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Lack of significant long-term effect of dietary carbohydrates on hepatic glucose-6-phosphatase expression in rainbow trout (*Oncorhynchus mykiss*)

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Hepatic glucose-6-phosphatase (G6Pase) plays an important role in glucose metabolism because it catalyzes the release of glucose to the circulatory system in the processes of glycogenolysis and gluconeogenesis. The present study was initiated to analyze the regulation of hepatic G6Pase expression by dietary carbohydrates in rainbow trout. The first step in our study was the identification of a partial G6Pase cDNA in rainbow trout that was highly homologous to that of mammals. Hepatic G6Pase activities and mRNA levels were measured in trout fed one of the experimental diets, with or without carbohydrates. We found no significant effect of intake of dietary carbohydrates on G6Pase expression (mRNA and activity) 6 hours and 24 hours after feeding. These results suggest that there is no control of G6Pase synthesis by dietary carbohydrates in rainbow trout and that the lack of regulation of gluconeogenesis by dietary carbohydrates could at least partially explain the postprandial hyperglycemia and the low dietary glucose utilization observed in this species. (J. Nutr. Biochem. 11:22–29, 2000)

Keywords:glucose-6-phosphatase expression; fish nutrition; rainbow trout; dietary carbohydrates

Introduction

Glucose plays a key role in mammalian energetics but its importance as a metabolic fuel in fish is not fully understood.[1] Even though most of the enzymes involved in glucose metabolism have been detected in fish, the regulation of carbohydrate metabolism differs from that of mammals[2] in several ways: (1) glucose contributes minimally to the oxidative fuel demands of locomotory muscle in rainbow trout (*Oncorhynchus mykiss*) and the glucose turnover rates are below the levels detected in mammals[3,4]; (2) amino acids seem to be more potent stimulators of insulin secretion than glucose[5] (under in vitro conditions or when administered at supraphysiologic levels); (3) glucose appears to be a poor substrate for glycogen synthesis in isolated trout hepatocytes[6]; (4) there is no apparent effect of glucose on hepatocyte gluconeogenesis[7]; and (5) the low capacity of glucose utilization as an energy source in fish muscle is suspected to be due to low levels of insulin receptors,[8] possible lack of glucose transporters,[9] or supposed low levels of hexokinase enzymes.[10] All of these characteristics can explain why oral administration of glucose and high levels of dietary carbohydrates cause persistent postprandial hyperglycemia[11,12] associated with low dietary glucose utilization in rainbow trout.[10] Our objective is to achieve an understanding of the nutritional regulation of the glucose metabolism in fish because improvement of dietary carbohydrate utilization is an important challenge in aquaculture.[10]

Liver is known to play a central role in glucose homeostasis by extracting the absorbed dietary glucose and by reducing the release of endogenous glucose (gluconeogenesis).[13] Salmonids are carnivorous and rely on hepatic gluconeogenesis for glucose production.[14] Thus, we hypothesized that the lack of such a regulation of endogenous hepatic glucose production by dietary carbohydrate can partially explain the postprandial hyperglycemia observed in salmonids as suggested previously.[7] In the whole animal, this would mean that newly synthesized glucose can be added to the one resulting from absorption and already in

The Genbank accession number for the rainbow trout G6Pase sequence is AF120150.

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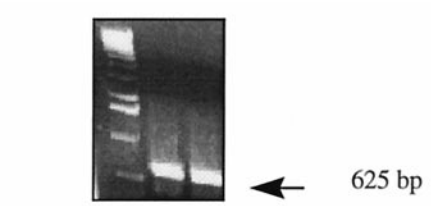
Table 1 Formulation and chemical composition of the experimental diets for rainbow trout

Diets	20% (+) Carbohydrates	(−) Carbohydrate	8% Carbohydrates	12% Carbohydrates
Dry matter (DM; %)	85.5	92.7	83.6	84.3
Crude protein (%DM)	39.5	54.8	43.1	42.2
Crude lipid (%DM)	16.6	18.3	18.0	17.8
Digestible starch (%DM)	20.4	<0.2	8.0	11.7
Gross energy (kJ/g DM)	22.1	23.1	22.1	22.3

circulation, resulting in hyperglycemia. To our knowledge, the nutritional regulation of enzymes involved in hepatic glucose production in fish is poorly documented, especially at a molecular level.

One key step in hepatic glucose production is catalyzed by the microsomal glucose-6-phosphatase (G6Pase) system because this enzyme catalyzes the hydrolysis of glucose-6-phosphate (coming from gluconeogenesis and glycogenolysis) in glucose.[15] There is now convincing molecular evidence in favor of the substrate model of G6Pase in



A

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     S   G   H   A   M   G   S   S   G   V   W   Y   V   M   I   T   A    17
CT TCA GGT CAT GCC ATG GGC TCG TCT GGG GTG TGG TAC GTG ATG ATA ACA GCT    53

 V   F   S   V   A   T   E   R   R   F   P   P   L   L   Y   R   F   L    35
GTC TTC TCA GTG GCG ACA GAA AGG CGG TTC CCC CCT CTC CTG TAC AGG TTC TTG   107

 Q   V   G   L   W   M   L   L   C   T   V   E   L   L   V   C   M   S    53
CAG GTG GGG CTC TGG ATG CTG CTG TGT ACA GTA GAG CTG TTG GTG TGC ATG TCC   161

