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# Resistance of protein and glucose metabolism to insulin in denervated rat muscle

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Denervated (1–10 days) rat epitrochlearis muscles were isolated, and basal and insulin-stimulated protein and glucose metabolism were studied. Although basal rates of glycolysis and glucose transport were increased in 1–10-day-denervated muscles, basal glycogen-synthesis rates were unaltered and glycogen concentrations were decreased. Basal rates of protein degradation and synthesis were increased in 1–10-day-denervated muscles. The increase in degradation was greater than that in synthesis, resulting in muscle atrophy. Increased rates of proteolysis and glycolysis were accompanied by elevated release rates of leucine, alanine, glutamate, pyruvate and lactate from 3–10-day-denervated muscles. ATP and phosphocreatine were decreased in 3–10-day-denervated muscles. Insulin resistance of glycogen synthesis occurred in 1–10-day denervated muscles. Insulin-stimulated glycolysis and glucose transport were inhibited by day 3 of denervation, and recovered by day 10. Inhibition of insulin-stimulated protein synthesis was observed only in 3-day-denervated muscles, whereas regulation by insulin of net proteolysis was unaffected in 1–10-day-denervated muscles. Thus the results demonstrate enhanced glycolysis, proteolysis and protein synthesis, and decreased energy stores, in denervated muscle. They further suggest a defect in insulin's action on protein synthesis in denervated muscles as well as on glucose metabolism. However, the lack of concurrent changes in all insulin-sensitive pathways and the absence of insulin-resistance for proteolysis suggest multiple and specific cellular defects in insulin's action in denervated muscle.

## INTRODUCTION

Contractile activity markedly affects the metabolism of glucose as well as the response to insulin in skeletal muscle. During contraction, glucose uptake is enhanced and this increased glucose transported into muscle is primarily metabolized through the glycolytic pathway (Nesher *et al.*, 1985). Immediately after contractile activity, glucose uptake remains elevated and is channelled primarily toward the replenishment of glycogen stores (Davis *et al.*, 1986). Although insulin is not required to increase glucose uptake during contraction (Nesher *et al.*, 1985), after contractile activity the insulin dose required for half-maximum stimulation is decreased, suggesting an increase in insulin-sensitivity, and the maximal response to insulin is increased (Davis *et al.*, 1986). In contrast, a decrease in muscle activity owing to denervation markedly decreases the ability of insulin to stimulate glycogen synthesis and glucose transport, by decreasing the sensitivity and maximal response to insulin (Burant *et al.*, 1984; Smith & Lawrence, 1985). Although the initial defect appears to be in the ability of insulin to stimulate glycogen synthase, the effect on the glycolytic pathway of a decrease in muscle activity owing to denervation has not been examined.

Contractile activity also alters muscle protein turnover, primarily by decreasing protein synthesis (Davis & Karl, 1986); yet it also enhances the responses of protein synthesis, protein degradation and amino acid transport to insulin (Davis & Karl, 1986; Zorzano *et al.*, 1985). Denervation results in atrophy of muscle, probably owing to an increase in protein degradation, since protein synthesis was reported to decrease initially, and then to increase, after denervation (Goldspink, 1976). Denervation also has been reported to decrease the insulin-stimulated uptake of α-aminoisobutyrate (Forsayeth & Gould, 1982; Turinsky, 1987). However, the effect of denervation on the response of protein synthesis and degradation to insulin has not been studied. The determination of whether insulin-resistance is present in insulin-stimulated pathways other than glycogen synthesis and glucose transport is particularly important, since this would be useful in the identification of the specific defect(s) in insulin's action on denervated muscle (Kahn, 1978).

In the present study, the effects of 1–10 days of denervation on the utilization of glucose via glycolysis (glycolytic utilization), glycogen synthesis, glucose transport, protein synthesis, protein degradation, amino acid metabolism and high-energy-phosphate contents, as well as the response of these parameters to insulin in skeletal muscle, were investigated. The results demonstrate enhanced glycolysis, glucose transport, proteolysis, and protein synthesis and decreased energy stores in denervated muscle. These results further provide evidence that denervation-induced insulin resistance in muscle develops with different time courses, dependent on the insulin-stimulated function examined, and suggest that insulin-dependent functions are under separate sets of control.

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## METHODS

### Animals

Female Sprague–Dawley rats were anaesthetized with chloral hydrate (36 mg/100 g body wt., intraperitoneally), and one epitrochlearis muscle from each rat was denervated by sectioning the ulnar nerve. Nerves to contralateral epitrochlearis muscles were left intact. Muscles from normal rats not subjected to surgery also served as controls. After 1, 3, 5, 7 and 10 days of denervation, rats were decapitated, and muscles were removed quickly and incubated.

The entire experiment was conducted twice, and 6–12 rats were used for each time point at each experiment. Muscles from all animals used in each experiment were incubated on the same day (between 08:00 and 13:00 h). The animals weighed 120–140 g at the initiation of the experiment and 160–180 g on the day of incubation. Although feed intake was not measured, growth rates were relatively constant (approx. 4.0 g/day) throughout the study, except on day 1 after denervation (approx. 2.5 g/day).

### Incubation of muscles

Epitrochlearis muscles were incubated in 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) (Umbreit, 1957), containing 5.5 mM-glucose, 5 mM-Hepes buffer, 0.3% (w/v) purified bovine serum albumin (Sigma, St. Louis, MO, U.S.A.), 5 $\mu$Ci of [5-$^3$H]glucose (Amersham, Arlington Heights, IL, U.S.A.)/ml, 0.05 $\mu$Ci of [U-$^{14}$C]-phenylalanine (New England Nuclear, Boston, MA, U.S.A.)/ml, and 0.5 mM-phenylalanine. Muscles were incubated in the absence (basal) or the presence of insulin (10 munits/ml; purified glucagon-free pig insulin, kindly supplied by Eli Lilly, Indianapolis, IN, U.S.A.). During the 2 h of incubation at 37 °C, muscles were gassed continuously with $O_2/CO_2$ (19:1). After incubation, muscles and media were immediately frozen in Freon, cooled with liquid $N_2$.

