} | 36 ± 1 | 35 ± 1 |
| 0.007 ml·min^{-1}·kg AA^{-1} | 47 ± 3 | 64 ± 4† |
| 0.015 ml·min^{-1}·kg AA^{-1} | 36 ± 4 | 80 ± 7† |

Venous phenylalanine, mM

| 0.004 ml·min^{-1}·kg AA^{-1} | 41 ± 2 | 33 ± 1† |
| 0.007 ml·min^{-1}·kg AA^{-1} | 52 ± 3 | 58 ± 5 |
| 0.015 ml·min^{-1}·kg AA^{-1} | 40 ± 5 | 73 ± 9† |

Values are means ± SE. AA, amino acid. †P < 0.01 for basal vs. insulin infusion period.

Table 2. Forearm blood flow and the balances for glucose, lactate, and oxygen across forearm muscle in the basal period (~30 to 0 min) and over the last 30 min of insulin infusion (210–240 min)

<table>
<thead>
<tr>
<th>Blood flow, ml·min^{-1}·100 ml^{-1}</th>
<th>Basal</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004 ml·min^{-1}·kg AA^{-1}</td>
<td>3.2 ± 1.3</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>0.007 ml·min^{-1}·kg AA^{-1}</td>
<td>3.0 ± 0.3</td>
<td>7.4 ± 1.7†</td>
</tr>
<tr>
<td>0.015 ml·min^{-1}·kg AA^{-1}</td>
<td>4.7 ± 0.8</td>
<td>9.4 ± 3.0</td>
</tr>
</tbody>
</table>

Glucose balance, µmol·min^{-1}·100 ml^{-1}

| 0.004 ml·min^{-1}·kg AA^{-1} | 0.3 ± 0.1 | 7.0 ± 2.0† |
| 0.007 ml·min^{-1}·kg AA^{-1} | 1.0 ± 0.3 | 7.7 ± 1.8 |
| 0.015 ml·min^{-1}·kg AA^{-1} | 0.9 ± 0.3 | 12.7 ± 3.8† |

Lactate balance, µmol·min^{-1}·100 ml^{-1}

| 0.004 ml·min^{-1}·kg AA^{-1} | ND | ND |
| 0.007 ml·min^{-1}·kg AA^{-1} | −0.3 ± 0.1 | −1.7 ± 0.5† |
| 0.015 ml·min^{-1}·kg AA^{-1} | ND | ND |

Oxygen balance, µmol·min^{-1}·100 ml^{-1}

| 0.004 ml·min^{-1}·kg AA^{-1} | 7.6 ± 1.4 | 12.1 ± 1.8† |
| 0.007 ml·min^{-1}·kg AA^{-1} | 7.1 ± 0.5 | 11.6 ± 0.9† |
| 0.015 ml·min^{-1}·kg AA^{-1} | ND | ND |

Values are means ± SE. ND, not determined. *P < 0.05 and †P < 0.01 for basal vs. insulin infusion period.

Forearm glucose, lactate, and oxygen balances and blood flows. Forearm glucose uptake significantly increased in each study group during the experimental period (Table 2). Forearm lactate release increased in each of the subjects in whom it was measured. Blood flow in the insulin-infused arm increased markedly in the three study groups (average 230%, Table 2). In response to the high-dose insulin, forearm oxygen uptake increased by 158 and 162% in subjects given 0.004 and 0.007 ml·min^{-1}·kg AA^{-1} amino acid, respectively.

Forearm phenylalanine kinetics. There was a net release of phenylalanine by the forearm in each of the three groups at baseline (Fig. 2). Phenylalanine's only metabolic fate in muscle is its incorporation into and release from muscle protein, as it is not concentrated by muscle (i.e., intracellular and extracellular concentrations are nearly equal; see Ref. 8). Therefore, the net postabsorptive release of phenylalanine indicates a net loss of muscle protein.

Phenylalanine balance across the arm converted from a net release to a net uptake in each group during the experimental period (Fig. 2). Protein synthesis rose significantly in each of the three study groups (Fig. 2).
In the entire group of 14 subjects, there was a positive linear correlation \((P < 0.05)\) between both the arterial and venous phenylalanine concentrations and the rate of protein synthesis \((r = 0.63 \text{ and } 0.58, \text{ respectively})\). Thus extreme concentrations of insulin acted comparably irrespective of the precursor pool model used for tracing protein synthesis.