 R   V   Y   M   A   A   H   F   P   H   Q   V   I   S   G   V   I   T    71
AGA GTC TAC ATG GCT GCC CAC TTC CCA CAC CAG GTC ATC AGT GGG GTC ATC ACA   215

 G   I   M   V   A   E   A   F   S   R   V   Q   W   I   Y   G   A   S    89
GGT ATC ATG GTG GCT GAG GCC TTC TCC AGA GTG CAG TGG ATC TAT GGA GCC AGT   269

 L   K   K   Y   F   Y   T   T   L   F   L   L   S   F   A   V   G   F   107
CTG AAG AAG TAC TTC TAC ACC ACC CTC TTT CTG CTC TCC TTT GCT GTG GGC TTC   323

 Y   E   L   L   K   A   I   G   V   D   L   L   W   S   L   E   K   A   125
TAC GAG CTA CTG AAA GCT ATA GGC GTG GAC CTG CTG TGG TCC CTG GAG AAA GCC   377

 Q   K   W   C   V   R   A   E   W   V   Y   M   D   S   T   P   F   A   143
CAG AAG TGG TGT GTG AGA GCC GAG TGG GTC TAC ATG GAC TCC ACT CCT TTC GCC   431

 I   L   L   R   N   M   G   T   L   F   G   L   G   L   G   L   H   S   161
ATC CTC CTG CGC AAC ATG GGC ACC CTG TTT GGC CTG GGC CTG GGC CTG CAC TCA   485

 P   L   Y   T   E   N   K   N   S   S   I   P   F   R   V   G   C   I   179
CCC CTC TAC ACC GAG AAC AAG AAC AGC AGC ATC CCC TTC AGG GTG GGG TGT ATC   539

 T   V   S   L   L   L   L   Q   I   L   D   G   L   T   F   S   S   R   197
ACT GTC TCT TTA TTG TTG CTA CAG ATT TTG GAT GGC TTG ACG TTC TCC TCG AGA   593

 D   Q   A   M   F   Y   T   L   S   F                                   207
GAC CAG GCA ATG TTC TAC ACC CTT TCC TTT GG                              625
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B

Figure 1 Partial cloning of the glucose-6-phosphatase (G6Pase) gene in rainbow trout. (*A*) Reverse transcriptase-polymerase chain reaction on hepatic total RNA using degenerated primers chosen in the conserved region of known G6Pase. M, molecular weight marker X (Boerhinger, Roche Molecular Biochemicals, Germany). (*B*) Nucleotide and deduced amino acid sequences of the rainbow trout G6Pase clone. Underlined letters correspond to the primer sequences.

