Adaptive Nature of Glycogen Synthetase Activity in Rat Adipose Tissue. Requirement for Insulin and Energy[1] (35670)

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Glycogen accumulation in adipose tissue of animals trained to consume their food in a single daily 2-hr meal (meal-fed) proceeds at a rate approximately 50 times that occurring in tissue of fasted–refed control animals (1). The increased capacity for glycogen storage produced by meal-feeding is accompanied by an increased activity of all enzymes required for the conversion of glucose to glycogen (2). In addition, UDPG-glycogen-transglucosylase (glycogen synthetase), presumably the rate-limiting enzyme for glycogen synthesis in adipose tissue, increases further in the meal-fed animal following the ingestion of the daily meal. *In vivo* studies have indicated that the enzyme activity increases with time after meal initiation until about 5 hr afterwards and the increase is only partially blocked by puromycin administration (2). Adipose tissue glycogen synthetase activity also increases in fasted *ad libitum*-fed animals as a result of food ingestion, but the increase is less than that seen in meal-fed rats (2).

The activity of glycogen synthetase in adipose tissue of fasted rats, particularly in tissue of meal-fed animals, has been found to increase during *in vitro* incubation under appropriate conditions. This system has been employed to study the influence of various substrates and insulin on the increase in enzyme activity. The results of these experiments form the basis for this report.

Materials and Methods. Male Sprague-Dawley rats, weighing approximately 250 g, were used in all experiments. Some of the animals were fed *ad libitum* (nibblers) and others were allowed access to food daily from 8 to 10 a.m. only (meal-fed). The animals were maintained on their respective feeding schedules for at least 3 weeks before use, since previously observed adaptations to meal-feeding have been shown to occur within this time (3). Animals were kept in a room maintained at 22° in individual metal cages having raised wire floors. Water was available at all times and the diet fed during the experimental period supplied approximately 19, 12, and 70% of the calories as casein, corn oil, and glucose, respectively. The composition of the diet has been given elsewhere (4). The effects of pattern of food intake on body weight and food consumption were similar to those previously described (5).

Animals were killed by decapitation and the epididymal fat pads were rapidly removed and placed in a 0.9% NaCl solution. Fat pads were cut into sufficient portions to provide one piece for assaying initial enzymatic activity and one piece for incubation under each of the various conditions included in a given experiment. The tissue used for determining initial enzymatic activity was homogenized[2] immediately in glycylglycine buffer (0.1 *M*, pH 8.3) containing 5 m*M* mercaptoethanol. Homogenates were centrifuged at 3000*g* for 15 min, and the supernatant was used for enzymatic analysis. The other portions of tissue were incubated in Ca^{2+}-free Krebs–Ringer bicarbonate (KRB) buffer (6) (pH 7.4), at 37°, under an atmosphere of 95% O_2–5% CO_2. Other additions to the incubation buffers are indicated in Tables

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[2] Sorvall Omnimixer, Ivan Sorvall, Inc., Norwalk, Conn.

I–III. After the appropriate incubation time, tissues were rinsed in 0.9% NaCl, homogenized, and centrifuged as described for the control tissues. Enzymatic activity was assayed as previously described (2) except that assays were conducted at pH 8.3 rather than 7.4, and in the presence of 10 m*M* dithioerythritol. The final glycogen precipitate was heated at 100° in 1 ml of water until dissolved and the total volume was then transferred to a vial and 15 ml of a triton-toluene[3] scintillation solution[4] were added. Radioactivity was determined as previously described (2). Protein determinations were conducted on the supernatants used for the enzyme assays according to the method of Lowry *et al.* (7). One experiment was designed to study protein synthesis from lysine-U-^{14}C and the oxidation of glucose-U-^{14}C and its incorporation into fatty acids by isolated adipose tissue. Adipose tissue protein was isolated as previously described (8). The method used for CO_2 collection, isolation of fatty acids and determining radioactivity have also been described (9). Differences between groups were tested for significance by the *t* test. Puromycin dihydrochloride[5] and UDP-glucose-U-^{14}C[6] were purchased, as were all other biochemicals.[7]

Results. The results presented in Table I demonstrate that glycogen synthetase activity in adipose tissue of fasted rats increases during *in vitro* incubation in the presence of glucose and insulin. In tissue of meal-fed animals the increase appears to be maximal after 2.5 hr of incubation. The greatest increase occurs during this same time in tissue of nibbling rats but a further increase in enzyme activity occurs between 2.5 and 5 hr of incubation. The observed increase in glycogen synthetase activity was almost exclusively in the assay for total activity, *i.e.*, in the presence of glucose-6-phosphate (G-6-*P*).

