ALTERATION OF GLYCOGEN SYNTHASE ACTIVATION BY INSULIN IN SOLEUS MUSCLES OF OBESE MICE

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1. Introduction

Skeletal muscle from obese, insulin-resistant rodents is characterized by an impaired ability to increase glycogen formation in response to insulin in vivo [1,2] and in vitro [3–5]. Insulin stimulates glycogen synthesis in muscle by activating both glucose transport and the enzyme glycogen synthase [6,7]. In skeletal muscle of the genetically obese *ob/ob* mouse, the basal activities of the two forms (phosphorylated and dephosphorylated) of glycogen synthase and glycogen phosphorylase have been reported to be essentially unaltered [8]. It was thus assumed that the marked insulin resistance of muscle glycogen synthesis in obesity resulted only from impaired glucose transport, a defect which has been well documented in obese mice [3,5]. However, the effect of insulin on glycogen synthase in muscle from obese animals has not been specifically examined.

Here we have directly investigated the activation of glycogen synthase by insulin in soleus muscles of goldthioglucose (GTG)-obese mice. Since it has been shown that this activation in skeletal muscle occurs via a mechanism independent of insulin effect on glucose transport [6,9,10], the present studies were performed in the absence of extracellular glucose. The results strongly suggest that the activation of glycogen synthase, an intracellular step of insulin action, is impaired in soleus muscles of obese mice.

2. Materials and methods

Soleus muscles were isolated from lean or GTG-obese mice [5,6] and preincubated for 15 min as in [3,5]. They were then incubated for 90 min at 37°C in Krebs-Ringer bicarbonate buffer containing 20 mg defatted bovine albumin/ml and 2 mM pyruvate, followed by a 30 min incubation in the presence of varying concentrations of insulin, or by a 10 min incubation with 1 μM epinephrine. No glucose was present in the extracellular medium throughout the incubations. At the end of the incubation, muscles were freeze-clamped in liquid nitrogen and homogenized as in [11]. Homogenization buffers were 50 mM Tris–HCl (pH 8.2), 5 mM EDTA, 100 mM KF for glycogen synthase; 50 mM morpholino ethane sulfonic acid (pH 6.5), 100 mM EDTA, 100 mM KF for glycogen phosphorylase. Glycogen synthase activity and glycogen content were measured as in [11]. Glycogen synthase active (I) form is expressed as a percentage of total (I + D) synthase activity. Glycogen phosphorylase was measured as in [12]. Phosphorylase active (*a*) form is expressed as % of total activity. Total enzymatic activities are expressed as nanomoles of substrate incorporated into glycogen per minute and per milligram of muscle protein.

3. Results

Soleus muscles were isolated from lean and GTG-obese mice whose characteristics are presented in table 1. Following GTG injection mice became markedly obese and moderately hyperglycemic, despite hyperinsulinemia. In this model of acquired obesity, insulin resistance has been demonstrated in vivo and in vitro [3,4]. Muscle protein and glycogen contents were identical in obese and control mice. While in soleus muscles of GTG-obese mice, glycogen synthase total activity was slightly increased, glycogen phosphorylase was not significantly different from controls.

Table 1
Characteristics of experimental mice

	n	Lean	n	GTG-obese	P
Weight (g)	22	37.5 ± 1.0	36	56.2 ± 0.7	< 0.001
Plasma					
Glucose (mg/100 ml)	18	185.5 ± 4.7	36	232.0 ± 4.4	< 0.001
Insulin (ng/ml)	18	2.1 ± 0.2	34	7.2 ± 1.1	< 0.005
Soleus muscle					
Protein (mg/muscle)	12	2.33 ± 0.06	12	2.21 ± 0.03	n.s.
Glycogen (μg/mg protein)	6	16.7 ± 1.8	6	19.2 ± 1.7	n.s.
Glycogen synthase (nmol . mg protein^{-1} . min^{-1})	9	23.1 ± 1.2	11	28.0 ± 1.5	< 0.025
Glycogen phosphorylase (nmol . mg protein^{-1} . min^{-1})	32	276 ± 8	28	302 ± 11	n.s.

Plasma glucose and insulin were determined from blood samples obtained immediately before muscle isolation [3]. Protein and glycogen contents, and total enzymatic activities, were measured in muscles at the end of incubations. Values are means ± SE with the number of *n* determinations. Statistical significance was assessed by *t*-test for unpaired comparisons. n.s., not significant

The activation of glycogen synthase following muscle exposure to varying insulin concentrations for 30 min in the absence of extracellular glucose is shown in fig.1; this duration (30 min) of muscle exposure to insulin had been shown to result in a maximal activation of glycogen synthase [11]. In muscles from lean mice, insulin increased the activity ratio of glycogen synthase from 31% to 50%. Half-maximal effect occured at 1.5 nM insulin. In muscles from GTG-obese mice, insulin was much less effective than in muscles from controls in activating glycogen synthase, both at submaximal and maximal concentrations (fig.1). Basal glycogen synthase activity (measured in the absence of insulin) was not statistically different in muscles from obese and lean animals. The defective insulin response in muscles from obese mice could not be explained by an increase in muscle glycogen content or by a decrease in the total activity of the enzyme (table 1).

