Tissue-Specific Regulation of Mitochondrial and Cytoplasmic Protein Synthesis Rates by Insulin

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In vivo studies have reported conflicting effects of insulin on mixed tissue protein synthesis rates. To test the hypothesis that insulin has differential effects on synthesis rates of various protein fractions in different organs, we infused miniature swine (n = 8 per group) with saline, insulin alone (at 0.7 mU/kg^{-1} · min^{-1}), or insulin plus an amino acid mixture for 8 h. Fractional synthesis rate (FSR) of mitochondrial and cytoplasmic proteins in liver, heart, and skeletal muscle, as well as myosin heavy chain (MHC) in muscle, were measured using L-[1-^{13}C]leucine as a tracer. The FSR of mitochondrial and cytoplasmic proteins were highest in liver, followed by heart and then muscle. Mitochondrial FSR in muscle was higher during insulin and insulin plus amino acid infusions than during saline. Insulin had no significant effect on FSR of MHC in muscle. In contrast, FSR of both mitochondrial and cytoplasmic proteins were not stimulated by insulin in liver. Insulin also did not increase FSR of mitochondrial in heart, whereas insulin and amino acid stimulated FSR of cytoplasmic protein. In conclusion, insulin stimulates the synthesis of muscle mitochondrial proteins, with no significant stimulatory effect on synthesis of sarcoplasmic and MHC. These results demonstrate that insulin has different effects on synthesis rates of specific protein fractions in the liver, heart, and skeletal muscle. *Diabetes* 50:2652–2658, 2001

Insulin is a major regulator of muscle mass, as clearly demonstrated by reversal of emaciation in patients with type 1 diabetes after insulin replacement (1). The mechanism of this anticatabolic effect of insulin remains to be clearly defined. In vitro (2) and in vivo experiments in growing rodents (3) demonstrated that insulin stimulates muscle protein synthesis. In contrast, whole-body studies in humans demonstrated that insulin deprivation in patients with type 1 diabetes results in an increase of both protein breakdown and synthesis, and that a greater increment in protein breakdown than synthesis results in net protein loss (4–7). Recent studies using arteriovenous tracer balance techniques across splanchnic and muscle tissue beds further demonstrated that insulin regulates protein turnover in a tissue-specific manner (8,9). These organ balance studies demonstrated that while insulin decreased protein breakdown in all tissues, it inhibited splanchnic protein synthesis, without similar effect on muscle protein synthesis, This specificity also extends to individual liver proteins as it was shown that insulin deprivation inhibited albumin synthesis but stimulated synthesis of fibrinogen, another exported hepatic protein (10).

Almost all human studies, with one exception (11), have reported that insulin infusion given either systemically (8,12,13) or regionally (14) does not stimulate muscle protein synthesis. Supraphysiological insulin administration, however, has been shown to stimulate muscle protein synthesis based on an arteriovenous model (15). There are no well-validated explanations for the differences among human, rodent, and in vitro studies. All of the previous human and animal studies have measured the synthesis rate of total (mixed) muscle proteins, which is a weighted average of all individual muscle proteins. Synthetic rates vary among the individual proteins in skeletal muscle (16,17), but it is unknown whether rates of synthesis of subcellular protein fractions, i.e., mitochondria or sarcoplasmic proteins, are also related to the metabolic activity of the different organs, such as the liver, the heart, or the muscle. It is also unclear whether insulin action differs among the mitochondrial and sarcoplasmic proteins within the tissues and between the different tissues. The specific action of insulin on mitochondrial and sarcoplasmic protein synthesis rate in the skeletal muscle, heart, and liver in vivo has not been previously investigated. Mitochondria are major functional components in all of these tissues, because they are the intracellular sites for fuel oxidation and ATP production. Insulin-mediated changes in the activity or synthesis rates of mitochondrial proteins may affect tissue function in accordance with the nutritional state (fasting, feeding). Therefore, the present study was performed to determine in vivo the effect of insulin on the fractional synthesis rate (FSR) of mitochondrial proteins and sarcoplasmic proteins in the liver, the heart, and together with the FSR of myosin heavy chain (MHC) in the muscle. The specific effect of amino acids in conjunction with insulin was also assessed in these tissues to consider the possible confounding effect of hypoaminoacidemia during insulin administration.

RESEARCH DESIGN AND METHODS

Animals. Twenty-four mature male Hanford miniature swine that weighed 35.0 ± 2.9 kg (mean ± SD) were obtained from Charles River Lab (Wilmington, MA). The study protocol was approved by the Institutional Animal Care

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AA, group treated with amino acid; ANOVA, analysis of variance; BCAA, branched-chain amino acids; FSR, fractional synthesis rate; GC-MS, gas chromatograph–mass spectrometer; INS, group treated with insulin; INS+AA, group treated with insulin plus amino acid; KIC, ketoisocaproate; MHC, myosin heavy chain; S, group treated with saline.

and Use Committee before starting. The care and handling of the animals were in accordance with the National Institutes of Health guidelines for the use of experimental animals.

Materials. L-[1-^{13}C]leucine (99 MPE) was purchased from Cambridge Isotope Laboratories (Woburn, MA). Isotopic and chemical purity of leucine were checked by gas chromatography mass spectrometry. Solutions of tracers were tested for sterility and pyrogenicity before use and were prepared in sterile apyrogenic water. Purified pork insulin (Regular Iletin II; Eli Lilly, Indianapolis, IN) was used for the hyperinsulinemic-euglycemic clamps. Blood glucose was maintained at baseline level with a constant adaptation of glucose delivery by 20% dextrose (Baxter Healthcare, Deerfield, IL). A crystalline amino acid solution of 10% Travasol was obtained from Baxter Healthcare.

Experimental design. All 24 animals were randomized to three study groups of 8 each. Studies were performed after 2 days of acclimatization to the laboratory environment. The animals were kept on a 12:12 h light:dark cycle and received identical standard diet (Lean Grow 93; O'Lakes, Fort Dodge, IA). On the morning of the experimental day, after a 12-h fast with ad libitum access to water, the animals were administered an intramuscular ketamine injection (12.5 mg/kg body wt, Ketaset; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine injection (2 mg/kg body wt, Rompur; Miles, Shawnee Mission, KS) as a tranquilizer and as an anesthesia. Anesthesia was then maintained by continuous inhalation of Isoflurane (Abbott Laboratories, Chicago, IL), delivered by a mouth-mask in oxygen at 5 l/min. A Harvard Respirator (Apparatus Dual Phase Control; Harvard Apparatus, South Natick, MA) was used for mechanical ventilation throughout the study and was adjusted to achieve a normal carbon dioxide tension. Continuous electrocardiograph monitoring was performed. A heating blanket maintained body temperature. Polyethylene catheters were inserted into the femoral vein for tracers, insulin, glucose, and amino acid infusions and into the femoral or carotid artery for blood samplings.

