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SHORT REPORT

Skimmed milk as a determinant of vitamin A deficiency

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Objective: To compare the levels of vitamin A in ultra-high temperature treated (UHT) whole milk (3.5% fat) and UHT skimmed milk (0.5% fat) using UV-visible light spectrophotometry and to compare the contribution of whole milk and skimmed milk to the recommended dietary allowance (RDA) for vitamin A.
Design: Paired samples of liquid whole milk and liquid skimmed milk were used. Sampling and analysis were performed by different individuals to achieve a randomised blind design.
Outcome measures: Thirty paired samples ($n = 30$) of whole milk and skimmed milk were evaluated for vitamin A content using UV-visible light spectrophotometry at 328 nm.
Results: Absolute concentration of vitamin A was reduced from 208.830 ± 0.083 µg/L in whole milk to 35.855 ± 0.046 µg/L in skimmed milk. The 85.7% reduction in butterfat content from 3.5% in whole milk to 0.5% in skimmed milk was accompanied by an 82.824 ± 3.51% (mean ± SD) reduction in retinol content.
Conclusion: The contribution of milk to the RDA for vitamin A was reduced from the standard 7.6% for whole milk to 1.30% for skimmed milk with 0.5% fat. The results emphasise the need for fortification of skimmed milk with vitamin A in order to augment the prevention of vitamin A deficiency diseases in developing countries.

Keywords: recommended dietary allowance, skimmed milk, vitamin A, whole milk

Introduction

Amongst all natural foods and beverages milk is unique in that it contains all known water-soluble and fat-soluble vitamins. The fat-soluble vitamins A (retinol), D (1,25 dihydroxycholecalciferol), E (α-tocopherol) and K (2-methyl-3-phytal-1,4-naphthoquinone) are normally associated with the butterfat content of milk. In addition, butterfat contains β-carotene, which upon ingestion serves as a precursor for synthesis of vitamin A.[1] Extraction of butterfat from milk during skimming is therefore expected to result in substantial loss of fat-soluble vitamins. However, the healthy organism is able to synthesise vitamin D beginning with the action of ultraviolet light on cholesterol in the skin. Likewise vitamin K can be obtained from synthetic activities of symbiotic bacteria resident in the colon. Vitamin A and vitamin E, on the other hand, have to be obtained from external sources. A daily serving of 244 ml of cow's whole milk contributes 7.6% to the recommended dietary allowance for vitamin A and only 1% to the RDA for vitamin E.[2] Despite the lesser nutritional contribution, vitamin E in milk helps to slow down peroxidation of lipids[3] and thus enhances the bioavailability of vitamin A as well as other fat-soluble vitamins. According to previous reports, whole milk contains on average 310 µg/L[4] of vitamin A (1 IU = 0.3 µg/L). Important seasonal variations have, however, been documented such that the vitamin A content of whole milk ranges between 200 and 480 µg/L.[5] Although the above values are expected to be significantly reduced in skimmed milk, public literature is inundated with the health benefits of fat-free milk, as portrayed in advertisements in various newspapers and magazines. A dearth of scientific studies and data overshadows the effects of skimming on the status of fat-soluble vitamins. The objective of the present study was to quantify the reduction in vitamin A content when bovine standardised whole milk (3.5% butterfat) is converted to skimmed milk (0.5% butterfat). Measurements of this nature help in providing a quantitative basis for universal fortification and prevention of vitamin A deficiency diseases in developing nations.

Method

Thirty paired samples of the same brand of UHT (ultra-high temperature treated) whole milk (3.5% butterfat) and UHT skimmed milk (0.5% butterfat) of Zimbabwean origin were randomly acquired from commercial sources. In accordance with the regulations of the country, all milk samples acquired were within the expiry dates of the products i.e. nine months from the date of manufacture. Only milk from light-proof packages was used, to protect vitamin A from oxidation by light.[6] No external antioxidant was added to milk samples because whole milk contains on average 800 µg/L[7] of vitamin E, which protects against peroxidation of unsaturated double bonds in vitamin A.[8] The principles of extracting fat-soluble vitamins from milk for analysis are well established.[9] All the reagents used in the vitamin A measurements were of analytical grade. In the current investigation, the first step in determination of fat-soluble vitamin A in the milk samples involved disruption of fat, casein and whey protein by addition of 1.7 ml aliquots of methanol (Fisher Scientific, Loughborough, UK) to 5 ml samples of whole milk and 5 ml samples of skimmed milk, respectively. Next, alkaline saponification was carried out to break down lipid globules in which the fat-soluble vitamins were bound. To this end, 3.3 ml of 10% w/v KOH (Fisher Scientific, Loughborough, UK) were added to the samples, after which they were vortex-mixed for 1 min and placed in a water bath at 70°C for 30 min. The samples were then removed from the water bath and cooled in ice for 10 min. The third step involved extraction of fat-soluble vitamins from the milk. Exactly 1.7 ml of diethyl ether (Skylabs, Johannesburg, South Africa) was added to the samples and vortex-mixed for 1 min. The samples were then centrifuged using a bench centrifuge (Centaur 2, MSE UK Ltd, London, England) at 3 000 rpm/1818 x *g* for 10 min. Separation yielded a lipophilic supernatant containing the fat-soluble constituents of milk and a bottom aqueous emulsion containing the rest of the milk components. In the fourth and final step, the levels of vitamin A in the supernatant were determined using a UV-visible light spectrophotometer