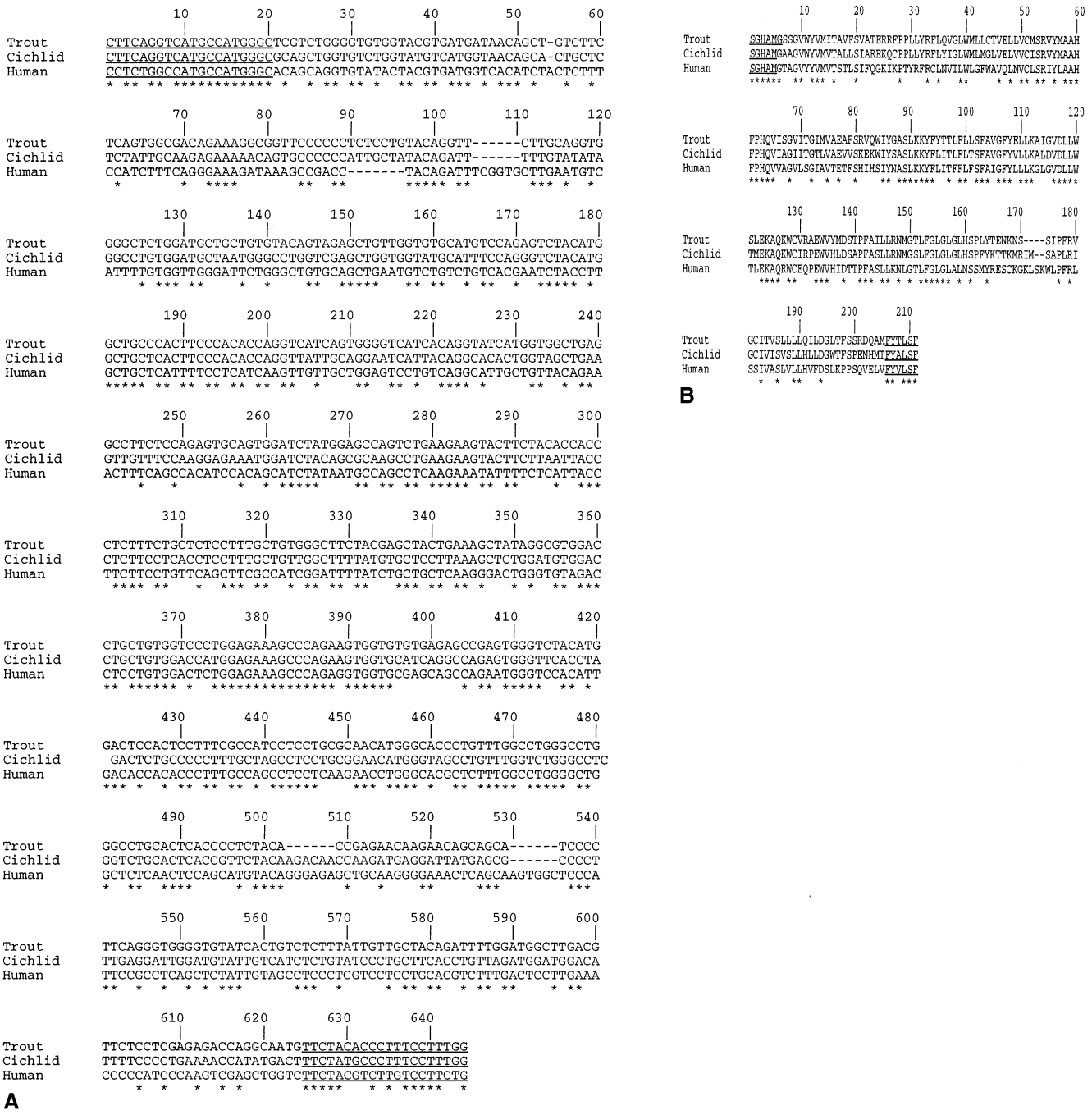

Figure 2 Alignments of the partial glucose-6-phosphatase cDNA clone of the rainbow trout with human; (Genbank accession number UO1120) and cichlid fish (*Haplochromis nubilus* Genbank accession number AF008945). Underlined letters correspond to the primer sequences. (*A*) Alignment of nucleotide sequences. (*B*) Alignment of amino acid sequences.

mammals; that is, a protein complex involving a phosphohydrolase [catalytic subunit protein, G6Pase (E.C.3.1.3.9), the object of the present study] with its active site oriented toward the lumen of the endoplasmic reticulum, and transporter proteins for glucose-6-phosphate (T1), phosphate (T2), and glucose (T3).[16] The expression of G6Pase in the liver is thought to play an important role in glucose homeostasis,[15] as illustrated by an increased G6Pase activity and the corresponding mRNA levels in type II noninsulin-dependent diabetes mellitus.[17–20] Regulation of G6Pase mRNA abundance is a major control of G6Pase activity: It has long been recognized that hepatic G6Pase synthesis is markedly regulated by changes in hormones (G6Pase gene transcription is stimulated by glucagon and glucocorticoids and inhibited by insulin) and nutritional status (starvation-refeeding).[21–26] In fish, little is known about the regulation of this enzyme by dietary factors. In some nonsalmonids, such as the "omnivorous" common carp *Cyprinus carpio*,

Table 2 G6Pase activities in livers of rainbow trout fed with 20% of carbohydrates or without carbohydrates

Diets	G6Pase activities (mU/mg protein)	
	6 hours	24 hours
+ Carbohydrates*	22 ± 6	20 ± 1
− Carbohydrate	15 ± 5	18 ± 3

Data (means ± SD) are from two pools of microsomes (1 pool = 3 fish).
*20% of digestible starch.
G6Pase–glucose-6-phosphatase.

the existence of G6Pase activity in liver and kidney has been clearly shown,[27–31] but the regulation of its expression remains unclear.

In light of the mammalian data and to understand the mechanisms of the low dietary glucose utilization by fish in general and by salmonids in particular, the objective of the present paper was to analyze the nutritional regulation of G6Pase expression (mRNA and activity) in livers of rainbow trout. The first step of our study was to characterize the G6Pase at a molecular level in rainbow trout. We then attempted to analyze the control of G6Pase expression (mRNA and activity) by dietary levels of carbohydrates.

Methods and materials

Fish and diets

Triplicate groups of juvenile immature rainbow trout were reared in our experimental fish farm at 18°C with the respective diets for 10 weeks during spring under natural photoperiod. Twice a day fish were fed one of the experimental diets containing 8%, 12%, or 20% digestible starch (supplied as dehulled extruded peas or extruded wheat) or without starch to near satiation (*Table 1*). At the end of 10 weeks, after a 24-hour fast, fish (weighing approximately 150 g) were fed once, and 9 fish from each group were sacrificed 6 and 24 hours after the meal. Whole liver, kidney, heart, intestine, brain, and a small piece of dorsal muscle tissue were sampled clamp frozen in liquid nitrogen and stored at −80°C.

RNA isolation and reverse transcription

Total RNA was extracted from rainbow trout tissues as described by Chomczinski and Sacchi.[32] cDNA was obtained by annealing 2 μg of total RNA with 1 μg of random primers and incubating with AMV reverse transcriptase (Boehringer, Roche Molecular Biochemicals, Germany) for 1 hour at 42°C.

Cloning of partial G6Pase cDNA

G6Pase sequences from human (Genbank accession number UO1120), rat (Genbank accession number RNU07993), and cichlid fish (*Haplochromis nubilus*) (Genbank accession number AF008945) were compared using the Clustal-W miltiple alignment algorithm.[33] Primers were chosen that corresponded to the most conserved coding regions of G6Pase at positions 426-445 and 1043-1062 of the human gene. The sequence of the upstream primer was 5′-CTTCAGGTCATGCCATGGGC-3′ and that of the downstream degenerate primer was 5′-CMRAARGAMAR GRYRTAGAA-3′, where Y = C/T, M = A/T, and R = A/G. cDNA (1 μL) was amplified by polymerase chain reaction (PCR) using 100 pmol of the degenerate primers in a reaction mixture containing 2 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl, 0.25 mM dNTP, and 2.5 U Taq polymerase (Boehringer). Thirty-five cycles of denaturation at 94°C, for 1 minute, annealing at 50°C for 40 seconds, and extension at 72°C for 1 minute were performed. PCR products were subjected to electrophoresis in 1% agarose gels and fragments of the expected size range were purified (Micropure System, Amicon, Bedford, MA USA). The purified DNA fragments were inserted into the pCR II plasmid and used for transformation of One Shot competent cells (Invitrogen, Carlsbad, CA USA). Inserts were detected by *Eco*RI digestion of the extracted plasmid DNA. Clones with inserts were sequenced (Sequenase-2 sequencing kit, Amersham, Buckinghamshire, England).

