Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Isoform specific expression of Δ9 desaturases in two brain regions of common carp

Beáta Balla and Edit Hermesz*

Department of Biochemistry and Molecular Biology, Faculty of Science, University of Szeged, Szeged, Hungary

ABSTRACT An appropriate ratio of saturated and unsaturated fatty acids contributes to membrane fluidity. The Δ9 stearoyl-CoA desaturases are key enzymes involved in the regulation of the lipid composition of cellular membranes. The expressions of the Δ9 stearoyl-CoA desaturase genes are regulated by many environmental factors, such as temperature changes and metal exposure, mostly at the levels of transcription and mRNA stability. The present study analyzes the effects of cold shock and Cd^{2+} treatment on the regulation of two Δ9 desaturase genes in the olfactory lobe and the cerebellum of common carp. The effect of Cd^{2+} was also followed on the expressions of the Δ9 desaturase genes of cold-shocked animals. Sudden cold shock or Cd^{2+} exposure induced Δ9 desaturase expressions in a time-, brain region- and isoform-specific manner. In contrast, both Δ9 desaturase genes were repressed by Cd^{2+} in cold-shocked animals.
Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

brain
carp
cold
shock
Δ9
desaturase
heavy metal

Diverse physiological and environmental stresses (*e.g.* hyper- and hypothermia, oxidative injury and heavy metals) produce multiple intracellular changes. Organisms respond to environmental challenges by adaptive responses. Maintenance of the appropriate membrane fluidity is an important adaptive process during temperature shock. The ability of cells to modulate the physical characteristics of the membrane lipids is mainly determined by the action of desaturases. Desaturases are enzymes essential for introducing double bonds into fatty acids (Lee et al. 1990; Nakamura et al. 2002). Δ9 stearoyl-CoA desaturase (Scd, Cds, Δ9 desaturase) is responsible for the introduction of the first *cis* double bond at position Δ9. *Scd* cDNA has been isolated from a variety of vertebrate species. Three variants of the *Scd* gene have been found in rodents (Thiede et al. 1986; Ntambi et al. 1988; Kaestner et al. 1989; Ideta et al. 1995). A single Δ9 desaturase gene has been identified from grass carp (*Ctenopharyngodon idella*), milkfish (*Chanos chanos*) (Chang et al. 2001; Hsieh et al. 2001; Hsieh et al. 2005), thilapia (*Oreochromis mossambicus*) (Hsieh et al. 2004) and zebrafish (*Danio rerio*) (Hsieh et al. 2003). In common carp, (*Cyprinus carpio*) two Δ9 desaturase (*Cds*) genes (*Cds1* and *Cds2*) have been reported (Tiku et al. 1996; Trueman et al. 2000; Polley et al. 2003).

The tissue distribution of the Δ9 desaturases has been extensively studied in a number of fish species. In milkfish, Δ9 desaturase was detected in the liver, brain, kidney, gill, heart and muscle (Hsieh et al. 2001), whereas the Δ9 desaturase transcript is highly expressed in the liver of grass carp, common carp, tilapia and zebrafish, but is not or only barely detectable in the brain (Hsieh et al. 2003; Polley et al. 2003).

To maintain homeostasis under cold shock, ectothermal teleosts increase the membrane fluidity through an increase in the proportion of unsaturated fatty acids in the cell membranes (Cossins et al. 2002; Valko et al. 2005). In common carp, it has been demonstrated that progressive cooling causes a large increase in Δ9 desaturase expression in the liver and the *Cds2* gene is transiently upregulated a few days after cold treatment (Tiku et al. 1996). It has also been shown that the carp liver possesses a latent, inactive form of Δ9 desaturase proteins that is activated shortly after the onset of progressive cooling, before the synthesis of the new Δ9 desaturase (Trueman et al. 2000).

Membrane phospholipids of aerobic organisms are continually subjected to oxidant challenges from endogenous and exogenous sources. Toxic chemical pollutants (especially heavy metals) are important sources of reactive oxygen species in biological systems. It is possible that metal ions cause changes in membrane fluidity; stimulating lipid peroxidation by oxidizing poly-unsaturated fatty acids and causing other damage (Kudo and Waku 1996; Cavaletto et al. 2002). Cadmium itself is unable to generate free radicals directly, but its indirect generation of superoxide and hydroxyl radicals and nitric oxide has been reported (Waisberg et al. 2003; Valko et al. 2005).

