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Effects of Sublethal Copper Exposure on Copper Accumulation, Food Consumption, Growth, Energy Stores, and Nucleic Acid Content in Common Carp

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Abstract. Juvenile common carp were exposed for 28 days to three different sublethal copper concentrations (0.20 μM, 0.55 μM, and 0.80 μM). Food consumption was monitored on a daily basis during the exposure period, while growth, copper accumulation, energy stores, and nucleic acid contents were assessed weekly. Copper exposure to 0.80 μM affected both growth and feeding behavior in common carp. At 0.55 μM, growth was affected despite normal food consumption. Even at the lowest copper concentration (0.20 μM), metabolic demand for the fish increased, challenging the carp with an increased demand for food. Copper accumulation mainly occurred in the liver, reaching an equilibrium between uptake and excretion after 1 month of exposure. Substantial biochemical changes were observed at the two highest copper exposure concentrations, but the correlation between growth rate and RNA:DNA ratio was poor considering the substantial differences in growth rate. The use of the RNA:DNA ratio as a sensitive biomarker is questioned.

Growth of an organism is generally used as a sensitive and reliable endpoint in chronic toxicological investigations. Sublethal levels of a wide variety of toxicants have been found to slow the growth of fish larvae or juveniles (see Woltering 1984 for review). This can be due to a reduced food intake, but also due to increased metabolic expenditure for detoxification and maintenance of the normal body functions. Sublethal copper exposure has proven to reduce appetite as well as growth in different fish species (Drummond *et al.* 1973; Benoit 1975; Lett *et al.* 1976; Buckley *et al.* 1982). Even at copper concentrations where food consumption is not affected, copper exposure has been known to slow growth (Collvin 1985).

Before changes in growth occur, changes, in biochemical composition should become apparent. The use of energy stores (glycogen, fat) might be initiated, and protein synthesis might decrease. Protein is a major component of an organism's body mass, and RNA is necessary for the synthesis of protein. Consequently, a positive relationship between the concentration of RNA and the rate of protein synthesis has been suggested. Typically, maximum RNA:DNA ratios should occur during peaks of protein production. Based on these observation, protein, RNA and DNA relationships have been implied as a promising biomarker of reduced growth (Giesy and Graney 1989; Niimi 1990; Jobling 1994; Heath 1995). The RNA:DNA ratio has been useful in some cases to detect starvation as well as toxic effects (Bucley 1979; Barron and Adelman 1984; Cleveland *et al.* 1986; Steinhardt and Eckmann 1992; Malloy and Targett 1994). Other studies do not confirm the RNA:DNA ratio as such a sensitive indicator of growth (Jürss *et al.* 1987; McKee *et al.* 1989; Pinkney *et al.* 1990; Benton *et al.* 1994). The aim of the present study was to evaluate the effects of three sublethal copper concentrations on growth and feeding of common carp, *Cyprinus carpio*, and compare these effects with RNA:DNA ratios in liver and muscle tissue. Copper accumulation in brain, muscle, and liver tissues was also followed. Determination of the biochemical composition included measurement of the energy stores of glycogen and fat, as well as determination of the levels of protein.

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Materials and Methods

Animal Holding and Copper Exposure

Juvenile (1 month old) common carp, *Cyprinus carpio,* were obtained from the fish hatchery at the Agricultural University of Wageningen, The Netherlands. They were grown at the University of Antwerp at the optimal temperature of 25°C (Elliott 1981) in softened Antwerp city tap water (0.875 mM Ca, 0.145 mM Mg, pH 7.0–8.0). Two weeks before starting the experiments, four groups of 40 carp weighing 18 ± 2 g (means ± SD) were transferred into four 150-l aquariums filled with standard moderately hard fresh water (FW) according to standard methods (American Public Health Association 1989: 0.348 mM $CaSO_4 \cdot 2H_2O$; 0.5 mM $MgSO_4 \cdot 2H_2O$; 1.143 mM $NaHCO_3$; 0.054 mM KCl; pH 7.8–8.0). The FW was well aerated for at least 24 h before use. The photoperiod was set at 14-h light, 10-h dark period and temperature remained at 20°C. Carp were fed once a day, with ''Pond Sticks'' (Tetrapond, Henckel). After 15 min, the remaining food was removed. When remaining food could be recovered from all four aquariums (n = 4–6 days a week), it was dried overnight at 60°C and weighed to compare mean food consumption. Water quality was checked daily for pH, ammonia, nitrite, and nitrate and 50% of the water was renewed twice a week.

