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Body lipid composition modulates acute cadmium toxicity in Daphnia magna adults and juveniles

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HIGHLIGHTS

- Dietary liposome supplementation protects adult daphnids against acute Cd challenge.
- Offspring tolerance to Cd is influenced by neutral lipid richness in ALA and 18:0.
- Daphnids absorb, incorporate and transfer ingested EPA to their offspring.
- EPA supplementation does not confer an additional protection against Cd.
- Maternal transfer of body lipids is influenced by the diet and the brood number.

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ABSTRACT

Long chain polyunsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) affect zooplankton fitness and ability to cope with environmental stressors. However, the impact of LC-PUFAs on zooplankton sensitivity to chemical stressors is unknown. Here, we aimed to document the interaction between EPA and cadmium (Cd), as model chemical stressor, in *Daphnia magna*. A life-history experiment was performed in which daphnid neonates were raised into adulthood on three diets of different lipid composition: (i) algae mix; (ii) algae mix supplemented with control liposomes; (iii) algae mix supplemented with liposomes containing EPA. Juveniles (3rd, 4th and 5th brood) released by daphnids during this life-history experiment were sampled, challenged with Cd during 48 h and their immobility was assessed. At the end of this life-history experiment, another immobilisation test was performed with adults from each treatment. Daphnids absorbed, incorporated and transferred ingested EPA to their offspring. Liposome feeding increased adult tolerance to Cd. The presence of EPA in liposomes did not increase adult tolerance to Cd. Offspring's tolerance to Cd was influenced by the brood number and the maternal diet. It was positively correlated with the PUFA level in body neutral lipids, especially alpha-linolenic acid (ALA, 18:3n-3) and negatively correlated with the saturated fatty acid level in body neutral lipids, especially stearic acid (18:0). Overall, these results emphasize the importance of dietary lipids and maternal transfer of body lipids in *D. magna* sensitivity to Cd and highlight the need to take into account these parameters in ecotoxicological studies and risk assessment.

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal that has been extensively extracted from the Earth crust and released in the environment as a consequence of its direct or indirect (waste-product) use in various industrial applications (Pan et al., 2010). At the individual level, Cd impairs growth, reproduction and survival

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Abbreviation

α	type 1 error
AA	arachidonic acid
ALA	alpha-linolenic acid
Cd	Cadmium
DOC	dissolved organic carbon
CT	control
D[4,3]	volumetric diameter
DHA	docosahexaenoic acid
EC50	median effect concentration
EPA	eicosapentaenoic acid
FAME(s)	fatty acid methyl ester(s)
LA	linoleic acid
LC-PUFA(s)	long chain polyunsaturated fatty acid(s)
MUFA(s)	monounsaturated fatty acid(s)
POPC	1-palmitoyl-2-oleoyl-phosphatidylcholine
POPG	1-palmitoyl-2-oleoyl-phosphatidylglycerol
RTL-W1	rainbow trout liver- Waterloo 1 cell line
SFA(s)	saturated fatty acid(s)
TK-TD	Toxicokinetic-toxicodynamic

of aquatic organisms (McGeer et al., 2011). Cd toxicity is commonly linked to its potency to (i) alter ion homeostasis, (ii) bind sulfhydryl groups and modulate the properties of many proteins and enzymes and (iii) generate oxidative stress (Barata et al., 2005b; Connon et al., 2008; McGeer et al., 2011; Matović et al., 2015).

Lipids, and their constitutive fatty acids, have important biological roles in aquatic organisms as they (i) provide a significant part of the energy required for somatic growth, locomotion and reproduction, (ii) constitute the biological membranes and maintain them in an appropriate structure for a good functioning (iii) are precursors of signalling molecules (e.g. the eicosanoids) (Tocher, 2003; Heckmann et al., 2008; Parrish, 2009). Some fatty acids can be synthesized *de novo* in zooplankton and fish but others cannot and must therefore be assimilated from their diet and are called essential fatty acids (Parrish, 2009). Linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), two essential fatty acids, are exclusively synthesized by primary producers and some heterotrophic protists (Desvilettes and Bec, 2009; Parrish, 2009). These two fatty acids can be further desaturated and elongated by heterotrophic organisms to produce the valuable long chain polyunsaturated fatty acids (LC-PUFAs): arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Bell and Tocher, 2009). However, these biotransformation steps being of poor efficiency in zooplankton (Farkas et al., 1981; von Elert, 2002; Martin-Creuzburg et al., 2010), the LC-PUFA content of these organisms is highly dependent on the lipid composition of their food, i.e. the primary producers (Brett et al., 2009). The fatty acid profile of these primary producers has been shown to vary a lot among taxa (Brett et al., 2009; Lang et al., 2011) and is modulated by environmental conditions, such as temperature (e.g. Hixson and Arts (2016)) and water pollution (e.g. Chia et al. (2013)).

