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Short- and long-term nutritional modulation of acetyl-CoA carboxylase activity in selected tissues of rainbow trout (*Oncorhynchus mykiss*)

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Acetyl-CoA carboxylase (ACoAC) catalyses the carboxylation of acetyl-CoA into malonyl-CoA. This product plays a pivotal role in the regulation of energy metabolism since it is both a substrate for fatty acid synthesis and an inhibitor of the oxidative pathway. The present study was initiated to analyse the modulation of ACoAC activity in liver and selected extrahepatic tissues of rainbow trout (*Oncorhynchus mykiss*) by dietary changes as a contribution to the understanding of the nutritional control of lipid metabolism in fish. Short-term effects of food intake were studied by measuring ACoAC activity in the liver and dorsal white muscle at different time intervals after a meal. Only slight variations were observed in the muscle during the period 2–72 h after the meal. The long-term effects of an increase in dietary lipids or carbohydrates levels were examined by measuring ACoAC activity in the liver, adipose tissue, intestine, kidney, red muscle, dorsal and ventral white muscles of trout after 3 months of feeding with different diets. ACoAC activity is stimulated by a high-digestible starch diet in the abdominal adipose tissue and the white muscle. A high-lipid diet decreases ACoAC activity in the liver and the intestine, but not in other tissues. Contrary to mammals, a rapid adaptation of ACoAC activity to food supply is not effective in rainbow trout. However, a long-term nutritional control of ACoAC activity does occur in this species, but the target tissue differs with the predominant non-protein energy sources in the diet. The present results suggest the potential existence of two ACoAC isoforms with different tissue distribution as has been observed in mammals and birds.

Acetyl co-enzyme A carboxylase: Nutritional regulation: Rainbow trout

Acetyl-CoA carboxylase (ACoAC) catalyses the ATP- and bicarbonate-dependent carboxylation of acetyl-CoA into malonyl-CoA. This reaction is generally considered as the rate-limiting step of the fatty acid synthesis (Wakil *et al*. 1983; Iritani *et al*. 1984). Malonyl-CoA is the key metabolic signal for the control of both fatty acid synthesis and fatty acid oxidation in response to dietary changes. Indeed, it is a substrate for fatty acid synthase and it modulates the transport of fatty acids into mitochondria by inhibiting carnitine palmitoyltransferase-1 (Brown & McGarry, 1997). ACoAC links fatty acid and carbohydrate metabolism through the shared intermediate acetyl-CoA, the product of the pyruvate dehydrogenase and the donor of all except two of the C for the synthesis of long-chain fatty acids (Hillgartner *et al*. 1996). Thus, ACoAC represents a key enzyme in the regulation of energy homeostasis in animals and plays a major role in lipid deposition in different body compartments through its product malonyl-CoA. In this context, the study of the modulation of this carboxylase by macronutrients is a particularly important issue in the control of flesh quality in animal production, including fish.

In mammals, ACoAC is highly regulated by diet, hormones and other physiological factors (Pape *et al*. 1988; Mabrouk *et al*. 1990; Iritani, 1993; Kim, 1997). Short-term changes in activity are mediated by allosteric and covalent modification mechanisms (Kim *et al*. 1989). ACoAC activity is also controlled by long-term changes in enzyme concentrations (Evans & Witters, 1988; Pape *et al*. 1988; Takai *et al*. 1988; Foufelle *et al*. 1992). Food intake increases ACoAC activity, while starvation

Abbreviations: ACoAC, acetyl-CoA carboxylase; HL, low-digestible starch, low-fat diet; HS, high-digestible starch, low-fat diet; M, low-digestible starch, low-fat diet.

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or diabetes mellitus decreases activity (Kim & Tae, 1994; Abu-Elheiga *et al*. 2001).

