Insulin sensitivity and responsiveness of epitrochlearis and soleus muscles from fed and starved rats

Recognition of differential changes in insulin sensitivities of protein synthesis and glucose incorporation into glycogen

William S. STIREWALT,* Robert B. LOW and Jeffrey M. SLAIBY
Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405, U.S.A.

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1. The insulin sensitivity of protein synthesis and glucose incorporation into glycogen by the soleus and epitrochlearis muscles from fed rats and 24h-starved rats was determined *in vitro* during the first and second hours of incubation after isolation of the muscles. 2. Rates of protein synthesis by both muscles from fed rats in the first hour of incubation were 2-fold higher than in the second hour and were not increased by insulin. 3. Rates of protein synthesis during the first hour in the presence of 6000 μ units of insulin/ml were increased in soleus, but not in epitrochlearis, muscles from starved rats. 4. Rates of protein synthesis in both muscles from fed and starved rats were increased significantly by insulin during the second hour. 5. High concentrations of insulin caused a marked stimulation of the rates of glucose incorporation by both muscles from fed and starved rats in both the first and second hours of incubation. 6. The insulin sensitivity of glucose incorporation during the second hour, defined as the concentration of insulin causing half-maximal stimulation, was increased 10-fold for both muscle types from starved rats (soleus, 65 μ units/ml; epitrochlearis, 45 μ units/ml) relative to muscles from fed rats (soleus, 600 μ units/ml; epitrochlearis, 500 μ units/ml). 7. The insulin sensitivity of protein synthesis in the second hour was greater for soleus muscles from starved rats (65 μ units/ml) than from fed rats (500 μ units/ml). In contrast, the insulin sensitivity of protein synthesis in epitrochlearis muscles from starved rats was significantly decreased (225 μ units/ml) compared with fed rats (25 μ units/ml). Maximal rates achieved by high concentrations of insulin were not different from those in the same muscle from fed rats. 8. It is suggested that protein synthesis, in distinction to glucose utilization, may be resistant to insulin stimulation during periods of acute starvation in muscles with fibre compositions similar to the epitrochlearis, but not in muscles with fibre compositions similar to the soleus. Partial reversal of the resistance observed *in vitro* for epitrochlearis muscles from starved rats may be due to the loss of factors which suppress the effect of insulin *in vivo*.

Insulin is considered to play a major role in the regulation of protein and carbohydrate metabolism in skeletal muscle (Jefferson, 1980; Rennie & Edwards, 1981). Analysis of the qualitative effects of the hormone in isolated muscle preparations have indicated that the hormone stimulates the rate of protein synthesis acutely, by increasing the translational efficiency of ribosomes (Jefferson *et al.*, 1974), and accelerates glucose incorporation

into glycogen predominantly by activation of glycogen synthase (Chiaasson *et al.*, 1981).

After food deprivation of rats, circulating concentrations of insulin are decreased and the rates of protein synthesis and glucose utilization in selected skeletal muscles are diminished (Li *et al.*, 1979; Owen & Reichard, 1971). This suggests a role for insulin in the metabolic adaptations of skeletal muscles to starvation and feeding. The extent to which insulin influences the two target metabolic processes in different muscles *in vivo*, however, is

* To whom reprint requests should be addressed.

poorly understood, primarily owing to the difficulty in separating the effects of the hormone from other factors which may influence the rates of these processes. It is likely that the influence of insulin is a function of both its concentration and the sensitivity of the two metabolic pathways to stimulation by the hormone, and that changes in both parameters occur during absorptive and postabsorptive periods.

Starvation appears to increase insulin sensitivity of glucose uptake and disposal by rat hindlimb preparations (Goodman & Ruderman, 1979) and by isolated soleus muscles from rats (Brady *et al.*, 1981) and mice (LeMarchand-Brustel & Freychet, 1979). In contrast, the insulin sensitivity of protein synthesis by gastrocnemius muscles of rats *in vivo* appears to be decreased by starvation (Garlick *et al.*, 1983). Thus restoration of insulin concentrations to those found in fed animals by infusion of either insulin or glucose fails to cause any change in protein synthesis, though a significant effect is observed when higher concentrations of the hormone are achieved.