To investigate whether in muscles of obese mice glycogen synthase and glycogen phosphorylase could be phosphorylated by endogenous cyclic AMP-dependent protein kinase, the activity of both enzymes was measured following muscle exposure to epinephrine. Fig.2 shows that basal activities were not statistically different in muscles of lean and obese mice. Following a 10 min exposure of muscle to 1 μM epinephrine, conditions under which a maximal effect of epinephrine was observed (not shown), glycogen synthase was

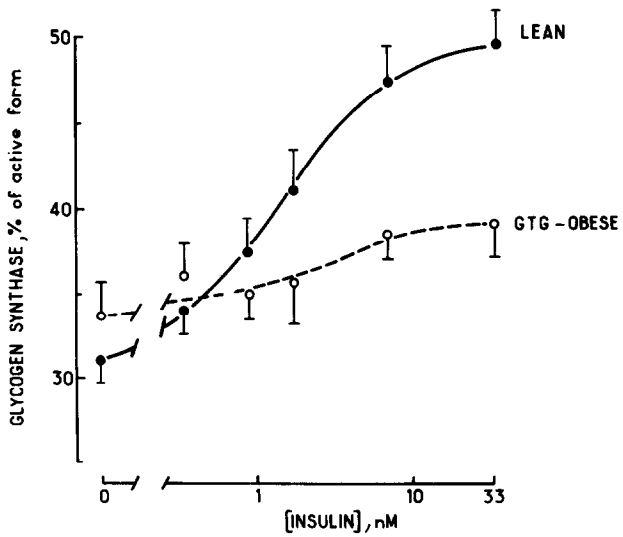

Fig.1. Insulin effect on glycogen synthase in soleus muscles of lean and obese mice. Muscles isolated from lean and gold-thioglucose (GTG)-obese mice were incubated as in section2, in the absence or presence of insulin. At the end of the incubation, glycogen synthase activity was measured in muscle extracts as in section 2, in the absence (I form) or presence (I + D form) of 6.7 mM glucose 6-phosphate. The data are expressed as the percent of active form (I/I + D) × 100) of glycogen synthase. Each point is the mean ± SE of 8 (GTG-obese) or 20 (lean) muscles.

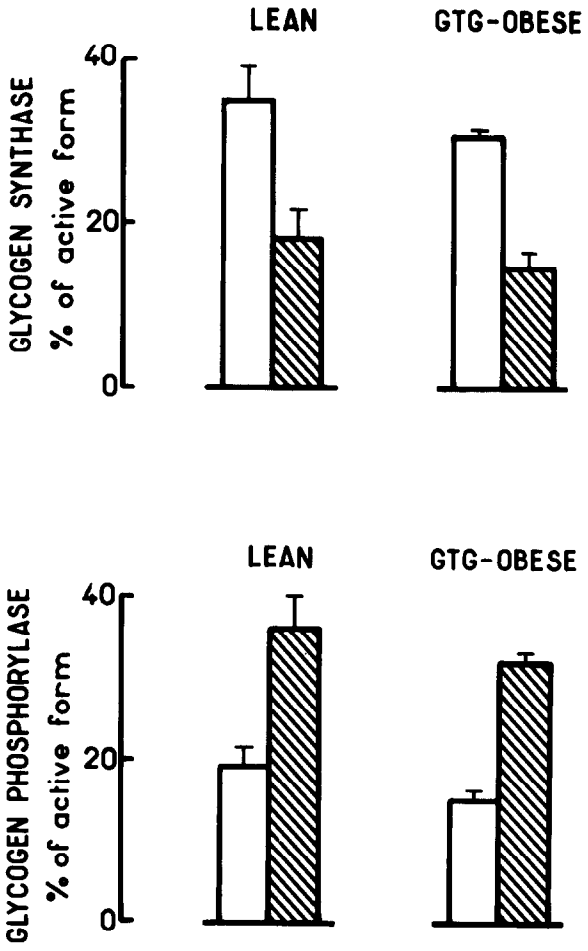

Fig.2. Epinephrine effect on glycogen synthase and glycogen phosphorylase in soleus muscles of lean and obese mice. Muscles from lean and GTG-obese mice were incubated as in section 2, without (open bars) or with (hatched bars) 1 μM epinephrine. Enzyme activities were measured as in fig.1 and section2. The data are expressed as % of active form of glycogen synthase and of glycogen phosphorylase, and represent the means ± SE of 5 muscles for each condition.

inactivated and glycogen phosphorylase was activated with the same magnitude in muscles from obese and from lean animals (fig.2).