Copper exposure was started by adding 250, 125, 62.5, or 0 μg copper/L FW by adding copper nitrate solution ($Cu(NO_3)_2 \cdot 2H_2O$

1 g/L) (Merck, Darmstadt). Water was well aerated and mixed for at least 24 h. In the aquariums, water was filtered with Eheim filter, filled with Rivalon synthetic filter wadding. Twice a week 75% of the water was renewed with standard water containing copper. Five times a week the exact amount of copper levels in the aquariums was determined using an atomic absorption spectrophotometer and nominal copper levels were 0.80 ± 0.38 µM, 0.55 ± 0.15 µM, and 0.20 ± 0.08 µM, respectively. When extrapolating from the results of a study by Peres and Pihan (1991) and considering the hardness of the water used in this study (85 mg/L as $CaCO_3$), a 48-h LC50 value for common carp of 3.41 µM was calculated. Marek *et al.* (1991) found a 100% survival for carp with a mean weight of 19 g at copper concentrations up to 1.57 µM during a 10-day exposure period, and 90% survival at a copper concentration of 7.87 µM under the same circumstances. Therefore, the copper concentrations used in this study are considered as sublethal concentrations.

Sampling of Tissue

Liver, white muscle, and brain tissue were sampled before and after 1, 2, 3, and 4 weeks of exposure to copper. On the day the tissues were sampled, fish were not fed in the morning because sampling started at their normal feeding time. During sampling, eight fish were removed from each exposure group. The fish were quickly anesthetized with MS 222, weighed, and decapitated. Liver and white muscle tissues were dissected for biochemical and copper determinations, brain tissue was dissected into three parts (telencephalon, hypothalamus, and brain stem) for copper determination. Tissues were frozen in liquid nitrogen within 5 min of decapitation and stored in an eppendorf tube at −80°C. The remaining fish were weighed for determination of growth rate and fed afterwards. Because fish were sacrificed during the 4-week sampling period in each exposure group, the number of fish for which we could determine growth rate decreased from 32 after 1 week of copper exposure to eight fish after 4 weeks of copper exposure.

Analytical Procedure

The procedure for isolation has been described by McKee and Knowles (1986). Samples were analyzed for protein content by the method of Bradford (1976) by VIS spectrophotometry at a wavelength of 595 nm, for glycogen with the anthrone reagent (Roe and Dailey 1966) by VIS spectrophotometry at a wavelength of 620 nm, for RNA by UV absorption spectrophotometry at a wavelength of 260 nm (Dagg and Littlepage 1972), and for DNA by the method of Vytášek (1982) by fluorescence spectrophotometry at an emission wavelength of 520 nm. Since earlier measurements of lipid content following the procedure described by McKee and Knowles did not give good results, lipid content of liver and white muscle was determined gravimetrically using a Soxtec System (Soxtec System 1047 Hydrolysation Unit and Soxtec System 1043 Extraction Unit). In order to obtain a sufficient amount of tissue for the extraction procedure, samples from the eight fish were pooled by exposure group and day. Therefore, no replicates are available for the lipid determinations and no statistics could be performed.

Statistics

All values are given as means ± SD. Statistics were performed with GraphPad InStat, using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparisons test if significant differences were found. If conditions for ANOVA were not fulfilled, the Kruskal-Walis and Dunn's test were used. For statistical analysis of food consumption, two-way analysis of variance was used, followed by a Tukey HSD comparisons test.

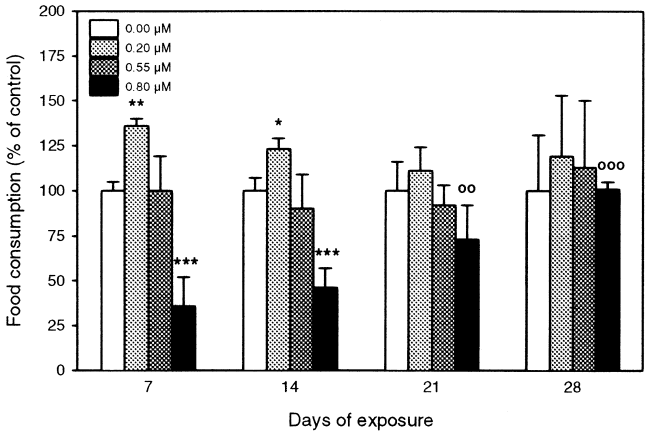

Fig. 1. Food consumption as % of control in common carp during copper exposure to different copper concentrations (mean ± SD, n = 4–6). Significant differences compared to control group are indicated with * (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), significant differences compared to the first week are indicated with o (oo, $p < 0.01$; ooo, $p < 0.001$)