Protocol design was identical for all animals except that they were divided in three distinct groups receiving saline, insulin and glucose, or insulin and glucose and amino acids. A prime (1 mg/kg body wt) and continuous infusion of L-[1-^{13}C]leucine (1 mg · kg body wt^{-1} · h^{-1}) was administered via the femoral vein. Eight animals were studied during a euglycemic-hyperinsulinemic clamp (INS group) by infusing purified pork insulin at a rate of 0.7 mU · kg body wt^{-1} · min^{-1}. Variable doses of 20% dextrose were infused to maintain the baseline blood glucose level. The infusion rate of dextrose was adjusted based on blood glucose checked every 15 min with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA). A second group of eight animals was given insulin at an identical infusion rate (0.7 mU · kg body wt^{-1} · min^{-1}) with 20% dextrose, but an infusion of amino acid mixture (10% Travasol) was added at a rate of 2 ml · kg body wt^{-1} · h^{-1} (INS+AA group). A control group of eight animals received saline (S group) alone at a rate of 5–10 ml · kg^{-1} · h^{-1} to match the other two groups. Arterial blood samples were collected before and during the tracer infusions for analysis of leucine isotopic enrichment, amino acid concentrations, and plasma insulin levels at baseline (T0) and at time points 4, 5, 6, 7, and 8 h. Through a skin incision performed in the midthigh, a muscle biopsy was taken at 8 h and myocardium pieces were also taken at 8 h before tracer interruption. Liver samples were taken through an open abdominal incision at 4 and 8 h. All tissue samples were immediately frozen in a liquid nitrogen bath and stored at −80°C until further analysis. At the completion of the study, animals were killed by an intravenous lethal dose of sodium pentobarbital (Sleepaway, Fort Dodge Laboratories).

Analysis. A 100- to 150-mg piece of each tissue was used for the isolation of mitochondrial, sarcoplasmic proteins, and, in muscle only, MHC (16,18), as previously described (17). The purity of the MHC fractions was checked by analytic SDS-PAGE on a 5% gel followed by silver staining. All tissue proteins were hydrolyzed (6 mol/l HCl for 24 h at 110°C), and the constituent amino acids in the hydrolysate were purified by cation exchange chromatography (Dowex 50W 8X; Bio-Rad Laboratories, Hercules, CA). Amino acids were eluted in 4 ml of 4 mol/l NH_4OH. Leucine in the protein hydrolysates was evaporated to dryness, methylated in a mixture of methanol/HCl for 60 min at 85°C, and acylated in acetone-trimethylamine-acetic anhydride for 45 min at 85°C. The product was then dissolved in ethyl acetate in a new vial before injection in the mass spectrometer. Muscle tissue fluid was collected as previously described from a small muscle sample (19,20). tRNA in liver tissue was isolated as previously described (19,20), and amino acids were hydrolyzed from tRNA.

Mass spectrometry. Isotopic enrichment of plasma [^{13}C]ketoisocaproate (KIC) and [^{13}C]leucine in both tissue fluid amino acyl tRNA and tissue proteins was determined as previously described (19). Isotopic enrichment of plasma [^{13}C]KIC was measured with a gas chromatograph–mass spectrometer (GC-MS) as the quinoxalinol-trimethylsilyl derivative. Leucine in plasma and leucyl tRNA was derived as its N-heptafluorobutyryl methyl ester for tissue fluid analysis, and isotopic enrichment was measured by GC-MS. Selective mass-to-charge (m/z) fragment ions for leucine and KIC were monitored. Carbon dioxide enrichment from the carboxyl group of leucine in tissue proteins was measured using an on-line gas chromatography/combustion isotope-ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) as previously described (21).

Plasma amino acid concentrations were measured by a high-performance liquid chromatography system as previously described (22). Plasma insulin was measured by chemiluminescent sandwich assay (Sanofi Diagnostics, Chaska, MN). Plasma glucose concentrations were measured enzymatically with an autoanalyzer (Beckman Instruments).

Calculations. FSR of specific proteins (MHC, sarcoplasmic, and mitochondrial proteins) in the three tissues (muscle, heart, and liver) were calculated by measuring the time-related changes in protein-bound ^{13}C-leucine enrichments, divided by the enrichment in the precursor pool, i.e., ^{13}C-KIC or ^{13}C-Leu in plasma, and ^{13}C-leucine in tissue fluid in all tissues, and [^{13}C]leucyl tRNA in case of liver (19,23,24).

Statistical analysis. All data are presented as the means ± SE. Analysis of variance (ANOVA) was used to calculate the differences among treatment groups for each of the three tissues. When ANOVA was statistically significant, an unpaired t test was applied to locate pairwise differences between groups. A rank difference test was used to compare the relative treatment effects on mitochondrial versus sarcoplasmic protein synthesis rate in muscle. In this procedure, mitochondrial and sarcoplasmic protein synthesis values in the S and INS or S and INS+AA groups were independently ranked, and then the difference between the mitochondrial and sarcoplasmic rank for each animal was obtained. An unpaired t test was used to compare the mean rank value of S versus INS or INS+AA. The same procedure was used to compare the relative treatment effects on mitochondrial versus MHC synthesis rate in muscle. The threshold for significant differences was set at $\alpha = 0.05$.

RESULTS

Plasma glucose and insulin concentrations. Plasma glucose was maintained at baseline level within the normal range in all groups throughout the study (86.6 ± 5.1 vs. 90.8 ± 3.4 vs. 91.4 ± 5.5 mg/dl, S versus INS versus INS+AA groups, respectively; NS). Plasma insulin concentration was not different in the INS and INS+AA groups but was significantly higher than in the S group (1.78 ± 0.28 vs. 36.47 ± 2.43 vs. 40.32 ± 2.39 μU/ml, S versus INS versus INS+AA, respectively; $P < 0.01$ versus S).