Skeletal muscles of different fibre-type distributions respond differently to starvation in terms of alterations in the rates of protein synthesis (Goodman *et al.*, 1981; Frayn & Maycock, 1979) and glucose utilization (Maizels *et al.*, 1977). Taken together with the aforementioned effects of starvation on insulin sensitivity, this suggests that the response of the metabolic processes to hormones and other factors may differ in different muscle types. In no case, however, has the insulin sensitivity for both protein synthesis and glucose utilization been measured simultaneously in muscles of different fibre types (see above).

In the present study, we have investigated the response of protein and glycogen synthesis to insulin in the isolated epitrochlearis (composed of 65% fast-twitch white fibres, 20% fast-twitch red fibres and 15% slow-twitch intermediate fibres; Nesher *et al.*, 1980) and soleus muscle (composed of over 70% slow-twitch intermediate fibres; Gutmann *et al.*, 1971; Ianuzzo *et al.*, 1976) from fed and 1-day-starved rats, in order to determine whether differential changes in insulin sensitivities, implied from previous studies, can be recognized *in vitro*. By using isolated muscles, it was possible to measure simultaneously in the same muscle the rates of both protein synthesis and extracellular glucose incorporation into glycogen (Stirewalt & Low, 1983). Measurements were made in the presence of a range of insulin concentrations necessary to define the biological responses in terms of both the maximum response that can be achieved at high concentrations of the hormone and the sensitivity of the response, i.e. the concentration of hormone that gives a half-maximal response (Kahn, 1978).

Methods

Animals

Female rats (Sprague-Dawley; 70–90 g) obtained from Charles River (Montreal, Quebec, Canada) were housed on a 12h-light/12h-dark cycle and provided with Purina laboratory chow and water *ad libitum*. Food was removed from rats designated 'starved' at 11:00h on the day before muscles were incubated. Each experiment involved incubation of muscles from 20–30 rats. Fed rats were killed between 10:30h and 13:00h by cervical dislocation, and the lumbar vertebral column was crushed to prevent muscle spasm. The hindlimbs were detached to permit simultaneous removal, by two operators, of the soleus from the hindlimb and the epitrochlearis from the forelimb.

Experimental procedures

Muscles were removed intact and trimmed of adherent fat under a dissecting microscope while immersed at room temperature in oxygenated (O_2/CO_2 , 19:1) bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932) modified to contain 1.2 mM- Ca^{2+} (Harter *et al.*, 1979) and containing 7.5 mM-glucose and amino acids at normal plasma concentrations (Morgan *et al.*, 1980). This procedure required approx. 5 min. The muscles were then transferred to plastic tubes containing 700 μ l of the same buffer, supplemented to include 5 mM-Hepes buffer, pH 7.4, and 0.2% (w/v) gelatin (pig skin; type I; catalogue no. G-2500; Sigma, St. Louis, MO, U.S.A.) with or without insulin (Iletin; Eli Lilly and Co., Indianapolis, IN, U.S.A.). Incubation of muscles for metabolic studies was at 37°C in a shaking water bath (70 oscillations/min) with continuous exposure at the liquid surface to a stream of O_2/CO_2 (19:1). For measurements designated 'first hour', muscles were preincubated for 15 min with or without insulin at the concentrations indicated, and then transferred to identical medium containing [3H]leucine (final sp. radioactivity 800–1500 d.p.m./pmol) and [^{14}C]glucose (final sp. radioactivity 0.8–2.0 d.p.m./pmol) for a subsequent 1 h incubation. For measurements designated 'second hour', muscles were preincubated for 15 min without insulin, transferred to identical medium, incubated for an additional 1 h and then transferred again to medium containing radioisotopes for 1 h. At 15 min before the last transfer, insulin was added in a volume of 50 μ l to a final concentration as indicated. Thus all muscles incubated in the presence of insulin were exposed *in vitro* to the hormone at the designated concentration for 15 min before incubation for a period of 1 h in medium containing radioisotopes and the hormone.