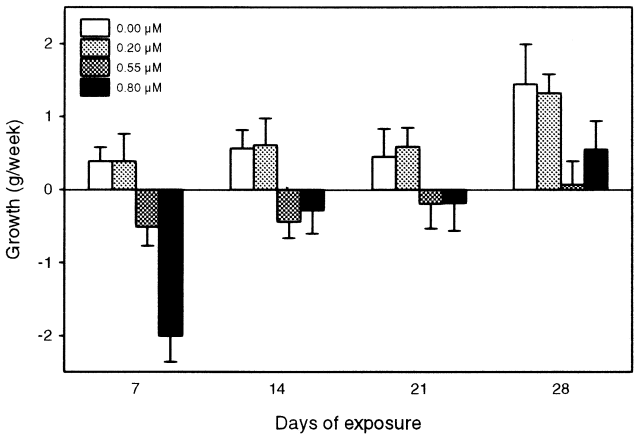

Fig. 2. Mean growth in g/week of common carp exposed to different copper concentrations (mean ± SD, n = 32 at day 7, n = 24 at day 14, n = 16 at day 21, n = 8 at day 28)

Results

From the first day of copper exposure, changes in food consumption could be seen (Figure 1). Fish exposed to 0.80 µM of copper became apathic and slow, and consumed significantly less food during the first 2 weeks of the exposure period. The effect was most striking during the first week, when food consumption dropped to 36% of control values. Food consumption then slowly recovered to control values. Food consumption in the lowest exposure group (0.20 µM) appeared to be stimulated. No changes in food consumption were seen in the group exposed to 0.55 µM of copper.

During the fourth week of the experiment, growth rate in the control group increased compared to the previous weeks,

Table 1. Copper accumulation in brain, muscle, and liver tissues of common carp exposed to 0.00, 0.20, 0.55, and 0.80 µM of copper (means ± SD, n = 8)

	Copper in Water (µM)	Copper Concentrations in Different Tissues (µg/g)				
		Day 0	Day 7	Day 14	Day 21	Day 28
Telencephalon	0.00	1.11 ± 0.12	1.24 ± 0.26	1.18 ± 0.14	1.24 ± 0.20	1.10 ± 0.20
	0.20	1.12 ± 0.13	1.33 ± 0.23	1.32 ± 0.27	1.13 ± 0.11	1.06 ± 0.16
	0.55	1.24 ± 0.28	1.39 ± 0.12	1.39 ± 0.28	1.19 ± 0.16	1.18 ± 0.15
	0.80	1.29 ± 0.16	1.14 ± 0.27	1.44 ± 0.24	1.30 ± 0.22	1.12 ± 0.16
Hypothalamus	0.00	2.11 ± 0.31	1.84 ± 0.23	1.85 ± 0.24	1.60 ± 0.25	1.64 ± 0.31
	0.20	1.89 ± 0.11	1.88 ± 0.19	1.98 ± 0.29	1.58 ± 0.31	1.64 ± 0.09
	0.55	1.85 ± 0.20	1.96 ± 0.23	1.79 ± 0.36	1.70 ± 0.31	1.61 ± 0.27
	0.80	1.94 ± 0.25	2.12 ± 0.18	1.96 ± 0.38	1.76 ± 0.30	1.74 ± 0.44
Brain stem	0.00	1.73 ± 0.33	1.63 ± 0.24	1.70 ± 0.14	1.62 ± 0.15	1.80 ± 0.10
	0.20	1.76 ± 0.14	1.71 ± 0.18	1.74 ± 0.14	1.60 ± 0.10	1.88 ± 0.16
	0.55	1.68 ± 0.15	1.71 ± 0.12	1.80 ± 0.13	1.80 ± 0.21	1.71 ± 0.12
	0.80	1.60 ± 0.04	1.80 ± 0.03	1.89 ± 0.11*	1.99 ± 0.08***	1.89 ± 0.10
Muscle	0.00	0.44 ± 0.14	0.48 ± 0.11	0.40 ± 0.19	0.42 ± 0.06	0.46 ± 0.15
	0.20	0.33 ± 0.07	0.39 ± 0.10	0.44 ± 0.08	0.41 ± 0.05	0.46 ± 0.07
	0.55	0.35 ± 0.06	0.42 ± 0.08	0.41 ± 0.04	0.38 ± 0.08	0.49 ± 0.10
	0.80	0.39 ± 0.06	0.38 ± 0.10	0.41 ± 0.06	0.39 ± 0.06	0.36 ± 0.06
Liver	0.00	19.91 ± 4.78	18.94 ± 5.38	21.93 ± 6.76	25.04 ± 4.96	19.18 ± 3.94
	0.20	20.46 ± 4.10	25.42 ± 6.65	29.29 ± 8.68	29.29 ± 5.58	25.65 ± 4.76
	0.55	19.03 ± 4.76	24.06 ± 3.99	37.66 ± 11.25**	45.09 ± 13.46***	50.26 ± 10.08***
	0.80	19.66 ± 2.95	38.85 ± 8.05***	51.92 ± 8.83***	62.66 ± 7.21***	65.16 ± 11.94***

Significant differences were indicated when accumulation of the copper was significantly higher than controls of the same day (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

presumably because less fish were present in the aquariums. Whereas food consumption in the lowest exposure group was elevated compared to the control group, growth remained very similar to the control group during the entire exposure period, including the fourth week (Figure 2). In the group exposed to 0.55 µM of copper, growth was reduced, and fish even lost weight during the first 3 weeks of exposure, despite the normal food intake. In the highest exposure group, weight loss of the fish was considerable, certainly during the first week of exposure. Both of these groups started to gain weight again during the last week of copper exposure, but growth rates remained lower than those in the control group during the fourth week.

