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Effect of dietary cadmium on lipid metabolism and storage of aquatic bird *Cairina moschata*

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Abstract In environment, birds often fast in connection with breeding, migration or drastic climatic conditions and need to mobilize lipid reserves during these periods. The impairment of lipid metabolism by cadmium (Cd; 1 mg kg^{-1} added in diet) was investigated on palmiped *Cairina moschata*. Expression levels of genes involved in lipid metabolism, mitochondrial metabolism and detoxification were investigated in liver and muscle of ducks. Lipid content in muscle and liver were analysed and plasma triglycerides were quantified. After 20 days, ducks exposed to Cd displayed a lower body weight and lower lipid content in liver than controls. In muscle, the increase of lipid content was only significant for control ducks but not for exposed ducks. Exposed ducks appeared unable to sufficiently transport and store lipids into peripheral tissues. Cd impairs lipid metabolism by several ways. First, Cd triggered the down-regulation of fatty acids synthesis in liver even if the NADPH production and the mitochondrial metabolism are enhanced, suggesting a stronger energy needs. Secondly, the associated decrease of plasma triglycerides and lipoprotein lipase activity with Cd are consistent with impairment of lipids storage in peripheral tissues.

Keywords Cadmium · Aquatic bird · *Cairina moschata* · Gene expression · Lipid metabolism

Introduction

Cadmium (Cd) is one of the most abundant non-essential metal present in environment due to its large use in industrial activities. This toxic metal is known to have various adverse effects on organisms, such as lesions in the kidney or intestinal tissues, induction of reactive oxygen species, DNA damage, impact on hepatic enzymes activities and lipid peroxidation (Mayack et al. 1981; Jordan et al. 1990; Wang et al. 2004; Erdogan et al. 2005; Berzina et al. 2007). Moreover, Cd impairment on lipid metabolism has also been demonstrated on fish and mammalian species (Pierron et al. 2007; Larregle et al. 2008). This effect observed in European eels was mainly explained by an increased utilisation of triglycerides for the renewal of damaged lipid or to provide energy under Cd exposure (Pierron et al. 2007). On the other hand, in rat exposed to Cd, the fatty acids content increased in cytosol and a hypertriglyceridemia appeared in relation to the decreased activity of lipoprotein lipase (LPL) (Larregle et al. 2008).

Various bird species need to accumulate lipid reserves before fasting in relation to breeding, migration or adverse climatic conditions (Pilo and George 1983). Moreover, birds need to mobilize these reserves during these periods. Three metabolic phases have been described to reflect the variation in body mass during fasting (Le Maho et al. 1981; Cherel et al. 1988). Phase I is an adaptation period, marked by a high fat mobilization. Phase II represents a phase of

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economy, during which more than 90% of the energy is provided by lipids. And finally, during phase III the remaining lipid reserves are used as well as proteins. Moreover, some authors have shown that Cd could affect the profile of lipoprotein in pigeon (Revis et al. 1980). Despite the fact that in environment aquatic birds are subjected to multiple contamination sources, little is known about Cd impairment on lipid metabolism in these species.

