

# Glucose 6-Phosphate Regulates Hepatic Glycogenolysis Through Inactivation of Phosphorylase

Susan Aiston,<sup>1</sup> Birgitte Andersen,<sup>2</sup> and Loranne Agius<sup>1</sup>

**High glucose concentration suppresses hepatic glycogenolysis by allosteric inhibition and dephosphorylation (inactivation) of phosphorylase-*a*. The latter effect is attributed to a direct effect of glucose on the conformation of phosphorylase-*a*. Although glucose-6-phosphate (G6P), like glucose, stimulates dephosphorylation of phosphorylase-*a* by phosphorylase phosphatase, its physiological role in regulating glycogenolysis in intact hepatocytes has not been tested. We show in this study that metabolic conditions associated with an increase in G6P, including glucokinase overexpression and incubation with octanoate or dihydroxyacetone, cause inactivation of phosphorylase. The latter conditions also inhibit glycogenolysis. The activity of phosphorylase-*a* correlated inversely with the G6P concentration within the physiological range. The inhibition of glycogenolysis and inactivation of phosphorylase-*a* caused by 10 mmol/l glucose can be at least in part counteracted by inhibition of glucokinase with 5-thioglucose, which lowers G6P. In conclusion, metabolic conditions that alter the hepatic G6P content affect glycogen metabolism not only through regulation of glycogen synthase but also through regulation of the activation state of phosphorylase. Dysregulation of G6P in diabetes by changes in activity of glucokinase or glucose 6-phosphatase may be a contributing factor to impaired suppression of glycogenolysis by hyperglycemia. *Diabetes* 52:1333–1339, 2003**

**L**iver glycogen has a major role in the maintenance of blood glucose homeostasis. Its synthesis and degradation are determined by the phosphorylation state of glycogen synthase and phosphorylase and by allosteric mechanisms (1).

Glycogen synthase is regulated by multisite phosphorylation, which results in inactivation (2,3). Two sites at the NH<sub>2</sub>-terminal designated sites 2 and 2a are phosphorylated in vitro by cAMP-dependent protein kinase, phosphorylase kinase, calmodulin-dependent protein kinase, and protein kinase C, and five sites at the COOH-terminal designated sites 3a, 3b, 3c, 4, and 5 are phosphorylated by glycogen synthase kinase-3 and casein kinase-II. Several metabo-

lites are involved in the allosteric regulation of glycogen synthase (4), of which glucose 6-phosphate (G6P) is considered to have the predominant role (5). In addition to being a potent allosteric activator, G6P makes the enzyme a better substrate for dephosphorylation by synthase phosphatase. Accordingly, the activation state of glycogen synthase correlates with the hepatocyte content of G6P, both in vivo and in vitro (5–7). Another important mechanism in the regulation of glycogen synthase involves the allosteric inhibition of glycogen synthase phosphatase by phosphorylase-*a* (1). Mechanisms that cause depletion of phosphorylase-*a* by dephosphorylation relieve the inhibition of glycogen synthase phosphatase (1).

Phosphorylase is converted from an inactive *b* form to an active *a* form by phosphorylation of a serine residue at the NH<sub>2</sub>-terminal, catalyzed by phosphorylase kinase (1). Phosphorylase exists as two conformational states designated R (relaxed) and T (tense). The R state is promoted by phosphorylation of the NH<sub>2</sub>-terminal or by the allosteric activator AMP, which binds to a nucleotide activation site. The effect of AMP is counteracted by ATP and G6P (8,9). Glucose is an allosteric inhibitor. It promotes the T-state, which is a better substrate for dephosphorylation by phosphorylase phosphatase (8). The effects of glucose on conformation and function are mimicked by caffeine, which binds to a distinct inhibitory site (10).

It is generally accepted that the phosphorylation state of liver phosphorylase is determined by hormones through regulation of phosphorylase kinase and phosphatase and by glucose, which makes phosphorylase-*a* a better substrate for dephosphorylation (1). Phosphorylase has therefore been described as the “glucose-sensor” of the liver (1), whereas glycogen synthase is regarded as the “G6P sensor” (5).

Although glucose is thought to be the main metabolite that determines the phosphorylation state of phosphorylase in liver cells (1,11), its potency at dephosphorylating phosphorylase in intact cells is greater than can be explained by kinetic studies on the effects of glucose and AMP on the purified enzyme (10). Because the effect of glucose in vitro is counteracted by physiological concentrations of AMP but is synergistic with caffeine, it has been proposed that there may be endogenous ligands for the purine-inhibitory site, which potentiate the effect of glucose in vivo (10). Alternatively, the presence of ligands that compete for the AMP site, such as G6P, might also explain the potency of glucose in vivo. There is in vitro evidence that G6P can stimulate dephosphorylation of phosphorylase-*a* by phosphorylase phosphatase (12–14) and inhibit phosphorylation of phosphorylase-*b* by phosphorylase kinase (15,16) by substrate-directed mecha-

From the <sup>1</sup>Department of Diabetes, University of Newcastle upon Tyne, the Medical School, Newcastle upon Tyne, U.K.; and <sup>2</sup>Diabetes Biochemistry and Metabolism, Novo Nordisk A/S, Måløv, Denmark.

Address correspondence and reprint requests to Loranne Agius, Department of Diabetes, School of Clinical Medical Sciences, The Medical School, Newcastle upon Tyne, NE2 4HH, U.K. E-mail: loranne.agius@ncl.ac.uk.

Received for publication 15 January 2003 and accepted in revised form 19 February 2003.

S.A. and L.A. have received funds from Norvo Nordisk. G6P, glucose 6-phosphate.

© 2003 by the American Diabetes Association.

nisms. However, the putative physiological role of G6P in regulating the phosphorylation state of phosphorylase in liver cells has not been tested.

The hepatocyte content of G6P is markedly dependent on the activities of glucokinase and glucose 6-phosphatase (17,18). Accordingly, if G6P had a regulatory role on the phosphorylation state of phosphorylase, then changes in the activities of glucokinase or glucose 6-phosphatase might be a contributing factor to the impaired suppression of hepatic glucose production by hyperglycemia in type 2 diabetes through changes in the activation state of phosphorylase.

In this study, we provide supporting evidence for the hypothesis that the activation state of phosphorylase in hepatocytes is dependent on the cellular content of G6P. This has important implications for understanding the mechanism(s) underlying impaired suppression of hepatic glucose production by hyperglycemia in type 2 diabetes.