Plasma amino acid concentration. The concentration of plasma total amino acids decreased during insulin infusion in the INS group in comparison with the S group (3,541 ± 384 vs. 4,316 ± 476 μmol/l, INS versus S, respectively; $P < 0.05$) and for branched-chain amino acids (BCAA; 625 ± 77 vs. 967 ± 130 μmol/l, INS versus S, respectively; $P < 0.05$). The infusion of amino acid solution in the INS+AA group induced an elevation of BCAA and total amino acid levels above the other two groups (2,035 ± 104 and 7,375 ± 713 μmol/l for BCAA and total amino acids, respectively; $P < 0.01$ versus S and INS).

Leucine enrichments in plasma, tissue fluids, and tissue proteins. The average plasma [^{13}C]KIC enrichments from 4 to 8 h, in mole % excess, were 3.68 ± 0.09 (S), 3.97 ± 0.21 (INS), and 2.65 ± 0.06 (INS+AA). Plasma leucine enrichment from 4.8 h was 5.05 ± 0.20 (S), 5.68 ± 0.40 (INS), and 3.22 ± 0.12 (INS+AA). Plasma KIC and leucine enrichments in the INS+AA group were significantly lower ($P < 0.001$) than in both of the other two groups. [^{13}C]leucine enrichment in tissue fluid in the INS+AA group was significantly lower ($P < 0.05$) than in the S group in liver and muscle and lower than in the INS group alone in liver and heart (Table 1). Enrichment of Leu-tRNA in liver was 3.53 ± 0.25 (S), 3.86 ± 0.21 (INS), and 2.73 ± 0.17 (INS+AA). Liver tRNA enrichment in the INS+AA group was significantly lower ($P < 0.025$) than in both of the other two groups. Isotopic enrichment of leucine in mitochondrial proteins was lower in the INS+AA

TABLE 1
[^{13}C]leucine enrichments in tissue fluid and bound in mitochondrial and sarcoplasmic proteins in each tissue

Tissue	Tissue fluid (mole % excess)	Mitochondrial protein (atom % excess)	Sarcoplasmic protein (atom % excess)
Liver			
Saline	4.95 ± 0.37	0.132 ± 0.010	0.079 ± 0.015
Insulin	5.27 ± 0.36	0.102 ± 0.014	0.091 ± 0.011
INS+AA	3.91 ± 0.26*†	0.070 ± 0.007*	0.054 ± 0.008†
Heart			
Saline	3.98 ± 0.28	0.081 ± 0.004	0.085 ± 0.005
Insulin	4.50 ± 0.24*	0.094 ± 0.003*	0.105 ± 0.004*
INS+AA	2.88 ± 0.04†	0.068 ± 0.003*†	0.079 ± 0.004†
Muscle			
Saline	2.96 ± 0.17	0.020 ± 0.003	0.011 ± 0.002
Insulin	2.63 ± 0.23	0.036 ± 0.005*	0.017 ± 0.004
INS+AA	2.22 ± 0.10*	0.025 ± 0.003	0.010 ± 0.002

Data are means ± SE. Significant differences ($P < 0.05$) versus control (*) and insuline (†) groups. INS+AA, insulin in conjunction with amino acids.

group than in the S group in the liver and heart but was higher in the INS group than in the S group in the muscle and the heart. In the sarcoplasmic proteins, leucine enrichment was lower in the INS+AA than in the INS group in the heart and the liver. The enrichments in MHC protein in skeletal muscle, in atom % excess, were 0.0111 ± 0.0016 (S), 0.0150 ± 0.0036 (INS), and 0.0115 ± 0.0026 (INS+AA), with no significant differences among groups.

FSR of muscle-, heart-, and liver-specific proteins. We calculated FSR in all tissues using plasma [^{13}C]KIC, plasma [^{13}C]leucine, and [^{13}C]leucine tissue fluid enrichment as precursor pools, and in liver, Leu-tRNA was also used. Our previous studies clearly demonstrated that tissue fluid enrichment best represents amino acyl tRNA enrichment in different tissues (19,20). In nearly all tissue fractions, the effects of the treatments on FSR were similar, regardless of which precursor was used.

Basal synthesis rates of specific proteins in muscle, heart, and liver. FSR of mitochondrial and sarcoplasmic proteins in the three tissues of the saline group calculated using [^{13}C]leucine tissue fluid enrichment as precursor are shown in Fig. 1. Mitochondrial FSR was higher in liver than in heart and higher in heart than in muscle (all comparisons, $P < 0.001$). The sarcoplasmic protein FSR

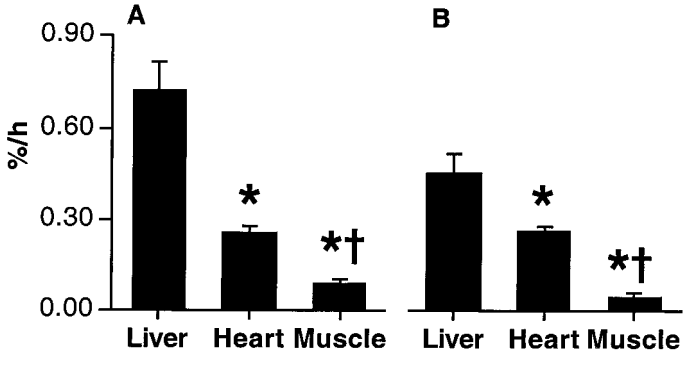

FIG. 1. FSR of mitochondrial (*A*) and sarcoplasmic/cytoplasmic (*B*) proteins in liver, heart, and skeletal muscle in control animals (saline), estimated using tissue fluid [^{13}C]leucine enrichment as precursor pool. *Less than liver ($P < 0.01$); †less than heart ($P < 0.01$).

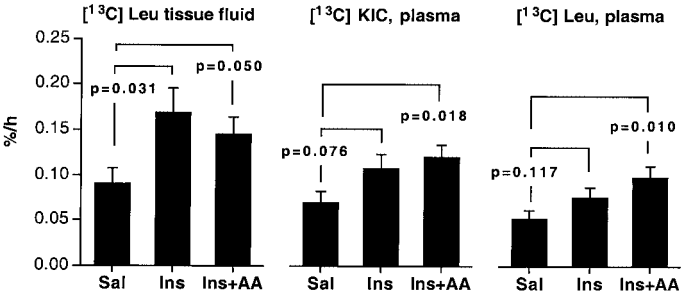

FIG. 2. Skeletal muscle mitochondrial protein FSR. Synthesis rates were calculated using the three precursor pool enrichments shown. *P* values reflect comparisons (*t* test) between S and either Ins or Ins+AA. There were no significant differences between Ins and Ins+AA ($P > 0.19$).

followed the same pattern because it was (in %/h) 0.723 ± 0.089 in liver, 0.266 ± 0.011 in heart, and 0.0372 ± 0.0065 in muscle using tissue fluid [^{13}C]leucine as precursor (all comparisons, $P < 0.001$). Similar differences between tissues were observed with plasma [^{13}C]KIC or [^{13}C]leucine as precursor.

