Review

In vivo regulation of muscle glycogen synthase and the control of glycogen synthesis

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Contributed by R. G. Shulman, June 6, 1995

ABSTRACT The activity of glycogen synthase (GSase; EC 2.4.1.11) is regulated by covalent phosphorylation. Because of this regulation, GSase has generally been considered to control the rate of glycogen synthesis. This hypothesis is examined in light of recent in vivo NMR experiments on rat and human muscle and is found to be quantitatively inconsistent with the data under conditions of glycogen synthesis. Our first experiments showed that muscle glycogen synthesis was slower in non-insulin-dependent diabetics compared to normals and that their defect was in the glucose transporter/hexokinase (GT/HK) part of the pathway. From these and other in vivo NMR results a quantitative model is proposed in which the GT/HK steps control the rate of glycogen synthesis in normal humans and rat muscle. The flux through GSase is regulated to match the proximal steps by “feed forward” to glucose 6-phosphate, which is a positive allosteric effector of all forms of GSase. Recent in vivo NMR experiments specifically designed to test the model are analyzed by metabolic control theory and it is shown quantitatively that the GT/HK step controls the rate of glycogen synthesis. Preliminary evidence favors the transporter step. Several conclusions are significant: (i) glucose transport/hexokinase controls the glycogen synthesis flux; (ii) the role of covalent phosphorylation of GSase is to adapt the activity of the enzyme to the flux and to control the metabolite levels not the flux; (iii) the quantitative data needed for inferring and testing the present model of flux control depended upon advances of in vivo NMR methods that accurately measured the concentration of glucose 6-phosphate and the rate of glycogen synthesis.

Recent theoretical and experimental advances have allowed improved quantitative understanding of flux control in vivo. The theoretical advances have been formulated by metabolic control theory, which provides a systematic framework for evaluating quantitatively the control an enzyme in a pathway exerts on the flux (1, 2). This evaluation requires a well-characterized pathway in which the relevant substrates and allosteric effectors are known and on which comprehensive measurements can be performed under in vivo conditions. Despite the appeal of metabolic control theory, experimental applications have lagged because of the limited availability of in vivo data. Experimental advances of in vivo NMR measurements have recently started to help elucidate metabolic control by allowing simultaneous measurements of metabolic fluxes, substrates, and effectors in vivo (3–6). A principal focus of these investigations has been glycogen synthase (GSase; EC 2.4.1.11), which often has been assumed to control the rate of glycogen synthesis. This review describes the use of NMR results for understanding the control of muscle glycogen synthesis in rat and human muscle. The understanding is consolidated by a model based upon a control theory analysis, which gives satisfactory agreement with a wide range of data. The model shows that GSase does not, in fact, control the flux of muscle glycogen synthesis. In addition to its consequences for this specific pathway, the present results call into question the contemporary paradigm that kinase and/or phosphatase activities explain flux control. The general consequences of these results in which enzymatic phosphorylations control intermediates, rather than fluxes, will be presented subsequently.

The pathway of glycogen synthesis in the muscle is pictured schematically in Fig. 1. Glucose is transported into the muscle by diffusion through the GTs and the G3 is phosphorylated by HK to G6P from where it goes via two more intermediates to GSase, which adds glucose to the glycogen polymer. Steps believed to be stimulated by insulin are indicated by the solid arrows while the positive allosteric effect of G6P upon GSase activity is indicated by a dashed arrow.

Two recently developed in vivo NMR measurements have made these evaluations of the flux control possible. In the first 13C NMR peaks of glycogen have been shown in a series of experiments (7–9) to be ~100% NMR visible and to measure muscle glycogen concentrations continuously, accurately, and noninvasively in vivo (9), thereby providing glycogen concentrations and synthesis rates. The second advance came from 31P NMR measurements of G6P in vivo. Basal values of human muscle concentrations of G6P were shown to be ~100 μM and to increase substantially during glycogen synthesis after intense exercise (6) or at hyperinsulinemic clamps (5). These basal levels were lower than in many biopsy determinations (10–12), in which higher G6P concentrations presumably arose from postexercise glycogenolysis. Accurate in vivo concentrations of G6P have allowed us to understand GSase activity in vivo by establishing conditions for in vitro assays that reflect in vivo values. These NMR advances have been used to compare muscle glycogen synthesis in patients with non-insulin-dependent diabetes mellitus (NIDDM) and their age-weight-matched controls during hyperglycemic/hyperinsulinemic clamps that simulated postprandial conditions (3). The 13C NMR results showed that under these conditions muscle glycogen synthesis was the major pathway of glucose disposal accounting for >80% of the glucose uptake. (This near-equivalence of muscle glycogen synthesis and glucose uptake is used below for converting glucose uptake to glycogen synthesis flux in some reports.) The 13C NMR glycogen synthesis rates in patients with NIDDM and matched controls showed that in the patients the rate was ~50% of the normals. These differences were investigated by comparing the G6P concentrations, measured by 31P

Abbreviations: GSase, glycogen synthase; G6P, glucose 6-phosphate; GT, glucose transporter; HK, hexokinase; μU, microunit(s); NIDDM, non-insulin-dependent diabetes mellitus.