## RESEARCH DESIGN AND METHODS

**Hepatocyte monolayer culture.** Hepatocytes were isolated by collagenase perfusion of the liver (19) from male Wistar rats (240–340 g body wt) obtained from B & K (Hull, U.K.). They were suspended in minimum essential medium containing 7% newborn calf serum and seeded in multiwell plates. After cell attachment (~4 h), the medium was replaced by serum-free medium containing 10 nmol/l dexamethasone. Unless otherwise indicated, the medium contained 5 mmol/l glucose.

**Overexpression of glucokinase or glucose 6-phosphatase.** To modulate the cell content of G6P independent of glucose concentration, glucokinase and glucose 6-phosphatase were overexpressed using recombinant adenoviruses. After cell attachment (~2 h), the serum-containing medium was replaced by serum-free medium containing adenoviruses for expression of glucokinase (AdCMV-LGK) (20) or glucose 6-phosphatase (AdCMV-G6Pase) (21). Two adenoviral titers were used for AdCMV-LGK that resulted in enzyme overexpression by twofold and fourfold relative to endogenous activity (22) and one adenoviral titer of AdCMV-G6Pase that caused twofold overexpression relative to endogenous activity (23). Two sets of controls were used that were either untreated or treated with AdCMV- $\beta$ GAL at the same viral titer as AdCMV-G6Pase. After 2 h of incubation with the adenoviruses, the medium was replaced by serum-free medium containing 5 mmol/l glucose and 10 nmol/l dexamethasone.

**Incubations for determination of G6P, phosphorylase *a*, and glycogenolysis.** After overnight culture (16–18 h), the medium was replaced and the monolayers were incubated for 1 h with the additions indicated. Parallel incubations were performed for determination of G6P or phosphorylase-*a*. For determination of glycogenolysis, the hepatocyte monolayers were cultured overnight (16–18 h) in medium containing 25 mmol/l glucose, 10 nmol/l insulin, and [U-<sup>14</sup>C]glucose (1.5  $\mu$ Ci/ml; Perkin Elmer). They were then incubated for 1 h in fresh medium containing the substrates indicated. Cellular glycogen was determined by ethanol precipitation (19), and glycogenolysis was calculated from the decrease in the glycogen content during 1 h of incubation and is expressed as nanomoles of glucosyl units degraded per hour per mg protein. G6P was determined fluorimetrically in neutralized perchlorate extracts as previously described (23). For determination of phosphorylase-*a*, hepatocyte monolayers were snap-frozen in liquid nitrogen and stored at -80°C. They were extracted as described previously (24), and activities were determined in either the whole homogenates or after sedimentation of the extracts at 13,000g (15 min). Phosphorylase-*a* was determined either spectrometrically (25) or, where indicated, radiochemically (26). Enzyme activity determined in the whole homogenate or 13,000g supernatant or pellet fractions is expressed as milliunits per milligram of total homogenate protein, where 1 mU is the amount converting 1 nmol of substrate per minute.

Results are expressed as means  $\pm$  SE for the number of experiments indicated. Statistical analysis was by the Student's paired *t* test.

## RESULTS

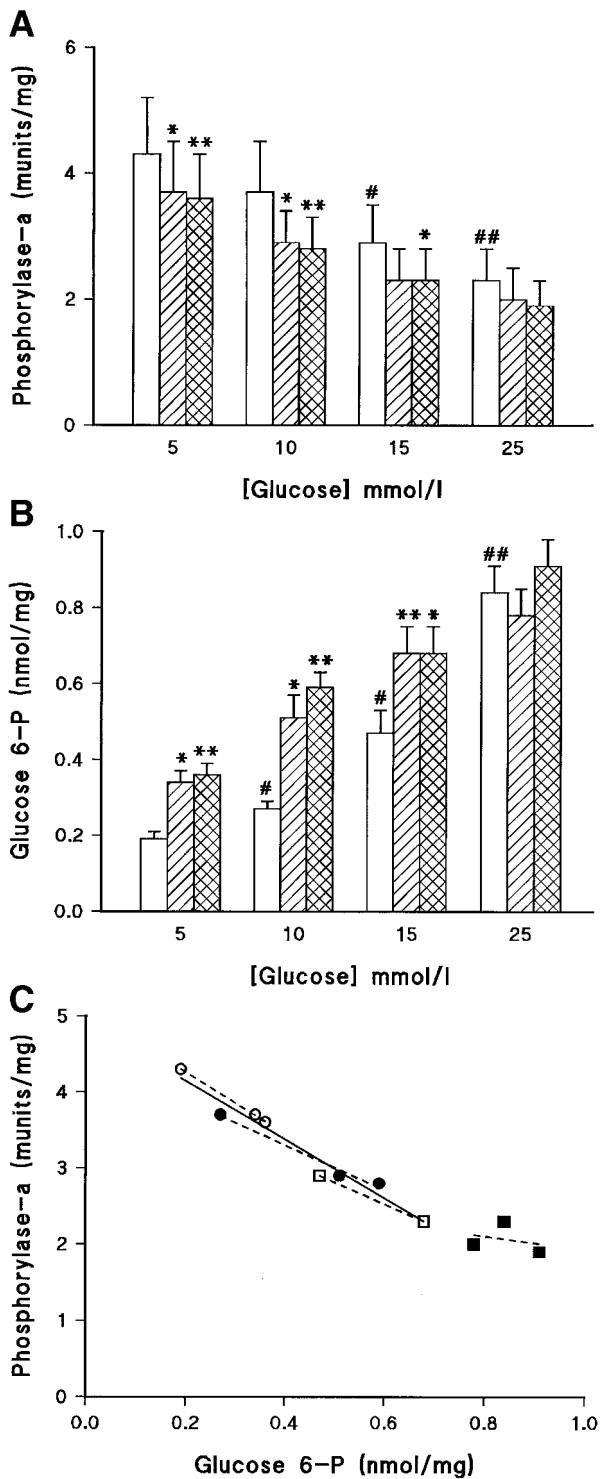
**Octanoate and dihydroxyacetone inactivate phosphorylase and increase G6P.** In preliminary studies, we tested the effects of incubation of hepatocytes with various substrates including gluconeogenic precursors, amino

acids, and octanoate on the activation state of phosphorylase. Significant inactivation of phosphorylase ( $P < 0.05$ ) occurred with octanoate, which has been shown previously to increase G6P (27), and with dihydroxyacetone (2 mmol/l), which also increases G6P (Figs. 1A and 2A). Octanoate decreased the activity of phosphorylase-*a* (Fig. 1A) and increased the G6P content (Fig. 1B) at glucose concentrations of 5, 10, and 15 mmol/l but had no significant effect on either parameter at 25 mmol/l glucose. Dihydroxyacetone and octanoate had additive effects on the cell content of G6P (Fig. 2B), and there was an apparent inverse correlation between the activity of phosphorylase-*a* and G6P at concentrations  $<1$  nmol/mg (Figs. 1C and 2C). We confirmed that at the concentrations of dihydroxyacetone used in this study, there were no changes in cellular ATP or ADP (results not shown).