It has been observed that the Δ9 desaturase activity in rat hepatocytes is suppressed by Cd^{2+} exposure (Kudo et al. 1991). However, Cd^{2+} itself does not inhibit microsomal Δ9 desaturase activity *in vitro*. It has been suggested that the reduction of the Δ9 desaturase is due to a secondary effect,

Accepted June 19, 2009
*Corresponding author. E-mail: hermesz@bio.u-szeged.hu

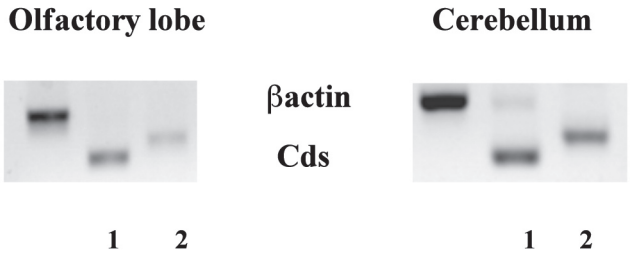

Figure 1. Expressions of carp *Cds1* and *Cds2* genes in the olfactory lobe and cerebellum of untreated animals. A representative result of the RT-PCR amplification. For *Cds1* primer pair Cds11F-CdsR was used, while primers Cds2F/CdsR amplified the *Cds2*-specific sequences. In parallel with the *Cds* isoform-specific transcripts, β-actin mRNA was amplified and used as an internal control to determine the relative levels of the two *Cds* transcripts.

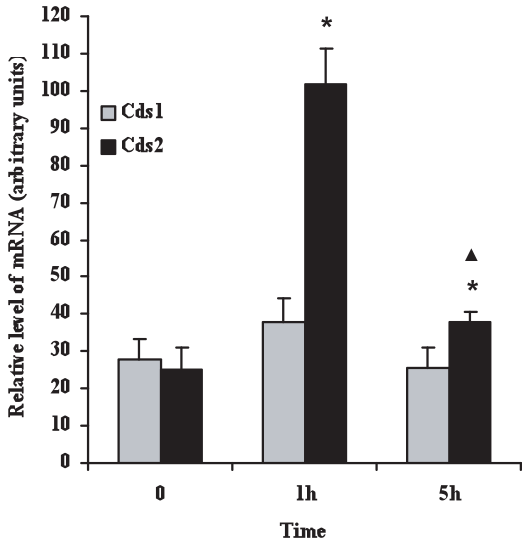

Figure 2. Time courses of expressions of the *Cds1* and *Cds2* genes in the olfactory lobe, followed by hypothermia. 1h and 5h indicate the duration of cold treatments. All data are means ± S.D. of the results of measurements on 4 animals at each time point. Significant difference: ∗, between the control (0) and the value at a given time point; ▲, between the values at consecutive time points of treatments.

brought about through changes in other factors, such as the insulin or glucose level.

One aim of the present study was to learn the consequences of a sudden temperature drop on the expressions of the two the Δ9 desaturase genes in the brain. We also investigated the regulation of the Δ9 desaturase genes of common carp exposed to Cd^{2+} under physiological and cold-shocked conditions, in brain regions with different levels of protection by the blood-brain barrier.

Materials and Methods

Animals and treatments

Carp were acclimatized under fasting conditions over a 3-week period at 12°C. In cold shock experiments fish were transferred from 12°C to 5°C for 1 and 5 h and samples were taken from the tissues immediately after the cold treatment. For Cd exposure the carp were transferred into 100 l water tanks containing 10 mg/l Cd^{2+} ($Cd(CH_3COO)_2x2H_2O$, Sigma-Aldrich Germany, Steinheim) for up to 126 h under static conditions. In the combined experiment, the fish were first kept at 5°C for 1 or 5 h and subsequently exposed to Cd^{2+} at 10 mg/l immediately after the cold shock. In all experiments, 4 animals were sacrificed for organ harvesting at each time point.

RNA extraction, reverse transcription and PCR amplification

Brain was homogenized in RNAzol B reagent (Tel-Test, Inc. USA, Texas) and total RNA was prepared according to the procedure suggested by the manufacturer. Total RNA was routinely treated with 100 U RNAse-free DNAseI (Fermentas, Lithuana, Vilnius) to avoid any DNA contamination.

To detect carp *Cds* specific mRNAs, an RT-PCR-based strategy was employed as earlier described (Hermesz et al. 2001).