TABLE I. Effect of Incubation Time on *in Vitro* Adaptation of Adipose Tissue Glycogen Synthetase.[a]

Incubation time (hr)	Glycogen synthetase; (nmoles of substrate utilized/mg of protein/min)	
	—G-6-*P*	+G-6-*P*
	Meal-fed	
0	0.4 ± 0.05[b]	5.3 ± 0.6
2.5	0.3 ± 0.04	9.2 ± 0.4
5.0	0.2 ± 0.1	8.0 ± 0.2
	Nibbling	
0	0.2 ± 0.04	2.2 ± 0.1
2.5	0.2 ± 0.09	4.3 ± 0.4
5.0	0.4 ± 0.14	5.8 ± 0.3

[a] Tissues obtained from animals maintained without food for 22 hr was incubated in a buffer containing 5 μmoles of glucose, and 0.1 U of insulin/ml.

[b] Mean for 5 animals ± SEM.

It should be noted that the increase in total activity was not accompanied by an increase in the activity measured in the absence of G-6-*P*, *i.e.*, the independent form of the enzyme.

The next experiments were designed to determine whether the observed increase in glycogen synthetase activity represented an in-

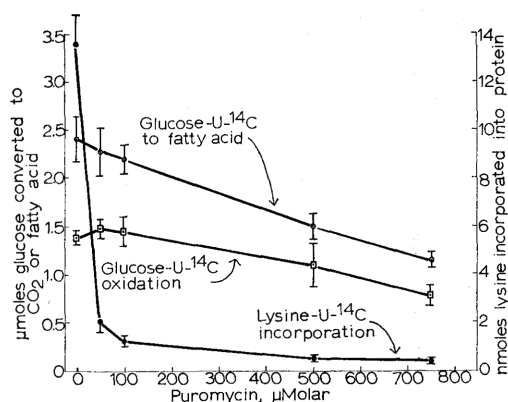

FIG. 1. Influence of puromycin concentration on glucose-U-^{14}C and L-lysine-U-^{14}C utilization by isolated adipose tissue. Adipose tissue was incubated in 3 ml of bicarbonate buffer containing per ml: glucose, 5 μmoles; L-lysine, 5 μmoles; insulin, 0.1 unit; and glucose-U-^{14}C or L-lysine-U-^{14}C, 0.3 μCi. Each point represents the mean for five animals. The vertical bars represent ± SEM.

[3] Ready-Solv, purchased from Beckman Instruments, Inc., Fullerton, Cal.

[4] Omnifluor, purchased from New England Nuclear Corp., Boston, Mass.

[5] Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

[6] New England Nuclear Corp., Boston, Mass.

[7] Sigma Biochemical Corp., St. Louis, Mo.

crease in *de novo* protein synthesis. Puromycin was selected as an inhibitor of protein synthesis (10, 11) for these studies. The experiment summarized in Fig. 1 was conducted to select a level of puromycin which would inhibit protein synthesis without interfering with other cellular activities. As shown, puromycin concentrations between 100 and 500 μM caused a 90–95% reduction in lysine-U-^{14}C incorporation into protein by isolated adipose tissue without significantly ($p > 0.05$) affecting glucose-U-^{14}C oxidation or incorporation into fatty acids. Thus a puromycin concentration of 200 μM was selected for subsequent experiments. This concentration of puromycin inhibited the *in vitro* increase in glycogen synthetase activity in adipose tissue of meal-fed and nibbling animals by less than 25% (Table II). These data imply that the increase in glycogen synthetase activity was not due to *de novo* enzyme synthesis.

The experiments summarized in Table III were conducted to determine the effect of various substrates and insulin on the *in vitro* increase in glycogen synthetase activity. Adipose tissue from meal-fed rats was employed since it showed a greater response than tissue from nibbling animals. Glucose plus insulin produced the greatest increase in enzyme activity. Glucose alone at a concentration of 5 or 100 mM produced much less of an increase in glycogen synthetase activity and

TABLE II. Effect of Puromycin on *in Vitro* Adaptation of Adipose Tissue Glycogen Synthetase from Meal-Fed and Nibbling Animals.[a]

Incubation time[b] (hr)	Puromycin, 200 M	Glycogen synthetase[c]; (nmoles/min/mg of protein[d])	
		Meal-fed	Nibbling
0	—	5.1 ± 0.6	2.5 ± 0.2
2.5	—	9.4 ± 0.1[e]	4.5 ± 0.3[e]
	+	8.4 ± 0.7[e]	4.1 ± 0.3[e]

[a] All animals maintained without food for 22 hr prior to killing.

[b] Incubation medium as described for Table I.

[c] All assays conducted in the presence of G-6-P.

[d] Mean for 4 (meal-fed) or 5 (nibblers) animals ± SEM.

[e] Significantly different from control ($p < .02$).

insulin alone was likewise relatively ineffective. Pyruvate and fructose were about as effective as glucose in stimulating glycogen synthetase activity and a further increase was observed when these substrates were added in the presence of insulin.

