VITAMIN CONTENT OF HUMAN MILK

by

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ABSTRACT

Fifteen lactating women served as milk donors for this study. Analyses were made for vitamin A, thiamine, and riboflavin. Samples were grouped into those which had been stored at -10 C for several months and those which had been stored approximately one week. Subgroups were made for each individual donor within each group. It was noted that a large variation occurred in vitamin A content of human milk both between donors and between samples from the same donor. This variation was presumed to be due at least partly to variation in dietary intake of this vitamin. Another source of variation may have been fluctuation in fat content of the milk due to time of sampling. No differences were noted due to length of storage. There was not nearly as great a difference between samples from the same donor or between donors in thiamine content as was the case with vitamin A. Length of storage appeared to have no effect on thiamine levels. There were greater variations in riboflavin content found in human milk in this study than in the case of thiamine. This agrees with earlier work which reported that dietary intake of riboflavin does affect the levels appearing in milk. Long-term storage had no effect on riboflavin content. In all three vitamins studied the levels found were very comparable to those levels reported in much earlier work indicating that our changing dietary habits have not changed the levels of these vitamins appearing in human milk.

INTRODUCTION

Vitamin A, thiamine, and riboflavin are three of the most important vitamins needed by the human for normal growth and health (8). There is a lack of recent comprehensive information giving the content and variability of these three vitamins in human milk. Since many believe that human milk is better for a newborn infant than milk analogs or cow's milk, it was decided to undertake the task of determining the content of these three vitamins in human milk. It was felt that more recent information would be useful in view of possible dietary changes resulting from wide differences in eating habits and in food product developments. The purpose was to obtain values which would be useful in helping those desiring to compare the nutritional value of human milk with simulated infant formulas or milk analogs of today. In this work, milk samples from donors were analyzed and average values compared for variance for each donor as well as between donors. Samples stored frozen for several months were compared with samples frozen for only a few days.

REVIEW OF LITERATURE

Over the years, an extensive amount of data has been published concerning the quantities of the vitamins present in human milk (15). Data have been obtained through the application of one or more of these methods: biological, microbiological, and chemical. These methods have been adequately described by others and will be mentioned only briefly in this review.

Since 1934, several studies have been made of the vitamin A content of human milk. The majority of these studies utilized the Carr-Price reaction (9). However, a much newer method using trichloroacetic acid (TCA) has been recently described (1).

Kon and Mawson (15) found that human milk samples which were rich in fat had a particularly high content of vitamin A. They also found that the content of vitamin A in milk fat was increased by meals containing liver and other foods rich in vitamin A. Other factors affecting the vitamin A content of human milk were influence of stage of lactation, milk yield, season of the year, and age of the mother (15). It was also noted that as fat percentage in milk increased vitamin A content also increased. Hartman and Dryden (11) in a review article reported that the average vitamin A content of human milk was 189.8 I.U. per 100 ml of milk for 16 references that were consulted.

Because of vitamin A's alcohol group, it can readily form esters and in normal milk, most is present in the ester form (16, 19, 21).

Thiamine occurs in milk and other natural foods either as free thiamine or in a form in which it is phosphorylated and complexed with protein (9). In normal milk, the total thiamine consists of 50% to 70% free thiamine and 5% to 17% protein-bound thiamine (3, 4). DeJong (5) found that the phosphorylated form was thiamine monophosphate.

The content of thiamine in milk remains fairly constant with little variation occurring from day to day in the milk of individual women. Much of the variation may be due to the rather unsatisfactory nature of the thiochrome method of estimation. Hartman and Dryden (11) reported an average value for human milk to be 16 µg per 100 ml.

Several tests have been utilized for thiamine analysis. The first method developed was by animal assay, with another being the microbiological method (9). The other two principal types of chemical analysis were: (a) a determination on thiochrome and (b) a measurement of the color produced by coupling an amine, such as p-aminoaceto-phenone, with thiamine (17, 20). All methods yielded similar data for most products (2, 7).