### Analytical procedures

All measurements of glucose and protein metabolism, as well as release rates and intracellular contents of amino acids, glycolytic intermediates, ATP and phosphocreatine were done on each muscle. Glycogen synthesis was determined by measuring the incorporation of [5-$^3$H]glucose into glycogen as previously described (Stauffacher & Renold, 1969). The flux of extracellular glucose through the glycolytic pathway was determined by measuring the amount of $^3H_2O$ formed during the aldolase–triosephosphate isomerase reaction by muscles incubated with [5-$^3$H]glucose (Davis *et al.*, 1986) and is designated 'glycolytic utilization'. Because muscle is virtually lacking in the pentose phosphate pathway (Green & Landau, 1965; Nesher *et al.*, 1985), the glucose transported into muscle is utilized via glycolysis or the synthesis of glycogen, and thus glucose transport can be calculated as the sum of glycogen synthesis and glycolytic utilization. Glucose transport determined by summing these two parameters agrees with values obtained by using 2-deoxyglucose (results not shown).

Protein synthesis was calculated from the [$^{14}$C]-phenylalanine radioactivity in the trichloroacetic acid-precipitable protein and the extracellular specific radioactivity (Fulks *et al.*, 1975). Previous studies have shown that the intracellular, extracellular and aminoacyl-tRNA specific radioactivities are similar when high concentrations of the unlabelled amino acid are present in the media (Bylund-Fellenius *et al.*, 1984; Davis *et al.*, 1985; McKee *et al.*, 1978). Tyrosine release and intracellular concentration were determined by using a tRNA method as previously described (Harter *et al.*, 1979). Since both tyrosine and phenylalanine are not snythesized or catabolized by muscle (Goldberg & Chang, 1978), the release of tyrosine from each muscle, plus any change in the intracellular tyrosine concentration (final minus initial concentration), were measured to determine net protein degradation (Fulks *et al.*, 1975). Total protein degradation was calculated as the sum of net protein degradation and protein synthesis in each muscle. Previous studies from our laboratory have shown that the intracellular contents as well as the rates of release of phenylalanine and tyrosine from epitrochlearis muscle are comparable (Davis *et al.*, 1985).

Protein content in muscle was determined by the method of Lowry *et al.* (1951). Glycogen content was determined from the hydrolysis of glycogen and the fluorimetric measurement of glucose formed (Huijing, 1970). Intracellular substrate concentrations and/or release rates of alanine, pyruvate, glutamate, glutamine, lactate, ATP and phosphocreatine were measured by using enzymic fluorimetric techniques as previously described (Harter *et al.*, 1979).

### Statistics

Since there were no statistically significant differences between the two experiments as determined by analysis of variance (Snedecor & Cochran, 1967), the results of both experiments were combined. To determine differences among days of denervation, analysis of variance was conducted. Student's *t* test was used for comparisons between denervated and contralateral muscles in either the absence or the presence of insulin, as well as to test for the effect of insulin in denervated, contralateral and normal muscles. Repeated-measures analysis of variance was used to test whether there were differences between denervated and contralateral muscles in their response to insulin. Probability levels of less than 0.05 were considered statistically significant. All data are expressed on the basis of wet weight, because percentages of water and protein did not differ between denervated and contralateral muscles.

## RESULTS

### Muscle weight and protein content

Epitrochlearis muscle weights were decreased by 15% after 3 days of denervation, and decreased progressively to 48% of contralateral muscle weights by 10 days (Table 1). The protein concentration of epitrochlearis muscles did not change throughout the 10 days of denervation, but the total protein content per muscle declined in proportion to the decrease in muscle weight. Results from muscles of animals not subjected to surgery are presented in all Tables and Figures as 'day 0' and did not differ significantly from those in contralateral muscles in their weight, protein content or other parameters studied. This lack of significant differences between muscles from rats not subjected to surgery and muscles from controls suggests that the surgery itself had little effect on the parameters studied.

**Table 1. Effect of denervation on weight and protein content of epitrochlearis muscle**

Values are means ± S.E.M. for six determinations. Data presented for day 0 are from normal animals not subjected to surgery. *Contralateral differed significantly from denervated, $P < 0.05$.

| | Period of denervation (days) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 7 | 10 |
| Weight (mg) | | | | | | |
| Contralateral | 30.3±0.9 | 30.4±1.2 | 31.6±1.1 | 28.4±2.5 | 31.0±1.3 | 32.2±2.0 |
| Denervated | – | 30.4±0.9 | 26.8±1.6* | 20.3±1.4* | 20.7±1.3* | 16.7±1.4* |
| Protein (mg/100 mg) | | | | | | |
| Contralateral | 18.5±1.3 | 19.3±0.5 | 18.6±1.1 | 17.2±1.3 | 17.2±1.7 | 16.8±1.6 |
| Denervated | – | 17.4±1.7 | 18.9±1.6 | 17.2±2.3 | 16.7±2.1 | 15.3±1.2 |
| Protein (mg/muscle) | | | | | | |
| Contralateral | 5.6±0.4 | 5.8±0.3 | 5.9±0.5 | 4.8±0.4 | 5.3±0.5 | 5.4±0.5 |
| Denervated | – | 5.2±0.4 | 5.0±0.4 | 3.4±0.3* | 3.4±0.2* | 2.5±0.2* |

## Glucose metabolism

Basal glycogen contents in epitrochlearis muscles after 3–10 days of denervation were significantly less than those in contralateral muscles (Table 2). Insulin increased glycogen by approx. 50% in normal and contralateral muscles. The response to insulin was less in denervated than in contralateral muscles at 1, 3 and 5 days, and tended to be less at 7 days ($P < 0.07$). After 10 days of denervation, the increase in glycogen by insulin was comparable with that in contralateral muscles, although the absolute values remained markedly lower. When total glycogen content of each muscle incubated in the absence or presence of insulin was determined, a similar decrease in glycogen content as a result of denervation was found (results not shown). Glycogen contents determined in muscles that were frozen before incubation were similar to the basal values after incubation for 2 h (results not shown).