(Li-295, Lasany International, Panchkula, Haryana, India). Use of spectrophotometry in determination of fat-soluble vitamins in biological fluids has been previously described.[10] In the present investigation, 3 ml of the supernatant were transferred from each centrifuge tube to a quartz cuvette and the optical density of vitamin A measured at the wavelength corresponding to the peak absorbance. The absorption spectrum of vitamin A is between 300 and 350 nm,[11] with peak absorbance at 328 nm. The standard was prepared by dissolving 600 µg/L of vitamin A (Norbrook, Newtry, Northern Ireland) in a mixture of methanol and diethyl ether as for the milk samples. The molar extinction coefficient of vitamin A in the methanol/diethyl ether mixture was 1.345×10^{-6} L/cm/mol. Filtered distilled water was used as a blank. Vitamin A is fat-soluble and hence certainly absent in pure water. All experiments were carried out in duplicate and the results expressed as mean ± standard deviation (SD). Optical density was converted to vitamin A concentration as follows:

(Optical density of sample/Optical density of standard) × Concentration of vitamin A in standard.

Modern spectrophotometers are equipped with a photomultiplier that ensures adequate sensitivity of the instrument in the entire UV-visible region of the electromagnetic spectrum. The detection limit of the instrument in the present study was 0.001 absorbance units, equivalent to a vitamin A concentration of 0.213 µg/L.

The measurements of vitamin A levels in milk were carried out in the Analytical Chemistry Laboratory of the Department of Preclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe. Two suitably qualified and trained technicians performed the analysis. The coefficient of variation of the extractions and spectrophotometry analyses, as computed from the formula, standard deviation/mean, was 0.276 for skimmed milk compared with 0.084 for whole milk. Thus sensitivity of UV-visible light spectrophotometry decreased with decreasing vitamin concentrations. Student's *t*-test for comparison of two means was used to test for differences in vitamin A content between whole milk and skimmed milk. The level of significance was set at 95% confidence limits ($p < 0.05$).

Results

In spectrophotometry the concentration of a substance is directly proportional to its optical density, or absorbance. It was thus possible to quantify the loss of vitamin A from whole milk upon skimming by comparing the optical density of the nutrient in whole milk and skimmed milk. In all cases, consistently lower levels were recorded for skimmed milk compared with full cream milk. Figure 1 shows the optical density of vitamin A in skimmed, whole milk and the standard, depicting the reduction in concentration of vitamin A in skimmed milk relative to whole milk.

The average loss of vitamin A from skimmed milk expressed as a percentage of whole milk was 82.824 ± 3.51% as computed from the formula:

$$\frac{\text{Optical density of vitamin A in whole milk} - \text{Optical density of vitamin A in skimmed milk}}{\text{Optical density of vitamin A in whole milk}} \times 100$$

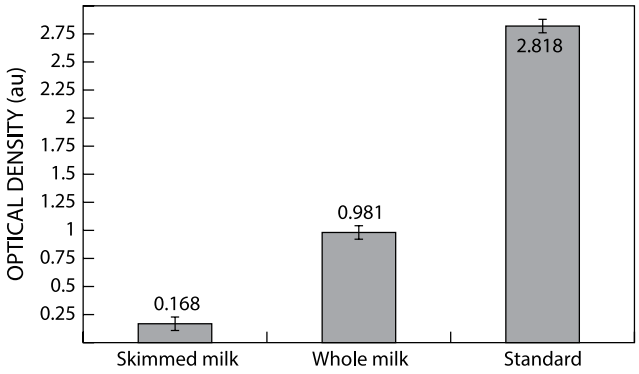

Figure 1: Mean optical density of vitamin A in skimmed milk with 0.5% fat, whole milk with 3.5% fat and the standard with 600 µg/L of vitamin A.

