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Plasticity in Skeletal, Cardiac, and Smooth Muscle Selected Contribution: IGF-I antibody prevents increases in protein synthesis in epitrochlearis muscles from refed, diabetic rats

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Fedele, Mark J., Thomas C. Vary, and Peter A Farrell. Selected Contribution: IGF-I antibody prevents increases in protein synthesis in epitrochlearis muscles from refed, diabetic rats. *J Appl Physiol* 90: 1166–1173, 2001.—The purpose of this study was to examine whether immune neutralization of muscle-produced insulin-like growth factor I (IGF-I) would prevent an appropriate anabolic response to refeeding in diabetic rats. Male Sprague-Dawley rats were made diabetic by partial pancreatectomy and were randomly assigned to be either control-fed, fasted, or fasted-refed (n = 7–8 per group). Diabetes decreased rates of protein synthesis and increased rates of protein degradation in incubated epitrochlearis muscles ($P < 0.05$). In both groups of rats, fasting lowered protein synthesis and increased proteolysis and subsequent refeeding returned both parameters to near basal values ($P < 0.05$). Neutralization of muscle IGF-I by the addition of IGF-I antibody to the incubation medium reduced protein synthesis an average of 22% for all groups ($P < 0.05$). However, rates of protein degradation were not affected. In nondiabetic rats, refeeding increased protein synthesis in both control and antibody-treated muscles ($P < 0.05$). Refeeding also increased protein synthesis in the control muscles from diabetic rats ($P < 0.01$). In contrast, muscles from diabetic rats that were incubated with anti-IGF-I did not increase protein synthesis in response to refeeding. These data suggest that immune neutralization of muscle IGF-I in hypoinsulinemic rats negated the ability of endogenous IGF-I to promote protein synthesis and thereby prevented an appropriate anabolic response.

nutritional status; hypoinsulinemia; growth factors

BOTH ANABOLIC HORMONES, insulin and insulin-like growth factor I (IGF-I), and amino acids are important controllers of skeletal muscle protein synthesis in vivo and in vitro. Therefore, a fundamental determinant in the regulation of protein synthesis is the nutritional status of an animal, given that it affects the plasma concentration of anabolic hormones and amino acids. The perturbations of starvation and feeding on rates of protein synthesis in skeletal muscle have been previously documented (23, 29, 38). In general, starvation lowers rates of protein synthesis and subsequent refeeding restores protein synthesis to near basal values. Moreover, starvation has been found to result in a disproportionate degradation of myofibrillar protein compared with total protein (26). Thus skeletal muscle protein may serve as a major reservoir of amino acids and nitrogen during periods of nutritional flux.

In addition to insulin, the IGF-I system is sensitive to changes in nutritional status and various anabolic stimuli (17, 19, 32). Like insulin, IGF-I can stimulate protein synthesis and inhibit protein degradation in skeletal muscle (2, 7, 18, 30, 31, 35, 36). Previous studies have shown that there is an increase in the concentrations of plasma and gastrocnemius IGF-I in moderately diabetic rats following acute resistance exercise (13). We suggested that this increase is a compensatory adaptation that allows the hypoinsulinemic rats to elevate rates of protein synthesis in response to the anabolic stimulus. In support of this hypothesis, we have shown that when moderately diabetic rats were passively immunized against IGF-I the normal exercise-induced increases in protein synthesis rates were negated (unpublished observations).

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The relative importance of hepatic-produced endocrine IGF-I vs. locally produced paracrine and/or autocrine IGF-I in controlling skeletal muscle protein metabolism and the mechanisms distinguishing between the two are not fully elucidated. Systemically administered anti-IGF-I antibody successfully neutralized circulating IGF-I (unpublished observations) but may have been less effective at neutralizing locally produced intramuscular IGF-I. In the present study, we wanted to determine whether immune neutralization of locally produced IGF-I could likewise prevent an anabolic response to refeeding in diabetic rats. The approach taken to answer this question was to neutralize endogenously produced IGF-I by incubating epitrochlearis muscles with IGF-I antibodies.