In mammals and birds, ACoAC is expressed in many extra-hepatic tissues including some non-lipogenic tissues (heart, kidney, muscle, etc.) (Bianchi *et al*. 1990; Iverson *et al*. 1990; Thampy & Koshy, 1991). The importance of different tissues for lipogenesis varies among species. In rat, both liver and adipose tissue are important, the latter is the major lipogenic site in ruminant animals, whereas the liver dominates in chicken (Vernon *et al*. 1999). In most fish species including salmonids, the liver is the major site for lipogenesis, the adipose tissue being another site of lesser importance (Henderson & Sargent, 1981; Likimani & Wilson, 1982; Sargent *et al*. 1989).

In teleosts, ACoAC has been studied mainly for its role on fatty acids biosynthesis, so studies on this enzyme were conducted on the liver or primary cultures of hepatocytes (Iritani *et al*. 1984; Gnoni & Muci, 1990; Segner & Böhm, 1994; Dias *et al*. 1998; Alvarez *et al*. 2000). Information on the regulation of ACoAC by dietary factors is also restricted to a small number of fish species (Poston & McCartney, 1974; Gnoni & Muci, 1990; Dias *et al*. 1998). Until now, the roles of ACoAC in energy metabolism in lipogenic and non-lipogenic tissues remain largely unexplored in these animals.

The present work was undertaken to explore the short- and long-term nutritional modulation of ACoAC activity in selected extra hepatic tissues in comparison with the liver in rainbow trout (*Oncorhynchus mykiss*). In the present study, 'short-term modulation' should be understood as the changes in ACoAC activity taking place within the hours following a meal and 'long-term modulation' as the changes in ACoAC activity attributable to the increase in dietary starch or in dietary lipid in trout fed the experimental diet for 3 months. We first studied the postprandial modulation of ACoAC activity in the liver and in a non-lipogenic tissue, the white muscle. In mammals, it has been reported that food intake after a moderate fasting period increases hepatic ACoAC activity within hours after the meal (Kim & Tae, 1994). Our first hypothesis was that it could be different in fish. Indeed, previous studies in fish have shown that several weeks were needed to observe significant diet induced changes of the activity of some hepatic lipogenic enzymes such as glucose-6-phosphate dehydrogenase, malic enzyme, isocitrate dehydrogenase, ATP-citrate lyase and fatty acid synthase (Lin *et al*. 1977*a*,*b*). We also studied whether ACoAC was involved in the nutritional control of fat metabolism (lipogenesis and lipolysis) in the different body compartments of rainbow trout. We examined the consequence of controlled changes of dietary levels of lipids and carbohydrates on ACoAC activity in lipogenic and non-lipogenic tissues of trout adapted to different dietary regimens for 3 months. In terrestrial animals, two isoforms of ACoAC with distinct tissue distributions and functions have been reported and both have been demonstrated to be regulated by diet (Abu-Elheiga *et al*. 2001). Our second hypothesis was that ACoAC activity could be nutritionally modulated in the trout liver, but also in some extra hepatic tissues of this species.

Material and methods

Experimental diets

Experimental dry pelleted diets were formulated with practical ingredients to contain different sources and concentrations of non-protein energy. Ingredients and proximate composition of the experimental diets are presented in Table 1. Concentrations of proteins, essential fatty acids, vitamins and minerals in the diets were chosen to meet the requirements of rainbow trout (National Research Council, 1993). Diet HS was high in digestible starch and low in lipids whereas diet HL was low in digestible starch and high in lipids. Diet M was lower in digestible starch than HS and lower in lipids than diet HL. Protein levels were similar in all diets, but energy level was higher in diet HL in comparison with diets HS and M due to the higher lipid level. All ingredients were finely ground, well mixed and pressed as pellets, which were then dried in a fluid air dryer at 37°C until the moisture was reduced to $<10\,\%$. Proximate composition analyses

Table 1. Main ingredients, chemical composition and gross energy of diets for rainbow trout (*Oncorhynchus mykiss*) containing high-digestible starch, low-fat levels (HS), low-digestible starch, high-fat levels (HL) or low-digestible starch, low-fat levels (M)

