Influence of Age on Insulin Stimulation of Amino Acid Uptake in Rat Diaphragm*

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SUMMARY

1. The transport of o-amino[14C]isobutyric acid by intact rat diaphragm was studied in vitro over a 60-min course of incubation, after a 180-min prior incubation period. Insulin stimulated o-aminoisobutyric acid accumulation by muscle from 10-, 25-, 37-, 50-, and 100-day-old animals by 41, 147, 109, 54, and 23%, respectively.

2. Insulin stimulated uptake by increasing the maximum velocity of active transport (Vmax) in muscle from 10-day-old animals. In contrast, insulin increased the apparent affinity (Km) of the carrier mechanism for o-aminoisobutyric acid in muscle from 25- and 50-day-old animals.

3. Puromycin dihydrochloride inhibited uptake by resting, nonstimulated muscle from 10-, 25-, and 37-day-old animals when the antibiotic was added 180 min before o-aminoisobutyric acid. This inhibitory effect decreased with increasing age. Puromycin dihydrochloride did not inhibit o-aminoisobutyric acid uptake by muscle from 50- and 100-day-old animals.

4. Puromycin had no effect on insulin stimulation of o-aminoisobutyric acid transport in muscle from 10-day-old; reduced uptake in muscle from 25-day-old; but completely abolished subsequent insulin stimulation in muscle from 50-day-old animals.

5. These studies show that insulin stimulates o-aminoisobutyric acid uptake by different mechanisms in skeletal muscle from rats of different ages. The results suggest that insulin affects its stimulatory response in two distinct ways: directly by interacting with membrane carrier mechanisms; and indirectly by initiating the synthesis of a specific protein(s) which enhance transport. Muscle from 10-day-old rats responds only to the direct stimulatory mechanism. Muscle from the 25-day-old rats responds to both direct and indirect stimulation. Muscle from 50-day-old rats has lost responsibility to direct stimulation and relies completely on indirect stimulation through new protein synthesis.

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Several groups of investigators have demonstrated that insulin stimulates the accumulation of the nonutilizable amino acid, o-aminoisobutyric acid, by intact rat diaphragm muscle (1-3). Insulin also stimulates the incorporation of naturally occurring amino acids into new protein (4). After 180 or 120 min of exposure to inhibitors of protein synthesis before initiating transport studies, an association of these dual stimulatory effects was seen (5, 6). However, a different relationship was found between stimulation of amino acid transport and protein synthesis by insulin when diaphragm muscles from 25-day-old (60 to 90 g) and 50-day-old (220 to 250 g) rats were compared. In the younger animals, prolonged inhibition of protein synthesis inhibited both resting o-aminoisobutyric acid uptake and the stimulatory effect of insulin. In the older animals, exposure to inhibitors of protein synthesis did not affect resting transport, but abolished insulin stimulation of subsequent transport. It was postulated that insulin stimulated o-aminoisobutyric acid transport by skeletal muscle in two ways: directly by affecting a plasma membrane carrier protein; and indirectly by stimulating the synthesis of specific protein(s), which then enhanced transmembrane transport (7). The present studies explore further the relationship of age to the mechanisms by which insulin stimulates amino acid transport.

METHODS AND MATERIALS

Six groups of male, Sprague-Dawley, albino rats were obtained from Charles River Laboratories (Wilmington, Mass.) with the following growth specification:

| Age (days) | Weight (g) |
|-----------|------------|
| 10        | 19-21      |
| 17        | 34-35      |
| 25        | 60-90      |
| 37        | 126-145    |
| 50        | 220-250    |
| 100       | 300-310    |

The 10-day-old pups were received as sucklings with their mother. The older rats were fed Purina rat chow ad libitum until they were killed by stunning and decapitation. Intact whole diaphragms or hemidiaphragms were prepared by previously described techniques and placed in 10 or 20 ml of Krebs-Ringerbicarbonate buffer (pH 7.4), gassed with 95% oxygen 5% carbon...
Table I