The aim of the current study was to evaluate the possible impact of a daily administration of Cd (1 mg kg^{-1}) in diet of palmiped *Cairina moschata* on lipid metabolism and lipid storage into peripheral tissue. This species has indeed the ability to store important lipid reserves. Cd concentration was chosen to investigate the metal effects at field level. This concentration could be encountered in ecosystem characterized by Cd contamination of waters and sediments, such as the Gironde estuary (Baudrimont et al. 2005). Cd bioaccumulation was investigated at the organ level. In order to examine its effects, 8 genes encoding for proteins involved in lipid metabolism, lipid storage, mitochondrial metabolism and detoxification were selected. Gene expressions were measured in liver, the major site of de novo lipogenesis (Hermier 1997), by real-time PCR and normalized according to expression of the reference gene: β-actin. Lipid metabolism and storage were investigated through the expressions of genes encoding for glucokinase (*gk*), NADP-dependent malic enzyme (*me*), acetyl-CoA carboxylase (*acc*), fatty acid synthase (*fas*), and lipoprotein lipase (*lpl*). In parallel, activities of two fundamental enzymes were also studied: glucokinase (GK), the first and obligatory step for glucose utilization after transport of the sugar into the cell (Iynedjian 1993) and LPL, the major enzyme responsible for hydrolysis of triglyceride molecules found in circulating lipoproteins (Goldberg 1996). The production of this enzyme is tissue-specific (Auwerx et al. 1992). LPL is anchored on the luminal surface of the capillary endothelial cells where it hydrolyzes triglycerides to regulate their plasma levels with the production of free fatty acids. These generated free fatty acids are then assimilated by muscle and adipose tissue and can serve either as a direct energy source or can be stored (Zechner 1997). To complete these genetic and enzymatic measures, level of triglycerides in plasma and lipid content in tissues were investigated.

Materials and methods

Experimental design

Twenty-five male ducks were raised in a breeding structure belonging to the French National Institute for Agricultural Research (INRA) at the "domaine d'Artiguères", Landes, France. The biological model was chosen according to its facility of access and its stable evolution in a controlled system. The present work was carried out in agreement with the French legislation on animal experimentation and with the authorization from the French Ministry of Agriculture (Animal Health and Protection Directorate). From the first day of life to 4 weeks of age, males were fed ad libitum with small granules (length of 2.5 mm) enriched in protein. Between 4 and 12 weeks of age, ducks were fed ad libitum with growth food (granule with length of 4 mm) less enriched in protein.

Animals were weighed prior to the experiment. Duck were placed in individual cages and two exposure conditions were studied: ducks not exposed to Cd which constituted control animals for analysis and exposed ducks with 1 mg kg^{-1} of Cd. Capsules of Cd were made by adding Cd to crushed granules in the form of $CdCl_2$. One capsule per day was added to 200 g of granule which contained 14% water, 15.7% protein, and 2.3% fat and were given to animals. Concentrations of Cd in food were checked: 0.04 ± 0.01 and 1.03 ± 0.01 mg kg^{-1} for control ducks and exposed ducks, respectively (mean ± standard error of the mean, $n = 5$). Feeding of ducks was carefully monitored to make sure that animals eat all the granules. At time 0 (before addition of Cd), five ducks were removed to determine the lipid content of liver and muscle prior to the experiment. Five ducks per condition were removed after 10 and 20 days. Intravenous injection of 400 UI/kg of heparin was carried out in order to measure plasma activity of LPL released into general circulation by heparin. Blood was retrieved 10 min later by an occipital venous puncture and centrifuged ($3{,}000 \times g$ for 10 min at 4°C) to collect plasma and frozen at −20°C until further biochemical analyses (measure of LPL activity and quantification of plasma triglycerides level). Animals were then killed in a slaughterhouse by exsanguination under stunning. From each animal dissected, pectoral muscle, liver and kidneys were sampled. The liver and muscle were divided into two parts. The first part was stored at −80°C for genetic and enzymatic (liver only) analyses. The second part was stored at −20°C for measure of lipid content and Cd determination (liver, muscle and kidneys). The total weight of the body, the liver and the kidneys were recorded.

Metal determination

Metal determinations were made on five replicates per experimental condition. Tissues (0.1 g) were dried (45°C, 48 h) and digested with 3 mL of nitric acid at 65% (Fluka; Buchs, Switzerland) added in tubes and incubated at 100°C for 3 h. After a 6-fold dilution of the digestates with ultrapure water (MilliQ, Bedford; MA, USA), Cd

concentrations were measured by electrothermic atomic absorption spectrophotometry with Zeeman correction, using a graphite tube atomizer (EAAS Thermoptec M6Solaar). Samples of 20 μL were mixed before atomisation with 4 μg of Palladium (analyte modifier) and 3 μg of $Mg(NO_3)_2$ (matrix modifier). The detection limit was 0.1 μg Cd L^{-1} (3 × standard deviation of the reagent blanks). The analytical methods were simultaneously validated for each sample series by the analysis of standard biological reference materials (Tort-2: lobster hepatopancreas and Dolt-2: dogfish liver from NRC-CNRC; Ottawa, Canada). Values were consistently within the certified ranges (data not shown).