Sequence analysis

Nucleotide sequences (excluding the primer sequences) were compared with DNA sequences from the Genbank database with the basic local alignment search tool (BLAST) algorithm.[34] Sequence alignments and percentage of amino acid conservation were assessed with the Clustal-W multiple alignment algorithm[31] using the cloned fish sequence and other G6Pase sequences corresponding to the amplified regions from databases.

Northern analysis

Twenty micrograms of extracted total RNA samples were electrophoresed in 1% agarose gels containing 5% formaldehyde and capillary was transferred onto nylon membrane (Hybond-N^+, Amersham). Membranes were hybridized with [^{32}P]-DNA probes labeled by random priming (Stratagene, La Jolla, CA USA) that recognized rainbow trout G6Pase cDNA. Membranes were also hybridized with a common carp 16 S ribosomal RNA probe (the 3021-3100 bp fragment, Genbank accession number MICCCG) to confirm equivalency of loading and specificity of response. After stringent washing, the membranes were exposed to X-ray film and the resulting images were quantitated using Visio-Mic II software (Genomic, Grenoble, France).

PCR analysis

cDNAs were amplified by PCR using specific primers chosen in the partial rainbow trout G6Pase cDNA sequence: 5′-TCAGTG GCGACAGAAAGGCG-3′ and 5′-CAGCAGGTCCAGGCCTA TAG-3′. The PCR reaction was carried out in a final volume of 25 μl containing 1.5 mM $MgCl_2$, 4 pmol of each primer, 2 μl cDNA, and 1 U Taq polymerase (Boehringer). The number of cycles was 35 and includes 20 seconds for hybridization (at 59°C), 20 seconds for elongation (at 72°C), and 20 seconds for denaturation (at 94°C). The PCR products were characterized by hybridization with the labeled [^{32}P]-DNA rainbow trout G6Pase probe.

Enzyme assays

Microsomes were obtained from rainbow trout livers as described by Mol et al.[35] The final preparation, which was stored at −80°C, averaged 3 to 6 mg protein/ml and was used in the spectrophotometric assays. Microsomes were suspended in the buffer (NaH_2PO_4 100 mM, Na_2HPO_4 25 mM, EDTA 2 mM, DTT 1 mM, pH 7) without further treatment. The standard procedure followed was as described by Alegre et al.,[36] monitoring the increase in absorbance (NADH production) using glucose dehydrogenase (Sigma Chemical Co., St. Louis, MO USA) in excess as coupling enzyme. One unit of G6Pase activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of glucose-6-phosphate per minute under the specified conditions (30°C). The latency (percentage of the activity of fully disrupted microsomes that is not expressed in microsomes not treated with detergents)