In some experiments, one soleus and one epi-

trochlearis muscle from each rat served as a zero-insulin control for the contralateral muscle from the same animal. In other experiments one muscle was incubated for the first hour and the contralateral muscle incubated for the second hour.

At the end of the incubation muscles were removed, rinsed quickly to remove gelatin (Stirewalt & Low, 1983), frozen in isopentane cooled to the temperature of liquid N₂, and stored at -80°C until analysis.

Protein and glycogen synthesis

The fractional rate of protein synthesis (k_s , expressed as %/day) was calculated from the specific radioactivities of leucine in protein and leucine acylated to tRNA (Airhart *et al.*, 1979; Stirewalt & Low, 1983) by using the latter as the precursor specific radioactivity, according to the following formula:

$$k_s = \frac{S_b \times 100}{S_t \times t}$$

where S_b and S_t are the specific radioactivities of protein-bound leucine and leucine acylated to tRNA, and t is the time of incubation in the presence of radioisotope (days). The rate of incorporation of glucose into glycogen was calculated by dividing the total radioactivity incorporated into glycogen per unit wet weight of tissue by the specific radioactivity of glucose in the incubation medium (Stirewalt & Low, 1983). RNA was measured by the procedure described by Fleck & Munro (1962) as modified by Wool *et al.* (1968). Protein was measured by the method of Bradford (1976), or alternatively was calculated from the protein leucine content of the tissue by dividing the total radioactivity incorporated into protein by the specific radioactivity of leucine in protein.

Analysis of data

Variability of rates of protein synthesis and glucose conversion into glycogen measured for each muscle type was the same within each experiment, between experiments and with or without pairing data for muscles from the same animal. Also, mean values for a given experimental condition were not significantly different between experiments. Accordingly significance tests were on the combined data from all experiments, with an adjustment for multiple comparisons (Duncan, 1955; Miller, 1981).

Results

Basal rates and insulin responsiveness

Protein synthesis. Muscles isolated from fed and 24h-starved rats were preincubated for either

15 min or 75 min. They were then incubated for an additional 1 h in the presence of radiolabelled precursors, with or without insulin (see detailed protocol in the Methods section) at a concentration determined to effect a maximum response on the measured parameter (see below).

Rates of protein synthesis were calculated by using the specific radioactivity of tRNA-bound leucine as precursor. The ratio of the specific radioactivities of leucine acylated to tRNA to that of free leucine in the incubation medium was determined to be 0.62 (± 0.07 S.E.M., $n = 22$) for soleus muscles and 0.49 (± 0.04 S.E.M., $n = 28$) for epitrochlearis. These ratios were the same after all incubation times and in muscles from fed and starved animals.

The results of measurements of protein synthesis are shown in Table 1. In the absence of insulin the fractional rates of protein synthesis in soleus from fed or starved rats were faster than the corresponding values for epitrochlearis from the same animals during the first 1 h (Table 1; $P < 0.05$). The higher rates for soleus appeared to be due to a higher concentration of ribosomes, as reflected by differences in the RNA content. Thus the two muscles synthesized protein with the same efficiency (g of protein synthesized/g of RNA per day; Table 1) for each nutritional condition during this time interval. This was true also for soleus and epitrochlearis muscles from starved rats during the second hour. However, during this interval the efficiency of protein synthesis in soleus muscles from fed rats was greater than in epitrochlearis muscles from the same animals (Table 1; $P < 0.05$).

Comparisons of rates of protein synthesis in muscles from fed with those from starved rats indicated that fractional rates in both muscles from starved rats were less than half those of muscles from fed rats during the first hour ($P < 0.001$). This was also the case when the results were expressed as protein synthesized per amount of RNA, indicating that the efficiency of protein synthesis was decreased ($P < 0.001$) in muscles from starved animals.

During the second hour of incubation, rates of protein synthesis in muscles from fed rats were significantly slower than for the preceding time interval ($P < 0.001$), though the decline in the rate for soleus was less than that for the epitrochlearis. In contrast, there was no significant difference in the rates of protein synthesis in the first and the second hour interval for either muscle from starved rats.