Sequencing of genes

Partial sequences of three genes were determined for lipoprotein lipase (*lpl*), malic enzyme (*me*) and glucokinase (*gk*). A quantity of 40 mg of fresh liver was homogenized to extract total RNAs using Absolutely Total RNA Miniprep kit (Stratagene, Netherlands), according to the manufacturer's instructions. The quality of all RNAs extracted was evaluated by electrophoresis on a 1% agarose-formaldehyde gel, and their concentration determined by spectrophotometry. First-strand cDNA was synthesized from 5 μg of previously extracted total RNA with AffinityScript Multiple temperature cDNA synthesis kit (Stratagene, Netherlands), according to manufacturer's instructions. The unspecific primers pairs, used for PCR to obtain amplified cDNA fragment of the different genes, were determined after multiple sequence alignment of birds or mammalian species using Clustal W software (Infobiogen) (Table 1). Amplified products were cloned into pGEM-T vector and sequenced (Millegen, France).

For actin and acetyl-CoA carboxylase (*acc*), multiple sequence alignments of corresponding sequences from birds were used to determine primer pairs usable to amplify these two genes in *C. moschata*. Alignments between the resulting sequences of *C. moschata* and those of another duck *Anas platyrhynchos* have demonstrated that primer pairs chosen in conserved regions could be used for both species during qPCR analysis (Table 2).

Table 1 Unspecific primers used to clone lipoprotein lipase (*lpl*), malic enzyme (*me*) and glucokinase (*gk*) cDNA

Gene name	Unspecific primers (5′–3′)
lpl	GCTGGTCCCACCTTTGAGTATGC[a]
	GTCCACCAGTCTGACCAGCTGAAG[b]
me	TTGAAAAGTTCATGCCTATTGTTTA[a]
	AAGAGTGACAGGATCAAAAGGAC[b]
gk	ATTGTGCATCAGGTCTTTCCC[a]
	TGTGCCAATGCACCACTGGCT[b]

[a] Forward primers

[b] Reverse primers

Real-time PCR

After extraction and reverse transcription (see above), real-time PCR reactions were performed in a Mx3000P (Stratagene) following the manufacturer's instructions (1 cycle at 95°C for 10 min, and 40 amplification cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s). Each 25 μL reaction contained 1 μL of reverse transcribed product template, 12.5 μL of Brilliant master mix including the SyberGreen I fluorescent dye (Stratagene), enabling the monitoring of the PCR amplification, and the gene-specific primer pairs at a final concentration of 200 nM for each primer. Gene-specific primer pairs were determined using the LightCycler probe design software (version 1.0, Roche) (Table 2).

Reaction specificity was determined for each reaction from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SyberGreen fluorescence level during a gradual heating of the PCR products from 60 to 95°C. Relative gene expression level was normalized according to the β-actin gene expression. For each gene expression level, mean value and the associated standard deviation ($n = 5$) were determined. Induction or repression factors were obtained with the comparison of each mean value between contaminated and control ducks.