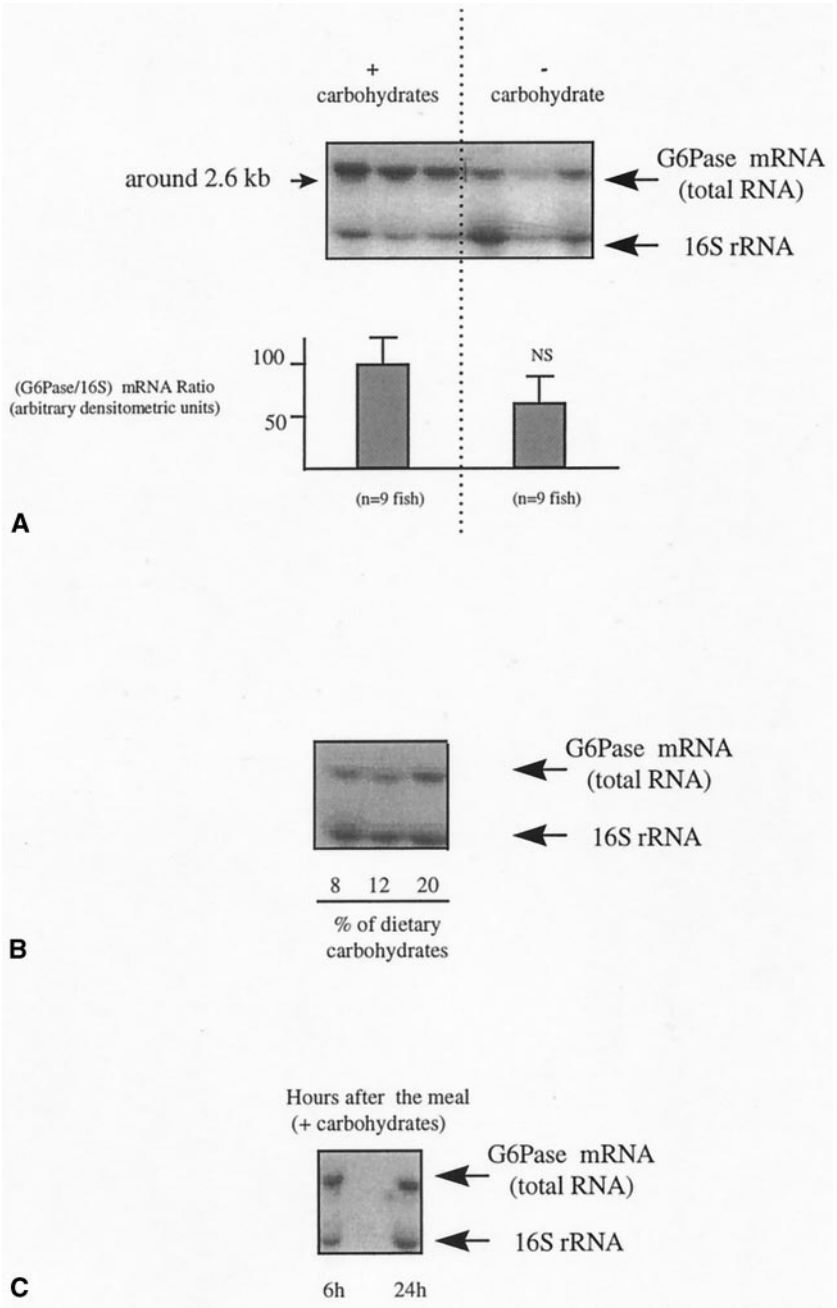

Figure 3 Glucose-6-phosphatase (G6Pase) gene expression in livers of fish. (*A*) Fish fed with 20% of carbohydrates (+) or without (−) carbohydrates (Northern blotting) 6 hours after feeding. Each band is from a different fish. The 16S rRNA served as an internal control of sample loading. An analysis by densitometry of G6Pase mRNA levels for 9 fish from each treatment group weighed by 16S rRNA values was performed (Visio-Mic II software). NS–nonsignificant (Student *t*-test). (*B*) Fish fed with two intermediary levels of dietary carbohydrates (8%, 12%) 6 hours after feeding. (*C*) Fish fed with carbohydrates 6 hours and 24 hours after feeding.

was 33% in rat microsomes[36] and can be estimated to be relatively similar in trout microsomes.

Data analysis

Results are expressed as means ± SD. Statistical analysis between two series of data was determined using an unpaired two-tailed Student's *t*-test (Statview software, SAS Inst. Inc., Cary, NC). Differences were considered significant with a *P*-value of less than 0.05.

Results

The available G6Pase cDNA sequences were aligned and highly conserved regions from cichlid fish (*Haplochromis nubilus*; Genbank accession number AF008945) to humans (Genbank accession number UO1120) were identified (data not shown). A set of primers (the reverse primer was degenerated) was designed and made it possible to amplify a fragment of approximately 630 bp. Reverse transcriptase (RT)-PCR was performed on hepatic total RNA extracted from fish fed without carbohydrates. PCR conditions were optimized and a major amplification product of the expected size was obtained (*Figure 1A*). The fragments were purified, cloned, and sequenced. The cDNA sequence of 625 bp was very similar to those of genes from other G6Pase genes (BLAST algorithm, $P = 10^{-77}$–10^{-33}). The corresponding amino acid sequences were deduced from the cDNA sequences showing an open reading frame of 207 codons (*Figure 1B*) that were highly homologous to G6Pase

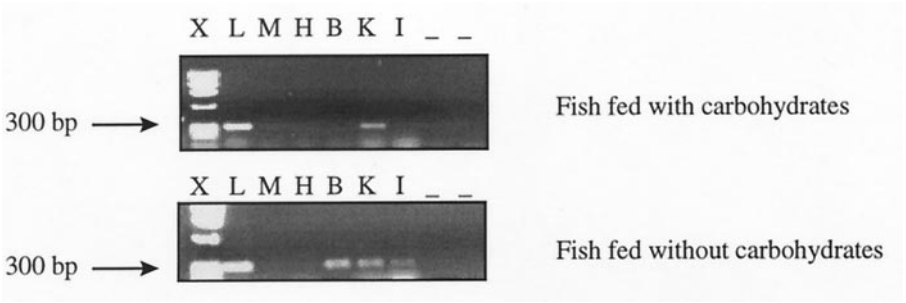

Figure 4 Tissue specificity of glucose-6-phosphatase (G6Pase) gene expression in rainbow trout fed with 20% of carbohydrates or without carbohydrates (6 hours after feeding). Analysis by reverse transcriptase-polymerase chain reaction RT-PCR; (n = 2 fish per treatment). X, molecular weight marker phiX174 DNA/HaeIII (Promega, Madison, WI USA); L, liver; M, muscle; K, Kidney; I, intestine; H, heart; B, Brain; -, negative controls (RT-PCR reactions performed without RNA and without RT). The exact length of the G6Pase fragment (300 bp) is determined following the known G6Pase gene sequence.

(BLAST algorithm, $P = 10^{-63}$–10^{-43}; *Figure 2*). The highest level of homology (73% for the nucleotide sequence and 67% for the deduced amino acid sequence) was observed with the G6Pase sequence of the cichlid fish.

