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Role of glucose and insulin in regulating glycogen synthase and phosphorylase activities in rainbow trout hepatocytes

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Abstract This study, using ^{13}C nuclear magnetic resonance spectroscopy showed enrichment of glycogen carbon (C1) from ^{13}C-labelled (C1) glucose indicating a direct pathway for glycogen synthesis from glucose in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. There was a direct relationship between hepatocyte glycogen content and total glycogen synthase, total glycogen phosphorylase and glycogen phosphorylase *a* activities, whereas the relationship was inverse between glycogen content and % glycogen synthase *a* and glycogen synthase *a*/glycogen phosphorylase *a* ratio. Incubation of hepatocytes with glucose (3 or 10 mmol·l^{-1}) did not modify either glycogen synthase or glycogen phosphorylase activities. Insulin (porcine, 10^{-8} mol·l^{-1}) in the medium significantly decreased total glycogen phosphorylase and glycogen phosphorylase *a* activities, but had no significant effect on glycogen synthase activities when compared to the controls (absence of insulin). In the presence of 10 mmol·l^{-1} glucose, insulin increased % glycogen synthase *a* and decreased % glycogen phosphorylase *a* activities in trout hepatocytes. Also, the effect of insulin on the activities of % glycogen synthase *a* and glycogen synthase *a*/glycogen phosphorylase *a* ratio were more pronounced at low than at high hepatocyte glycogen content. The results indicate that in trout hepatocytes both the glycogen synthetic and breakdown pathways are active concurrently *in vitro* and any subtle alterations in the phosphorylase to synthase ratio may determine the hepatic glycogen content. Insulin plays an important role in the regulation of glycogen metabolism in rainbow trout hepatocytes. The effect of insulin on hepatocyte glycogen content may be under the control of several factors, including plasma glucose concentration and hepatocyte glycogen content.

Key words Glycogen · Hepatocyte · Insulin · ^{13}C NMR · Rainbow trout, *Oncorhynchus mykiss*

Abbreviations ^{13}C NMR ^{13}C nuclear magnetic · resonance spectroscopy · GPase glycogen phosphorylase · GSase glycogen synthase · LDH lactate dehydrogenase · PCA perchloric acid

Introduction

Salmonids are generally considered glucose intolerant because they do not regulate plasma glucose concentrations in response to exogenous glucose load [see Buddington and Hilton (1987); Mommsen and Plisetskaya (1991) for references]. Hepatic glycogen stores, however, increase when fish are fed high carbohydrate diets (Hilton and Atkinson 1982; Buddington and Hilton 1987), suggesting a stimulation of glycogen synthesis by glucose. Also, rainbow trout hepatocytes showed incorporation of ^{14}C-glucose into glycogen supporting the concept that glucose may play a role in glycogen repletion (Pereira et al. 1995). Glycogen synthesis from glucose can occur either by a direct pathway (glucose → glucose 6-phosphate → glucose 1-phosphate → UDP-glucose → glycogen) or by an indirect pathway (glucose → lactate/pyruvate → glucose 6-phosphate → glycogen), and *in vivo* studies using ^{13}C NMR indicate that the majority of glycogen production in rats is by the indirect route (Shulman et al. 1985; Shulman and

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Landau 1992). The preferential route for glucose incorporation into glycogen in fish is not known.

High dietary carbohydrate levels and exogenous glucose loads have been shown to increase plasma insulin concentrations in salmonids [review: see Mommsen and Plisetskaya (1991)]. Insulin is a known anabolic hormone in fish and several studies have shown that insulin decreased glycogen breakdown and increased glycogen deposition in salmonid hepatocytes (Plisetskaya et al. 1984; Foster and Moon 1989; Vijayan et al. 1993a, b). It is, therefore, possible that the glucose effect on glycogen metabolism is mediated indirectly by the increase in plasma insulin concentrations, although our companion study (Pereira et al. 1995) did not sflhow an effect of insulin on glyconeogenesis from glucose in trout hepatocytes *in vitro*. To date few studies have actually addressed the role of insulin in the regulation of glycogen metabolism in fish.

The two enzymes involved in glycogen metabolism *viz* glycogen synthase (GSase) and glycogen phosphorylase (GPase) have been shown to be active in trout liver (see Vijayan and Moon 1992). These enzymes exist in active (GSase *a* and GPase *a*) and less active (GSase *b* and GPase *b*) forms and can be readily interconverted, both *in vitro* and *in vivo* by phosphorylation-dephosphorylation reactions. In mammals, especially rats, both glucose and insulin have been shown to regulate glycogen metabolism by exerting their effects on the phosphorylating-dephosphorylating mechanisms [review: Van de Werve and Jeanrenaud (1987)]. In addition, glucose may exert a modulating effect on insulin action on glycogen metabolism (Witters and Avruch 1978). The role of glucose and insulin in the regulation of glycogen metabolism in fish is not well understood. A recent study examined the effect of insulin on hepatic phosphorylase activity in the American eel (*Anguilla rostrata*) (Foster and Moon 1990), while most other studies examined the effect of either glucose or insulin on plasma glucose concentrations and liver glycogen content in fish. Liver glycogen content is highly variable in fish and may not be a particularly useful indicator of glycogen metabolism, unless accompanied by the activities of GSase and GPase.