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NMR, in the two groups under the same clamped conditions (5). The results showed G6P increased substantially in the normals but negligibly in the NIDDMs, indicating that the defect was proximal to G6P in the pathway (see Fig. 1). This led to the model of the muscle glycogen synthesis pathway explicitly proposed in this paper in which the flux is controlled by the GT/HK step. This model disagrees with the more generally accepted model in which control is exercised by GSase (13, 14).

GSase is often described as "the rate-limiting enzyme in the synthesis of glycogen" (15–17). This belief is supported partly from low GSase activity compared to the activities of HK, phosphoglucomutase, and UDP-glucose pyrophosphorylase and partly from the complex regulation of GSase, by allosteric modulation (18) and by covalent phosphorylation–dephosphorylation, (19, 20), suggesting a controlling role for GSase in the pathway (13, 17). On the other hand, exercise (21) and insulin (22) have been shown to stimulate glucose transport across the muscle plasma membrane through the recruitment of glucose transporters from an intracellular pool and also by an increase in the activity of the transporters (23). The transport data suggest that muscle glucose uptake under insulin stimulation or during postexercise recovery, and hence during rapid glycogen synthesis, is controlled by glucose transport. These implicitly conflicting claims of control would be reconciled if GSase activity were regulated by phosphorylation and substrate effector concentrations without it playing a significant role in the control of the flux through the glycogen synthesis pathway. In this review we show that such a model is supported by recent NMR experiments and is consistent with many observations.

Our scope is first to highlight which of the numerous mechanisms influencing the GSase activity in vitro are relevant for a quantitative description of the glycogen synthesis rates under two conditions of rapid glycogen synthesis—e.g., hyperinsulinemia and recovery following intense exercise. Second, the central role of G6P as a link between activation of GT/HK and enhanced GSase activity in vivo is examined in light of more accurate NMR measurements of its concentration in vivo. Finally, a model is proposed in which flux control in the muscle glycogen synthesis pathway is primarily exercised before GSase, at the GT/HK step, while the regulation of GSase is determined by its degree of phosphorylation and its allosteric control by G6P. In this model G6P acts as a "feed forward" activator of GSase, as originally proposed by Piras and Staneloni (18), which helps match the rate of glycogen synthesis to the rate of glucose transport.

**GSase Phosphorylation–Dephosphorylation:** 

In vivo net glycogen synthesis is determined by the balance between GSase and glycogen phosphorylase activities. The limited number of in vivo studies of muscle glycogen turnover during net glycogen synthesis (24, 25) support the assumption that during rapid glycogen synthesis glycogenolysis can be neglected in contrast with hepatic glycogen, where NMR studies have shown continual turnover (26). Since muscle concentration of UDP-glucose, the direct substrate of GSase, remains nearly constant for a wide range of glycogen synthesis rates (18) or even decreases as the glycogen synthesis rate increases (24), changes in the flux through GSase in vivo have to be related to changes in enzyme activity, not substrate level. Since GSase total activity in the muscle does not vary in response to short-term stimulation by insulin (27) or exercise (28), the activity changes must be caused by allosteric effects in concert with changes in the degree of phosphorylation.

**Intermediate States of GSase Phosphorylation.** It was subsequently demonstrated that GSase can exist in several intermediate phosphorylated forms that have depressed activity ratios but have enhanced sensitivities to G6P (30, 31) concentration. Fractional activities or velocities (FVx) are now usually reported to describe the activation state of the GSase instead of the activity ratio I/(I + D). FVx is defined as the ratio of the GSase activity at a physiological G6P concentration x to the total activity at high G6P concentrations of >6 mM (30, 31). In the physiological range of GSase phosphorylation, this parameter provides a better correlation with in vivo rates of glycogen synthesis than the I/(I + D) ratio, although, as described later in this review, the agreement is not quantitative.

**Mechanisms for Changes in Phosphorylation State.** The interconversion between various species of GSase, phosphorylated at different sites, can be catalyzed by the opposing activities of several site-specific GSase kinases and phosphatases identified in vitro (32, 33). However, the specific intracellular events and mechanisms responsible for the dephosphorylation of GSase during insulin stimulation are still unclear. One mechanism involving the activation of the protein phos-
phatase 1, through phosphorylation of its regulatory subunit by an insulin-stimulated protein kinase, has recently been proposed by Dent et al. (34).

