The following is the abstract of the article discussed in the subsequent letters:

Shearer, Jane, Rhonda J. Wilson, Danielle S. Battram, Erik A. Richter, Deborah L. Robinson, Marica Bakovic, and Terry E. Graham. Increases in glycogenin and glycogenin mRNA accompany glycogen resynthesis in human skeletal muscle. Am J Physiol Endocrinol Metab 289: E757–E759, 2005. First published May 3, 2005; doi:10.1152/ajpendo.00532.2005.—Glycogenin is the self-glycosylating protein primer that initiates glycogen granule formation. To examine the role of this protein during glycogen resynthesis, eight male subjects exercised to exhaustion on a cycle ergometer at 75% \( \dot{V}O_2 \) \( \text{max} \) followed by five 30-s sprints at maximal capacity to further deplete glycogen stores. During recovery, carbohydrate (75 g/h) was supplied to promote rapid glycogen repletion, and muscle biopsies were obtained from the vastus lateralis at 0, 30, 120, and 300 min postexercise. At time 0, no free (deglycosylated) glycogenin was detected in muscle, indicating that all glycogenin was complexed to carbohydrate. Glycogenin activity, a measure of the glycosylating ability of the protein, increased at 30 min and remained elevated for the remainder of the study. Quantitative RT-PCR showed elevated glycogenin mRNA at 120 min followed by increases in protein levels at 300 min. Glycogenin specific activity (glycogenin activity/relative protein content) was also elevated at 120 min. Proglycogen increased at all time points, with the highest rate of resynthesis occurring between 0 and 30 min. In comparison, macroglycogen levels did not significantly increase until 300 min postexercise. Together, these results show that, during recovery from prolonged exhaustive exercise, glycogenin mRNA and protein content and activity increase in muscle. This may facilitate rapid glycogen resynthesis by providing the glycogenin backbone of proglycogen, the major component of glycogen synthesized in early recovery.

Glycogenin, proglycogen, and glycogen biogenesis: what’s the story?

To the Editor: The regulation of glycogen biogenesis is an important area of research that has implications for understanding polymer synthesis, diseases associated with carbohydrate metabolism, and muscle performance. In a recent study, Shearer et al. (10) examined the roles of glycogenin, the self-glycosylating primer for glycogen biosynthesis, and “proglycogen” in glycogen synthesis in human skeletal muscle. I wish to address two issues that pertain to this study: 1) the method used to analyze glycogenin activity and 2) the term proglycogen.

To assess glycogenin activity, Shearer et al. measured the ability of muscle extracts to catalyze glucose transfer from UDP-glucose to the synthetic substrate \( n \)-dodecyl-\( \beta \)-d-maltoside (DBM), based on a method originally described by Meezan et al. (6). Classically, glycogenin activity is measured by following the ability of an extract to catalyze Mn\( ^{2+} \)-dependent glucose transfer from UDP-glucose into an endogenous, TCA-precipitable, protein with a M\( _r \) of \( \sim 38 \) kDa, which is the M\( _r \) of nonglucosylated and active glycogenin (3, 9, 13). Although the TCA precipitation method remains the only practical assay of the glucosylation of glycogenin itself (i.e., autoglucosylation), this is considered laborious and to result in low product yield (6). Moreover, in skeletal muscle extracts, autoglucosylation is often not detectable, probably owing to insufficient free glycogenin (2, 4, 12). Therefore, the method chosen by Shearer et al. would appear to be a useful alternative. Shearer et al. stated that their measurement of glycogenin activity reflects the “protein’s self-glycosylating ability.” The basis for this statement is not provided; indeed, its correctness is questionable considering that glycogenin-mediated glucosylation of exogenous substrates (including DBM) is independent of the degree of glycogenin autoglucosylation (1). Thus one can debate whether measuring the glucosylation of exogenous substrates by glycogenin vs. the autoglucosylation of glycogenin is useful in terms of understanding the physiological function of the enzyme. A more important concern is that, in addition to glycogenin, glycogen synthase also catalyzes the transfer of glucose from UDP-glucose to DBM (6–8). Shearer et al. do not report results from control experiments demonstrating that the activity measured was solely attributable to glycogenin and not to another glucosyltransferase. To illustrate the importance of such experiments, the following can be considered. We (2) attempted measurements of glycogenin in human muscle extracts by using the classical TCA method. Whereas we always found incorporation of radiolabeled glucose into TCA-precipitable material under conditions that favor activation of glycogenin but not glycogen synthase (2), we could never demonstrate incorporation of glucose into free glycogenin by autoradiography (i.e., incorporation of labeled glucose into a 38-kDa protein). Ultimately, we demonstrated that the glucosylation of the endogenous, TCA-precipitable proteins that we were measuring was fully accounted for by glycogen synthase (2).