In an attempt to better understand the regulation of glycogen metabolism in salmonids, we examined the effects of both glucose and insulin either alone or in combination on *in vitro* GSase and GPase activities in rainbow trout hepatocytes. Also, the preferential route of incorporation of glucose into glycogen in trout hepatocytes was determined by ^{13}C NMR. The advantage of ^{13}C NMR over radioisotope techniques lies in the fact that NMR permits the visualization of six carbons of the glycosyl units of glycogen. Consequently, it provides information regarding glucose label distribution and thereby, on the pathway of glucose incorporation into glycogen. This technique has been used widely in mammals both *in vivo* and *in vitro* to study the route of glycogen synthesis (Cohen 1984; Shulman et al. 1985; Spence and Koudelka 1985; Shulman and Landau 1992).

Materials and methods

Animals

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Linwood Acres Farm (Campbellcroft, Ontario, Canada) and maintained in indoor tanks (300 l) supplied with flowing dechlorinated Ottawa tap water at 12 °C. The photoperiod was maintained at 12L:12D regime and the fish were fed to satiety (Purina Trout Chow) every second day. The fish were acclimated in the holding tanks for more than 2 months and were deprived of food 48 h prior to sampling .

Hepatocyte isolation and incubations

The fish (approximate body wt 300 g) were netted and anaesthetized in a 1:10000 solution of neutralized (with sodium bicarbonate) 3-aminobenzoic acid ethyl ester (MS222, Sigma Chemical Co., Mo., USA). The hepatic portal vein was cannulated for liver perfusion and the hepatocytes were isolated by collagenase treatment according to Moon et al. (1985). Cells were suspended in a final incubation medium containing (in $mmol \cdot l^{-1}$): 110 NaCl, 3 KCl, 1.25 KH_2PO_4, 5 $NaHCO_3$, 0.6 $MgSO_4$, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 1% fatty acid-free bovine serum albumin, and adjusted to pH 7.63. All hepatocyte incubations were carried out at 8 °C. The cells were preincubated for 4 h, centrifuged and resuspended in fresh medium, before the final incubation. The cell wet weight ranged from 70 to $80 mg \cdot ml^{-1}$.

Cells (1-ml aliquots) were incubated in medium containing either no glucose or D-glucose (Sigma) at 3 or 10 $mmol \cdot l^{-1}$ and with or without 10^{-8} $mol \cdot l^{-1}$ bovine insulin (Eli Lilly, Indianapolis, Ind., USA). Prior to the final incubation, samples were taken for determining hepatocyte glycogen content, measured enzymatically after amyloglucosidase hydrolysis according to Perry et al. (1988). After 60 min of incubation the cell suspension was centrifuged (10000 *g*) for 10 s and the pellet stored frozen at −80 °C for enzyme determination.

Sample preparation and enzyme activity

The frozen pellets were sonicated in a phosphorylation-dephosphorylation "stopping buffer" (0.3 ml) containing (in $mmol \cdot l^{-1}$): 50 imidazole, 15 β-mercaptoethanol, 100 KF, 5 EDTA, 5 EGTA, and 0.1 PMSF, adjusted to pH 7.5. The homogenates were centrifuged at 10000 *g* for 5 min and the supernatant was then passed through a 3 ml syringe packed with Sephadex G-25 that had been equilibrated with the stopping buffer. The columns were centrifuged (1500 *g*) for 3 min and the eluate was used for measuring enzyme activities at 8 °C (Moon et al. 1989).

(i) *Glycogen phosphorylase assay*

GPase activities were estimated spectrophotometrically using the method of Moon et al. (1989). GPase *a* was assayed in a phosphate buffer containing 10 $mmol \cdot l^{-1}$ caffeine, while total GPase (*a* + *b*) was assayed in the absence of caffeine but with 2 $mmol \cdot l^{-1}$ AMP. Activity was coupled to the reduction of $NADP^+$ by glucose 6-phosphate dehydrogenase. Percent GPase *a* represents the ratio of GPase *a* to total GPase × 100.