Another mechanism for the activation of GSase was proposed in 1965 by Danforth (29) to explain the rapid glycogen synthesis observed during recovery from intense exercise. This feedback mechanism invokes the inhibition of protein phosphatase 1 when bound to glycogen, and the removal of the inhibition as glycogen is depleted by exercise and the phosphatase is released (35–37). This mechanism has been widely accepted but has been questioned by some authors (38, 39).

A third mechanism that can contribute to the GSase dephosphorylation rate in vivo, after exercise and during insulin stimulation, is the stimulation of the rate of GSase dephosphorylation by increased intracellular G6P (16, 40), which binds to the enzyme and exposes the phosphorylated sites to phosphatase action.

In summary, more than three mechanisms are known to change the phosphorylation state of GSase following insulin stimulation or exercise and have been summarized by Harris (32). No data have distinguished the quantitative contributions of these individual mechanisms in vivo. Regardless of the mechanism responsible, the degree of phosphorylation is a parameter which can readily be determined by in vitro assays.

Does Phosphorylation Explain the in Vivo Regulation of GSase? Numerous studies have tried to correlate the FV₄ of GSase extracted from biopsied muscle tissue with either direct or indirect measurements of muscle glycogen synthesis. Correlations have been observed between FV₄ and the inferred rate of muscle glycogen synthesis in conditions of insulin stimulation (24, 27, 41) and in postexercise glycogen resynthesis (42, 43). However, these correlations have not been quantitative in that FV₄ was not proportional to the rate of glycogen synthesis, and several experiments have demonstrated a change in the in vivo glycogen synthesis rate with no change in FV₄ (27, 41) as reviewed below.

Quantitative measurements of the dependence of muscle glycogen synthesis on glucose and insulin concentration have used the glucose clamp method in which both the plasma glucose and insulin concentration are “clamped” at a constant value by variable infusions (44). In many studies the change in glycogen concentration could not be measured directly and had to be inferred from either muscle or whole body glucose uptake or storage. Using ¹³C NMR we showed in 1990 that at high insulin concentrations almost 90% of whole body glucose storage was accounted for by muscle glycogen synthesis (3). On this basis, it will be assumed in the studies discussed below that at high insulin concentrations nonoxidative glucose storage is proportional to muscle glycogen synthesis.

Yki-Järvinen et al. (41) measured the dependence of muscle glucose uptake and GSase FV₄ on glucose and insulin concentrations with G6P concentration set in the assay at 140 μM. At high glycemia (~400 mg/dl) GSase FV₄, reached maximum at an insulinemia of ~160 microunits (μU)/ml. During a further increase of the insulinemia to ~1700 μU/ml GSase FV₄ remained constant. However, the higher insulin induced an almost 2-fold increase of the muscle glucose uptake in the forearm, which in these hyperinsulinemic/hyperglycemic conditions approximately equals the glycogen synthesis rate (3). Hence, under high insulin conditions there was no correlation between the constant FV₄ and the 2-fold increase in flux. In the same study for all insulin concentrations above 160 μU/ml a considerable increase in muscle glucose uptake was observed as a function of glucose concentration without a change in GSase FV₄.

By combining glucose, O₂, and CO₂ balance across the leg in humans, Mandarino et al. (45) determined muscle glucose storage during hyperinsulinemic clamps (400–500 pmol/liter) at two different plasma glucose levels. When glycemia was raised from 6 to 12 mmol/liter, glucose storage increased from 2.4 to 5.0 μmol/min·100 ml of leg, but GSase FV₁₀₀ was unchanged. Since the increased glucose storage can be inferred to be primarily glycogen synthesis, GSase activity was uncoupled from the flux. Similarly, in the rat muscle (27), increasing the glycemia from 6 to 10 mmol/liter at constant insulinemia (~140 μU/ml) doubled the glycogen synthesis rate (measured directly by freeze-clamping the muscle) while GSase FV₁ (1 + D) was unchanged. These results show that the GSase phosphorylation state alone does not explain variations of the glycogen synthesis rates under hyperinsulinemic conditions.

Immediately after an intense exercise, inducing a severe depletion of muscle glycogen stores, the glycogen repletion rate becomes very rapid and then progressively decreases as muscle glycogen is replenished (46). In vivo ¹³C NMR spectroscopy has allowed the rapid repletion of muscle glycogen after exercise to be measured noninvasively in the same subject. Price et al. (4) reported for the human gastrocnemius a 10-fold drop (from 27 ± 5 to 2.9 ± 0.8 mmol/liter of muscle) in the glycogen synthesis rate between the early period (first half-hour) and the late period (after 1 hr) of recovery. This large variation contrasts with the minor changes (~2-fold) in the GSase 1/(1 + D) or FV₄ from early to late postexercise recovery reported by others (42). By combining in vivo NMR spectroscopy and GSase enzymatic assay in a rat model, Bloch et al. (25) demonstrated a 4-fold drop in the gastrocnemius glycogen synthesis rate between early and late postexercise recovery (at elevated insulin levels), while GSase 1/(1 + D) decreased by a factor of only 1.8.