In the above experiments, phosphorylase-*a* was assayed spectrometrically in the 13,000g supernatant. When the assays were performed on the whole homogenate, supernatant, and pellet fractions, there was little or no detectable activity in the pellet fraction, and the activity in the whole homogenate was slightly lower than in the supernatant and showed a similar fractional decrease with octanoate as in the supernatant (results not shown). The lack of detectable activity in the pellet by the spectrometric assay could be due to interference from either glucose 6-phosphatase or NADPH oxidase associated with the particulate fraction. To check whether the decrease in activity in the supernatant can be explained by translocation to the particulate fraction, we determined phosphorylase-*a* radiochemically in the direction of glycogen synthesis (Fig. 3). The activity of phosphorylase-*a* in the whole homogenate was significantly decreased by both octanoate and dihydroxyacetone (Fig. 3), confirming that these substrates cause an overall decrease in the activation state of phosphorylase. The activity in the pellet was 4% of that of the homogenate in control incubations and 19% in incubations with dihydroxyacetone.

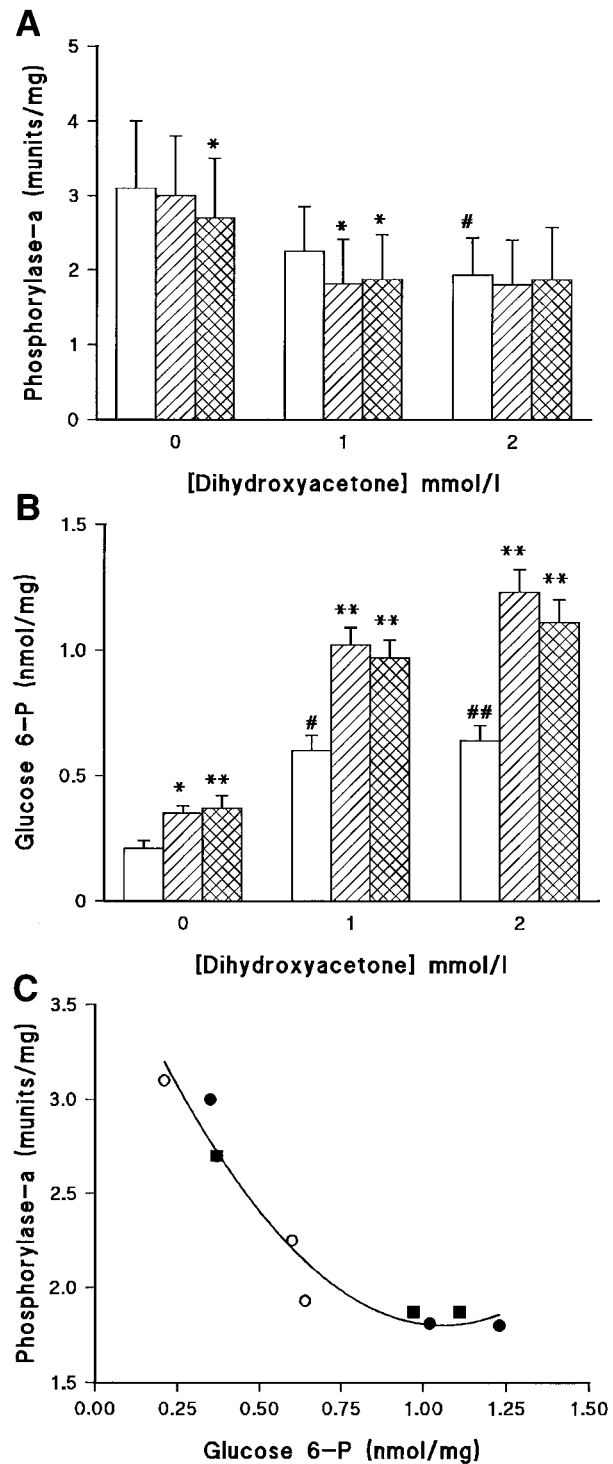
**Inhibition of glycogenolysis by octanoate and dihydroxyacetone.** The above experiments were performed on cells that were precultured with 5 mmol/l glucose. Additional experiments were performed on cells that were precultured with 25 mmol/l glucose for repletion of glycogen and subsequent determination of glycogenolysis at 5 mmol/l glucose. Octanoate and dihydroxyacetone inhibited glycogenolysis by 20–33% (Fig. 4). This inhibition of glycogenolysis cannot be explained by stimulation of glycogen synthesis because the incorporation of [U-<sup>14</sup>C]glucose into glycogen, determined in parallel experiments in which the <sup>14</sup>C label was added during the final incubation, was negligible (control 1.5; octanoate 2.1 nmol  $\cdot$  h<sup>-1</sup>  $\cdot$  mg<sup>-1</sup>) relative to the rate of glycogenolysis (~400 nmol  $\cdot$  h<sup>-1</sup>  $\cdot$  mg<sup>-1</sup>; Fig. 4). The activity of phosphorylase-*a* determined spectrometrically in the whole homogenate, in parallel incubations, was decreased by octanoate and dihydroxyacetone (from 14.6  $\pm$  0.8 to 7.2  $\pm$  0.6 and 7.1  $\pm$  0.7 mU/mg,  $n = 4$ , respectively).

