Fish Physiol Biochem (2009) 35:519–539
DOI 10.1007/s10695-008-9259-5

Nutritional regulation of hepatic glucose metabolism in fish

P. Enes · S. Panserat · S. Kaushik · A. Oliva-Teles

Received: 3 May 2008 / Accepted: 27 July 2008 / Published online: 14 September 2008

Abstract Glucose plays a key role as energy source in the majority of mammals, but its importance in fish appears limited. Until now, the physiological basis for such apparent glucose intolerance in fish has not been fully understood. A distinct regulation of hepatic glucose utilization (glycolysis) and production (gluconeogenesis) may be advanced to explain the relative inability of fish to efficiently utilize dietary glucose. We summarize here information regarding the nutritional regulation of key enzymes involved in glycolysis (hexokinases, 6-phosphofructo-1-kinase and pyruvate kinase) and gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase) pathways as well as that of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. The effect of dietary carbohydrate level and source on the activities and gene expression of the mentioned key enzymes is also discussed. Overall, data strongly suggest that the liver of most fish species is apparently capable of regulating glucose storage. The persistent high level of endogenous glucose production independent of carbohydrate intake level may lead to a putative competition between exogenous (dietary) glucose and endogenous glucose as the source of energy, which may explain the poor dietary carbohydrate utilization in fish.

Keywords Carbohydrate · Fish · Gluconeogenesis · Glycogen · Glycolysis · Nutrition

P. Enes (✉) · A. Oliva-Teles
Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal
e-mail: enes@portugalmail.com

P. Enes · A. Oliva-Teles
CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, 4050-123 Porto, Portugal

S. Panserat · S. Kaushik
INRA, UMR1067 Nutrition Aquaculture & Genomique, Pôle d'Hydrobiologie, CD918, 64310 St-Pee-sur-Nivelle, France

Introduction

Glucose plays a key role as energy source in the majority of mammals, but its importance in fish appears limited (Wilson 1994; Hemre et al. 2002; Stone 2003). Thus, in fish and particularly in carnivorous species, a prolonged postprandial hyperglycemia is generally observed after feeding a carbohydrate-rich diet (Cowey and Walton 1989; Wilson 1994; Moon 2001). Until now, the physiological basis for such apparent glucose intolerance in fish has not been fully understood.

Several hypotheses were advanced to explain this low dietary glucose utilization in fish, such as the stronger effect of dietary amino acids than glucose as stimulators of insulin secretion (Mommsen and

Plisetskaya 1991); the relatively low number of insulin receptors in fish muscle as compared to the rat (Párrizas et al. 1994); a low glucose phosphorylation capacity (Cowey and Walton 1989) and low number of glucose transporters (Wright et al. 1998) in fish muscle and an inadequate regulation of glucose homeostasis attributable to an imbalance between hepatic glucose uptake (glycolysis) and production (gluconeogenesis) (Panserat et al. 2001a).

This review focus on the last hypothesis and summarizes information regarding the nutritional regulation of key enzymes involved in glycolysis [hexokinases, 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase (PK)] and gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase) and glucose-6-phosphatase (G6Pase)] pathways. Special attention is given to the effect of dietary carbohydrate level and source on the activities and gene expression of the mentioned enzymes.

Data on gene expression and activities of the key glycolytic and gluconeogenic enzymes are limited to the liver, since this organ plays a key role in regulating body metabolism in response to nutritional status (Pilkis and Granner 1992; Klover and Mooney 2004). Most of the regulatory effects on nutrient utilization and metabolism initially occur in the liver, consequently affecting the cascade of events in other tissues and organs.

Glucose homeostasis

Once in the blood, glucose moves across the hepatocyte membrane mainly through facilitative glucose transporter (GLUT2) (Pilkis and Granner 1992). This transporter maintains the same glucose concentration in the extra and intracellular media; because of its low affinity for glucose (high Km), it allows fast glucose diffusion into the hepatocyte when blood glucose levels are high and a weak diffusion into the plasma when glycemia is low. Recently, a functional GLUT2 transporter was reported in rainbow trout *Oncorhynchus mykiss* liver (Krasnov et al. 2001; Panserat et al. 2001c), and the GLUT2 gene was shown to be highly expressed irrespective of the fish nutritional status (Panserat et al. 2001c).

Under aerobic conditions, glucose is catabolized through the glycolytic pathway, Krebs cycle, and respiratory chain for ATP production (Fig. 1) or through the pentose phosphate pathway leading to the production of NADPH for lipid biosynthesis purposes and ribose 5-phosphate required for nucleotide synthesis. Excess glucose may be stored as glycogen (glycogenesis) or converted to lipids (Fig. 1). Under fasting, glucose requirements for metabolic purposes may be satisfied by glycogen degradation into glucose (glycogenolysis) or by de novo glucose synthesis through gluconeogenesis (Fig. 1). Thus, a balance between glucose storage and glucose production, which is accomplished by nutritional and hormonal factors, is of utmost importance for maintaining glucose homeostasis, and it is mainly dependent on the regulation of activity and expression of key enzymes involved in the glycolysis and gluconeogenesis pathways (Pilkis and Granner 1992).

Glycolysis

Glycolysis is the only route of glucose catabolism in all organisms, including fish (Cowey and Walton 1989), and consists of a progressive oxidation of one molecule of glucose (6C) into two molecules of pyruvate (3C) (Fig. 2). All enzymes involved in this pathway were reported to exist in fish (Walton and Cowey 1982). The regulation of the glycolytic pathway is dependent on the activities of key enzymes such as hexokinases, PFK-1 and PK.

Hexokinase (HK; EC 2.7.1.1) (Fig. 2) catalyze the first reaction of glycolysis, which consists on the phosphorylation of glucose to glucose-6-phosphate, a molecule that may be used in other metabolic pathways, such as glycogenesis and the pentose-phosphate pathway. Thus, HKs play a key role in intermediary metabolism. In rats, HKs are a family of four closely related enzymes designated HK I–IV according to their relative mobility following starch gel electrophoresis. HK I–III share several common properties, including a high molecular weight (about 100 kDa), a relatively high affinity for glucose (low Km), and inhibition by high glucose-6-phosphate concentrations (Printz et al. 1993). However, they differ in tissue distribution. Therefore, HK-I is ubiquitous, but with high levels found in the brain and the kidney. HK-II is found in muscle, mammary gland, and adipocytes, whereas HK-III is mainly expressed in the spleen and in liver, although it is also

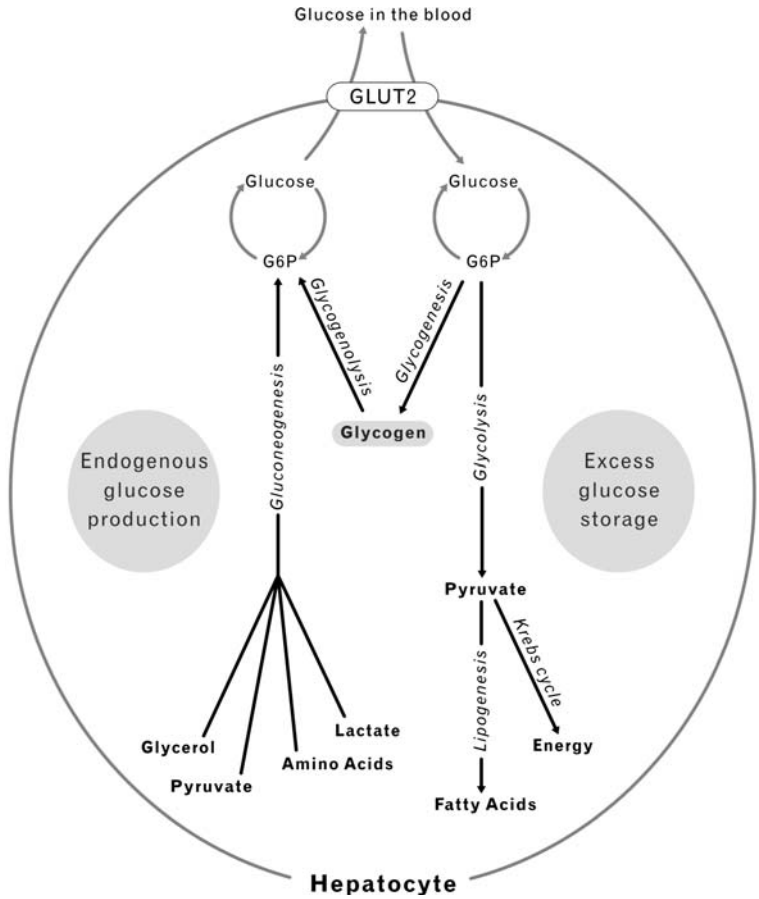

Fig. 1 Schematic representation of the main metabolic pathways responsible for maintaining glucose homeostasis

found in the kidney, the lung, and the brain. HK-IV, also known as glucokinase (GK; EC 2.7.1.2), is characterized by a low affinity for glucose (high Km), lack of inhibition by glucose-6-phosphate, and a molecular weight of about 50 kDa (Printz et al. 1993). It is mainly expressed in the hepatic parenchyma cells and in the pancreatic B-cells. In rats, GK expression is under both nutritional and hormonal control (Printz et al. 1993): GK gene expression is stimulated by a high insulin/glucagon ratio, which occurs after a carbohydrate-rich meal, whereas it is repressed by a low insulin/glucagon ratio, which occurs during fasting or after a protein or lipid-rich meals.

In fish liver, Nagayama and Ohshima (1974) isolated by DEAE-cellulose column chromatography four types of hexokinase isozymes named A, B, C, and D. However, the presence of these four isozymes seemed to be species-specific. Thus, isozymes A and B were found in common carp *Cyprinus carpio*; isozyme B in grass carp *Ctenopharyngodon idella*; isozymes B and C in silver carp *Hypophthalmicthys molitrix*; isozymes B, C and D in Japanese eel *Anguilla japonica*; isozymes C and D in yellowtail *Seriola quinqueradiata*; and isozyme D in rainbow trout. Based on the elution patterns and Km values for glucose and ATP, the authors reported that fish isozymes A and D resemble mammalian isoenzymes I and III, respectively, while isozymes B and C resemble isoenzyme II. However, an isozyme resembling mammalian isoenzyme IV (glucokinase) was not at that time detected in fish liver (Nagayama and Ohshima 1974; Cowey et al. 1975, 1977).