As to the term proglycogen, in the introduction of their paper, Shearer et al. defined proglycogen as a small glycogen granule that has a low carbohydrate content, and they then cite several studies to support the existence of this entity. All of these citations ultimately lead back to the studies of Whelan’s group [Lomako and colleagues (4, 5)], who originally coined the term proglycogen and defined it as a species of glycogen that is acid precipitable, has a discrete M\( _r \) of \( \sim 400 \) kDa, and contains glycogenin. They suggested that proglycogen production is catalyzed by proglycogen synthase (a glycogen synthase-like enzyme) and branching enzyme using primed glycogenin (i.e., glucosylated glycogenin containing \( \sim 10 \) glucose residues) as substrate. Proglycogen, in turn, serves as the substrate for formation of high-molecular-mass glycogen that is acid soluble (i.e., mature glycogen, M\( _r \) \( \sim 10^7 \) kDa). Mature glycogen formation would then be catalyzed by the well-established glycogen synthase and branching enzyme. Shearer et al. then go on to use the term proglycogen repeatedly in their discussion, leaving the impression that this is now an accepted concept (at least in the eyes of a reader who is not familiar with the topic). Unfortunately, nowhere in their paper do they consider the data that speak against the existence of proglycogen as a discrete species. By using a continuous buffer system as well as two-dimensional gel electrophoresis, Skurat et al. (11) demonstrated a heterogeneous and continuum of glycogen intermediates. The implication of these findings is that proglycogen is not a discrete species but is, rather, an artifact of discontinuous electrophoresis. An alternative to the proglycogen concept is that classical glycogen synthase uses primed glycogenin to form mature glycogen (which is the traditional view). From this perspective, the term glycogen would apply to any oligo/poly saccharide formed by glycogen synthase, regardless of the number of attached glucosyl residues. This would obviate the need to use different terms for glycogen chains of varying lengths.
Currently our understanding of glycogen biogenesis is incomplete. Thus all research that advances our understanding of this process is of benefit. To further the advancement of knowledge, however, studies should interpret data on the basis of methods that are sufficiently validated as well as provide balanced discussions.

REFERENCES


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Rebuttal to Abram Katz’s Letter To The Editor

To the Editor: We thank Dr. Katz for his long letter regarding our recent paper (8). Dr. Katz presented two issues: one a methodological point and one concerning proglucogen (PG).

With respect to PG, Dr. Katz points out that this acid-insoluble form of glycogen (PG) is not a “discrete species”. We can find no place in our paper (8) in which we suggested that this is so; in fact, we suggested the opposite. In our previous work (1, 7), we proposed that PG is one of two fractions of the glycogen granules and that they exist as a continuum. Our electron microscopy work (5) shows this conclusively, and our review (6) makes the point very clearly. We believe that the work from Whalen’s laboratory [Lomako et al. (3)] cited by Dr. Katz was pioneering and insightful; we also believe that the results reported in those studies are open to alternative interpretations. As noted above, we questioned this finding some years ago and did research to resolve it. It should be noted that Dr. Whalen’s group recently wrote (4) that their investigation (3) in 1991 “estimated, very roughly [emphasis mine], that the M_r of this TCA-soluble glycogen . . . was 400 kDa.” Nowhere in our recent work (8) do we state or imply that PG is a separate species of glycogen. It appears that Dr. Katz has misinterpreted our discussion in this paper. Our previously published work (5), which he does not cite, demonstrates very clearly that the granules are a continuum, ranging in diameter from approximately 8 to 42 nm, and we state strongly that PG cannot be a discrete species of glycogen granule.

Dr. Katz’s other issue involves one of our three main measurements, that of the in vitro activity of glycogenin. He points out that we have used a published method that is different from that of his work (2). It is difficult to compare our results with those of his study, as it appears that the muscle samples used in his study may not be comparable to those in our investigation on the basis of sampling sites and duration of storage.

We agree with Dr. Katz that our description in the discussion that the activity assay measures the “self-glycosylating ability of GN-1” (glycogenin) is misleading. Rather, we should have reiterated that our activity assay measures the transglycosylation of n-dodecyl-β-d-maltoside by glycogenin, which is clearly stated in our MATERIALS AND METHODS section. The “activity” assay was used as an indirect measurement of glycogenin protein rather than for use in the classical sense.

Dr. Katz states that we do not report results from control experiments demonstrating that the activity measured was solely attributable to glycogenin. In our present (unpublished) work, the large majority of the transglycosylation appears to occur following amylase treatment of the sample, which would support the reaction’s occurring via glycogenin. Of course, we cannot rule out the possibility that other enzymes may contribute; however, their contributions appear to be minimal (<10 mU·mg protein−1·min−1).

REFERENCES


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