In these studies the changes in glycogen synthesis rates in vivo were consistently greater than those predicted by in vitro assays of GSase 1/(1 + D) or FV₄. Hence, factors in addition to the phosphorylation state of the enzyme must be considered to establish a quantitative match between GSase activity and glycogen flux during hyperinsulinemia and after intense exercise. We propose below that the in vivo concentration of G6P, acting as an allosteric effector, is the major factor that can reconcile this discrepancy.

Allosteric Regulation by G6P of GSase in Vivo. A biochemical basis for a more quantitative description of the glycogen synthesis regulation in vivo has been established by the early work of Piras et al. (18, 47) and Roach et al. (19, 48). Several phosphate compounds present in the myocytes, especially ATP, but also ADP, AMP, phosphocreatine, and Pi, were shown to be inhibitors of GSase regardless of its phosphorylation state. The inhibition is reversed by G6P; thus, in the presence of these inhibitors the more active GSase I form, minimally independent of G6P, becomes G6P dependent. Under these conditions, the I and D forms are stimulated by G6P, with the former still the more active form. This provides an additional regulatory mechanism that can modulate GSase activity in vivo, in which the inhibition of GSase by physiological levels of ATP (and other phosphorylated compounds) is partly reversed by concentrations of G6P in the physiological range.

The effectiveness of this mechanism in vivo had been demonstrated in the pioneering work of Piras and Staneloni (18), who studied glycogen repletion after a short tetanic contraction of the rat thigh that created very high G6P concentrations. The delay in accepting the importance of G6P allosteric control in vivo was partly due to reports of resting muscle G6P concentrations, especially in humans, which were overestimated most likely due to artifactual degradation of glycogen during biopsy (10–12), thereby overwhelming the smaller effective changes in vivo.

3¹P NMR allows phosphorylated metabolites to be measured without tissue removal and has been used in our laboratory to determine noninvasively the muscle G6P concentrations during animal (6, 25) and human studies (5, 50, 4), in combination with ¹³C NMR measurements of glycogen synthesis rates. Bloch et al. (25) followed the
time courses of the glycogen synthesis rate and of the G6P concentration after a glycogen-depleting exercise in the rat gastrocnemius. Their data are shown in Fig. 2. GSase I/(1 + D) and allosteric activation by G6P were also measured in vivo on muscles sampled 10 min (early recovery) and 100 min (late recovery) after exercise. The ATP concentration in the assay was matched to in vivo values determined by in vivo 31P NMR.

From early recovery to late recovery, the in vivo glycogen synthesis rate dropped from 0.46 to 0.11 mmol/kg of wet tissue-min, the G6P concentration dropped from 0.83 to 0.32 mmol/kg of wet tissue, and I/(1 + D) dropped from 83% to 47%. The combination of the changes in G6P concentration and in GSase I/(1 + D) together quantitatively describe the 4-fold decrease in glycogen synthesis rate observed from early to late recovery. These results demonstrate that in this case phosphorylation, determining GSase I/(1 + D), and allosteric control of GSase by G6P contribute approximately equally to the regulation of the postexercise GSase activity in vivo. Preliminary studies in humans (4, 7) have shown that the rapidly decreasing rate of glycogen resynthesis after heavy exercise correlates with a rapid decrease in muscle G6P concentration from values as high as 1.1 mmol/liter of intact tissue immediately after exercise (%).

Using rapid biochemical assays, so as to minimize errors in G6P concentration, Rossetti and Giaccari (51) reported a 2.5-fold increase in the muscle G6P of euglycemic, conscious rats when insulin was raised from 63 to 403 μU/ml, while muscle glycogen synthesis rate increased 6.6-fold. The authors did not interpret their data in terms of allosteric regulation of GSase by G6P and did not provide data about the activation state of the enzyme. However, in a follow-up study on the same animal preparation, Farrace and Rossetti (15) observed a 2.6-fold increase in GSase FV100 when insulin was raised from 29 to 495 μU/ml at euglycemia. Using this ratio, we propose the following interpretation of their data. Neither the change in G6P concentration (2.5-fold) nor the change in GSase FV100 (2.6-fold) alone accounts for the change in the glycogen synthesis rate between high physiologic and maximally stimulating insulinemia. However, the product of increases stimulated by the two mechanisms does quantitatively describe the 6.6-fold increase of the glycogen synthesis rate, with concomitant dephosphorylation and allosteric activation making approximately equal contributions. A recent study by Rossetti et al. (52) reports good agreement between the direct measurement of glycogen synthesis and the GSase activity determined at the in vivo G6P concentrations in human muscle biopsies using a fast freezing method to minimize autolysis.