Nagayama et al. (1980) subsequently purified the hexokinases from rainbow trout liver and, in contrast to what was reported in a previous study (Nagayama and Ohshima 1974), found two isozymes, C and D, of which isozyme D was predominant and similar to mammalian isoenzyme III. Since then, hexokinase activities have been reported in several tissues of rainbow trout, cod *Gadus morhua* and plaice

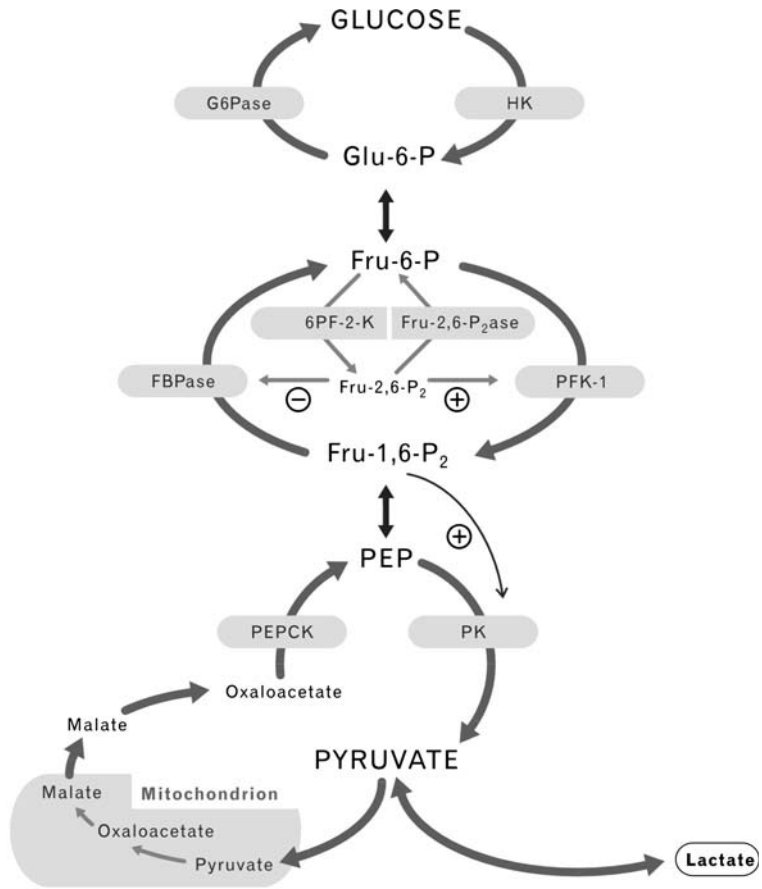

Fig. 2 Substrate cycles in the glycolytic/gluconeogenic pathways. The key enzymes of the glycolytic pathway are hexokinase (HK), 6-phosphofructo-1-kinase (PFK-1), and pyruvate kinase (PK), whereas the key gluconeogenic enzymes are phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphatase (G6Pase). The bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PF-2-K/Fru-2,6-P_2ase) is also represented. Adapted from Pilkis and Granner (1992)

Pleuronectes platessa, with the highest levels detected in the heart (Knox et al. 1980); kidney, gills, and brain also had appreciable hexokinase activities, whereas liver and skeletal muscle presented the smallest levels. It was suggested that the prolonged hyperglycemia observed in fish after glucose tolerance tests or after an administration of high levels of dietary carbohydrates might result from the low level of glucose phosphorylation by HKs, mainly in liver and skeletal muscle, and/or to the absence of an inducible hepatic GK expression (Nagayama et al. 1973; Nagayama and Ohshima 1974; Cowey et al. 1977; Walton and Cowey 1982). Later studies using biochemical analysis detected however the presence of a glucokinase-like enzyme in the liver of Atlantic salmon *Salmo salar* (Borrebaek et al. 1993; Tranulis et al. 1996), in the Brockmann bodies of Atlantic halibut *Hippoglossus hippoglossus* (Tranulis et al. 1997), and in the liver of gilthead sea bream *Sparus aurata*, rainbow trout, and common carp (Panserat et al. 2000b).

The cloning and sequencing of partial HK cDNA from the liver of rainbow trout, common carp, and gilthead sea bream showed the presence of a HK-IV-like sequence (glucokinase) in all the three teleosts studied, whereas HK-I- and II-like sequences were only reported in common carp and gilthead sea bream, and HK-III-related sequences were not detected at all (Blin et al. 1999). In common carp, the expression of HK-I gene is ubiquitous, being detected in brain, liver, heart, kidney, and muscle, whereas the GK gene is specifically expressed in the hepatopancreas (Blin et al. 2000). The isolation and characterization of a full-length cDNA coding for GK in the liver of rainbow trout, common carp, and gilthead sea bream were also accomplished, as well as the GK mRNA expression in different tissues (Panserat et al. 2000a). Thus, GK mRNA expression is highly specific to the liver in all three species studied, being also detected in rainbow trout whole brain. Recently, also in rainbow trout, two different HK-I cDNAs were identified with different tissue

distribution (Soengas et al. 2006). One transcript named heart or H-HK-I was observed in the four brain regions assessed (hypothalamus, midbrain, hindbrain, and telencephalon), white muscle, kidney, and gills, but not in the liver or the erythrocytes. A second transcript named liver or L-HK-I was found in all brain regions assessed, white muscle, kidney, gills, intestine, erythrocytes, and heart. GK mRNA was only identified in the liver and the four brain regions.

Although the activity of the low Km HK (HK I–III) does not seem to be under nutritional regulation (Panserat et al. 2000b; Kirchner et al. 2005; Enes et al. 2006a, b, 2008a, b; Moreira et al. 2008), several studies reported that dietary carbohydrates promote changes in both GK (HK IV) activity (Table 1) and gene expression. Thus, GK seems to be the dietary regulated isoform. Borrebaek et al. (1993) observed in Atlantic salmon liver a gradual increase of GK activity with the increase of dietary starch from 0% to 30%. Also in this species, hepatic GK activity was 1.6 fold higher in fish fed diets with 25% digestive energy from starch comparatively to fish fed a carbohydrate-free diet (Tranulis et al. 1996). An increase of GK activity was also reported in the liver of perch *Perca fluviatilis* following feeding a diet with 23% carbohydrate (Borrebaek and Christophersen 2001; Borrebaek et al. 2003). Induction of both GK gene expression and activity by high dietary carbohydrates (>20% digestible starch) was also observed in the liver of rainbow trout, gilthead sea bream, and common carp (Panserat et al. 2000b). In rainbow trout, a single meal containing 24% of glucose was sufficient to induce hepatic GK expression at both enzymatic and molecular levels (Panserat et al. 2001a). Gilthead sea bream fed high carbohydrate/low protein diets presented higher hepatic GK gene expression, while it was hardly detected in fish fed low carbohydrate/high protein diets (Caseras et al. 2002; Metón et al. 2004). Capilla et al. (2003) provided the first evidence that GK activity and mRNA level increased in rainbow trout liver in proportion to dietary starch content. Plasma glucose concentration and GK activity in common carp liver were not affected by the content (30 or 46%) or origin (wheat or peas) of digestible starch (Capilla et al. 2004). However, 6 h after feeding, GK activity was higher in fish fed carbohydrate diets comparatively to fish fed a carbohydrate-free diet. The latter authors also reported an absence of relation between GK activity and post-prandial insulin levels. Thus, hepatic GK activity increases with the presence of dietary carbohydrates independently of the levels of plasma insulin, which were elevated after ingestion of either a carbohydrate or a carbohydrate-free diet. Similar results were previously observed in rainbow trout and perch (Borrebaek et al. 2003; Capilla et al. 2003). Overall, these results suggest that in fish insulin could be necessary, but not sufficient to induce GK activity. Recently, in European sea bass *Dicentrarchus labrax* and gilthead sea bream a significant increase in hepatic GK activity was noticed with the increase of dietary starch level from 10% to 20% (Enes et al. 2006a, 2008a; Moreira et al. 2008). However, in the liver of European sea bass, there was a lack of further increase of GK activity with the increase of carbohydrate level from 20% to 30%, suggesting that 20% dietary starch is probably near the maximum tolerable level for metabolic utilization by that species (Moreira et al. 2008). Further, both in European sea bass and gilthead sea bream a higher induction of hepatic GK activity by dietary glucose than by dietary starch was also shown (Enes et al. 2006b, 2008b).

Starvation (Caseras et al. 2000; Kirchner et al. 2003a, 2005; Metón et al. 2004; Soengas et al. 2006) as well as energy restriction (Caseras et al. 2000) strongly decreased hepatic GK mRNA and activity levels in rainbow trout and gilthead sea bream, whereas re-feeding increased GK expression above that of regularly fed fish (Metón et al. 2004; Soengas et al. 2006). In contrast, in European sea bass, short starvation (9 days) had no effect on hepatic GK activities (Pérez-Jiménez et al. 2007). Caseras et al. (2000) observed that GK gene expression in the liver of gilthead sea bream exhibited a delayed onset during the daily feeding rhythm. Thus, while in rat, the liver GK gene expression increases immediately after food intake, in gilthead sea bream no induction of GK gene expression was observed until 4 h after the intake of food. Both GK mRNA levels and activity reached a peak at a postprandial time of 4–8 h. According to the last authors, the delayed induction of GK gene expression observed in gilthead sea bream after food intake may contribute to explaining the prolonged hyperglycemia recorded.

6-Phosphofructo-1-kinase (EC 2.7.1.11) (Table 1; Fig. 2) catalyzes the phosphorylation of fructose-6-phosphate into fructose-1,6-bisphosphate. In fish,

Table 1 Effect of carbohydrate type and level on hepatic key enzymes activities (mU/mg protein) of glycolytic (HK, hexokinase; GK, glucokinase; PFK-1, 6-phosphofructo-1-kinase; PK, pyruvate kinase) and gluconeogenic (FBPase, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase) pathways in selected fish species

Fish species	Water temperature (°C)	Temperature of enzyme assays (°C)	Carbohydrate type	Processing method	Inclusion content (%)	HK	GK	PFK-1	PK	FBPase	G6Pase	References
European sea bass (*Dicentrarchus labrax*)	25	37	–	–	0	0.5	0.4	–	62.8	29	24.1	Enes et al. (2006a)
			Maize starch	Native	10	0.3	1.3	–	73.2	28	26.5	
			Maize starch	Waxy	10	0.3	0.4	–	76.3	25.3	24.2	
			Maize starch	Native	20	0.3	2.8	–	84.3	24.9	23.7	
			Maize starch	Waxy	20	0.5	1.5	–	90.1	23.2	26.2	
	25	37	Maize starch	Waxy	20	0.5	2.6	–	66.7	28	15.5	Enes et al. (2006b)
			Glucose	–	20	0.6	13.1	–	110.3	24.6	16.3	
	18	37	Maize starch	Waxy	20	0.4	1.7	–	68.9	23.5	16.8	
			Glucose	–	20	0.6	4.3	–	90	20.1	16.1	
	25	37	Maize starch	Pregelatinized	10	0.4	1.6	–	–	10.8	–	Moreira et al. (2008)
			Maize starch	Pregelatinized	20	0.2	6.6	–	–	11.8	–	
			Maize starch	Pregelatinized	30	0.3	6.6	–	–	11.4	–	
	18	37	Maize starch	Pregelatinized	10	0.2	4.7	–	–	12	–	
			Maize starch	Pregelatinized	20	0.3	4.8	–	–	10.8	–	
			Maize starch	Pregelatinized	30	0.2	3.1	–	–	10.8	–	
	25	37	–	–	0	–	0.6[a]	–	–	–	16.1[a]	Enes et al. (2008c)
			Glucose	–	20	–	9.6[a]	–	–	–	18.2[a]	
	18	37	–	–	0	–	0.6[a]	–	–	–	15.9[a]	
			Glucose	–	20	–	4.7[a]	–	–	–	18.1[a]	
Gilthead sea bream (*Sparus aurata*)	20	20	Maize starch	Not mentioned	12	–	–	20	264	94	–	Bonamusa et al. (1992)
			Maize starch	Not mentioned	16	–	–	24	296	112	–	
			Maize starch	Not mentioned	35	–	–	28	394	124	–	
			Maize starch	Not mentioned	50	–	–	36	357	80	–	
	20	30	Maize starch	Gelatinized	10	–	–	30.7	300	123	–	Metón et al. (1999b)
			Maize starch	Gelatinized	13	–	–	36	382	119	–	
			Maize starch	Gelatinized	17	–	–	33	513	144	–	
			Maize starch	Gelatinized	26	–	–	42.5	784	140	–	
			Maize starch	Gelatinized	32	–	–	49.2	567	142	–	
	25	37	–	–	0	2	1	–	–	–	–	Panserat et al. (2000b)
			Peas starch	Extruded	20	2.3	29.9	–	–	–	–	