**Effects of glucokinase and glucose 6-phosphatase overexpression.** To test the role of G6P in the regulation of phosphorylase independent of substrate availability, we determined the effects of overexpression of glucokinase or glucose 6-phosphatase. In agreement with previous stud-



**FIG. 1.** Effects of octanoate at varying glucose on phosphorylase-*a* and G6P. Hepatocytes were incubated for 1 h with the glucose concentrations indicated without (open bars) or with 0.2 mmol/l (single hatch) or 1.0 mmol/l (crossed hatch) octanoate. Parallel incubations were performed for determination of phosphorylase-*a* (assayed spectrometrically; A) and G6P (B). C: Phosphorylase-*a* versus respective G6P: 5 mmol/l (○), 10 mmol/l (●), 15 mmol/l (□), and 25 mmol/l (■) glucose. Means  $\pm$  SE for eight experiments. \* $P < 0.05$ , \*\* $P < 0.005$  octanoate relative to respective control; # $P < 0.05$ , ## $P < 0.005$  relative to 5 mmol/l glucose.

ies (17,18), the cell content of G6P was increased by glucokinase overexpression and decreased by glucose 6-phosphatase expression (Fig. 5B). This was associated with inverse changes in the activity of phosphorylase-*a*



**FIG. 2.** Combined effects of dihydroxyacetone and octanoate on phosphorylase-*a* and G6P. Hepatocytes were incubated for 1 h with 5 mmol/l glucose and the concentrations of dihydroxyacetone indicated without (□) or with 0.2 mmol/l (▨) or 1.0 mmol/l (▩) octanoate. Parallel incubations were performed for determination of phosphorylase-*a* (assayed spectrometrically; A) or G6P (B). C: Phosphorylase-*a* versus respective G6P: no octanoate (○), 0.2 mmol/l octanoate (●), and 1.0 mmol/l octanoate (■). Means  $\pm$  SE for four experiments. \* $P < 0.05$ , \*\* $P < 0.005$  octanoate relative to no octanoate; # $P < 0.05$ , ## $P < 0.005$  dihydroxyacetone relative to control.

(Fig. 5A). When the activity of phosphorylase-*a* was plotted against the corresponding G6P content, there was a decrease in activity at moderate increases in G6P, which reached a plateau at  $\sim 1$  nmol G6P/mg protein (Fig. 5C).

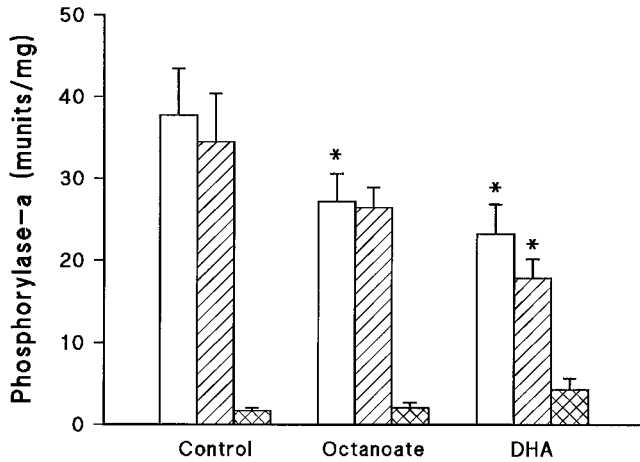


FIG. 3. Phosphorylase-a activity in homogenate, supernatant, and pellet fractions. Phosphorylase-a activity was determined by the radiochemical assay in the whole homogenate (□), supernatant (▨), or pellet (▩) fractions after the cells were incubated for 1 h with 15 mmol/l glucose without (control) or with 0.2 mmol/l octanoate or 2 mmol/l dihydroxyacetone (DHA). Means ± SE for four experiments. \*P < 0.05 relative to control.

Cells that were treated with adenovirus encoding β-galactosidase showed no change in G6P or phosphorylase-a activity (results not shown).

The effects of glucokinase overexpression on the sub-cellular distribution of phosphorylase-a were determined using the radiochemical assay in incubations with 15 mmol/l glucose. Glucokinase expression (fourfold) decreased the activity of phosphorylase-a in the homogenate (34.3 ± 3.5 to 27.7 ± 3.0 mU/mg; n = 7; P < 0.01) and supernatant (32.2 ± 3.3 to 21.8 ± 3.1 mU/mg; n = 7; P < 0.01) and increased it in the pellet (1.7 ± 0.6 to 5.1 ± 1.0 mU/mg; n = 7; P < 0.005) as observed also with dihydroxyacetone (Fig. 3). This suggests that in these, conditions there is both inactivation of phosphorylase-a in the whole homogenate and partial translocation from the supernatant to the pellet.

**The role of G6P in the inactivation of phosphorylase by glucose.** To determine whether G6P is involved in the

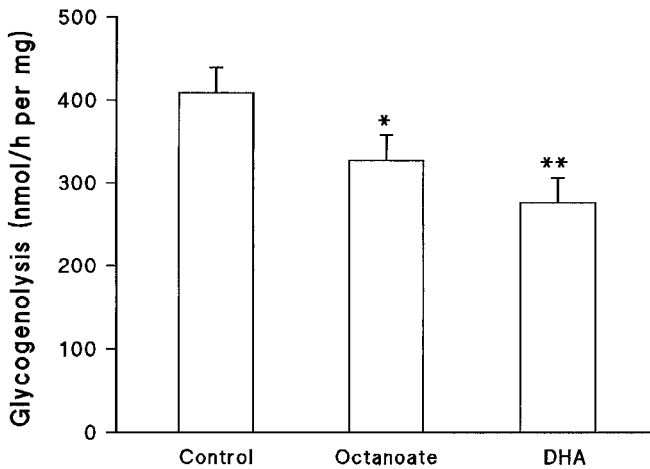


FIG. 4. Octanoate and dihydroxyacetone inhibit glycogenolysis. Hepatocytes were precultured with 25 mmol/l glucose and [U-14C]glucose and then incubated for 1 h in fresh medium containing 5 mmol/l glucose without (control) or with 0.2 mmol/l octanoate or 2 mmol/l dihydroxyacetone (DHA). Means ± SE for n = 5 experiments. \*P < 0.05, \*\*P < 0.005 relative to control.