The incubated epitrochlearis preparation has routinely been used to analyze the effects of various hormones (7, 35) and metabolic inhibitors (5) on protein turnover and glucose metabolism. The epitrochlearis muscle is also unique in that swimming (8, 9) and voluntary wheel running (unpublished observations) do not increase rates of protein synthesis, despite causing increases in glucose uptake and insulin sensitivity. Therefore, we chose response of protein metabolism to fasting and refeeding as a means to examine the role of locally produced IGF-I in accelerating protein accretion. Unlike the response to swimming and voluntary wheel running, protein metabolism in incubated epitrochlearis muscle is responsive to the effects of fasting and refeeding (29, 38). This approach consistently results in a decrease in protein synthesis during the fast followed by an increase back to near basal levels following refeeding. This experimental model thus provides the necessary increases in rates of protein synthesis to determine whether locally produced IGF-I is compensatory for insulin by facilitating the anabolic response to refeeding in hypoinsulinemic rats. Thus the purpose of this study was to investigate the effects of immune neutralization of IGF-I in vitro in controlling skeletal muscle protein turnover in diabetic rats in response to fasting and refeeding.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in all experiments. They were individually housed in wire-bottom cages in temperature- and humidity-controlled holding facilities with lights on at 0700 and off at 1900. Rats were fed ad libitum a standard rodent diet (diet 5001, PMI Feeds, Richmond, IN) that contained 24% protein, 12% fat, 50% carbohydrate, 7% ash, 6% fiber, and vitamins. The experimental design was a 2×3 comparison designated as follows: nondiabetic or diabetic $\times$ control fed or 48-h fasted or 48-h fasted and refed.

Partial pancreatectomy. On the basis of previous work (11), a partial pancreatectomy (PPX) procedure was used; the method was modified to include rats weighing 110–140 g as opposed to the weight (90–110 g) suggested by Folgia (16). We find that a larger percentage (~80%) of the animals become diabetic when heavier rats are pancreatectomized (unpublished observations). We also used a microcauterizer to eliminate small pancreatic blood vessels and to reduce bleeding during surgery. Rats were anesthetized using isoflurane and placed on a heated surgical pad, and sterile surgery was performed. The procedure requires the physical removal of pancreatic tissue from the splenic, duodenal, and pyloric regions, while leaving the major blood vessels intact. This is accomplished using sterile cotton Q-tips. Pancreatic tissue between the bile duct and the duodenum, ~10% of the original total pancreatic tissue, is not removed. At the conclusion of surgery, rats were given ampicillin subcutaneously (5 mg/100 g body wt; Sigma Chemical, St. Louis, MO) as an antimicrobial agent. Two weeks after PPX, a tail vein blood sample was obtained in the fed state to determine the plasma glucose concentration using a Beckman glucose analyzer 2. Rats that were either not diabetic (<300 mg/dl) or too severely diabetic (>600 mg/dl) were eliminated from the study. Age-matched nondiabetic rats were housed and handled in a manner identical to that for diabetic rats, with the exception of surgery and tail vein sampling to verify diabetic status. Our laboratory previously determined that there was no difference in the rate of muscle protein synthesis and plasma insulin concentrations between rats undergoing the sham pancreatectomy procedure and naïve control animals (12). Therefore, we have subsequently used rats with no surgical manipulation for nondiabetic controls.

Fasting and refeeding. Nondiabetic and diabetic rats were randomly assigned to either fasted, fasted and refed, or control-fed groups. The fasted rats had food removed from their cages 48 h before tissue procurement, and they had access to water ad libitum. In these experiments, the food was removed at 10 AM, and all physiological variables were analyzed at 10 AM 48 h later. The fasted and refed rats were fasted for 48 h and then were refed their standard chow ad libitum for 10 h before death. This group of rats had food removed at 12 AM, were refed at 12 AM 48 h later, and were analyzed 10 h later at 10 AM. Food consumption was also measured for the 10-h refeeding period. Body weights were measured before and after the 48-h fast and 10-h refeeding periods. The control-fed rats had continuous access to food and water.