Table 1 continued

Fish species	Water temperature (°C)	Temperature of enzyme assays (°C)	Carbohydrate type	Processing method	Inclusion content (%)	HK	GK	PFK-1	PK	FBPase	G6Pase	References
Gilthead sea bream (*Sparus aurata*)	20	30	Maize starch	Gelatinized	10	–	0.4	–	–	–	55	Caseras et al. (2002)
			Maize starch	Gelatinized	13	–	0.4	–	–	–	56	
			Maize starch	Gelatinized	26	–	1.2	–	–	–	60	
			Maize starch	Gelatinized	32	–	1.7	–	–	–	54	
	25	37	–	–	0	0.2	0.2	–	73.6	23.8	–	Enes et al. (2008a)
			Maize starch	Native	10	0.3	3.5	–	61.2	19	–	
			Maize starch	Waxy	10	0.3	2.2	–	73.2	19.9	–	
			Maize starch	Native	20	0.3	12.7	–	44.8	21.8	–	
			Maize starch	Waxy	20	0.3	13.6	–	46	19.9	–	
	25	37	Maize starch	Waxy	20	0.4	9.7	–	50.2	–	6.4	Enes et al. (2008b)
			Glucose	–	20	0.4	22.5	–	59.4	–	6.4	
	18	37	Maize starch	Waxy	20	0.2	3.1	–	52.3	–	7.3	
			Glucose	–	20	0.3	8	–	43.3	–	7.1	
	25	37	–	–	0	–	0.3[a]	–	–	–	13.4[a]	Enes et al. (2008c)
			Glucose	–	20	–	14.3[a]	–	–	–	12.8[a]	
	18	37	–	–	0	–	0.3[a]	–	–	–	13.6[a]	
			Glucose	–	20	–	8.0[a]	–	–	–	13.1[a]	
	21	30	Maize starch	Gelatinized	5	–	–	30	214.2	112.1	–	Fernández et al. (2007)
			Maize starch	Gelatinized	18	–	–	37.9	463.1	100.4	–	
			Maize starch	Gelatinized	26	–	–	56.6	740.1	129.2	–	
Rainbow trout (*Oncorhynchus mykiss*)	12	Not mentioned	Dextrose	Dextrinized	26	0.4	–	12.5	200	–	–	Fideu et al. (1983)
			Dextrose	Dextrinized	77	0.6	–	13.8	200	–	–	
	18	37	–	–	0	0.4	3.3	–	–	–	–	Panserat et al. (2000b)
			Peas starch	Extruded	20	2.1	36.7	–	–	–	–	
	18	30	–	–	0	–	–	–	–	–	15	Panserat et al. (2000c)
			Peas starch	Extruded	20	–	–	–	–	–	22	

Table 1 continued

Fish species	Water temperature (°C)	Temperature of enzyme assays (°C)	Carbohydrate type	Processing method	Inclusion content (%)	HK	GK	PFK-1	PK	FBPase	G6Pase	References
	8	37	–	–	0	2.3	6.2	–	–	–	–	Capilla et al. (2003)
			Wheat starch	Extruded	20	1.9	14.3	–	–	–	–	
			Wheat starch	Extruded	40	2.3	18.8	–	–	–	–	
			Peas starch	Extruded	20	3.2	27.5	–	–	–	–	
			Peas starch	Extruded	40	4.9	44.8	–	–	–	–	
	18	37	–	–	0	2.8	1.4	–	–	–	–	
			Wheat starch	Extruded	20	3.7	11.8	–	–	–	–	
			Wheat starch	Extruded	40	4	29.5	–	–	–	–	
			Peas starch	Extruded	20	8.6	29.4	–	–	–	–	
			Peas starch	Extruded	40	6.8	33	–	–	–	–	
Atlantic salmon (*Salmo salar*)	Not mentioned	30	–	–	0	–	5.2	–	–	13.9	–	Borrebaek et al. (1993)
			Starch	Not mentioned	5	–	5.8	–	–	14.2	–	
			Starch	Not mentioned	10	–	8.8	–	–	15	–	
			Starch	Not mentioned	20	–	10.7	–	–	11.8	–	
			Starch	Not mentioned	30	–	13.5	–	–	12.3	–	
	11	30	–	–	0	5.4	6	–	–	10.8	–	Tranulis et al. (1996)
			Starch	Not mentioned	25	5.4	9.4	–	–	10.7	–	
Common carp (*Cyprinus carpio*)	18	37	–	–	0	7.2	1.8	–	–	–	–	Panserat et al. (2000b)
			Peas starch	Extruded	20	4.8	9.7	–	–	–	–	
	24	37	–	–	0	4.1	0.5	–	–	–	–	Capilla et al. (2004)
			Wheat starch	Extruded	30	4.1	1.7	–	–	–	–	
			Wheat starch	Extruded	46	4.8	2.5	–	–	–	–	
			Peas starch	Extruded	30	5.1	2.3	–	–	–	–	
			Peas starch	Extruded	46	6.6	3	–	–	–	–	
Perch (*Perca fluviatilis*)	15	30	–	–	0	–	7.1	–	22.6	101	–	Borrebaek and Christophersen (2000)
			Digestible carbohydrate		14	–	27.7	–	65.5	95.2	–	

Table 1 continued

Fish species	Water temperature (°C)	Temperature of enzyme assays (°C)	Carbohydrate type	Processing method	Inclusion content (%)	HK	GK	PFK-1	PK	FBPase	G6Pase	References
Tilapia (*Oreochromis niloticus* × *O. aureus*)	26	25	Maize starch	Not mentioned	40	47.7	–	92.5	–	–	–	Lin and Shiau (1995)
			Glucose	–	40	40.1	–	81.5	–	–	–	
	26	25 (HK/PFK-1)	Maize starch	Not mentioned	44	22.9	–	43.7	–	117.5	8	Tung and Shiau (1991)
		37 (FBPase/ G6Pase)	Dextrin	Dextrinized	44	23.3	–	47.5	–	151.6	7.1	
			Glucose	–	44	28	–	43	–	145.6	10.6	

[a] Fish reared at 25°C and 18°C received the same feed levels

PFK-1 activity was found in several tissues of cod, plaice, and rainbow trout, such as liver, red and white muscle, kidney, gills, heart, and brain (Knox et al. 1980): skeletal muscle presented the highest activity levels, followed by the heart and brain, whereas liver, kidney, and gills had the lowest PFK-1 activities. Fish PFK-1 resembles that of mammals in being activated by fructose-1,6-bisphosphate and AMP and in being inhibited by ATP (Cowey and Walton 1989). As in rats, fish fed on high carbohydrate/low protein diets showed higher hepatic PFK-1 activity compared to those given low carbohydrate/high protein diets (Fideu et al. 1983; Walton 1986; Bonamusa et al. 1992; Metón et al. 1999b; Fernández et al. 2007). In contrast, starvation resulted in a decrease of hepatic PFK-1 activity (Fideu et al. 1983; Bonamusa et al. 1992; Metón et al. 1999b, 2003). However, in gilthead sea bream, short-term refeeding (8 h to 2 days) following 18 days of starvation rapidly restored liver PFK-1 activity levels (Metón et al. 1999b, 2003). According to the last authors, the rapid rise in PFK-1 activity may result from increased levels of its allosteric activator, fructose-2,6-bisphosphate, whose synthesis and degradation is catalyzed by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PF-2-K/Fru-2,6-P_2ase). In fact, previous results showed that short-term refeeding of 8 h was enough to markedly increase hepatic fructose-2,6-bisphosphate levels in gilthead sea bream following 18 days of starvation (Metón et al. 1999a). Meal frequency (daily ration subdivided into two or six equal feedings per day) also seems to significantly affect hepatic PFK-1 activities. Indeed, in hybrid tilapia *Oreochromis niloticus* × *O. aureus* higher PFK-1 activities were observed in fish fed a 44% starch diet six times a day than in fish fed the same diet twice a day (Tung and Shiau 1991). Regarding carbohydrate complexity, no differences in hepatic PFK-1 activities were observed between hybrid tilapia fed glucose or starch (Lin and Shiau 1995). In common carp, hepatic PFK-1 activity was higher in fish fed diets containing 30% of starch or glucose compared to fish fed a high protein diet without carbohydrates, whereas supplementation with 30% galactose depressed PFK-1 activity (Shikata et al. 1994).

Pyruvate kinase (EC 2.7.1.40) (Fig. 2) is another key glycolytic enzyme that catalyzes the last step in glycolysis, the conversion of phosphoenolpyruvate (PEP) to pyruvate. In mammals, PK exists as four isoenzymes (L-, R-, M_1-, and M_2-) encoded by two

genes: PKL and PKM (Yamada and Noguchi 1999). The hepatic PK activity and gene expression are largely attributed to the L-type isoenzyme, and decreases during starvation or diabetes and increases as the result of high carbohydrate feeding or insulin administration (Pilkis and Granner 1992). Glucagon, acting via cyclic AMP (cAMP), inhibits the synthesis of L-PK mRNA by decreasing gene transcription.

In fish, the distribution of PK has been investigated in cod, plaice, and rainbow trout tissues with higher activities being reported in skeletal muscle, heart, and brain and relatively lower activities being detected in the liver, kidney, and gills (Knox et al. 1980). Recently, partial cloning of PK gene was accomplished in rainbow trout liver, and PK gene expression was also detected in the intestine and kidney (Panserat et al. 2001c).

In rainbow trout, Cowey et al. (1977) reported that an intraperitoneal injection of insulin in fish fed a high carbohydrate diet had no effect on hepatic PK activity. Similarly, no effect of insulin on PK activity was found in sea raven *Hemitripterus americanus* hepatocytes, following a 45 and 180 min incubation (Foster and Moon 1990a). In contrast, glucagon decreases the affinity of PK for its substrate (PEP) and increases its sensitivity to ATP inhibition in both rainbow trout and sea raven hepatocytes (Mommsen and Suarez 1984; Petersen et al. 1987; Foster and Moon 1990a).

Regarding nutritional regulation, Panserat et al. (2001c) observed in rainbow trout that dietary carbohydrate did not induce any specific effect on hepatic PK gene expression. Thus, similar PK mRNAs levels were observed 6 and 24 h after feeding in the livers of fish fed with or without carbohydrates. These data suggest that the induction of PK activity by dietary carbohydrates reported in several fish species (Table 1) (Metón et al. 1999b; Borrebaek and Christophersen 2000; Enes et al. 2006a; Fernández et al. 2007) is not mainly due to transcriptional mechanisms, but is possibly linked to post-transcriptional regulation or to qualitative enzyme modifications, such as by phosphorylation/dephosphorylation of PK (Panserat et al. 2001c). This subject requires further clarification as in several other studies with fish no induction of PK activity by dietary carbohydrates was observed (Hilton and Atkinson 1982; Fideu et al. 1983; Bonamusa et al. 1992; Suarez et al. 2002; Dias et al. 2004; Enes et al. 2008a).