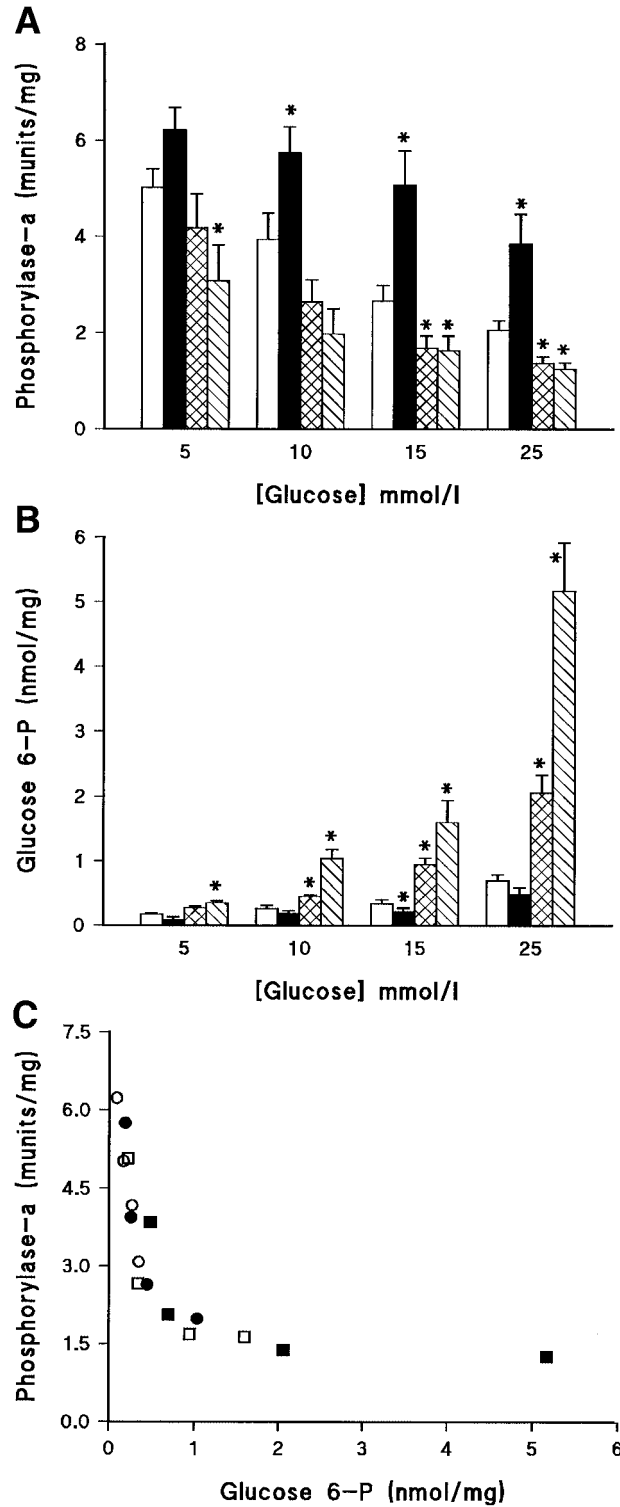


FIG. 5. Inactivation of phosphorylase by glucokinase overexpression. Hepatocytes were either untreated (□) or treated with recombinant adenovirus for overexpression of glucose 6-phosphatase by twofold (■) or glucokinase by twofold (▨) or fourfold (▩) relative to endogenous activity as described in the RESEARCH DESIGN AND METHODS. After preculture for 16 h, they were then incubated for 1 h with the glucose concentrations indicated. Parallel incubations were performed for determination of phosphorylase-a (A) or G6P (B). C: Phosphorylase-a versus respective G6P: 5 mmol/l (○), 10 mmol/l (●), 15 mmol/l (□), and 25 mmol/l (■) glucose. Means ± SE for four experiments. \*P < 0.05, \*\*P < 0.005 relative to controls.