Response of synthesis rates of specific proteins to insulin in liver, heart, and muscle. In comparison with the saline group (0.0906 ± 0.0170%/h), muscle mitochondrial FSR was 86% higher in INS and 60% higher in INS+AA when using [^{13}C]leucine enrichment in tissue fluid as precursor pool. Similar differences were observed using either plasma [^{13}C]KIC or [^{13}C]leucine as the precursor pool (Fig. 2). However, the FSR of both muscle sarcoplasmic proteins and MHC were not different among the three groups irrespective of the precursor pool used (Figs. 3 and 4). When the effects of the INS or INS+AA treatments on mitochondrial FSR were compared to treatment effects on sarcoplasmic or MHC FSR, using the rank difference test, there was no significant difference.

Heart mitochondrial and sarcoplasmic protein FSRs were not affected by insulin infusion alone (Figs. 5 and 6), but sarcoplasmic protein FSR, based on [^{13}C]leucine in tissue fluid, was significantly higher than S and INS when INS+AA was infused. Similar trends were evident when the other precursors were used to calculate FSR of heart proteins. In contrast to the stimulatory effects observed in muscle and heart, liver mitochondrial protein FSR tended to be lower in the INS and INS+AA groups versus S group using the various precursors, reaching statistical significance in most cases (Fig. 7). However, synthesis rates of

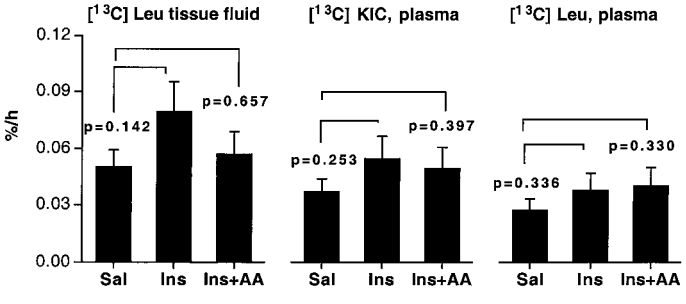

FIG. 3. Skeletal muscle sarcoplasmic protein FSR. Synthesis rates were calculated using the three precursor pool enrichments shown. *P* values reflect comparisons (*t* test) between S and either Ins or Ins+AA. There were no significant differences between Ins and Ins+AA ($P > 0.29$).

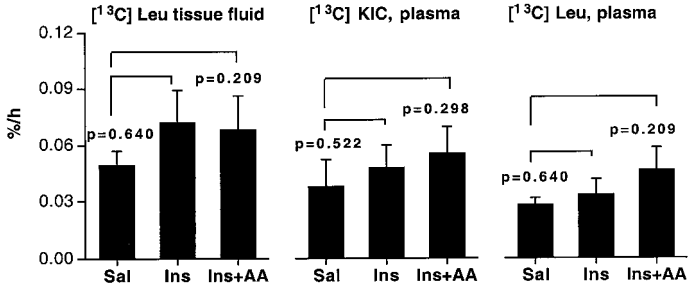

FIG. 4. Skeletal muscle MHC protein FSR. Synthesis rates were calculated using the three precursor pool enrichments shown. *P* values reflect comparisons (*t* test) between S and either Ins or Ins+AA. There were no significant differences between Ins and Ins+AA ($P > 0.42$).

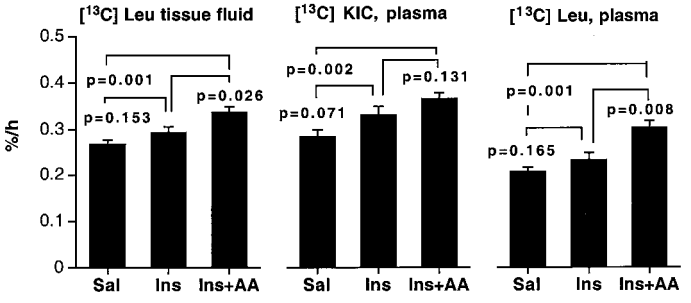

FIG. 6. Heart sarcoplasmic protein FSR. Synthesis rates were calculated using the three precursor pool enrichments shown. *P* values reflect comparisons (*t* test) among groups.

liver cytoplasmic proteins were not significantly different among the treatment groups (Fig. 8).

DISCUSSION

The current study demonstrated for the first time that insulin stimulates muscle mitochondrial protein synthesis, with no significant effect on synthesis rate of sarcoplasmic proteins and MHC. This effect of insulin is independent of circulating amino acids because infusing an amino acid mixture along with insulin had no additional stimulatory effect on mitochondrial protein synthesis. The effect of insulin was also found to be tissue-specific because insulin did not increase the synthesis rate of mitochondrial protein in heart and liver. The tissue-specific effects of insulin on protein synthesis are further demonstrated by the increased sarcoplasmic protein synthesis in heart by insulin plus amino acids.

The results from the current study could potentially resolve some of the controversies in the literature on in vivo effects of insulin on muscle protein synthesis (3,11,14,25–29). All of these previous studies are based on total (mixed) muscle protein synthesis, representing an average of several individual muscle proteins. The demonstration of differential effects of insulin on individual muscle protein fractions indicates that the measurement of an average synthesis rate of these different fractions may not be sufficiently sensitive to detect an insulin effect. It is likely that when mitochondrial content is high in a muscle, the changes related to mitochondrial protein synthesis are reflected in mixed muscle protein synthesis of that muscle. In young (9- to 10-week-old) mice, the mixed protein synthesis rate of the highly oxidative soleus muscle is 2–3 times higher than glycolytic muscles such as the plantaris or gastrocnemius (30). This increased mixed muscle protein synthesis in soleus is likely to reflect the higher mitochondrial content in this highly oxidative muscle. Of note, it has been reported that insulin has a stimulatory effect on mixed muscle protein synthesis in growing animals, whereas such stimulatory effect is attenuated by maturation and aging (29,31). The relatively lower mitochondrial content of aging muscle (32) and the decrease in mitochondrial protein synthesis that occurs with aging (16) render support to the above explanation for the discrepancy between studies performed in young and older animals. Lack of demonstrable effect of insulin on mixed muscle protein synthesis in humans is based on studies performed in mature adults (8,11–14). The differences between these human studies and studies in growing animals must therefore be cautiously interpreted, and the discrepancies could be resolved by studies based on measurements of synthesis rates of individual muscle proteins. The current study was performed in sexually mature miniature pigs and is comparable to young human adults.