**Influence of age on stimulation by insulin of a-aminoisobutyric acid transport**

Intact diaphragms from rats 10 to 100 days of age were previously incubated for 3 hours in Krebs-Ringer-bicarbonate buffer (pH 7.4) at 37° in 95% O2-5% CO2 atmosphere. Tissues were then transferred to fresh buffer containing a-amino[14C]isobutyric acid (0.1 mM) and where indicated insulin (0.4 unit per ml) for a subsequent 60-min incubation. Results are expressed as the mean distribution ratio ± 1 S.E. with the number of observations in parentheses.

| Age (days) | Control | Insulin | Stimulation | Statistical analysis *
|-----------|---------|---------|-------------|-------------------|
| 10        | 6.67 ± 0.50 (13) | 9.42 ± 0.78 (13) | 41 ± 12 | p < 0.01 |
| 25        | 1.47 ± 0.14 (6) | 3.68 ± 0.17 (6) | 147 ± 12 | p < 0.01 |
| 37        | 0.85 ± 0.06 (10) | 1.77 ± 0.14 (9) | 109 ± 18 | p < 0.01 |
| 50        | 1.09 ± 0.09 (9) | 1.68 ± 0.14 (9) | 54 ± 13 | p < 0.01 |
| 100       | 0.76 ± 0.06 (9) | 0.93 ± 0.06 (8) | 23 ± 13 | 0.10 < p < 0.20 |

* p values were obtained with Student’s t test.

Table II

**Influence of age on inhibition of a-aminoisobutyric acid transport by puromycin**

Experimental conditions were identical with those in Table I. Puromycin (0.55 mM) was added where indicated throughout the 3-hour prior incubation period. Insulin was not present during the subsequent incubation period with a-amino[14C]isobutyric acid (0.1 mM). Results are expressed as in Table I.

| Age (days) | Control | Puromycin | Inhibition | Statistical analysis *
|-----------|---------|-----------|------------|-------------------|
| 10        | 6.67 ± 0.50 (13) | 1.46 ± 0.09 (9) | 78 ± 1 | p < 0.01 |
| 25        | 1.47 ± 0.14 (6) | 0.86 ± 0.07 (6) | 41 ± 4 | p < 0.01 |
| 37        | 0.85 ± 0.06 (10) | 0.76 ± 0.04 (11) | 29 ± 5 | 0.02 < p < 0.05 |
| 50        | 1.09 ± 0.09 (9) | 0.94 ± 0.12 (9) | 14 ± 11 | 0.40 < p < 0.50 |
| 100       | 0.76 ± 0.06 (9) | 0.63 ± 0.09 (9) | 17 ± 12 | 0.20 < p < 0.30 |

Active velocity of transport by a saturable mechanism (Y) was estimated at initial times of uptake (60 min) with the previously described mathematical techniques of Akebo and Christensen (10). Sixty-min time points were found to represent initial rates of transport for all four conditions in 10-, 25-, and 50-day-old animals (7). An apparent diffusion constant (Ko) was calculated from the expression (1 - e-2ko) given by the ordinate intercept of a plot relating the intracellular concentration (A) divided by the medium concentration (A) to the reciprocal of the medium concentration (1/A). Y was calculated after subtracting these estimations of nonsaturable transport for each age group and experimental condition.

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1. The duration of incubation prior to addition of insulin or labeled substrate will subsequently be referred to as the prior incubation period.
2. Units per ml designates units of insulin per ml of medium.

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dioxide, and incubated for 180 min in a Dubnoff metabolic shaker at 37° prior to initiating uptake studies (8). Puromycin (0.55 mM) was present throughout this prior incubation period where indicated.1 The duration of the prior incubation period, tissues were transferred to flasks containing 10 or 20 ml of fresh buffer, puromycin, insulin (0.4 units per ml) where indicated, and a-amino[14C]isobutyric acid. The flask was gassed again with 95% O2-5% CO2 and incubated for 60 min. At the end of the incubation period, the diaphragms were dissected from surrounding tissue, rinsed in 0.9% sodium chloride solution, blotted, weighed, and homogenized in 10% trichloroacetic acid. After centrifugation at 200 × g for 10 min, 0.2 ml aliquots of tissue and medium supernatant were prepared for liquid scintillation spectrometry. Tissue water, estimated by comparing the wet weight to dry weight, was 79.7 ± 0.2% for all age groups. Extracellular space determinations obtained with [14C]insulin were 26.4 ± 1.2% of wet weight. The distribution ratio for a-amino[14C]isobutyric acid (counts per min per ml of intracellular fluid to counts per min per ml of incubation medium) was calculated as described previously (7).