PK activities are also affected by the nature of dietary carbohydrate. Thus, in European sea bass, a higher induction of liver PK activity by glucose than by starch was observed (Enes et al. 2006b), whereas in gibel carp *Carassius auratus* and Chinese longsnout catfish *Leiocassis longirostris*, PK activity was significantly higher in fish fed diets containing sucrose than glucose, dextrin or starch (Tan et al. 2006). In common carp, comparatively to fish fed a high protein diet without carbohydrates, dietary supplementation with 30% of starch, glucose, or fructose had no effect on hepatic PK activity, whereas supplementation with 30% of galactose depressed PK activity (Shikata et al. 1994).

Hepatic PK activity in rainbow trout and gilthead sea bream significantly decreases with starvation (Fideu et al. 1983; Bonamusa et al. 1992; Metón et al. 1999b, 2003; Kirchner et al. 2003a). However, no difference in PK gene expression between food-deprived and fed fish was observed in rainbow trout liver (Panserat et al. 2001c; Kirchner et al. 2003a). In gilthead sea bream, short-term refeeding (8 h to 2 days) following 18 days of starvation was not sufficient to produce significant changes on hepatic PK activity (Metón et al. 2003). These results suggested that the initial effect of dietary nutrients is to restore glycogen levels, which were recovered in 8 h to 2 days, rather than being metabolized by glycolysis. A total restoration of PK activity levels was only attained at day 20 of refeeding, which denotes a longer term stimulation of glycolysis in order to metabolize the excess of glucose towards pyruvate production (Metón et al. 2003).

Gluconeogenesis

The gluconeogenesis pathway involves the synthesis of glucose from non-glycosidic substrates, such as lactate, glycerol, or α-ketoacids (Fig. 1). All enzymes involved in gluconeogenesis are reported to exist in fish (Walton and Cowey 1982; Cowey and Walton 1989). The regulation of the gluconeogenesis is dependent on the activities of key enzymes, such as PEPCK, FBPase, and G6Pase.

Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (Fig. 2) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, the first step in gluconeogenesis. PEPCK exists as two isoforms, a cytosolic

(PEPCK-C) and a mitochondrial (PEPCK-M) isoform, with similar kinetic properties and approximately the same molecular weight, but encoded by separate genes (Hanson and Reshef 1997). The proportion of these two isoforms in liver cells is species dependent, being 100% cytosolic in rat, 100% mitochondrial in pigeon, and having dual localization in other species. In rats, PEPCK is an important rate-limiting enzyme involved in the synthesis of glucose in the liver and the kidney, and of glyceride-glycerol in the white adipose tissue and the small intestine. PEPCK is not allosterically or post-transcriptionally modified. Thus, transcriptional regulation of PEPCK gene expression is the major control of PEPCK enzyme activity. The gene for the cytosolic form of PEPCK is under nutritional and hormonal regulation, whereas the mitochondrial form of hepatic PEPCK is constitutively expressed. Major factors that increase PEPCK-C gene expression in rat include glucagon (through cAMP), glucocorticoids, and thyroid hormone, whereas it is inhibited by insulin and glucose (Hanson and Reshef 1997).

In fish, PEPCK activity is highly specific to gluconeogenic tissues, such as the liver and the kidney (Knox et al. 1980; Suarez and Mommsen 1987). Thus, in rainbow trout, cod, and plaice, no PEPCK activity was detected in the heart, brain, or skeletal muscle, and only trace levels of activity were found in gills (Knox et al. 1980). Recently, Panserat et al. (2001b) cloned a full-length PEPCK cDNA in rainbow trout liver coding for a mitochondrial isoform and found it to be highly homologous to the mammalian PEPCKs. The authors also reported that in rainbow trout PEPCK-M gene was expressed in the liver, kidney, and intestine.

In rainbow trout liver, higher PEPCK activities were reported in fish fed a high protein diet than in fish fed a high carbohydrate diet (Cowey et al. 1977; Hilton and Atkinson 1982; Walton 1986). Regarding hormonal regulation, Cowey et al. (1977) observed in rainbow trout that insulin reduced hepatic PEPCK activity in the high protein fed group, whereas in the high carbohydrate fed group, insulin had no effect on PEPCK activity. Also, no effect of insulin on PEPCK activity was found in sea raven hepatocytes, following 45 and 180 min incubation, whereas glucagon incubation increased PEPCK activities (Foster and Moon 1990a).

The effect of dietary carbohydrate complexity on PEPCK activity was studied in the liver of gibel carp and Chinese longsnout catfish (Tan et al. 2006). While in gibel carp higher PEPCK activities were observed in fish fed dextrin than glucose and starch, in Chinese longsnout catfish, PEPCK activity was higher in fish fed glucose and sucrose than dextrin or starch. According to the latter authors, the discrepancy of results observed between the two species may be related to plasma glucose levels. Thus, in gibel carp plasma glucose levels were higher in fish fed dextrin compared to fish fed glucose or starch, whereas in Chinese longsnout catfish plasma glucose levels were higher in fish fed the glucose diet than in fish fed the dextrin or starch diets. The significant correlation observed between plasma glucose levels and liver PEPCK activities in both species ($r = 0.6355$ in gibel carp and $r = 0.6073$ in Chinese longsnout catfish) supports this hypothesis (Tan et al. 2006).

Opposite to rats, PEPCK gene expression in rainbow trout liver was always high, independently of the nutritional status of the animals: fed or unfed, or fed with or without carbohydrates (Panserat et al. 2001b). The absence of PEPCK gene expression control by nutritional status suggests that the PEPCK gene cloned in rainbow trout liver is mainly mitochondrial. This is further supported by previous data showing that in rainbow trout the location of PEPCK is predominantly mitochondrial (>80%) (Walton and Cowey 1979).

Partial cloning of PEPCK gene was also achieved in common carp and gilthead sea bream livers (Panserat et al. 2002b). In the mentioned study the 5′ region of PEPCK gene, which allows the identification of cytosolic or mitochondrial isoform, was not cloned, and thus it was impossible to identify the PEPCK isoform encode by PEPCK gene. In common carp, PEPCK gene expression was significantly lower 6 h than 24 h after feeding dietary carbohydrates, whereas in gilthead sea bream, PEPCK gene was expressed at the same level independently of the postprandial time or diet composition (Panserat et al. 2002b). In rainbow trout liver, mitochondrial PEPCK activity was significantly higher in food-deprived than in fed fish, while cytosolic PEPCK activity was similar in both groups (Kirchner et al. 2003a). However, in contrast to enzyme activity, no difference was observed in mitochondrial PEPCK at the molecular level. This suggests that the mitochondrial PEPCK gene is constitutively expressed in rainbow trout. Also in rainbow trout, hepatic PEPCK gene

expression and activity were unaffected by increasing dietary protein level (Kirchner et al. 2003b). However, no distinction between cytosolic and mitochondrial PEPCK forms was made in that study. Thus, a nutritional regulation of the expression of cytosolic PEPCK could have been masked, as PEPCK activity in rainbow trout is mainly mitochondrial and only the cytosolic form is acutely regulated by the diet.

Overall, the issue of PEPCK isoforms is an important aspect to take into account before studying the effect of dietary carbohydrates or nutritional status on PEPCK gene expression. As the proportion of the two PEPCK isoforms in liver cells is species-specific, it will be necessary to first define the proportion of each isoform for each species and to be certain that the gene under study is the one that encodes the PEPCK-C isoform, which is the only one that is nutritionally regulated; the mitochondrial form is constitutively expressed and thus can mask results.

Fructose-1,6-bisphosphatase (EC 3.1.3.11) (Fig. 2) catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate. Several FBPase isoforms, all cytosolic, were reported in different species. In rainbow trout, cod, and plaice, FBPase activity is higher in the liver, followed by the kidney and skeletal muscle (Knox et al. 1980). In rats, FBPase gene expression is induced by high plasma glucagon levels (or intracellular cAMP), characteristic of the fasting condition, and is repressed by high plasma insulin levels, which occur after a carbohydrate-rich meal (Pilkis and Granner 1992). In rainbow trout fed a high carbohydrate or a high protein diet, Cowey et al. (1977) reported that an intraperitoneal injection of insulin had no significant effect on hepatic FBPase activity. In contrast, in common carp insulin administration significantly decreased plasma glucose levels as well as hepatic FBPase activity, whereas glucagon had the opposite effect (Sugita et al. 1999, 2001b).

The partial cloning of an FBPase gene was reported in rainbow trout, common carp, and gilthead sea bream livers (Panserat et al. 2001c, 2002b). In rainbow trout FBPase genes are expressed in the liver, intestine, and kidney (Panserat et al. 2001c). However, there is no apparent nutritional effect on the regulation of FBPase gene expression in the liver of rainbow trout. Thus, no significant differences on FBPase gene expression were observed between food-deprived and fed fish (Panserat et al. 2001c). In addition, the authors were unable to demonstrate a significant decrease in hepatic FBPase gene expression in response to dietary carbohydrates. This is in accordance with the absence of inhibition of FBPase activity by dietary carbohydrates (Table 1) observed in other species such as perch (Borrebaek and Christophersen 2000), Atlantic salmon (Tranulis et al. 1996), and European sea bass (Enes et al. 2006a; Moreira et al. 2008). In common carp liver, Panserat et al. (2002b) also reported no differences in FBPase gene expression between fish fed with or without carbohydrates, whereas in gilthead sea bream liver, FBPase gene expression was depressed 6 h after feeding a 20% carbohydrate diet. However, other studies with gilthead sea bream reported no effect of dietary carbohydrate content on FBPase activity (Fernández et al. 2007; Enes et al. 2008a).

Tung and Shiau (1991) observed similar FBPase activities in hybrid tilapia fed diets with starch, dextrin, or glucose, which suggests that carbohydrate complexity has no relevant effect on enzyme activity. Enes et al. (2006b) showed however that in European sea bass reared at 18°C, but not at 25°C, a starch-based diet led to higher FBPase activity than a glucose-based diet. Tung and Shiau (1991) also reported no effect of meal frequency (daily ration subdivided into two or six equal feedings per day) on FBPase activity. In common carp, diet supplementation with either 30% of starch, glucose, or galactose depressed hepatic FBPase activity in comparison to those fed a high protein diet without carbohydrates, whereas the same level of dietary fructose had no effect on FBPase activity (Shikata et al. 1994).

Further, some evidence exists that the major factor involved in gluconeogenic enzyme regulation in fish may be dietary protein and not carbohydrates. Indeed, several studies reported a positive effect of dietary protein in hepatic FBPase activity and/or gene expression in rainbow trout and European sea bass (Cowey et al. 1977; Hilton and Atkinson 1982; Walton 1986; Kirchner et al. 2003b, 2005; Enes et al. 2006a), though in gilthead sea bream such an effect was not observed (Enes et al. 2008a).

