The Effects of Food Deprivation and Re-Feeding on Bovine Adipose-Tissue Glycogen Synthase

By Ronald D. EICHNER* and Robert J. ARNOLD

Roman L. Hruska U.S. Meat Animal Research Center, Science and Education Administration,
Agricultural Research, U.S. Department of Agriculture, Clay Center, NE 68933, U.S.A.

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Bovine adipose-tissue glycogen metabolism was studied during food deprivation and re-feeding. Changes in the specific activity of adipose-tissue glycogen synthase paralleled changes in tissue glycogen content: both parameters increased during food deprivation and remained so during the first 10 days of re-feeding. The values for the $A_{0.5}$ (activation constant) for glucose 6-phosphate of the freshly isolated enzyme from adipose tissue from fed and starved steers were 2.9 ± 0.1 mM and 0.90 ± 0.05 mM respectively. Additionally, whereas incubation of adipose-tissue extracts from fed steers did not activate endogenous glycogen synthase (through a presumed phosphoprotein phosphatase mechanism), the enzyme from starved or re-fed (up to 3 days re-feeding) steers was reversibly activated as measured by changes in the value for the $A_{0.5}$ for glucose 6-phosphate. Thus activation of bovine adipose-tissue glycogen synthase during food deprivation appears to be related to expression of glycogen synthase phosphatase activity. These effects of food deprivation on bovine glycogen metabolism contrast markedly with the effects observed in rat adipose tissue.

Starving and re-feeding of monogastric animals result in diminishing and supranormal deposition of adipose-tissue glycogen respectively (Villar-Palasi & Lerner, 1960; Frerichs & Ball, 1962; Steinberg *et al.*, 1975; Gutman & Shafir, 1964). Glycogen synthase (UDP-glucose-glycogen 4- α -glucosyltransferase, EC 2.4.1.11), which catalyses the rate-limiting step in mammalian glycogen synthesis (Ryman & Whelan, 1971), exists in multiple interconvertible forms differing in covalent phosphate content and kinetic properties, including the K_m for UDP-glucose and $A_{0.5}$ for glucose 6-phosphate (Soderling, 1976; Roach *et al.*, 1976; Brown *et al.*, 1977; Eichner, 1976; Kaslow *et al.*, 1979). Phosphorylation of the enzyme increases both the value of the $A_{0.5}$ and its dependence on glucose 6-phosphate for activity. Interconversion is thought to occur through dephosphorylation by glycogen synthase phosphatase(s) and phosphorylation by glycogen synthase cyclic AMP-dependent and -independent kinases (Lerner & Villar-Palasi, 1972; Nimmo & Cohen, 1974; Schlender & Reimann, 1975; Soderling, 1975; Huang *et al.*, 1975; Nimmo *et al.*, 1976).

Adipose-tissue glycogen synthase has been characterized kinetically in the fed mature ruminant animal (Eichner & Arnold, 1979). The bovine enzyme, which exists apparently in a single discrete kinetic species,

has an elevated value for the $A_{0.5}$ for glucose 6-phosphate (2.8 mM) and a low –glucose 6-phosphate/+glucose 6-phosphate ratio (<0.02). Attempts to activate the enzyme in cell-free extracts (presumably through a phosphoprotein phosphatase-catalysed reaction) have not succeeded; however, addition of exogenous sources of glycogen synthase phosphatase to these cell-free preparations resulted in activation of the bovine enzyme (Eichner & Arnold, 1979). We have demonstrated that the refractory nature of adipose tissue from fed steers to activate the endogenous glycogen synthase appears to be due to the absence of glycogen synthase phosphatase activity and not to alteration in the substrate, glycogen synthase. We now report that food deprivation causes expression of an apparent bovine glycogen synthase phosphatase activity. These results are in marked contrast with those obtained with adipose tissue from fed and starved rats, where starvation resulted in diminished activity of adipose-tissue glycogen synthase phosphatase (Eichner, 1976; Steinberg *et al.*, 1975).