Growth rates of rainbow trout fed with (20%) or without carbohydrates were comparable: Daily growth coefficients were 3.31 ± 0.09 and 3.36 ± 0.06, respectively. Because all fish were fed nutritionally adequate diets, it was possible to perform a comparative analysis on the effect of dietary carbohydrates on the regulation of G6Pase expression between fish groups fed different carbohydrate levels. Six hours after feeding, glycemia of rainbow trout fed with (20%) and without carbohydrates were significantly ($P < 0.01$), different: 10.5 ± 2.5 mM and 4.3 ± 0.6 mM, respectively. Hepatic G6Pase activities measured in microsomal samples were similar (*Table 2*) between fish groups fed with and without carbohydrates 6 hours and 24 hours after feeding.

G6Pase gene expression was analyzed in fish livers by Northern blotting (*Figure 3*). A unique G6Pase mRNA of approximately 2.6 kb was found. We found a high and constant G6Pase gene expression in the livers of fish fed with and without carbohydrates, indicating that hepatic G6Pase gene expression was not dependent on the presence of dietary carbohydrates (*Figure 3A*). G6Pase gene expression did not depend on the levels of dietary carbohydrates (8–20% of digestible starch; *Figure 3B*) and the time interval after feed intake (6 *versus* 24 hours; *Figure 3C*). By using RT-PCR performed with specific rainbow trout G6Pase primers, G6Pase mRNA could be detected in liver and kidney (the gluconeogenic tissues) but not in muscle and heart, irrespective of the composition of the diet (*Figure 4*). In contrast, G6Pase gene expression was observed in the intestine and brain only in fish fed without carbohydrates (*Figure 4*).

Discussion

G6Pase belongs to a family of enzymes such as L-type pyruvate kinase (E.C.2.7.1.40), phosphoenolpyruvate carboxykinase (E.C.4.1.1.32), and fatty acid synthetase (E.C.2.3.1.85) whose expression has been shown to be regulated by dietary carbohydrates in mammals.[37] In fish, the data in this area is scarce. Although formal proof awaits the cloning of the full-length cDNA sequence, the high similarity (up to 73%) between the cDNA sequence of G6Pase in rainbow trout and the G6Pase sequences previously characterized in other vertebrates strongly suggest that this sequence corresponds to a functional enzyme.

As in mammals, the main gluconeogenic tissues (liver and kidney) in rainbow trout express a higher level of G6Pase mRNAs than other tissues.[15] G6Pase gene expression in the intestine of fish fed a diet lacking carbohydrates is comparable to that found in mammals.[16] G6Pase expression in the brain of rainbow trout is more intriguing[15] and requires further studies to be confirmed. Our data are also in accordance with the observation of Shimeno and Ikeda,[28] who showed an absence of G6Pase activities in the muscle and heart and a high level of G6Pase activity in the liver.

The low hepatic G6Pase activity observed in this study (approximately 20 mU/mg protein) compared with mammalian data (approximately 400 mU/mg protein)[36] can be explained by the low glucose turnover rates in fish.[3,4] The relatively low need for glucose as an energy source, which necessitates a lower level of endogenous glucose production in fish than in mammals, has been previously observed.[3,4,10] A comparison with published data on carp[29–31] is rather difficult to make because the methods of measurement of G6Pase activity were quite different.

With regard to the regulation of G6Pase by dietary factors in rainbow trout, our data show that G6Pase expression (mRNA and activity) is neither affected by the dietary carbohydrate levels tested here nor apparently modified between 6 and 24 hours after feeding. This is in contrast with our own data (from the same samples) on glucokinase (E.C.2.7.1.1) expression in rainbow trout livers: Glucokinase, which catalyzes the phosphorylation of glucose to glucose-6-phosphate, is significantly induced by dietary carbohydrates[38] (Panserat et al., unpublished studies). Studies of nutritional regulation of G6Pase activity in common carp have shown dietary supplementation with starch, glucose, or fructose seems to depress the activities of G6Pase (and the other gluconeogenic enzymes) in common carp,[29–31] as in mammals.[15,39] Thus, there is an apparent absence of nutritional control of G6Pase activity and synthesis in rainbow trout in contrast to common carp. Further studies using ex vivo analysis on hepatocytes and checking the existence of putative allosteric controls (not tested in this study) involved in G6Pase expression in fish are needed to definitive conclusions can be drawn regarding the absence of nutritional control of G6Pase synthesis. In mammals, some products of lipid metabolism such as long chain fatty acyl coenzyme A potentially inhibit G6Pase[15,40,41] without modifying G6Pase production. Knowledge of po-

tential endocrine control (insulin and/or glucagon) on G6Pase is also lacking in fish. To understand the possible physiologic significance of the absence of nutritional regulation of G6Pase expression in rainbow trout, comparative studies on molecular regulation of G6Pase gene expression in species such as common carp are required.