Starvation also significantly increased FBPase activity in rainbow trout and gilthead sea bream liver (Morata et al. 1982b; Metón et al. 1999b, 2003; Kirchner et al. 2003a). In gilthead sea bream starved for 18 days, FBPase activity remained elevated

during the early periods of refeeding (8 h to 2 days) and returned to the values observed in fed fish after 21 days of refeeding (Metón et al. 2003). According to the last authors, the high levels of FBPase activity recorded during the early stages of refeeding may be related with an indirect pathway for restoring hepatic glycogen from three-carbon compounds via gluconeogenesis as observed in rats (Pilkis and Granner 1992).

Glucose-6-phosphatase (EC 3.1.3.9) (Fig. 2) is a microsomal enzyme that plays a key role in blood glucose homeostasis by catalyzing the dephosphorylation of glucose-6-phosphate to glucose, the last step of gluconeogenesis and glycogenolysis. In all cell types, G6Pase activity is tightly linked to the endoplasmic reticulum membrane (van de Werve et al. 2000). In rats, hepatic G6Pase expression is under both nutritional and hormonal regulation. Thus, starvation and hormones that increase cAMP, such as glucagon and glucocorticoids, stimulate G6Pase expression, whereas refeeding and insulin inhibit G6Pase expression (Pilkis and Granner 1992). Similarly, in common carp, insulin administration significantly decreases plasma glucose levels as well as hepatic G6Pase activities, whereas glucagon had the opposite effect (Sugita et al. 1999, 2001b).

Panserat et al. (2000c) in rainbow trout detected high G6Pase gene expression in the liver and the kidney (the main gluconeogenic tissues) and no G6Pase gene expression in the muscle and heart, which is in accordance with the early observations by Shimeno and Ikeda (1967) on G6Pase activity.

A partial cloning of G6Pase gene was also reported in rainbow trout, common carp, and gilthead sea bream livers (Panserat et al. 2000c, 2002b), whereas Metón et al. (2004) isolated a cDNA encoding the full-length G6Pase catalytic subunit from gilthead sea bream liver. The cDNA encodes a 350-amino acid protein, with low homology to the mammalian G6Pase, but containing most of the key residues required for catalysis. Regarding regulation of G6Pase by dietary factors (Table 1), Panserat et al. (2000c) reported that in rainbow trout liver G6Pase expression (mRNA and activity) was neither affected by the dietary carbohydrate level tested (0%, 8%, 12%, or 20% digestible starch) nor modified between 6 and 24 h after feeding. Also in rainbow trout, Panserat et al. (2001a) further observed a low inhibitory effect of dietary glucose on hepatic G6Pase gene expression, but without affecting G6Pase activity. In gilthead sea bream, Caseras et al. (2002) observed that dietary carbohydrate did not regulate hepatic G6Pase gene expression and activity, although Panserat et al. (2002b) reported lower G6Pase gene expression 6 h after feeding a diet with 20% digestible carbohydrates compared to a carbohydrate-free diet. In European sea bass, both dietary carbohydrate level (10% or 20%) and nature (native versus waxy maize starch) had no effect on G6Pase activity (Enes et al. 2006a). Lin and Shiau (1995) in hybrid tilapia and Enes et al. (2006b) in European sea bass found no differences in hepatic G6Pase activity between fish fed starch or glucose. Also in hybrid tilapia, Tung and Shiau (1991) observed similar hepatic G6Pase activities in fish fed starch, dextrin, or glucose. The authors also reported no effect of meal frequency (daily ration subdivided into two or six equal feedings per day) on G6Pase activity.

With regard to the regulation of G6Pase gene expression and activity by dietary lipid and protein, Panserat et al. (2002a) reported that in rainbow trout liver a high fat (25%) diet led to significantly higher G6Pase mRNA expression at 3, 6, and 12 h after feeding and enzyme activity 6 h after feeding. Similarly, Kirchner et al. (2003b) also in rainbow trout liver observed that 6 h after feeding activities and mRNA levels of G6Pase increased with increasing dietary protein.

Long-term starvation also increases hepatic G6Pase activity and gene expression in rainbow trout and in gilthead sea bream (Morata et al. 1982b; Caseras et al. 2002; Kirchner et al. 2003a; Metón et al. 2004). In gilthead sea bream, 1 day of refeeding following 19 days of starvation was sufficient to decrease the hepatic G6Pase mRNA levels to values similar to those observed in fed fish (Metón et al. 2004). Although in gilthead sea bream, hepatic G6Pase expression increased in energy-restricted fish (Caseras et al. 2002), in the common carp, ration size did not affect G6Pase activity (Shikata et al. 1993).

Bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase

The bifunctional enzyme, 6PF-2-K/Fru-2,6-P_2ase (EC 2.7.1.105/EC 3.1.3.46) (Fig. 2), catalyzes the synthesis and degradation of fructose-2,6-bisphosphate

(Fru-2,6-P_2), a key allosteric regulator of glycolysis/gluconeogenesis through its action on PFK-1 and FBPase (Pilkis and Granner 1992). In rats, the concentration of Fru-2,6-P_2 is modulated by hormones, mainly insulin and glucagon, and by the nutritional status (Pilkis and Granner 1992). Starvation leads to an increase in plasma glucagon levels and thus to an increase of cAMP. The phosphorylation of 6PF-2-K/Fru-2,6-P_2ase by the cAMP-dependent protein kinase results in the inactivation of 6PF-2-K and in the activation of Fru-2,6-P_2ase, leading to a decrease of Fru-2,6-P_2 level. This leads to the inhibition of PFK-1 and to an enhanced FBPase activity, thus increasing the gluconeogenic flux. The increase in gluconeogenesis is also favored by the inactivation of PK through its phosphorylation. In contrast, high carbohydrate diets lead to high plasma glucose and insulin levels, which decrease cAMP. The dephosphorylation of 6PF-2-K/Fru-2,6-P_2ase leads to high Fru-2,6-P_2 levels due to an increase in the 6PF-2-K/Fru-2,6-P_2ase ratio. As Fru-2,6-P_2 stimulates PFK-1 activity and inhibits FBPase, glycolysis is activated, while gluconeogenesis is kept low. The increase of fructose-1,6-biphosphate levels, an allosteric activator of PK, also favors the glycolytic flux.

In fish, partial cloning of the 6PF-2-K/Fru-2,6-P_2ase gene was reported in rainbow trout and in gilthead sea bream livers (Metón et al. 1999a; Panserat et al. 2001c). In rainbow trout 6PF-2-K/Fru-2,6-P_2ase gene expression is ubiquitous, being detected in the liver, intestine, kidney, and brain (Panserat et al. 2001c). Fructose-2,6-bisphosphate has been found in several tissues in different fish species (Foster et al. 1989; Foster and Moon 1990a; Garcia de Frutos et al. 1990, 1991; Bonamusa et al. 1992; Metón et al. 2000) and may act as an allosteric regulator of the PFK-1 and FBPase fish enzymes (Baanante et al. 1991). Thus, in gilthead sea bream liver and in isolated hepatocytes of sea raven and American eel *A. rostrata*, PFK-1 was allosterically activated by Fru-2,6-P_2 (Foster et al. 1989; Foster and Moon 1990a; Bonamusa et al. 1992). Furthermore, Fru-2,6-P_2 showed an inhibitory effect over the FBPase activity in the liver of gilthead sea bream (Baanante et al. 1991).

Diet composition also has a strong effect on Fru-2,6-P_2 concentration in the liver of gilthead sea bream (Garcia de Frutos et al. 1990). Thus, fish fed on a high carbohydrate diet showed the highest Fru-2,6-P_2 values, which were 30-fold higher than values reported in rat. In contrast, the lowest Fru-2,6-P_2 values were found in fish fed either with high protein or high lipid diets. The authors also found that 6PF-2-K activity values in the liver of gilthead sea bream were higher than in the rat, with the highest values being attained in fish fed the high carbohydrate diet. Thus, a high carbohydrate diet enhanced 6PF-2-K activity and Fru-2,6-P_2 levels, which in turn could activate the rate of glycolysis. In American eel hepatocytes, porcine insulin increases the sensitivity of PFK-1 to ATP, but it had no effect on Fru-2,6-P_2 levels, the principal modulator of PFK-1 (Foster et al. 1989). The incubation of sea raven hepatocytes with glucagon lead to a decrease in Fru-2,6-P_2 concentration, presumably through changes in the phosphorylation of the bifunctional enzyme that would directly reduce PFK-1 activities (Foster and Moon 1990a).

A metabolic adaptation to high carbohydrate diets was observed in gilthead sea bream, through increasing mRNA abundance, protein content, and kinase activity of 6PF-2-K/Fru-2,6-P_2ase, leading to an increase of Fru-2,6-P_2 levels and subsequent stimulation of glycolysis and simultaneous inhibition of gluconeogenesis in the liver (Metón et al. 2000). Panserat et al. (2001c) in rainbow trout liver also reported an induction of 6PF-2-K/Fru-2,6-P_2ase gene expression by feeding carbohydrates.

In contrast to rat, Fru-2,6-P_2 level in gilthead sea bream liver remains high after 24 h of starvation, being similar to values found in fed fish (Garcia de Frutos et al. 1990, 1991). After 48 h of starvation Fru-2,6-P_2 diminished to 38% of the control values and continued to decrease until at least 20 days of starvation, by then reaching 1.2% of the control values (Garcia de Frutos et al. 1990). Liver glycogen content followed a pattern similar to Fru-2,6-P_2 (Garcia de Frutos et al. 1990). The fall in Fru-2,6-P_2 and glycogen levels in fish liver under starvation conditions suggests an increase of gluconeogenesis; this is further supported by the simultaneous lower PFK-1 activities and the decrease of PFK-1/FBPase ratios (Garcia de Frutos et al. 1990). In gilthead sea bream, Metón et al. (1999a) also reported that the kinase enzyme activity, the protein content, the mRNA levels of 6PF-2-K/Fru-2,6-P_2ase, as well as the Fru-2,6-P_2 levels decreased in the liver of starved fish. These authors hypothesized that the decrease in liver 6PF-2-K enzyme activity could be caused by a

specific phosphorylation and allosteric modulation of 6PF-2-K/Fru-2,6-P_2ase, and by a mechanism that involves a decrease in both mRNA and protein levels. Metón et al. (2000) observed that the expression of the bifunctional enzyme and Fru-2,6-P_2 levels in the liver of gilthead sea bream were dependent on the quantity of feed supplied.

After 18 days of starvation, short-term refeeding (8 h) was sufficient to markedly increase (10-fold) Fru-2,6-P_2 levels in the liver of gilthead sea bream (Metón et al. 1999a). Moreover, a rapid recovery of the active/total 6PF-2-K ratio to 75% of the values recorded in fed fish was also observed, maybe due to dephosphorylation and allosteric modulation of the enzyme. In contrast, the mRNA levels of the bifunctional enzyme were only slightly increased. Long-term regulation of the bifunctional enzyme activity seems to be modulated by an increase in the levels of both mRNA and protein content (Metón et al. 1999a).