Materials and Methods

UDP-[U- 14 C]glucose was obtained from Amer-sham/Searle Corp., Arlington Heights, IL, U.S.A.; bovine liver glycogen, further purified by a modification (Eichner & Arnold, 1979) of the Somogyi (1957) method, glucose 6-phosphate and dithiothreitol were from P-L Biochemicals, Milwaukee, WI, U.S.A.; glycerol guaiacolate was from Summit Hill

*Present address: U.S. Department of Agriculture Food Safety and Quality Service, Science, Western Laboratory, Chemistry Division, 630 Sansome Street, San Francisco, CA 94111, U.S.A.

Laboratories, Avalon, NJ, U.S.A; sodium thiopental was from Diamond Laboratories, Des Moines, IA, U.S.A; all other chemicals were of reagent grade. Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

Tissue glycogen was determined as described previously (Eichner & Arnold, 1979).

Glycogen synthase activity was measured by the method of Thomas *et al.* (1968). Preparation of enzyme solutions and activation conditions and methods for determining kinetic parameters have been described (Eichner & Arnold, 1979). Glycogen synthase activity ratios were calculated from the ratio of the activity determined in the absence to that in the presence of 10mM-glucose 6-phosphate ('-/+ glucose 6-phosphate'). One unit of activity is defined as the incorporation of 1nmol of glucose from UDP-glucose/min. The concentration of UDP-glucose was 0.5mM, approximately 5 times the apparent K_m for the enzyme for UDP-glucose. The activation constant for glucose 6-phosphate ($A_{0.5}$) and the Hill coefficient were determined graphically from Hill plots [$\log [(V-V_0)/(V_{max}-V)]$ versus $\log [\text{glucose 6-phosphate}]$, where V_0 is the velocity observed in the absence of effector; V_{max} was determined by extrapolation from double-reciprocal plots of $V-V_0$ versus $[\text{glucose 6-phosphate}]$]. Conversion of the enzyme in adipose-tissue extracts from starved steers required addition of ATP (1mM) and Mg^{2+} (10mM); samples were removed at various times, diluted (10-fold into 0.2M-sucrose/10mM-Tris/1mM-EDTA/5mM-dithiothreitol, pH 8.0, 4°C), and the $A_{0.5}$ values for glucose 6-phosphate determined.

Samples of subcutaneous adipose tissue from five Hereford steers (350–375 kg, maintained on a 11.1 MJ/kg dry wt. diet *ad libitum*) were removed as

described previously (Eichner & Arnold, 1979); intravenous administration of a combination of glycerol guaiacolate (10 %, w/v) and sodium thiopental (0.4 %, w/v) produced a satisfactory state of sedation. Samples were removed as follows: one before food deprivation; the next after food deprivation for 4 days; and one each after 3, 10, 17 and 24 days of re-feeding.

Statistical analysis was by Student's paired *t* test to determine the probability of the Null Hypothesis for the difference between means (Brewer *et al.*, 1974). Data are presented as means $\pm$ S.E.M.

Results

Adipose-tissue glycogen content increased during re-feeding of mature steers without exhibiting a

Fig. 1. Adipose-tissue glycogen content and glycogen synthase activity in starved and re-fed steers

Between 09:00 and 10:00h on the indicated days, tissue samples (one from each of five animals) were excised, and glycogen synthase activity (●) and glycogen content (○) were determined (see the Materials and Methods section). The levels of significance are illustrated by asterisks: * $P < 0.05$; ** $P < 0.01$.

Table 1. Effects of food deprivation and re-feeding on kinetic parameters of bovine adipose-tissue glycogen synthase. Adipose-tissue samples were removed from live steers on the indicated days of the experiment. The freshly prepared enzyme solutions were then used to determine the $A_{0.5}$ for glucose 6-phosphate and -/+ glucose 6-phosphate activity ratio as outlined in the Materials and Methods section. Initial and final values $\pm$ S.E.M. ($n = 5$) after a 60 min incubation at 20°C of the enzyme solutions are presented.