The absence of regulation of G6Pase enzyme in salmonids such as rainbow trout is probably not surprising given that salmonids are carnivorous and that glucose is mainly produced through gluconeogenesis even in the fed state because of the low glucose availability from their natural prey.[14] Glucose, which is in constant demand, is provided mainly, if not exclusively, by the liver in rainbow trout.[14] This means that the need for a rapidly responding system to transient periods of high plasma glucose is reduced, because they do not occur naturally. Therefore, it is apparent that trout liver is not adapted to respond with a rapid change in gluconeogenic rates when challenged with unusually high glycemic conditions. Supporting data are provided by studies on trout hepatocytes that show a weak regulation of glycogen metabolism in response to glucose and insulin.[6] However, control of hepatic glucose production can be realized by metabolic reactions other than the one catalyzed by G6Pase[13]: Gluconeogenesis seems to be affected by the nutritional status with an increase in the activities of the gluconeogenic enzymes [fructose 1,6-biphosphatase (E.C.3.1.3.11) and phosphoenolpyruvate carboxykinase] in fish fed a low carbohydrate diet compared with fish fed a carbohydrate rich diet.[42]

In conclusion, our data strongly suggest a lack of control of hepatic G6Pase expression by dietary carbohydrates in rainbow trout. In addition to possible abnormal hormonal (insulin/glucagon ratio) response to dietary carbohydrates[5] and tissue resistance to insulin,[8–10] poor carbohydrate utilization by rainbow trout also may be linked to a poor regulation of hepatic gluconeogenesis. Interspecific comparative studies are warranted to fully elucidate differences in dietary carbohydrate utilization between species.

Acknowledgments

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References

1 Moon, T.W. and Foster, G.D. (1995). Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. In *Biochemistry and Molecular Biology of Fishes* (Hochachka, P.W. and Mommsen, T.P., eds.), pp. 65–100, Elsevier, New York, NY, USA

2 Cowey, C.B. and Walton, M.J. (1989). Intermediary metabolism In *Fish Nutrition* (E. Halver, ed.), pp. 259–329. Academic Press, New York, NY, USA

3 West, T.G., Arthur, P.G., Suarez, R.K., Doll, C.J., and Hochachka, P.W. (1993). In vivo utilization of glucose by heart and locomotory muscles of exercising rainbow trout (*Onchorhynchus mykiss*). *J. Exp. Biol.* **177,** 63–79

4 Haman, F., and Weber, J.M. (1997). Continuous tracer infusion to measure in vivo metabolite turnover rates in trout. *J. Exp. Biol.* **199,** 1157–1162

5 Mommsen, T.P., and Plisetkaya, E.M. (1991). Fish insulin: History, structure and metabolic regulation. *Rev. Aquat. Sci.* **4,** 225–259

6 Pereira, C., Vijayan, M.M., Storey, K.B., Jones, R.A., and Moon, T.W. (1995). Role of glucose and insulin in regulating glycogen synthase and phosphorylase activities in rainbow trout hepatocytes. *J. Comp. Physiol. [B]* **165,** 62–70

7 Pereira, C., Vijayan, M.M., and Moon, T.W. (1995). In vitro hepatocyte metabolism of alanine and glucose and the response to insulin in fed and fasted rainbow trout. *J. Exp. Zool.* **271,** 425–431

8 Parrizas, M., Planas, J., Plisetskaya, E.M., and Gutierrez, J. (1994). Insulin binding and receptor tyrosine kinase activity in skeletal muscle of carnivorous and omnivorous fish. *Am. J. Physiol.* **266,** R1944–R1950

9 Wright, J.R., O'Hali, W., Yuang, H., Han, X., and Bonen, A. (1998). Glut-4 deficiency and severe peripheral resistance to insulin in the teleost fish tilapia. *Gen. Comp. Endocrinol.* **111,** 20–27

10 Wilson, R.P. (1994). Utilisation of dietary carbohydrate by fish. *Aquaculture* **124,** 67–80

11 Palmer, T.N., and Ryman, B.E. (1972). Studies on glucose intolerance in fish. *J. Fish Biol.* **4,** 311–319

12 Bergot, F. (1979). Effects of dietary carbohydrates and of their mode of distribution on glycaemia in rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **64A,** 543–547

13 Pilkis, S.J., and Granner D.K. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis of glycolysis. *Annu. Rev. Physiol.* **54,** 885–909

14 Suarez, R.K., and Mommsen, T.P. (1987). Gluconeogenesis in teleost fishes. *Can. J. Zool.* **65,** 1869–1882

15 Mithieux, G. (1997). New knowledge regarding glucose-6-phosphatase gene and protein and their roles in the regulation of glucose metabolism. *Eur. J. Endocrinol.* **136,** 137–145

16 Foster, J.D., Pederson, B.A., and Nordlie, R.C. (1997). Glucose-6-phosphatase structure, regulation and function: An update. *Proc. Soc. Exp. Biol. Med.* **215,** 314–332

17 Barzilai, N., and Rossetti, L. (1993). Role of glucokinase and glucose-6-phosphatase in the acute and chronic regulation of hepatic glucose fluxes by insulin. *J. Biol. Chem.* **268,** 25019–25025

18 Liu, Z., Barret, E.J., Dalkin, A.C., Zwart, A.D., and Chou, J.Y. (1994). Effect of acute diabetes on rat hepatic glucose-6-phosphatase activity in and its messenger RNA level. *Biochem. Biophys. Res. Commun.* **205,** 680–686

19 Trinh, K.Y., O'Doherty, R.M., Anderson, P., Lange, A.J., and Newgard, C.B. (1998). Perturbation of fuel homeostasis caused by overexpression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. *J. Biol. Chem.* **273(47),** 31615–31620

20 Mevorach, M., Giacca, A., Aharon, A., Hawkins, M., Shamoon, H., and Rosetti, L. (1998). Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. *J. Clin. Invest.* **102(4),** 744–753