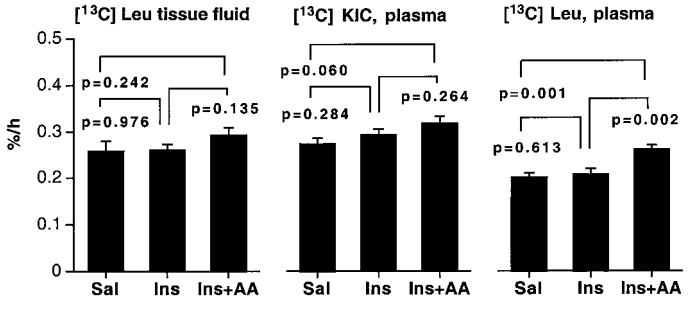

FIG. 5. Heart mitochondrial protein FSR. Synthesis rates were calculated using the three precursor pool enrichments shown. *P* values reflect comparisons (*t* test) among groups.

Because insulin is the predominant postprandial hormone and muscle is the major site of postprandial substrate disposal (33), it is not surprising that mitochondria, the cellular organelle responsible for oxidative energy metabolism, is affected by insulin. Previous studies have demonstrated that insulin has profound effects on energy metabolism (34). For enhanced mitochondrial functions, especially for oxidative phosphorylation, it is crucial that all mitochondrial protein complexes be present in sufficient quantity. It has been reported that increased availability of mitochondrial proteins in mitochondria is closely associated with enhanced mitochondrial respiration (35, 36). By stimulating mitochondrial protein synthesis, insulin may increase mitochondrial protein content and could thus enhance mitochondrial respiration.

The current study demonstrated an important regulatory role for insulin in determining muscle mitochondrial function. Several in vitro and animal studies have demonstrated that insulin stimulates various steps in protein synthesis process (37,38). Insulin's action at the posttranscriptional level especially in phosphorylating the elongation factors (eIF2, eIFB, eIF3, and eIF4) and phosphorylated heat- and acid-stable insulin-stimulated protein (eukaryotic initiation factor binding protein) (38,39) have been well characterized. The previous studies, however, were not designed to determine whether insulin's stimulatory effect is global to all muscle proteins or specific to certain

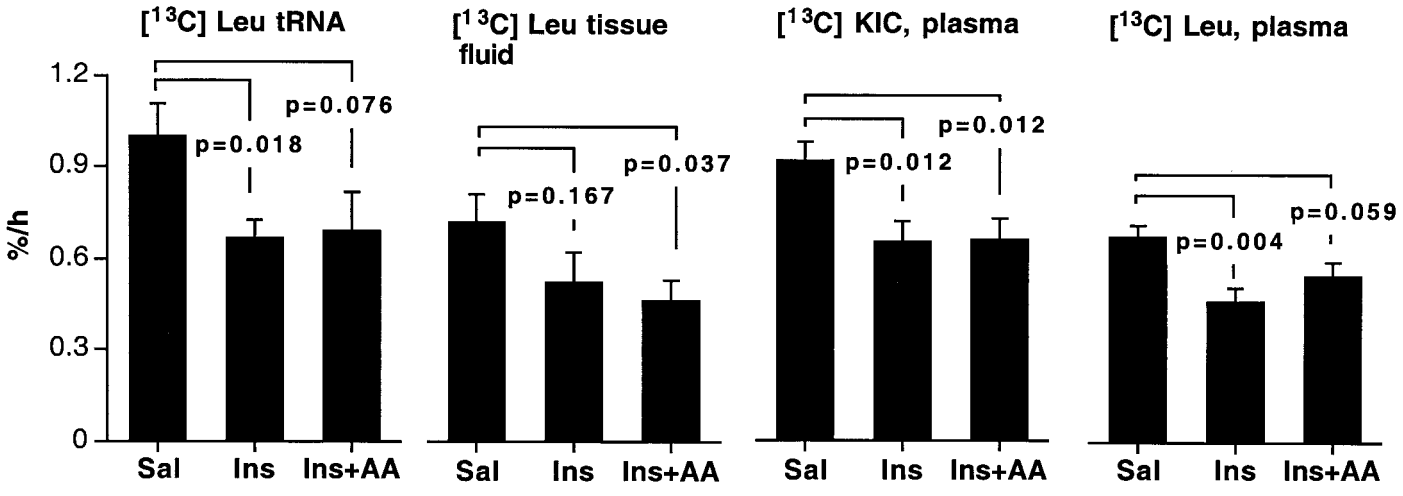

FIG. 7. Liver mitochondrial protein FSR. Synthesis rates were calculated using the four available precursor pool enrichments shown. *P* values reflect comparisons (*t* test) between S and either Ins or Ins+AA. There were no significant differences between Ins and Ins+AA ($P > 0.23$).

proteins. The current study clearly demonstrated that insulin does not stimulate the synthesis of all muscle proteins equally. The stimulatory effect of insulin on mitochondrial protein synthesis is consistent with the recent observation that insulin upregulates the expression of some mitochondrial genes in skeletal muscle (40).

The absence of a significant effect of insulin on muscle MHC synthesis rate is compatible with the previous observation that synthesis rate of MHC was not affected by insulin withdrawal in patients with type 1 diabetes (41). It is also possible that the relatively slower increase in isotope enrichment in MHC, a slow–turning-over muscle protein, makes the measurement of the increment of the isotope in muscle less precise than that of mitochondrial protein. The absence of any stimulatory action of insulin on MHC also does not exclude the effect of insulin on specific isoforms of MHC because MHC isolation does not differentiate between the various myosin isoforms. This is particularly important because insulin action in muscle has been reported to be influenced by muscle fiber type (42), and a recent study demonstrated that insulin infusion stimulated myosin isoform type 2X expression after 3 h of exposure to insulin (43). In addition, there is a growing body of evidence that muscle fiber composition is modified in the skeletal muscle of patients with type 2 diabetes (44). It is possible that insulin may have stimulated the synthesis rate of specific myosin isoforms while inhibiting synthesis of others, resulting in no net change.