The effects of a high dose of insulin (6000 μ units/ml) on the fractional rates of protein synthesis expressed per amount of RNA during the two incubation intervals are shown in Table 1. Rates of protein synthesis for both muscles from

Table 1. *Rates of protein synthesis in muscles from fed and starved rats*

Rates of protein synthesis were calculated from incorporation of label during a 60 min interval after preincubation for 15 or 75 min by using the steady-state specific radioactivity of tRNA-bound leucine as precursor as described in the Methods section. Values are shown as means $\pm$ S.E.M., with numbers of determinations in parentheses: * $P < 0.001$ for values for each muscle between the first and second time interval in the absence of insulin; † $P < 0.05$ for values during the first time interval between the soleus and epitrochlearis; ‡ $P < 0.05$ for values between the soleus and epitrochlearis from fed rats during the second time interval; § $P < 0.001$ for values for each muscle in the presence of insulin (6000 μ units/ml) compared with zero-insulin controls during the same interval. See the text for other statistical comparisons.

State of animal	Muscle	RNA content (mg/g of protein)	Incubation interval (min)	Insulin	Protein synthesis	
					k_s (%/day)	(g of protein/g of RNA per day)
Fed	Epitrochlearis	13.3 ± 0.4 (6)	15-75	0	7.35 ± 0.47 (14)	5.6 ± 0.4
				+	8.52 ± 0.60 (13)	6.4 ± 0.5
			75-135	0	2.99 ± 0.33* (37)	2.3 ± 0.3
				+	5.95 ± 0.35 (22)	4.5 ± 0.3§
	Soleus	15.9 ± 0.3 (6)	15-75	0	8.76 ± 0.54† (14)	5.5 ± 0.4
				+	10.03 ± 0.64 (13)	6.3 ± 0.4
			75-135	0	4.89 ± 0.16* (32)	3.1 ± 0.2‡
				+	7.94 ± 0.35 (21)	5.0 ± 0.2§
Starved	Epitrochlearis	11.3 ± 0.7 (6)	15-75	0	2.69 ± 0.15 (16)	2.4 ± 0.1
				+	3.19 ± 0.25 (14)	2.8 ± 0.2
			75-135	0	2.43 ± 0.12 (45)	2.2 ± 0.1
				+	5.95 ± 0.50 (12)	5.3 ± 0.4§
	Soleus	14.9 ± 0.3 (6)	15-75	0	3.67 ± 0.30 (16)	2.5 ± 0.2
				+	5.85 ± 0.34 (14)	3.9 ± 0.2§
			75-135	0	3.38 ± 0.09 (50)	2.3 ± 0.1
				+	7.21 ± 0.49 (19)	4.8 ± 0.3§

fed rats were unresponsive to this concentration of insulin during the first hour of incubation. However, exposure of muscles to the high dose of insulin for the same amount of time, during the second hour, significantly increased the rate of protein synthesis in muscles from fed animals, by approx. 70% for soleus and 100% for epitrochlearis ($P < 0.001$).

The high dose of insulin significantly increased rates of protein synthesis by soleus muscles from starved animals during the first hour of incubation ($P < 0.001$), but this was not true for epitrochlearis from the same animals. This insulin dose, however, caused a doubling of synthesis rates for both muscles from starved animals during the second hour ($P < 0.001$). In this case, the hormone restored values for the efficiency of protein synthesis for muscles from both fed or starved rats near to those values observed in the first hour for muscles from fed animals incubated with or without insulin.

Glucose incorporation into glycogen. In contrast with changes in the rates of protein synthesis between the first and second hourly incubation intervals in muscles from fed rats, rates of glucose incorporation into glycogen measured simultaneously in the same muscles were unchanged as a function of incubation time (Table 2). Rates of glucose incorporation into glycogen in the absence of insulin in

soleus muscles from fed and starved rats were not significantly different from each other. Glucose incorporation in epitrochlearis muscles was greater than in soleus for both time intervals, and also was increased in muscles from starved rats. In the presence of the high concentration of insulin (6000 μ units/ml), incorporation of glucose into glycogen in both muscles was increased significantly and to a similar rate for each muscle type in both nutritional conditions.