	Time (days)	$A_{0.5}$ for glucose 6-phosphate (mM)		Activity ratio	
		Initial	After incubation	Initial	After incubation
Control	0	2.9 $\pm$ 0.1	3.0 $\pm$ 0.2	0.022 $\pm$ 0.008	0.029 $\pm$ 0.008
Starved	4	0.90 $\pm$ 0.07	0.39 $\pm$ 0.04	0.069 $\pm$ 0.041	0.16 $\pm$ 0.02
Re-fed	7	1.6 $\pm$ 0.1	0.49 $\pm$ 0.04	0.012 $\pm$ 0.002	0.009 $\pm$ 0.002
	14	3.2 $\pm$ 0.2	2.7 $\pm$ 0.2	0.014 $\pm$ 0.004	0.013 $\pm$ 0.004
	21	2.8 $\pm$ 0.4	2.7 $\pm$ 0.3	<0.01	<0.01
	28	2.8 $\pm$ 0.4	2.5 $\pm$ 0.3	0.01 $\pm$ 0.005	<0.01

characteristic decrease during starvation (Fig. 1). A significant supranormal deposition of glycogen ($P < 0.01$) occurred on day 10 of re-feeding; continued re-feeding resulted in a subsequent decrease in tissue glycogen content. Synchronous changes in total glycogen synthase activity also occurred during starvation and re-feeding. Expressing the data as either activity/g wet tissue wt. or activity/mg of protein did not change this result.

The $A_{0.5}$ for glucose 6-phosphate of glycogen synthase was monitored during starvation and re-feeding. Starvation decreased the $A_{0.5}$ for glucose 6-phosphate from 2.9 ± 0.1 to 0.90 ± 0.05 mM (Table 1). After 10 days of re-feeding, the $A_{0.5}$ returned to the control value and remained constant. No concomitant significant changes occurred in the value of the $-/+$ glucose 6-phosphate activity ratio. The Hill coefficient, a measure of co-operativity between glucose 6-phosphate-binding sites, remained near unity during the experiment (results not shown). Incubations for 60 min at 20°C of cell-free extracts of adipose tissue from mature beef cattle fed *ad libitum* produced no changes in the $A_{0.5}$ for glucose 6-phosphate or the

$-/+$ glucose 6-phosphate activity ratio of endogenous glycogen synthase. However, incubation of extracts from starved or re-fed (3 days) animals decreased the $A_{0.5}$ for glucose 6-phosphate, but did not substantially change the $-/+$ glucose 6-phosphate activity ratio (Table 1).

A 20 min incubation of extracts from adipose tissue of starved steers with ATP (1.0 mM) and Mg^{2+} (10 mM) resulted in a time-dependent increase in the value of $A_{0.5}$ for glucose 6-phosphate from 0.9 to 3.1 mM (Fig. 2). The same incubation did not cause a change in the $A_{0.5}$ for glucose 6-phosphate for the enzyme in extracts from steers fed *ad libitum* (results not shown). Incubation with ATP and Mg^{2+} of extracts (previously incubated for 60 min at 20°C with no additions) from starved steers increased the $A_{0.5}$ for glucose 6-phosphate 10-fold and decreased the $-/+$ glucose 6-phosphate activity ratio by approx. 50 % (Fig. 2). The presence of cyclic AMP had little effect on the rates of change of these parameters. Food deprivation and re-feeding produced no alteration in the reported value for the apparent K_m for UDP-glucose (0.13 mM) (Eichner & Arnold, 1979). Incubation with ATP and Mg^{2+} of extracts from starved steers also had no effect on this parameter.

Discussion

We have found that bovine adipose-tissue glycogen metabolism responds to food deprivation and re-feeding in a manner that contrasts markedly with effects reported for adipose tissue from other species (Villar-Palasi & Larner, 1960; Frerichs & Ball, 1962; Steinberg *et al.*, 1975; Gutman & Shafir, 1964; Kaslow & Mayer, 1979). For example, depriving steers of food caused a small increase in adipose-tissue glycogen content, whereas starving rats causes adipose-tissue glycogen content to decline dramatically. In addition, on re-feeding, the supranormal accumulation of bovine adipose-tissue glycogen was modest (approx. 180 %) compared with the rat.