	Diets		
	HS	M	HL
Ingredients (g/kg)			
Fish meal*	368·2	334·5	400·0
Soluble fish protein concentrate†	18	5·7	106·7
Soyabean meal‡	0	250	0
Extruded peas§	0	200	235·3
Maize gluten‖	250	0	0
Fish oil¶	85·8	105·8	228
Raw starch	0	74	0
Gelatinised starch	248	0	0
Vitamin mixture**	10	10	10
Mineral mixture††	10	10	10
Binder	10	10	10
Chemical composition			
DM (g/kg)	948	941	946
Crude protein (N × 6·25) (g/kg DM)	438	402	405
Crude fat (g/kg DM)	129	142	283
Starch (g/kg DM)	264	192	116
Digestible starch (g/kg DM)	242	130	93
Gross energy (MJ/kg DM)	21·8	21·0	24·3

* Norwegian herring meal (Norseamink, Sopropêche, Boulogne-sur-Mer, France): 700 g crude protein (N×6·25)/kg.
† CPSP G (Sopropêche): 740 g crude protein (N × 6·25)/kg.
‡ Provisoy (Vamo Mills, Izegem, Belgium): 520 g crude protein (N×6·25)/kg.
§ Aquatex 8071 (Sotexpro, Hermonville, France): 237 g crude protein (N×6·25)/kg.
‖ Glutalys (Roquette Frères, Lestrem, France): 600 g crude protein (N×6·25)/kg.
¶ Feedoil (Sopropêche).
** Vitamin mixture contained (/kg diet). DL-α-tocopheryl acetate 100 mg, sodium menadione bisulfate 10 mg, retinyl acetate 10 mg, DL-cholecalciferol 25 mg, thiamin 1 mg, riboflavin 4 mg, pyridoxine 3 mg, cyanocobalamin 0·01 mg, nicotinic acid 10 mg, pteroylmonoglutamic acid, ascorbic acid 50 mg, inositol 300 mg, biotin 0·2 mg, calcium panthotenate 20 mg, choline 1000 mg.
†† Mineral mixture contained (/kg diet): calcium carbonate 2·15 g, magnesium oxide 1·24 g, ferric sulfate 0·2 g, potassium iodide 0·4 mg, zinc sulfate 40 mg, copper sulfate 30 mg, manganese sulfate 30 mg, dibasic calcium phosphate 5 g, cobalt sulfate 0·2 mg, sodium selenite 0·3 mg, sodium fluoride 10 mg, KCl 0·9 g, NaCl 0·4 g.

of the diets were carried out following the procedures of the Association of Official Analytical Chemists (1990).

The feeding trial was conducted with rainbow trout (initial body weight 120 g) for a period of 3 months at the Institut National de la Recherche Agronomique experimental fish farm (Donzacq, Landes, France) at a constant water temperature of 17±1°C.

Sample collection

Short-term effect of feeding. Five fish (mean weight 250 (SD 47) g) fed the diet M were killed by a blow to the head 2, 5, 8, 12, 18, 24 and 72 h after the meal (08·30 hours). Whole liver and a piece of dorsal white muscle were removed, chopped, immediately frozen in liquid N_2 and stored at −80°C prior to ACoAC activity measurements. Dorsal white muscle was sampled from the anterior body part below the operculum above the lateral line.

Long-term effect of the diets. Five fish from each dietary treatment were killed by a blow to the head 5 h after the meal. Whole liver, whole kidney and samples of dorsal white muscle, ventral white muscle, red muscle, abdominal adipose tissue and intestine were taken, chopped, frozen in liquid N_2 and stored at −80°C until ACoAC activity measurements. Red muscle samples were dissected at the level of the lateral line. Ventral white muscle samples were removed under the lateral line of the fish at the base of the pectoral fins. Intestine samples (about 20 mm) were taken just after the pyloric caeca and briefly washed with demineralised water in order to avoid faecal contamination. At the time of sampling, the mean weights of the fish fed the diet HS, M and HL were 274 (SD 42), 280 (SD 45) and 338 (SD 29) g respectively.