**DISCUSSION**

In this study, the very high insulin concentrations previously shown to stimulate adult skeletal muscle protein synthesis in vitro provoked a similar action in the adult human forearm in vivo. Protein synthesis was strongly stimulated when amino acids were infused at any of the three doses tested. At the lowest dose infused, arterial amino acid concentrations were not changed, but venous amino acid concentrations fell. The latter mimics the cellular concentrations \((14)\), which might also be expected to fall. Despite this, protein synthesis rates doubled \((\text{Table 2 and Fig. 2})\). This contrasts to findings with physiological hyperinsulinemia and euaminoacidemia, which leaves forearm protein synthesis unchanged or even decreased \((38, 41)\). High concentrations of insulin \((>1,000 \mu\text{U/ml})\) were also achieved in the study by Denne and colleagues \((15)\). They observed that leg muscle proteolysis declined but saw no increase in protein synthesis. However, plasma amino acid concentrations were not maintained in that study, and the decline in amino acid concentrations with hyperinsulinemia may have obscured an effect.

**Fig. 2.** Protein kinetics. Protein balance, degradation, and synthesis rates are shown \((\text{measured in nmol phenylalanine \cdot min}^{-1} \cdot 100 \text{ ml}^{-1})\). Values represent means \(\pm\) SE for the basal (open bars) and last 30 min of the insulin infusion \(\text{rates (in ml \cdot min}^{-1} \cdot \text{kg}^{-1}\text{)} (\ast P < 0.05 \text{ and } \ast\ast P < 0.01 \text{ for basal vs. infusion period}).

In the entire group of 14 subjects, there was a positive linear correlation \((P < 0.05)\) between both the arterial (Fig. 3) and venous phenylalanine concentrations and the rate of protein synthesis \((r = 0.63 \text{ and } 0.58, \text{ respectively})\). Phenylalanine release from muscle protein degradation was similar during the basal period in each group. Degradation appeared to decline in each group during the insulin infusion, but the change was only statistically significant when the data from all subjects were pooled \((54 \pm 8 \text{ vs. } 45 \pm 5 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}, \text{ P } < 0.05)\).

The kinetics of forearm protein turnover were also analyzed using arterial phenylalanine as the index pool for tracing protein turnover \((\text{see METHODS, data not shown})\). Use of the arterial phenylalanine specific activity as the precursor pool for estimating muscle protein synthesis and degradation yielded results comparable to those presented above based on use of the venous precursor pool. Specifically, phenylalanine rate of disappearance \((\text{like } S, \text{ an index for protein synthesis})\) increased with insulin at each amino acid infusion dose \((P < 0.02, 0.01, \text{ and } 0.01 \text{ for the } 0.004, 0.007, \text{ and } 0.015 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{ amino acid infusions, respectively})\). Thus extreme concentrations of insulin acted comparably irrespective of the precursor pool model used for tracing protein synthesis.

**Fig. 3.** Protein synthesis. Regression line \((\text{solid line})\) for the current study demonstrates that protein synthesis rates are positively correlated with increasing phenylalanine \((\text{PHE})\) concentrations \((r = 0.63, \text{ P } < 0.02)\). Mean \(\pm\) SE values are depicted for both variables in each of the 3 amino acid infusion groups along the regression line. For comparison, we have graphed \((\text{dashed line})\) protein synthesis rates with physiological insulin \((\sim 80 \mu\text{U/ml})\) during euaminoacidemia \((38)\) and hyperaminoacidemia \((21)\).
In the present study, steady-state extreme hyperinsulinemia shifted forearm muscle phenylalanine balance from negative to strongly positive. This shift is more marked than reported with physiological hyperinsulinemia in human forearm or leg muscle, where insulin’s inhibition of protein degradation accounted for the entire effect (21, 30, 38, 40, 41). In the forearm, raising insulin to four different levels throughout the physiological range (20–125 µU/ml) during euaminoacidemia shifted phenylalanine balance to modestly positive values (−10 nmol·min⁻¹·100 ml⁻¹) in all groups, suggesting a plateau effect of physiological hyperinsulinemia to retard proteolysis (38). The greater shift in the current study (even in groups 1 or 2 when plasma amino acid concentrations remained at basal) was primarily due to the marked stimulation of protein synthesis that was not previously seen at physiological insulin concentrations.

In only one human study was muscle protein synthesis reported to increase in response to physiological concentrations of insulin (9). In that study, protein synthesis was measured in leg muscle of six subjects, using both an arterial-venous difference method (as employed here) as well as a tissue biopsy technique. Good internal agreement was obtained between results seen with the arterial-venous difference method and the biopsy technique, both showing an enhancement of muscle protein synthesis by physiological hyperinsulinemia. Contrary to previous (6, 15, 30, 38, 41) or subsequent reports (21), the authors found no evidence for an effect of insulin to restrain proteolysis. No previous human limb balance study (including more than 60 subjects) has seen a stimulation of muscle protein synthesis by physiological hyperinsulinemia using the arterial-venous difference method (21, 27, 38, 41). Most have seen an effect of insulin on whole body and muscle proteolysis. In addition, other studies in both humans (40) and rodents (59) using muscle biopsies and estimating muscle protein synthesis using a variety of methods (including determining the labeling of the aminocyl-tRNA) have also failed to show an effect of physiological hyperinsulinemia. Thus the results of Biolo and colleagues (9), although internally consistent, are divergent with all other in vivo studies, and we can not currently reconcile their unique findings.