Enzyme activities determination

Post-heparin LPL assay

Enzyme activities of LPL were determined in plasma by a colorimetric enzymatic method (NEFA C kit, Wako), according to the manufacturer's instructions. This technique is based on the in vitro quantification of plasmatic free fatty acids released under LPL activity. Analysis of LPL was performed on five replicates per experimental condition. A total of 0.4 mL of plasma was added to 0.1 mL of 0.1 M NH_4Cl/NH_4OH (pH 8.6) and 0.5 mL of substrate (0.1 M NH_4Cl/NH_4OH (pH 8.6) containing 3% bovine serum albumin (fraction V, Sigma, St Louis, MO) and 15% Intralipid (AB-Kabivitrum, Stockholm, Sweden) beforehand activated at 4°C for 30 min with 20% duck serum). Enzyme activity quantification is the result of the difference observed between fatty acids already present in plasma before analysis and fatty acids resulting from substrate hydrolysis after 60 min-incubation at 37°C.

Table 2 Specific primers and accession numbers or reference of genes used for qPCR

Gene name	Accession number/reference	Specific primers (5′–3′)
act	EF667345[a]	ACAGGAAGTTACTCGCCTCT[b]
		TGGGGAACACAGCCCGC[c]
cox1	EU755254	GCACAGCACTCAGCCTAC[b]
		AATGCCATGTCGGGGGC[c]
nad5	EU755254	ATTCGCCGCCACATGC[b]
		ATGGACCCGGAGCATAGGAA[c]
acc	EF990143[a]	GTCCTCCAAGCCAAGCAATGTG[b]
		GGCCTTGATCATGACAGGGTAGCC[c]
mt	U34230	TGGACCCCCAGGACTGC[b]
		CCGGCTATTTACAGGCGGA[c]
gk	EU598458	CCGGGCCTCTGATCTCAC[b]
		CTCTATCTGGGAGGTCACAAACT[c]
me	EU598457	ATCAAGGCTATTGTGGTGACAG[b]
		ATTCTCTTGTGTCTCAGCCC[c]
lpl	EU598459	TGCCGCAAGAACCGCT[b]
		ACTCTCATCTAAAGTGCCATACAG[c]
fas	AY613443[a]	GGCTAAACGCATCTATGCTACAA[b]
		ATTTACCTCCTGTGGATCTCCAA[c]

act actin, *cox1* cytochrome C oxidase subunit 1, *nad5* NADH dehydrogenase subunit 5, *acc* acetyl-CoA carboxylase, *mt* metallothionein, *gk* ADP-dependent glucokinase, *me* NADP-dependent malic enzyme, *lpl* lipoprotein lipase, *fas* fatty acid synthase

[a] Accession number/reference of *Anas platyrhynchos*

[b] Forward primers

[c] Reverse primers

GK assay

The activity of this enzyme was measured in fresh liver at 37°C by coupling the production of ribulose-5-phosphate from glucose-6-phosphate to the reduction of NADP using purified glucose-6-phosphate dehydrogenase (Sigma) and 6-phosphogluconate dehydrogenase (Sigma) as coupling enzymes. One unit of enzyme activity was defined as the amount that phosphorylates 1 µmol of glucose/min. Activity of GK was estimated by subtracting NADPH formed (at 340 nm) in the presence of 0.5 mM glucose (scoring low-*K*m hexokinase activities) from NADPH produced in the presence of 100 mM glucose (scoring total hexokinase activities) (13).

Liver pieces (0.5 g) were homogenised (dilution 1/10) in ice-cold buffer consisting of 80 mM Tris, 5 mM EDTA, 2 mM Dithiothreitol (DTT), 1 mM Benzamidine, and 1 mM 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF), adjusted to pH 7.6. The resulting homogenate was centrifuged for 10 min at 1,000 rpm at 4°C. The clear supernatant fraction was removed and used for protein and enzyme activity determination. Protein concentration was determined using the Bradford method with bovine serum albumin as standard. Measure of $NADP^+$ dependent enzymes activity was realised in 80 mM Tris, 8 mM $MgCl_2$, 5 mM EDTA, 1 mM KCl, 1 mM KH_2PO_4, 2 mM $NaHCO_3$, 2.5 mM DTT, 2 mM NADP, 7 mM ATP, 0.1 U/mL glucose-6-phosphate Dehydrogenase and 0.1 U/mL 6-phosphogluconate dehydrogenase. Reaction rates were assayed spectrophotometrically at 340 nm following for 10 min the appearance of NADPH.