Acetyl-CoA carboxylase assays

Tissue samples were homogenised in 3 vol. ice-cold buffer (0·02 M-Tris–HCl, 0·25 M-mannitol, 2 mM-EDTA, 0·1 M-NaF, 0·5 mM-phenylmethylsulfonyl fluoride, 0·01 M-β-mercaptoethanol, pH 7·4). The fluoride ions were included in the homogenisation buffer to inhibit the phosphatases, and therefore, to conserve the phosphorylation state of ACoAC (Segner & Böhm, 1994). We used this approach to estimate the actual ACoAC activity in the tissues. Homogenates were centrifuged at 30 000 ***g*** at 4°C for 20 min. The soluble protein content of tissue homogenates was determined by the method of Bradford (1976), using bovine serum albumin as the standard. ACoAC activity was assayed on supernatant fractions using an isotopic method (Holland *et al*. 1984). The assay conditions were optimised for trout tissues. Briefly, assays were performed in capped 2 ml centrifuge tubes at 30°C in a final volume of 500 µl. A sample of the supernatant fraction (50 µl) was added to 450 µl temperature-adjusted assay mixture in order to start the reaction. The assay mixture consisted of (final concentrations) 50 mM-Tris–HCl (pH 7·8), 10 mM-$MgCl_2$, 10 mM-potassium citrate, 125 µM-acetyl-CoA, 3·6 mM-glutathione, 1·5 mg bovine serum albumin/ml, 3 mM-ATP and 12·6 mM-sodium [^{14}C]bicarbonate (final specific activity ≥9·2 kBq/µM) and was adjusted to pH 7·4. Blank values were determined by omitting acetyl-CoA from the reaction mixture and routinely gave negligible values. The reaction was stopped after 20 min by addition of 100 µl 5 M-HCl and mixed thoroughly. The acidified samples were left on ice for 15 min, then they were centrifuged to remove the precipitated protein. Samples (400 µl) of the clear supernatant fraction were transferred into scintillation vials and dried overnight at 60°C, according to Hardie & Guy (1980). The dried samples were then dissolved in 400 µl distilled H_2O and 4 ml scintillation liquid (Ultima Gold™; Packard Instrument, Rungis, France) was added before the radioactivity was measured. ACoAC activity units (U), defined as µmol substrate converted to product/min at the assay temperature (30°C), were expressed per mg soluble protein (specific activity) or per g tissue (wet weight).

Statistical analysis

Results are presented as the mean values for each group with the standard deviation and the significance levels of the main effects. One-way ANOVA was used to test the differences between mean values. When appropriate, mean values were compared by the Student–Newman–Keuls test. Statistical significance was tested at a 0·05 probability level. An individual fish was the experimental unit for analysis of all data. All statistics were performed as described in Sokal & Rohlf (1995).

Results

Postprandial changes in activity of acetyl-CoA carboxylase

In the liver, the activity of ACoAC was significantly greater ($P<0·05$) 72 h after feeding compared with other sampling times after the meal (Table 2). However, expressed in terms of specific activity, there was no significant difference between values. In the dorsal white muscle, a maximum specific activity was observed 5 h after feeding, but it was not significantly different from the specific activity observed 24 and 72 h after the meal. A minimum specific activity was detected 18 h after feeding, but it was not significantly different from the values observed 2, 8 and 12 h after the meal. The specific activity of ACoAC was about 200-fold greater in the liver than in the dorsal white muscle, irrespective of the time after the meal (Table 2).

Activity of acetyl-CoA carboxylase in different tissues

Compared with the other tissues, the liver had the highest specific activity of ACoAC, irrespective of the dietary treatments (Table 3). Activities were not significantly different in the adipose tissue, intestine, red muscle, kidney, and ventral and dorsal white muscles, except in trout fed the HS diet where the activity was significantly greater ($P<0·001$) in the adipose tissue than in the other extra-hepatic tissues.