Under similar experimental conditions, the intracellular accumulation and incorporation of L-[U-14C]lysine into tissue protein was investigated by incubating diaphragm preparations with 0.1 mM concentrations of this amino acid for 60 min after the 180 min prior incubation period. Tissues were homogenized in 10% trichloroacetic acid and acid-precipitable tissue protein was prepared by the method of Steinberg et al., as modified by Manchester and Young (9). Tissue protein was weighed and dissolved in hyamine, and its specific activity was calculated. Incorporation of L-[U-14C]lysine into protein was expressed as disintegrations per min per mg of tissue protein. The active velocity of transport by a saturable mechanism (Y) was estimated at initial times of uptake (60 min) with the previously described mathematical techniques of Akebo and Christensen (10). Sixty-min time points were found to represent initial rates of transport for all four conditions in 10-, 25-, and 50-day-old animals (7). An apparent diffusion constant (Ko) was calculated from the expression (1 - e-2ko) given by the ordinate intercept of a plot relating the intracellular concentration (A) divided by the medium concentration (A) to the reciprocal of the medium concentration (1/A). Y was calculated after subtracting these estimations of nonsaturable transport for each age group and experimental condition.

a-Amino[1-14C]isobutyric acid (specific activity 3.97 mCi per mmole), L-[U-14C]lysine (specific activity 116 mCi per mmole), and [carboxyl-14C]insulin (specific activity 3.0 mCi per g) were obtained from New England Nuclear. Glucagon-free bovine
insulin (23.8 units per mg) was a gift from Eli Lilly and Company (Indianapolis, Indiana). Unlabeled α-aminoisobutyric acid was purchased from Calbiochem. Unlabeled L-lysine and puromycin dihydrochloride were obtained from Nutritional Biochemicals.

RESULTS

Influence of Age on Amino Acid Transport in Resting and Insulin-stimulated Muscle—Control, non-insulin-stimulated distribution ratios decreased with increasing age (Table I). Diaphragm muscle from suckling 10-day-old animals accumulated α-aminoisobutyric acid against a concentration gradient to 6.67 ± 0.50 times the medium during the 60-min incubation period. The mean resting control distribution ratio fell to 1.47 ± 0.14 in 25-day-old animals, 0.85 ± 0.06 in 37-day-old animals, and 0.76 ± 0.06 in 100-day-old animals. The response to insulin stimulation rose and then fell with increasing age. Insulin stimulation was 147% in the 25-day-old group, whereas transport by the younger 10-day-old animals was increased by only 45%. Note that the lower response to insulin was associated in the youngest group with the highest control distribution ratios. Peak insulin stimulation occurred in the 25-day-old animals. Further increase in age was associated with decreasing insulin responsiveness. The 37- and 50-day-old animals responded with 109 and 54% stimulation, respectively, and the oldest animals (100-day-old) responded least with 23% stimulation. Doubling the insulin concentration to 0.8 unit per ml failed to cause further stimulation in 50- or 100-day-old animals.

Influence of Age on Inhibition of α-Aminoisobutyric Acid Transport by Puromycin—In previous studies with 25-day-old animals, 180 or 120 min of previous exposure to puromycin inhibited subsequent amino acid transport. In 50-day-old animals, however, puromycin failed to inhibit α-aminoisobutyric acid uptake (7). The data in Table II describe the influence of age on the inhibition of resting, non-insulin-stimulated α-aminoisobutyric acid transport. The inhibitory response to puromycin decreased with increasing age. In diaphragms from the youngest age group (10-day-old) the per cent inhibition was greatest (78% inhibition), whereas in muscle from the older 50- and 100-day-old animals, puromycin failed to inhibit α-aminoisobutyric acid uptake. In separate experiments not presented here puromycin did not impair transport by diaphragm muscle from 50-day-old animals even after 6 hours of prior incubation, or when present in twice the concentration (1.1 mM).