(ii) Glycogen synthase (GSase) assay

GSase was assayed spectrophotometrically using the method of Passonneau and Rottenberg (1973). The active form (GSase *a*) was assayed with 20 mmol·l^{-1} UDPG, while total GSase (*a* + *b*) was assayed with 5 mmol·l^{-1} G6P + 1.5 mmol·l^{-1} UDPG. Other components of the assay were 100 mmol·l^{-1} KCl, 10 mmol·l^{-1} $MgCl_2$, 2 mg dialysed glycogen·ml^{-1}, 5 mmol·l^{-1} phosphoenolpyruvate (PEP), 0.15 mmol·l^{-1} NADH, and 10 units each of pyruvate kinase – lac-tate dehydrogenase (PK/LDH) in 50 mmol·l^{-1} imidazole buffer (pH 7.8).

NMR experiment

Hepatocytes from four fish were pooled and suspended in 60 ml of the final incubation medium and pre-incubated for 4 h. This cell suspension was centrifuged and the cells resuspended in 40 ml of fresh incubation medium to a final cell concentration of 69.7 mg·ml^{-1}. The suspension was divided into two equal fractions; to one, porcine insulin (10^{-8} mol·l^{-1}) and D-glucose (10 mmol·l^{-1}) were added, while to the other ^{13}C-enriched (99% at C1 position) D-glucose (10 mmol·l^{-1}) was substituted for unlabelled glucose. Insulin was added to both the fractions in order to prevent the excessive glycogen breakdown that is normally seen in fish hepatocyte incubations *in vitro* (Vijayan et al. 1993b). The cells were incubated for 60 min and the incubation terminated by the addition of 0.5 ml 70% PCA. The acidified sample was sonicated followed by 10 min centrifugation at 5000 rpm (Sorvall RC-285, SS34 rotor). The supernatant was neutralized with KOH and the precipitate removed by centrifugation (the loss of glycogen in the precipitate was less than 10%). The glycogen was extracted from the supernatant overnight with 0.1% LiCl in 99% ethanol containing 1% Na_2SO_4 (50:1). The glycogen precipitate was washed three times with 20 ml of 95% ethanol and left to dry overnight at 50 °C to remove any trace of ethanol. The glycogen was then redissolved in 1 ml H_2O + 1 ml D_2O and the ^{13}C spectrum of glycogen (approx. 90 µmol glycosyl units) was obtained on a 9.4 T Bruker spectrometer (carbon frequency of 100.6 MHz). The acquisition parameters were a 1-s repetition time, an acquisition time of 0.62 s and 35000 averages were performed. The resulting signals were Fourier transformed and manually phase corrected. The areas under the peaks were obtained by plotting the spectra, cutting out the peaks and weighing them. The difference in the peaks between the two spectra indicates ^{13}C enrichment and were expressed as percent change from unlabelled spectrum. As the ^{13}C NMR technique has been widely used in mammalian studies to determine glucose incorporation into glycogen (Canioni et al. 1983; Sillerud and Shulman 1983; Cohen 1984), no internal standards were necessary to characterize the glycogen spectrum.

Statistical analysis

The effect of glucose and insulin on GSase and GPase activities were determined using a two-way analysis of variance (ANOVA). Where *F* indicated significance, means were compared using a Student *t*-test (Sigmastat). There was no effect of glucose on any of the parameters measured and therefore, all tests for linear regression were carried out with data from 0 mmol·l^{-1} glucose. The effect of insulin was compared to their respective control (no insulin) using a paired *t*-test (Sigmastat).

Results

NMR

The difference in intensity between corresponding peaks from the NMR spectra B (^{13}C enriched) and A (un-enriched) indicates the preferential incorporation of label into each of the positions (Fig. 1). The signals from the glycogen carbons in positions 2–6 have a similar chemical shift as the carbons 2–6 of free glucose molecules. Hence, signal enhancement in these positions can represent incorporation of the label into either glucose or glycogen. However, in the case of the C1 position, the signals from the glycogen and glucose are well resolved. The appearance of the peaks at 91 and 95 ppm in spectrum B are due to traces of labelled glucose remaining after the extraction, while the peak at 98.6 ppm is that of C1 position of glycogen. It is likely that a similar contamination of unlabelled glucose occurred in spectrum A, but the peaks were undetectable because of the low natural abundance of ^{13}C (1.1%) compared to the 99% enrichment of the labelled glucose.

The area of the peak corresponding to the C1 position of glycogen (98.6 ppm) in spectrum B was 33% larger than in spectrum A, indicating incorporation of glucose carbon in that position (Fig. 1). There was no increase in the area under the peak for the other carbons (C2–C6), and the differences in the area between the spectra for these carbons were less than the noise level of 9%.