Table 2. Activity of acetyl-CoA carboxylase 2, 5, 8, 12, 18, 24 and 72 h after the meal in the liver and the dorsal white muscle of rainbow trout (*Oncorhynchus mykiss*)*

(Mean values and standard deviations for five fish per group)

	Tissues							
	Liver				Dorsal white muscle			
	mU/g tissue		mU/mg protein		mU/g tissue		mU/mg protein	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Time after meal (h)								
2	226·7^{b}	68·8	4·21	1·24	0·98^{abc}	0·04	0·018^{bc}	0·002
5	209·8^{b}	34·8	4·40	0·76	1·13^{ab}	0·20	0·024^{a}	0·005
8	229·0^{b}	27·3	4·34	0·46	0·98^{abc}	0·16	0·018^{bc}	0·003
12	173·2^{b}	30·8	3·55	0·67	0·85^{bc}	0·05	0·018^{bc}	0·002
18	205·3^{b}	46·9	3·94	1·05	0·77^{c}	0·05	0·016^{c}	0·001
24	201·2^{b}	74·5	3·48	1·38	1·01^{abc}	0·28	0·022^{ab}	0·003
72	314·2^{a}	52·2	4·81	0·61	1·29^{a}	0·29	0·022^{ab}	0·003
Statistical significance of effect (*F*): *P*	0·007		>0·05 (NS)		0·004		0·01	

^{a,b,c}Mean values within a column with unlike superscript letters were significantly different (one-way ANOVA and Student–Newman–Keuls test, $P<0·05$).

* For details of diets and procedures, see Table 1 and p. 805.

Long-term nutritional modulation of acetyl-CoA carboxylase in different tissues

A significant effect of the diets on the ACoAC activity was observed in liver, adipose tissue, intestine and in the ventral and dorsal white muscles of trout fed the experimental diets for 3 months, but not in the red muscle or the kidney (Table 3). The increase in digestible starch in the diet (diet HS *v.* diet M, Table 1) led to a significant increase of the ACoAC specific activity only in the adipose tissue and the dorsal and ventral white muscles. A notable increase was also observed in the liver, but it was not significant. The increase in the dietary lipid content (diet HL *v.* diet M, Table 1) led to a considerable and significant decrease in ACoAC specific activity in the liver (−51 %), but also in the intestine (−95 %, Table 3). The changes were not significant in the other tissues.

Discussion

Based on literature on the tissue distribution of glucose-6-phosphate dehydrogenase, malic enzyme and isocitrate dehydrogenase, Segner & Böhm (1994) concluded that these dehydrogenases producing NADPH for fatty acid synthesis are ubiquitous in fish. Surprisingly, ACoAC has received much less attention than the other enzymes involved in *de novo* lipogenesis in fish. To our knowledge, the present study is the first to examine the tissue distribution of ACoAC and the influence of dietary factors on the ACoAC activity in the extrahepatic tissues of fish. We were able to measure a detectable activity of ACoAC in all the tissues studied but the liver was clearly predominant. This is in accordance with earlier studies showing that the liver is the main site of *de novo* lipogenesis in salmonids (Lin *et al.* 1977*a*; Henderson & Sargent, 1981;

Table 3. Specific activity of Acetyl-CoA carboxylase (mU/mg protein) in the tissues of rainbow trout (*Oncorhynchus mykiss*) fed for a 3-month period a high-digestible starch–low-fat diet (HS), a low-digestible starch–high-fat diet (HL) and a low-digestible starch–low-fat diet (M)*†

(Mean values and standard deviation for five fish per group)

