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Original Article

Plasma vitamin A concentrations in captive African penguins (*Spheniscus demersus*) supplied with graded levels of dietary vitamin A supplements

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Abstract

Both deficiency and toxicosis of vitamin A have been reported in captive penguins. Our previous study showed potential hypervitaminosis A in a clinically healthy African penguin (*Spheniscus demersus*) that was regularly fed jack mackerels with vitamin A supplement since the plasma retinol and retinyl palmitate concentrations increased to beyond the criterial levels. To clarify the relationship between plasma vitamin A concentration and excessive intake of vitamin A, in this study, we investigated the effect of graded levels of vitamin A supplementation on plasma retinol and retinyl palmitate concentrations in penguins. Four adult male African penguins were fed jack mackerels, which naturally contain high levels of vitamin A, and these were supplemented with an additional 0, 840, or 1,680 IU/day retinyl acetate for one month. Thus, the total averaged vitamin A intake for the penguins was 1,640, 2,480 and 3,320 IU/day, respectively. Plasma samples were collected before the morning meal on the last day of each treatment. The results showed that plasma retinyl palmitate concentration was significantly higher in the penguins that were provided with the higher dose of vitamin A than in those that did not receive vitamin A supplements. However, plasma retinol concentration was not significantly affected by vitamin A supplementation. These results suggest that in African penguins, plasma retinyl palmitate sensitively responds to high vitamin A intake but plasma retinol does not. Similar to humans, in penguins, plasma retinyl palmitate concentration in fasting can, therefore, be used as a biomarker of excess vitamin A.

Introduction

Vitamin A deficiency has been reported in captive penguins that are fed clupeid fishes containing small amounts of vitamin A[1)]. However, another study has shown the possibility of vitamin A toxicosis induced by a vitamin A supplemented diet containing extremely high level of vitamin A (205,000 IU/kg dry matter (DM)) in captive penguins[2)].

Retinol is transported from the liver to other tissues as a complex with retinol binding protein 4 (RBP4) and transthyretin in birds[3)] and mammals[4)]. Retinol release from the liver is homeostatically controlled by RBP4 synthesis in humans, and subsequently, circulating levels of retinol are relatively stable unless vitamin A is deficient[4)]. As a result of this control mechanism, diets rich in vitamin A cause an increase in hepatic retinyl ester concentrations[4)]. However, in cases of chronic excess in vitamin A consumption in humans, studies have shown that circulating concentrations of retinyl esters, such as retinyl palmitate, increase owing to retinyl esters “spilling out” from the liver into the blood stream[4)]. An increase in circulating retinyl esters has also been observed postprandially in humans; thus, after a period of fasting, blood concentration of retinyl esters can be a useful biomarker of excess vitamin A[4)].

In penguins, Crissey *et al.*[5)] reported that a reduction of dietary vitamin A concentration resulted in a decrease in both retinyl palmitate and retinol concentrations, even though the reduced dietary vitamin A level was still much higher than the recommended vitamin A requirement tentatively proposed by Crissey *et al.*[6)]. Wallace *et al.*[7)] suggested that potential hypervitaminosis A in penguins can be diagnosed as a circulating retinol level of over

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200 μg/100 ml, with a circulating retinyl palmitate level of over 10 μg/100 ml. Akashi *et al.*[8] indicated that plasma retinol and retinyl palmitate concentrations were beyond these diagnostic levels, showing potential hypervitaminosis A in one of 16 clinically healthy African penguins (*Spheniscus demersus*) that were fed a basal diet of jack mackerels with an additional vitamin A supplement.

The objective of this experiment was to clarify whether different levels of vitamin A supplementation affect plasma retinol and retinyl palmitate concentrations in penguins fed jack mackerels containing more vitamin A than the tentative recommended requirement[6].

Materials and Methods

All animal experiments were conducted in accordance with the Code of Ethics of the Japanese Association of Zoos and Aquariums.

The African penguins at Kyoto aquarium regularly hand-fed whole jack mackerels as three equal portions in a day. The penguins are also given one or two tablet(s) of vitamin pre-mix (Mazuri Vita-Zu Small Bird Tablet 5M25, MN), which contains vitamin A as retinyl acetate at 840 IU/tablet, with the morning meal.