Enzyme activities

The initial hepatocyte glycogen content in the present study was 55.6 ± 13.3 µmol·g wet wt^{-1} of hepatocytes (mean ± SEM; $n = 8$). Total GSase and GPase activities showed strong linear relationships with initial glycogen content of hepatocytes (Fig. 2A, C). GPase *a* activity also showed a significant linear relationship with glycogen content (Fig. 2D), whereas there was no relationship between GSase *a* and hepatocyte glycogen content (Fig. 2B). The % GSase *a* showed a significant inverse relationship with glycogen content (Fig. 3A), whereas the ratio of GSase *a* to GPase *a* showed a significant inverse relationship with glycogen content only in the presence of insulin (Fig. 3C). Since the addition of insulin modifies the slope of the % GSase *a* and the GSase *a* to GPase *a* ratio to glycogen content (Fig. 3A, C), insulin appears to be favouring the synthetic pathway over breakdown, especially at low glycogen content. There was no significant relationship between glycogen content and % GPase *a* activity (Fig. 3B).

There was no effect of glucose or insulin added to the incubation medium on total GSase (overall mean 0.185 ±0.02 U·g^{-1}), GSase *a* (overall mean 0.105 ±0.02 U·g^{-1}) or % GSase *a* activities (glucose alone). The addition of insulin to the medium had no significant effect on % GSase *a* activity at 0 mmol·l^{-1} (56 ±18% no insulin and 64 ±14% with insulin) or 3 mmol·l^{-1} glucose (61 ±13% no insulin and 65 ± 15% with insulin), but insulin significantly

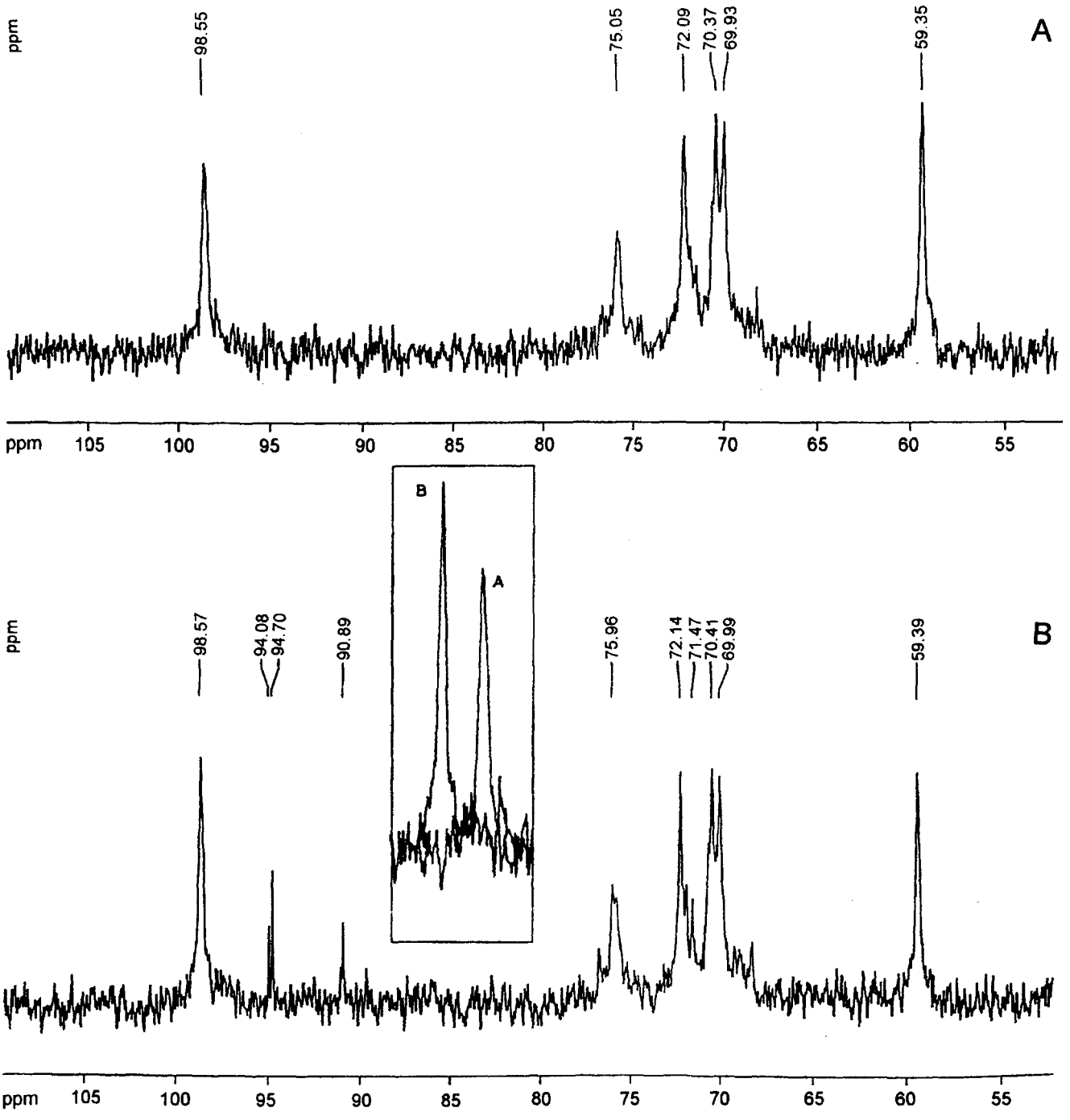
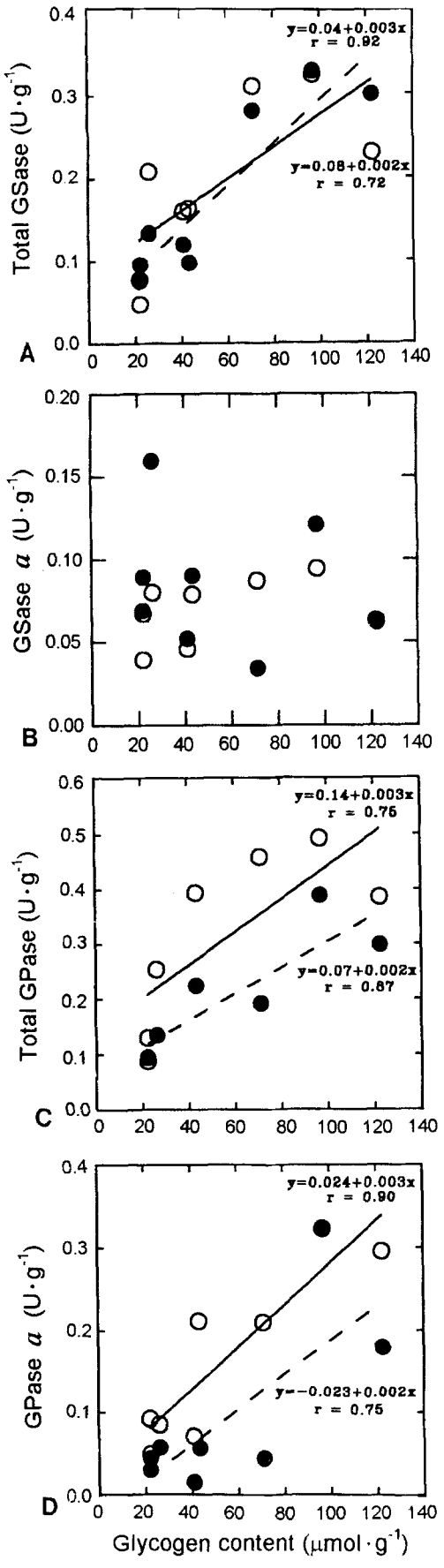