Despite the absence of an inhibitory effect of puromycin on α-aminoisobutyric acid uptake in the older animals, inhibition of lysine incorporation into protein was as rapid and as complete as in younger animals (Table III). This antibiotic inhibited lysine incorporation into protein by 99% in the 10-day-old,

### Table III

**Absence of effect of age on inhibition of protein synthesis by puromycin**

| Duration of prior incubation with puromycin | Inhibition of lysine incorporation into protein |
|--------------------------------------------|-----------------------------------------------|
|                                            | 10-day-old | 25-day-old | 50-day-old |
| 0                                          | %          | %          | %          |
| 10                                         | 99.1       | 94.6       | 93.2       |
| 60                                         | 99.6       | 98.1       | 94.5       |
| 120                                        | 99.6       | 94.1       | 94.4       |
| 180                                        | 99.4       | 96.1       | 95.1       |

* Duration of prior incubation with puromycin refers to the time of exposure to puromycin (0.55 mM) during the 3-hour prior incubation period. Puromycin was present throughout the subsequent 60-min incubation period. Results are expressed as the per cent inhibition of at least duplicate observations.

Effect of insulin on incorporation of lysine into diaphragm muscle protein

| Age | Lysine incorporation | Stimulation |
|-----|----------------------|-------------|
|     | Control              | Insulin     |
| days| dpm/mg/hr            | %           |
| 10  | 1769 ± 167 (6)       | 0           |
| 25  | 481 ± 67 (5)         | 46±         |

* p < 0.01.

### Table IV

**Influence of age on insulin stimulation of α-aminoisobutyric acid transport in absence of protein synthesis**

Experimental conditions were identical with those described in Tables I and II. Puromycin was present throughout the 3-hour prior incubation period. Insulin (0.4 unit per ml) where indicated and α-aminoisobutyric acid were present only during the final 1-hour incubation period.

| Age | Distribution ratio | Stimulation | Statistical analysis |
|-----|--------------------|-------------|----------------------|
|     | Puromycin          | Insulin plus puromycin | % | p < 0.01 |
| days|                    |              |                      |
| 10  | 1.46 ± 0.09 (9)    | 4.60 ± 0.47 (9) | 215 ± 32 | p < 0.01 |
| 25  | 0.86 ± 0.07 (6)    | 1.70 ± 0.08 (6) | 97 ± 10 | p < 0.01 |
| 37  | 0.60 ± 0.04 (11)   | 1.12 ± 0.06 (9) | 86 ± 9 | p < 0.01 |
| 50  | 0.94 ± 0.12 (9)    | 1.01 ± 0.08 (9) | 8 ± 9 | 0.60 < p < 0.70 |
| 100 | 0.63 ± 0.00 (9)    | 0.71 ± 0.06 (9) | 13 ± 9 | 0.40 < p < 0.50 |

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TABLE VI

Summary of kinetic analysis of \(\alpha\)-aminoisobutyric acid transport

\(K_D\) was determined from the ordinate intercept \((1 - e^{-K_D\theta})\) of a plot relating the distribution ratio to the reciprocal of \(\alpha\)-aminoisobutyric acid concentration in the external medium for each age group. \(V_{\text{max}}\) and \(K_m\) were observed from Lineweaver-Burk plots (\(1/A_f\) versus \(1/Y\)) and confirmed with the equation: \(Y = V_{\text{max}}A_f/K_m + A_f\).