For this study, we used four adult male African penguins in the colony at Kyoto Aquarium; the birds were clinically healthy. During the experiment, the birds were hand-fed whole jack mackerels, at approximately 220 g fresh weight/day, according to their normal feeding regime. The birds were randomly grouped based on whether they received vitamin A supplement with the morning meal as follows: 0 IU/day, one tablet of vitamin pre-mix containing no vitamin A (Mazuri Vita-Zu Small Bird Tablet 5TLC, MN); 840 IU/day, one tablet of the ordinary vitamin pre-mix containing vitamin A; or 1,680 IU/day, two tablets of the ordinary vitamin pre-mix containing vitamin A. Each bird was tested for one month with each of the above supplement levels, but the order varied among birds. Two birds were treated in the order 0, 840, and 1,680 IU vitamin A/day; one was treated in the order 840, 1,680, and 0 IU vitamin A/day; and one was treated in the order 1,680, 0, and 840 IU vitamin A/day. When the treatment involved a lower dose of vitamin A than that in the immediately preceding period, a one-month washout phase was followed, with a dietary regime of the basal diet of jack mackerels and the vitamin pre-mix without vitamin A.

Blood was collected from the medial metatarsal vein in a heparinized tube before the morning meal on the last day of each dietary treatment. The plasma samples were stored at −80℃ until analysis. Retinyl esters mainly exist as retinyl palmitate in plasma[9], and circulating concentration of retinyl palmitate has been reported in penguins[5,7,8]. Both plasma retinol and retinyl palmitate concentrations were determined using an HPLC method as detailed in Akashi *et al.*[8].

Data are expressed as the mean ± standard deviation. The effect of the different dietary treatments was evaluated using the MIXED Procedure of SAS (version 9.1, SAS Institute, NC). The differences between means were evaluated using the Tukey method. Differences were considered statistically significant at $P < 0.05$.

Results

All birds were clinically healthy during the experiment. The vitamin A content in the jack mackerel was approximately 25,700 IU/kg DM on average[10]. Averaged vitamin A intake was 1,640, 2,480 and 3,320 IU/day in penguins supplied with 0, 840 and 1,680 IU vitamin A/day, respectively. The dietary vitamin A concentration can, therefore, be calculated as 25,700, 38,800, and 51,900 IU/kg DM, respectively, if the weight of vitamin pre-mix tablets is considered negligible. Thus, the dietary vitamin A concentration in all three dietary regimes was much higher than the tentative recommended requirement (3,500 IU/kg DM)[6], even in the diet without vitamin A supplementation.

Plasma retinol concentrations were numerically higher in the birds supplied with both doses of vitamin A than in those without vitamin A supplementation; however, this effect of vitamin A supplementation on plasma retinol concentrations was not significant (Table 1). The reference range of plasma retinol concentration in penguins has been reported to be between 68 and 130 μg/100 ml[7]. All measured plasma retinol concentrations in all birds in this study exceeded the lower limit of this reference range, and plasma retinol concentration was over the higher limit of the reference range in one bird without vitamin A supplementation, in two birds supplied with the lower dose of vitamin A, and in one bird supplied with the higher dose of vitamin A. All birds, however, had lower plasma retinol concentrations than the criterial level for potential hypervitaminosis A[7], irrespective of whether they were given vitamin A supplementation or not.

Plasma retinyl palmitate concentrations were significantly higher in the penguins supplied with the higher dose of vitamin A than in those without vitamin A supplementation (Table 1). The birds supplied with the lower dose of supplementary vitamin A had concentrations of

Table 1 Plasma vitamin A concentrations in penguins given graded levels of vitamin A supplement

		Vitamin A supplementation (IU/day)			Effect
		0	840	1,680	(P =)
Retinol	μg/100 ml	111.7 ± 27.7	137.4 ± 25.9	133.7 ± 26.2	0.063
Number of birds*		0	0	0	
Retinyl palmitate	μg/100 ml	8.98 ± 2.73^{b}	11.39 ± 2.65^{ab}	11.50 ± 1.27^{a}	0.037
Number of birds*		1	3	3	

Means ± SD for four birds
a,b Different letters indicate significant difference ($P < 0.05$).
* Number of birds having plasma vitamin A concentration beyond the criterial level for potential hypervitaminosis A[7].

plasma retinyl palmitate at a level that was intermediate between the penguins without vitamin A supplementation and those supplied with the higher dose. Plasma retinyl palmitate concentrations were higher than the criterial level for potential hypervitaminosis A[7] in one bird without vitamin A supplementation and in three birds in each of the diets which supplied supplementary vitamin A at both doses.