Zooplankton has a key role in aquatic food webs as it transfers biomass and macronutrients, such as lipids, from the primary producers to higher trophic levels. Among the freshwater zooplankton, the large water flea, *Daphnia magna*, can be found in many lakes and ponds of temperate regions and is commonly used as model species in ecotoxicological studies as it (i) has a short generation time, (ii) can easily be handled and maintained in controlled laboratory conditions and (iii) has a parthenogenetic reproduction cycle, which ensures genetic stability of the clones. As other filter feeders, *D. magna* does not select its food based on its nutrient richness but rather based on its size (DeMott, 1986). The natural diet of *D. magna* is thus composed of a wide variety of microorganisms and particulate suspended matter with size ranging from 0.6 to 40 μm (Geller and Müller, 1981). This property can be used in the laboratory to study the impact of a wide variety of food sources on the organism fitness. Particularly, the biological roles of specific fatty acids can be studied through feeding *Daphnia* spp. with liposomes loaded with specific fatty acids. For example, using that technique, it has been shown that EPA and AA influence *Daphnia* spp. somatic growth and/or reproductive success (Ravet et al., 2003; Martin-Creuzburg et al., 2009, 2010, 2012; Sperfeld and Wacker, 2012; Schlotz et al., 2013, 2014) and modulate their ability to cope with environmental stressors, such as temperature (Martin-Creuzburg et al., 2012), predation (Brzeziński and von Elert, 2015) or infection (Schlotz et al., 2013, 2014). Several mechanisms have been suggested to explain these interactions. It has first been suggested that, in ectotherms with poor fatty acid biotransformation efficiencies, such as *D. magna*, limitations in dietary LC-PUFAs could prevent the organisms from performing the homeoviscous adaptation required to maintain membrane fluidity in case of cold temperature shocks, which can happen either during winter or during diel vertical migrations (Martin-Creuzburg et al., 2012; Brzeziński and von Elert, 2015). Besides, it has also been suggested that dietary fatty acids with 20 carbons could modulate the immune response to parasite infection through influencing the eicosanoid cascade induction (Schlotz et al., 2013, 2014).

Following a similar reasoning, we hypothesized that dietary LC-PUFAs could also influence the ability of aquatic organisms to cope with other environmental stressors, such as chemical pollutants. Indeed, the membrane unsaturation level could modulate chemical uptake, excretion, cellular/subcellular partitioning and/or body burden, which, in turn, could modulate the organism general sensitivity to the chemical. In addition, lipid signalling could enhance the organism defences against chemical toxicity. However, such interactions have been poorly investigated. Still, there is evidence from *in vitro* studies that specific fatty acids can modulate the sensitivity to heavy metals such as Cd. For example, ALA, EPA and DHA given either alone (Ferain et al., 2016) or in combination (Linhartova and Sampels, 2015) to rainbow trout liver cells or human hepatocellular cells have been shown to increase the cellular tolerance to an acute Cd challenge, while supplementation with either LA or AA did not have any impact (Ferain et al., 2016). However, the influence of specific fatty acids on sensitivity to Cd has, to our knowledge, never been investigated *in vivo*. We therefore aimed at investigating this interaction using *D. magna* as experimental model and EPA as targeted fatty acid.

We hypothesized that EPA would protect *D. magna* against an acute Cd challenge. To test this hypothesis, we carried out a life history experiment *with D. magna* Straus raised into adulthood on diets specifically enriched in EPA, using the liposome supplementation technique. Adult daphnids from each dietary treatment as well as the offspring they have released during the life-history experiment (3rd to 5th brood) were sampled and challenged with Cd. After 48 h of exposure, the immobilisation of the daphnids was assessed as toxicity endpoint. *D. magna* are known to have a fatty acid composition that globally reflects that of their diet. In addition, they preferentially drain PUFA to their progeny and have a better fitness under EPA feeding. We therefore expected that 21-day old daphnids fed EPA and their juvenile progeny would contain a higher EPA body level and be more tolerant to an acute Cd challenge than those fed no EPA and their progeny, respectively.