Table 1: Concentration of vitamin A in skimmed milk compared with whole milk (Mean ± SD)

Sample	Concentration of vitamin A (µg/L)
Skimmed milk	35.855 ± 0.046
Whole milk	208.830 ± 0.083

Reduction in butterfat content from 3.5% to 0.5% caused loss of milk fat equivalent to 85.714%. Hence the correlation between milk fat content and vitamin A content was 97.28%.

Table 1 shows the actual concentrations of vitamin A in skimmed milk and whole milk as calculated from the optical density of skimmed milk, whole milk and the vitamin A standard.

Discussion

The present study elucidated a significant reduction of vitamin A in skimmed milk in comparison with whole milk. Milk skimming involves extraction of butterfat from whole milk for the manufacture of butter as well as various kinds of cream. Inevitably, fat-soluble vitamins partitioned in the fat portion of milk were also removed during the skimming process. Therefore apart from a low fat content, skimmed milk also happens to be low in fat-soluble vitamins. In developing parts of the world, deficiency of vitamin A in skimmed milk has received very little attention with the effect that the milk is marketed without any attempt to replace lost vitamins. In the present investigation, the contribution of skimmed milk with 0.5% fat to the RDA for vitamin A was a paltry 1.30%, compared with the standard 7.6% for whole milk with 3.5% fat. The results emphasise the need for fortification of skimmed milk with vitamin A in order to augment the prevention of vitamin A deficiency diseases in Zimbabwe as well as other countries where fortification is not mandatory.

The RDA for vitamin A ranges from 400 µg/day in infants below 6 months old, through 700 µg/day for adult females, to 900 µg/day for adult males.[12] Whole milk is a good source of vitamin A.[2]

The physiological functions of vitamin A and its derivatives have been extensively reviewed[1] and are summarised as follows. Retinol is critical in vision. Retinoic acid functions in maintenance of the integrity and innate immunity in epithelia (cornea, respiratory tract, digestive tract and reproductive tract). It is also involved in specific immunity by influencing differentiation of T cells. Other functions of retinoic acid include cell growth and development. With reduction in vitamin A content in skimmed milk, the contribution of milk to the RDA for vitamin A was reduced by well over 80%. Consumption of skimmed milk in poorly resourced settings therefore should be seen as a determinant of poor vitamin A status. In a worldwide survey by the World Health Organisation,[13] 190 million children under the age of five years and 19.1 million pregnant women had biochemical vitamin A deficiency, i.e. low serum retinol concentrations. Of this, 2.55 million children and 3 million expectant mothers suffered from night blindness, an indicator of clinical vitamin A deficiency. Other manifestations of vitamin A deficiency include xerophthalmia, permanent blindness, hyperkeratosis, anaemia and nutritionally acquired immune deficiency.[14] It is clear that any factor that contributes to a reduction in the dietary intake of vitamin A in vulnerable groups is highly undesirable, including plain skimmed milk. Although controlled studies on consumption patterns of skimmed milk in developing countries are lacking, intake of skimmed milk is likely to be higher in women of child-bearing age than other population groups, fuelled by the desire to 'shed' weight. As a result, offspring from vitamin A-deficient mothers may be at risk of hypovitaminosis A. Another factor that may increase consumer preference for skimmed milk, particularly among the health-conscious population groups, is the low cholesterol content of the milk.

As shown in Figure 1 vitamin A exists in milk chiefly as retinol. Results from the current investigation affirm that sufficient levels of vitamin A are normally present in whole milk from intake of fresh forages or silages.[15] As in previous studies, variations occurred in concentrations of vitamin A from sample to sample. Absolute concentrations of vitamin A in milk vary according to season, with increased levels in the summer months.[16] Variations in concentrations of vitamin A in milk also occur with diet[17] and with the stage of lactation.[18] In spite of the limitations to the study posed by the diverse sources of variation, the present results fell within the previously reported range of vitamin A concentration in whole milk. The average concentration was marginally lower than reported for whole milk prior to processing, pointing to a possible depletive effect of ultra-high temperature treatment. The principal limitation of UV-visible light spectrophotometry is that it does not distinguish between two substances that absorb light at identical wavelengths. For this reason, an empirically determined wavelength of 328 nm was used in the spectrophotometric measurements, the validity of which was confirmed from the standard solution of vitamin A. In conclusion, reduction in vitamin A content in skimmed milk relative to whole milk was a consistent finding. This study represents the first scientific report on vitamin A concentrations in skimmed milk. Further studies are clearly essential to fully explore the impact of the depletion, and its consequences on nutrient status, particularly of vitamin A and other fat-soluble vitamins at various levels of skimming.

Conflict of interest – There is no conflict of interest to declare.

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