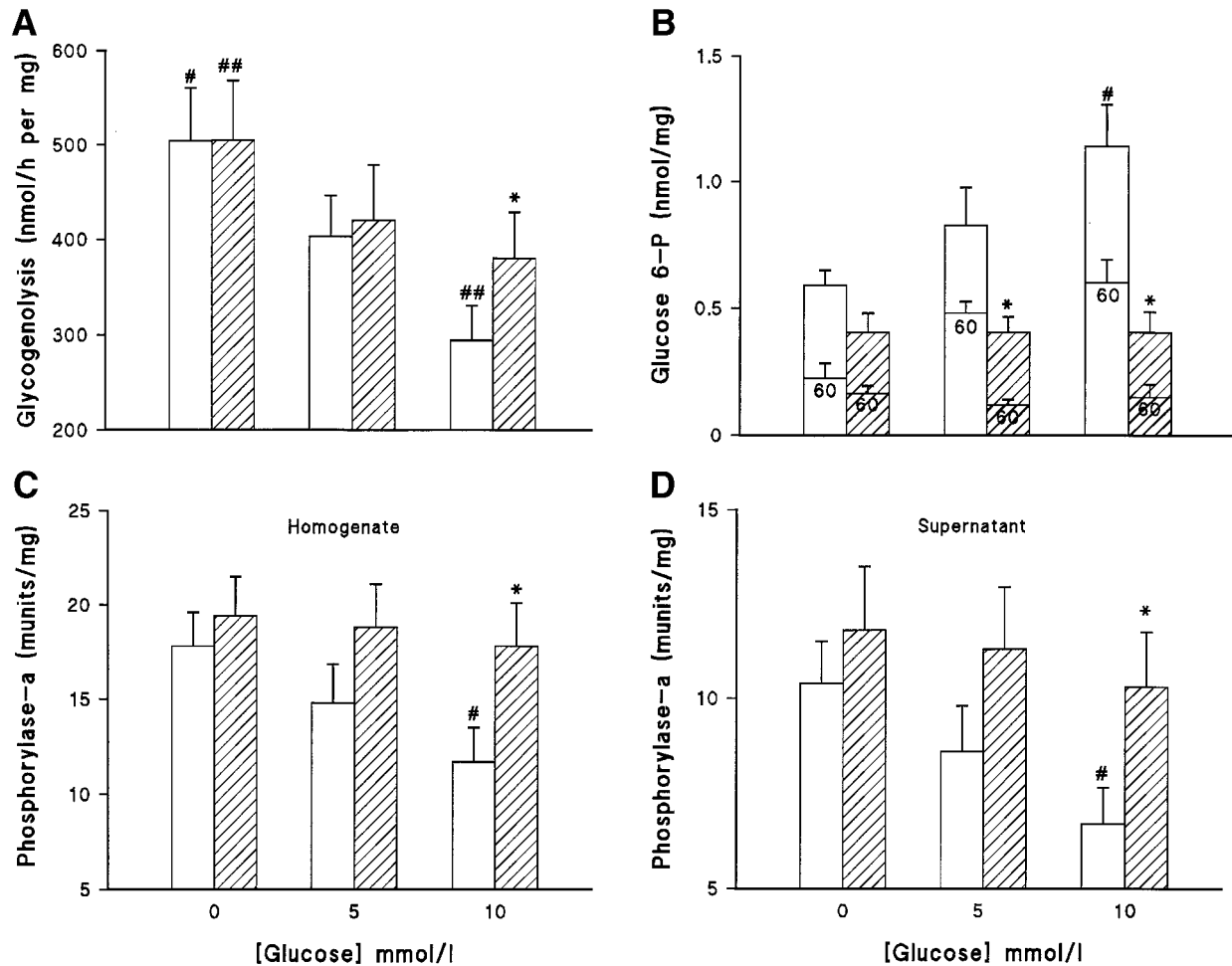


FIG. 6. 5-Thioglucose partially counteracts the effects of glucose. Hepatocytes were precultured with 25 mmol/l glucose (A–D) and [ $^{14}$ C]glucose label (A) and then incubated for 30 or 60 min in fresh medium with the glucose concentrations indicated in either the absence (□) or presence (▨) of 3 mmol/l 5-thioglucose. A: Glycogen degradation determined during 60 min of incubation. B: G6P determined after 30 min (upper bar) or 60 min (lower bar). Values at time zero  $1.1 \pm 0.2$  nmol/mg protein. Phosphorylase-*a* activity determined after 60 min in the whole homogenate (C) or 13,000g supernatant (D). Means  $\pm$  SE for five (A), three (B), or four (C and D) experiments. \* $P < 0.05$ , 5-thioglucose relative to respective control; # $P < 0.05$ , ## $P < 0.005$  relative to values at 5 mmol/l glucose.

inactivation of phosphorylase and inhibition of glycogenolysis caused by glucose, we used 5-thioglucose, a potent inhibitor of glucokinase (28). In cells precultured at 5 mmol/l glucose, 5-thioglucose (3 mmol/l) counteracted the increase in G6P caused by incubation with 25 mmol/l glucose for 60 min (5 mmol/l glucose,  $0.24 \pm 0.04$ ; 5 mmol/l + 5-thioglucose,  $0.14 \pm 0.04$ ; 25 mmol/l glucose,  $0.68 \pm 0.09$ ; 25 mmol/l glucose + 5-thioglucose,  $0.15 \pm 0.04$ , means  $\pm$  SE,  $n = 4$  nmol G6P/mg protein).

The effect of 5-thioglucose on glycogenolysis was tested in cells that were precultured overnight with 25 mmol/l glucose to replete glycogen stores and then incubated for 1 h with 0, 5, or 10 mmol/l glucose in the absence or presence of 5-thioglucose (Fig. 6). Parallel incubations were performed for determination of phosphorylase *a* (after 60 min) and G6P (after 30 and 60 min). Glycogenolysis was inhibited by 20 and 40% at 5 and 10 mmol/l glucose, respectively, relative to the rate in glucose-free medium (Fig. 6A). In cells incubated with 10 mmol/l glucose, 5-thioglucose counteracted the inhibition of glycogenolysis (Fig. 6A) and the inactivation of phosphorylase-*a* in the homogenate (Fig. 6C) and supernatant (Fig. 6D). However, it had no effect on glycogenolysis or

phosphorylase-*a* activity in the glucose-free medium. 5-Thioglucose lowered ( $P < 0.05$ ) the G6P content in cells that were incubated with glucose by  $\sim 50\%$  after both 30 and 60 min of incubation (Fig. 6B). These results are consistent with the hypothesis that the inhibitory effects of 10 mmol/l glucose on glycogenolysis and inactivation of phosphorylase can be at least in part explained by the higher cell content of G6P formed from glucose.

## DISCUSSION

It is currently accepted that G6P is the main metabolite regulator of the phosphorylation state of glycogen synthase (5), whereas glucose is the main regulator of the phosphorylation state of phosphorylase (1,11). Nonetheless, there is evidence from in vitro studies that G6P stimulates not only synthase phosphatase activity but also phosphorylase phosphatase activity by a substrate-mediated mechanism (12–14), and it also inhibits phosphorylase kinase activity by a substrate-directed mechanism (15,16). Although several studies have provided evidence for a correlation between the activation state of glycogen synthase and the hepatocyte content of G6P (5–7), the

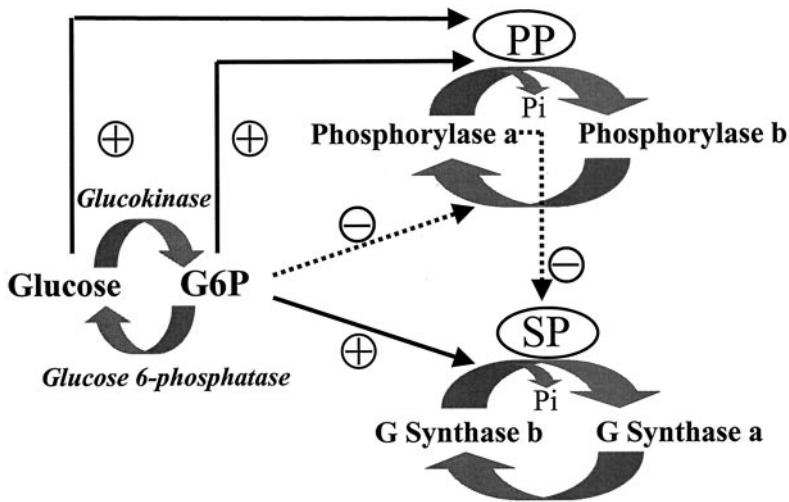


FIG. 7. Model showing mechanisms by which G6P may alter the phosphorylation state of phosphorylase and glycogen synthase. Glucose and G6P stimulate dephosphorylation of phosphorylase-*a* by phosphorylase phosphatase (PP) and G6P may also inhibit phosphorylation of phosphorylase-*b*. G6P stimulates the dephosphorylation of glycogen synthase by synthase phosphatase (SP) by a substrate-directed mechanism and by depletion of phosphorylase-*a*, which is a potent inhibitor of synthase phosphatase.

putative physiological role of G6P in regulating the activation state of phosphorylase in hepatocytes has not previously been tested.