**Table 2. Effect of denervation on glycogen contents in epitrochlearis muscles**

Values (in μmol/g wet wt.) are means ± S.E.M., with numbers of determinations in parentheses. Day-0 data are from normal rats not subjected to surgery. Muscles were incubated in the absence (basal) or the presence of 10 munits of insulin/ml. Glycogen contents in muscles that were fast-frozen and not incubated were similar to basal values in incubated muscles. Values differ significantly ($P < 0.05$) for *denervated versus contralateral, †basal versus insulin in either denervated or contralateral muscles, and ‡response to insulin between denervated and contralateral muscles.

| Time (days) | Denervation | Glycogen | |
|---|---|---|---|
| | | Basal | Insulin |
| 0 | – | 27.4±1.7 (8) | 40.0±2.0† (8) |
| 1 | – | 24.2±1.8 (6) | 35.6±3.5† (6) |
| | + | 23.2±2.1 (6) | 21.8±2.0*‡ (6) |
| 3 | – | 29.2±1.7 (12) | 41.3±1.7† (12) |
| | + | 13.9±0.8 (12)* | 16.0±1.3*‡ (12) |
| 5 | – | 28.3±2.8 (6) | 44.4±3.2† (6) |
| | + | 15.0±0.8 (6)* | 17.7±2.0*‡ (6) |
| 7 | – | 30.1±2.5 (12) | 39.6±2.6† (12) |
| | + | 13.3±0.7 (12)* | 16.6±1.1*† (12) |
| 10 | – | 28.3±2.3 (6) | 43.4±1.3† (6) |
| | + | 20.1±2.1 (6)* | 33.4±3.6*† (6) |

Despite the decrease in basal (absence of insulin) glycogen contents in 3–10-day-denervated muscles, the basal rate of incorporation of [5-$^3$H]glucose into glycogen did not differ between denervated and contralateral muscles at any time after surgery (Fig. 1*a*). Fig. 1(*b*) illustrates the absolute change in the rates of glucose incorporation into glycogen in response to insulin in denervated and contralateral muscles as calculated from the differences between the mean rates in the absence and presence of insulin. The response of glycogen synthesis to insulin was less in denervated than in contralateral muscles by 1 day of denervation and throughout the period of study, indicating the presence of insulin resistance in denervated muscle. The most marked degree of resistance appeared to occur on day 3 of denervation.

The basal rate of utilization of glucose in the glycolytic cycle (glycolytic utilization) was increased in 1–10-day-denervated compared with contralateral muscles (Fig. 1*c*). Despite the elevated basal values, in the presence of insulin glycolytic utilization was not significantly different between denervated and contralateral muscles at 3 and 5 days. As illustrated in Fig. 1(*d*), the response to insulin was less in 3- and 5-day-denervated than in contralateral muscles. The lack of response to insulin in denervated muscles at 3 days was not due to a maximum having been reached in the absence of insulin, since glycolytic utilization rose from 76 nmol/min per g at 3 days to 119 nmol/min per g at 10 days in denervated muscles in response to insulin. These data suggest resistance of glycolytic utilization to insulin by 3 days of denervation, but an apparent recovery of insulin-stimulated glycolytic utilization in the 7–10-day-denervated muscles.

Since glucose transport is determined by summing glycogen synthesis and glycolytic utilization, the defects noted will be a combination of these two parameters. The basal rate of glucose transport in muscle was increased by 1 day in denervated as compared with contralateral muscles (Fig. 1*e*). In muscles incubated in