Glycogen metabolism

Liver glycogen contents are extremely variable in fish, representing 1–12% of liver fresh weight (Kaushik 1999). In fish, the importance of glycogen use as energy source during fasting appears to be species-specific. Fish such as European sea bass, gilthead sea bream, rainbow trout, or brown trout *Salmo trutta fario* mobilize glycogen during the initial phase of fast, showing a significant depletion of liver glycogen content (Table 2) (Navarro and Gutiérrez 1995; Metón et al. 2003; Soengas et al. 2006; Pérez-Jiménez et al. 2007). In contrast, fish such as common carp, American eel, and Atlantic salmon rely essentially on lipid stores during food deprivation, whereas glycogen reserves change little (Table 2) (Nagai and Ikeda 1971; Moon 1983; Sundby et al. 1991). A rapid recovery of liver glycogen content after refeeding occurs in species like European sea bass, gilthead sea bream, and rainbow trout (Table 2) (Metón et al. 2003; Soengas et al. 2006; Pérez-Jiménez et al. 2007).

As in mammals, glycogen synthesis (glycogenesis) and breakdown (glycogenolysis) in fish liver are catalyzed by glycogen synthase (GSase) and glycogen phosphorylase (GPase), respectively. These enzymes are sensitive to hormonal control via phosphorylation and dephosphorylation reactions. An increase in glucagon/insulin ratio leads to the activation of adenylyl cyclase and thus to an increase in the formation of intracellular cAMP, which in turn binds to a protein kinase activating it. The activated protein kinase catalyzes the phosphorylation of several proteins, including GPase and GSase. Phosphorylation of GPase results in its activation and subsequent breakdown of glycogen, whereas phosphorylation of GSase results in its inactivation and thus in the inactivation of glycogen synthesis. In contrast, a decrease in glucagon/insulin ratio results in dephosphorylation of GPase and GSase and thus in a drop of glycogen breakdown.

In common carp hepatopancreas GPase-a (active form) activity and cAMP concentration increased, while glycogen content decreased 1 h after intraperitoneal glucagon administration (Sugita et al. 2001b). Similar results were also obtained in this species 2 h after intraperitoneal epinephrine administration (Sugita et al. 2001a). The hormones epinephrine, norepinephrine, and prostaglandin E-2 (PGE2) led to a rapid conversation of existing GPase into the active GPase-a form in rockfish *Sebastes caurinus* and brown bullhead *Ictalurus nebulosus* hepatocytes (Moon et al. 1999). Busby et al. (2002) further observed in rockfish hepatocytes that the intracellular targets for PGE2 were adenylyl cyclase, protein kinase A, and GPase. Moreover, PGE2 activates plasma membrane adenylyl cyclase and GPase in a dose-dependent manner. Incubation of rainbow trout hepatocytes with glucagon and glucagon-like peptide (GLP) induced an increase in cAMP, GPase-a activity, and glucose release, and a decrease in glycogen level (Puviani et al. 1990). In American eel hepatocytes, the magnitude of bovine glucagon effects was dependent on the initial glycogen content of the cells; thus, glucagon increased % GPase-a (represents the ratio of GPase-a to total GPase) only at glycogen concentrations lower than 70 μmol g^{-1} (Foster and Moon 1990b). A direct relationship between hepatocyte glycogen content and total GSase, total GPase, and GPase-a activities was reported in rainbow trout hepatocytes (Pereira et al. 1995). In contrast, an inverse relationship was observed between glycogen content and % GSase-a and the GSase-a/GPase-a ratio (Pereira et al. 1995). These authors further reported that incubation of

Table 2 Liver glycogen content in selected fish species

Fish species	Feeding status	Glycogen	Units of measurement	References
European sea bass (*Dicentrarchus labrax*)	Fed (22 days)	115	mg g^{-1} liver	Pérez-Jiménez et al. (2007)
	Fasted (1 day)	94		
	Fasted (3 days)	67		
	Fasted (9 days)	17		
	Refed (1 day)	4		
	Refed (3 days)	127		
	Refed (12 days)	101		
Gilthead sea bream (*Sparus aurata*)	Fed (17 days)	180	mg g^{-1} liver	Metón et al. (2003)
	Fasted (8 days)	50		
	Fasted (18 days)	0		
	Refed (8 h)	55		
	Refed (2 days)	150		
	Refed (8 days)	170		
	Refed (21 days)	210		
Atlantic salmon (*Salmo salar*)	Fed (7 weeks)	20.2	mg g^{-1} liver	Sundby et al. (1991)
	Fasted (7 weeks)	14.8		
Brown trout (*Salmo trutta fario*)	Fed (1 day)	46	mg g^{-1} liver	Navarro and Gutiérrez (1995)
	Fed (15 days)	39		
	Fed (30 days)	39		
	Fed (50 days)	25		
	Fasted (8 days)	9		
	Fasted (30 days)	9		
	Fasted (50 days)	6		
Common carp (*Cyprinus carpio*)	Fed (10 days)	85	mg g^{-1} liver	Nagai and Ikeda (1971)
	Fasted (2 days)	122		
	Fasted (4 days)	107		
	Fasted (10 days)	88		
	Fasted (15 days)	111		
	Fasted (22 days)	107		
	Fasted (101 days)	16		
Hybrid tilapia (*Oreochromis niloticus* × *O. aureus*)	Fed (8 weeks)	102	mg g^{-1} liver	Hsieh and Shiau (2000)
	Fasted (1 weeks)	89		
	Fasted (2 weeks)	86		
	Fasted (3 weeks)	79		
Cod (*Gadus morhua*)	Fed (8 weeks)	47	mg g^{-1} liver	Sundby et al. (1991)
	Fasted (4 weeks)	34		
American eel (*Anguilla rostrata*)	Fed (6 months)	49	μmol glucosyl	Moon (1983)
	Fasted (6 months)	53	Units g^{-1} liver	
Rainbow trout (*Onchorhynchus mykiss*)	Fed (14 days)	35	μmol glucosyl	Soengas et al. (2006)
	Fasted (14 days)	10	Units g^{-1} liver	
	Refed (7 days)	193		

rainbow trout hepatocytes with insulin (10^{-8} mol l^{-1}) significantly decreased total GPase and GPase-a activities, but had no effect on GSase activities. Similarly, in American eel hepatocytes, insulin decreased cAMP levels and total GPase and % GPase-a activities (Foster and Moon 1990b). In

contrast, in Atlantic salmon hepatic GPase-a activity increased, whereas GPase b (less active form) activity and glycogen content decreased 30 h after an intraperitoneal injection of insulin (Sundby et al. 1991). However, the high levels of plasma cortisol recorded in the mentioned study suggested that fish were stressed, which could interfered with the obtained results. In fact, Morata et al. (1982a) observed in the liver of rainbow trout an increase of GPase activity in response to a severe physical disturbance.

In European perch, the liver GPase activity was considerably lower in fish fed a diet with 14% digestible carbohydrate as compared to fish fed a carbohydrate-free diet, while glycogen content was significantly higher in the first case (Borrebaek and Christophersen 2000). Both in American eel and cod, hepatic GPase activity and liver glycogen content changed little during feed deprivation (Foster and Moon 1989; Sundby et al. 1991), whereas in fasted Atlantic salmon an increase of GPase-a activity and a decrease of liver glycogen content were observed comparatively to the control-fed group (Sundby et al. 1991; Soengas et al. 1996). Furthermore, in the liver of Atlantic salmon total GSase activity was lower in unfed than in fed fish, whereas the % GSase-a was only significantly lower after 42 days of fasting (Soengas et al. 1996). To our knowledge no studies on glycogen metabolic enzymes have so far been undertaken at the molecular level.

Conclusions and perspectives

Available data support the hypothesis that the relatively low capacity of fish, particularly carnivorous species, to efficiently utilize dietary carbohydrates is partially related to an inadequate regulation of hepatic glucose utilization (glycolysis) and production (gluconeogenesis). Glucokinase, which is the first enzyme of the glycolytic pathway, plays a key role in intermediary metabolism by catalyzing the phosphorylation of glucose into glucose-6-phosphate, an intermediate metabolite that may be used in different catabolic metabolic pathways (glycogenesis and pentose-phosphate pathway). In species such as rainbow trout, gilthead sea bream, and common carp, hepatic GK activity is highly induced after feeding dietary carbohydrates, and this induction is related to a high GK gene expression (Panserat et al. 2000b, 2001a; Caseras et al. 2002; Capilla et al. 2003; Metón et al. 2004). The cloning of GK cDNA as well as its nutritional regulation were the first demonstrations of an adaptation of fish to dietary carbohydrates by mechanisms similar to those in rats. Furthermore, data suggested the existence of a rat-type induction of 6PF-2-K/Fru-2,6-P_2ase gene expression by dietary carbohydrates in the liver of species such as rainbow trout and gilthead sea bream (Metón et al. 2000; Panserat et al. 2001c) in association with high levels of PK gene expression (Panserat et al. 2001c). Thus, an ineffective regulation of the glycolytic pathway does not seem to explain the low capacity for dietary carbohydrate utilization by fish.

Gluconeogenesis and glycogenolysis are the metabolic pathways involved in the production of endogenous glucose by the liver. In rats, gene expression of the key enzymes of the gluconeogenetic pathway (PEPCK, FBPase and G6Pase) are induced by fasting and repressed by feeding a carbohydrate-rich diet. Opposite to rats, in rainbow trout liver, PEPCK and FBPase were always highly expressed independently of fish nutritional status (Panserat et al. 2001b, c). Furthermore, in rainbow trout, gilthead sea bream, European sea bass, and Atlantic salmon liver, no modification of PEPCK, FBPase, and G6Pase gene expression and/or activities due to dietary carbohydrates were observed (Tranulis et al. 1996; Panserat et al. 2000c, 2001a, b, c; Caseras et al. 2002; Enes et al. 2006a; Moreira et al. 2008). This suggests that there is a persistent high level of endogenous glucose production in the fish liver due to a lack of regulation of gluconeogenesis by dietary carbohydrates. The use of substances like metformin (an anti-diabetic drug), which is a potential inhibitor of gluconeogenesis, may be useful to test if there is any competition between endogenous and exogenous glucose production.

A possible way to reduce the expression of the key enzymes of gluconeogenic pathway when fish are fed dietary carbohydrates is through the modification of its genome by creating transgenic animals. However, transgenic animals and plants are not well accepted by the consumers. Another possibility is to modify the response of key enzymes of the gluconeogenic pathway to dietary carbohydrates by nutritional programming. Several studies showed that dietary influences exerted at critical development stages early in ontogeny may have long-term consequences

on physiological functions in later life stages (Lucas 1998; Patel and Srinivasan 2002). Indeed, Geurden et al. (2007) applied the concept of nutritional programming to rainbow trout at two early life stages and found that molecular adaptations of the carbohydrate digestive enzymes occurred without, however, any effect on the genes involved in glucose transport (SGLT1) or metabolism (G6Pase). Further studies need to be pursued in this domain.

