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Secretion pattern of retinol-binding protein in blood of goats: Effect of vitamin A, provitamin A and their dosing schedule

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Summary: Studies were conducted on the secretion of retinol-binding protein (RBP) in blood of goats given different treatments of preformed vitamin A, β-carotene and plant carotenoids. Administration of these sources either in a single massive dose or massive dose split into four equal doses, markedly increased the secretion of RBP in blood. The secretion of RBP in blood occurred at least in two phases, one at early periods and the other at later periods.

Zusammenfassung: Die Sekretion von retinolbindendem Protein (RBP) ins Blut von Ziegen wurde nach unterschiedlicher Verabreichung von Vitamin A (Retinylacetat), β-Carotin und einer Mischung von pflanzlichen Carotinoiden untersucht. Die Zufuhr dieser Verbindungen – entweder in einer einzelnen hohen Dosis oder in der gleichen Menge gleichmäßig auf 4 Tagesdosen verteilt – führte zu einem deutlichen Anstieg der RBP-Sekretion. Diese Sekretion erfolgte in zwei Phasen, von denen eine früh und eine später einsetzte.

Key words: RBP secretion; vitamin A; carotenoids; goat

Schlüsselwörter: RBP-Sekretion; Vitamin A; Carotinoide; Ziege

Introduction

A number of studies on vitamin A metabolism have been done and a great deal of knowledge has emerged concerning the mechanism of absorption, storage, and transport (1, 2). Goodman et. al. showed for the first time that vitamin A is circulated as retinol bound to a specific protein called Retinol-binding protein (RBP) (3). On demand by the target tissue, the retinol (vitamin A) is secreted from its storage site in the liver to the blood circulation, and bound to RBP in the form of a 1:1 molar complex. The presence of receptor/translocation protein for vitamin A has earlier

Table 1. Dosing schedule for subject goats.

Group 1	single massive dose of vitamin A (retinyl acetate), i.e., 3.3 mg/kg body wt/goat and also 2 μCi 3H-retinyl acetate (specific activity 29 Ci/mmol) (SMDS);
Group 2	given 3.3 mg vitamin A into four split doses, i.e., 0.825 mg/kg body wt/goat daily for four days; also given 2 μCi 3H-retinyl acetate once (SDS);
Group 3	single massive dose of beta-carotene, i.e., 19.8 mg/kg body wt/goat. The vitamin A content of beta-carotene was calculated as 6 μg beta-carotene = 1 μg all-trans-retinol (12) (SMDS);
Group 4	given 4.95 mg beta-carotene/kg body wt/goat daily for four days (SDS);
Group 5	single dose of plant carotenoids, i.e., 36 · 6 mg/kg body wt/goat (SMDS). The vitamin A content of carotenoids was calculated as 12 μg mixed carotenoids = 1 μg all-trans-retinol (11);
Group 6	9.9 mg carotenoids/kg body wt/goat daily for four days (SDS);
Group 7	39.6 mg carotenoids/kg body wt/goat daily for four days (MMDS).

SMDS = single massive dosing schedule, SDS = split dosing schedule, MMDS = multiple massive dosing schedule

been reported in target tissues (4–6). Under physiological conditions, the RBP mediated transport of retinol in blood is predominant and the delivery of retinol from blood to target tissue has been shown to be RBP dependent (7–9). In the present investigation, we studied the secretion of RBP in blood of goats at different periods after treatment with vitamin A or provitamin A. We have also studied the effect of dosing schedule in this regard.

Materials and methods

Goats (body wt 15–23 kg) were purchased locally and housed individually in iron bar cabins. After acclimatization from farm conditions to animal house conditions, they were changed from green grass diet to concentrate mixture diet. The composition of concentrate mixture was (g/kg)-wheat straw 500, wheat 385 deoiled groundnut cake 100 NaCl 5 and mineral mixture 10. The mineral mixture contained (%)-Ca 22, P 9, NaCl 22, Co o. 2, Cu o. 1, Fe o. 6, Mn o. 12, I o. 1 and F o. 3.

The blood serum vitamin A and serum RBP levels of goats were in the range of 30–40 μg/100 ml and 2.6–2.7 mg/100 ml, respectively. Goats had free access to tap water.

Plant carotenoids from raddish *(Raphanus sativus)* leaves were extracted according to Britton and Goodwin (10) and stored under nitrogen atmosphere at –20 °C until used. The biological potency of these carotenoids was determined using vitamin A deficient chicks according to parameters of growth, liver and plasma vitamin A-levels, as described earlier (11). Goats were divided into seven groups (three in each group) and given treatments as shown: in Table 1.