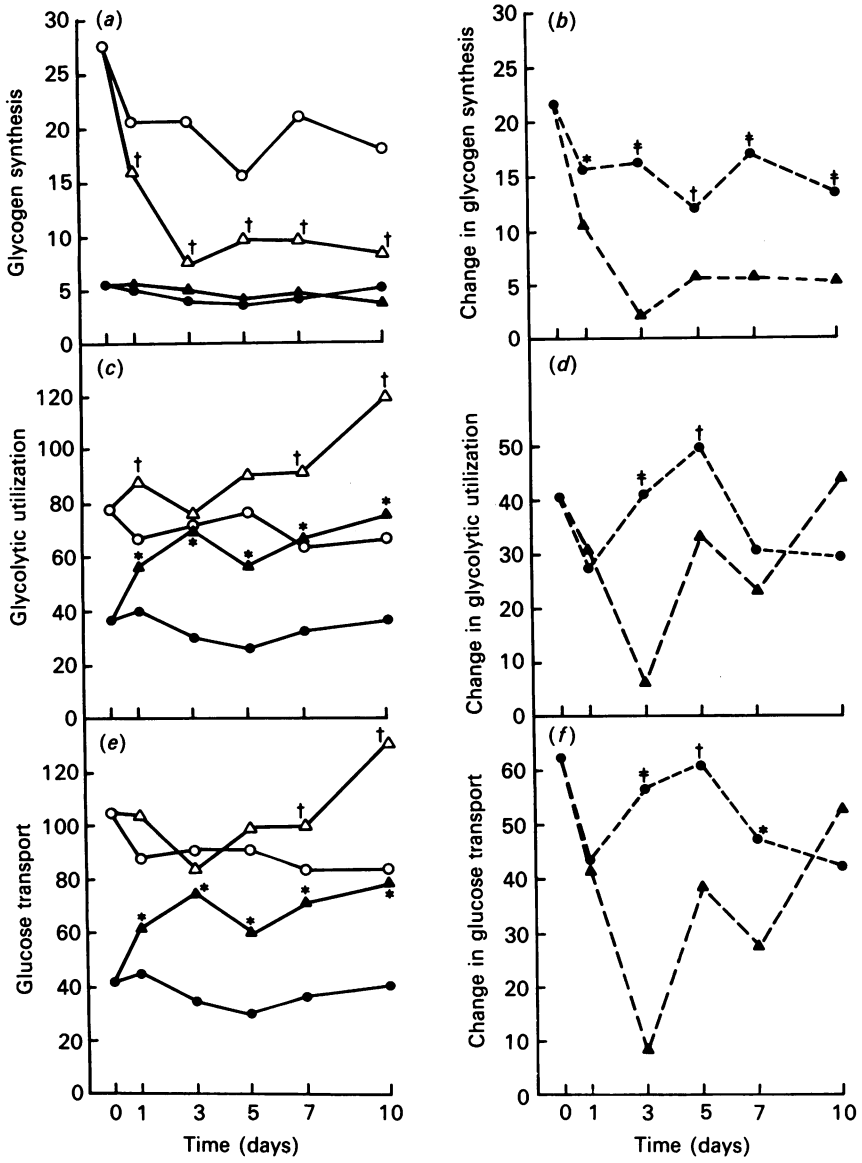


**Fig. 1. Effect of insulin on glycogen synthesis, glycolytic utilization and glucose transport in 1–10-day-denervated muscle**

Data for 0 days of denervation are from muscles incubated in the absence (black symbols) or the presence (white symbols) of 10 munits of insulin/ml from normal rats not subjected to surgery. Panels (*a*), (*c*) and (*e*) show data from denervated (△, ▲) and contralateral (○, ●) epitrochlearis muscles incubated in the absence (●, ▲) or the presence (○, △) of 10 munits of insulin/ml. Values in these panels differed significantly ($P < 0.05$) between denervated and contralateral muscles in the absence (*) and presence (†) of insulin. The absolute changes in rates in response to insulin in denervated (▲) and contralateral (●) muscles are shown in panels (*b*), (*d*) and (*f*). These were calculated from the differences between the mean rates in the absence and presence of insulin, and statistical significance of the differences between denervated and contralateral muscles in their response to insulin is shown by $*P < 0.05$, $\dagger P < 0.01$ and $\ddagger P < 0.001$. All values are in nmol/min per g. Numbers of muscles in each group at each time point were 6–12, and S.E.M. values were < 10% of the mean. Values are expressed on the basis of wet weight.

the presence of insulin, however, denervation did not increase glucose transport until day 7. This lack of response of glucose transport to insulin in 3–7-day-denervated muscles (Fig. 1*f*) suggests resistance of glucose transport to insulin by 3 days of denervation, with a return to normal insulin response by day 10 of denervation.

### Protein turnover

The basal rate of incorporation of [$^{14}$C]phenylalanine into muscle protein was significantly greater in denervated than in contralateral muscle on all days of study (Fig. 2*a*). When expressed as incorporation per total muscle (results not shown), basal rates of protein synthesis were significantly greater in denervated than in contralateral muscles only on days 1 and 3. On subsequent days, denervated and contralateral muscles did not differ significantly in basal protein synthesis per total muscle, owing to the marked decrease in muscle weight in denervated muscles. In the presence of insulin, protein synthesis was greater in denervated than in contralateral muscle, except on day 3 (Fig. 2*a*). Insulin

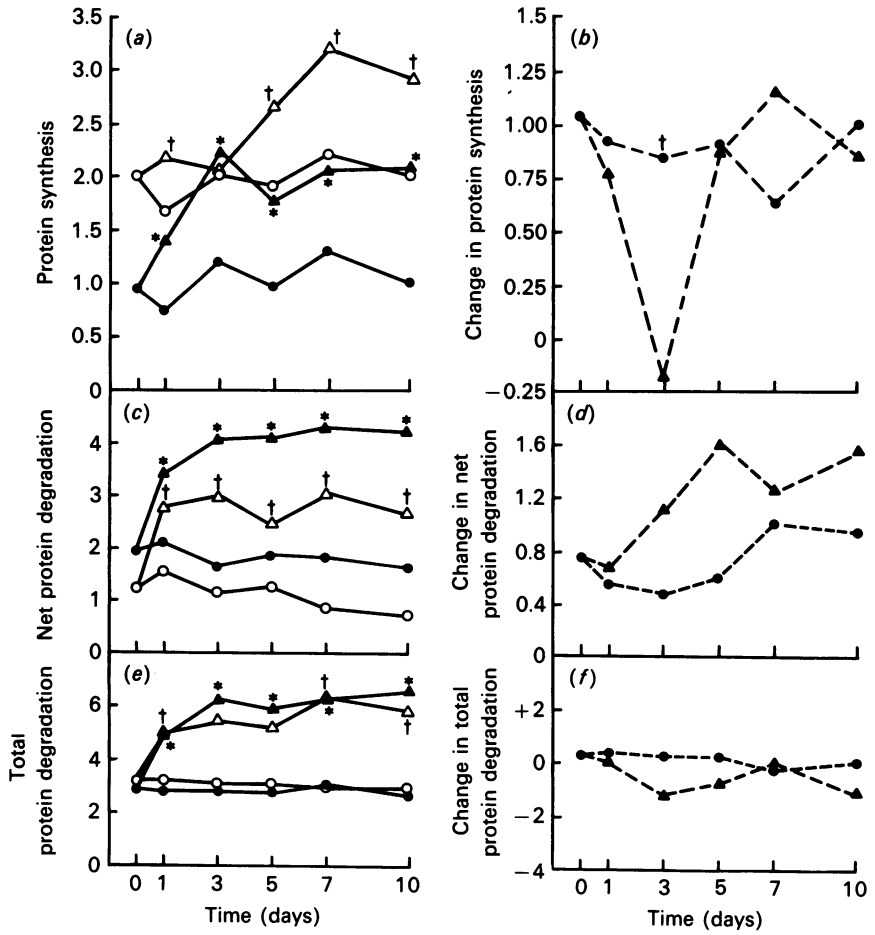


**Fig. 2. Effect of insulin on protein synthesis, net protein degradation and total protein degradation in 1–10-day-denervated muscles**