Epitrochlearis incubations. The rats were anesthetized with isoflurane, and the skin on both forelimbs was removed. Both epitrochlearis muscles were excised intact and immediately placed in Krebs-Henseleit bicarbonate (KHB) buffer. With the use of stainless steel minibrads and plastic tubing, the tendons were then pinned at resting length, as opposed to flaccid, which is a factor shown to affect protein balance (1, 21). The muscles were quickly transferred to borosilicate glass tubes containing 3 ml of KHB buffer. After the epitrochlearis muscles were removed, the right gastrocnemius muscle was excised for the measurement of muscle IGF-I concentration. Blood was withdrawn directly from the left ventricle using a 20-gauge needle for the measurements of plasma glucose, insulin, and IGF-I. The epitrochlearis incubations were performed in a 37°C environmental chamber with gentle shaking and continuous gassing with 95% O_2-5% CO_2, as described previously (35). The KHB buffer consisted of (in mM) 110 NaCl, 25 $NaHCO_3$, 3.4 KCl, 1 $CaCl_2$, 1 $MgSO_4$, and 1 KH_2PO_4 (pH 7.4), supplemented with 5.5 glucose, 5 HEPES, 0.5 phenylalanine, 0.2 valine, 0.17 leucine, 0.1 isoleucine, and 0.01% (wt/vol) BSA.

Epitrochlearis muscles were first preincubated for 30 min. One muscle was preincubated in the presence of anti-IGF-I (sc-7144; Santa Cruz Biotechnology, Santa Cruz, CA) in the media, whereas the epitrochlearis from the contralateral limb was preincubated with an equal amount of nonspecific

IgG (sc-2028; Santa Cruz). The anti-IGF-I is an affinity-purified polyclonal antibody specific for human, rat, and mouse IGF-I as determined by epitope mapping to the carboxy terminus of the human IGF-I precursor. The antibodies were first dialyzed in 0.9% NaCl, and the concentration of antibody in the media was 1 μg/ml. Preliminary studies determined that this concentration of antibody was effective in achieving the maximal inhibition of protein synthesis. Also, pilot experiments showed no difference in terms of its effects on protein synthesis, whether the muscle was preincubated with the antibody for 30 or 60 min. After preincubation with anti-IGF-I antibody, the muscles were quickly rinsed in buffer and transferred to 3 ml of fresh buffer and incubated for 2 h. The KHB incubation buffer did not contain antibodies, and it was supplemented with 5 μCi/ml of L-[2,3,4,5,6-^{3}H]phenylalanine (Amersham Life Science, Arlington Heights, IL). At the end of the incubation, the muscles were quickly removed from the buffer, blotted dry, weighed, and frozen in liquid nitrogen. Muscles and incubation media were stored at −80°C until analysis.

Protein metabolism. In vitro rates of protein synthesis, expressed as nanomoles phenylalanine incorporated per milligram protein per hour, were measured by the incorporation of radioactive phenylalanine from the incubation medium into the epitrochlearis as previously described (35). Muscles were homogenized in 2 ml of 10% trichloroacetic acid (TCA) using a Polytron type T25-S1 (Janke and Kunkle) at 75% of maximum speed. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was decanted, and the pellet was washed two additional times with 10% TCA to eliminate any acid-soluble radioactivity. The final pellet was suspended in 1.5 ml of 1 N NaOH and incubated at 40°C for at least 30 min with frequent vortexing until the pellet completely dissolved. Aliquots were assayed for total protein using a Lowry protein determination assay kit (Sigma Chemical) and measured for radioactivity by liquid scintillation counting (Beckman model LS-6500) with appropriate correction for quench. An aliquot of the incubation medium was also measured to determine the specific radioactivity of phenylalanine. Rates of protein synthesis were calculated by dividing the amount of radioactive phenylalanine incorporated into TCA-precipitable protein over the 2-h period by the specific radioactivity of phenylalanine in the incubation medium.

In vitro rates of protein degradation, expressed as nanomoles of tyrosine released per milligram of protein per hour, were measured by the accumulation of tyrosine in the incubation medium as previously described (6, 7, 33, 35). Tyrosine in the medium was measured fluorometrically as described previously (34, 37). Because tyrosine is neither synthesized nor metabolized by skeletal muscle, the accumulation of tyrosine in the incubation medium reflects net protein balance. Total protein degradation was determined as the sum of tyrosine accumulation in the incubation medium plus the amount of tyrosine equivalents incorporated into the muscle during the 2-h incubation period. The amount of tyrosine incorporated into muscle was determined by multiplying the amount of phenylalanine incorporated by 0.77, which is the molar ratio of tyrosine to phenylalanine in mixed skeletal muscle proteins (6, 7, 35).