Measurement of *Cds* mRNA levels

For amplification of carp *Cds* mRNAs, primers Cds11F, Cds2F and CdsR were used. The sequences of the primers were derived from the common carp *Cds* sequences (GenBank Accession Nos.: AJ249259 and U31864). Primers Cds11F and Cds2 are specific to *Cds1* and *Cds2*, respectively, and were used in pairs with CdsR recognizing both *Cds1* and *Cds2* sequences. The sequences of primers β-actin3 and 4 were derived from GenBank entry M24113 and used to amplify β-actin mRNA for internal standard.

Primers: Cds11F: 5'ccgcgtgcgctacattcgct3', Cds2F: 5'ttcttgtgtttctcagatca3', CdsR: 5'acgagtccatacagagctccg3', β-actin3: 5'gcaagagaggtatcctgacc3', β-actin4: 5'ccctcgtagatgggcacagt3'.

Images of ethidium bromide-stained agarose gels were digitized with a GDS 7500 Gel Documentation System and analyzed with GelBase/GelBlot™ Pro Gel Analysis Software (UVP). The relative levels of *Cds* mRNAs are expressed as ratios [*Cds*/β-actin x100].

Statistical analysis

For each time point of the experiments, 4 fish were used. RT-PCR reactions for each sample were performed in triplicate to increase the reliability of the measurements. Statistical differences were calculated with one-way analysis of variance (ANOVA) (MedCalc Statistical Software version 9.4.2.0,

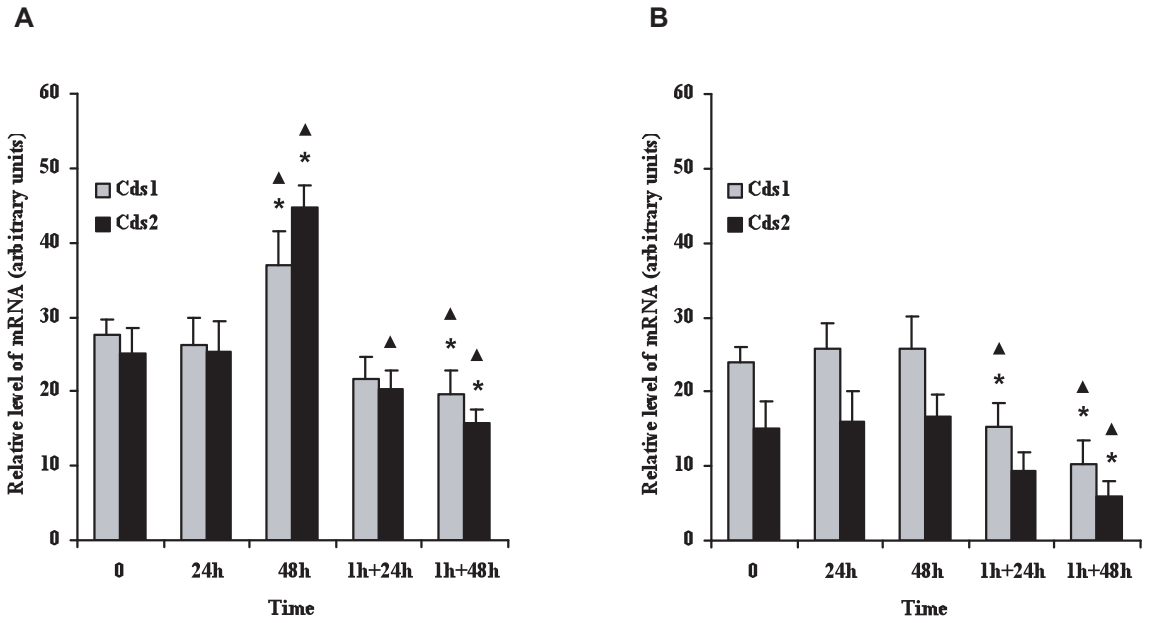

Figure 3. Effects of 10 mg/l Cd^{2+} on the expressions of the *Cds1* and *Cds2* genes in the (A) olfactory lobe and (B) in the cerebellum. 24h and 48h indicate the time period of Cd^{2+} treatment. 1h+24 and 1h+48 indicate the duration of cold shock followed by a 24h and 48h of Cd^{2+} treatments at the acclimatization temperature. All data are means ± S.D. of the results of measurements on 4 animals at each time point. Significant difference: ∗, between the control (0) and the value at a given time point; ▲, between the values at consecutive time points of treatments.

Broekstraat, Belgium) with a Student-Newman-Keuls follow-up test. Significant difference was accepted at $P<0.05$.

