Insulin and Insulin-like Growth Factor I

Effects on Protein Synthesis in Isolated Muscles from Lean and Goldthioglucose-Obese Mice

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SUMMARY

The effects of insulin and insulin-like growth factor I (IGF-I) on protein synthesis were compared in muscle isolated from lean and goldthioglucose (GTG)-obese mice. Two types of skeletal muscles, the red soleus and the white extensor digitorum longus (EDL) muscles, were studied. In muscles from lean mice, 6.7 nM insulin and 50 nM IGF-I caused a similar maximal stimulation of tyrosine incorporation in total proteins (40% increase). However, the potency of IGF-I was only 5–10% that of insulin both in soleus and in EDL muscles ($EC_{50} \cong 6$ nM for IGF-I and 0.5 nM for insulin). Basal rate of protein synthesis was identical in muscles from GTG-obese and lean mice. Similarly, a comparable increase in the rate of protein synthesis was obtained using maximally effective concentrations of insulin and IGF-I in both lean and GTG-obese animals. SDS-polyacrylamide gel electrophoresis analysis of proteins labeled with ^{35}S-methionine confirmed that, in muscles from lean and GTG-obese animals, insulin and IGF-I increased overall protein synthesis in a similar manner. These results suggest that the protein synthesis machinery is not impaired in GTG-induced obesity, which is therefore not associated with resistance to insulin for its effect on protein metabolism. DIABETES *32*:392–397, May 1983.

Protein metabolism in muscle is under the control of various metabolites and hormones. Insulin appears to play a major role since it has been shown to stimulate the synthesis and to inhibit the degradation of proteins in muscle from normal animals both in vivo and in vitro.[1–3] Consistent with these observations, the loss of muscular mass and of body protein content observed in insulinopenic diabetes can be reversed after insulin administration.[1] However, other observations made in genetically[4–9] or experimentally obese animals[10] seem difficult to reconcile with those mentioned above. Indeed, in these animals, despite a marked hyperinsulinemia, a loss of muscular mass is also observed, presumably because obese animals consume more protein and more energy than the lean controls but deposit less nitrogen in their carcasses than lean animals.[5,7,8]

Besides insulin, insulin-like growth factors have been reported to stimulate protein synthesis in cultured fibroblasts and chondrocytes.[11] In the muscle in vitro, a similar role for growth factors has been suggested by two studies.[12,13] Unfortunately, they were performed with a partially purified preparation of human somatomedin[12] and with an undefined human serum factor,[13] which precluded any definite conclusion toward a regulatory role of these factors as well as a comparison with the effects of insulin.

In the present study, we investigated the effects of insulin and of a pure preparation of insulin-like growth factor (IGF-I)* on protein metabolism in isolated muscles. Since turnover rates of protein are dependent on the type of muscles,[3,14,15] two leg muscles have been used: the soleus muscle, which is continuously active and is composed almost exclusively of dark fibers, and the extensor digitorum longus (EDL) muscle, which consists primarily of pale fibers.[16] Those studies have been performed both in normal lean mice and in goldthioglucose-obese, hyperinsulinemic mice.

MATERIALS AND METHODS

Animals. Male Swiss Albino mice (7–8 wk old) were provided laboratory chow (Usine d'Alimentation Rationelle, Vil-

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*The following abbreviations are used: IGF-I, insulin-like growth factor I; GTG, goldthioglucose; EDL, extensor digitorum longus; TCA, trichloroacetic acid; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis.

lemoisson, Epinay/Orge, France) ad libitum and maintained at 23°C on a 12-h light cycle until the time of death (9–11 a.m.). Some mice were rendered obese by goldthioglucose (GTG) injection at 3 wk of age and were used at 25–30 wk when obesity had reached a plateau.[17,18] In experiments with GTG-obese mice, age-matched lean control animals were used. Before muscle isolation, a blood sample was taken up and used for determination of glucose and immunoreactive insulin as previously described.[18]

Incubation methods. Mice were killed by cervical dislocation and the two muscles (soleus and extensor digitorum longus, EDL) were isolated and incubated as previously described.[14,18] After a 15-min preincubation, muscles were incubated in 1 ml Krebs-Ringer bicarbonate buffer (pH 7.3) containing 2 mM pyruvate, 2 mg/ml defatted bovine serum albumin, branch chain amino acids (0.2 mM leucine, 0.1 mM isoleucine, 0.2 mM valine), and insulin or IGF-I as indicated in the legends to figures or tables.