The increases in protein synthesis seen with the higher rates of amino acid infusion (Figs. 2 and 3) indicate that raising plasma amino acid concentrations alone augments protein synthesis. This is in agreement with several previous human studies showing increased rates of whole body (10, 28) or skeletal muscle (7, 8, 46) protein synthesis in response to raised amino acid concentrations with or without added insulin. As these studies augmented amino acid concentrations simultaneously with physiological hyperinsulinemia, it was not possible to assess whether insulin had an independent effect from that of amino acids. We subsequently addressed this experimentally using a double forearm cannulation method. We observed that superimposing local, physiological hyperinsulinemia in one forearm during systemic amino acid infusion did not further stimulate muscle protein synthesis in the insulin-infused arm (21). The amino acid infusion rate (0.015 ml·min⁻¹·kg⁻¹) in that study was the same as the highest dose used in the present study, and the arterial phenylalanine concentrations were higher than in the present study (113 ± 4 vs. 80 ± 7 µM; see Fig. 3). Therefore, the stimulation of protein synthesis to 90–150% above basal rates with marked hyperinsulinemia in the present study both under basal and hyperaminoacidemic conditions suggests that marked hyperinsulinemia has an effect separate from that of amino acids.

Protein degradation declined modestly in each of the three study groups in the current study (Fig. 2). This change (~20% decline overall) was statistically significant when observations for all three groups were pooled. In the previous human forearm studies, insulin was found to retard proteolysis by 25–40%. As proteolysis is estimated from the dilution of phenylalanine specific activity across the muscle bed, the high blood flows (vide infra) seen during the very high insulin infusion used here likely added variability to this measurement. That protein synthesis, the primary outcome variable, and net phenylalanine balance were significantly stimulated in each of the three study groups attests to a quantitatively greater (~100%) effect on synthesis with the use of high insulin concentrations.

Although the relative change in protein kinetics was similar in all three groups in the current study, the absolute rates of basal protein synthesis and degradation were lower in the 0.004 ml·min⁻¹·kg⁻¹ group. Gender differences could partially explain this, as all four study subjects were female in the 0.004 group, whereas the 0.007 and 0.015 ml·min⁻¹·kg⁻¹ groups had a mix of male and female subjects. However, each subject was compared with his/her own baseline value, and a consistent and significant effect on protein synthesis was still observed in all three groups. If anything, our results may underestimate the effect of marked hyperinsulinemia to stimulate protein synthesis with euaminoacidemic conditions because of the large proportion of females in two of the three groups.

It has been suggested that a stimulation of protein synthesis in vivo has been difficult to demonstrate in humans because basal insulin has already maximally stimulated protein synthesis. Three observations suggest that this may not be the case. First, in the current study, very high insulin concentrations do further stimulate protein synthesis. Second, as infusion of growth hormone (20) or of insulin-like growth factor I (IGF-I; see Refs. 19 and 21) acutely increases forearm muscle protein synthesis, synthesis rates are clearly not at a maximum. Third, in studies of insulin-deficient diabetic humans, acute replacement of insulin did not increase whole body or muscle protein synthesis (44, 45).

1 In 76 healthy postabsorptive adult subjects (40 males, 36 females), protein synthesis rates averaged 24 ± 2 nmol phenylalanine·min⁻¹·100 ml⁻¹ in females and 36 ± 3 in males (P < 0.005; see Ref. 31a).
Collectively, these data suggest that physiological increments in insulin do not augment bulk protein synthesis in humans.

In insulin-withdrawn streptozotocin diabetic rats, replacement of insulin rapidly restores protein synthetic rates in both heart and skeletal muscle (1, 2). Before treatment, these animals are severely catabolic (insulin withdrawn for up to 5 days), and it is quite possible that restoring basal insulin does affect protein synthesis in this setting. This may be a direct effect of insulin or an indirect effect achieved by restoring sensitivity to other growth factors such as IGF-I (31).

Marked hyperinsulinemia combined with amino acid infusion stimulated blood flow more than twofold (100–160%) in each of the three groups in the current study. Both insulin (4) and amino acids likely contributed to this, as generalized hyperaminoacidemia (21) or infusion of arginine (29) increases forearm blood flow. Importantly, physiological hyperinsulinemia in the presence of hyperaminoacidemia does not stimulate blood flow more than hyperaminoacidemia alone (21).