Data for 0 days of denervation are from muscles incubated in the absence (black symbols) or the presence (white symbols) of 10 munits of insulin/ml from normal rats not subjected to surgery. Panels (*a*), (*c*) and (*e*) show data from denervated (△, ▲) and contralateral (○, ●) epitrochlearis muscles incubated in the absence (●, ▲) and presence (○, △) of 10 munits of insulin/ml. Values in these panels differed significantly ($P < 0.05$) between denervated and contralateral muscles in the absence (*) and presence (†) of insulin. The absolute changes in rates in response to insulin in denervated (▲) and contralateral (●) muscles are shown in panels (*b*), (*d*) and (*f*). These were calculated from the differences between the mean rates in the absence and presence of insulin, and statistical significance of the differences between denervated and contralateral muscles in their response to insulin is shown by $*P < 0.05$, $\dagger P < 0.01$ and $\ddagger P < 0.001$. All values are in nmol/min per g. Numbers of muscles in each group at each time point were 6–12, and S.E.M. values were < 10% of the mean. Values are expressed on the basis of wet weight.

increased protein synthesis in all groups at each time point except in 3-day-denervated muscle. This apparent insulin-resistance with respect to protein synthesis in 3-day-denervated muscles can be seen more clearly in Fig. 2(*b*), where the absolute change in response to insulin is presented. The decreased response to insulin on day 3 of denervation was followed by recovery of insulin-stimulated protein synthesis during days 5–10 of denervation.

Net protein degradation, measured by tyrosine release plus any change in the tyrosine intracellular pool, was increased in 1–10-day-denervated muscles incubated in the absence or presence of insulin (Fig. 2*c*). There were no differences in the decrease by insulin in net degradation in 1–10-day-denervated and contralateral muscles (Fig. 2*d*), and thus resistance of net protein degradation to insulin was not readily apparent on any days of study. Rates of net protein degradation per total muscle in either the absence or the presence of insulin were also increased in 1–10-day-denervated muscles, but, owing to the decrease in muscle weight, this increased proteolysis was less marked on days 5–10 of denervation (results not shown).

The rate of total protein degradation was elevated in denervated muscles throughout the period of study (Fig. 2*e*). Protein degradation per total muscle was also elevated in denervated muscle on all days of study, but the degree of elevation was less on days 5–10, owing to the decrease in muscle weight (results not shown). Insulin had no significant effect on total protein degradation in all muscles (Figs. 2*e* and 2*f*), perhaps owing to the short incubation period (Jefferson *et al.*, 1977; Stirewalt & Low, 1983; Davis & Karl, 1986).

### Release rates and intracellular concentrations of amino acids, glycolytic products and high-energy phosphates

To examine amino acid metabolism and its interrelationship with glucose and protein metabolism in denervated muscle, the release rates of leucine, alanine, glutamine, glutamate, pyruvate and lactate were examined. Release of leucine, alanine, glutamate, pyruvate

**Table 3. Effect of denervation on the release of amino acids and glycolytic products from epitrochlearis muscle**

Values (nmol/min per g) are means ± S.E.M. for *n* determinations. Data presented for day 0 are from normal animals not subjected to surgery. *Contralateral differed from denervated, $P < 0.05$.

| | | Release | | | | | |
|---|---|---|---|---|---|---|---|
| | Denervated | Day ... 0 | 1 | 3 | 5 | 7 | 10 |
| | | *n* ... 8 | 6 | 12 | 6 | 12 | 6 |
| Leucine | − | 3.9 ± 0.4 | 3.4 ± 0.4 | 3.8 ± 0.3 | 4.4 ± 0.2 | 3.4 ± 0.1 | 4.0 ± 0.3 |
| | + | − | 4.2 ± 0.4 | 7.7 ± 0.4* | 9.9 ± 0.8* | 12.1 ± 1.2* | 10.4 ± 0.9* |
| Alanine | − | 16.7 ± 1.0 | 18.1 ± 1.5 | 16.7 ± 0.5 | 19.0 ± 1.8 | 16.9 ± 1.2 | 16.1 ± 0.9 |
| | + | − | 20.8 ± 1.8 | 29.6 ± 1.2* | 29.4 ± 1.0* | 25.6 ± 1.2* | 30.6 ± 1.4* |
| Glutamine | − | 34.0 ± 2.0 | 33.7 ± 2.5 | 33.5 ± 1.4 | 34.6 ± 3.4 | 33.3 ± 1.7 | 33.1 ± 1.5 |
| | + | − | 31.5 ± 1.6 | 34.3 ± 2.3 | 32.0 ± 2.5 | 27.2 ± 1.4* | 34.7 ± 3.7 |
| Glutamate | − | 8.6 ± 0.3 | 8.6 ± 0.7 | 7.4 ± 0.4 | 6.7 ± 0.6 | 7.6 ± 0.6 | 7.1 ± 0.6 |
| | + | − | 7.4 ± 0.6 | 10.1 ± 0.6* | 10.8 ± 1.2* | 13.8 ± 0.6* | 15.0 ± 1.5* |
| Pyruvate | − | 12.5 ± 0.7 | 13.4 ± 1.5 | 13.2 ± 0.8 | 12.9 ± 1.1 | 12.4 ± 1.1 | 12.7 ± 0.7 |
| | + | − | 17.0 ± 1.2 | 17.3 ± 1.1* | 18.7 ± 1.8* | 17.5 ± 1.5* | 26.6 ± 1.4* |
| Lactate | − | 130 ± 7 | 137 ± 13 | 135 ± 5 | 120 ± 15 | 122 ± 12 | 119 ± 7 |
| | + | − | 140 ± 7 | 160 ± 10* | 173 ± 16* | 163 ± 10* | 229 ± 12* |
| Lactate/pyruvate ratio | − | 10.5 ± 0.3 | 10.2 ± 1.0 | 10.2 ± 0.7 | 9.1 ± 0.6 | 9.9 ± 0.6 | 9.4 ± 0.4 |
| | + | − | 8.3 ± 0.6 | 9.3 ± 0.8 | 8.7 ± 0.4 | 9.3 ± 0.9 | 8.7 ± 0.6 |