Measurement of protein synthesis and protein degradation. The rates of protein synthesis, estimated by [^{3}H]tyrosine incorporation in TCA-precipitable material, were measured as described by Fulks et al.[19] Muscles were incubated in the medium described above, supplemented with L-[side-chain-2,3-^{3}H]tyrosine (0.1 mM, 1 μCi/ml, 16 Ci/mmol) and [^{14}C]tyrosine (1.25 μCi/ml, 400 mCi/mmol). At the end of the 3-h incubation, a condition under which the rates of protein synthesis were shown to be linear (personal observation[14]), muscles were freeze-clamped and homogenized;[19] TCA-precipitable proteins were measured as previously described.[14,19] Aliquots of supernatant following TCA precipitation were used for tyrosine assay,[20] and for determination of ^{14}C- and ^{3}H-radioactivity. In each muscle, the intracellular specific radioactivity of tyrosine was calculated[19] by using the extracellular sucrose space. Neither insulin nor IGF-I affected intracellular specific activity of tyrosine (data not shown).

Protein degradation was estimated by measuring tyrosine released in the incubation medium in the presence of 0.5 mM cycloheximide to block protein synthesis.[14,19] All results were expressed per mg of muscle protein.[21]

Polyacrylamide gel electrophoresis (PAGE). Muscles were incubated with [^{35}S]methionine (50 μCi/ml, 1000 Ci/mmol) in the same medium as described above, except that branch chain amino acids were omitted, and insulin or IGF-I was included when required. Muscles were then lysed by sonication in a boiling SDS solution [3% SDS (wt/vol), 10% glycerol]. The samples were heated at 100°C for 5 min after addition of 2-mercaptoethanol (2%, vol/vol) and bromophenol blue (0.01%, wt/vol). Proteins contained in SDS extracts were analyzed by one-dimensional gel electrophoresis,[22] using a 5–15% linear gradient of acrylamide as a resolving gel and a 3% stacking gel. The gels were stained with Coomassie blue [0.25% solution in 50% (wt/vol) trichloroacetic acid], destained in 7% (vol/vol) acetic acid, vacuum dried, and exposed to Kodak X-Omat films. The autoradiograms were scanned in a Gelman microdensitometer. The proteins used as molecular weight markers were: myosin heavy chain (200 K), β-galactosidase (116 K), phosphorylase B (94 K), bovine serum albumin (67 K), ovalbumin (43 K), carbonic anhydrase (30 K), soybean trypsin inhibitor (20 K), and lysozyme (14 K).

Statistical methods. In each experiment the two soleus or the two EDL muscles were isolated from the same animal. One muscle was incubated with the peptide, the other one was used as a control. Statistical significance was assessed using the paired Student *t* test.[23]

Materials. IGF-I is a pure preparation (biological SA, 340 mU/mg, mol wt, 7500) kindly supplied by Dr. R. E. Humbel (Zurich, Switzerland). Porcine monocomponent insulin was a gift from Novo Research Institute (Copenhagen, Denmark). Radioactive substances were purchased from the Radiochemical Centre (Amersham, England) or New England Nuclear (Boston, Massachusetts). Chemicals (analytical grade) were from Merck, A. G. (Darmstadt, West Germany) or from Sigma Chemical Co. (St. Louis, Missouri). All reagents for SDS-gel electrophoresis were from Bio-Rad Laboratories (Richmond, California).