The reported stimulation of limb blood flow with physiological hyperinsulinemia ranges from 10 to 125% (5, 13, 30, 36, 38, 45, 52, 54). This variability involves many factors such as insulin infusion (local vs. systemic and single dose vs. sequential increments in insulin infusion dose), methodology (capacitance plethysmography vs. thermodilution used as well as variability between laboratories in employing a particular method), locale (forearm vs. leg), and muscle content of the limb studied (4, 54). Most studies reporting a stimulation of blood flow above 50% with physiological hyperinsulinemia have used a sequential increase of insulin infusion in the same subject (5, 36, 52).

As our previous studies have extensively used the same procedure of capacitance plethysmography in the forearm, it seems most logical to contrast our current blood flow results with our past results. We previously observed that insulin at five different concentrations within the physiological range did not stimulate blood flow >25%, and this was only in the high physiological range (27). Additionally, we studied the effects of hyperaminoacidemia (0.015 ml·kg⁻¹·min⁻¹ balanced amino acid infusion) with or without insulin on forearm flow. Physiological hyperinsulinemia did not stimulate blood flow beyond hyperaminoacidemia alone. Of interest, in that study and several others, we noted that local IGF-I strongly stimulated flow when amino acids were elevated or remained at basal levels (21). Thus the two- to threefold stimulation of blood flow in the current study with extreme hyperinsulinemia even when amino acids remained at basal levels is unlike physiological hyperinsulinemia and similar to flow changes provoked by local forearm IGF-I infusion (19, 21).

The stimulation of protein synthesis in the current study is consistent with previous in vitro studies of isolated muscle incubated with insulin at high concentrations. As comparable effects of insulin have generally not been seen in vivo with physiological insulin concentrations (27, 30, 38, 41, 40, 59), our results suggest that very high insulin concentrations may be required. An exception is in young rats in which in vivo studies have demonstrated a stimulatory action of physiological insulin concentrations on muscle protein synthesis (25, 26). However, these effects are not seen in older animals (3, 11, 39) or, with one exception (9), in adult humans. Thus the muscle's sensitivity to insulin's stimulatory action on protein synthesis may decline with aging.

In the current study, the effects of extreme hyperinsulinemia to stimulate protein synthesis, enhance oxygen consumption, markedly increase blood flow, and induce a strongly positive phenylalanine balance are each similar to the actions of locally infused IGF-I in human forearm (19, 21) and differ from the effects seen with physiological hyperinsulinemia concentrations (27, 30, 38, 41). As the insulin concentrations achieved in the current study were 4–7 × 10⁻⁸ M (100-fold above the physiological range), stimulation of the IGF-I receptor by insulin is entirely plausible (dissociation constant (Kₐ) of insulin for the IGF-I receptor ~10⁻⁸ compared with Kₐ 10⁻¹⁰ for IGF-I (58)). Furthermore, as the mitogenic effect of insulin (10⁻⁶) in vitro is mediated via the IGF-I receptor rather than the insulin receptor (55), an analogous effect with protein synthesis is possible. If this is the case, our results introduce a necessary caution to the interpretation of the many in vitro studies documenting an effect of high concentrations of insulin to increase bulk protein synthesis in adult animals (see Refs. 31 and 35 for reviews). With few exceptions (18, 50, 51), the insulin concentrations used in these studies (generally ≥2 mU/ml) are in a range in which effects of insulin mediated by the IGF-I receptor or hybrid IGF-I/insulin receptors (48, 49) could complicate data integration. As the current study's aim was only to determine if extreme hyperinsulinemia could reproduce in vivo the stimulation of protein synthesis seen with similar concentrations in vitro, further research is needed to elucidate if protein synthesis is indeed being stimulated via IGF-I signaling pathways.

In summary, these results indicate that insulin at high concentrations strongly stimulates muscle protein synthesis in the human forearm. This effect is quite consistent with the action of insulin described in a number of in vitro studies using similar concentrations of insulin but distinct from what is observed with physiological hyperinsulinemia. Therefore, much of the discrepancy previously reported between insulin action on protein synthesis in vivo vs. in vitro may result directly from differences in insulin concentrations used.

In addition to stimulating protein synthesis, high-dose insulin resembles the action of IGF-I observed previously (19, 21). As IGF-I receptors can be stimulated by high concentrations of insulin, the present results together with findings from in vitro studies raise the possibility that some or all of insulin's actions to stimulate protein synthesis may be mediated by pathways other than the insulin receptor.

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