Fig. 1A, B ^{13}C NMR spectra of hepatocyte glycogen and glucose carbon after a 60-min incubation either with unlabelled glucose (**A**) or ^{13}C glucose (**B**) and in the presence of 10^{-8} mol·l^{-1} porcine insulin. The chemical shifts for glycogen carbons are 98.6 (C1), 70.0 (C2), 72.1 (C3), 70.4 (C5) and 59.4 (C6), and for glucose carbons are 94.8 (C1*a*), 90.9 (C1*b*), 70.4 (C2,C5) and 71.8 (C3*a*). *Inset* shows the superimposed (slightly offset) C1 peaks of glycogen (98.6) with unlabelled glucose (**A**) or ^{13}C glucose (**B**)

increased ($P < 0.05$, paired t-test) % GSase *a* activity at 10 mmol·l^{-1} glucose concentration (59 ± 14% no insulin and 75 ± 19% with insulin).

There was no effect of glucose in the incubation medium on GPase activities (Fig. 4); however the addition of insulin to the medium significantly decreased ($P < 0.05$) total GPase and GPase *a* activities (Fig. 4A, B). There was a significant effect ($P < 0.05$) of insulin on % GPase *a* activity only in the 10 mmol·l^{-1} glucose concentration and not in the other two groups (Fig. 4C). The ratio of GSase *a* to GPase *a* activities were significantly increased ($P < 0.05$) with insulin in



all the groups when compared to the control (no insulin) regardless of glucose concentration in the medium (Fig. 4D).

Discussion

Our study is the first to utilize ^{13}C NMR spectroscopy in an attempt to elucidate the pathway of glycogen synthesis from glucose in fish hepatocytes. When ^{13}C-enriched glucose (C1 labelled) is converted directly to glycogen (glucose → glucose-6-P → glucose-1-P → UDP-glucose → glycogen) all the label appears in the C1 position of glycogen. Alternatively, if the labelled glucose is first catabolized to triose phosphates and then converted to glycogen, equal amounts of label appear in the C1 and C6 positions of glycogen. Lastly, if the sources for glycogen synthesis are lactate/pyruvate derived from the utilized glucose, equal amounts of ^{13}C label will occur in positions C1, C2, C5 and C6 (Shulman et al. 1985). The last two alternatives are called the indirect pathway of glycogen synthesis. The enrichment of the C1 carbon of glycogen indicates that the direct pathway of glycogen synthesis is present in trout hepatocytes (Fig. 1).