RESULTS

Insulin and IGF-I effects on protein synthesis in muscles of lean mice. We have studied the concentration dependence of insulin and IGF-I effect on protein synthesis. To overcome problems due to daily and to interanimal variations, results have been expressed in percent increase above basal (within each pair of muscles). Figure 1 shows that maximal

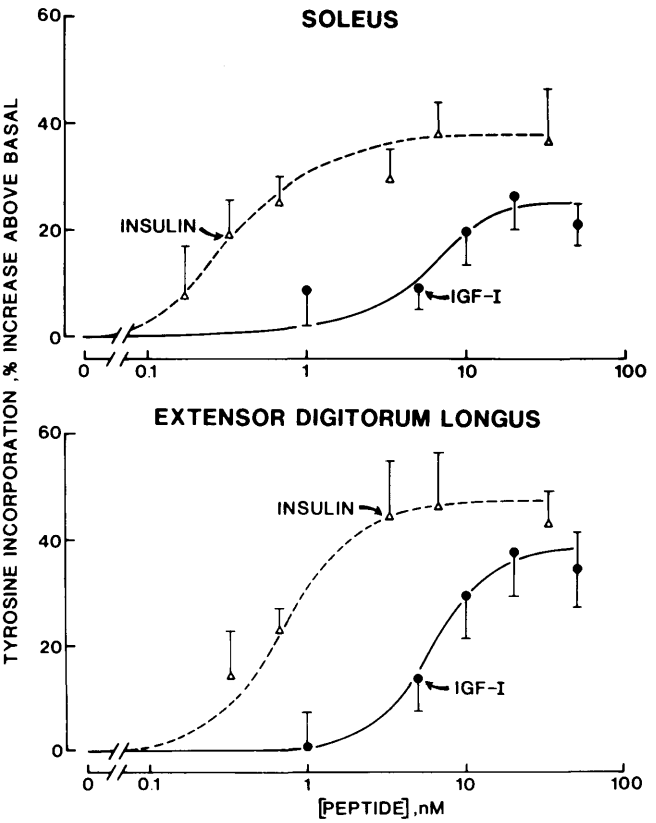

FIGURE 1. Dose-response curves of insulin and IGF-I effects on protein synthesis in soleus and EDL muscles of 8-wk-old lean mice. Muscles were incubated for 3 h in Krebs-Ringer bicarbonate buffer containing 2 mg/ml defatted bovine albumin, 2 mM pyruvate, branch chain amino acids, and 0.1 mM ^{3}H-tyrosine with various concentrations of insulin or IGF-I. Tyrosine incorporation in TCA-precipitable proteins was measured as described in METHODS. Results for each pair of muscles are expressed as percent increase over basal (stimulated value-basal value/basal value). Points are means ± SEM of 8–18 pairs of muscles.

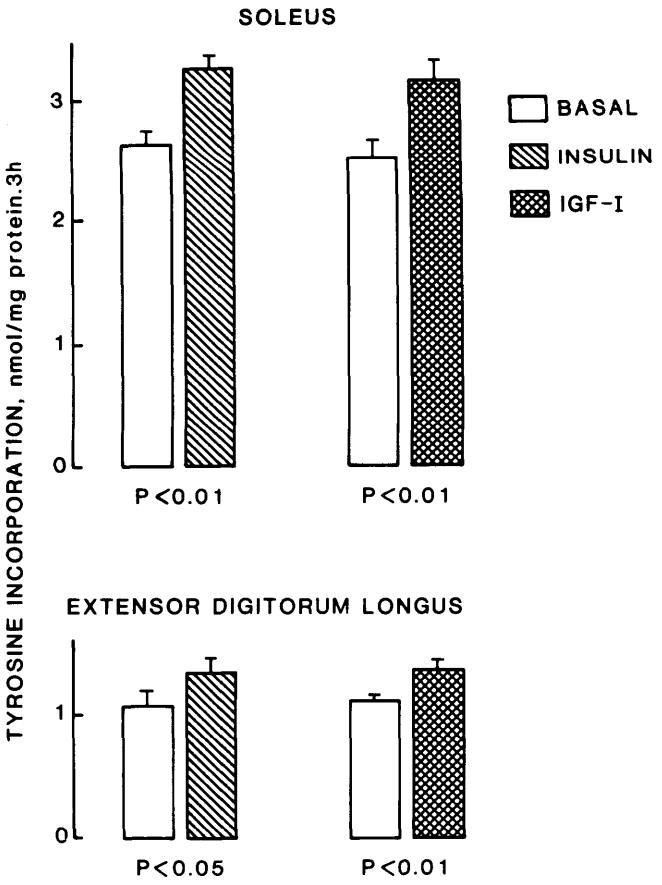

FIGURE 2. Effect of insulin and IGF-I on the rate of protein synthesis in soleus and EDL muscles of 8-wk-old lean mice. Muscles were incubated without or with insulin (6.7 nM) or IGF-I (50 nM) as described in Figure 1. Values are means ± SEM of 4–5 muscles. The statistical significance of the peptides' effect was assessed using Student's *t* test for paired comparisons.