All treatments were given orally in groundnut oil in gelatin encapsulated form. Blood samples were drawn at 6, 12 and 24 h and then daily up to 14 days after treatment. Serum was prepared by centrifuging at 2200 rpm for 20 min in a Sorvall refrigerated centrifuge (model RC-5B). The total RBP level in serum was estimated using rocket electro-immunoassay (13); for this, goat serum RBP was purified to homogeneity as described earlier (14). The anti-RBP-sera was produced in male rabbits (body wt 1.5 kg). The standardized volume of anti-RBP-sera was mixed with 1% agarose gel solution. Gel was then coated on glass plates (gel thickness 1.5 mm).

Round wells of appropriate size were made at one end of the gel plate. In each well 10 μl serum sample was applied and the electrophoresis was carried out in a

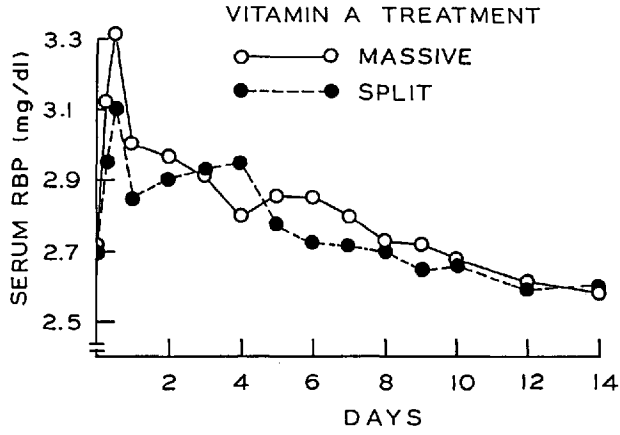

Fig. 1. Serum RBP levels at different periods in goats given preformed vitamin A.

cold room. The immunoprecipitates were formed in rocket shapes. The height of each rocket was measured under simple microscope with mm scale attachment. The amount of RBP was calculated using standard curve drawn between purified RBP concentrations and rocket height of the immunoprecipitates. The 3H-vitamin A radioactivity bound to serum RBP was determined by immunoprecipitation method as described earlier (15).

Results and discussion

The aim of this study was, first, to determine the secretion pattern of RBP in response to different vitamin A treatments at different periods,

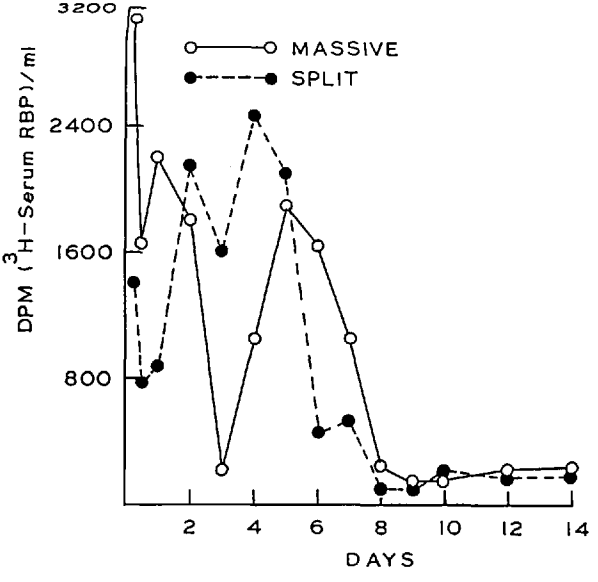

Fig. 2. Secretion of RBP associated 3H-vitamin A radioactivity in serum of goats at different periods after vitamin A treatment.

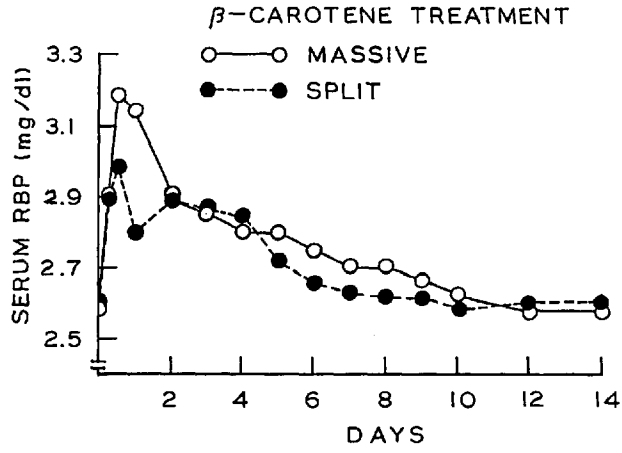

Fig. 3. Serum RBP levels at different periods in goats given beta-carotene.

and secondly, to study the effect of dosing schedule on the secretion process. Therefore, the different treatments were given in, i) single massive dose and ii) massive dose, split into four equal doses given on consecutive days, and, iii) massive doses for four consecutive days.