Lipid analysis

Lipid content was determined in liver and muscle using the method of Folch et al. (1957). The biological pieces (liver: 10 g; muscle: 20 g) were homogenised in 100 mL of methanol–chloroform (1:2) using Ultra-Turrax and settle overnight. After 20 h, the lower organic phase was then separated and evaporated. Lipids were weighed and results are expressed as lipid percentage.

Plasma triglycerides quantification

Triglycerides were quantified at 550 nm by colorimetric enzymatic methods using kit (Sobioda, France), according to the manufacturer's instructions.

Statistical analysis

One-way analysis of variance was applied to assess differences in Cd accumulation, genes expression levels, enzymatic activities, lipid contents and plasmatic triglycerides between control and exposed ducks. The effect of exposure time was also determined. Post-hoc test was used if significant differences ($P < 0.05$) appeared between data to identify which group differed (Tukey HSD test). Normality was assumed and homogeneity of variance necessary for the use of this parametric test was verified. If this assumption was not achieved despite $\log_{10}(x + 1)$ transformation (Cochran C test), non-parametric analysis of variance was applied (Mann–Whitney *U*-test, Statistica 7.1).

Table 3 Mean Cd concentrations ± SE measured in liver, kidney, and muscle tissues prior to the beginning of the experiment (day 0) and after 10 or 20 days for control and exposed ducks to diets containing 1 mg kg^{-1} Cd ($n = 5$ each)

Organs	Day 0	10 days		20 days	
	Control	Control	Exposed	Control	Exposed
[Cd] mg kg^{-1} (dw)					
Liver	0.55 ± 0.12	0.79 ± 0.10	1.70 ± 0.21**	0.84 ± 0.09	1.72 ± 0.07**
Kidneys	1.24 ± 0.20	1.62 ± 0.11	4.33 ± 0.21**	1.47 ± 0.12	4.38 ± 0.45**
Muscle	0.03 ± 0.02	0.04 ± 0.01	0.09 ± 0.03	0.02 ± 0.004	0.57 ± 0.05*

Comparison between control and exposed group was notified at 10 and 20 days in each tissue with *P* level indication at the level $\alpha = 0.05$ (* $P < 0.05$; ** $P < 0.01$)

Results

Cd bioaccumulation

After 10 and 20 days, exposed ducks had greater Cd concentrations in liver and kidney tissues compared to control ducks (Table 3). However, the concentrations in these organs did not significantly increase between these two periods. Despite an increase in Cd concentrations in muscle of exposed ducks after 10 days of experimentation, no significant difference was observed compared to control ducks. Significant difference only appeared after 20 days of exposure.

Cd impact on body condition of ducks

Total body weight of control animals was the same throughout the experiment (control 0 vs. control 20, Fig. 1). In contrast, Cd exposed group significantly lost weight compared to the beginning of the experiment (control 0 vs. exposed 20; $P = 0.045$). Moreover, after 20 days, the metal triggered a significant lower body weight in exposed ducks compared to control sampled at the same time (exposed 20 vs. control 20; $P = 0.028$). No differences were observed for liver and kidneys weight between the two groups.