References

Baanante IV, Garcia de Frutos P, Bonamusa L, Fernandez F (1991) Regulation of fish glycolysis-gluconeogenesis: role of fructose-2,6-P2 and PFK-2. Comp Biochem Physiol 100B:11–17

Blin C, Panserat S, Médale F, Gomes E, Brèque J, Kaushik S et al (1999) Teleost liver hexokinase and glucokinase-like enzymes: partial cDNA cloning and phylogenetic studies in rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*). Fish Physiol Biochem 21:93–102. doi:10.1023/A:1007748204428

Blin C, Panserat S, Kaushik S, Krishnamoorthy R (2000) Partial molecular cloning and tissue distribution of hexokinase I cDNA in common carp. J Fish Biol 56:1558–1561. doi:10.1111/j.1095-8649.2000.tb02166.x

Bonamusa L, Garcia de Frutos P, Fernandez F, Baanante IV (1992) Nutritional effects on key glycolytic-gluconeogenic enzyme activities and metabolite levels in the liver of the teleost fish *Sparus aurata*. Mol Mar Biol Biotechnol 1:113–124

Borrebaek B, Christophersen B (2000) Hepatic glucose phosphorylating activities in perch (*Perca fluviatilis*) after different dietary treatments. Comp Biochem Physiol 125B:387–393

Borrebaek B, Christophersen B (2001) Activities of glucose phosphorylation, glucose-6-phosphatase and lipogenic enzymes in the liver of perch, *Perca fluviatilis*, after different dietary treatment. Aquacult Res 32:221–224. doi:10.1046/j.1355-557x.2001.00018.x

Borrebaek B, Waagbo R, Christophersen B, Tranulis MA, Hemre GI (1993) Adaptable hexokinase with low affinity for glucose in the liver of Atlantic salmon (*Salmo salar*). Comp Biochem Physiol 106B:833–836

Borrebaek B, Christophersen B, Sundby A (2003) Metabolic function of hepatic hexokinase in perch, *Perca fluviatilis*. Aquacult Res 34:235–239. doi:10.1046/j.1365-2109.2003.00809.x

Busby ER, Cooper GA, Mommsen TP (2002) Novel role for prostaglandin E-2 in fish hepatocytes: regulation of glucose metabolism. J Endocrinol 174:137–146. doi:10.1677/joe.0.1740137

Capilla E, Médale F, Navarro I, Panserat S, Vachot C, Kaushik S et al (2003) Muscle insulin binding and plasma levels in relation to liver glucokinase activity, glucose metabolism and dietary carbohydrates in rainbow trout. Regul Pept 110:123–132. doi:10.1016/S0167-0115(02)00212-4

Capilla E, Médale F, Panserat S, Vachot C, Rema P, Gomes E et al (2004) Response of hexokinase enzymes and the insulin system to dietary carbohydrates in the common carp, *Cyprinus carpio*. Reprod Nutr Dev 44:233–242. doi:10.1051/rnd:2004027

Caseras A, Metón I, Fernández F, Baanante IV (2000) Glucokinase gene expression is nutritionally regulated in liver of gilthead sea bream (*Sparus aurata*). Biochim Biophys Acta 1493:135–141

Caseras A, Metón I, Vives C, Egea M, Fernández F, Baanante IV (2002) Nutritional regulation of glucose-6-phosphatase gene expression in liver of the gilthead sea bream (*Sparus aurata*). Br J Nutr 88:607–614. doi:10.1079/BJN2002701

Cowey CB, Walton MJ (1989) Intermediary metabolism. In: Halver JE (ed) Fish nutrition. Academic Press, San Diego, pp 260–329

Cowey CB, Adron JW, Brown DA (1975) Studies on the nutrition of marine flatfish. The metabolism of glucose by plaice (*Pleuronectes platessa*) and the effect of dietary energy source on protein utilization in plaice. Br J Nutr 33:219–231. doi:10.1079/BJN19750026

Cowey CB, Knox D, Walton MJ, Adron JW (1977) The regulation of gluconeogenesis by diet and insulin in rainbow trout (*Salmo gairdneri*). Br J Nutr 38:463–470. doi:10.1079/BJN19770111

Dias J, Rueda-Jasso R, Panserat S, da Conceição LEC, Gomes EF, Dinis MT (2004) Effect of dietary carbohydrate-to-lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis*, Kaup). Aquacult Res 35:1122–1130. doi:10.1111/j.1365-2109.2004.01135.x

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2006a) Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles. Comp Biochem Physiol 143A:89–96

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2006b) Rapid metabolic adaptation in European sea bass (*Dicentrarchus labrax*) juveniles fed different carbohydrate sources after heat shock stress. Comp Biochem Physiol 145A:73–81

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2008a) Growth performance and metabolic utilization of diets with native and waxy maize starch by gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture 274:101–108. doi:10.1016/j.aquaculture.2007.11.009

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2008b) Hepatic glucokinase and glucose-6-phosphatase responses to dietary glucose and starch in gilthead sea bream (*Sparus aurata*) juveniles reared at two temperatures. Comp Biochem Physiol 149A:80–86

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2008c) Rearing temperature enhances hepatic glucokinase but not glucose-6-phosphatase activities in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) juveniles fed with the same level of glucose. Comp Biochem Physiol 150A:355–358

Fernández F, Miquel AG, Cordoba M, Varas M, Metón I, Caseras A et al (2007) Effects of diets with distinct

protein-to-carbohydrate ratios on nutrient digestibility, growth performance, body composition and liver intermediary enzyme activities in gilthead sea bream (*Sparus aurata*, L.) fingerlings. J Exp Mar Biol Ecol 343:1–10. doi:10.1016/j.jembe.2006.10.057

Fideu MD, Soler G, Ruiz-Amil M (1983) Nutritional regulation of glycolysis in rainbow trout (*Salmo gairdneri* R.). Comp Biochem Physiol 74B:795–799

Foster GD, Moon TW (1989) Insulin and the regulation of glycogen metabolism and gluconeogenesis in American eel hepatocytes. Gen Comp Endocrinol 73:374–381. doi:10.1016/0016-6480(89)90194-9

Foster GD, Moon TW (1990a) Control of key carbohydrate-metabolizing enzymes by insulin and glucagon in freshly isolated hepatocytes of the marine teleost *Hemitripterus americanus*. J Exp Zool 62:254–255

Foster GD, Moon TW (1990b) The role of glycogen phosphorylase in the regulation of glycogenolysis by insulin and glucagon in isolated eel (*Anguilla rostrata*) hepatocytes. Fish Physiol Biochem 8:299–309. doi:10.1007/BF00003425

Foster GD, Storey KB, Moon TW (1989) The regulation of 6-phosphofructo-1-kinase by insulin and glucagon in isolated hepatocytes of American eel. Gen Comp Endocrinol 73:382–389. doi:10.1016/0016-6480(89)90195-0

Garcia de Frutos P, Bonamusa L, Fernandez F, Baanante IV (1990) Fructose-2, 6-bisphosphate in liver of *Sparus aurata*: influence of nutritional state. Comp Biochem Physiol 96B:63–65

Garcia de Frutos P, Bonamusa L, Baanante IV (1991) Metabolic changes in fish liver during the starved-to-fed transition. Comp Biochem Physiol 98A:329–331. doi:10.1016/0300-9629(91)90541-J

Geurden I, Aramendi M, Zambonino-Infante JL, Panserat S (2007) Early feeding of carnivorous rainbow trout (*Oncorhynchus mykiss*) with a hyperglucidic diet during a short period: effect on dietary glucose utilisation in juveniles. Am J Physiol Regul Integr Comp Physiol 292:R2275–R2283. doi:10.1152/ajpregu.00444.2006

Hanson RW, Reshef L (1997) Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. Annu Rev Biochem 66:581–611. doi:10.1146/annurev.biochem.66.1.581

Hemre G-I, Mommsen TP, Krogdahl Å (2002) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquacult Nutr 8:175–194. doi:10.1046/j.1365-2095.2002.00200.x

Hilton JW, Atkinson JL (1982) Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets. Br J Nutr 47:597–607. doi:10.1079/BJN19820071

Hsieh SL, Shiau SY (2000) Effects of diets containing different carbohydrates on starved condition in juvenile tilapia *Oreochromis niloticus* × *O. aureus*. Fish Sci 66:32–37. doi:10.1046/j.1444-2906.2000.00004.x

Kaushik S (1999) Nutrition glucidique: intérêt et limites des apports de glucides. In: Guillaume J, Kaushik S, Bergot P, Métailler R (eds) Nutrition et alimentation des poissons et crustacés. INRA Editions, France, pp 171–186

Kirchner S, Kaushik S, Panserat S (2003a) Effect of partial substitution of dietary protein by a single gluconeogenic dispensable amino acid on hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol 134A:337–347

Kirchner S, Kaushik S, Panserat S (2003b) Low protein intake is associated with reduced hepatic gluconeogenic enzyme expression in rainbow trout (*Oncorhynchus mykiss*). J Nutr 133:2561–2564

Kirchner S, Seixas P, Kaushik S, Panserat S (2005) Effects of low protein intake on extra-hepatic gluconeogenic enzyme expression and peripheral glucose phosphorylation in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol 140B:333–340

Klover PJ, Mooney RA (2004) Hepatocytes: critical for glucose homeostasis. Int J Biochem Cell Biol 36:753–758. doi:10.1016/j.biocel.2003.10.002

Knox D, Walton MJ, Cowey CB (1980) Distribution of enzymes of glycolysis and gluconeogenesis in fish tissues. Mar Biol (Berl) 56:7–10. doi:10.1007/BF00390588

Krasnov A, Teerijoki H, Molsa H (2001) Rainbow trout (*Onchorhynchus mykiss*) hepatic glucose transporter. Biochim Biophys Acta 1520:174–178

Lin JH, Shiau SY (1995) Hepatic enzyme adaptation to different carbohydrates in juvenile tilapia *Oreochromis niloticus* × *O. aureus*. Fish Physiol Biochem 14:165–170

Lucas A (1998) Programming by early nutrition: an experimental approach. J Nutr 458:401S–406S

Metón I, Caseras A, Mediavilla D, Fernández F, Baanante IV (1999a) Molecular cloning of a cDNA encoding 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase from liver of *Sparus aurata*: nutritional regulation of enzyme expression. Biochim Biophys Acta 1444:153–165

Metón I, Mediavilla D, Caseras A, Canto E, Fernández F, Baanante IV (1999b) Effect of diet composition and ration size on key enzyme activities of glycolysis-gluconeogenesis, the pentose phosphate pathway and amino acid metabolism in liver of gilthead sea bream (*Sparus aurata*). Br J Nutr 82:223–232

Metón I, Caseras A, Fernández F, Baanante IV (2000) 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene expression is regulated by diet composition and ration size in liver of gilthead sea bream, *Sparus aurata*. Biochim Biophys Acta 1491:220–228

Metón I, Fernández F, Baanante IV (2003) Short- and long-term effects of refeeding on key enzyme activities in glycolysis-gluconeogenesis in the liver of gilthead seabream (*Sparus aurata*). Aquaculture 225:99–107. doi:10.1016/S0044-8486(03)00281-3

Metón I, Caseras A, Fernández F, Baanante IV (2004) Molecular cloning of hepatic glucose-6-phosphate catalytic subunit from gilthead sea bream (*Sparus aurata*): response of its mRNA levels and glucokinase expression to refeeding and diet composition. Comp Biochem Physiol 138B:145–153

Mommsen TP, Plisetskaya EM (1991) Insulin in fishes and agnathans: history, structure and metabolic regulation. Rev Aquat Sci 4:225–259