| Condition              | 10 days | 25 days | 50 days |
|------------------------|---------|---------|---------|
|                        | \(K_D\) | \(V_{\text{max}}\) | \(K_m\) | \(K_D\) | \(V_{\text{max}}\) | \(K_m\) | \(K_D\) | \(V_{\text{max}}\) | \(K_m\) |
| Control                | 0.69    | 18.2    | 2.2     | 0.29    | 10.0    | 6.7    | 0.36    | 10.0    | 20.0    |
| Puromycin              | 0.40    | 2.6     | 1.3     | 0.29    | 5.0     | 6.7    | 0.36    | 10.0    | 20.0    |
| Insulin                | 0.69    | 22.2    | 2.2     | 0.29    | 10.0    | 6.7    | 0.36    | 10.0    | 6.5     |
| Insulin and puromycin  | 0.69    | 0.7     | 1.3     | 0.29    | 6.5     | 4.0    | 0.36    | 10.0    | 20.0    |

**Fig. 1.**

*a*, plot of distribution ratio versus the reciprocal of medium \(\alpha\)-aminoisobutyric acid (\(AIB\)) concentration \((1/A_f)\). Intact hemidiaphragms from 10-day-old rats were compared under conditions identical with those described in Tables I, II, and IV. Each point represents the mean of at least triplicate observations. \(K_D\) was calculated from the ordinate intercept \((1 - e^{-K_D\theta})\).

*b*, plot of distribution ratio versus medium concentration \((1/A_f)\). Intact hemidiaphragms from 25- and 50-day-old rats were compared under conditions identical with those described in Table IV. \(K_D\) was calculated as described in *a* and was presented in Table 6.
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Influence of Age and Insulin on Amino Acid Transport: Relationship to New Protein Synthesis—The effect of age on insulin responsiveness, independent of new protein synthesis, was investigated and is depicted in Table IV. Despite 3 hours of exposure to puromycin, insulin stimulated a-aminoisobutyric acid uptake into 10-day-old rat diaphragm by 215%. Insulin stimulation in the presence of puromycin decreased to 97 and 86% in 25- and 35-day-old animals. By 50 and 100 days of age, no significant insulin stimulation was seen after previous exposure to puromycin. In the 10-day-old group, insulin stimulated transport more in the presence of puromycin than in its absence (cf. Table I). The influence of age on insulin stimulation of new protein synthesis was then investigated. As seen in Table V, insulin unexpectedly failed to stimulate lysine incorporation into muscle protein from 10-day-old rats, but effected an expected stimulatory response in muscle from 25- and 50-day-old animals. In the younger animals a higher control rate of incorporation (1769 dpm per mg per hour) was seen which remained unchanged in the presence of insulin. In the older animals insulin increased the rate of lysine incorporation from 451 to 703 dpm per mg per hour, a 63% stimulatory effect.

Influence of Age on Transport Kinetics—The age-related differences in puromycin inhibition and insulin stimulation of a-aminoisobutyric acid transport were subjected to kinetic analysis. Akebo and Christensen (10) previously demonstrated two distinct processes in diaphragm from 25- to 37-day-old rats: (a) a saturable process was subject to insulin stimulation; (b) a nonsaturable process was unaltered by insulin. Using their mathematical formalism, we defined the diffusion constant ($K_D$) as a parameter of the second, nonsaturable uptake process (Table VI). Under control conditions the $K_D$ was 0.69, 0.29, and 0.36 per hour in diaphragm muscle from 10-, 25-, and 50-day-old rats, respectively. These values were unaltered by puromycin and insulin in the older animals, but puromycin reduced the $K_D$ in 10-day-old muscle to 0.40 per hour (Fig. 1a). Representative data for the calculations of $K_D$ in 25- and 50-day-old animals are presented in Fig. 1b. Neither puromycin alone nor puromycin and insulin changed either the distribution ratio or the $K_D$ in 50-day-old animals. The absent effect of these conditions on the $K_D$ for a-aminoisobutyric acid uptake in diaphragms from 25-day-old animals has been reported previously (7). With the observed values for $K_D$ the apparent active velocity of transport ($V_{max}$) was calculated and plotted to obtain the maximum transport velocity ($V_{max}$) and the apparent affinity ($K_m$). In the absence of insulin, puromycin reduced the $V_{max}$ from 18.2 to 2.6 mmoles per liter per hour in 10-day-old and from 10.0 to 5.0 mmoles per liter per hour in 25-day-old animals. Puromycin had no effect on the $V_{max}$ in 50-day-old animals or on the $K_m$ in either 25- or 50-day-old animals. Control values for $K_m$ rose with increasing age from 2.2 to 6.7 to 20.0 mM at 10, 25, and 50 days.