Copper accumulation was followed during the 28-day exposure period in three different brain parts, muscle, and liver tissue (Table 1). The most pronounced copper accumulation was found in the liver. After 1 week of exposure to 0.80 µM of copper, the level of accumulated copper had increased from 20 µg per gram of wet liver tissue to 39 µg. This increase continued: 65 µg copper per gram of wet tissue was measured after 28 days of exposure. At a copper concentration of 0.55 µM, a significant copper accumulation in the liver appeared after 2 weeks. After 4 weeks of continuous exposure, levels had increased to 50 µg/g of wet liver tissue. No significant copper accumulation occurred in muscle tissue; in telencephalon or hypothalamus a significant rise in copper levels was also absent. In brain stem, a significant rise in copper concentration was observed at the highest copper exposure concentration after 2 and 3 weeks only, not after 4 weeks of exposure.

Protein content of white muscle remained stable during the first 3 weeks of exposure (Figure 3), but after 4 weeks of copper exposure protein content dropped in the exposure group of 0.55 µM as well as in the exposure group of 0.80 µM. In liver tissue, a drop in protein levels only occurred at the highest exposure concentration, where levels were significantly reduced after 2 and 3 weeks of copper exposure. After 4 weeks of copper exposure, liver protein levels had returned to a normal level. In muscle tissue, copper exposure had a pronounced effect on glycogen levels (Figure 4). At all three exposure copper concentrations glycogen levels dropped during the second and third week of exposure, although the drop was not significant at the lowest copper concentration. During the fourth week of exposure, glycogen levels in muscle tissue returned to normal. In the liver, on the contrary, glycogen concentrations tended to rise, the effect being significant at the highest copper concentration from the second week forward. In the liver, lipid levels were rather stable and remained roughly between 1.5 and 2% of the wet tissue weight (Figure 5), whereas in muscle lipid levels varied between 0.4 and 1%, except at the highest copper concentration, where lipid levels were elevated during the second half of the exposure time. Due to the pooling of the samples for each group, no statistics could be performed. Both in white muscle and in liver tissue (Figure 6), RNA levels were stable during the entire exposure period. DNA levels (Figure 7) differed only in white muscle after 4 weeks of copper exposure, where they were significantly increased at all three copper exposure concentrations. Therefore, RNA:DNA ratios were quite stable and no good correlation was obtained between growth rate and RNA:DNA ratio, either in muscle or liver tissue (Figure 8). Also protein:RNA or protein:RNA:DNA ratios failed to give a significant indication of growth rates (data not shown).

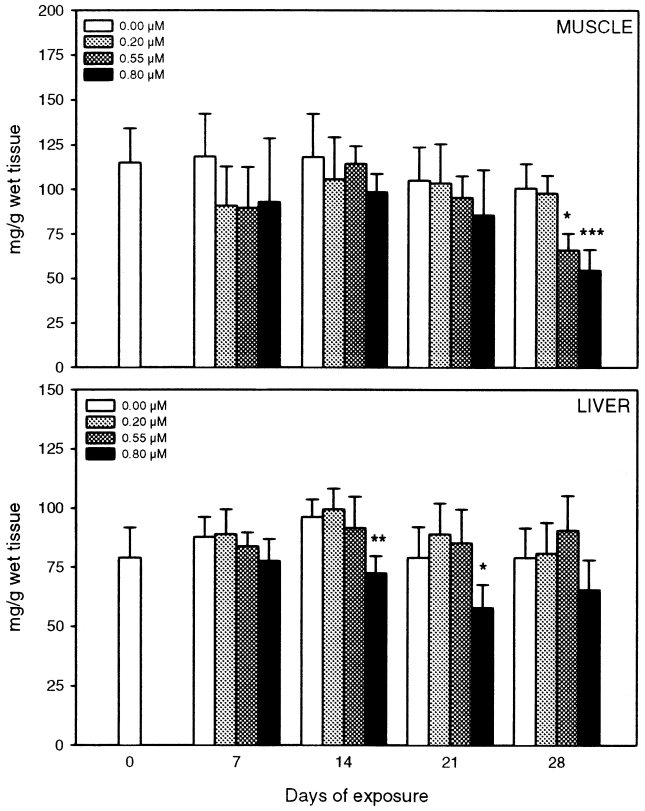

Fig. 3. Protein content of muscle and liver tissue from copper exposed carp (means ± SD, n = 8), significant differences compared to control value at the same day are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