4. Discussion

These studies have indicated that in soleus muscles of obese mice the activation of glycogen synthase by insulin is markedly diminished. Previous studies have shown that muscle is the first tissue to develop insulin resistance in GTG-induced obesity [4] or in genetically obese *ob/ob* [1] or *mdb/mdb* [13] mice. The results reported here suggest that the pronounced decrease in insulin-stimulated glycogen synthesis does not stem merely from a decrease in insulin-activated glucose transport [3,5], but also results from a diminished activation of glycogen synthase. Glycogen controls its own synthesis by affecting the interconversion of the two forms of glycogen synthase [14]. This mechanism is unlikely to be the cause of the altered insulin responsiveness since glycogen content was not increased in muscles from obese animals. This observation of a normal glycogen content in GTG-obese muscles despite hyperglycemia (and thus increased substrate availability) and hyperinsulinemia is a further indication that muscle glycogen synthesis is insulin-resistant in obese mice in vivo.

The number of insulin receptors is decreased by ~30% in muscles of GTG-obese mice [3]. This modest defect is unlikely to account for the markedly decreased insulin responsiveness, particularly at maximally effective concentrations since insulin exerts maximal effects in soleus muscles when only a fraction of total receptors is occupied [3]. The recent observation [10] that insulin-like growth factor (IGF-I) was much less effective in activating glycogen synthase in muscles of obese mice than in lean controls, whereas IGF-I binding was unaltered, also suggests that the alteration of glycogen synthase activation is a post-receptor defect. A recent report [15] has suggested that insulin would dephosphorylate a protein phosphatase inhibitor, resulting in an activation of the glycogen synthase phosphatase and therefore of the glycogen synthase. One step in this cascade is apparently defective in muscles of obese mice.

The effect of epinephrine was unaltered in muscles of obese mice (fig.2). This observation indicates that cyclic AMP-dependent protein kinase is normally responsive to epinephrine in muscles from obese mice. This is in contrast with the impaired response of adenylate cyclase to epinephrine in adipocyte plasma membranes from *ob/ob* mice [16]. Our data also suggest that in muscles of obese mice glycogen synthase is still capable of being modulated by changes in endogenous cyclic AMP levels.

Glycogen synthase activation by insulin is markedly altered in muscles of obese mice. This abnormality points to an intracellular defect of insulin action. In [17], insulin increased the concentration of a low

molecular weight substance in rat skeletal muscle. Whether the defective activation of glycogen synthase by insulin in muscles of obese mice may result from a defective emergence or effectiveness of this putative 'mediator' remains to be investigated.

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References

[1] Stauffacher, W. and Renold, A. E. (1969) Am. J. Physiol. 216, 98–105.
[2] Czech, M. P., Richardson, D. K., Becker, S. G., Walters, C. G., Gitomer, W. and Heinrich, J. (1978) Metabolism 27, 1967–1981.
[3] Le Marchand-Brustel, Y., Jeanrenaud, B. and Freychet, P. (1978) Am. J. Physiol. 234, E348–E358.
[4] Le Marchand, Y., Freychet, P. and Jeanrenaud, B. (1978) Endocrinology 102, 74–85.
[5] Cuendet, G. S., Loten, E. G., Jeanrenaud, B. and Renold, A. E. (1976) J. Clin. Invest. 58, 1078–1088.
[6] Larner, J. and Villar-Palasi, C. (1971) Curr. Top. Cell. Regul. 3, 195–236.
[7] Cohen, P. Antoniw, J. F., Nimmo, H. G. and Yeaman, S. J. (1976) in: Polypeptide hormones: Molecular and cellular aspects. Ciba Found. Symp. 41, pp. 281–295, Elsevier/Excerpta Medica, Amsterdam, New York.
[8] Das, I. and Hems, D. A. (1974) Horm. Metab. Res. 6, 40–44.
[9] Le Marchand-Brustel, Y. and Freychet, P. (1979) J. Clin. Invest. 64, 1505–1515.
[10] Poggi, C., Le Marchand-Brustel, Y., Zapf, J., Froesch, E. R. and Freychet, P. (1979) Endocrinology 105, 723–730.
[11] Le Marchand-Brustel, Y., Cohen, P. T. W. and Cohen, P. (1979) FEBS Lett. 105, 235–238.
[12] Gilboe, D. P., Larson, K. L. and Nuttall, F. Q. (1972) Anal. Biochem. 47, 20–27.
[13] Le Marchand-Brustel, Y. and Jeanrenaud, B. (1978) Amer. J. Physiol. 234, E568–E574.
[14] Danforth, W. H. (1965) J. Biol. Chem. 240, 588–593.
[15] Foulkes, J. G., Jefferson, L. S. and Cohen, P. (1980) FEBS Lett. 112, 21–24.
[16] Laudat, M. H. and Pairault, J. (1975) Eur. J. Biochem. 56, 583–589.
[17] Larner, J., Galasko, G., Cheng, K., De Paoli-Roach, A. A., Huang, L., Daggy, P. and Kellogg, J. (1979) Science 206, 1408–1410.