Insulin affected transport kinetics differently in the different age groups. In the 25- and 50-day-old animals insulin stimulated a-aminoisobutyric acid transport by lowering the apparent $K_m$ (Table VI). In muscle from the 50-day-old animals, insulin

94.8% in the 25-day-old, and 93.2% in the 50-day-old rats when present only during the final 60-min incubation period. Thus, the inability of puromycin to inhibit non-insulin-stimulated a-aminoisobutyric acid transport in older animals was not a result of this inability to inhibit protein synthesis.

Figs. 2. Double reciprocal plots of mediated velocity ($Y$) and medium a-aminoisobutyric acid concentration ($A_o$). Intact hemidiaphragms from 50-day-old rats were treated as described in Table IV. a-Aminoisobutyric acid concentrations ranged from 0.1 to 20.0 mM. Each point represented the mean of at least quadruplicate observations. The results observed at the five highest concentrations were indicated on the expanded scale above. Values for $Y$ were expressed in millimoles per liter per hour. Values for $A_o$ were expressed in millimoles per liter.
lowered the resting $K_m$ of 20.0 to 6.7 mM. No change in transport kinetics was seen when puromycin was added 180 min before the initiation of uptake studies, but as seen in Fig. 2, puromycin abolished the expected reduction in $K_m$ by insulin. Insulin could still lower the apparent affinity in diaphragm muscle from 25-day-old animals, although this stimulatory response was partially impaired. In this age group, despite 3 hours exposure to puromycin, insulin lowered the $K_m$ from 6.7 to 4.0 mmoles per liter per hour, but not to 3.0 mmoles per liter per hour noted in the absence of puromycin. In the 10-day-old animal, insulin evoked a quite different kinetic response (Fig. 3). Insulin raised the resting $V_{max}$ from 18.2 to 22.2 mmoles per liter per hour. In the presence of puromycin, insulin increased the $V_{max}$ from 2.6 to 6.7 mmoles per liter per hour. In contrast to the 25 and 50-day-old animals in this age group, insulin did not affect the $K_m$ in either the presence or absence of puromycin. That the $V_{max}$ was observed at saturation and was significantly raised by insulin is shown in the legend to Fig. 3. $Y$ values of 16.8, 14.9, and 16.7 mmoles per liter per hour were observed at 5, 10, and 20 mM $\alpha$-aminoisobutyric acid in the absence of insulin. Insulin elevated these values to 28.3, 26.3, and 24.2 mmoles per liter per hour.

**DISCUSSION**

This paper describes the developmental changes with aging for a well defined mechanism of insulin action, the acceleration of $\alpha$-aminoisobutyric acid transport into rat diaphragm muscle.

**Fig. 3.** Plot of mediated velocity ($Y$) and medium $\alpha$-aminoisobutyric acid concentration ($A_i$) in intact hemidiaphragms from 10-day-old rats. Each point represented at least quadruplicate observations. $Y$ was expressed in millimoles per liter per hour and $A_i$ in millimoles per liter. The mean $\pm$ 1 S.E. are indicated below. $p$ values were derived using the paired $t$ test.

![Diagram](https://example.com/diagram.png)

**Fig. 4.** Influence of age on the mechanism of insulin stimulation of $\alpha$-aminoisobutyric acid transport ($AIB$) 0000, messenger RNA, ribosome complex; $\rightarrow$, membrane transport site; $\rightarrow\rightarrow$, direct stimulatory effect, independent of new protein synthesis; $\rightarrow\rightarrow\rightarrow$, indirect stimulatory effect, dependent on new protein synthesis.