Results

Basal level of expression

For the detection and semiquantitative determination of *Cds1* and *Cds2* transcripts, isoform-specific primers were designed and used in RT-PCR reactions. In the control animals, both *Cds1*- and *Cds2*-specific mRNAs were readily detected in the two brain regions examined. The amount of *Cds2* mRNA was always less than that of *Cds1* mRNA in the olfactory lobe, with a ratio *Cds1*/*Cds2* of ~2:1, while in the cerebellum a modest excess of *Cds1* was detected; *Cds1*/*Cds2* ~1.2-1 (Fig. 1).

Effect of hypothermia

The *Cds1* and *Cds2* expressions demonstrated time-dependent and brain region-specific induction when the carp were exposed to a sudden 7°C decrease in temperature.

In the olfactory lobe, *Cds2* was transiently induced. A 4-fold increase in the level of *Cds2* mRNA was detected after 1 h of cold treatment. This induced level had dropped to 150% of the control value by 5 h of cold shock. The *Cds1* gene was less inducible; after 1 h of cold treatment, a modest (but not significant) 1.5-fold increase in mRNA level was measured (Fig. 2). In the cerebellum, only the *Cds2* gene was induced by the temperature drop; the mRNA level was increased ~1.5-fold after the 1-h cold exposure, but this change was not significant (data not shown).

Effect of Cd

Cd^{2+} at 10 mg/l proved to be an inefficient inducer of the *Cds* genes in the two brain regions examined. In the olfactory lobe, there were at most 1.5- and 2-fold increases in the levels of the *Cds1* and *Cds2* transcripts, respectively, with a maximum at 48-96 h. By 120 h of treatment, the expressions of both *Cds* genes had returned to the control level (data not shown and Fig. 3A). In the cerebellum, no significant changes in *Cds* transcript level were measured during the 120-h exposure (data not shown and Fig. 3B). A striking difference was found in the expressions of the *Cds* genes in the combined experiment when cold-shocked carp were exposed to Cd^{2+} at 10 mg/l for 24 and 48 h at 12°C, immediately after 1 h or 5 h of cold shock; the expressions of both *Cds* genes were suppressed. After 24 h of Cd^{2+} challenge, 20% and 10% decreases in the *Cds1* and *Cds2* mRNA levels were detected, respectively, in the olfactory lobe of cold-shocked carp. Cd^{2+} exposure for 48 h caused a further reduction (25-30%) in both mRNA levels (Fig. 3A). In the cerebellum, the downregulation of the *Cds* genes was more prominent after the combined exposure; 40% and 60% decreases in both mRNA levels were detected after 24 h and 48 h of Cd^{2+} exposure of cold-shocked animals, respectively (Fig. 3B). A 24-h or 48-h Cd^{2+} challenge following

5 h of cold exposure resulted in very similar suppression rates of both *Cds* genes; the data did not differ significantly from those measured after 1 h of cold shock.

Discussion

We report here the transcriptional regulation of two *Cds* genes in two brain regions of carp exposed to Cd^{2+} under physiological and cold-shocked conditions. We also show that both *Cds1*- and *Cds2*-specific mRNAs are readily detectable in the brain of the common carp and the distribution of the mRNAs exhibits isoform- and brain region-specificity. The tissue distribution of the Δ9 desaturases has previously been extensively studied in many fish species, and in most cases their expressions proved to be barely or not detectable at all in the brain (Hsieh et al. 2003; Polley et al. 2003). Differences in the expression profiles of the Δ9 desaturase genes between vertebrates possibly reflect species-specific differences in the requirements of these proteins in physiological adaptation (Chang et al. 2001).

No data have been published on the expressions of the Δ9 desaturase genes under stress conditions in the fish brain. We now report the first evidence that sudden cold shock induces the expressions of the *Cds* genes in the brain of the common carp. This expression is regulated spatially and temporally in an isoform-specific manner. The effect of cold shock on the *Cds* expression was investigated earlier in the carp liver (Schünke et al. 1983; Tiku et al. 1996; Polley et al. 2003). The hepatic Δ9 desaturase transcript was transiently upregulated a few days after slow progressive cooling treatment from 30°C to 23, 17 and 10 °C. While modest cooling (to 23°C) during 1 day had no effect on the *Cds* expression, the most severe temperature drop (to 10°C) led to a large increase in *Cds* mRNA level. We found that the expression of *Cds2*, but not *Cds1*, was significantly affected by cold shock. In the liver, the two Cds genes were differentially regulated; the Cds2 gene was transiently upregulated by progressive cooling. The peak expression was measured during the cooling procedure to 10°C, but after exposure to 10°C for a day the level of the *Cds2* transcript was decreased dramatically. The control of desaturase expression seems to be very complex and may be related to the extent of cooling (Trueman et al. 2000).