stimulation was obtained with 6.7 nM insulin and 50 nM IGF-I; half-maximal effects (EC_{50}) were obtained with insulin at 0.4 nM or 0.6 nM for soleus and EDL muscles, respectively, and with 6 nM IGF-I in both types of muscles (Figure 1). The difference between maximal effects of insulin and IGF-I was not significant. Indeed, maximally effective concentrations of insulin (6.7 nM) and IGF-I (50 nM) tested in the same experiment (Figure 2), induced a similar stimulation of tyrosine incorporation in soleus as well as in EDL muscles. Note that basal rate of protein synthesis was 2–3 times higher in the soleus than in the EDL.

Insulin and IGF-I effects on protein synthesis in muscles of GTG-obese mice. Some of the main features of mice rendered obese by GTG treatment are summarized in Table 1. Body weight, plasma glucose, and insulin were consistently higher than in controls. While in soleus muscle, protein content was unchanged, EDL muscle from GTG-obese mice contained less protein than muscle from lean mice.

The ability of muscles from GTG-obese mice to synthesize proteins was compared with that of lean mice (Table 2). Because lean mice used in these experiments were age-matched with GTG-obese mice (25–30 wk old), the values reported in Table 2 are lower than those presented in Figure 2. No differences were observed between the two groups of mice in basal rate of tyrosine incorporation in proteins; similarly, the ability of insulin to maximally stimulate protein synthesis was unaltered. In contrast, no significant effect was obtained in EDL muscles of GTG-obese mice with 0.67 nM insulin, while the response of the soleus muscles to the same insulin concentration was not affected. As observed in muscles of lean mice (Figure 2), IGF-I (50 nM) and insulin (6.7 nM) caused a similar stimulation of protein synthesis in muscles of GTG-obese mice (Figure 3).

Protein degradation was studied in muscles of lean and GTG-obese mice. As previously reported,[14,21] the rate of protein degradation measured in vitro exceeded the rate of synthesis. Obesity did not modify basal protein breakdown. Insulin slightly reduced protein degradation in soleus and EDL muscles from lean or GTG-obese mice (Table 3), but this decrease was not statistically significant.

To look for qualitative changes in protein synthesis, ^{35}S-labeled proteins from muscles incubated without or with a maximally effective insulin concentration were analyzed by SDS-PAGE. For each type of muscle, Coomassie blue-stained protein patterns were similar in control and obese mice incubated in the absence or presence of insulin (data not shown). In contrast, the autoradiograms presented in Figure 4 revealed marked differences between the patterns of labeled proteins in soleus and EDL muscles. Insulin stimulated overall ^{35}S-methionine incorporation in muscular proteins by 80–90% as estimated by measuring the radioactivity present in acetone-ammoniac precipitates. However, the effect was more important for some bands, such as the 43,000-dalton protein (a threefold increase). This protein represented one of the major bands stained by Coomassie blue and corresponded very likely to actin. Comparable results were obtained with IGF-I (data not shown).