The administration of preformed vitamin A either in a single massive dose or in split doses, profoundly increased the secretion of RBP in blood serum of goats at 12 h after treatment (Fig. 1) The peak increase in RBP was about 22 % in SMDS and about 15 % in SDS; thereafter RBP levels started declining and reached the basal values after seven days (Fig. 1). In addition, the secretion of RBP bound 3H-vitamin A activity at various periods occurred mostly in early periods, i.e., up to two days (Fig. 2). The secretion was also observed at later periods. The secretion pattern of RBP in blood serum of goats given beta-carotene, is shown in Fig. 3. The peak increase in RBP was about 23 % in SMDS and about 14 % in SDS at 12 h after treatment. However, in SMDS a continuous gradual decrease in secretion of RBP was noted, but in SDS the RBP levels declined 12 h after the treatment followed by an increase on the second which sustained up to the fourth day; thereafter the RBP levels decreased and reached the basal values (Fig. 3).

The effect of administration of plant carotenoids on the secretion of RBP in blood serum is depicted in Fig. 4. The peak increase was about 24 % in SMDS and about 15 % in SDS at 12 h after treatment. However, in the case of multiple massive dosing schedule (MMDS) of carotenoids, the secretion of RBP occurred for longer periods (Fig. 5). The RBP levels increased rapidly 12 h after treatment and then decreased on the first day, followed by a maximum level up to the fourth day; after that the secretion process slowed down and RBP levels gradually reached basal values (Fig. 5).

On the basis of these results, it can be observed that secretion of RBP into blood circulation is influenced by preformed or provitamin A. Further, the dosing schedule appears to be very important in influencing

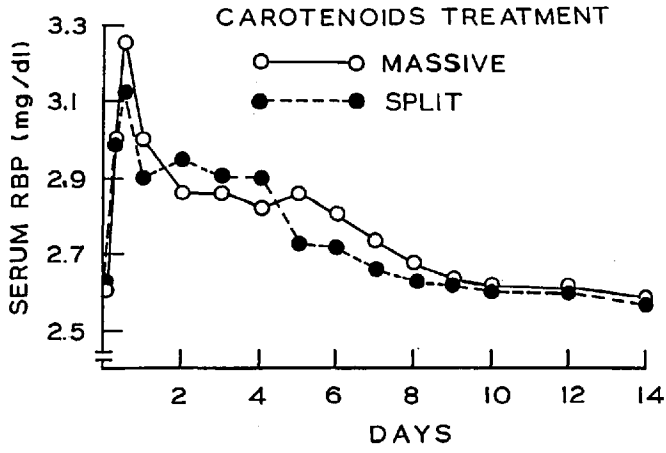

Fig. 4. Serum RBP levels at different periods in goats treated with carotenoids.

the secretion phenomena. These results also show that secretion of RBP occurred in several phases. This may be explained on the basis that more than one body pool of vitamin A are present in the body – one a rapid turnover pool of newly dosed vitamin A, a slow turnover pool of endogenous storage vitamin A, and another extra-hepatic pool. The presence of such pools in rat tissue was earlier suggested (16, 17).

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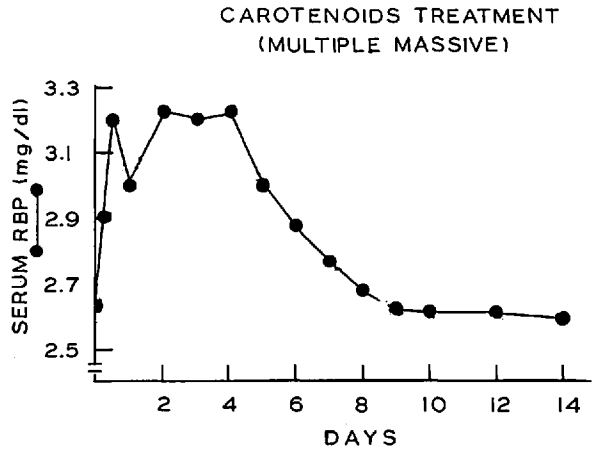

Fig. 5. Effect of multiple massive dosing schedule of carotenoids on serum RBP levels at different periods.

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