Insulin sensitivity

The absence of responsiveness of protein synthesis to stimulation by insulin in the first hour of incubation for all but soleus muscles from starved rats precluded determination of the insulin sensitivity for this target response during this interval. However, the dose-response relationships for glucose incorporation into glycogen, as reflected by responses to a low concentration of insulin (60 μ units/ml), were found to be similar to those determined in the second hour for each muscle from fed and starved rats (Table 2).

Tables 2 and 3 show the results of experiments designed to assess the insulin sensitivity of glucose incorporation into glycogen and protein synthesis during the second hour of incubation, an interval when the two target processes in both muscles from

Table 2. *Effects of insulin on glucose incorporation into glycogen in muscles from fed and starved rats*
 Rates were calculated by using as precursor the specific radioactivity of [^{14}C]glucose in the incubation medium.
 Values are means $\pm$ S.E.M., with numbers of determinations in parentheses: * $P < 0.001$ and † $P < 0.005$ for values versus zero insulin for the same interval.

Incubation interval (min)	Insulin ($\mu\text{units/ml}$)	Glucose incorporation into glycogen ($\mu\text{mol/g}$ of muscle in 1 h)			
		Epitrochlearis		Soleus	
		Fed	Starved	Fed	Starved
15–75	0	1.02 $\pm$ 0.11 (11)	2.31 $\pm$ 0.12 (14)	0.62 $\pm$ 0.11 (10)	0.56 $\pm$ 0.06 (12)
	60	1.80 $\pm$ 0.30 (6)	4.94 $\pm$ 0.85 (6)*	0.74 $\pm$ 0.29 (6)	2.19 $\pm$ 0.53 (6)†
	6000	6.74 $\pm$ 0.90 (8)*	6.90 $\pm$ 0.34 (9)*	4.50 $\pm$ 0.34 (12)*	3.73 $\pm$ 0.13 (12)*
75–135	0	1.19 $\pm$ 0.10 (46)	2.28 $\pm$ 0.13 (46)	0.48 $\pm$ 0.05 (41)	0.48 $\pm$ 0.04 (40)
	6	1.21 $\pm$ 0.20 (4)	—	—	0.41 $\pm$ 0.07 (10)
	18	—	3.60 $\pm$ 0.35 (4)*	—	—
	60	2.05 $\pm$ 0.20 (16)*	5.57 $\pm$ 0.42 (26)*	0.57 $\pm$ 0.05 (14)	2.73 $\pm$ 0.28 (23)
	180	—	6.21 $\pm$ 1.24 (3)*	—	—
	600	3.12 $\pm$ 0.29 (5)*	7.97 $\pm$ 0.87 (11)*	1.87 $\pm$ 0.12 (4)*	5.09 $\pm$ 0.41 (8)*
	6000	6.28 $\pm$ 1.08 (15)*	7.68 $\pm$ 0.60 (17)*	3.70 $\pm$ 0.29 (20)*	4.84 $\pm$ 0.36 (19)*
	12000	5.90 $\pm$ 0.76 (5)*	—	3.90 $\pm$ 0.75 (5)*	—

Table 3. *Effects of insulin on the rate of protein synthesis in muscles from fed and starved rats during the 75–135 min incubation interval*

Values are shown as means $\pm$ S.E.M., with numbers of determinations in parentheses: * $P < 0.001$ and † $P < 0.005$ for values versus zero insulin.