Summary. In this section studies were reviewed that demonstrated that the in vivo rate of GSase cannot be explained by the phosphorylation state of GSase alone. In the study of Bloch et al. (25), quantitative agreement between the in vivo rate and the in vitro assay was achieved by taking into account the GSase phosphorylation state and the concentrations of G6P and ATP. We believe similar agreement will be found in future studies if the in vitro GSase assay is performed under in vivo concentrations of G6P and ATP, and recent experiments support this view (52).

Model of Glycogen Synthesis Control

The present data on the in vivo regulation of GSase suggest that the in vivo flux through this enzyme can be quantitatively predicted from knowledge of the concentration of G6P and the phosphorylation state of the enzyme. However, a quantitative understanding of the regulation of GSase does not by itself provide a quantitative understanding of the control of the glycogen synthesis rate. In this section we present a model for the control of muscle glycogen synthesis using metabolic control analysis in order to make quantitative predictions. The predictions of the analysis are then compared with the results from selected experiments where the analysis is applicable. The application of the analysis is shown below to support glucose transport or HK as being the flux controlling steps under conditions of rapid glycogen synthesis.

Metabolic Control Analysis. Metabolic control analysis has been developed in order to provide quantitative measures of metabolic control and to develop methods for the determination of control in vivo. As described in the initial papers on metabolic control analysis, the control of flux is a distributed property of all enzymes in a pathway (1, 2). The enzyme with the major role in determining the flux rate is characterized by having the highest flux control coefficient, which is defined as the fractional change in pathway flux J for a fractional change in the concentration of enzyme Ei:

$$C_i = \frac{\partial J}{\partial E_i} \frac{E_i}{J}$$

[1]

where the partial derivatives indicate that all other enzyme activities are held constant. A flux control coefficient of unity would indicate that the flux is proportional to the activity of the enzyme, which corresponds to the older qualitative definition of rate limiting. The more general case, derived by control theory, shows that \( \sum C_i = 1 \) allowing for control to be distributed throughout the metabolic pathway (1).

Metabolic Control Analysis of the Muscle Glycogen Synthesis Pathway. In order to interpret the existing data, the metabolic control analysis of the glycogen synthesis pathway is reduced to the following pathway:

$$\text{glucose} \rightarrow \text{G6P} \rightarrow \text{Glycogen}$$

[2]

The enzymes proximal to G6P (GT/HK) are treated as having a single control coefficient as are distal enzymes (GSase, etc.). As shown by top-down metabolic control analysis (53), this approach is valid provided G6P is the only shared substrate between the grouped enzymes and intracellular glucose is not an allosteric effector of the GSase-grouped steps (16). The glycolytic flux is neglected since under the
condition of rapid glycogen synthesis it accounts for only a small fraction of muscle glucose uptake and phosphorylation (3). In applying control analysis to this system it is assumed that except for G6P, allosteric effectors of GT/HK and GSase such as ATP as well as the total activity and phosphorylation state of the enzymes are held constant.

A major conceptual change in the understanding of metabolic regulation introduced by metabolic control analysis is that an enzyme that is highly sensitive to allosteric regulation by a proximal pathway intermediate will have a low control coefficient, if the enzyme proximal to the intermediate in the pathway is insensitive to the concentration of this intermediate. This conclusion follows from the connectivity theorem of metabolic control analysis that relates the relative flux control coefficients of enzymes in a pathway to the elasticity with respect to a shared substrate or effector (1, 2). This relation allows a second experimental evaluation of the control coefficients. The elasticity coefficient of an enzyme i to a substrate S is defined by

$$ e_i^S = \frac{\partial V_i}{\partial S} S $$

where $V_i$ is the enzyme velocity under the in vivo condition that is determined by $E_i$ and concentration of substrate and allosteric effectors $S$. For a two-enzyme system such as shown in Eq. 2, the connectivity theorem is given by $C_k^S = -C_k^2$, which indicates the enzyme that is more sensitive to $S$ (high elasticity coefficient) exerts less control on the flux. Since the GSase velocity is highly sensitive to G6P concentration in vivo, this principle suggests that for the proximal steps to have the highest flux control coefficients for the pathway, the activities of glycogen transport and HK must be insensitive to G6P concentration.

There must be several methods by which metabolic control coefficients can be determined from experimental data for the glycogen synthesis pathway. The most direct method would be to selectively modulate the total activity of either the GT/HK or GSase steps of the pathway and measure the resultant change in glycogen synthesis rate. The result of this experiment would directly give the value of the flux control coefficient of the modulated enzyme and from the summation theorem would determine the control coefficient of the other step. An alternate method proposed by Kacser and Burns (1) for determining control coefficients is to vary the concentration of an external effector (Q) that acts on an enzyme in the pathway. The response of the flux to the external effector they define as the combined response coefficient $R^Q_k$, where

$$ R^Q_k = \frac{\partial F}{\partial Q}/Q $$

and show that

$$ R^Q_k = C_k k_e Q $$

where $k_e$ is the elasticity coefficient describing the effect of changing $Q$ upon the activity of the enzyme in vivo. The major external effectors that influence the activities of GT/HK and GSase are plasma glucose and insulin concentration. Eqs. 4 and 5 cannot be applied to a change in plasma insulin concentration because this will alter the activity of the GT/HK and GSase steps in the pathway. However, under conditions of high plasma insulin the phosphorylation state of GSase is insensitive to changes in plasma glucose (41), which allows application of Eqs. 4 and 5 to determine the flux control coefficient of GT/HK (41). This alters the flux control coefficient of GT/HK from the fractional change in glycogen synthesis rate in response to change in plasma glucose concentration ($\partial G/G$).