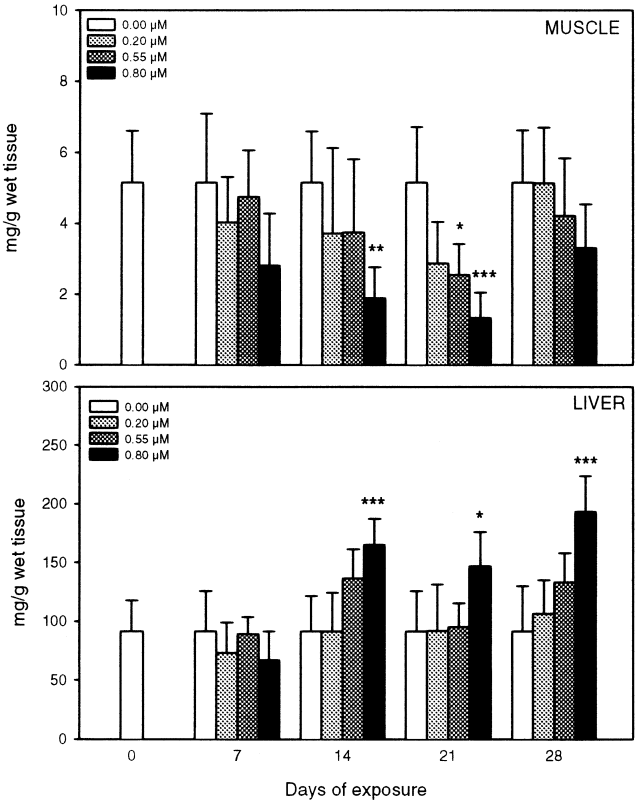

Fig. 4. Glycogen content of muscle and liver tissue from copper exposed carp (means ± SD, n = 8), significant differences compared to control value at the same day are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

Discussion

One of the first marked changes when exposing common carp to sublethal copper concentrations was the immediate reduction of feeding in fish exposed to 0.80 µM of copper; thereafter the feeding rate slowly recovered to control levels during the next weeks of exposure. Consequently, growth was also reduced in this high exposure group, and carp lost weight during the first 3 weeks of exposure. Loss of appetite and reduced growth rate followed by recovery to rates approaching control levels have also been seen in salmonid fishes exposed to copper (Drummond *et al.* 1973; Lett *et al.* 1976; Buckley *et al.* 1982). Whereas feeding levels of the carp exposed to 0.55 µM of copper remained unchanged, growth did not. The fish in this exposure group lost weight during the first 3 weeks of copper exposure; thereafter they slowly started gaining weight again. Despite the increased food consumption of the lowest exposure group, no increase in growth rate was observed. Thus, it appears that the copper-exposed carp spent more energy sustaining their normal metabolism, leaving less energy available for growth. This effect has also been observed in copper-exposed perch (Collvin 1985) and coho salmon (Buckley *et al.* 1982). Presumably, the increase in metabolic rate may have been associated with tissue repair and development of defense and copper-excreting mechanisms.

Tissue damage may be at least partially a cause of the reduced appetite seen in copper-exposed fish. Low-level copper exposure in rainbow trout revealed degeneration of the olfactory system (Julliard *et al.* 1993) and responses to a stimulus (L-alanine) on the olfactory receptors of Atlantic salmon appeared to be disturbed under copper exposure (Winberg *et al.* 1992; Bjerselius *et al.* 1993). In the study by Julliard *et al.* (1993), signs of neural regeneration were reported during the course of the exposure, indicating some form of acclimation, which agrees with the recovery of feeding rate seen. Other neural mechanisms may also be involved in copper-induced loss of appetite: copper exposure has been noted to reduce acetylcholinesterase activity in carp, the consequent increase in acetylcholine contents in nerve endings may disrupt synaptic transmissions between neurones (Nemcsók *et al.* 1984; Nemcsók and Hughes 1988). Also, indications for the involvement of monoamine neurotransmitters exist (De Boeck *et al.* 1995a).