Discussion. The major objective of these experiments was to elucidate the factors which trigger the diurnal increase in glycogen synthetase activity in adipose tissue of meal-fed rats (1, 2). The fact that the activity of this enzyme could be increased in adipose tissue incubated *in vitro* provided a convenient system to systematically study several factors which could be anticipated to be involved in the response.

Previous *in vivo* studies (2) had suggested that the increase in enzyme activity could not be entirely attributed to *de novo* protein synthesis. However, interpretation of these results was complicated by the fact that we had no evidence that the dose of puromycin administered had completely abolished protein synthesis. The results obtained with the *in vitro* system have shown that in the presence of a level of puromycin which almost completely blocked lysine-U-^{14}C incorporation into protein without significantly altering glucose utilization, the increase in activity must represent the conversion of a form of the enzyme which is not active in the usual assay procedure to an assayable form. Our results are in accord with the scheme proposed by Vardanis (12), which suggests that glycogen synthetase exist in three forms, a form dependent upon G-6-P for activity (D), a second form independent (I) of G-6-P (13) and a third inactive form which is convertable to the assayable D and I forms of the enzyme. Evidence in support of such a scheme has been presented for glycogen synthetase in rat muscle (14), liver (15), and in mouse liver (12). The present report would add the rat adipose tissue enzyme to this list.

The combined results presented in Table III imply two requirements for the observed adaptive increase in glycogen synthetase activity, namely, a need for an energy source and for insulin. The availability of energy alone can potentiate an increase in enzyme activity although not a maximal response.

TABLE III. Effect of Various Substrates on *in Vitro* Adaptation of Adipose Tissue Glycogen Synthetase.[a]

Substrate	Expt.:	Glycogen synthetase[b]; (nmoles substrated/min/mg of protein) 1	2
Control		3.8 ± 0.2[c]	2.3 ± 0.3
Glucose, 5 m*M*		4.5 ± 0.5	4.1 ± 0.2[d]
100 m*M*		4.9 ± 0.4[d]	4.2 ± 0.3[d]
5 m*M* + insulin, 0.1 U/ml		6.7 ± 0.6[d]	6.1 ± 0.7[d]
Fructose, 10 m*M*		4.3 ± 0.4	4.9 ± 0.7[d]
+ insulin, 0.1 U/ml		6.1 ± 0.1[d]	4.9 ± 0.3[d]
Pyruvate, 10 m*M*		5.4 ± 0.3[d]	4.5 ± 0.5[d]
+ insulin, 0.1 U/ml		6.1 ± 0.4[d]	4.8 ± 0.9[d]
Insulin, 0.1 U/ml		4.1 ± 0.3	2.7 ± 0.3

[a] Adipose tissue from meal-fed rats removed 22 hr after the last meal and incubated 2.5 hr in 10 ml of KRB containing the substrates indicated.
[b] Assays conducted in the presence of G-6-*P*.
[c] Mean for 5 animals ± SEM.
[d] Significantly different from control ($p < .05$).

Insulin, on the other hand, is ineffective in the absence of an energy source. The results presented also demonstrate that insulin has an effect which is unrelated to its role in stimulating glucose transport. Thus, glucose uptake by adipose tissue would have been equivalent when the medium concentration was 100 m*M* in the absence of insulin and when the concentration was 5 m*M* in the presence of the hormone (16). Yet the increase in glycogen synthetase activity was greater in the presence of insulin than in its absence. Furthermore, in all but one case, when adipose tissue was incubated with fructose or pyruvate, greater increase in glycogen synthetase activity was observed in the presence of insulin than in its absence, in spite of the fact that the cellular transport of these substrates is not stimulated by the hormone. Also, active glycogen synthesis is apparently not a prerequisite for the increase in glycogen synthetase activity. Pyruvate, which is not converted to glycogen in adipose tissue (17), was essentially as effective as glucose or fructose in stimulating an increase in enzyme activity. Thus we would conclude that the maximal increase in glycogen synthetase in rat adipose tissue is dependent upon the simultaneous availability of an energy source and the presence of insulin.

Summary. Total glycogen synthetase activity in adipose tissue from fasted meal-fed or nibbling rats increased in activity when the tissue was incubated for 2.5 hr in a buffer containing 5 m*M* glucose and insulin. Inclusion of puromycin in the incubation medium at a concentration which depressed lysine-U-^{14}C incorporation into protein by more than 90%, depressed the increase in glycogen synthetase activity by less than 25%. These data indicate that protein synthesis was responsible for no more than a small part of the overall increase in enzyme activity. Glycogen synthetase activity also increased when adipose tissue was incubated with glucose, fructose, or pyruvate without insulin, but the increase was less than when insulin was included in the incubation medium. The greatest increase in enzyme activity occurred in tissue incubated in the presence of both glucose and insulin. The results are consistent with the hypothesis that the increase in glycogen synthetase activity results from the conversion of a form of the enzyme which is inactive under the usual assay conditions to an assayable form.

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