In order to determine $C_{GT/HK}$ from experimental data and Eq. 4 it is necessary to know $k_{GSase}$, which is the elasticity of the GT/HK combined step to plasma glucose. Direct calculation of $k_{GSase}$ is not possible since the detailed kinetic properties of glucose transport and HK in vivo are not known. However, it can be shown with minimal assumptions about in vivo enzyme kinetics that the combined elasticity of the GT/HK step is less than unity (see Appendix). Therefore a minimum estimate of the flux control coefficient for GT/HK ($C_{GT/HK}$) is obtained by setting $k_{GSase}$ to 1 or expressed as an inequality $C_{GT/HK} \geq R^Q_k$.

**Model of Flux Control and Its Predictions.** Based on application of control analysis to studies performed by ourselves and other groups we propose that GT/HK has a control coefficient close to unity under conditions of rapid glycogen synthesis. The velocity of GSase is coupled to GT/HK activity by the concentration of G6P. When transporter activity is increased by recruitment to the plasma membrane (21–23), the flux into the G6P pool increases. The increased flux raises the G6P concentration, which increases, by allosteric control, the activity of GSase. In addition, the activity of GSase is increased by dephosphorylation. Provided HK is not sensitive to G6P inhibition—i.e., that its elasticity with respect to G6P is low—the concentration of G6P will increase until at steady state the flux through GSase matches the rate of net glucose uptake. The role of dephosphorylation of GSase is to increase the sensitivity to G6P so that the concentration of G6P does not have to rise to unphysiologically high levels to match the flux from GT/HK. This can be seen in Fig. 2 by noting that G6P concentrations would need to be several millimolar to match the flux immediately after glycogen-depleting exercise if the intrinsic activity of GSase did not increase. Hence, GSase is being regulated by phosphorylation in order to adapt to the flux but is not controlling the flux. In the context of control analysis specific predictions of this model are as follows: (i) The glycogen synthesis flux during constant insulin concentration is proportional to the activity of GT/HK. This prediction follows from the definition of the flux control coefficient. (ii) GSase is sensitive to G6P allosteric regulation while GT/HK is relatively insensitive to this mechanism. This prediction follows from the elasticity theorem as applied to the muscle glycogen synthesis pathway.

**Evidence for the Model.** In this section we review studies that bear upon the model's prediction. According to metabolic control analysis, GT/HK would be flux-controlling if an isolated increase in the activity of this step induced a proportional increase in glycogen synthesis. In this event by the summation theorem control coefficients would be zero for all other enzymes of the pathway. Difficulties in modulating the glucose transport activity in vivo independently of other changes in the pathway (in particular GSase activity), as well as unfamiliarity with control theory have led to incomplete strategies being used to address metabolic control of glucose disposal/glycogen synthesis. Therefore, the experimental data that can be used to test the model are often incomplete and in some cases the authors have interpreted their data differently. However, the predictions of the model do explain many of the existing data as follows:

(i) Studies of the relationship of glycogen synthesis rate to the activity of GT/HK. To test this proportionality, changes in GT/HK activity must be induced in the absence of changes in GSase activity. The proportionality of GT/HK and flux has been evaluated in three ways: Measurement of the relationship of plasma glucose concentration and glycogen synthesis rate, the correlation of unidirectional glucose transport with glycogen synthesis rate, and the correlation between transporter activity and glycogen synthesis rate.

(ii) Proportionality of plasma glucose concentration and glycogen synthesis rate. A method of altering the activity of the GT/HK step and comparing it with the glycogen synthesis flux is to change the extracellular glucose concentration under conditions of high insulin. Studies have shown that under conditions of high insulin the phosphorylation state of GSase is independent of plasma glucose concentration (41), which fulfills all of the requirements for applying control analysis. As described above in Eq. 5, this measurement may be used to calculate the flux control coefficient for GT/HK.