It is intriguing that the effects of insulin on protein synthesis are highly tissue-specific. Mitochondrial protein synthesis in liver tends to be inhibited by insulin. These results are consistent with the previous interorgan studies demonstrating insulin's inhibitory effect on splanchnic protein synthesis (8,9). Studies performed in patients with diabetes suggest that these changes in protein synthesis do not occur in the gut (45) and thus occur in the liver. Studies in rodents also suggest that insulin deprivation or insulin administration has no effect on liver protein synthesis (3,46,47). However, the lack of insulin effect on liver protein synthesis is not universally supported in animal studies, especially when these studies focus on the specific action of insulin on albumin synthesis rate (10). These experiments suggest that insulin's effect on fixed liver tissue proteins and secreted plasma proteins may not be the same. It is clear, however, that liver, with substantially higher mitochondrial content and mitochondrial protein

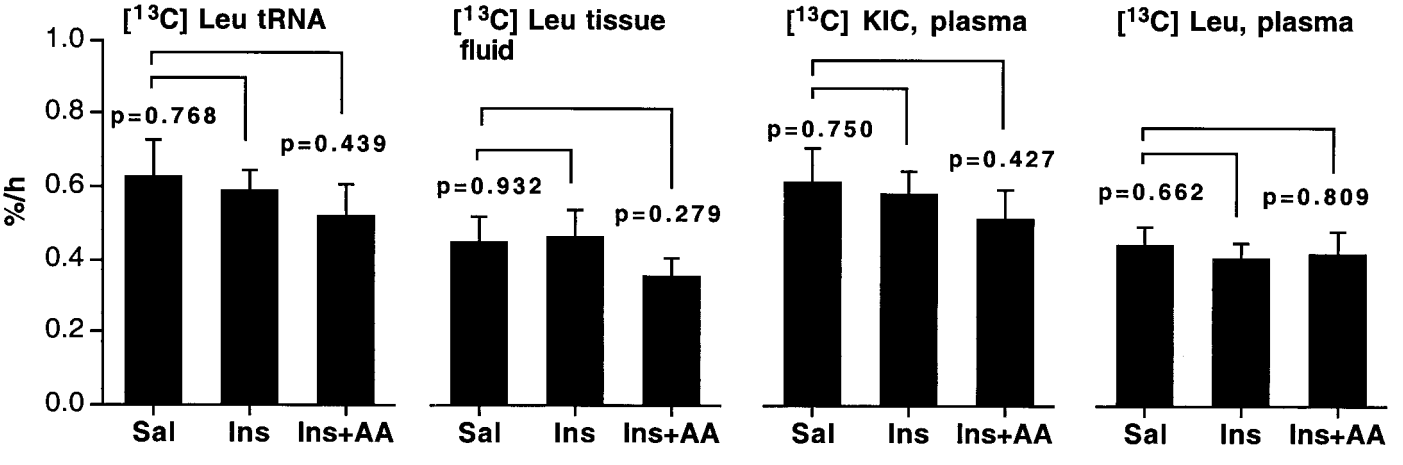

FIG. 8. Liver cytoplasmic protein FSR. Synthesis rates were calculated using the four available precursor pool enrichments shown. *P* values reflect comparisons (*t* test) between S and either Ins or Ins+AA. There were no significant differences between Ins and Ins+AA ($P > 0.26$).

synthesis than muscle (Fig. 1), responded differently to insulin than the muscle. During insulin infusion, the flux of substrates such as nonesterified fatty acids and amino acids decreases, thus reducing the overall liver substrate metabolism (48). However, replacement of amino acids alone failed to reverse the inhibitory effect of insulin on liver mitochondrial protein synthesis. Other potential factors that affect liver energetics include a suppression of gluconeogenesis in liver by insulin (48). At the postreceptor level, the insulin signaling pathway has been demonstrated to differ between the muscle and the liver. The potential differences in intracellular signaling events caused by insulin receptor substrates 1 and 2 in muscle and liver, respectively, may underlie the tissue-specific changes in protein synthesis seen in these two organs (49).

Another intriguing finding of the current study is that amino acids do not have any stimulatory effect on synthesis rates of mitochondrial proteins or cytoplasmic proteins in liver and any of the protein fractions in skeletal muscle. Amino acids, especially BCAA, have been shown to inhibit liver protein breakdown (50,51), thus achieving net protein anabolism in liver. A high-protein diet does not increase liver tissue size, thus arguing against simultaneous increase in liver protein synthesis and inhibition of liver protein breakdown, which would result in net protein accretion in liver tissue. It is more likely that liver protein balance is constantly maintained after a meal and between meals. The amino acids necessary for liver protein synthesis between meals is provided by breakdown of protein in peripheral tissues, especially in skeletal muscle (9). It is not clear whether this inhibitory effect of amino acids on protein breakdown is universal to all proteins in liver or specific to any particular proteins. The current study and our previous report (52) demonstrated that amino acids do not stimulate liver protein synthesis. Orally administered leucine has been shown to stimulate mixed muscle protein synthesis in skeletal muscle by enhancing eIF4F formation independent of insulin (53). It has also been shown that amino acid imbalance (e.g., high levels of glutamine, tyrosine, and leucine) inhibits protein synthesis by modulating translation initiation factors (54). It is possible that the composition of amino acids in commercially available mixtures results in an imbalance of amino acids profile in the circulation, which by itself may prevent protein synthesis. All available evidence (53–58) indicates that the effect of amino acids on protein synthesis is complex and depends on the type of amino acid mixture administered. In addition, the duration of administration of amino acids may be a factor that determines the effect of amino acids on protein synthesis. Most of the previous studies demonstrating stimulation of mixed muscle protein synthesis by amino acids were conducted after acute elevation of amino acids (53,54,59), whereas in the current study, amino acids were continuously infused for 8 h. All of this indicates that the effect of amino acids on synthesis rates of various fractions of proteins in various tissues requires detailed future investigations.