Hormone and glucose assays. Plasma insulin concentrations were determined by radioimmunoassay (RIA) (27). The antibody (no. 1013, Linco Research, St. Charles, MO) used in the rat insulin assay also recognizes other mammalian insulin isoforms but does not cross-react with glucagon, pancreatic polypeptide, somatostatin, or IGF-I. Total plasma IGF-I was also determined by RIA after first being extracted using a modified acid-ethanol (0.25 N HCl-87.5% ethanol) procedure with cryoprecipitation (10, 24). The IGF-I antibody (lot no. AFP4892898, National Hormone and Pituitary Program) does not cross-react with either insulin or IGF-II, and the assay has a sensitivity of 0.03–0.08 ng/tube. Gastrocnemius muscles used for IGF-I determinations were extracted using acid homogenization and Sep-Pak C18 extraction (10, 24) and then measured by RIA. Plasma glucose was measured on a Beckman glucose analyzer model 2.

Statistical analysis. Statistical differences among groups were assessed by repeated-measures ANOVA using the PROC GLM procedure of SAS. For the analysis of physical characteristics and hormone concentrations, the design was a two (nondiabetic/diabetic) by three (control fed/48-h fasted/48-h fasted and refed) group by treatment comparison. For the analysis of in vitro epitrochlearis metabolism, the design was a two (nondiabetic/diabetic) by three (control fed/48-h starved/48-h starved and refed) by two (anti-IGF-I preincubation/nonspecific IgG preincubation) comparison. Each variable was tested separately with all groups included that allowed for comparisons on the effect of diabetes, feeding, and anti-IGF-I separately and combined. The number of comparisons was limited by the degrees of freedom based on the number of groups and the number of animals tested. When significant F ratios were present, a Student-Newman-Keuls post hoc procedure was used to evaluate differences among means. $P < 0.05$ was chosen a priori as statistically significant. Values are presented as means ± SE.

RESULTS

Table 1 provides the physical and physiological characteristics of the rats in the study. Fasted rats weighed significantly less ($P < 0.05$) than the control and refed rats for both the nondiabetic and diabetic groups. The nondiabetic refed rats weighed less (320 ± 4 g) than the nondiabetic controls (365 ± 5 g, $P < 0.05$). Diabetic rats had higher plasma glucose concentrations than the nondiabetic rats ($P < 0.01$). Fasted diabetic rats had lower plasma glucose concentrations (336 ± 16 mg/dl) compared with the control (574 ± 56 mg/dl) and refed (572 ± 58 mg/dl) diabetic rats ($P < 0.01$). In the nondiabetic group, fasting also lowered plasma glucose concentrations; however, this reduction did not reach statistical significance. Plasma insulin concentrations were lower in the diabetic compared with that in the nondiabetic rats ($P < 0.05$). The nondiabetic fasted rats had lower ($P < 0.01$) plasma insulin (76 ± 7 pmol/l) than the nondiabetic control (371 ± 74 pmol/l) and refed rats (539 ± 168 pmol/l). The diabetic fasted rats also had lower plasma insulin concentrations (54 ± 13 pmol/l) compared with the refed diabetic rats (186 ± 44 pmol/l, $P < 0.05$) but were not significantly lower than the control diabetics. Both groups of rats had similar weight losses during the 48-h fasting period, with the nondiabetic rats losing an average of 18 ± 1% of their body weight and the diabetic rats losing 20 ± 1%. There was no significant difference between the amount of food consumed during the 10-h refeeding period in diabetic (0.073 ± 0.005 g food/g body wt) and nondiabetic (0.062 ± 0.003 g food/g body wt) rats.

Diabetic rats had significantly lower ($P < 0.01$) plasma IGF-I concentrations than nondiabetic rats (Fig. 1). In the nondiabetic rats, the control-fed ani-

Table 1. *Physical and physiological characteristics of the rats*

Group	*n*	Weight, g	Plasma Glucose, mg/dl	Plasma Insulin, pmol/l	Weight Loss, %body wt	Food Consumption, g/g body wt
Nondiabetic						
Control Fed	7	365 ± 5[a]	300 ± 11[a]	371 ± 74[a]		
Fasted	8	284 ± 2[a,b]	236 ± 17[a]	76 ± 7[a,e]	18 ± 1	
Fasted-Refed	7	320 ± 4[a,c]	276 ± 8[a]	539 ± 168[a]		0.062 ± 0.003
Diabetic						
Control Fed	7	263 ± 24	574 ± 56	110 ± 43		
Fasted	8	213 ± 20[b]	336 ± 16[d]	54 ± 13	20 ± 1	
Fasted-Refed	7	262 ± 13	572 ± 58	186 ± 44[f]		0.073 ± 0.005