In this study, we used three approaches, involving incubation with substrates that raise the hepatocyte content of G6P (27), overexpression of glucokinase (17), and inhibition of glucokinase with 5-thiogluconate (28), to test whether changes in cellular G6P within the physiological range regulate the activation state of phosphorylase. In incubations with octanoate and dihydroxyacetone or in cells overexpressing glucokinase, there was an inverse correlation between the activity of phosphorylase-*a* and the cell content of G6P over the physiological range of concentrations of this metabolite (<1 nmol/mg protein, (29). Because octanoate inactivated phosphorylase only in incubation conditions associated with an increase in G6P, the inactivation is unlikely to be due to a direct effect of octanoate. The increase in G6P caused by octanoate is most likely due to inhibition of glycolysis and/or stimulation of gluconeogenesis. In the experiments with glucokinase overexpression, in which the cell content of G6P was increased by up to threefold above the physiological range (29), the lack of further inactivation of phosphorylase at high concentrations of G6P suggests saturation of the response. In metabolic conditions associated with an increase in the cell content of G6P within the physiological range, there was a decrease in activity of phosphorylase-*a* in both the whole homogenate and in the high-speed supernatant fraction. The fractional decrease in activity of phosphorylase-*a* was similar when determined by the spectrometric assay, which measures activity in the direction of glycogenolysis, or the radiochemical assay, which measures the reverse reaction. It is unlikely, therefore, that the decrease in activity by the spectrometric assay is due to enzymes that interfere with the coupling system of the glycogenolytic assay. Although in experiments with dihydroxyacetone and glucokinase overexpression there was a small increase in the activity of phosphorylase-*a* associated with the pellet fraction, the decrease in activity in the supernatant could not be explained by enzyme translocation alone, as shown by the decrease in activity of phosphorylase-*a* in the whole homogenate. The physiological significance of the inactivation of phosphorylase in the incubations with dihydroxyacetone or octanoate is supported by a similar

fractional inhibition of the rate of glycogenolysis. The significance of the increase in phosphorylase-*a* in the pellet fraction during glucokinase overexpression is unclear but may be analogous to the translocation of glycogen synthase to the pellet fraction in incubation conditions associated with accumulation of G6P (30).

At least three mechanisms can be invoked to explain the inhibition of glycogenolysis by hyperglycemia: direct allosteric inhibition of phosphorylase-*a* by glucose, increased dephosphorylation of phosphorylase-*a* by phosphorylase phosphatase by a glucose-mediated mechanism, and increased dephosphorylation of phosphorylase-*a* by a G6P-mediated mechanism. The finding that 5-thiogluconate, a potent inhibitor of glucokinase (28), counteracted the inactivation of phosphorylase by 10 mmol/l glucose is supportive evidence for a role for G6P (or a downstream metabolite) in either mediating or potentiating the effect of glucose on the dephosphorylation of phosphorylase-*a* and is consistent with the finding that glucokinase overexpression potentiates the inactivation of phosphorylase by glucose. These results do not exclude an additional role for allosteric inhibition of phosphorylase by glucose, but they suggest that the G6P is a component of the inactivation of phosphorylase by glucose.

In summary, this study shows that the activity of phosphorylase-*a* in hepatocytes is decreased in metabolic conditions associated with an increase in the cell content of G6P, irrespective of whether this is derived from glucose phosphorylation or from gluconeogenesis. Furthermore, the inhibition of glycogenolysis by octanoate and dihydroxyacetone is evidence for a physiological role for the decrease in activity of phosphorylase-*a* in regulating glycogen degradation. We conclude that G6P has a more complex regulatory role in hepatic glycogen metabolism than has hitherto been recognized (1). This effect may be due to either stimulation of phosphorylase phosphatase (12–14) or inhibition of phosphorylase kinase (15,16) activity.

There has been a long-standing debate on the relative roles of G6P as activator of glycogen synthase phosphatase (11) as opposed to phosphorylase-*a* as allosteric inhibitor of synthase phosphatase (1,31) in determining the activity ratio of glycogen synthase. Further evidence for a role for phosphorylase-*a* in regulating the phosphor-

ylation state of glycogen synthase has emerged from studies using novel inhibitors of phosphorylase (32), which cause inactivation (dephosphorylation) of phosphorylase-*a* and sequential activation of glycogen synthase (24,32–35). The present study introduces a new link in the control of glycogen metabolism, whereby G6P generated by glucokinase during hyperglycemia downregulates phosphorylase-*a* (Fig. 7). Thus, G6P regulates glycogen synthase phosphatase by reversal of the inhibition by phosphorylase-*a* as well as by substrate-directed stimulation of dephosphorylation by synthase phosphatase.

Two major determinants of the hepatocyte G6P content are the activities of glucokinase and glucose 6-phosphatase (17,18). Disease states associated with altered activities of either of these enzymes would therefore be expected to show dysregulation of phosphorylase and hepatic glycogenolysis. Recent studies have demonstrated the therapeutic potential of phosphorylase inhibitors for normalizing blood glucose in animal models of type-2 diabetes and in human diabetes (32). It is currently not known whether the activation state of phosphorylase is altered in type 2 diabetes. However, a decreased activity of glucokinase in type 2 diabetes has been reported (36). It can be speculated that the impaired suppression of hepatic glucose production by hyperglycemia in type 2 diabetes (37,38) could be in part due to dysregulation of phosphorylase as a result of a decreased cellular content of G6P.

#### ACKNOWLEDGMENTS

We thank Dr. Chris Newgard for the kind gifts of the recombinant adenoviruses, Dr. Viggo Diness for useful discussions, and Diabetes U.K. for support.