The specificity of insulin's effect on different organs is further demonstrated by the lack of insulin effect on mitochondrial protein synthesis in the heart. However, insulin plus amino acids stimulated sarcoplasmic protein synthesis in the heart. Previous studies demonstrated that amino acids (specifically BCAA) stimulate myocardial mixed muscle protein synthesis in vitro (60,61). The current study demonstrated that when circulating amino acid levels were increased, myocardial sarcoplasmic protein synthesis increased, suggesting that the previous observation of increased myocardial protein synthesis by BCAA (61) is related to the sarcoplasmic protein pool. The functional implications of this finding remain to be determined.

In conclusion, insulin is a major regulatory hormone for mitochondrial protein synthesis in skeletal muscle, the major site of postprandial fuel metabolism. The effect of insulin, however, is highly tissue-specific, as demonstrated by the lack of stimulation of mitochondrial protein synthesis by insulin in liver and heart. This tissue-specific action of insulin has important implications in understanding the role of insulin in the regulation of substrate metabolism and the potential relationship between altered mitochondrial functions and the pathogenesis of type 2 diabetes.

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REFERENCES

1. Geyelin HR, Du Bois EF: A case of diabetes of maximum severity with marked improvement: a study of blood, urine and respiratory metabolism. *JAMA* 66:1532–1534, 1916
2. Jefferson LS, Kimball SR: *Insulin Regulation of Protein Synthesis. Insulin Action*. New York, Alan R. Liss, 1989, p. 133–142
3. Pain VM, Garlick PJ: Effect of streptozotocin diabetes and insulin treatment on the rate of protein synthesis in tissues of the rat in vivo. *J Biol Chem* 249:4510–4514, 1974
4. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D: Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia* 25:400–403, 1983
5. Luzi L, Castellino P, Simonson DC, Petrides AS, DeFronzo RA: Leucine metabolism in IDDM: role of insulin and substrate availability. *Diabetes* 39:38–48, 1990
6. Umpleby AM, Boroujerdi MA, Brown PM, Carson ER, Sonksen PH: The effect of metabolic control on leucine metabolism in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 29:131–141, 1986
7. Tessari P, Biolo G, Inchiostro S, Sacca L, Nosadini R, Boscarato MT, Trevisan R, De Kreutzenberg SV, Tiengo A: Effects of insulin on whole body and forearm leucine and KIC metabolism in type 1 diabetes. *Am J Physiol* 259:E96–E103, 1990
8. Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J: Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. *J Clin Invest* 95:2926–2937, 1995
9. Meek SE, Persson M, Ford GC, Nair KS: Differential regulation of amino acid exchange and protein dynamics across splanchnic and skeletal muscle beds by insulin in healthy human subjects. *Diabetes* 47:1824–1835, 1998
10. De Feo P, Gaisano MG, Haymond MW: Differential effects of insulin deficiency on albumin and fibrinogen synthesis in humans. *J Clin Invest* 88:833–840, 1991