Values are means ± SE; *n*, no. of rats per group. Fasted rats were starved for 48 h before measurements. Fasted-refed rats were starved for 48 h and then refed for 10 h before measurements. Weight loss is the percentage of body weight lost during the 48-h fast. Food consumption is the amount of food eaten during the 10-h refeeding period. [a]Nondiabetic groups are significantly different from the corresponding diabetic group ($P < 0.05$). [b]Fasted rats weighed less than respective control and refed rats ($P < 0.05$). [c]Refed rats weighed less than respective control rats ($P < 0.05$). [d]Fasting lowered plasma glucose concentrations in diabetic rats ($P < 0.01$). [e]Nondiabetic fasted rats had lower plasma insulin concentrations than the other nondiabetic groups ($P < 0.01$). [f]Diabetic refed rats had higher plasma insulin concentrations than diabetic fasted rats ($P < 0.05$).

mals had higher ($P < 0.01$) plasma IGF-I concentrations (1,180 ± 88 ng/ml) than either the fasted (501 ± 44 ng/ml) or refed animals (522 ± 42 ng/ml). The diabetic control rats had higher plasma IGF-I concentrations (403 ± 51 ng/ml) than the diabetic fasted rats (253 ± 38 ng/ml, $P < 0.05$) and the diabetic refed rats (315 ± 50 ng/ml), but, in this later comparison, the difference was not significant. There were no significant differences in plasma IGF-I concentrations between the fasted and refed groups for both the nondiabetic and diabetic rats.

Gastrocnemius IGF-I protein content is illustrated in Fig. 2. There was a significant reduction in muscle IGF-I in the control-fed ($P < 0.01$) and fasted ($P < 0.05$) diabetic rats compared with their respective nondiabetic groups. Fasting resulted in a diminished muscle IGF-I content compared with control-fed animals in both nondiabetic and diabetic rats ($P < 0.01$). With either nondiabetic or diabetic animals, refeeding did not cause muscle IGF-I content to return to values observed in fed animals. There were no significant differences in muscle IGF-I concentrations between the fasted and refed groups for both the nondiabetic and diabetic rats.

Figure 3 shows in vitro rates of protein synthesis in incubated epitrochlearis muscles (expressed as nmol phenylalanine·mg protein^{-1}·h^{-1}). Compared with respective IgG-treated muscles, preincubation of muscles with anti-IGF-I lowered rates of protein synthesis an average of 22% for all treatment groups. The differences in protein synthesis between the anti-IGF-I and respective IgG-treated muscles were significant ($P < 0.05$) for all groups with the one exception of the diabetic fasted rats. For all groups, rates of protein synthesis in muscles from nondiabetic animals were higher than the respective diabetic animals ($P < 0.01$). Fasting reduced protein synthesis in both nondiabetic and diabetic rats ($P < 0.01$), and refeeding restored rates of protein synthesis to values obtained in fed animals. This finding is consistent with observations in

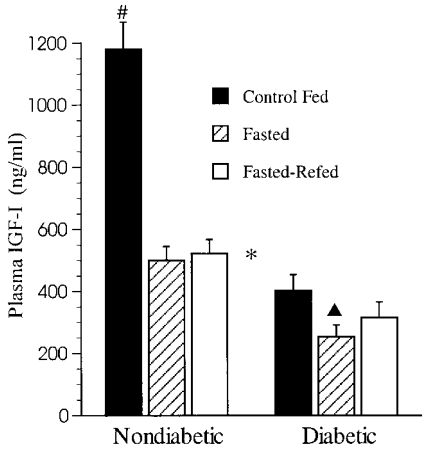

Fig. 1. Total plasma insulin-like growth factor I (IGF-I) concentrations. Values are means ± SE; n = 7–8 rats per group. Fasted rats were starved for 48 h before measurements. Fasted-refed rats were starved for 48 h and then refed for 10 h before measurements. *Values for all diabetic groups are lower than respective nondiabetic groups ($P < 0.01$ for control-fed animals and $P < 0.05$ for fasted and fasted-refed animals). #Nondiabetic fasted and fasted-refed rats are lower than nondiabetic control-fed rats ($P < 0.01$). ▲Diabetic fasted rats are lower than diabetic control-fed rats ($P < 0.05$).