**Table 4. Effect of denervation on tissue concentrations of amino acids, glycolytic products and high-energy phosphates in epitrochlearis muscle**

Values are means ± S.E.M. for *n* determinations, and are in μmol/g, except for leucine, which is in nmol/g. Day-0 data are from normal animals not subjected to surgery. *Contralateral differed from denervated, $P < 0.05$.

| | | Concn. | | | | | |
|---|---|---|---|---|---|---|---|
| | Denervated | Day ... 0 | 1 | 3 | 5 | 7 | 10 |
| | | *n* ... 8 | 6 | 12 | 6 | 12 | 6 |
| Leucine | − | 71.5 ± 5.8 | 73.1 ± 6.4 | 62.6 ± 4.7 | 79.5 ± 11.1 | 68.9 ± 6.9 | 79.7 ± 15.4 |
| | + | − | 83.8 ± 6.4 | 148.6 ± 18.6* | 109.0 ± 11.9* | 132.9 ± 12.2* | 150.4 ± 8.9* |
| Alanine | − | 1.00 ± 0.07 | 0.94 ± 0.10 | 0.92 ± 0.05 | 0.94 ± 0.08 | 1.04 ± 0.12 | 0.95 ± 0.09 |
| | + | − | 1.34 ± 0.12* | 1.31 ± 0.14* | 1.16 ± 0.15 | 1.13 ± 0.08 | 1.05 ± 0.07 |
| Glutamine | − | 3.29 ± 0.35 | 3.66 ± 0.37 | 2.76 ± 0.29 | 3.04 ± 0.24 | 2.94 ± 0.20 | 3.08 ± 0.17 |
| | + | − | 3.02 ± 0.30 | 2.11 ± 0.20* | 1.87 ± 0.17* | 2.39 ± 0.13* | 3.20 ± 0.21 |
| Glutamate | − | 0.77 ± 0.07 | 0.70 ± 0.05 | 0.92 ± 0.03 | 0.95 ± 0.07 | 0.92 ± 0.07 | 0.96 ± 0.04 |
| | + | − | 0.84 ± 0.05 | 1.42 ± 0.14* | 1.26 ± 0.05* | 1.16 ± 0.10* | 1.24 ± 0.25 |
| Lactate | − | 1.17 ± 0.21 | 1.25 ± 0.15 | 1.42 ± 0.25 | 1.23 ± 0.27 | 1.16 ± 0.16 | 1.04 ± 0.12 |
| | + | − | 1.93 ± 0.39 | 1.43 ± 0.14 | 1.17 ± 0.12 | 1.02 ± 0.11 | 0.92 ± 0.15 |
| ATP | − | 4.9 ± 0.3 | 5.0 ± 0.4 | 5.5 ± 0.2 | 5.2 ± 0.3 | 5.7 ± 0.4 | 4.8 ± 0.2 |
| | + | − | 5.7 ± 0.3 | 4.7 ± 0.3 | 3.9 ± 0.2* | 3.5 ± 0.2* | 3.2 ± 0.2* |
| Phosphocreatine | − | 16.2 ± 1.3 | 15.9 ± 1.7 | 18.9 ± 0.7 | 18.8 ± 0.7 | 17.6 ± 1.3 | 22.8 ± 2.7 |
| | + | − | 17.3 ± 1.0 | 14.3 ± 2.0* | 12.2 ± 1.0* | 10.9 ± 0.6* | 12.7 ± 1.4* |

and lactate from muscle was significantly increased in 3–10-day-denervated muscles (Table 3). However, glutamine release from denervated muscle was comparable with that in contralateral muscles, except for the small decrease on day 7. The ratio of lactate to pyruvate did not differ between denervated and contralateral muscles, indicating that denervated muscles were viable. When expressed as release per total muscle, leucine release was significantly increased in 3–10-day-denervated muscle, but alanine, pyruvate, and lactate release were significantly increased only on day 3 (results not shown). Glutamate release per total muscle was increased in 3–7-day-denervated muscles, and glutamine release per total muscle was decreased in 5–10-day-denervated muscles.

To determine if the increased release of amino acids was due to leakage from the intracellular pool, intracellular concentrations were determined (Table 4). Intracellular leucine was increased in 3–10-day-denervated muscles, whereas alanine was increased at 1 and 3 days. Glutamate was increased and glutamine was decreased in denervated muscles on days 3–7 of study. Lactate concentrations in muscle were unaffected by denervation. Before incubation, alanine and lactate concentrations did not differ between denervated and

contralateral muscles (results not shown). However, leucine and glutamate were increased and glutamine was decreased in denervated muscles before incubation.

To determine whether the insulin-resistance of glucose and protein metabolism in denervated muscle is related to a depletion of energy stores, ATP and phosphocreatine were determined. Denervation decreased ATP and phosphocreatine on days 3–10 of study (Table 4). This decrease in high-energy phosphates was present even when the decrease in muscle weight with denervation was considered, and occurred also in muscles frozen immediately before incubation (results not shown).

