IMPACT OF FASTING/LACTATION ON THE LEVELS OF PCBs, DDTs AND FAT-SOLUBLE VITAMINS IN NORTHERN ELEPHANT SEAL TISSUES: SIMILARITIES, DISCREPANCIES AND RECOMMENDATIONS FOR FUTURE STUDIES

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Introduction

Several studies in marine and terrestrial animals have shown that vitamin A, and to a lesser extent, vitamin E status can be disrupted by organochlorines[1-4]. Vitamins A and E may thus be useful biomarkers to measure exposure as well as effects of environmental contamination in wildlife. However, the physiological status (fast, lactation) and body condition of the animals may have an impact on the concentrations of pollutants and vitamins encountered in the tissues examined. If it is the case, this parameter should be taken into account in the interpretation of the results.

In the present study, we developed a comparative approach of the impact of fasting and lactation on the dynamics of fat-soluble vitamins (vitamins A and E) and fat-soluble pollutants (PCBs and DDTs) in the northern elephant seal (NES) (*Mirounga angustirostris*). As in most phocid seal species, lactation is very short and intense in NES and females fast entirely while secreting fatty milk. Twenty NES mother-pup pairs were captured and sampled at day 4 and day 21 of lactation in order to get longitudinal samples of blubber, serum and milk from the mothers and serum from their pups. Samples were analysed for vitamins A and E, 19 PCB congeners, DDT and its metabolites. Results in maternal blubber and milk are presented here. This study attempts to bring new information about the potential mechanisms of transfer of organochlorines and vitamins and to recommend parameters to take into consideration when using vitamins as biomarkers for contaminant effects.

Material and Methods

- *Sample collection*. The study was conducted on the colony of Año Nuevo, CA, USA (37°06'30''N, 122°20'10''W) in January and February 2005. Techniques of sample collection are described elsewhere[5].

- *Contaminant and vitamin analyses.* Blubber biopsies (approximately 6 cm long) were cut into 3 equal parts. Inner (closest to the muscle) and outer (closest to the skin) layers were analyzed separately for vitamins and contaminants. PCBs (IUPAC 52, 101, 105, 110, 118, 128, 138, 143, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209) as well as DDTs (*p,p'*DDT, *p,p'*DDE and *p,p'*DDD) were analysed by gas chromatography whereas vitamin A (retinol and retinyl esters) as well as vitamin E (α-tocopherol and α-tocopheryl esters) were analyzed by HPLC. The details of sample preparation, extraction, clean-up as well as quality assurance are provided elsewhere[6-9].

- *Data analyses.* Results were analysed using the GLM procedure (Statistica 7.1). Models used for PCBs and DDTs are detailed elsewhere[5]. The same models were used for vitamins A and E.

Results

Milk lipids - Milk lipid content was 28.2±5.0% at day 4 and 45.8±6.3% at day 21.

PCBs and DDTs - In all compartments, the major PCB congeners quantified were PCB-101, -118, -128, -138, -149, -153, -156, -170, -180, -183, -187, which accounted for more than 95% of total PCB concentrations. *p,p'*DDE accounted for more than 95% of all forms (*p,p'*DDT, *p,p'*DDD and *p,p'*DDE). PCB and DDT levels in inner blubber increased significantly between early and late lactation ($p < 0.01$) whereas they remained constant in outer blubber ($p = 1.00$) (Fig. 1A&B). At early lactation, inner blubber was significantly less contaminated by PCBs and DDTs than outer blubber ($p < 0.01$). At late lactation, the situation changed: PCB levels in inner blubber were significantly higher than in outer blubber ($p < 0.01$) whereas DDT levels were similar in both layers ($p=0.68$). In milk, PCB and DDT concentrations increased significantly between early and late lactation ($p < 0.01$) (Fig.2A&B).