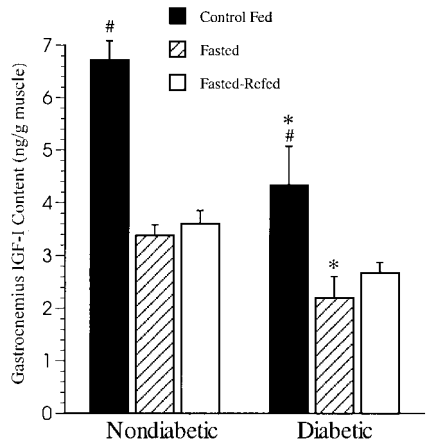

Fig. 2. Gastrocnemius muscle IGF-I peptide concentrations. Values are means ± SE; n = 7–8 rats per group. Fasted rats were starved for 48 h before measurements. Fasted-refed rats were starved for 48 h and then refed for 10 h before measurements. *Values for diabetic rats are lower than respective nondiabetic rats ($P < 0.01$ for control-fed and $P < 0.05$ for fasted). #Control-fed rats are higher than corresponding fasted and fasted-refed rats ($P < 0.01$).

vivo in which refeeding restored rates of protein synthesis, indicating that the epitrochlearis muscle incubated in vitro responds in a manner analogous to the in vivo setting. However, unlike muscles from nondiabetic rats, refeeding did not increase protein synthesis rates in muscles incubated with anti-IGF-I antibodies from diabetic rats. Furthermore, in the refed diabetic rats, rates of protein synthesis were reduced by 36% in muscles treated with anti-IGF-I compared with values from non-antibody-treated muscles.

In vitro rates of proteolysis in incubated epitrochlearis muscles (expressed as nmol tyrosine·mg protein^{-1}·h^{-1}) are shown in Fig. 4. Unlike protein synthesis, preincubation with anti-IGF-I did not significantly change proteolysis in any group of rats. Diabetes increased proteolysis in both the fed and refed groups ($P < 0.05$). Rates for fasted diabetic rats were not significantly higher than those for fasted nondiabetics. Fasted rats had higher rates of proteolysis compared with the fed and refed groups ($P < 0.05$), except for the diabetic anti-IGF-I preincubated muscles (2.67 ± 0.20), which were not significantly higher than values obtained in the antibody-treated fed group (2.29 ± 0.10).

DISCUSSION

The present study was undertaken because our laboratory has previously demonstrated that IGF-I may be compensatory by facilitating an anabolic response during diabetes-induced hypoinsulinemia (13). The findings from this study demonstrate that the increased rate of protein synthesis in epitrochlearis muscle normally observed after refeeding is negated in diabetic rats if the muscle is preincubated with a neutralizing antibody against IGF-I. In an analogous manner, when hypoinsulinemic rats are passively immunized against IGF-I, the normal exercise-induced increases in plasma and gastrocnemius IGF-I concentrations and corresponding elevations in rates of protein synthesis are negated (unpublished observations). However, passive immunization does not cause the same effect in nondiabetic rats despite lowering plasma IGF-I concentrations. This is perhaps because IGF-I is not functioning as a compensatory factor in normoinsulinemic rats.

Another study (unpublished observations) and the present findings suggest that the effects of immune neutralization of IGF-I appear to be particularly detrimental to diabetic animals during conditions when protein synthesis is normally elevated. Systemic administration of IGF-I-specific antibodies successfully decreased circulating IGF-I concentrations. However, it is uncertain as to whether antibodies to IGF-I were effective in neutralizing locally produced IGF-I. The in vitro analysis used in this study allowed for the effects of IGF-I treatment to be limited exclusively to IGF-I associated with muscle tissue because exogenous IGF-I was not added to the incubation medium. This enabled us to differentiate between the actions of hepatic IGF-I, which functions in an endocrine manner, and the paracrine and/or autocrine function of locally produced IGF-I.