The effect of Cd^{2+} loading on the *Cds* expression has previously been investigated only in the liver and in cultured rat hepatocytes (Kudo et al. 1990; Kudo et al. 1991). It has been shown that exposure to Cd^{2+} causes changes in the fatty acid composition of the phospholipids; the extent of formation of the 18:1 unsaturated fatty acid is reduced, while that of the 20:4 acid is slightly increased, as a result of the suppression of Δ9 and the induction of Δ6 desaturases (Kudo and Waku 1996). No data are available concerning the expressions of the *Cds* genes following metal exposure in fish. The present study has reveled that Cd^{2+} exposure induces the expressions of the *Cds* genes in the brain of the common carp. The two brain regions exhibited characteristic differences in sensitivity to Cd^{2+} and the effects of metal treatment were time-dependent. While the cerebellum proved to be unaffected by Cd^{2+}, in the olfactory lobe significant increases were measured in the expressions of both *Cds* genes. The olfactory lobe is not perfectly protected by the blood-brain barrier (Wong and Klaass 1982), and therefore its possible Cd^{2+} content might be an explanation for the elevated *Cds* mRNA level. This result contrasts with the effects of Cd^{2+} on the *Cds* activities in the rat liver and cultured hepatocytes (Kudo et al. 1990), where the Δ9 desaturase activities are reduced due to the direct action of Cd^{2+} on the liver.

We also followed the effects of Cd^{2+} on the *Cds* regulation in the brain of cold-shocked animals. In this combined treatment, the expressions of both *Cds* genes were suppressed in an isoform- and brain region-specific manner. It is interesting that the level of suppression correlated with the extent of Cd^{2+} exposure, whereas it was independent of the period of cold treatment. It has previously been shown that in the liver of Cd^{2+}-challenged rat the changes in Δ9 desaturase activity are greater in Zn^{2+}-deficient animals (Kudo et al. 1990). The cerebellum displays an altered *Cds* expression similar to that measured in the olfactory lobe. Since the cerebellum is protected by the blood-brain barrier, an indirect effect must be involved in the regulation of the *Cds* expression of cold-shocked animals. Measurement of the Zn^{2+} concentration in different tissues of cold-shocked animals revealed that the Zn^{2+} content in the brain was dramatically decreased (~ 50%) after 1 and 5 h of cold exposure (Hermesz, unpublished). The Δ9 desaturase expression is known to be regulated at the transcriptional level in response to various biological factors, such as the levels of essential fatty acids, insulin and glucose in the blood (Waters and Ntambi 1994). Since the Zn^{2+} concentration influences the insulin and glucose levels in the blood *in vivo*, we suggest that the reduction in the level of *Cds* mRNA is due to a secondary effect of Cd^{2+} exposure through changes in Zn^{2+} content and other factors, such as the insulin or glucose level.

In conclusion: The two Δ9 desaturase genes examined in different brain regions in this work exhibited unique basal expressions and characteristic sensitivities to Cd^{2+} treatment and a temperature drop. A sudden short cold shock influences the *Cds2* expression similarly as measured during progressive cooling. Cd^{2+} exposure of cold-shocked animals suppresses the *Cds* expression in a brain region-independent manner.

References

Chang BE, Hsieh SL, Kuo CM (2001) Molecular cloning of full-length DNA encoding delta-9 desaturase through PCR strategies and its genomic organization and expression in grass carp (*Ctenopharyngodon idella*). Mol Rep Devel 58:245-254.

Cavaletto M, Ghezzi A, Burlando B, Evangelisti V, Ceratto N, Viarengo A (2002) Effect of hydrogen peroxide on antioxidant enzymes and metallothionein level in the digestive gland of *Mytilus galloprovincialis*. Comp Biochem Physiol C 131:447-455.

Cossins AR, Murray PA, Gracey AY, Logue J, Polley S, Caddick M, Brooks S, Postle T, Maclean N (2002) The role of desaturases in cold-induced lipid restructuring. Biochem Soc Trans 30:1082-1086.