Copper accumulation at the two highest copper concentrations (0.55 and 0.80 µM) clearly occurred mainly in liver tissue. This corroborates the view that this organ is the major storage and regulatory organ in copper homeostasis. After induction by an environmental metal exposure, major concentrations of metallothionein can be found in the liver (Roesijadi 1992). After the first week of exposure to the highest copper concentration, copper concentrations in the tissue were doubled; after the third week, copper concentrations in the tissue were almost tripled. Then, the accumulation rate appeared to slow down, and copper levels after 4 weeks of exposure are comparable to those after 3 weeks of exposure. In brown bullhead, equilibrium

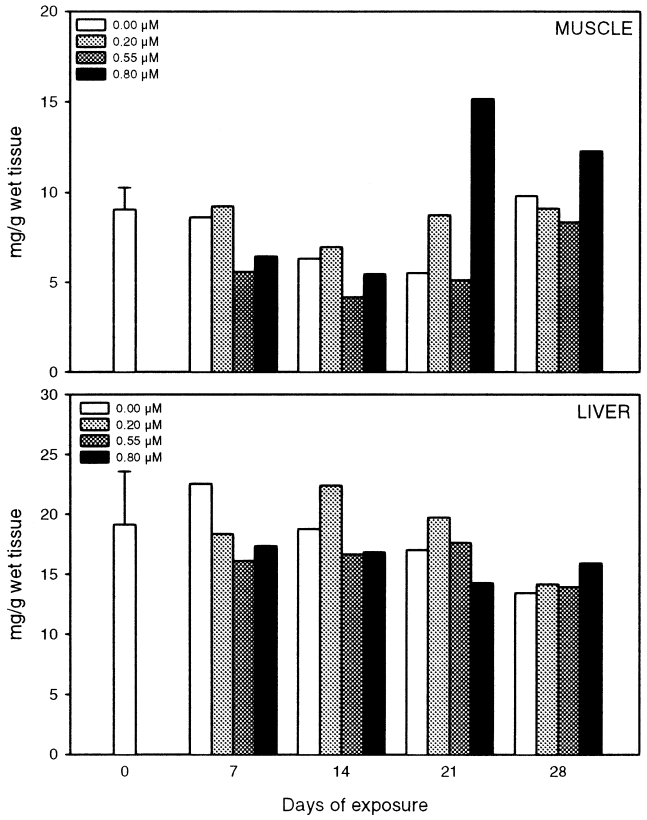

Fig. 5. Lipid content of muscle and liver tissue from copper exposed carp (means from pooled samples of eight fish)

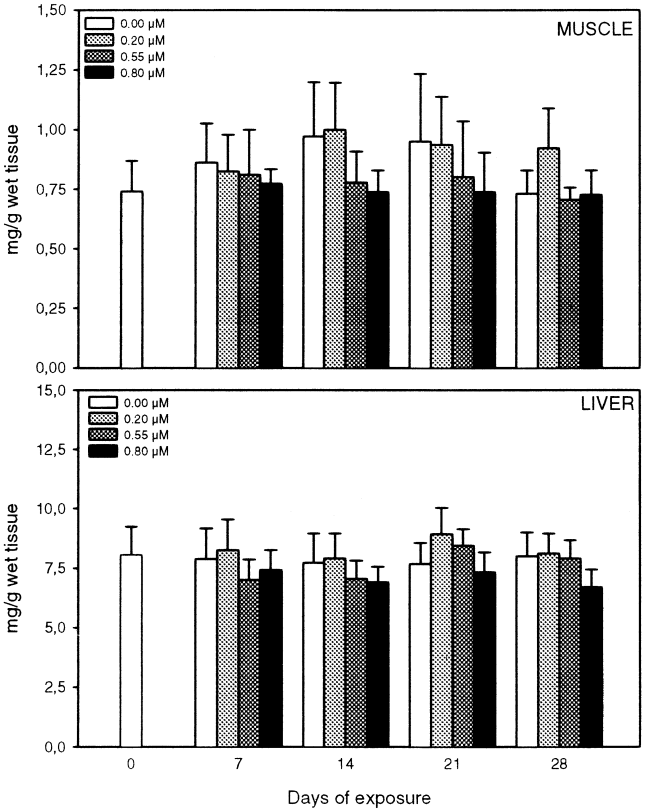

Fig. 6. RNA content of muscle and liver tissue from copper exposed carp (means ± SD, n = 8), no significant differences were found

concentrations of copper in the liver were also reached in 30 days and no great difference was apparent between tissue levels after 30 days or 20 months at copper exposure concentrations of 0.77 to 1.00 µM (Brungs *et al.* 1973). In coho salmon, this equilibrium was coincident with recovery in growth rate (Buckley *et al.* 1982), a fact confirmed by our results.

Copper accumulation in white muscle tissue has been seen in common carp exposed to copper (Nemcsók *et al.* 1987; Marek *et al.* 1991), usually at higher copper exposure concentrations than the ones used here (0.80 to 626 µM). The fact that no rise in white muscle copper levels has been seen here agrees with the view that accumulation in muscle becomes important only when the maximum storage capacity of the liver is reached (Laurén and McDonald 1987). The blood–brain barrier seems to protect the brain rather well from copper toxicity; no rise in copper levels was seen in telencephalon or hypothalamus, and only a temporary increase was noted in brain stem.