Compared with IgG-treated muscles, incubation with anti-IGF-I lowered rates of protein synthesis in

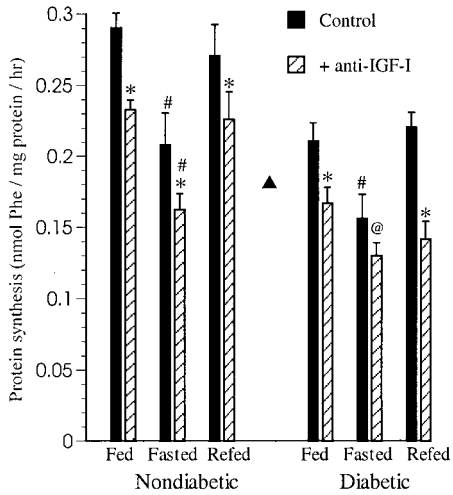

Fig. 3. Epitrochlearis in vitro rates of protein synthesis. Values are means ± SE; n = 7–8 per group. Phe, phenylalanine. Fasted rats were starved for 48 h before measurements. Refed rats were starved for 48 h and then refed for 10 h before measurements. +Anti-IGF-I refers to muscles that were preincubated with anti-IGF-I antibodies. Control muscles were preincubated with nonspecific IgG. *Preincubation with anti-IGF-I lowered rates of protein synthesis ($P < 0.05$ for nondiabetic fasted, nondiabetic refed, and diabetic fed; $P < 0.01$ for nondiabetic fed and diabetic refed). ▲Values for all diabetic groups are lower than the respective nondiabetic groups ($P < 0.01$). #Protein synthesis in fasted muscles is lower than the fed and refed muscles ($P < 0.01$). @Protein synthesis in anti-IGF-I-treated muscles from fasted diabetic rats is lower than the respective muscles from control-fed diabetic rats ($P < 0.05$).

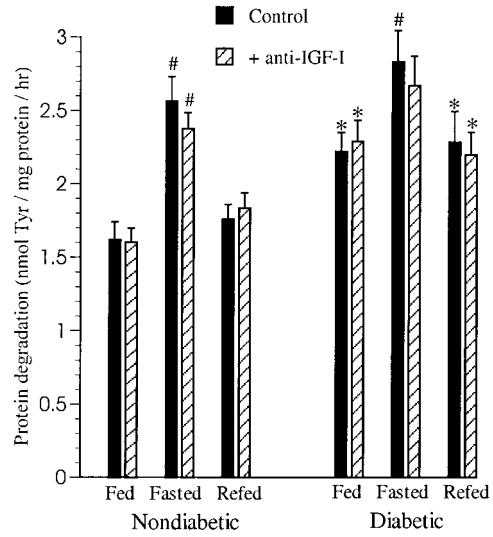

Fig. 4. Epitrochlearis in vitro rates of protein degradation. Values are means ± SE; n = 7–8 per group. Tyr, tyrosine. Fasted rats were starved for 48 h before measurements. Refed rats were starved for 48 h and then refed for 10 h before measurements. *Proteolysis is higher in the diabetic compared with the corresponding nondiabetic rats ($P < 0.05$ for refed nondiabetic vs. diabetic rats; $P < 0.01$ for control-fed nondiabetic vs. diabetic rats). #Proteolysis is higher in fasted rats compared with control-fed and refed rats ($P < 0.05$ for diabetic rats; $P < 0.01$ for nondiabetic rats).

all groups except the diabetic fasted rats (Fig. 3). Thus the effect of immunoneutralizing muscle IGF-I to limit protein synthesis rates is exhibited in both nondiabetic and diabetic rats. In contrast to protein synthesis, incubating the muscles with anti-IGF-I did not significantly change in vitro rates of proteolysis (Fig. 4). We expected proteolysis to increase in muscles treated with the IGF-I antibody. Koea et al. (22) demonstrated in vivo that an intravenous bolus of IGF-I antiserum caused a transient (1 h) increase in whole body net protein catabolism as evidenced by increased endogenous urea production. Moreover, supplementation of the incubation medium with IGF-I has been shown to increase protein synthesis and decrease proteolysis in epitrochlearis muscle (6, 7, 35).

In the present study, insulin and IGF-I were not added to the muscle incubation medium. This suggests that differences in rates of protein synthesis between the muscles from nondiabetic and diabetic rats are perhaps a result of a postreceptor defect. Rossetti and co-workers (28) demonstrated that IGF-I, but not insulin, is able to reverse some of the intracellular defects in insulin action in pancreatectomized rats. Thus IGF-I-mediated pathways are intact in diabetic rats, which can be considered additional support for the notion that IGF-I may compensate for insulin in diabetic animals. It is uncertain whether locally produced IGF-I exerts its physiological actions by being externalized and binding to its cell-surface receptor. Alternatively, it may bind to an internal receptor or have a direct intracellular effect. The particular antibody used in this study presumably bound to any endogenous IGF-I in the extracellular space irrespective of its origin. Therefore, the reductions in protein synthesis caused by the antibody treatment were perhaps a direct result of diminished ligand-receptor interactions.