DISCUSSION

To evaluate the effects of insulin and insulin-like growth factor I on protein synthesis in muscles of lean and obese mice, two different methods were used: (1) tyrosine incorporation in TCA-precipitable material, a method shown to be valid for measuring overall protein synthesis in soleus and EDL muscles;[14,15,24] (2) analysis of ^{35}S-methionine-labeled proteins after separation by polyacrylamide gel electrophoresis and autoradiography. This technique allows the comparison be-

TABLE 1
Characteristics of experimental animals

	Lean	GTG-obese
Weight (g)	46.0 ± 1.0	63.3 ± 0.7*
Plasma		
Glucose (mg/100 ml)	159 ± 4	177 ± 7†
Insulin (nM)	0.43 ± 0.07	6.5 ± 1.2*
Muscle protein (mg)		
Soleus	1.86 ± 0.06	1.84 ± 0.06
EDL	2.45 ± 0.07	2.07 ± 0.06*

Plasma glucose and insulin were determined from blood samples collected immediately before muscle isolation; muscle protein contents were determined at the end of incubation, after removal of tendons. Values are means ± SEM of 25–27 values.
*Values are significantly different with $P < 0.001$.
†Values are significantly different with $P < 0.02$.

TABLE 2
Insulin effect on protein synthesis in muscles of 25–30-wk-old lean and GTG-obese mice

	Tyrosine incorporation (nmol/mg protein, 3 h)					
Insulin (nM)	0	0.67	P	0	6.7	P
Soleus						
Lean	1.69 ± 0.08	1.89 ± 0.06	< 0.05	1.30 ± 0.09	1.83 ± 0.10	< 0.001
GTG-obese	1.41 ± 0.08	1.56 ± 0.10*	< 0.02	1.48 ± 0.10	1.88 ± 0.15*	< 0.001
Extensor digitorum longus						
Lean	0.72 ± 0.02	0.81 ± 0.03	< 0.01	0.61 ± 0.03	0.85 ± 0.04	< 0.01
GTG-obese	0.64 ± 0.04	0.68 ± 0.04	NS	0.62 ± 0.04	0.86 ± 0.04*	< 0.001

Two muscles from one mouse were incubated for 3 h as described in Figure 1, in the presence or the absence of insulin. Tyrosine incorporation into protein was measured as described in METHODS. Values are means ± SEM of 9–15 muscles. Statistical significance was assessed using Student's *t* test for paired comparisons. NS: not significant.
*The percent increase over basal was not statistically different between lean and GTG-obese mice.

tween different proteins, but is mostly restricted to a more qualitative aspect, because it requires the use of very low external concentration of methionine, which might become rate-limiting for protein synthesis. The results obtained by both approaches are comparable. Insulin stimulated tyrosine incorporation in total proteins by about 1.4 times. This result is in agreement with previous studies made in muscles isolated from rats and mice.[14,15,19,24] When methionine incorporation in total proteins was measured, the stimulatory effect of insulin was more important. This is probably due to the fact that insulin enhanced the entry of methionine into muscles, while it did not affect tyrosine uptake.[25] Measurement of the rate of amino acid incorporation into proteins reflects an equilibrium between synthesis and degradation. Under the in vitro conditions used, the rate of breakdown is higher than the rate of synthesis.[14,19,24] Since insulin did not significantly decrease tyrosine release, the stimulation of incorporation of labeled amino acid in proteins necessarily implies the stimulation of protein synthesis.

Insulin-like growth factor I significantly stimulated protein synthesis in both soleus and EDL muscles. Although IGF-I promotes α-amino-isobutyric acid transport in muscles (data not shown), its effect on protein synthesis is probably independent of an increase in intracellular amino acid concentration since it did not change tyrosine specific activity in the muscles. IGF-I was virtually as effective as insulin, but its potency was only 1/20 to 1/10 that of insulin. Such a biologic potency ratio between IGF-I and insulin is comparable to that found for their effects on glucose transport and metabolism in soleus muscle.[26] Specific IGF-I binding sites have been demonstrated in soleus muscle.[26] Since the affinity of IGF-I for the insulin receptor was low (less than 1% that of insulin) compared with its relative potency for stimulation of protein synthesis (5–10% that of insulin), it is likely that IGF-I exerts its effects, at least partly, through its own receptors. The range of concentrations where IGF-I was effective in stimulating protein synthesis in skeletal muscle suggests an "insulin-like" effect rather than a "growth-promoting" effect. This is at variance with the observations made