Discussion

Our study results indicate that vitamin A supplementation increased the plasma retinyl palmitate concentration when measured before the morning meal but did not significantly affect plasma retinol concentrations. This suggests that, in penguins, plasma retinyl palmitate sensitively responds to high vitamin A intake but plasma retinol does not.

Since absorbed vitamin A is transported as retinyl esters to the liver where vitamin A is stored, plasma retinyl ester concentration is postprandially increased in humans [11]. When vitamin A intake is excessive, however, the liver is no longer able to take up retinyl esters from the circulation, and retinyl esters are spilled out from the liver into the bloodstream in what is known as the post absorption phase[4]. Thus, plasma concentrations of retinyl esters such as retinyl palmitate are increased by excess vitamin A[12], even when a meal has not recently been consumed, and therefore circulating retinyl ester concentration in fasting humans is considered a useful biomarker for excess vitamin A[4].

Circulating retinol concentration is decreased by vitamin A deficiency. However, if humans are not deficient in vitamin A, varying vitamin A intake does not affect retinol concentrations in the blood because levels are homeostatically controlled by retinol binding protein 4 (RBP4) synthesis[4].

The present study reports that vitamin A supplementation increases plasma retinyl palmitate concentration but not plasma retinol concentration in fasting penguins which have been fed diets containing a large amount of vitamin A. These results differ from those presented by Crissey *et al.*[5], who reported that retinol concentrations in penguin plasma decreased with the reduction of dietary vitamin A concentrations from 59,800 IU/kg DM to 13,500 IU/kg DM. These dietary vitamin A levels are much higher than the tentative recommended vitamin A requirement[6]. The highest concentration of dietary vitamin A used by Crissey *et al.*[5] was almost the same as that used in this study. Wallace *et al.*[7] reviewed reports showing circulating retinol concentration in penguins, and indicated a positive correlation between plasma retinol concentration and vitamin A intake in penguins given diets rich in vitamin A; this conclusion also differs to the results shown in the present study.

The production of RBP4 is decreased by certain dietary conditions such as protein-energy malnutrition and zinc deficiency, which decreases circulating retinol concentrations[13]. The results of both Crissey *et al.*[5] and Wallace *et al.*[7] were based on varying vitamin A intake by feeding penguins with different kinds of fishes. The present study, however, controlled vitamin A intake by the supplementation of vitamin A to the otherwise unchanged basal diet. It is possible, therefore, that for Crissey *et al.*[5] and for Wallace *et al.*[7], it was other changes in the dietary regimes that affected RBP4 synthesis in their studies, rather than it being caused by changing vitamin A intake alone. However, it should be noted, that we only used four penguins in the present study and plasma retinol concentration was numerically increased by vitamin A supplementation, even though statistical significance was not achieved. The authors suggest that a further study using a larger number of penguins is necessary for confirming the effect of vitamin A supplementation on plasma retinol concentration in penguins.

According to the criteria proposed by Wallace *et al.*[7], none of the birds was diagnosed with potential hypervitaminosis A, because all birds had lower plasma retinol concentrations than the criterial level. However, plasma retinyl palmitate concentration was higher than the criterial level in one bird without vitamin A supplementation and in three birds each in the diets supplied with both doses of vitamin A. Since plasma retinyl palmitate sensitively responds to excess vitamin A in penguins, these penguins are considered to have excess vitamin A. However, no clinical problems were exhibited in these penguins. Wild penguins sometimes ingest fishes that are extremely rich in vitamin A, and penguins are therefore considered to be highly tolerant against excess vitamin A[5]. Vitamin A concentration has been reported to be 50,000 IU/kg DM in a representative diet for penguins at an aquarium[6], which is as high as the most highly supplemented diet in the present study. Therefore, we consider that there is only a very low risk of hypervitaminosis A in the penguins given jack mackerels with vitamin A supplementation. On the other hand, the present study also showed that plasma retinol concentration was higher than the lower limit of its reference range[7] even in the penguins which were not given vitamin A supplementation. These results support the conclusions of Akashi *et al.*[8], who suggested that vitamin A supplementation is meaningless for penguins that were fed jack mackerels, as they already receive adequate vitamin A from the fish in their basal diet. Therefore, we conclude that it is preferable not to provide supplementary vitamin A to penguins that are fed jack mackerels to avoid the risk of excess vitamin A.

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