The IGF-I system is extremely sensitive to changes in nutritional status. Starvation results in a reduction in circulating levels of growth hormone (19), IGF-I (20), and IGF-binding protein-3 (IGFBP-3) (32) and increased IGFBP-1 levels (19) and IGF-I receptor gene expression (25). Refeeding is capable of reversing these changes. These starvation-induced changes in IGF-I, IGF-I receptor, and binding protein concentrations are parallel to the changes in these factors stemming from diabetes (3, 4, 14). Circulating levels of IGFBPs were not measured in the present study. Because local concentrations of IGF-I and its bioavailabilty are influenced by the prevailing levels of IGFBPs, one cannot rule out the possibility that differences in binding proteins are influencing the changes that were observed in protein synthesis. In vitro protein turnover was measured in a medium that did not contain exogenous IGF-I or IGF-I binding proteins. Thus differences in the local concentration of these variables as well as their interaction with each other, which would affect protein turnover in incubated muscles, would be a result of changes in IGF-I and binding proteins present in the extracellular space. Therefore, it is conceivable that local concentrations of IGF-I and its interaction with binding proteins in vitro may be different from those occurring in vivo.

The 48-h fast lowered plasma insulin and glucose concentrations in the nondiabetic rats; however, the reductions in glucose were not significant (Table 1). In the hypoinsulinemic rats, fasting was effective in normalizing plasma glucose concentrations (Table 1). Differences in food consumption can potentially affect differences observed in skeletal muscle protein turnover between nondiabetic and diabetic rats. However, our laboratory has previously reported that pancreatectomized rats actually consume more food relative to body weight than nondiabetic rats, although the difference is not significant (11). Likewise, the refed diabetic rats in this study consumed more food (nonsignificant difference) than the nondiabetic rats during the 10-h refeeding period (Table 1). Thus the diabetic rats are not malnourished, and differences in food consumption between nondiabetic and diabetic rats are not likely to be a factor responsible for the differences in protein turnover between the two groups. Moreover, the whole body catabolic effect of fasting was similar between the two groups of rats because they both lost a similar relative amount of weight (Table 1).

Along with marked hypoinsulinemia in the pancreatectomized rats, there was also a concomitant decrease in both total plasma (Fig. 1) and gastrocnemius IGF-I peptide content (Fig. 2), a finding that our laboratory (13, 14) and others (28) have previously observed. In the present study, a 48-h fast lowered plasma and gastrocnemius IGF-I concentrations in both nondiabetic and diabetic rats. Ten hours of refeeding did not restore either plasma or gastrocnemius IGF-I concentrations to normal levels. Frystyk et al. (19) found similar changes in circulating IGF-I in rats that were fasted for 3 days. Moreover, it was not until 3 days after refeeding that plasma IGF-I concentrations returned to normal levels. The Frystyk et al. study did not measure protein turnover; however, those findings in addition to the current ones presented here suggest that the stimulation of skeletal muscle protein synthesis after refeeding is not due to changes in total circulating IGF-I. Likewise, the refeeding-induced increases in protein synthesis cannot be explained by changes in muscle IGF-I. Despite this, incubation with anti-IGF-I prevented the increases in rates of protein synthesis in the muscles from refed diabetic rats. Perhaps the postprandial increases in circulating insulin and/or amino acids may be responsible for facilitating the increases in protein synthesis. Nevertheless, our findings suggest that, in hypoinsulinemic rats, IGF-I may be obligatory for these increases in protein synthesis to occur.

In conclusion, incubation of epitrochlearis muscles with anti-IGF-I lowered in vitro rates of protein synthesis in nondiabetic and diabetic rats, whereas protein degradation rates were not changed as a result of the antibody. A 48-h fast lowered plasma and gastrocnemius IGF-I concentrations in both groups of rats,

and refeeding for 10 h was not sufficient in raising IGF-I concentrations from fasted levels. Fasting lowered protein synthesis rates for both groups of rats, and subsequent refeeding restored them to near basal rates. However, the muscles from the diabetic rats that were incubated with anti-IGF-I did not exhibit the refeeding-induced increase in protein synthesis. These data provide additional evidence that IGF-I is compensating for insulin in hypoinsulinemic rats to facilitate an anabolic response.

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