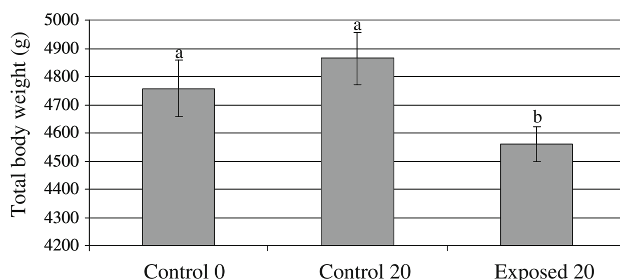

Fig. 1 Total body weight (mean ± SE, $n = 5$) of control and exposed ducks at the beginning of the experiment (control 0) and after 20 days of Cd exposure (control 20, exposed 20). Means designated with different *letters* (a and b) are significantly different at the level $\alpha = 0.05$

Gene expressions in response to Cd exposure

A partial malic enzyme cDNA of 1,043 bp was sequenced. The corresponding protein [347 amino acids (aa)] presented high similarities with *Anas platyrhynchos* malic enzyme (99% identity) and birds *Meleagris galopavo* (95% identity) and *Columba livia* malic enzyme. The partial fragment sequenced for *lpl* gene (712 bp, 237 aa) corresponded to LPL protein of birds *Anser anser domesticus* (99% identity) and *A. platyrhynchos* (99% identity). For the sequenced *gk* (439 bp; 146 aa), high similarity was found with the corresponding gene of *A. platyrhynchos* (100% identity) and *Gallus gallus* (95% identity).

After 10 days, genes involved in fatty acid production (*fas*, *acc*) and mitochondrial metabolism (*nad5*) were induced in liver of exposed ducks (Table 4). In contrast, in muscle, all genes involved in lipid metabolism (*gk*, *me*, *acc*, *fas*, *lpl*) were repressed. After 20 days, genes involved in lipid metabolism like *gk* and *me* were induced while *fas* was repressed in liver of exposed ducks. Moreover, mitochondrial metabolism was enhanced and *mt* gene implicated in detoxification process was more expressed

Table 4 Gene expression compared to control (induction or repression factors) in liver and muscle from *C. moschata* after 10 and 20 days of Cd exposure

Functions	Genes	10 days		20 days	
		Liver	Muscle	Liver	Muscle
Lipid metabolism	*gk*	/	1/10*	3*	/
	me	/	1/15*	6*	/
	acc	3*	1/10*	/	/
	fas	5*	1/50*	1/30*	1/10*
Peripheral lipid transfer	*lpl*	/	1/10*	/	1/10*
Mitochondrial metabolism	*cox1*	/	1/10*	2.5*	/
	nad5	6.5*	/	8**	/
Detoxification	*mt*	/	/	6*	/

Significant results are given as induction (>1) or repression (<1) factor. The *P* levels are indicated as * $P < 0.05$, ** $P < 0.01$. /: identical to control level

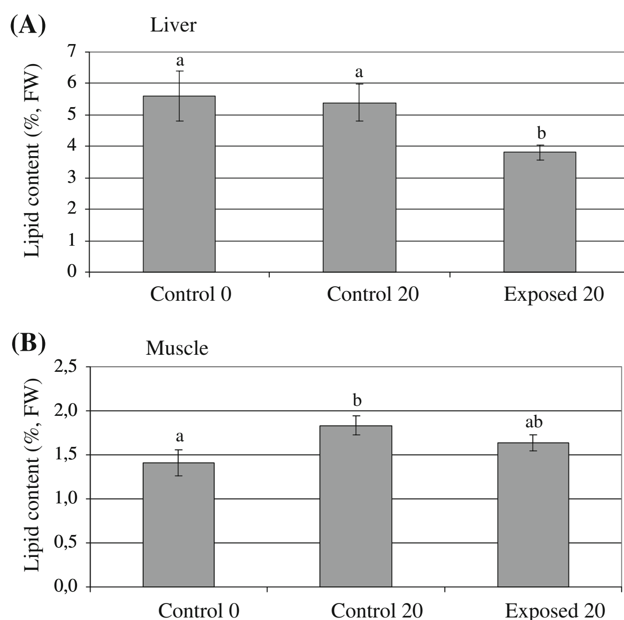

Fig. 2 Lipid content (mean ± SE, $n = 5$) expressed in percentage (FW: fresh weight) in liver (**a**) and muscle (**b**) at the beginning of the experiment (control 0) and after 20 days of Cd exposure (control 20, exposed 20). *Letters* (a and b) represent the significant differences between groups at the level $\alpha = 0.05$

following Cd exposure. In muscle, Cd triggered the repression of genes involved in lipid storage (*lpl*).