	Diets						
	HS		M		HL		Statistical significance of effect (*F*): *P*
	Means	SD	Mean	SD	Mean	SD	
Tissues							
Liver	12·70^{ax}	2·78	8·80^{ax}	4·29	4·30^{bx}	1·13	0·006
Adipose tissue	2·45^{ay}	0·84	1·03^{by}	0·43	0·81^{by}	0·58	0·002
Intestine	0·197^{az}	0·055	0·189^{ay}	0·026	0·009^{by}	0·015	0·001
Dorsal white muscle	0·014^{az}	0·002	0·011^{by}	0·002	0·010^{by}	0·001	0·017
Ventral white muscle	0·018^{az}	0·002	0·014^{by}	0·001	0·013^{by}	0·001	0·002
Kidney	0·090^{z}	0·016	0·082^{y}	0·017	0·077^{y}	0·011	>0·05 (NS)
Red muscle	0·136^{z}	0·037	0·126^{y}	0·025	0·128^{y}	0·050	>0·05 (NS)
Statistical significance of effect (*F*): *P*	0·0001		0·0001		0·0001		

* For details of diets and procedures, see Table 1 and p. 805.

† All tissues were sampled 5 h after the meal.

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different (the diet induced differences were assessed by one-way ANOVA and Student–Newman–Keuls test, $P<0·05$).

^{x,y,z}Mean values within a column with unlike superscript letters were significantly different (differences between tissues were assessed by one-way ANOVA and Student–Newman–Keuls test, $P<0·05$).

Sargent *et al*. 1989). The values of ACoAc specific activity found in the trout liver (4–12 mU/mg liver protein) are similar to the activities reported for rat and chicken liver (Iritani *et al*. 1984).

In terrestrial animals, including man, there are two isoforms of ACoAc, ACoAC1 and ACoAC2, which are encoded by separate genes and display distinct tissue distributions. ACoAC1 is ubiquitous, but is highly expressed in the tissues that account for the majority of whole-body lipogenesis (the liver and adipose tissue), whereas ACoAC2 is predominantly expressed in tissues that utilise fatty acids as an energy source (i.e. the skeletal muscle and heart), and to a lesser extent in the liver (Thampy, 1989; Bianchi *et al*. 1990; Iverson *et al*. 1990; Abu-Elheiga *et al*. 1995, 1997; Ha *et al*. 1996). Both ACoAC1 and ACoAC2 are regulated by diet (Abu-Elheiga *et al*. 2001). Malonyl-CoA, the product of ACoAC1 and ACoAC2, seems to exist in two independent pools that would differentially control fatty acid synthesis and fatty acid oxidation (Abu-Elheiga *et al*. 2001). The situation in fish is not known, but we found that ACoAC activity was modulated in a different manner in different trout tissues. This observation suggests that ACoAC could be under different control depending on body compartments in rainbow trout, as in mammals.

Until now, the analysis of changes in ACoAC activity after a meal had not been explored in fish. In rats that are subjected to starvation, hepatic ACoAC activity decreases quickly and virtually disappears within 24–48 h (Chilliard, 1993; Iritani, 1993; Kim & Tae, 1994). Upon re-feeding, ACoAC activity increases rapidly 12 h after feeding a fat-free diet and is completely restored 48 h after feeding (Kim & Tae, 1994). We have not been able to detect any significant postprandial changes of ACoAC specific activity in the liver of rainbow trout, even 72 h after feeding (Table 2). In fish, the time course of adaptation of lipogenic enzymes glucose-6-phosphate dehydrogenase, malic enzyme, isocitrate dehydrogenase, ATP-citrate lyase and fatty acid synthase to dietary changes have been reported (Nagayama *et al*. 1972; Lin *et al*. 1977*a*,*b*) to be much longer (several weeks) than in single-stomached terrestrial animals such as pig, rat and chicken. This delay can be linked to the lower body temperature of heterotherms. If we accept the assumption that, as reported for mammals, the nutritional regulation of activities of lipogenic enzymes in fish occurs at a transcriptional level, this longer time course of adaptation observed in fish could be due to the longer time in mRNA level modulation by nutritional stimuli.