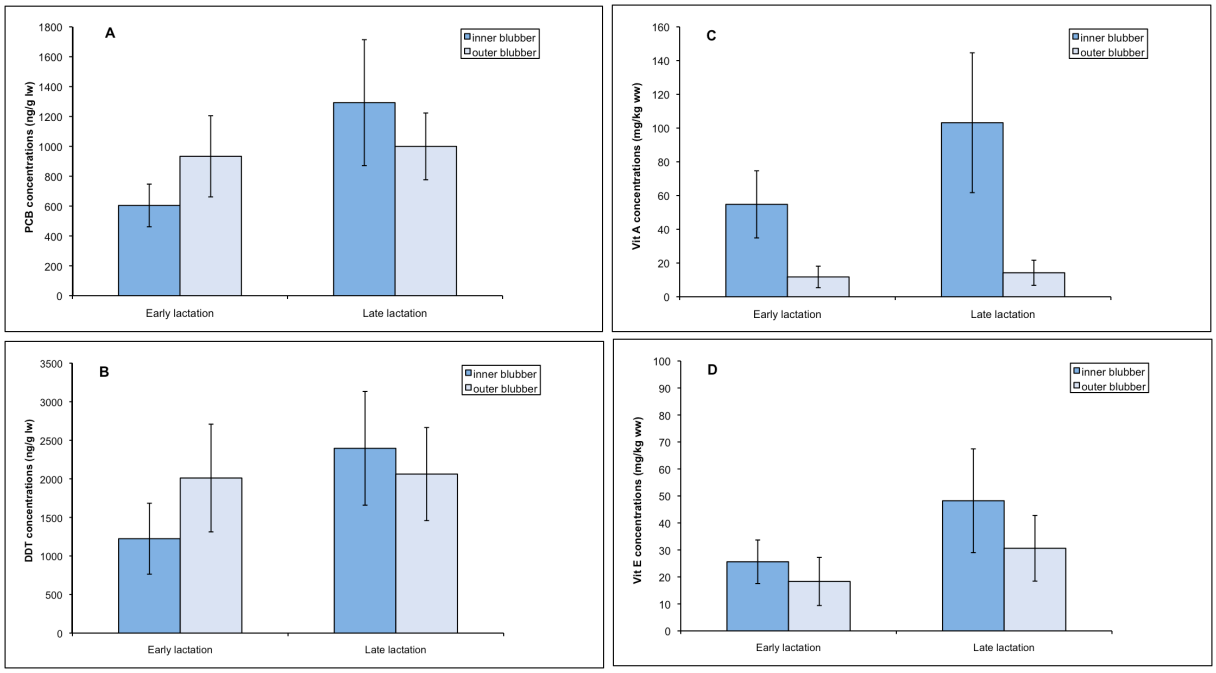

Fig 1: PCB (A), DDT (B), vitamin A (C) and vitamin E (D) concentrations in inner (dark blue) and outer (light blue) blubber of NES mothers at early and late lactation

The heterogeneous distribution of PCBs and DDT throughout the blubber layer was already observed in lactating grey seals as well as in NES pups during the post-weaning fast [8-10]. It might result from the fact that both blubber layers have different physiological properties that are among others reflected by differences of fatty acid profiles (results not shown). As lactation progresses, the freeing of fatty acids associated to triglycerides from inner blubber into the circulation induces the concentration of PCBs and DDTs in the remaining blubber layer. The fact that PCBs and DDTs seem to be less easily mobilised from blubber than fatty acids might originate in part from the lower affinity of lipophilic contaminants for circulating polar lipids as compared to blubber triglycerides[8,10,11]. In our NES females, the fatty acid profile of total serum lipid classes was dominated by PUFA which accounted for 50% of total fatty acid whereas they accounted for only 10% in inner blubber (results not shown). Several studies indicate that blubber is the main storage site (98-99%) of fat-soluble contaminant such as PCBs[11]. Considering a transfer from maternal stores, blubber can be reasonably considered as the main site of mobilisation and transfer in to the subsequent compartments (maternal circulation, mammary gland, milk and pup circulation). In milk, PCB and DDT concentrations increased significantly between early and late lactation

(p < 0.01) (Fig.2). This increase might result from a higher mobilisation rate of PCBs and DDTs from inner blubber at late lactation.