Effect of Cd on biochemical parameters

After 20 days, exposed ducks displayed lower lipid content in liver than controls (Mann–Whitney *U*-test, $P = 0.027$; Fig. 2a). In muscle, control ducks have significantly increased their lipid content since the beginning of the experiment (Mann–Whitney *U*-test, $P = 0.045$) whereas Cd exposed ducks did not (Fig. 2b). Discrepancies between the two groups were also observed for triglycerides. Indeed, after 20 days, exposed ducks significantly displayed lower plasmatic triglycerides levels than controls ($P = 0.043$; exposed: 0.44 ± 0.04 g/L; control: 0.62 ± 0.08 g/L).

Enzymatic activities were assayed in liver for GK and plasma for LPL. First, after 20 days, LPL activity was significantly lower for Cd exposed ducks (4.47 ± 0.24 mmol h^{-1} L^{-1}) compared to controls (5.46 ±0.16 mmol h^{-1} L^{-1}; $P = 0.021$). Second, in contrast, GK activity of exposed ducks (0.024 ± 0.004 µmol min^{-1} mg^{-1} of protein) was higher than controls (0.017 ± 0.001 µmol min^{-1} mg^{-1} of protein; $P = 0.025$). Protein concentrations were significantly higher for control ducks (188.20 ± 1.82 mg protein g^{-1} of liver) compared to exposed group (169.72 ± 2.68 mg protein g^{-1} of liver; $P = 0.048$).

Discussion

Cd bioaccumulation

Results of Cd bioaccumulation were consistent with previous studies conducted on bird species (Kumar et al. 2007; Kim et al. 2009; Lucia et al. 2008). Kidney represented the final target tissue for Cd. The levels of this toxic element after 10 and 20 days of exposure were higher in this organ than in liver as generally observed (Scheuhammer and Templeton 1990; Rodrigue et al. 2007). However, the liver is an important site for detoxification and lipid metabolism during fasting in connection with breeding, migration, or drastic climatic conditions (Le Maho et al. 1981; Cherel et al. 1988; Herzberg et al. 1988). The muscle displayed lower concentrations even if the contamination led to a significant increase of Cd concentration in that tissue. Although muscle is a minor site of accumulation, it has been previously described as a responsive organ for genes involved in antioxidant defences, DNA repair and apoptosis in case of Cd exposure (Gonzalez et al. 2006).

Cd impairment on body weight

Cd had an impact on the body weight of animals. Indeed, after 20 days, exposed ducks displayed significantly lower body weight than controls with an average difference of 304 g between the two groups. Duck were subjected to a weight loss under Cd exposure. The discrepancy of total body weight appeared to be mainly explained by the muscular compartment. The weight gain and then the general state of animals were impaired by this toxic metal. A previous study on wild common eiders (*Somateria mollissima borealis*) had reported the negative impact of Cd on capture mass (Wayland et al. 2003). However, the mechanism leading to this statement was not completely understood and could be due to impairment of lipid metabolism and lipid storage to peripheral tissues as previously observed for fish or mammalian species (Pierron et al. 2007; Larregle et al. 2008; Rogalska et al. 2009). Indeed, the current study observed a lower content of lipids in liver of exposed ducks compared to control group. Moreover, Cd impaired the lipid content growth of ducks in muscle. This is confirmed by the lower increase of lipid content in this tissue for exposed ducks compared to control ducks. In order to better understand this result, it is necessary to observe the cellular impact of Cd on genes and corresponding enzymes involved in lipid metabolism.