One of the first responses to a stressor such as copper exposure is the release of so-called stress hormones: adrenaline, noradrenaline, and cortisol (Wendelaar Bonga 1993). The release of these catecholamines and cortisol triggers a broad suite of biochemical and physiological changes known collectively as *secondary stress responses.* The metabolic effects may include hyperglycemia, hyperlacticemia, depletion of glycogen tissue reserves, lipolysis, and inhibition of protein synthesis. There may also be increased catabolism of muscle protein, and alterations in the plasma levels of amino acids, free fatty acids, and cholesterol (Jobling 1994). The cessation of feeding, accompanied by the catabolic effects of the catecholamines and corticosteroids on the energy reserves stored in the body tissues, must result in reduced growth in stressed fish. The changes seen in muscle tissue agree well with the general picture of secondary stress responses. The muscle glycogen store is depleted during the first 3 weeks of copper exposure. During the fourth week of exposure, when glycogen levels in the white muscle recover, a significant decrease in protein levels appears at the two highest copper concentrations. This protein catabolism is not unusual. Whereas catecholamines are thought to cause the initial elevation in plasma glucose levels by mobilizing the glycogen reserves (glycogenolysis), the corticosteroids may contribute to the maintenance of hyperglycemia via the stimulation of gluconeogenesis from amino acids and thus stimulate protein catabolism. In addition to maintaining hyperglycemia, this increase in synthesis of glucose from amino acids could, in the long run, also result in the restoration of glycogen levels (Jobling 1994), as was seen here for muscle tissue. Concerning this gluconeogenesis, even protein deficiency (necessitating amino acid conservation) does not suppress gluconeogenesis because starving fish can exhibit high rates of gluconeogenesis (Cowey and Sargent 1979). The fact that protein levels decrease during the fourth week of exposure to the two highest copper concentrations does not have to contradict the fact that growth rate recovered at this moment. Although protein content per gram of tissue decreased, total protein content of the organism might have increased. The rise in DNA in muscle tissue at this moment indicates that a reduction of cell size appeared, probably due to cell division considering the regained capacity for growth.

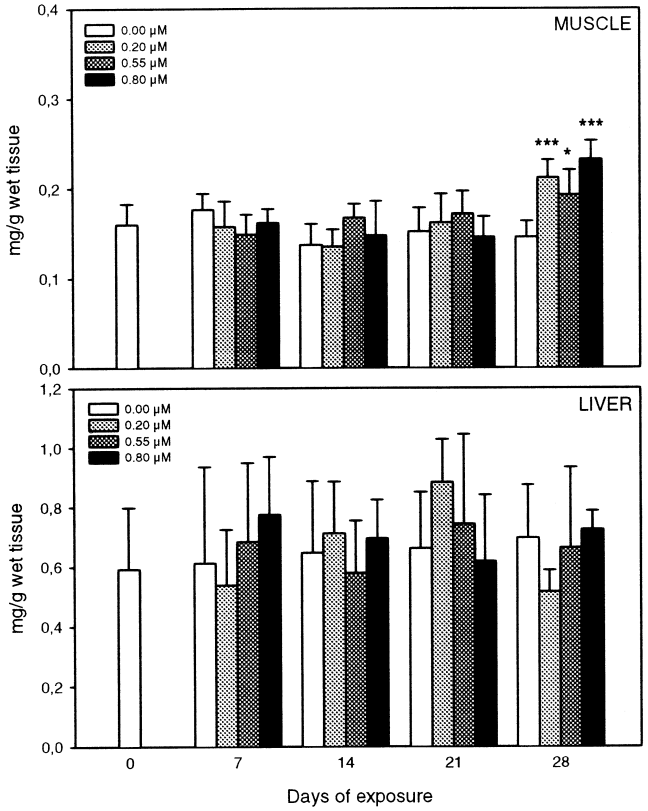

Fig. 7. DNA content of muscle and liver tissue from copper exposed carp (means ± SD, n = 8), significant differences compared to control value at the same day are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