Hermesz E, Ábrahám M, Nemcsók J (2001) Tissue-specific expression of two metallothionein genes in common carp during cadmium exposure and temperature shock. Comp Biochem Physiol C 128:457-456.

Hsieh SL, Liao WL, Kuo CM (2001) Molecular cloning and sequence analysis of stearoyl-CoA desaturase from milkfish (*Chanos chanos*). Comp Biochem Physiol B 130:467-477.

Hsieh SL, Liu RW, Wu CH, Kuo CM (2003) Nucleotide sequence of a cDNA clone coding for stearoyl-CoA desaturase and its embryo expression in zebrafish (*Danio rerio*). Mol Rep Dev 66:325-333.

Hsieh SL, Chang HT, Wu CH, Kuo CM (2004) Cloning, tissue distribution and hormonal regulation of stearoyl-CoA desaturase in tilapia, *Oreochromis mossambicus*. Aquaculture 230:527-546.

Hsieh SL, Kuo CM (2005) Stearoyl-CoA desaturase expression and fatty acid composition in milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) during cold acclimation. Comp Biochem Phys B 141:95-101.

Ideta R, Seki T, Adachi K (1995) Sequence analysis and characterization of FAR-17c, an androgen-dependent gene in the flank organs of hamsters. J Dermatol Sci 9:94-102.

Kaestner KH, Ntambi JM, Kelly TJ, Lane MD (1989) Differentiation-induced gene expression in 3T3-L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 264:14755-14761.

Kudo N, Nakagawa Y, Waku K (1990) The effect of cadmium on the composition and metabolism of hepatic fatty acids in zinc-adequate and zinc-deficient rats. Toxicol Lett 50(2-3):203-212.

Kudo N, Nakagawa Y, Waku K, Kawashima Y, Kozuka H (1991) Prevention by zinc of cadmium inhibitionof stearoyl-CoA desaturase in rat liver. Toxicology 68:133-142.

Kudo N, Waku K (1996) Cadmium suppresses Δ9 desaturase activity in rat hepatocytes. Toxicology 114:101-111.

Lee JA, Cossins AR (1990) Temperature adaptation of biological membranes: differential homoeoviscous responses in brush-border and basolateral membranes of carp intestinal mucosa. Biochim Biophys Acta 1026(2):195-203.

Macartney AI, Tiku PE, Cossins AR (1996) An isothermal induction of delta 9-desaturase in cultured carp hepatocytes. Biochim Biophys Acta 1302(3):207-216.

Nakamura MT, Nara TY (2002) Gene regulation of mammalian desaturases. Biochem Society Transact 30:1082-1086.

Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ, Lane MD (1988) Differentiation-induced gene expression in 3T3-L1 preadipocytes. Characterization of a differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 263:17291-17300.

Polley SD, Tiku PE, Trueman RT, Caddick MX, Morozov IY, Cossins AR (2003) Differential expression of cold- and diet-specific genes encoding two carp liver delta 9-acyl-CoA desaturase isoforms. Am J Physiol Regul Integr Comp Physiol 284:41-50.

Schünke M, Wodtke E (1983) Cold-induced increase of and desaturase activities in endoplasmic membranes of carp liver. Biochim Biophys Acta 734:70-75.

Thiede MA, Ozols J, Strittmatter P (1986) Construction and sequence of cDNA for rat liver stearoyl coenzyme A desaturase. J Biol Chem 261:13230-13235.

Tiku PE, Gracey AY, Macartney AI, Beynon RJ, Cossins AR (1996) Cold-induced expression of delta 9-desaturase in carp by transcriptional and posttranslational mechanisms. Science 271:815-818.

Trueman RJ, Tiku PE, Caddick MX, Cossins AR (2000) Thermal thresholds of lipid restructuring and delta 9-desaturase expression in the liver of carp (*Cyprinus carpio* L.). J Exp Biol 203:641-650.

Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12(10):1161-1208.

Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95-117.

Waters KM, Ntambi JM (1994) Insulin and dietary fructose induce stearoyl-CoA desaturase 1 gene expression of diabetic mice. Biol Chem 269(44):27773-27777.

Wodtke E, Cossins AR (1991) Rapid cold-induced changes of membrane order and activity in endoplasmic reticulum of carp liver: a time-course study of thermal acclimation. Biochim Biophys Acta 1064:343-350.

Wong KL, Klaassen CD (1982) Neurotoxic effects of cadmium in young rats. Toxicol Appl Pharmacol 63(3):330-337.