As regards the long-term modulation by diet composition, hepatic ACoAC activity was significantly reduced in trout fed the high-fat diet. This finding confirms previous studies showing that an increase in dietary fat level reduced the rate of fatty acid synthesis (Lin *et al*. 1977*c*) and depressed the activities of ACoAC (Poston & McCartney, 1974) and the other lipogenic enzymes (glucose-6-phosphate dehydrogenase, malic enzyme, ATP-citrate lyase and fatty acid synthase) in the liver of fish (Lin *et al*. 1977*a*; Likimani & Wilson, 1982; Jürss *et al*. 1985; Kheyyali *et al*. 1989; Arnesen *et al*. 1993; Shimeno *et al*. 1995*b*, 1996), providing that the increase in dietary lipid was high enough. Indeed, Henderson & Sargent (1981) found no relationship between the levels of dietary lipid in the range 2–10 % and the rate of fatty acid synthesis in rainbow trout liver slices. Results from Lin *et al*. (1977*a*) also show that the activity of lipogenic enzymes in coho salmon (*Oncorhynchus kisutch*) liver was not affected by an increase from 25 to 50 g dietary lipid/kg diet. Thus, an increase in the dietary lipid level inhibits *de novo* fatty acid synthesis in fish liver, but this effect is obvious only when the lipid level exceeds 100 g/kg DM. In the present experiment, the dietary lipid level was increased from 142 to 283 g/kg DM (Table 1), far greater than the previously reported threshold, but closer to commercial diets (180–350 g/kg DM). This situation clearly differentiates fish from most mammals, in which fatty acid synthesis is inhibited by 30–40 g dietary fat/kg (Chilliard, 1993). The significance of this difference is further enhanced by the fact that in fish diets, lipid are mainly provided as marine oils, which are rich in *n*-3 polyunsaturated fatty acids. Indeed, several studies have shown that polyunsaturated fatty acids were very efficient in inhibiting lipogenic enzymes gene expression (Iritani, 1993; Iritani *et al*. 1998). In the intestine, we observed a strong inhibition of ACoAC activity by the HL diet compared with M diet. The intestinal epithelium of teleosts fish is known to be a site of lipid synthesis since absorbed dietary fatty acids are re-esterified into triacylglycerols in this tissue (Sargent *et al*. 1989). Since palmitoyl-CoA is known to strongly inhibit ACoAC activity in eel (*Anguilla anguilla*) liver *in vitro* (Segner & Böhm, 1994), such an inhibition could also occur in the intestine of rainbow trout.

In mammals, hepatic enzymes involved in the lipogenic pathway are stimulated by feeding a carbohydrate-rich diet (Hillgartner *et al*. 1995; Towle *et al*. 1997). Glucose, as one of the precursors of acetyl-CoA, is the primary substrate for fatty acid synthesis in the liver (Newsholme & Start, 1973). A stimulation of lipogenic enzymes by high levels of dietary carbohydrate has also been reported in most of the fish species studied: rainbow trout (*Salmo gairdneri*; Hilton & Atkinson, 1982), sea bass (*Dicentrarchus labrax*; Dias *et al*. 1998), white sturgeon (*Acipenser transmontanus*; Fynn-Aikins *et al*. 1992), channel catfish (*Ictalurus punctatus*; Likimani & Wilson, 1982) and common carp (*Cyprinus carpio*; Shimeno *et al*. 1981, 1995*a*). Thus, the incorporation of available glucose from the diet into hepatic lipogenic routes seems to be a common feature in many species despite the difference in carbohydrates consumption. However, in the present experiment, the increase in dietary level of digestible starch (diet HS *v*. diet M) enhanced ACoAC specific activity in the liver (+44 %), but not significantly so. The response of ACoAC to dietary glucose could be dose-dependant. Studies conducted on isolated hepatocytes of rainbow trout (Alvarez *et al*. 2000) showed that activities of ACoAC, glucose-6-phosphate dehydrogenase, malic enzyme and ATP-citrate lyase were stimulated by an increase in glucose up to 20–25 mM. A further increase in glucose concentration (30 mM) markedly inhibited the activity of glucose-6-phosphate dehydrogenase and malic enzyme but still stimulated ACoAC activity. In contrast, the activity of fatty acid synthase was the highest with

10 mM-glucose and was inhibited by higher concentrations (Alvarez *et al*. 2000). Further experiments are needed to explain the observed differences.