Shulman et al. (1985) report that only one third of the rat liver glycogen is synthesized by the direct pathway *in vivo*, while the other two thirds come *via* the indirect pathway. The indirect pathway has also been reported as the main route of glycogen synthesis in hepatocytes of fasted rats, but not in fed rats kept in primary culture (Spence and Koudelka 1985). As the indirect pathway of glycogen synthesis is dependent on an active gluconeogenic pathway, the nutritional state of the animal may be a determining factor in the pathway utilized for glycogen repletion from glucose in mammals. In trout, however, the gluconeogenic pathway is active even in the fed state and is not influenced by either glucose or insulin in the medium (Pereira et al. 1995). Consequently, the enrichment of only the C1 carbon, but not the other carbons (C2–C6) of glycogen implies that the direct pathway is the preferred route of glucose utilization for glycogen synthesis in trout hepatocytes. The indirect pathway, if present, was very low and therefore not detectable in the present study.

Fig. 2A–D The relationship between initial glycogen content and Total GSase (**A**), GSase *a* (**B**), total GPase (**C**) and GPase *a* (**D**) activities in rainbow trout hepatocytes incubated either in the absence (*open circle*) or presence of insulin (*solid circle*). The *solid* (no insulin) and *dashed line* (with insulin) indicate the slope of the regression line, while *r* indicates the regression coefficient. There is a significant difference ($P < 0.05$) in the slopes of total GSase, total GPase and GPase *a*, but not in GSase *a* (hence slope or *r* not shown)