Mommsen TP, Suarez RK (1984) Control of gluconeogenesis in rainbow trout hepatocytes: role of pyruvate branchpoint and phosphoenolpyruvate-pyruvate cycle. Mol Physiol 6:9–18

Moon TW (1983) Metabolic reserves and enzyme activities with food deprivation in immature American eels *Anguilla rostrata* (LeSueur). Can J Zool 61:802–811

Moon TW (2001) Glucose intolerance in teleost fish: face or fiction? Comp Biochem Physiol 129B:243–249

Moon TW, Busby ER, Cooper GA, Mommsen TP (1999) Fish hepatocyte glycogen phosphorylase—a sensitive indicator for hormonal modulation. Fish Physiol Biochem 21:15–24. doi:10.1023/A:1007762229093

Morata P, Faus MJ, Perez-Palomo M, Sánchez-Medina F (1982a) Effect of stress on liver and muscle glycogen phosphorylase in rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol 72B:421–425

Morata P, Vargas AM, Sánchez-Medina F, Garcia M, Cardenete G, Zamora S (1982b) Evolution of gluconeogenic enzyme activities during starvation in liver and kidney of the rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol 71B:65–70

Moreira IS, Peres H, Couto A, Enes P, Oliva-Teles A (2008) Temperature and dietary carbohydrate levels effects on performance and metabolic utilisation of diets in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 274:153–160. doi:10.1016/j.aquaculture.2007.11.016

Nagai M, Ikeda S (1971) Carbohydrate metabolism in fish. I. Effects of starvation and dietary composition on the blood glucose level and the hepatopancreatic glycogen and lipid contents in carp. Bull Jpn Soc Sci Fish 37:404–409

Nagayama F, Ohshima H (1974) Studies on enzyme-system of carbohydrate-metabolism in fish. 1. Properties of liver hexokinase. Bull Jpn Soc Sci Fish 40:285–290

Nagayama F, Ohshima H, Takeushi T (1973) Activities of hexokinase and glucose dehydrogenase in fish liver. Bull Jpn Soc Sci Fish 39:1349

Nagayama F, Ohshima H, Suzuki H, Ohshima T (1980) A hexokinase from fish liver with wide specificity for nucleotides as phosphoryl donor. Biochim Biophys Acta 615:85–93

Navarro I, Gutiérrez J (1995) Fasting and starvation. In: Hochachka PW, Mommsen TP (eds) Metabolic biochemistry. Elsevier Science, Amsterdam, pp 393–434

Panserat S, Blin C, Médale F, Plagnes-Juan E, Brèque J, Krishnamoorthy J et al (2000a) Molecular cloning, tissue distribution and sequence analysis of complete glucokinase cDNAs from gilthead seabream (*Sparus aurata*), rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). Biochim Biophys Acta 1474:61–69

Panserat S, Médale F, Blin C, Brèque J, Vachot C, Plagnes-Juan E et al (2000b) Hepatic glucokinase is induced by dietary carbohydrates in rainbow trout, gilthead seabream, and common carp. Am J Physiol Regul Integr Comp Physiol 278:R1164–R1170

Panserat S, Médale F, Brèque J, Plagnes-Juan E, Kaushik S (2000c) Lack of significant long-term effect of dietary carbohydrates on hepatic glucose-6-phosphatase expression in rainbow trout (*Oncorhynchus mykiss*). J Nutr Biochem 11:22–29. doi:10.1016/S0955-2863(99)00067-4

Panserat S, Capilla E, Gutiérrez J, Frappart PO, Vachot C, Plagnes-Juan E et al (2001a) Glucokinase is highly induced and glucose-6-phosphatase poorly repressed in liver of rainbow trout (*Oncorhynchus mykiss*) by a single meal with glucose. Comp Biochem Physiol 128B:275–283

Panserat S, Plagnes-Juan E, Brèque J, Kaushik S (2001b) Hepatic phosphoenolpyruvate carboxykinase gene expression is not repressed by dietary carbohydrates in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 204:359–365

Panserat S, Plagnes-Juan E, Kaushik S (2001c) Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 204:2351–2360

Panserat S, Perrin A, Kaushik S (2002a) High dietary lipids induce liver glucose-6-phosphatase expression in rainbow trout (*Oncorhynchus mykiss*). J Nutr 132:137–141

Panserat S, Plagnes-Juan E, Kaushik S (2002b) Gluconeogenic enzyme gene expression is decreased by dietary carbohydrates in common carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*). Biochim Biophys Acta 1579:35–42

Párrizas M, Planas J, Plisetskaya EM, Gutiérrez J (1994) Insulin receptors and its tyrosine kinase activity in skeletal muscle of carnivorous and omnivorous fish. Am J Physiol 266:R1944–R1950

Patel S, Srinivasan M (2002) Metabolic programming: causes and consequences. J Biol Chem 277:1629–1632. doi:10.1074/jbc.R100017200

Pereira C, Vijayan MM, Storey KB, Jones RA, Moon TW (1995) Role of glucose and insulin in regulating glycogen synthase and phosphorylase activities in rainbow trout hepatocytes. J Comp Physiol 165B:62–70

Pérez-Jiménez A, Guedes MJ, Morales AE, Oliva-Teles A (2007) Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. Aquaculture 265:325–335. doi:10.1016/j.aquaculture.2007.01.021

Petersen TDP, Hochachka PW, Suarez RK (1987) Hormonal control of gluconeogenesis in rainbow trout hepatocytes: regulatory role of pyruvate kinase. J Exp Zool 243:173–180. doi:10.1002/jez.1402430202

Pilkis S, Granner DK (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 54:885–909. doi:10.1146/annurev.ph.54.030192.004321

Printz RL, Magnuson MA, Granner DK (1993) Mammalian glucokinase. Annu Rev Nutr 13:463–496. doi:10.1146/annurev.nu.13.070193.002335

Puviani AC, Ottolenghi C, Gavioli ME, Fabbri E, Brighenti L (1990) Action of glucagon and glucagon-like peptide on glycogen metabolism of trout isolated hepatocytes. Comp Biochem Physiol 96B:387–391

Shikata T, Kheyyali D, Shimeno S (1993) Effect of feeding rates on hepatopancreatic enzymes and body composition in common carp. Nippon Suisan Gakkaishi 59:835–839

Shikata T, Iwanaga S, Shimeno S (1994) Effects of dietary glucose, fructose, and galactose on hepatopancreatic enzyme activities and body composition in carp. Fish Sci 60:613–617

Shimeno S, Ikeda S (1967) Studies on glucose-6-phosphatase of aquatic animals. II. The enzyme's activities in fish tissues. Bull Jpn Soc Sci Fish 33:112–116

Soengas JL, Strong EF, Fuentes J, Veira JAR, Andres MD (1996) Food deprivation and refeeding in Atlantic Salmon, *Salmo Salar*—effects on brain and liver carbohydrate and ketone bodies metabolism. Fish Physiol Biochem 15:491–511. doi:10.1007/BF01874923

Soengas JL, Polakof S, Chen X, Sangiao-Alvarellos S, Moon TW (2006) Glucokinase and hexokinase expression and

activities in rainbow trout tissues: changes with food deprivation and refeeding. Am J Physiol Regul Integr Comp Physiol 291:R810–R821. doi:10.1152/ajpregu.00115.2006

Stone DAJ (2003) Dietary carbohydrate utilization by fish. Rev Fish Sci 11:337–369. doi:10.1080/10641260390260884

Suarez RK, Mommsen TP (1987) Gluconeogenesis in teleost fishes. Can J Zool 65:1869–1882

Suarez MD, Sanz A, Bazoco J, Garcia-Gallego M (2002) Metabolic effects of changes in the dietary protein: carbohydrate ratio in eel (*Anguilla anguilla*) and trout (*Oncorhynchus mykiss*). Aquacult Int 10:143–156. doi:10.1023/A:1021371104839

Sugita T, Shimeno S, Hosokawa H, Masumoto T (1999) Response of hepatopancreatic enzyme activities and metabolic intermediate concentrations to bovine insulin and glucose administration in carp *Cyprinus carpio*. Nippon Suisan Gakkaishi 65:896–900

Sugita T, Shimeno S, Ohkubo Y, Hsokawa H, Masumoto T (2001a) Response of enzyme activities and metabolic intermediate concentrations to epinephrine administration in hepatopancreas and muscle of carp. Fish Sci 67:281–286. doi:10.1046/j.1444-2906.2001.00231.x

Sugita T, Shimeno S, Ohkubo Y, Hsokawa H, Masumoto T (2001b) Response of enzyme activities and metabolic intermediate concentrations to glucagon administration in hepatopancreas and muscle of carp. Fish Sci 67:157–162. doi:10.1046/j.1444-2906.2001.00212.x

Sundby A, Hemre GI, Borrebaek B, Christophersen B, Blom AK (1991) Insulin and glucagon family peptides in relation to activities of hepatic hexokinase and other enzymes in fed and starved Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Comp Biochem Physiol 100B:467–470

Tan Q, Xie S, Zhu X, Lei W, Yang Y (2006) Effect of dietary carbohydrate sources on growth performance and utilization for gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther). Aquacult Nutr 12:61–70. doi:10.1111/j.1365-2095.2006.00382.x

Tranulis MA, Dregni O, Christophersen B, Krogdahl A, Borrebaek B (1996) A glucokinase-like enzyme in the liver of Atlantic salmon (*Salmo salar*). Comp Biochem Physiol 114B:35–39

Tranulis MA, Christophersen B, Borrebaek B (1997) Glucokinase in Atlantic halibut (*Hippoglossus hippoglossus*) Brockmann bodies. Comp Biochem Physiol 116B:367–370

Tung P-H, Shiau S-Y (1991) Effects of meal frequency on growth-performance of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, fed different carbohydrate diets. Aquaculture 92:343–350. doi:10.1016/0044-8486(91)90039-A

van de Werve G, Lange A, Newgard C, Mechin MC, Li YZ, Berteloot A (2000) New lessons in the regulation of glucose metabolism taught by the glucose 6-phosphatase system. Eur J Biochem 267:1533–1549. doi:10.1046/j.1432-1327.2000.01160.x

Walton MJ (1986) Metabolic effects of feeding a high protein/low carbohydrate diet as compared to a low protein/high carbohydrate diet to rainbow trout *Salmo gairdneri*. Fish Physiol Biochem 1:7–15. doi:10.1007/BF02309589

Walton MJ, Cowey CB (1979) Gluconeogenesis from serine in rainbow trout liver. Comp Biochem Physiol 62B:497–499

Walton MJ, Cowey CB (1982) Aspects of intermediary metabolism in salmonid fish. Comp Biochem Physiol 73B:59–79

Wilson RP (1994) Utilization of dietary carbohydrate by fish. Aquaculture 124:67–80. doi:10.1016/0044-8486(94)90363-8

Wright JJR, O'-hali W, Yang H, Han X, Bonen A (1998) GLUT-4 Deficiency and severe peripheral resistance to insulin in the teleost fish tilapia. Gen Comp Endocrinol 111:20–27. doi:10.1006/gcen.1998.7081

Yamada K, Noguchi T (1999) Nutrient and hormonal regulation of pyruvate kinase gene expression. Biochem J 337:1–11. doi:10.1042/0264-6021:3370001