In the white muscle of trout, specific activity of ACoAC was sensitive to food supply (short-term experiment) and was modulated by the dietary level of carbohydrate. In mammals, malonyl-CoA is generated primarily by ACoAC2 in this tissue (Thampy, 1989). Abu-Elheiga *et al*. (2001) reported a 30 % increase in the mitochondrial β-oxidation of fatty acids in the muscle of mutant mice lacking ACoAC2 compared with the wild-type mice. Thus, it appears that malonyl-CoA, synthesised by ACoAC2, controls the mitochondrial fatty acid β-oxidation in muscle through nutrient supply. In rainbow trout white muscle, which is not a site of *de novo* lipogenesis, the modulation of ACoAC by the HS diet could also be related to the control of β-oxidation. It is an important issue since it has been demonstrated that mitochondrial β-oxidation of fatty acids participates in the control of lipid storage in the tissues (Dobbins *et al*. 2001). Contrary to white muscle, we did not observe any effect of the diet on ACoAC activity in the red muscle of rainbow trout. This may be related to the very different functions and properties of these tissues. Red muscle is used for sustained swimming and for maintaining body position against current, whereas white muscle is used intermittently, for sudden movements in case of pursuit or escape (Love, 1970). Red muscle contains about 5-fold more lipid than white muscle and these lipid stores are utilised for the energy production *in situ*, especially during low-speed sustained swimming (van den Thillart & van Raaij, 1995). Variations in locomotion capacity with diet composition may not be tolerable. Other tissues like kidney, heart and brain are all highly capable of burning fatty acids, but their lipid contents are mostly moderate, supporting only local metabolism (van den Thillart & van Raaij, 1995). In the present study, we could not find any significant effect of diets on the ACoAC activity in the kidney. Thus, in catabolic tissues such as the red muscle or the kidney, ACoAC activity is not affected by diet in the long-term. Further studies will have to show if this is related to the presence of specific isoforms.

In the adipose tissue of mammals, ACoAC2 does not appear to be expressed to any appreciable extent (Iverson *et al*. 1990), suggesting that the ACoAC1 isozyme is mainly involved in *de novo* lipogenesis (Vernon *et al*. 1999). Interestingly, we did not find any detectable activity of carnitine palmitoyltransferase-1 in the abdominal adipose tissue of rainbow trout (S Gutieres, M Damon, S Panserat, S Kaushik and F Médale, personal communication), but there was an appreciable ACoAC activity. Moreover ACoAC activity in this tissue was modulated by HS diet. In channel catfish, Likimani & Wilson (1982) observed the stimulation of fatty acid synthase activity by an HS diet in the adipose tissue, but such induction was not found in coho salmon (Lin *et al*. 1977*a*). According to Vernon *et al*. (1999), ACoAC is considered to be the most carbohydrate dependent of the lipogenic enzymes. Our present results suggest that ACoAC is mainly involved in fatty acid biosynthesis in rainbow trout adipose tissue and could play a significant role in the modulation of fatty acid biosynthesis by dietary carbohydrates.

None of the studies conducted in fish examined the incidence of nutritional conditions on ACoAC activity in different extrahepatic tissues, making difficult any comparative analysis. In the present study, we showed that ACoAC activity is significantly stimulated by an HS diet in the adipose tissue and the white muscle. Furthermore, ACoAC activity is inhibited by an HL diet in the liver and the intestine, but not in other tissues. Further studies will have to clarify the roles of ACoAC and malonyl-CoA in the different fish body compartments, particularly as physiological regulators of mitochondria fatty acid oxidation, to search for possible tissue-specific isoforms as was demonstrated in higher vertebrates (Kim, 1997), and finally to explore the underlying mechanisms in the nutritional regulation of ACoAC in fish tissues.

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