11. Biolo G, Fleming RYD, Wolfe RR: Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest* 95:811–819, 1995
12. Tessari P, Inchiostro S, Biolo G, Vincenti E, Sabadin L, Vettore M: Effects of acute systemic hyperinsulinemia on forearm muscle proteolysis in healthy man. *J Clin Invest* 88:27–33, 1991
13. Pacy PJ, Nair KS, Ford C, Halliday D: Failure of insulin infusion to stimulate fractional muscle protein synthesis in type I diabetic patients: anabolic effect of insulin and decreased proteolysis. *Diabetes* 38:618–624, 1989
14. Gelfand RA, Barrett EJ: Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest* 80:1–6, 1987
15. Hillier TA, Fryburg DA, Jahn LA, Barrett EJ: Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in the human forearm. *Am J Physiol* 274:E1067–E1074, 1998
16. Rooyackers OE, Adey DB, Ades PA, Nair KS: Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc Natl Acad Sci U S A* 93:15364–15369, 1996
17. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS: Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 273:E790–E800, 1997
18. Rooyackers OE, Balagopal P, Nair KS: Measurement of synthesis rates of specific muscle proteins using needle biopsy samples. *Muscle Nerve Suppl* 5:S93–S96, 1997
19. Ljungqvist O, Persson M, Ford GC, Nair KS: Functional heterogeneity of leucine pools in human skeletal muscle. *Am J Physiol* 273:E564–E570, 1997
20. Baumann PQ, Stirewalt WS, O'Rourke BD, Howard D, Nair KS: Precursor pools of protein synthesis: a stable isotope study in a swine model. *Am J Physiol* 267:E203–E209, 1994
21. Balagopal P, Ford GC, Ebenstein DB, Nadeau DA, Nair KS: Mass spectrometric methods for determination of [^{13}C]leucine enrichment in human muscle protein. *Anal Biochem* 239:77–85, 1996
22. Hill DW, Walter FH, Wilson TD, Stuart JD: High performance liquid chromatographic determination of amino acids in the picomole range. *Ann Chem* 51:1338–1341, 1979
23. Nair KS: Assessment of protein metabolism in diabetes. In *Clinical Research in Diabetes and Obesity: Part I. Methods, Assessment, and Metabolic Regulation.* Draznin B, Rizza RA, Eds. Totowa, NJ, Humana Press, 1995, p. 137–170
24. Halliday D, McKeran RO: Measurement of muscle protein synthesis rate from serial muscle biopsies and total body protein turnover in man by continuous intravenous infusion of l-[1-N-15]lysine. *Clin Sci Mol Med* 49:581–590, 1975
25. Oppenheim RW, Prevette D, Qin-Wei Y, Collins F, MacDonald J: Control of embryonic motoneuron survival in vivo by ciliary neurotrophic factor. *Science* 251:1616–1618, 1991
26. McNurlan MA, Essen P, Thorell A, Calder AG, Anderson SE, Ljungqvist O, Sandgren A, Grant I, Tjader I, Ballmer PE, Wernerman J, Garlick PJ: Response of protein synthesis in human skeletal muscle to insulin: an investigation with L-[$^{2}H_{5}$]phenylalanine. *Am J Physiol* 267:E102–E108, 1994
27. Rooyackers OE, Nair KS: Hormonal regulation of human muscle protein metabolism. *Annu Rev Nutr* 17:457–485, 1997
28. Watt PW, Corbett ME, Rennie MJ: Stimulation of protein synthesis in pig skeletal muscle by infusion of amino acids during constant insulin availability. *Am J Physiol* 263:E453–E460, 1992
29. Baillie AGS, Garlick PJ: Attenuated responses of muscle protein synthesis to fasting and insulin in adult female rats. *Am J Physiol* 262:E1–E5, 1992
30. Bark TH, McNurlan MA, Lang CH, Garlick PJ: Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice. *Am J Physiol* 275:E118–E123, 1998
31. Wray-Chen D, Nguyen HV, Burrin DG, Beckett PR, Fiorotto ML, Reeds PJ, Wester TJ, Davis TA: Response of skeletal muscle protein synthesis to insulin in suckling pigs decreases with development. *Am J Physiol* 275:E602–E609, 1998
32. Shigenaga MK, Hagen TM, Ames BN: Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci U S A* 91:10771–10778, 1994
33. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J: Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* 76:149–155, 1985
34. Nair KS, Halliday D, Garrow JS: Increased energy expenditure in poorly controlled type I (insulin-dependent) diabetic patients. *Diabetologia* 27: 13–16, 1984
35. Takahashi M, Hood DA: Protein import into subsarcolemmal and intermyofibrillar skeletal muscle mitochondria. *J Biol Chem* 271:27285–27291, 1996
36. McKee EE, Grier BL: Insulin stimulates mitochondrial protein synthesis and respiration in isolated perfused rat heart. *Am J Physiol* 259:E413–E421, 1990
37. Kimball SR, Jefferson LS: Regulation of initiation of protein synthesis by insulin in skeletal muscle. *Diabetologia* 28:1–6, 1991
38. Kimball SR, Horetsky RL, Jefferson LS: Signal transduction pathways involved in the regulation of protein synthesis by insulin in L6 myoblasts. *Am J Physiol* 274:C221–C228, 1998
39. Kimball SR, Jefferson LS, Fadden P, Haystead TAJ, Lawrence JC Jr: Insulin and diabetes cause reciprocal changes in the association of eIF-4E and PHAS-I in rat skeletal muscle. *Am J Physiol* 270:C705–C709, 1996
40. Huang X, Eriksson K-F, Vaag A, Lehtovirta M, Hansson M, Laurila E, Kanninen T, Thuesen Olesen B, Kurucz I, Koranyi L, Groop L: Insulin-regulated mitochondrial gene expression is associated with glucose flux in human skeletal muscle. *Diabetes* 48:1508–1514, 1999
41. Charlton MR, Balagopal P, Nair KS: Skeletal muscle myosin heavy chain synthesis in type 1 diabetes. *Diabetes* 46:1336–1340, 1997
42. Lillioja S, Young AA, Cutter CL, Ivy JL, Abbott WGH, Zawadzki JK, Yki-Jarvinen H, Cristin L, Secomb TW, Bogardus C: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J Clin Invest* 80:415–424, 1987
43. Houmard JA, O'Neill DS, Zheng D, Hickey MSDGL: Impact of hyperinsulinemia on myosin heavy chain gene regulation. *J Appl Physiol* 86:1828–1832, 1999
44. Marin P, Andersson B, Krotkiewski M, Bjorntorp P: Muscle fiber composition and capillary density in women and men with NIDDM. *Diabetes Care* 17:382–386, 1994
45. Charlton M, Ahlman B, Nair KS: The effect of insulin on human small intestinal mucosal protein synthesis. *Gastroenterology* 118:299–306, 2000
46. Hay AM, Waterlow JC: The effect of alloxan diabetes on muscle and liver protein synthesis in the rat, measured by constant infusion of L-(C-14) lysine. *J Physiol* 191:111P–112P, 1967
47. Mosoni L, Houlier ML, Mirand PP, Bayle G, Grizard J: Effect of amino acids alone or with insulin on muscle and liver protein synthesis in adult and old rats. *Am J Physiol* 264:E614–E620, 1993
48. Turk D, Alzaid A, Dinneen S, Nair KS, Rizza R: The effects of non-insulin-dependent diabetes mellitus on the kinetics of onset of insulin action in hepatic and extrahepatic tissues. *J Clin Invest* 95:755–762, 1995
49. Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, Accili D: Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. *J Clin Invest* 105:199–205, 2000
50. Mortimore GE, Khurana KK, Miotto G: Amino acid control of proteolysis in perfused livers of synchronously fed rats. *J Biol Chem* 266:1021–1028, 1991
51. Mortimore GE, Poso AR: Lysosomal pathways in hepatic protein degradation: regulatory role of amino acids. *Fed Proc* 43:1289–1294, 1984
52. Ahlman B, Charlton M, Fu A, Berg C, O'Brien P, Nair KS: Insulin's effect on synthesis rates of liver proteins: a swine model comparing various precursors of protein synthesis. *Diabetes* 50:947–954, 2001
53. Anthony JC, Anthony TG, Kimball SR, Vary TC, Jefferson LS: Orally administered leucine stimulates protein synthesis in skeletal muscle or postabsorptive rats in association with increased eIF4F formation. *J Nutr* 130:139–145, 2000
54. Shah OJ, Antonetti DA, Kimball SR, Jefferson LS: Leucine, glutamine, and tyrosine reciprocally modulate the translation initiation factors eIF4F and eIF2B in perfused rat liver. *J Biol Chem* 274:36168–36175, 1999
55. Vary TC, Jefferson LS, Kimball SR: Amino acid-induced stimulation of translation initiation in rat skeletal muscle. *Am J Physiol* 277:E1077–E1086, 1999
56. Nair KS, Matthews DE, Welle SL, Braiman T: Effect of leucine on amino acid and glucose metabolism in humans. *Metabolism* 41:643–648, 1992
57. Nair KS, Schwartz RG, Welle S: Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol* 263:E928–E934, 1992
58. Louard RJ, Barrett EJ, Gelfand RA: Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. *Clin Sci* 79:457–466, 1990
59. Rennie MJ, Edwards RHT, Halliday D, Matthews DE, Wolman SL, Millward DJ: Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci* 63:519–523, 1982
60. Chua BH, Siehl DL, Morgan HE: A role for leucine in regulation of protein turnover in working rat hearts. *Am J Physiol* 239:E510–E514, 1980
61. Young LH, McNulty PH, Morgan C, Deckelbaum LI, Zaret BL, Barrett EJ: Myocardial protein turnover in patients with coronary artery disease: effect of branched chain amino acid infusion. *J Clin Invest* 87:554–560, 1991