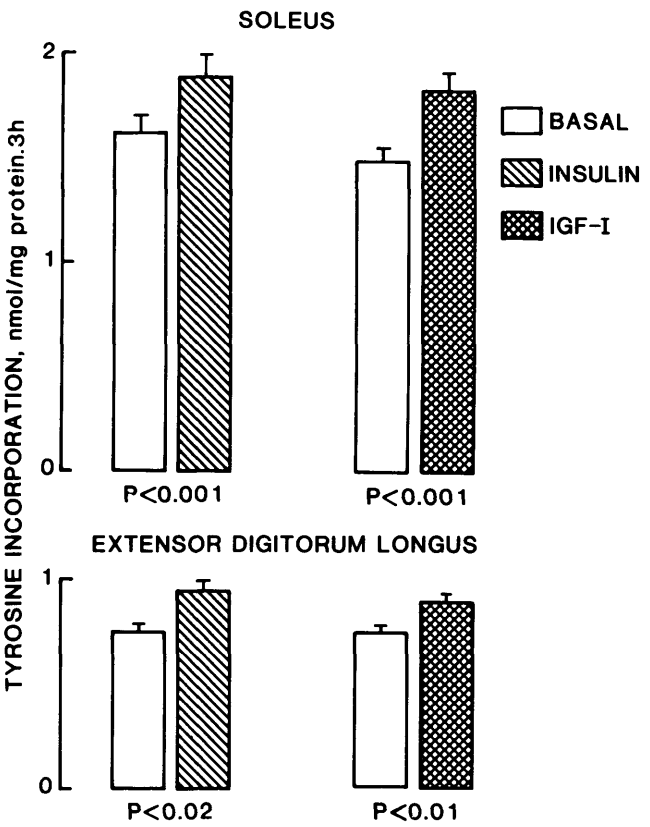

FIGURE 3. Effect of insulin and IGF-I on the rate of protein synthesis in soleus and EDL muscles of 25–30-wk-old GTG-obese mice. Muscles were isolated from GTG-obese mice and incubated as described in Figure 2. Values are means ± SEM of 13–20 muscles.

TABLE 3
Rate of protein degradation in muscles of 25–30-wk-old lean and GTG-obese mice

	Tyrosine release (nmol/mg protein, 3 h)	
Insulin (nM)	0	6.7
Soleus		
Lean	3.79 ± 0.20	3.30 ± 0.15
GTG-obese	3.45 ± 0.17	3.11 ± 0.19
Extensor digitorum longus		
Lean	3.18 ± 0.15	2.94 ± 0.34
GTG-obese	2.97 ± 0.16	2.58 ± 0.20

Muscles were incubated for 3 h in the presence of cycloheximide (0.5 mM); protein degradation was estimated from the amount of released tyrosine in the medium. Results are means ± SEM of 8–10 muscles. Insulin effect was not significant.

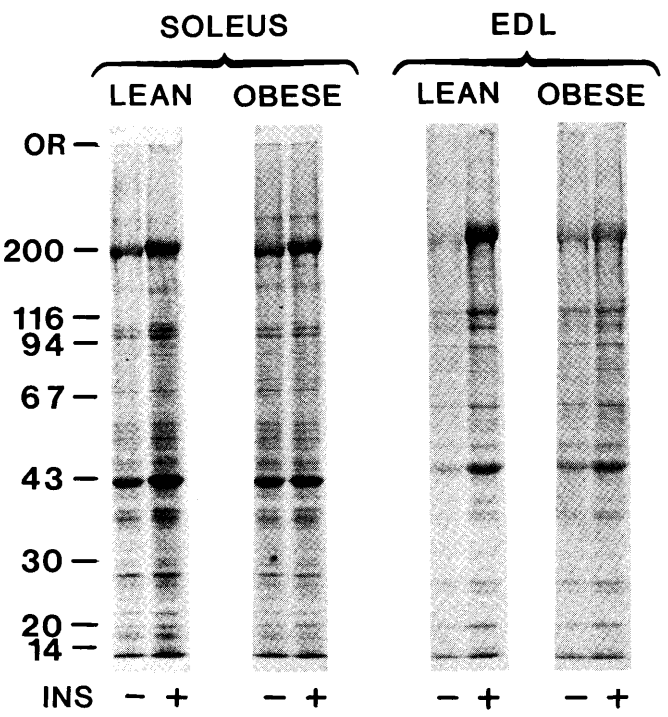

FIGURE 4. Polyacrylamide gel electrophoresis of ^{35}S-labeled proteins in soleus and EDL muscles of 25–30-wk-old lean and GTG-obese mice. Muscles were incubated for 3 h with ^{35}S-methionine in the absence or the presence of 6.7 nM insulin, lysed in boiling SDS; samples were analyzed by one-dimensional gel electrophoresis as described in METHODS.