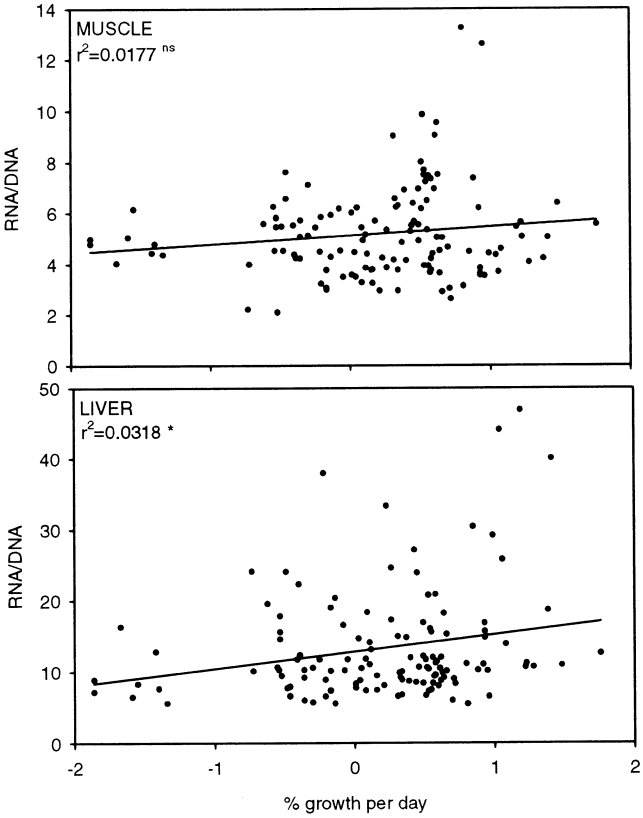

Fig. 8. RNA:DNA ratios of muscle and liver tissue compared to % growth per day measured for the week preceding the sampling of tissues

In the liver, however, a completely different pattern can be seen. After an insignificant decrease in glycogen content during the first week, glycogen levels start to rise at the highest copper exposure concentration. One possible explanation is that carp exposed to the highest copper concentration were experiencing some form of hypoxia. If a fish is kept in oxygen-deficient water for an extended period of time, it will become more hypoxia tolerant. This improved tolerance is attributable to several changes that occur during long-term exposure to nonlethal hypoxia. Besides an improved efficiency for extracting oxygen from the water, there may also be an increase in the tissue glycogen reserves and an elevation in the levels of several enzymes in the liver, leading to a higher gluconeogenic and anaerobic capacity following acclimation to hypoxia (Jobling 1994). Carp in the highest exposure group could very well have experienced hypoxia because copper has been shown to disrupt gill epithelia in carp and salmonids (Benedeczky *et al.* 1986; Marek *et al.* 1991; Kirk and Lewis 1993; Wilson and Taylor 1993). Collapse and fusion of lamellae, lifting of lamellar epithelium away from pillar cells, and swelling of the epithelial cells has been observed. In addition to this ultrastructural damage, an increase in the secretion of mucus and concomitant swelling of the mucus layer around the gill increases the diffusion distance for oxygen. Whereas structural gill damage repairs gradually, swelling of the epithelial cells and thickening of the mucus layer remain for a more extended period. Other experiments showed an impaired oxygen consumption in common carp exposed to similar copper concentrations (De Boeck *et al.* 1995b) and increased lactate levels were seen indicating an increase in anaerobic metabolism (De Boeck *et al.* 1995a). Increased liver glycogen levels could therefore be a consequence of a defense mechanism against this hypoxic condition.

Protein levels are slightly reduced in the liver after the second and third week of exposure to 0.80 µM. Possibly, this muscle catabolism was caused by the need for gluconeogenesis. Lipid stores in muscle and liver tissue do not appear to be a used extensively under these circumstances. Also Lett *et al.* (1976) found no changes in lipid content of rainbow trout during a 40-day exposure period to copper, though growth rates were also initially depressed and recovered subsequently.

In our study, a poor correlation was found between growth rate and RNA:DNA ratios and no correlation was found between growth and protein:RNA or protein:RNA:DNA ratios. Other studies have not been able to establish these relationships either. In rainbow trout liver, Jürss *et al.* (1987) found no relation between the RNA:DNA quotient and growth. Satomi and Tanaka (1973) also found no close correlation between the RNA:DNA quotient and the growth of rainbow trout. Protein synthesis is probably controlled faster and more comprehensively by translation than by the amount of RNA (Jürss *et al.* 1987), and levels of ribosomal RNA could mask changes in messenger RNA. Mathers *et al.* (1993) concluded that increased rates of protein synthesis were due to increased RNA efficiency, not increased RNA content. According to McKee *et al.* (1989) it

is important to measure the nucleic acids at or immediately preceding periods of rapid growth to allow maximum resolution of toxicant effects on RNA ratios, but they also acknowledge that from a practical point of view, this is virtually impossible.

In conclusion, we can say that copper exposure to 0.80 µM immediately affected growth as well as feeding behavior in common carp, where feeding and growth rates slowly recovered. At 0.55 µM, growth is affected despite the normal food consumption. Even at the lowest copper concentration (0.20 µM), metabolic demand for the fish increased, challenging the carp with an increased demand for food. Copper accumulation mainly occurred in the liver, reaching an equilibrium between uptake and excretion after one month of exposure. Substantial biochemical changes were observed at the two highest copper exposure concentrations, but no good correlation was found between growth rate and RNA:DNA ratio. Therefore, the use of the RNA:DNA ratio as a sensitive biomarker is questionable.

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