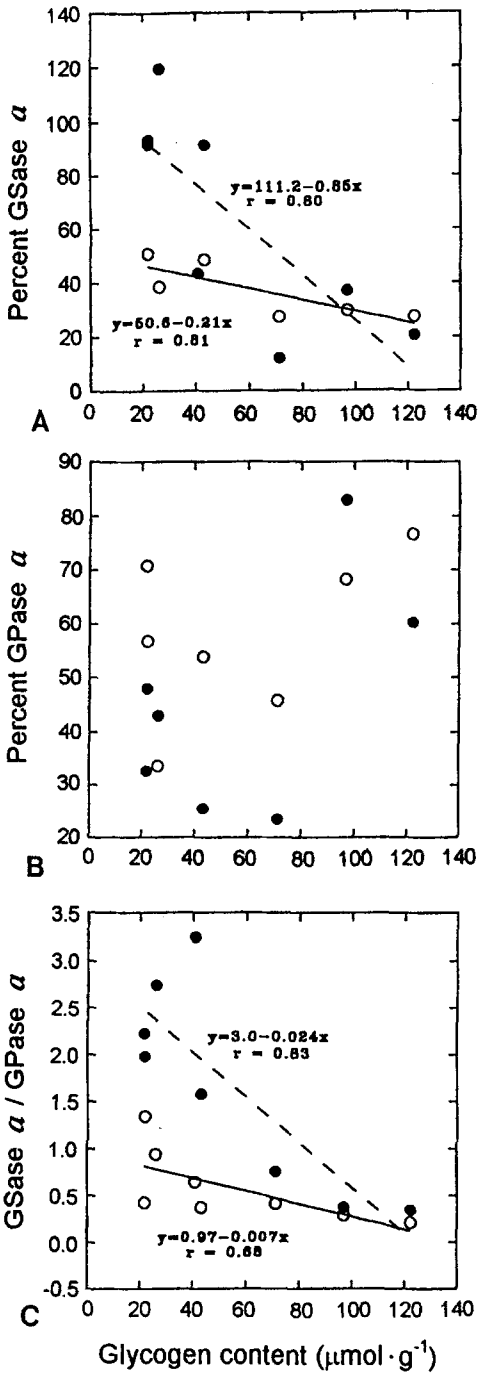


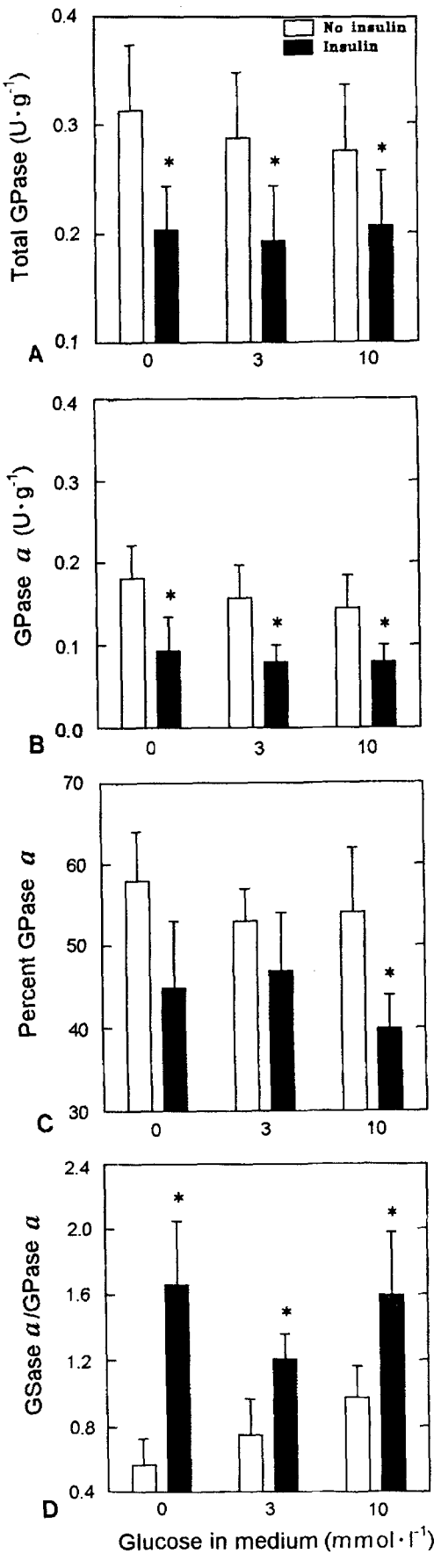
Fig. 3A–C The relationship between initial glycogen content and % GSase *a* (**A**) and % GPase *a* activities (**B**) and GSase *a* to GPase *a* ratio (**C**) in rainbow trout hepatocytes incubated either in the absence (*open circle*) or presence of insulin (*solid circle*). The *solid* (no insulin) and *dashed line* (with insulin) indicates the slope of the regression line, while *r* indicates the regression coefficient. There is a significant difference ($P < 0.05$) in the slope of % GSase *a* (both solid and dashed line) and GSase *a* to GPase *a* ratio (only dashed line), but not in % GPase *a* (hence slope or *r* not shown)

Liver glycogen is depleted in mammals (especially rats) after a 24-h fast, resulting in an active glycogen synthesis when substrates become available. In contrast, trout livers are not depleted of glycogen even after several months of food-deprivation (Pereira et al. 1995). Also, glycogen breakdown rate is very high *in vitro*, thereby masking any glycogen synthesis (Moon et al. 1985; Mommsen 1986). However, the ^{13}C NMR results (Fig. 1) indicate that, despite the high glycogen breakdown rate reported *in vitro*, the glycogen synthetic pathway is active concurrently in trout hepatocytes. Recent trout hepatocyte studies using ^{14}C glucose incubations also showed incorporation of radioactivity into glycogen (Pereira et al. 1995) supporting the above finding.

Initial glycogen content may exert an effect on glucose utilization as evidenced by the changes in GSase and GPase activities in trout hepatocytes (Fig. 2). A direct relationship between glycogen content and the activities of these enzymes (Fig. 2A, C) concur with those seen in mammals (Rognstad 1991) and amphibians (Scapin and Di Giuseppe 1994). Although total GSase increased with glycogen content, the activation of synthase (% GSase *a*) was inversely related (Fig. 3A) indicating that the de-phosphorylation of GSase *b* to GSase *a* occurs at low glycogen content in trout hepatocytes. This agrees with the findings in mammals showing an inverse relationship between glycogen content and GSase *a* activities (Hers 1976; Hems and Whittons 1980). GPase *a* showed a direct relationship to glycogen content (Fig. 2D) as in mammals (Hers 1976; Hems and Whitton 1980) and this dependence of GPase on glycogen content could explain the direct correlation observed between glycogen content and glycogen breakdown in salmonid hepatocytes *in vitro* (Mommsen 1986; Vijayan et al. 1994b). Obviously this correlation must be controlled *in vivo* to prevent significant fluctuations in liver glycogen. Several factors including metabolite levels, hormones and allosteric modulators may play a role in the control of glycogen metabolism (Ciudad et al. 1986; Nuttall and Gannon 1993).

Physiological concentrations of glucose did not modify GPase or GSase activities in rainbow trout hepatocytes. This finding is in marked contrast to mammalian systems where glucose greatly increases hepatic glycogen synthesis by promoting the dephosphorylation of GPase, releasing the inhibiting effect of GPase *a* on GSase and stimulating GSase *a* activity (Stalmans et al. 1970, 1971, 1974b). The absence of a glucose effect on GPase and GSase in trout hepatocytes suggests a block at the point of glucose entry into the cell. The maintenance of a high plasma glucose concentration after a glucose load has been attributed to the low hepatic hexokinase activity in trout (Cowey et al. 1977). The existence of a high K_m glucokinase in fish is controversial with most groups unable to demonstrate this enzyme (e.g., Cowey et al. 1977), although a recent study by Borrebaek et al. (1993) did report on adaptable hexokinase (glucokinase) in rapidly growing Atlantic salmon (*Salmo salar*).