in chick embryo fibroblasts where IGF-I acted as a growth factor since it was about 50 times more potent than insulin in stimulating DNA, RNA, and protein synthesis.[11]

A somewhat unexpected finding made in this study was the absence of alteration of basal and maximally insulin-stimulated rates of synthesis and of degradation of proteins in muscles from GTG-obese mice. Mice that have been injected with goldthioglucose develop a syndrome of obesity with insulin resistance detectable in vivo[17] and in vitro.[17,18] Previous in vitro studies performed in soleus muscles from these animals had revealed many abnormalities. Thus glucose and amino acid transport, both basal and maximally stimulated by insulin, were decreased.[18,26,27] Furthermore, insulin sensitivity was diminished together with the number of insulin receptors.[18] In the present study one would have also expected to find a loss of hormone sensitivity for stimulation of protein synthesis. However, using submaximally effective concentration of insulin, we were able to obtain an analogous effect of the hormone in the soleus muscle from both lean and obese animals. This suggests that the sensitivity of the soleus muscle for insulin was not affected.† In contrast, under identical conditions (submaximally effective insulin concentration), the EDL muscle from obese mice was insensitive to the hormone. Such a difference in sensitivity between the white EDL and the red soleus muscle toward protein synthesis has been observed in other circumstances. Indeed, fasting,[24] short-term insulinopenic diabetes[15] and treatment with glucocorticoids[28] induced a decrease in the rate of protein synthesis in fast-twitch (EDL) muscles, whereas slow-twitch (soleus) muscles were unaffected. This is presently not understood.

In conclusion, in GTG-obese mice, protein turnover is not markedly altered when studied in isolated muscles incubated in vitro. This suggests that the abnormalities that are observed in obese animals in vivo is a consequence of a complex abnormal hormonal profile and perhaps of a reduced physical activity rather than of an alteration intrinsic to the protein synthesis machinery.

†Dose-response curves for insulin were not performed in muscles from GTG-obese mice. Indeed, the small magnitude of the insulin effect that renders determination of EC_{50} inaccurate makes unrealistic the demonstration of a rightward shift in dose-response curves.

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REFERENCES

1 Wool, I. G., Castles, J. J., Leader, D. P., and Fox, A.: Insulin and the function of muscle ribosomes. *In* Handbook of Physiology, Section 7: Endocrinology. Vol. 1. Steiner, D. F., and Freinkel, N., Eds. Washington, American Physiological Society, 1972:385–94.

2 Jefferson, L. S.: Role of insulin in the regulation of protein synthesis. Diabetes 1980; 29:487–96.

3 Waterlow, J. C., Garlick, P. J., and Millward, D. J.: Protein Turnover in Mammalian Tissues and in the Whole Body. Amsterdam, New York, Oxford, North Holland Publishing Company, 1978.

4 Shapira, J. F., Kircher, I., and Martin, R. J.: Indices of skeletal muscle growth in lean and obese Zucker rats. J. Nutr. 1980; 110:1313–18.

5 Lin, P. Y., Romsos, D. R., and Leveille, G. A.: Food intake, body weight gain, and body composition of the young obese (ob/ob) mouse. J. Nutr. 1977; 107:1715–23.

6 Bergen, W. G., Kaplan, M. L., Merkel, R. A., and Leveille, G. A.: Growth of adipose and lean tissue mass in hindlimbs of genetically obese mice during preobese and obese phase of development. Am. J. Clin. Nutr. 1975; 28:157–61.

7 Dunn, M. A., and Hartsook, E. W.: Comparative amino acid and protein metabolism in obese and non-obese Zucker rats. J. Nutr. 1980; 110:1865–79.