We have recently (50) used 13C NMR to directly measure the muscle glycogen syn-
thesis rate in the human gastrocnemius at two different plasma glucose concentrations (9.5 mM and 5.0 mM), at a constant plasma insulin concentration (≈440 pM). The ratio of glycogen synthesis rates was 2.6, which is similar to the glucose concentration ratio of 1.9 showing that the glucose transport GT/HK contributes strongly to the control of flux. The non-perfect proportionality of glycogen synthesis rate to glucose may be due to the neglect of glycolysis at euaglycemia. In a branched metabolic pathway, transport may have a control coefficient >1 since the control coefficient of phosphofructokinase for glycogen synthesis is negative and the sum of all control coefficients must be unity (1). Other effectors of GSase external to the modeled pathway (Pi, ATP, pH, Mg++, and ADP) were measured in this study by 31P NMR and found not to change between euaglycemia and hyperaglycemia, validating the assumption made in the control analysis that external effectors other than glucose remained constant. As described below, the increase in GSase velocity with plasma glucose concentration was attributed to an increase in G6P concentration that was also measured in this study.

Several other studies have reported the dependence of glycogen synthesis rate on plasma glucose concentrations at constant insulin levels.

Yki-Järvinen and coworkers (54) measured the rates of glucose disposal in the whole body and also across the forearm in man at different insulin and glucose levels. At plasma insulin concentrations above 50 μU/ml, whole body and forearm glucose uptake were linear in the physiological glucose range (100–200 mg/dl), consistent with control by the glucose transport/HK step. This result is consistent with a Km of the 20 mM range, which has been recently reported (55–57) for the insulin recruited Glut 4 transporter. An apparent Ks of 7.4 mM was reported at basal insulin levels. They attributed the discrepancy between the Ks values at basal and hyperinsulinemic conditions to GSase control resulting in non-Michaelis–Menten uptake kinetics. However, the apparent insulin dependence of glucose uptake control kinetics may be explained by uptake at the non-insulin-sensitive Glut 1 transporters that dominate at basal insulin concentration.

Mandarino et al. (45) showed that during a hyperinsulinemic clamp when glycemia increased from 6.1 to 11.9 mmol/liter, the glucose storage increased by a factor of 2, showing a proportionate increase. GSase phosphorylation state remained constant.

A similar proportionality between plasma glucose concentration and muscle glycogen synthesis rate has been observed in several studies of rat muscle. Kruszynska et al. (27) reported that increasing glycemia from 6 to 10 mmol/liter at constant insulinemia doubled the glycogen synthesis rate while I/(I + D) of GSase remained constant. In two studies of the conscious rat, one at saturating insulinemia (58) and the other at high physiological insulinemia (15), the muscle glycogen synthesis rate was found to increase nearly proportionally to the glucose plasma level, while the GSase cofactor phosphorilation remained constant.

(ii) Correlation of unidirectional glucose transport and glucose uptake. Several studies have tried to directly measure glucose transport using radioisotope-labeled glucose analogues. Based on the uptake of radiolabeled glucose analogues 3-O-methyl-D-glucose and 2-deoxyglucose, the rates of glucose unidirectional transport and phosphorylation were measured in the anesthetized rat (59). Following a kinetic model of glucose phosphorylation, the authors defined a “transport limitation factor” whose value is unity when glucose transport is flux controlling for glucose disposal, and decreases toward zero as another step (phosphorylation in their model) increasingly contributes to the metabolic control. The data obtained on the red and white quadriceps showed that glucose transport is flux controlling during insulin stimulation (including saturating insulinemia) at euaglycemia. A slight decrease of the transport limitation factor (to 0.86 and 0.78 in the white and red muscle, respectively) was observed during hyperaglycemic (15.9 mM) and saturating insulin stimulation.

Using a double isotope method in human forearm, Bonadonna et al. (60) found reduced unidirectional glucose transport and net glucose uptake in NIDDM subjects. In a separate study (61) on non-diabetic subjects the rate of glucose uptake was found to increase in approximate proportionality to glucose uptake when the plasma insulin concentration was varied. Although the quantitative accuracy of this and the previous study described can be questioned due to the use of glucose analogues to assess transport, the results are consistent with transport having a high flux control coefficient for glycogen synthesis.

(iii) Correlation of glucose transport by direct assay activity and glucose uptake. Accurate determination of glucose transporter activity by direct assay of excised muscle has not been possible in humans and has been difficult to measure quantitatively even in the rat muscle. However, an increase in transporter number and activity following insulin infusion or intense exercise is well established (21–23). Transporter activity has been measured (23) in the rat hind limb muscle during recovery from an exercise protocol similar to that used by Bloch et al. (25). The 4-fold increase in transporter activity between early and late recovery agreed very well with the 4-fold increase in glycogen synthesis rate reported by Bloch et al.