#### REFERENCES

- Bollen M, Keppens S, Stalmans W: Specific features of glycogen metabolism in liver. *Biochem J* 336:19–31, 1998
- Lawrence JC, Roach PJ: New insights in the role and mechanism of glycogen synthase activation by insulin. *Diabetes* 46:541–547, 1997
- Ciudad C, Camici M, Ahmad Z, Wang Y, DePoali-Roach AA, Roach PJ: Control of glycogen synthase phosphorylation in isolated rat hepatocytes by epinephrine, vasopressin and glucagon. *Eur J Biochem* 142:511–520, 1984
- Nuttall FQ, Gannon MC: Allosteric regulation of glycogen synthase in liver. A physiological dilemma. *J Biol Chem* 268:13286–13290, 1993
- Villar-Palasi C, Guinovart JJ: The role of glucose 6-phosphate in the control of glycogen synthase. *FASEB J* 11:544–558, 1997
- Ciudad CJ, Carabaza A, Guinovart JJ: Glucose 6-phosphate plays a central role in the activation of glycogen synthase by glucose in hepatocytes. *Biochem Biophys Res Commun* 141:1195–1200, 1986
- Fernandez-Novell JM, Roca A, Bellido D, Vilaro S, Guinovart JJ: Translocation and aggregation of hepatic glycogen synthase during the fasted-to-refed transition in rats. *Eur J Biochem* 238:570–575, 1996
- Stalmans W, Laloux M, Hers HG: The interaction of liver phosphorylase a with glucose and AMP. *Eur J Biochem* 49:415–427, 1974
- Newgard CB, Huang PK, Fletterick RJ: The family of glycogen phosphorylases: structure and function. *Crit Rev Biochem Mol Biol* 24:69–99, 1989
- Kasvinski PJ, Shechosky S, Fletterick RJ: Synergistic regulation of phosphorylase a by glucose and caffeine. *J Biol Chem* 253:9102–9106, 1978
- Carabaza A, Ciudad CJ, Baque S, Guinovart JJ: Glucose has to be phosphorylated to activate glycogen synthase, but not to inactivate glycogen phosphorylase in hepatocytes. *FEBS Lett* 296:211–214, 1992
- Hurd SS, Teller D, Fischer EH: Probable formation of partially phosphorylated intermediates in the interconversions of phosphorylase a and b. *Biochem Biophys Res Commun* 24:79–84, 1966
- Martensen TM, Brotherton JE, Graves DJ: Kinetic studies of the activation of muscle phosphorylase phosphatase. *J Biol Chem* 248:8329–8336, 1973
- Cadefau J, Bollen M, Stalmans W: Glucose-induced glycogenesis in the liver involves the glucose-6-phosphate-dependent dephosphorylation of glycogen synthase. *Biochem J* 22:745–750, 1997
- Krebs EG, Love DS, Bratvold GE, Trayser KA, Meyer WL, Fischer EH: Purification and properties of rabbit skeletal muscle phosphorylase b kinase. *Biochemistry* 3:1022–1033, 1964
- Tu J-U, Graves DJ: Inhibition of the phosphorylase kinase catalysed reaction by glucose-6-P. *Biochem Biophys Res Commun* 53:59–65, 1973
- Seoane J, Gomez-Foix AM, O'Doherty RM, Gomez-Ara C, Newgard CB, Guinovart JJ: Glucose 6-phosphate produced by glucokinase, but not hexokinase I, promotes the activation of hepatic glycogen synthase. *J Biol Chem* 271:23756–23760, 1996
- Seoane J, Trinh K, O'Doherty RM, Gomez-Foix AM, Lange AJ, Newgard CB, Guinovart JJ: Metabolic impact of adenovirus-mediated overexpression of the glucose-6-phosphatase catalytic subunit in hepatocytes. *J Biol Chem* 272:26972–26977, 1997
- Agius L, Peak M, Alberti KGMM: Regulation of glycogen synthesis from glucose and gluconeogenic precursors by insulin in periportal and perivenous rat hepatocytes. *Biochem J* 266:91–102, 1990
- Becker TC, Noel RJ, Johnson JH, Lynch RM, Hirose H, Tokuyama Y, Bell GI, Newgard CB: Differential effects of overexpressed glucokinase and hexokinase I in isolated islets. Evidence for functional segregation of the high and low Km enzymes. *J Biol Chem* 271:390–394, 1996
- Trinh K, Minassian C, Lange AJ, O'Doherty RM, Newgard CB: Adenovirus-mediated expression of the catalytic subunit of glucose-6-phosphatase in INS-1 cells. Effects on glucose cycling, glucose usage, and insulin secretion. *J Biol Chem* 272:24837–24842, 1997
- Agius L, Peak M, Newgard CB, Gomez-Foix AM, Guinovart JJ: Evidence for a role of glucose-induced translocation of glucokinase in the control of hepatic glycogen synthesis. *J Biol Chem* 271:30479–30486, 1996
- Aiston S, Trinh KY, Lange AJ, Newgard CB, Agius L: Glucose-6-phosphatase overexpression lowers glucose 6-phosphate and inhibits glycogen synthesis and glycolysis in hepatocytes without affecting glucokinase translocation. Evidence against feedback inhibition of glucokinase. *J Biol Chem* 274:24559–24566, 1999
- Aiston S, Hampson L, Gomez-Foix AM, Guinovart JJ, Agius L: Hepatic glycogen synthesis is highly sensitive to phosphorylase activity: evidence from metabolic control analysis. *J Biol Chem* 276:23856–23866, 2001
- Aiston S, Agius L: Leptin enhances glycogen storage in hepatocytes by inhibition of phosphorylase and exerts an additive effect with insulin. *Diabetes* 48:15–20, 1999
- Gilboe DP, Larson KL, Nuttall FQ: Radioactive method for the assay of glycogen phosphorylases. *Anal Biochem* 47:20–27, 1972
- Morand C, Remesy C, Besson C, Demigne C: Control of glycogen metabolism by gluconeogenic and ketogenic substrates in isolated hepatocytes from fed rats. *Int J Biochem* 24:159–167, 1992
- Agius L, Stubbs M: Investigation of the mechanism by which glucose analogues cause translocation of glucokinase in hepatocytes: evidence for two glucose binding sites. *Biochem J* 346:413–421, 2000
- Niewoehner CB, Gilboe DP, Nuttall GA, Nuttall FQ: Metabolic effects of oral fructose in the liver of fasted rats. *Am J Physiol* 247:E505–E512, 1984
- Fernandez-Novell JM, Arino J, Vilaro S, Bellido D, Guinovart JJ: Role of glucose 6-phosphate in the translocation of glycogen synthase in rat hepatocytes. *Biochem J* 288:497–501, 1992
- Stalmans W, Cadefau J, Wera S, Bollen: New insight into the regulation of liver glycogen metabolism by glucose. *Biochem Soc Trans* 25:19–25, 1997
- Treadway JL, Mendys P, Hoover DJ: Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. *Expert Opin Investig Drugs* 10:439–454, 2001
- Bergans N, Stalmans W, Goldman S, Vanstapel F: Molecular mode of inhibition of glycogenolysis in rat liver by the dihydropyridine derivative BAY R3401. Inhibition and inactivation of glycogen phosphorylase by an activated metabolite. *Diabetes* 49:1419–1426, 2000
- Gustafson LA, Neef M, Reijngoud DJ, Kuipers F, Sauerwein HP, Romijn JA, Herling AW, Burger HJ, Meijer AJ: Fatty acid and amino acid modulation of glucose cycling in isolated rat hepatocytes. *Biochem J* 358:665–671, 2001
- Latsis T, Andersen B, Agius L: Diverse effects of two allosteric inhibitors on the phosphorylation state of glycogen phosphorylase in hepatocytes. *Biochem J* 368:309–316, 2002
- Caro JF, Triester S, Patel VK, Tapscott EB, Frazier NL, Dohm GL: Liver glucokinase: decreased activity in patients with type II diabetes. *Horm Metab Res* 27:19–22, 1995
- Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamon H, Rossetti L: Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. *J Clin Invest* 102:744–753, 1998
- Moore MC, Connolly CC, Cherrington AD: Autoregulation of hepatic glucose production. *Eur J Endocrinol* 138:240–248, 1998