21 Lange, A.J., Argaud, D., El-Magrabi, M.R., Pan, W., Maitra, S.R., and Pilkis, S.J. (1994). Isolation of a cDNA for the catalytic subunit of rat liver glucose-6-phosphatase: Regulation of gene expression in FAO hepatoma cells by insulin, dexamethasone and cAMP. *Biochem. Biophys. Res. Commun.* **201,** 302–309

22 Argaud, D., Zhang, Q., Pan, W., Maitra, S., Pilkis, S.J., and Lange, A.J. (1996). Regulation of rat liver glucose-6-phosphatase gene expression in different nutritional and hormonal states. *Diabetes* **45,** 1563–1571

23 Streeper, R.S., Svitek, C.A., Chapman, S., Greenbaum, L.E., Taub, R., and O'Brien, R.M. (1997). A multicomponent insulin response sequence mediates a strong repression of mouse glucose-6-phosphatase gene transcription by insulin. *J. Biol. Chem.* **272(18),** 11698–11701

24 Streeper, R.S., Eaton, E.M., Ebert, D.H., Chapman, S., Svitek, C.A., and O'Brien, R.M. (1998). Hepatocyte nuclear factor-1 acts as an accessory factor to enhance the inhibitory action of insulin on mouse glucose-6-phosphatase gene transcription. *Proc. Natl. Acad. Sci. USA* **95(16),** 9208–9213

25 Lin, B., Morris, D.W., and Chou, J.Y. (1997). The role of $HNF1_{\alpha}$, $HNF3_{\gamma}$ and cyclic AMP in glucose-6-phosphatase gene activation. *Biochemistry* **36,** 14096–14106

26 Dickens, M., Svitek, C.A., Culbert, A.A., O'Brien, R.M., and

Tavaré, J.M. (1998). Central role for phosphatidylinositide 3-kinase in the repression of glucose-6-phosphatase gene transcription by insulin. *J. Biol. Chem.* **273(32),** 20144–20149
27 Ikeda, S., and Shimeno, S. (1967). Studies on glucose-6-phosphatase of aquatic animals. I. Properties of hepatic glucose-6-phosphatase of fishes. *Bull. Jap. Soc. Sci. Fish.* **33(2),** 104–111
28 Shimeno, S. and Ikeda, S. (1967). Studies on glucose-6-phosphatase of aquatic animals. II. The enzyme's activities in fish tissues. *Bull. Jap. Soc. Sci. Fish.* **33(2),** 112–116
29 Furuichi, M. and Yone, Y. (1982). Changes in activities of hepatic enzymes related to carbohydrate metabolism of fishes in glucose and insulin-glucose tolerance tests. *Bull. Jap. Soc. Sci. Fish* **48,** 463–466
30 Shikata, T., Iwagana, S., and Shimeno, S. (1994). Effects of dietary glucose, fructose and galactose on hepatopancreatic enzyme activities and body composition in carp. *Fisheries Sci.* **60(5),** 613–617
31 Shimeno, S., Kheyyali, D., and Shikata, T. (1995). Metabolic response to dietary carbohydrate to protein ratios in carp. *Fisheries Sci.* **61(2),** 277–281
32 Chomczinski, P. and Sacchi, M. (1987). Single step method of RNA isolation by acid guanidium thiocyanate phenol chloroform extraction. *Anal. Biochem.* **162,** 156–159
33 Higgins, D. and Sharp, P. (1989). Fast and sensitive multiple sequence alignments on a microcomputer. *C.A.B.I.O.S.* **5,** 151–153
34 Atslush, S., Gish, W., Miller, W., Myers, E., and Lipman, S. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215,** 403–410
35 Mol, K.A., Van Der Geyten, S., Burel, C., Kuhn, E.R., Boujard, T., and Darras, V.M. (1998). Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five teleostean fishes. *Fish Physiol. Biochem.* **18,** 253–266
36 Alegre, M., Ciudad, C.J., Fillat, C., and Guinovart, J.J. (1988). Determination of glucose-6-phosphatase activity using the glucose dehydrogenase-coupled reaction. *Anal. Biochem.* **173,** 185–189
37 Rencurel, F., and Girard, J. (1998). Regulation of liver gene expression by glucose. *Proc. Nutr. Soc.* **57,** 265–275
38 Blin, C., Panserat, S., Médale, F., Gomes, E., Brèque, J., Kaushik, S, and Krishnamoorthy, R. (1999). Teleost liver hexokinase- and glucokinase-like enzymes: Partial cDNA cloning and phylogenetic studies in rainbow trout (*Onchorynchus mykiss*), common carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*). *Fish Physiol. Biochem.* **21(2),** 93–102
39 Newgard, C.B., Foster, D.W., and McGarry, J.D. (1984). Evidence for suppression of hepatic glucose-6-phosphatase with carbohydrate feeding. *Diabetes* **33(2),** 192–195
40 Mithieux, G., and Zitoun, C. (1996). Mechanisms by which fatty acyl-CoA esters inhibit or activate glucose-6-phosphatase in intact and detergent-treated rat liver microsomes. *Eur. J. Biochem.* **235,** 799–803
41 Mithieux, G., Daniele, N., Payrastre, B., and Zitoun, C. (1998). Liver microsomal glucose-6-phosphatase is competitively inhibited by the lipid products of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **273,** 17–19
42 Cowey, C.B., Knox, D., Walton, M.J., and Adron, J.W. (1977). The regulation of gluconeogenesis by diet and insulin in rainbow trout. *Br. J. Nutr.* **38,** 463–470