Insulin ($\mu\text{units/ml}$)	Protein synthesis (k_s , %/day)			
	Epitrochlearis		Soleus	
	Fed	Starved	Fed	Starved
0	2.99 $\pm$ 0.33 (37)	2.43 $\pm$ 0.12 (45)	4.89 $\pm$ 0.16 (32)	3.28 $\pm$ 0.09 (50)
6	3.29 $\pm$ 0.26 (11)	2.39 $\pm$ 0.14 (7)	4.60 $\pm$ 0.23 (10)	3.24 $\pm$ 0.32 (10)
60	5.14 $\pm$ 0.37 (22)*	3.21 $\pm$ 0.22 (21)†	4.84 $\pm$ 0.24 (22)	5.01 $\pm$ 0.28 (20)*
180	—	3.47 $\pm$ 0.68 (4)	—	—
600	6.68 $\pm$ 0.67 (14)*	6.09 $\pm$ 0.65 (7)*	6.54 $\pm$ 0.32 (16)*	6.95 $\pm$ 0.74 (7)*
6000	5.95 $\pm$ 0.35 (22)*	5.95 $\pm$ 0.50 (12)*	7.94 $\pm$ 0.35 (21)*	7.21 $\pm$ 0.49 (19)*

fed and starved rats were responsive to the hormone (Table 1). Insulin at a concentration of 60 $\mu\text{units/ml}$ significantly increased the fractional rate of protein synthesis in the epitrochlearis muscles from both fed and starved rats, though the effect in muscles from fed rats was substantially greater than in muscles from starved animals (Table 3). In contrast, this low concentration of insulin had no effect on the rate of protein synthesis in soleus muscles from fed rats, but had a significant effect on this parameter in soleus muscles from starved animals. A significant stimulation of glucose incorporation into glycogen occurred in epitrochlearis muscles from fed rats and in both the soleus and epitrochlearis muscles from starved rats, whereas the rate of this process in soleus muscles from fed rats was unaffected by a low concentration of insulin (60 $\mu\text{units/ml}$; Table 2). Finally, this dose of insulin stimulated protein synthesis to a greater extent in epitrochlearis from fed

than from starved animals, whereas the opposite was true for glucose incorporation into glycogen. In contrast, both metabolic processes were stimulated to a greater extent in soleus muscles from starved than from fed rats.

The relationships between the insulin sensitivities of the two target processes are seen more clearly when the data are plotted as shown in Fig. 1. The calculated half-maximal increases in protein and glycogen synthesis for soleus muscles from fed rats occurred at similar insulin concentrations (550 and 650 $\mu\text{units/ml}$ respectively; Fig. 1a). The concentration that caused half-maximal increases in the two parameters in soleus muscles from starved rats, however, was appreciably lower (60–70 $\mu\text{units/ml}$), indicating enhanced sensitivity of both metabolic processes in this muscle with starvation. In contrast, as shown in Fig. 1(b), the insulin sensitivity of protein synthesis in epitrochlearis muscles from fed rats (half-maximal in-

Fig. 1. Dose-response relationships of protein and glycogen synthesis in soleus and epitrochlearis muscles from fed and starved rats

Data from Tables 2 and 3 are plotted as percentage of maximal stimulation by insulin. Symbols: ●, fed, and ○, starved, for rates of glucose incorporation into glycogen; ▲, fed, and △, starved, for rates of protein synthesis. Continuous lines join values for muscles from fed rats, and broken lines join values for muscles from starved rats.

crease at 25 μ units/ml) was substantially greater than that of glycogen synthesis (half-maximal increase at 600–700 μ units/ml). The relative sensitivities of the two metabolic pathways were reversed, however, for epitrochlearis muscles from starved rats. This was due to both a decrease in the insulin sensitivity of protein synthesis and an increase in insulin sensitivity of glycogen synthesis.

Discussion

The present study has defined the insulin sensitivity of two distinct metabolic responses in two different skeletal muscles isolated from fed and acutely starved rats with the purpose of recognizing, *in vitro*, differences in the parameters which

might reflect the metabolic state of the muscles *in vivo*. The major advantage of the approach that we have used *in vitro* is the ability to measure the direct effects of insulin uncomplicated by the effects of alterations in other hormones and substrates which occur when insulin is administered *in vivo*. This permitted, for example, a demonstration of oppositely directed changes in the insulin sensitivities of two target responses in the epitrochlearis muscle and unidirectional changes in the insulin sensitivities of the same parameters in soleus muscles of starved compared with fed rats. However, the observed changes in both the basal rates and insulin-sensitivity of protein synthesis as a function of time *in vitro* indicates that at least some residual influences *in vivo* may be expressed only transiently *in vitro*, a circumstance that both facilitates and complicates interpretation of the results obtained *in vitro*.