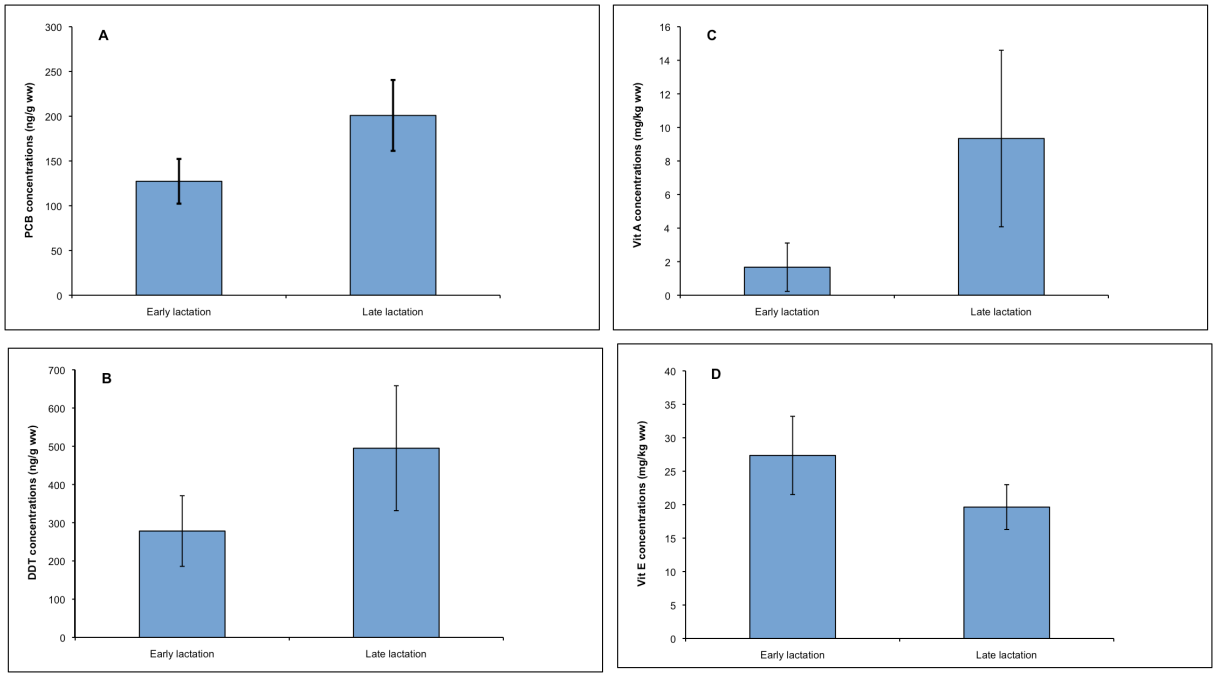

Fig 2 : PCB (A), DDT (B), vitamin A (C) and vitamin E (D) concentrations in the milk of NES mothers at early and late lactation

Vitamins A and E- There was a difference of vitamin A concentrations between inner and outer blubber, both at early and late lactation (p<0.01) (Fig. 1C). Inner blubber contained from 4.5 to 7.5 times higher vitamin A levels than outer blubber throughout lactation. Vitamin A being stored as retinyl esters in the adipose tissue, variations in the fatty acid profile might explain differences of vitamin A status between these 2 layers. The esterification and storage may indeed occur preferentially with some fatty acids compared to others. In addition, a higher expression of intracellular transporters (cellular retinol binding protein CRBP I and III[12]) as well as enzymes involved in the esterification of retinol into retinylesters might constitute a potential other explanation for the sharp difference of status between both layers.

As for PCBs and DDTs, vitamin A concentrations in inner blubber increased significantly between early and late lactation (p<0.01), whereas they remained stable in outer blubber (p=1.00) (Fig.1C). It means that triglycerides are more efficiently mobilised than vitamin A that accumulates in the remaining amount of blubber. However, at late lactation, the mobilisation rate from blubber might increase, seeing the dramatic rise of vitamin A concentrations observed in the milk (p < 0.01). On average, vitamin A levels were 6 times higher at late lactation (Fig. 2C). This pattern totally differed from terrestrial mammals. In the study of Schweigert and collaborators[13] in which the hepatic vitamin A content of dead grey seals shot at various lactating stages was analysed, levels seem to decrease during the first part of lactation followed by a stabilisation until the end of the nursing period. These observations combined to ours suggest that the liver is the first organ providing vitamin A to the mammary gland. However, once the hepatic stores are reduced, blubber becomes the main source of vitamin A for the milk.

The pattern was different for vitamin E. At early lactation, there was no significant difference of concentrations between inner and outer blubber (p=0.11). On the other hand, at late lactation, inner blubber concentrations were significantly higher than in outer blubber (p<0.01) (Fig. 1D). In both blubber layers, vitamin E concentration

increased significantly between early and late lactation ($p<0.01$). The increase of vitamin E in blubber might result from the drop of lipids in the blubber and the concentration of vitamin E in the remaining layer as lactation progresses. Part of inner blubber vitamin E might also migrate to the outer layer, resulting in an increase of concentrations in that layer too. Contrary to vitamin A, vitamin E does not seem to be mobilised from the blubber during lactation. The main source would rather be situated at the level of liver, as suggested by Schweigert and collaborators[13]. The authors have indeed noticed a drop of vitamin E concentration in that tissue during the lactating period.

In milk, the dynamics of vitamin E also differed from those of vitamin A. There was a significant drop of vitamin E concentration between day 4 and day 21 ($p<0.01$) (Fig.2D). The production of a colostrum with high levels of vitamin E as compared to mature milk is the usual pattern for vitamin E in mammals.

Conclusions

The changes of PCBs, DDTs and vitamin A levels in lactating NES exhibit intriguing similarities. A common dynamic of transfer from the blubber, and more precisely, from its inner layer, could explain this parallelism. Contrary to vitamin A, there was not parallelism between vitamin E and organochlorines. The fact that milk vitamin E might originate from the liver and not the blubber is probably one of the causes of these discrepancies. This study shows that lactation/fasting exerts significant impacts on the concentrations of both vitamins and organochlorines in tissues. It is therefore of utmost importance to account for physiological status of the animals in order to use vitamins as biomarkers of effects of organochlorines. It is also essential to be aware that those molecules are not homogeneously distributed throughout the blubber layer and, here again, this phenomenon must be taken into account in the process of assessing the possible effects of pollution on marine mammals.

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