8 Chee, K. M., Romsos, D. R., Bergen, W. G., and Leveille, G. A.: Protein intake regulation and nitrogen retention in young obese and lean mice. J. Nutr. 1981; 111:58–67.

9 Trostler, N., Romsos, D. R., Bergen, W. G., and Leveille, G. A.: Skeletal muscle accretion and turnover in lean and obese (ob/ob) mice. Metabolism 1979; 28:928–33.

10 Karakash, C., Rohner-Jeanrenaud, F., Hustvedt, B. E., and Jeanrenaud, B.: Nitrogen handling in adult hypothalamic obese rats. Am. J. Physiol. 1980; 238:E32–37.

11 Zapf, J., Froesch, E. R., and Humbel, R. E.: The insulin-like growth factors (IGF) of human serum: chemical and biological characterization and aspects of their possible physiological role. Curr. Top. Cell. Regul. 1981; 19:257–309.

12 Uthne, K., Reagan, C. R., Gimpel, L. P., and Kostyo, J. L.: Effects of human somatomedin preparations on membrane transport and protein synthesis in the isolated rat diaphragm. J. Clin. Endocrinol. Metab. 1974; 39:548–54.

13 Salmon, W. D., Jr., and Du Vall, M. R.: In vitro stimulation of leucine incorporation into muscle and cartilage protein by a serum fraction with sulfation factor activity: differentiation of effects from those of growth hormone and insulin. Endocrinology 1970; 87:1168–80.

14 Frayn, K. N., and Maycock, P. F.: Regulation of protein metabolism by a physiological concentration of insulin in mouse soleus and extensor digitorum longus muscles. Effects of starvation and scald injury. Biochem. J. 1979; 184:323–30.

15 Flaim, K. E., Copenhaver, M. E., and Jefferson, L. S.: Effects of diabetes on protein synthesis in fast- and slow-twitch rat skeletal muscle. Am. J. Physiol. 1980; 239:E88–95.

16 Goldberg, A. L., Martel, S. B., and Kushmerick, M. J.: In vitro preparations of the diaphragm and other skeletal muscles. Methods Enzymol. 1975; 39:82–94.

[17] Le Marchand, Y., Freychet, P., and Jeanrenaud, B.: Longitudinal study on the establishment of insulin resistance in hypothalamic obese mice. Endocrinology 1978; 102:74–85.

[18] Le Marchand-Brustel, Y., Jeanrenaud, B., and Freychet, P.: Insulin binding and effects in isolated soleus muscle of lean and obese mice. Am. J. Physiol. 1978; 234:E348–58.

[19] Fulks, R. M., Li, J. B., and Goldberg, A. L.: Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J. Biol. Chem. 1975; 250:290–98.

[20] Waalkes, T. P., and Udenfriend, S.: A fluorometric method for the estimation of tyrosine in plasma and tissues. J. Lab. Clin. Med.: 1957; 50:733–36.

[21] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 265:265–75.

[22] Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680–85.

[23] Snedecor, G. W., and Cochran, W. G.: Statistical Methods, 6th edit. Ames, Iowa State University Press, 1967.

[24] Li, J. B., and Goldberg, A. L.: Effects of food deprivation on protein synthesis and degradation in rat skeletal muscles. Am. J. Physiol. 1976; 231:441–48.

[25] Manchester, K. L.: The control by insulin of amino acid accumulation in muscle. Biochem. J. 1970; 117:457–65.

[26] Poggi, C., Le Marchand-Brustel, Y., Zapf, J., Froesch, E. R., and Freychet, P.: Effects and binding of insulin-like growth factor I in the isolated soleus muscle of lean and obese mice: comparison with insulin. Endocrinology 1979; 105:723–30.

[27] Le Marchand-Brustel, Y., Moutard, N., and Freychet, P.: Aminoisobutyric acid transport in soleus muscles of lean and gold thioglucose-obese mice. Am. J. Physiol. 1982; 243:E74–79.

[28] Rannels, S. R., and Jefferson, L. S.: Effects of glucocorticoids on muscle protein turnover in perfused rat hemicorpus. Am. J. Physiol. 1980; 238:E564–72.