Protein synthesis and acute starvation

A prominent role for insulin in the modulation of skeletal-muscle protein synthesis during absorptive and postabsorptive cycles is indicated by the parallel falls in the circulating concentration of insulin and in skeletal-muscle protein synthesis measured *in vivo* after short-term food deprivation, both of which are reversed by re-feeding (Millward & Waterlow, 1978). The alterations in protein-synthesis rates *in vivo* in starved rats are reflected *in vitro* in slower rates of protein synthesis in isolated muscle preparations from starved than from fed rats (Li *et al.*, 1979; Frayn & Maycock, 1979; Goodman *et al.*, 1981). Our present results demonstrate the same effect for both the isolated soleus and epitrochlearis muscles during the first hour of incubation *in vitro* when expressed either as a fractional synthetic rate or as rate of synthesis per amount of RNA as a measure of the efficiency of synthesis (Millward *et al.*, 1973). This difference is less distinct (soleus) or absent (epitrochlearis) during the second hour of incubation, during which rates of protein synthesis in muscles from fed rats fall to those characteristic of muscles from starved rats measured during either time interval (Table 1).

The fall in protein-synthesis rates *in vitro* has been observed also in extensor digitorum longus and soleus muscles from fed rats (Li & Goldberg, 1976) and in muscles of the perfused hindlimb of fed rats (Jefferson *et al.*, 1977; Li *et al.*, 1979). A simple explanation for these observations is that protein synthesis is sustained transiently by the residual effects of endogenous insulin, the concentrations of which fall together with falling synthesis rates as incubation is continued and resulting in a muscle preparation which is responsive then to added insulin (Jefferson *et al.*, 1977). This is consistent with the findings that protein synthesis

in perfused hindlimb muscles from fed rats is unresponsive to stimulation by added insulin during the first hour of incubation (Jefferson *et al.*, 1977; Flaim *et al.*, 1980) as was found in the present study for isolated muscles from fed rats (Table 1). If this explanation is correct, it would be predicted that concentrations of insulin comparable with those in fed rats would exert a near-maximal stimulation of protein synthesis. This was found to be the case for epitrochlearis muscle from fed rats, as determined in the second hour of incubation (Table 3; Fig. 1b). The finding that glucose incorporation into glycogen in epitrochlearis muscles from fed rats was fully as responsive to high concentrations of insulin in the first hour as in the second (Table 2) is also consistent with this explanation, since a concentration of insulin higher than that which might still be present in the first hour (60 μ units/ml) stimulated glucose incorporation by only 20% that of the maximum insulin response during both the first hour and the second hour (Table 2).

However, this argument does not hold for the soleus muscle from fed rats, since protein synthesis in this muscle was unchanged in the presence of a high physiological concentration (60 μ units/ml) of insulin (Table 3; Fig. 1a). Accordingly, slowing of the rate of protein synthesis in the soleus muscle in the second hour of incubation must have occurred for reasons other than the removal of the influence of endogenous insulin. The absence of a stretch or activity stimulus may have contributed to this decline in both muscles, in view of the ability of immobilization to decrease rapidly the rate of protein synthesis *in vivo* (Booth *et al.*, 1983) and of stretch *in vitro* to accelerate protein synthesis (Palmer *et al.*, 1981). The absence of these factors may also account for rates of protein synthesis in the first hour being lower than that measured for skeletal muscles of young rats *in vivo* (Waterlow *et al.*, 1978).