Previous investigators using 25- to 40-day-old animals defined this saturable, stereospecific, and energy-dependent concentrative process. In this age range insulin stimulated uptake by raising the apparent affinity of a saturable transport site for $\alpha$-aminoisobutyric acid (10). These characteristics of $\alpha$-aminoisobutyric acid transport suggested enzyme mediation across the plasma membrane. In bacterial transport systems, membrane carrier proteins or permeases have been isolated and characterized (11–13). Their existence as a component of the diaphragm muscle transport system remains speculative. Indirect evidence from our previous studies added evidence for the existence of such "protein carriers" in this system. When prolonged prior...
uptake by increasing the apparent affinity of the transport site independent of new protein synthesis. In both the presence and absence of puromycin, insulin stimulated α-aminoisobutyric acid uptake, suggesting two mechanisms: one protein dependent, and another independent of new protein synthesis. This observation suggests that with increasing age, muscle membranes lose their direct responsivity to insulin and rely instead on the initiation or activation of "specific" proteins which then accelerate membrane transport. Our kinetic analysis indicates that these specific proteins act, as in the 25-day-old group, by enhancing the affinity of the transport mechanism for α-aminoisobutyric acid. We are unaware of other observations indicating absolute dependence of hormonal stimulation of transport on protein synthesis. However, Garren, Ney, and Davis (18) demonstrated that prior treatment with protein synthesis inhibitors blocked the stimulatory effects of adrenocorticotrophic hormone on steroidogenesis. Similarly, Wool and Cavicchi (19) ablated the stimulatory effect of insulin on ribosomal protein synthesis by prior in vivo treatment with puromycin or cycloheximide. Our studies imply that the resistance to insulin stimulation which occurs with increasing age is associated with a progressive decrease in the turnover rate and loss of the direct effect of insulin on α-aminoisobutyric acid transport proteins.

Although the rat diaphragm muscle cannot be directly related to man, as a laboratory model for one mechanism of insulin action, it offers some pertinent points. In man, resistance to the effects of insulin develops with increasing age (20). Non-specific effects of obesity, inhibitors, and vascular impermeability to insulin are some of the mechanisms invoked (21). Obviously, in the rat diaphragm model, neither plasma antagonists nor vascular abnormalities can be implicated since neither are present. Perhaps the increased resistance to insulin expressed by aging muscle reflects a nonspecific effect of increased cell size and obesity. DiGirolamo and Rudman (22) found that an altered pattern of glucose utilization and resistance to insulin stimulation seen in epididymal adipose tissue from obese rats virtually disappeared with fasting. Reduction of muscle cell size through fasting is impossible without killing the animal, but several points mitigate against obesity as a cause for the observed differences in insulin stimulation of muscle transport at different ages. First, although that portion of uptake mediated through a nonsaturable, non-insulin-responsive diffusion process accounted for a greater portion of the distribution ratio with increasing age, this factor was eliminated when calculating the active velocity of uptake \( V \). With the use of these corrected \( V \) values, the apparent affinity of the carrier mechanism for its substrate decreased with increasing age. Second, the stimulatory effect of insulin exhibited different kinetic changes at different ages: in 10-day-old animals, insulin enhanced the capacity for α-aminoisobutyric acid uptake whereas in 25- and 50-day-old animals, the affinity was increased. Finally, insulin responsiveness did not change proportional to body weight. Ten-day-old animals demonstrate less insulin response than larger 25- or 37-day-old animals. Our findings in muscle suggest developmental mechanisms independent of obesity. In this regard, Stauffacher and Renold (23) found that medium glucose incor-
poration into glycogen by diaphragm muscle responded normally to insulin from mice the obesity of which was induced by gold thioglucone. In contrast, these investigators observed that muscle from genetically obese mice (ObOb and NZO) was resistant to insulin stimulation (23). In our results, normal development is associated with a rise and fall in the inherent sensitivity and a change in the mechanism by which insulin stimulates muscle membrane transport. Perhaps a similar change in end organ sensitivity to insulin develops as man ages resulting in the syndrome(s) now called “maturity onset diabetes.”

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