## DISCUSSION

The isolated epitrochlearis muscle preparation is an appropriate model for assessing metabolic activity and hormonal effects in mammalian skeletal muscle (Garber *et al.*, 1976*a*). ATP, phosphocreatine, ADP, AMP and glycogen concentrations in this isolated muscle preparation are similar to those in fast-frozen non-incubated muscle (Garber *et al.*, 1976*a*), and studies have demonstrated that the effects of hormonal and dietary perturbations on muscle metabolism are consistent with those seen *in vivo* (Karl *et al.*, 1976). The preparation was used to study glucose utilization and protein turnover in skeletal muscle, a major target tissue for insulin action in basal (no insulin present) and in maximal insulin-stimulated conditions in control and denervated tissue. Since both muscles, denervated and contralateral, were exposed *in vivo* to similar plasma insulin concentrations, the changes noted in intracellular concentrations of amino acids, glycolytic products and high-energy phosphates between the two muscles cannot be due to changes in the plasma concentration of the hormone. In this study, glucose utilization in the basal state mainly reflects non-insulin-mediated glucose uptake. Several lines of evidence for this assumption are: (1) evidence *in vivo* indicates that the half-life of insulin is approx. 30–50 min (Desbuquois & Aurbach, 1971; Felber *et al.*, 1983) and (2) studies *in vitro* on muscle (Duckworth & Kitabchi, 1981; Ryan *et al.*, 1985; Smith & Lawrence, 1985) have shown that insulin is rapidly degraded. If an insulin effect still existed in the isolated muscle as a result of exposure to insulin *in vivo* or after degradation of the insulin during incubation, one would expect the basal rate of glucose transport to be greater after a short incubation period (1 h) than a longer time period (2–3 h). We did not find this to be the case (results not shown); in fact, basal rates of glucose utilization were identical in the incubated muscles at 1 and 2–3 h (Davis *et al.*, 1986). Since there was no change between these two time points, even if some insulin effect was present in the excised muscles, the contribution of insulin-mediated glucose transport to total glucose transport would be minimal.

Denervation results in dramatic changes in the structure, biochemistry and contractility of muscle. These changes, which have been attributed to both a loss of a nerve-derived trophic factor and/or loss of stimulation by nerve (see Purves & Lichtman, 1985), may be quite different in direction, depending on the parameter studied. As seen in the present paper and in work by others, muscle mass decreases after denervation, yet glucose transport, glycolysis, and protein synthesis and degradation increase. In addition, the response to acetylcholine increases, owing to an increase in the number of acetylcholine receptors (Brockes & Hall, 1979). Similarly, the biochemical responses of denervated muscle to insulin change (Forsayeth & Gould, 1982; Burant *et al.*, 1984; Smith & Lawrence, 1984). Importantly, we report in the present paper that those changes in insulin response can be quite varied, depending on the parameter studied and the duration of denervation. Whereas resistance developed to the normal effects of insulin on glycolytic utilization, glycogen synthesis, glucose transpsort and protein synthesis, no resistance developed to net protein degradation. In addition, in some cases, the onset and duration of insulin resistance were different. These data suggest multiple and specific cellular defects in insulin's action in denervated muscle and, in addition, provide evidence that the varied effects of insulin are under different sets of controls.

### Basal glucose and protein metabolism

In the present study, sectioning of the ulnar nerve to the epitrochlearis muscle in rats did not alter the basal rate of glycogen synthesis. However, there appears to be a defect in basal glycogen synthesis. In most conditions, the content of glycogen in muscles in some way appears to determine the rate of glycogen synthesis. For example, after exercise in which muscle glycogen is decreased by 50%, the basal rate of glycogen synthesis from glucose is increased 2-fold (Davis *et al.*, 1986). In denervated muscle, basal contents of glycogen were decreased, but the rate of incorporation of glucose into glycogen was comparable with that of contralateral muscles. It should be noted that glycogen contents in the contralateral muscles were not significantly different from those in rats not subjected to surgery. Thus the increased glucose transported into denervated muscle is not used for replenishment of glycogen stores, but for glycolytic utilization. In addition, the 'normal' rate of glycogen synthesis from glucose and the low content of glycogen in denervated muscle also suggest an increase in glycogen phosphorylase activity in 3–10-day-denervated muscle.

The basal transport of glucose and flux of glucose through the glycolytic cycle were increased in denervated muscle. These changes were present by day 1 of denervation and throughout the 10 days of study. The increased release of pyruvate and lactate from denervated muscle also support the finding of increased glycolytic flux, although the increased proteolysis (see above) should also contribute to the elevated pyruvate and lactate concentrations (Garber *et al.*, 1976*b*). Both an increase (Shoji, 1986; Smith & Lawrence, 1984) and no change (Burant *et al.*, 1984) in basal glucose transport have been reported in denervated muscle, and these differences may be due, in part, to differences in fibre type (Turinsky, 1987). However, the effect of denervation on the flux of glucose through the glycolytic cycle has not been studied previously. The increase in glycolysis is particularly interesting, since decreases in the activities of enzymes of glycolysis and the oxidation of glucose also have been reported (DuBois & Max, 1983; Hogan *et al.*, 1965). However, these changes occurred during more prolonged periods of denervation (> 20 days) than in the present study. It has been suggested that pentose phosphate shunt activity, which is virtually lacking in normal muscle (Green & Landau, 1965), is increased in denervated muscle (Wagner & Max, 1979). However, other factors appear to be involved in the increased

utilization of glucose, since the $^3H_2O$ from the [5-$^3$H]-glucose is formed at the aldolase–triosephosphate isomerase reaction, which is distal to the point at which glycolysis and the pentose phosphate pathway diverge. Substrate recycling in denervated muscle could also increase the formation of $^3H_2O$, although little recycling of [5-$^3$H]glucose in normal muscle has been demonstrated previously (Hue & Hers, 1974). Thus further work may be required to determine the mechanism for the enhanced glycolytic flux in denervated muscle.

The results of the present study also demonstrate the profound effect of denervation on muscle protein turnover. Both protein synthesis and degradation in epitrochlearis muscle were increased by day 1 of denervation, and remained elevated throughout the 10 days of study. The increase in protein degradation was greater than the increase in protein synthesis, resulting in muscle atrophy by day 3. Moreover, when expressed per total muscle, degradation was elevated throughout the study, whereas synthesis was significantly increased only on days 1 and 3. What proportion of this increased synthetic rate is due to the synthesis of acetylcholine and other hormone receptors is not at present known. The results of this study are in contrast with that of Goldspink (1976), who reported a decrease in protein synthesis in the extensor digitorum longus for 2 days after denervation, followed by an increase in the synthesis rate. It was suggested that this stimulation in protein synthesis was due to an increase in ribosomal availability and efficiency. The difference in these two studies may be due to the different degrees of passive stretching or spontaneous activity and/or fibre type of the muscle. The mechanism(s) for the increase in protein degradation and synthesis in muscle is not known, but could involve changes in cytosolic $Ca^{2+}$, prostaglandins or phospholipids. Denervation increases intracellular $Ca^{2+}$ (Joffe *et al.*, 1981), and elevated $Ca^{2+}$ may increase both proteolysis and protein synthesis in normal muscle (Baracos *et al.*, 1986; Kameyama & Etlinger, 1979). Prostaglandins $E_2$ and $F_{2\alpha}$ have also been associated with increases in protein degradation and synthesis, respectively, in normal muscle (Rodemann *et al.*, 1982; Smith *et al.*, 1983). Although acute inhibition of prostaglandin $E_2$ production has been reported to have no effect on protein degradation in 16-day-denervated muscle (Turinsky, 1986), this does not negate the possible role of prostaglandins in the initiation of the increased protein turnover in denervated muscle.

