## SUPPLEMENTAL MATERIAL

*Glycogen synthase and glycogen phosphorylase activity assays*- Random fed male mice 3 months of age were sacrificed by cervical dislocation followed by decapitation. The heads were immediately dropped in liquid nitrogen and skeletal muscle was dissected and immersed in liquid nitrogen. Samples were stored at -80$^{o}$C until use. For skeletal muscle, samples were powdered under liquid nitrogen, homogenized in 10-volumes of buffer (50 mM Tris HCl, pH 7.8, 10 mM EDTA, 2 mM EGTA, 100 mM NaF, 2 mM benzamidine, 0.1 mM $N^{\alpha}$-p-tosyl-L-lysine chloromethyl ketone, 50 mM β-mercaptoethanol, 0.5 mM PMSF, 10 μg/ml leupeptin and 1 mM sodium orthovanadate) using a Tissue Tearor Model 285-370 (Biospec Products Inc.) at maximal speed for 20-30 seconds. The homogenate was centrifuged at 6,500 x g for 10 min, the supernatant (LSS) was removed and the low speed pellet (LSP) resuspended in the initial volume of buffer. An aliquot of the LSS was subjected to high speed centrifugation at 100,000 x g for 90 min. Pellets were resuspended in 20-25% of the original volume by brief sonication and analyzed by Western blotting with antibodies against PTG. For brain tissue, samples were homogenized in 10 volumes of the same homogenization buffer as above, except that 1 mg/ml glycogen and 0.2% Triton X-100 were also included and the homogenates were centrifuged at 10,000 x g for 10 min. The LSP's were resuspended in volumes smaller than the original homogenization volume because of the low protein content in the pellet fraction. Glycogen synthase (GS) and phosphorylase (Ph) activities were determined in the LSS and LSP by measuring the incorporation of [$^{14}$C]glucose from UDP-[U-$^{14}$C]glucose into glycogen as described by Thomas *et al.* (1) in the absence or presence of 7.2 mM glucose-6-phosphate (G6P) and by measuring the incorporation of [$^{14}$C]glucose from [$^{14}$C]glucose-1-phosphate into glycogen in the absence or presence of 2 mM AMP (2), respectively. Activity ratios represent the activity measured in the absence divided by that in the presence of the allosteric effectors G6P for glycogen synthase or AMP for phosphorylase and provide an index of the activation state, and hence, phosphorylation of the enzymes. Glycogen synthase activity is expressed as nmol/min/mg and phosphorylase activity as μmol/min/mg. Protein content was determined by the method of Bradford (3) using bovine serum albumin as a standard.

*Western Blotting*- Samples from the LSS and LSP described above were used for Western blot analyses. For skeletal muscle comparable amounts of LSS and LSP were loaded. The brain homogenates of the samples used for Westerns of laforin were prepared in the absence of added glycogen. Brain LSP's were enriched with respect to the LSS because of the low protein concentration in the fraction. However, all quantitations were normalized for comparable amounts of samples. After transfer, the nitrocellulose membranes were stained with Ponceau S to monitor loading. Various antibodies were used to probe different regions of the membrane, including antibodies against glycogen synthase, debranching enzyme (AGL), laforin, phospho- and non-phospho- GSK-3, phospho- and non-phospho-eIF2a, phospho and non-phospho-AMPK, glycogen targeting subunits of protein phosphatase 1 (PTG and $R_{GL}$), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glycogen synthase phospho-site 3a. Detection was performed by enhanced chemiluminescence followed by autoradiography. The levels of protein expression were quantitated by densitometric scanning of the autoradiograms using Quantity One software (BioRad).


1. Thomas, J. A., Schlender, K. K., and Larner, J. (1968) *Anal Biochem* **25**, 486-499
2. Gilboe, D. P., Larson, K. L., and Nuttall, F. Q. (1972) *Anal Biochem* **47**, 20-27
3. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254