Alterations in the kinetic properties of glycogen synthase correlated with the fluctuations of bovine adipose-tissue glycogen content. First, the specific activity of the enzyme increased with increasing tissue glycogen content. Second, the $A_{0.5}$ for glucose 6-phosphate decreased in tissue from starved steers. Additionally, and perhaps most importantly, starvation resulted in the demonstration of activation *in vitro* of glycogen synthase in cell-free extracts. Since adipose-tissue glycogen synthase from fed steers does serve as a substitute for exogenous phosphatase(s) (Eichner & Arnold, 1979), changes in glycogen synthase properties owing to food deprivation probably do not account for lack of conversion in these tissues. The ability then to demonstrate decreases in the $A_{0.5}$ for glucose 6-phosphate of glycogen synthase in adipose tissue from starved and re-fed (3 days) steers

Fig. 2. Effects of incubation *in vitro* of cell-free extracts of adipose-tissue from starved steers on kinetic parameters of endogenous glycogen synthase

Adipose-tissue extracts (see the Materials and Methods section) from starved steers were incubated for 60 min at 20°C in the presence of MgCl_2 (10 mM) and at the indicated time were assayed for the $-/+$ glucose 6-phosphate activity ratio (■) and $A_{0.5}$ for glucose 6-phosphate (●). After this 60 min incubation, ATP (1.0 mM) (closed symbols) or ATP (1.0 mM) and cyclic AMP (0.1 mM) (open symbols) were added to these extracts, which were then assayed (after dilution 20-fold) for the above parameters. The effects of incubation with ATP, Mg^{2+} and cyclic AMP on the $A_{0.5}$ for glucose 6-phosphate of the adipose-tissue extract before the 60 min incubation with MgCl_2 are illustrated (Δ).

appears to be the result, at least in part, of expression of glycogen synthase phosphatase. Alternatively, alterations in tissue contents of phosphoprotein phosphatase inhibitors (cf. Cohen *et al.*, 1978) may explain the appearance of glycogen synthase phosphatase activity in adipose tissue from starved steers. We have no direct measure of inhibitor amounts, although mixing experiments involving adipose tissue from fed and starved steers indicate that activation of glycogen synthase from fed steers occurs rapidly (Eichner & Arnold, 1979).

The results of these experiments differ from those for rat adipose tissue. For the rat, food deprivation decreases the specific activity of glycogen synthase (Kaslow & Mayer, 1979) and increases both the initial $A_{0.5}$ and the Hill coefficient of the glucose 6-phosphate activation of the enzyme (Eichner, 1976; Kaslow & Mayer, 1979). Furthermore, rat adipose-tissue glycogen synthase phosphatase activity, measured as the decrease in the $A_{0.5}$ for glucose 6-phosphate either in tissue extracts (Eichner, 1976) or in the intact tissue (Kaslow & Mayer, 1979), is decreased.

The kinetic properties of the enzyme from starved beef cattle and the ability to activate this enzyme in cell-free preparations similarly contrast with data from liver tissue from adrenalectomized starved rats (Mersmann & Segal, 1969; Tan & Nuttall, 1976; Gruhner & Segal, 1970), alloxan-diabetic rats (Gold, 1970) and diabetic dogs (Bishop, 1970); in these cases, the enzyme was either resistant to activation (as measured by increases in $-/+$ glucose 6-phosphate activity ratio) in cell-free preparations and/or possessed a higher $A_{0.5}$ for glucose 6-phosphate than did the corresponding enzyme from normal animals.

An analogous situation to the bovine system occurs in cultured choriocarcinoma cells (Huang *et al.*, 1978; Huang, 1978). The enzyme isolated from those cells grown in the presence of glucose possessed a high $A_{0.5}$ value for glucose 6-phosphate (3.6mM) and was not activated *in vitro*. However, the enzyme obtained from cells grown in the absence of glucose could be activated through a presumed phosphoprotein phosphatase-catalysed reaction. The authors concluded that perhaps during glucose starvation glycogen synthase is modified such that activation by dephosphorylation can occur.

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