The increase in protein degradation in denervated muscle is further confirmed by the increased rates of release of leucine, alanine and glutamate from denervated muscle. Tissue contents of these amino acids were either increased or unchanged in denervated muscles, indicating that their increased release was due to increased net formation of amino acids. The marked decrease in glutamine concentration in denervated muscle may indicate a diversion of amino nitrogen from glutamine to alanine formation, owing to the increased availability of pyruvate from the enhanced glycolytic flux or a decreased availability of free ammonia (Garber *et al.*, 1976*b*). However, the rate of release of glutamine during incubation was similar in denervated and contralateral muscles, suggesting an increased efflux of glutamine from denervated muscle. It is unlikely that this represents leakage, since this change was specific for glutamine, but it could indicate an alteration in the regulation of the specific transport system of glutamine, system N (Shotwell *et al.*, 1983).

**Insulin-resistance**

The resistance of glucose metabolism to insulin in denervated muscle, which was previously reported for glycogen synthesis and glucose transport (Burant *et al.*, 1984; Smith & Lawrence, 1984, 1985), was demonstrated in the present study to extend to the utilization of glucose in the glycolytic cycle. Insulin-resistance with respect to glycolysis is not surprising, since glucose transport is considered rate-limiting for glucose metabolism. Insulin stimulation of glycogen deposition as indicated from muscle glycogen contents was greater than that determined from the rate of incorporation of [5-$^3$H]glucose into glycogen (except in 1-day-denervated muscles). This is perhaps due to the decrease in glycogen breakdown by insulin and/or the formation of glycogen from sources other than glucose. However, this difference between the two measures of glycogen deposition was greater in contralateral than in 1–7-day-denervated muscles, suggesting that phosphorylase activity was greater in denervated muscles or that replenishment of glycogen from non-glucose sources was decreased.

Differences were observed in the time course of insulin resistance between glycogen synthesis compared with glycolytic utilization and glucose transport. The decreased ability of insulin to stimulate glycogen synthesis precedes the decrease in insulin-stimulated glucose uptake (Smith & Lawrence, 1984) and glycolysis. Moreover, there was an apparent recovery of insulin-stimulated glucose uptake and glycolytic flux by day 10 of denervation, whereas insulin resistance with respect to glycogen synthesis was present throughout the period of study. It is unlikely that the recovery of insulin-stimulated glycolysis and glucose transport were due to re-innervation, particularly since muscle weight progressively decreased during the 10 days of study. Thus the resistances of glycogen synthesis and glycolysis to insulin appear to be due to separate mechanisms; the defect in insulin-stimulated glycogen synthesis is primarily associated with a decreased activity of glycogen synthase, perhaps owing to a loss of the ability of insulin to stimulate dephosphorylation of glycogen synthase (Burant *et al.*, 1984; Smith & Lawrence, 1985; Smith *et al.*, 1988), and the resistance of glycolysis to insulin must be due to other factors, such as the decrease in insulin-stimulated glucose transport.

The decrease in insulin's ability to stimulate glycolysis and glucose transport in denervated muscle was accompanied by a decrease in ATP and phosphocreatine concentrations, and therefore may be related. Decreased ATP concentrations have been associated with a decrease in insulin-stimulated sugar transport as well as an increase in the basal rate of sugar transport in normal muscle incubated under anaerobic conditions (Yu & Gould, 1977). In the present study, high-energy phosphates were decreased from days 3–10 of denervation, whereas insulin-stimulated glycolysis and glucose transport appeared to recover by day 10. Thus it seems unlikely that the insulin-resistance of glucose metabolism in denervated muscle is due to a deficiency in cellular energy stores. Recently, no change in the high-energy-phosphate concentrations of 3-day-denervated calf muscle was reported (Turinsky, 1987).

Although the resistance of denervated muscle to insulin

involves numerous insulin-sensitive pathways, i.e. glucose transport, glycogen synthesis, glycolysis and amino acid uptake (Forsayeth & Gould, 1982; Turinsky, 1987), we found that the resistance of protein turnover to insulin was specific and transitory. Inhibition of insulin-stimulated protein synthesis was observed only on day 3 of denervation, whereas there was no apparent resistance of net protein degradation to insulin on any day of study. Since not all insulin-sensitive pathways are affected, and insulin binding appears not to be impaired in denervated muscle (Burant *et al.*, 1984; Smith & Lawrence, 1985), the defect(s) should lie distal to the receptor. Moreover, insulin-stimulated receptor tyrosine kinase activity was shown to be normal in 1-day-denervated muscle (Burant *et al.*, 1986). Whether the defect is in the generation of insulin mediator(s) or the control of specific intracellular pathways, or both, remains to be determined. It should be noted that there was recovery of insulin-stimulated protein synthesis, glycolysis and glucose transport in denervated epitrochlearis muscles. It would be interesting to determine if there is up-regulation of the insulin receptor after prolonged periods of denervation, particularly since acetylcholine receptors are increased (Brockes & Hall, 1979) and total protein synthesis is increased in denervated muscle. An alternative explanation to be considered is that the apparent differences in response to supraphysiological concentrations of insulin between some processes may be due to interaction with receptors for insulin-like growth factor.

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