Glucose carbon is incorporated into glycogen and oxidized in rainbow trout hepatocytes (Pereira et al.



1995), implying that glucose does enter the cell. It is likely, therefore, that the pathway for glucose entry is operating at maximum and does not respond to additional glucose, even in the presence of insulin. Low hepatocyte glycogen content (4 months of fasting) also did not modify the incorporation of glucose into glycogen in trout hepatocytes (Pereira et al. 1995), supporting the above argument. In rat hepatocytes several allosteric modulators including glucose 6-phosphate, nucleotides and cations have been shown to modify the activity of both GSase and GPase (Ciudad et al. 1986; Nuttall and Gannon 1993). It is not known if the absence of a glucose effect in trout hepatocytes is due to these allosteric effectors.

Plasma insulin titres have been shown to increase after a high carbohydrate meal or a glucose load in salmonids (Mommsen and Plisetskaya 1991). This rise in insulin may affect hepatocyte metabolism as insulin is a known anabolic hormone in fish (Mommsen and Plisetskaya 1991). Our results clearly show that insulin affects GPase activities in rainbow trout hepatocytes. A similar decrease in GPase activity with insulin treatment has been reported in American eel hepatocytes (Foster and Moon 1990). This decrease in GPase activity may be responsible for the lower glycogen breakdown with insulin observed *in vitro* in salmonid hepatocytes (Vijayan et al. 1993b). The mechanism of action of insulin in decreasing GPase activity in trout is not known. In rats the insulin effect on glycogen metabolism is mediated by several intracellular factors, including cAMP (Van de Werve and Jeanrenaud 1987). A decrease in cAMP levels due to insulin decreases the protein kinase activation of phosphorylase phosphatase kinase, resulting in decreased GPase activity and glycogen conservation (Van de Werve and Jeanrenaud 1987). The reduced levels of cAMP in eel hepatocytes treated with insulin suggests that a mechanism similar to that in mammals operate in fish (Foster and Moon 1990).

Our study is the first to examine the effect of insulin on GSase activities in isolated fish hepatocytes. The absence of an insulin effect of GSase activities in trout is contrary to findings in mammals. However, studies in mammals have shown that insulin stimulation of GSase activation is modulated by glucose concentrations (Witters and Avruch 1978; Nyfeler et al. 1981; Thomas et al. 1985), and several studies showed that it is glucose rather than insulin that induces GSase activity (Stalman et al. 1974a; Mulmed et al. 1979). Also, Van de Werve et al. (1977) reported that for GSase

Fig. 4A–D The effect of glucose and insulin on total GPase (**A**), GPase *a* (**B**), % GPase *a* activities (**C**) and in the ratio of GSase *a* to GPase *a* (**D**) in rainbow trout hepatocytes. Values are mean + SEM ($n = 7$–8). * Significantly different from the respective control values (no insulin) ($P < 0.05$, paired *t*-test)

activation to occur the % GPase *a* activity must be reduced to 10%. In our study, % GPase *a* seldom fell below 40% and this level may not have been sufficiently low to allow GSase activation. However, insulin does appear to have an effect on % GPase *a* and % GSase *a* activity at 10 mmol·l^{-1} glucose, suggesting that there is a threshold level (of glucose) rather than a dose response for insulin stimulation in trout hepatocytes.

The higher responsiveness to insulin observed in hepatocytes with lower glycogen content (Fig. 3A, C) indicates that the glycogen status of the cell may influence insulin stimulation for energy re-partitioning. This effect may be mediated through changes in enzyme activities (Fig. 4) and/or by altered hepatocyte responsiveness to insulin stimulation (Vijayan et al. 1993b). The insulin effect on hepatocyte glycogen metabolism appears to be modulated by several factors, including season (Mommsen et al. 1987; Foster and Moon 1987, 1989), nutritional status (Vijayan et al. 1993a), stress (Vijayan et al. 1994a) and glycogen content (Foster and Moon 1989; present study).

In conclusion, our study shows that the direct pathway of glucose utilization for glycogen repletion is active in trout hepatocytes. Both GSase and GPase activities co-exist in the active form, and the control of direction of flux is determined by the ratio of these two enzymes. Initial glycogen content exerts an effect on the enzyme system, while glucose has no effect on either GSase or GPase activities in trout hepatocytes. Insulin plays a role in the regulation of glycogen metabolism in rainbow trout hepatocytes. This effect of insulin may be modulated by both hepatocyte glycogen and plasma glucose concentrations.

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