Insulin sensitivity and acute starvation

The prompt restoration of skeletal-muscle protein-synthesis rates *in vivo* on re-feeding acutely starved rats is paralleled by increases in circulating concentrations of insulin (Garlick *et al.*, 1983). This correlation predicts that elevation of insulin concentrations in starved rats would restore protein-synthesis rates to those in the fed state. However, restoration of insulin concentrations in post-absorptive rats to those measured in fed rats by infusion of the hormone or glucose has been shown to have no effect on the rate of protein synthesis in gastrocnemius muscles (Garlick *et al.*, 1983). This indicates that factors in addition to the increase in insulin during re-feeding play a part in the restoration of protein-synthesis rates. Our results for epitrochlearis from starved rats are consistent with

this idea. During the first hour of incubation the rate of protein synthesis was low and was unresponsive to stimulation by even high concentrations of insulin (a complete insulin resistance). During the second hour protein synthesis was responsive, but the sensitivity was less than that from fed rats. This was not true, however, for soleus (Table 3). Accordingly, there may be a factor in starved rats which inhibits the insulin stimulation of protein synthesis in the epitrochlearis, but not in the soleus. The influence of the putative factor would diminish with time of incubation *in vitro*, resulting in an increase in insulin sensitivity, as we observed. *In vivo*, the decline in concentration of this factor together with the rise in insulin concentration as a consequence of re-feeding would contribute to the restoration of protein-synthesis rates in the affected muscles to those in fed rats.

One such factor may be corticosterone. Circulating concentrations of corticosterone are elevated in starved relative to fed normal rats and decline rapidly with re-feeding (Millward *et al.*, 1983). Odedra *et al.* (1982) have shown also that muscle protein synthesis in diabetic rats does not respond to infusion of insulin sufficient to elevate concentrations of the hormone to those measured in normal fed rats. Furthermore, protein synthesis in adrenalectomized diabetic rats is stimulated significantly by the same concentrations of hormone, though not after infusion of corticosterone (Odedra *et al.*, 1982).

In contrast with the lower insulin sensitivity of protein synthesis in epitrochlearis muscles from starved compared with fed rats, the insulin sensitivity of glucose incorporation into glycogen in the same muscles during the same incubation interval was increased. If insulin binds to a single class of membrane receptors (Czech, 1984) and binding is an obligatory event for both effects of the hormone, then modulation of insulin binding alone is unlikely to account for the oppositely directed changes in the insulin sensitivity of the two parameters. However, binding of insulin to its receptor at low concentrations of the hormone has been shown to be elevated in soleus muscle isolated from starved compared with fed mice and rats (LeMarchand-Brustel & Freychet, 1979), and this change may contribute to the increase in sensitivity that we observed for both glucose incorporation and protein synthesis in this muscle.

Protein-synthesis rates in muscles of rats fed ad libitum

In a previous study we were unable to detect a significant change in the rate of protein synthesis in epitrochlearis muscles from fed rats between the first and second hours of incubation (Stirewalt

& Low, 1983). The reason for this may be that rats were killed between 13:00 and 16:00h on the day of the experiment in our previous study, compared with 10:30–13:00h in the present study. Rates of muscle protein synthesis are slower during the later postabsorptive interval in rats feeding *ad libitum* (Preedy & Garlick, 1983). Accordingly, in the previous study (Stirewalt & Low, 1983), rats may have been in a postabsorptive state more nearly related to 1-day-starved rats in terms of the rate of muscle protein synthesis and sensitivity to insulin. In that case, no change in rate with incubation time would have been evident (Table 1). This could also explain the previously observed delay in the stimulatory effect of a low concentration of insulin on protein synthesis until the third hour of incubation, at which time sensitivity to insulin may have returned toward that of a true fed state (cf. Fig. 2 and Table 4 in Stirewalt & Low, 1983). In any event, the delay does not appear to be related to the time of exposure of the muscle to insulin, which in the present study was the same for both incubation intervals.

Coda

The changes in the basal rate and insulin sensitivity of protein synthesis as a function of time *in vitro* underscore the limitations to interpreting the meaning of such measurements in terms of their corresponding values *in vivo*. In addition, residual effects of hormones and other modulators present *in vivo* would complicate assessment of their effects on protein metabolism *in vitro*, at least at early times after isolation of the tissue. At the same time, recognition of such residual influences may contribute to an understanding of the state of the muscle *in vivo*. This would also facilitate identification of time intervals in which to examine the synergistic and antagonistic relationships between candidate modulators of skeletal-muscle protein metabolism in the absence of those effects.

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