(2) Studies of the elasticity of GT/HK and GSase to G6P. Direct measurement of the elasticity of GSase and GT/HK to G6P in vivo technically requires changing the G6P concentration and measuring the change in the enzyme velocities with all other effectors and enzyme total activities held constant. Studies in which plasma glucose concentration is varied with insulin held constant come closest to meeting these requirements since GT/HK total activity and GSase phosphorylation state remain constant. In the NMR study of Rothman et al. described above (50), the concentration of G6P increased by a factor of 1.5 in conjunction with a 2.6-fold increase in glycogen synthesis when plasma glucose concentration was doubled. Since other effectors of both GSases were measured by 31P NMR to remain constant, these data provide a direct in vivo estimate of GSase elasticity. Estimate of the elasticity of GT/HK to G6P is complicated by the increase in plasma glucose required to alter the glycogen synthesis rate, which violates the assumption of only G6P concentration changing. However, as shown in Appendix, the near-proportional increase in flux through the GT/HK step with plasma glucose concentration measured in vivo is the maximum theoretically possible. Therefore, any inhibition of the GT/HK step by G6P, and consequently its elasticity to G6P, was negligible. In contrast, the in vivo GSase activity was highly sensitive to an increase in G6P, which, by the connectivity relationship, indicates that steps prior to G6P have a much higher flux control coefficient consistent with the calculation of the high control coefficient from the responsivity of the flux to plasma glucose. The supralinear response in glycogen synthesis to G6P could be due either to an increase in UDP-glucose or to an experimental overestimation of the increase in G6P due to spectral overlap, which adds a constant intensity at both glucose levels (50).

Rossetti and Giaccari (51) reported a 2.5-fold increase in the muscle G6P concentration of euglycemic conscious rats when insulinemia was raised from 63 to 403 μU/ml, while muscle glycogen synthesis rate increased from 5.2 to 34.4 μg/min. As described above in a comprehensive discussion of their data, the change in G6P concentration was responsible for about one-half of the increase in GSase velocity with the rest due to a change in GSase phosphorylation state. Once again, the large increase in flux through GT/HK is not consistent with G6P being an effective allosteric inhibitor of GT/HK in this concentration range.

The finding of GT/HK being insensitive to G6P in vivo appears to be inconsistent with the in vitro studies in which G6P is a strong allosteric inhibitor of the HK reaction. The Ki reported in these early studies

Glucose transport/HK reactions held constant
\[ \frac{dv}{v} = k_{GT/HK} \frac{\partial G_0}{G_0} \]  \[ [A1] \]
At steady state the fractional velocity increases through the transport (GT) and HK steps must be the same. Written as total differentials
\[ \frac{dv}{v} = \frac{e_{GT} \partial G_0}{G_0} + \frac{e_{GT} \partial G_1}{G_1} \]  \[ [A2] \]
\[ \frac{dv}{v} = \frac{\partial G_2}{G_2} \]  \[ [A3] \]

G_i in Eqs. 2 and 3 is intracellular glucose, which must be explicitly included in the total differential of the isolated transport andHK enzymes. Substituting Eq. A2 into Eq. A3 to eliminate \( \frac{\partial G_i}{G_i} \) and using the definition of \( k_{GT/HK}^G \) in Eq. A1 gives
\[ k_{GT/HK}^G = \frac{e_{GT}}{1 - \frac{e_{GT}}{e_{HK}}} G_0 \]  \[ [A4] \]
As shown in ref. 1, general expressions may be derived for the elasticity of an enzyme to a substrate (S_i) and to a product (S_p)
\[ e_{S_i} = \frac{1}{1 - \rho} - Q_1 \]  \[ [A5] \]
\[ e_{S_p} = \frac{-\rho}{1 - \rho} - Q_2 \]  \[ [A6] \]
\( \rho \) is the “disequilibrium ratio,” which is the ratio of concentrations of the chemical reactants divided by the equilibrium constant for the reaction. The terms \( Q_1 \) and \( Q_2 \) are defined by
\[ Q_1 = \frac{S_1}{M_1 \left( 1 + S_1 + S_2 + \frac{S_3}{M_1} + \frac{S_4}{M_2} + \frac{S_5}{M_3} + \ldots \right)} \]  \[ [A7] \]
\[ Q_2 = \frac{S_2}{M_2 \left( 1 + \frac{S_1}{M_1} + \frac{S_2}{M_2} + \frac{S_3}{M_3} + \frac{S_4}{M_4} + \ldots \right)} \]  \[ [A8] \]
where \( M_i \) is the \( k_m \) to substrate \( i \). The \( in vivo \) HK reaction is far from equilibrium (\( \rho \rightarrow 0 \)) and since \( Q_1 < 1 \)
\[ e_{HK} \leq 1. \]  \[ [A9] \]
Setting \( e_{HK} = 1 \), Eq. A4 can be reexpressed as:
\[ k_{GT/HK}^G \leq \frac{e_{GT} G_0}{1 - e_{GT}} \]  \[ [A10] \]
Substituting Eq. A5 and Eq. A6 into Eq. A10 gives
\[ k_{GT/HK} \leq 1. \]  \[ [A12] \]

This work was supported by National Institutes of Health Grant DK43146.