Cd impact on production of fatty acids

Two phases were observed to explain the Cd impact on aquatic birds. In a first step, after 10 days of exposure with

this non-essential element, genes coding for enzymes involved in lipid metabolism were up-regulated (*acc*, *fas*). However, the stimulation of these genes such as *fas*, the last enzyme implicated in fatty acids production before their transformation into triglycerides, was not correlated with an increase of lipid content neither in the liver nor in the muscle. Cadmium could trigger the synthesis and the immediate mobilization of long chain fatty acids in order to provide sufficient amounts of ATP to counter oxidative stress. Indeed, one of the principal metabolic fates of long chain fatty acids is oxidation to yield ATP (Digel et al. 2009). This energy requirement hypothesis was also corroborated by the up-regulation of *nad5* which demonstrates that the presence of Cd stimulates mitochondrial activity *via* the electron transfer chain to produce energy as ATP. In this way, previous study on rat orally exposed to Cd (15 ppm for 8 weeks) have shown that, with contamination, the liver fatty acids synthesis increased, as determined by an increment of fatty acid synthetase (Larregle et al. 2008). Another explanation to this lipid production could be the renewal of lipids damaged by peroxidation of hepatocyte membrane under Cd action (Stohs and Bagchi 1995; Karmakar et al. 1999; Bertin and Averbeck 2006).

A second phase appeared after 20 days of exposure. Cd triggered the up-regulation of the *gk* and *me* genes and the stimulation of GK enzyme activity involved in the production of liver NADPH and then in lipogenesis. NADPH is a pivotal cofactor for lipogenesis (Rho et al. 2005) because it provides the reducing power for biosynthetic processes such as elongation of fatty acids, de novo synthesis of cholesterol (Rous and Lüthi 1968; Gaskin and Clayton 1972). Cd seems to stimulate the total potential of liver NADPH production of exposed ducks. However, this observation was in contrast with (1) the lower lipid content in liver of exposed ducks, (2) the down-regulation of gene coding for *fas* and (3) the similar expression between exposed and control ducks for *acc*, the first step for fatty acids synthesis and the rate-limiting enzyme of this process (Wakil et al. 1983; Iritani et al. 1984; Rollin et al. 2003). These arguments suggest that even if the amount of NADPH increased under Cd exposure, the metal impaired and reduced fatty acids production in liver. This paradox between the stimulation of enzymes involved in NADPH production and the lipid storage that did not increase was previously observed for the migrant European eel (Pierron et al. 2007).

Transport and storage of fatty acids

LPL is the rate-limiting enzyme responsible for hydrolysis of triglyceride molecules present in circulating lipoproteins into fatty acids. These free fatty acids are then incorporated in muscle and adipose tissue (Goldberg 1996; Merkel et al. 2002). It has been previously suggested that LPL played a predominant role in the determination of the body mass composition (Weinstock et al. 1997; André et al. 2007). The current study showed that control ducks increased the lipid content of their muscle in a significant way in contrast to Cd exposed ducks. This impairment of lipid storage could have several explanations. First, Cd triggered a decrease in plasma triglycerides concentrations certainly related to the reduced fatty acids production in liver. This decrease in plasmatic triglycerides led to the lower assimilation of fatty acids in muscle. Secondly, LPL enzyme activity appeared to be depleted in relation with the down-regulation in muscle of *lpl* gene. In this way, the lower level of triglycerides in plasma associated with the decreased activity of this essential enzyme for fatty acids assimilation led to the impairment of lipid storage and utilization.

The current study demonstrated that Cd not only triggered the impairment of fatty acids production in liver but also the poor integration of fatty acids in peripheral tissues. This observation could be of great interest considering that birds often reduce food intake during their migration or their nesting. The establishment of lipid reserves constitutes the main path for energy mobilization. The assimilation of lipids, fundamental for the surviving of the populations during these periods, is impaired by this metal. Further investigations should focus on Cd impact on lipids mobilization during fasting periods.

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