From 65% to 95% of the riboflavin in milk is in the free form (4, 10). According to Modi and Owen (18), the rest is present as flavin adenine dinucleotide (FAD), whereas none occurs as flavin mononucleotide (FMN).

Feer observed a greenish color in human milk which he attributed to riboflavin after the mother had eaten liver (15). Kayser (13) also examined the milk of women who had eaten liver and found that the riboflavin content was greatly increased. Donelson and Macy (6) found that the riboflavin content of milk was increased when the mother's diet was supplemented with yeast. It has been calculated by Hartman and Dryden (11), who consulted 16 research reports, that the average value for riboflavin in human milk was 36 µg per 100 ml.

Three general methods are satisfactory for the assay of riboflavin: fluorometric, microbiological, and animal. The fluorometric method is the most rapid and inexpensive.

MATERIALS AND METHODS

Fifteen lactating women served as milk donors for this study. Milk samples were collected by each donor in a sterilized bottle and stored at ca -10 C until analyzed.

After the milk sample had been thawed, it was divided into three portions: 75-100 ml for vitamin A analysis, 10-25 ml for thiamine analysis, and 8-10 ml for riboflavin analysis.

Vitamin A was determined in this experiment using TCA as the chromogenic agent (1). Results from this method are similar to those obtained with $SbCl_3$. Two disadvantages with the use of the TCA reagent are its corrosive nature which requires careful handling and the need for accurate timing due to the rapid fading of the blue color resulting from the reaction.

Prior to spectrophotometric measurement, the milk sample for vitamin A analysis was extracted by ether. The fat was then saponified and the unsaponifiable fraction was separated. By adding TCA to this fraction an unstable blue color was formed. Optical density of this blue solution was then read at 620 $m\mu$.

The thiochrome method (9) was used for thiamine analysis; however, the purification procedure was omitted in order to eliminate the possibility of any loss of thiamine due to incomplete adsorption and elution and to decrease errors of manipulation. Therefore, thiamine was determined directly from extracts of the milk sample. A Coleman Model 17C Fluorometer was used in this analysis.

The fluorometric method (9) was used for riboflavin analysis in this experiment.

RESULTS AND DISCUSSION

The results of vitamin A analysis of human milk are shown in Table 1. A large variation was noted both between donors and between samples from the same donor. This agrees with previous work (12, 14, 15) that has reported a major dietary influence on vitamin A content in milk. It is obvious from the large range in values (59.38 to 364.79 I.U. of vitamin A per 100 ml milk) that there was a very large variation in vitamin A intake by various donors. It should also be pointed out that a major source of error may occur during sample collection. Fat content of milk varies tremendously from first to last milk drawn from a single nursing (15). Consequently, vitamin A content (w/v) would vary according to the amount of fat in the milk sample. No attempt was made during the course of this experiment to control this variable. It is obvious that further study is necessary to determine adequately the exact cause of the variability of vitamin A in human milk.

While there was a high degree of variability of vitamin A in this study, it is interesting to note that average values of fresh milk and stored milk were nearly identical. It was also noted that a similar range and degree of variability occurred within each group. There was a slightly lower variability within the fresh milk samples than in the stored milk, but this was not considered significant. Thus, long-term, low-temperature storage appeared to have no adverse effects on vitamin A content.

Table 1. Mean Vitamin A Content of Human Milk

Donor	Number of Samples	Mean Value (I.U./100 ml)	Standard Deviation
Fresh Milk			
R	3	234.92	75.83
G	6	146.89	84.99
D	2	364.79	8.50
A	4	81.07	34.32
B	3	101.57	49.52
E	1	59.38	---
C	1	259.39	---
K	1	115.64	---
Subtotal		165.82	106.46
Stored Milk			
H	2	59.41	5.13
115	2	67.39	26.37
X	3	277.43	103.09
122	1	289.88	---
128	1	262.24	---
F	1	293.72	---
P	1	141.01	---
Subtotal		169.32	128.46
Total		166.72	110.29

Previous studies which were reviewed by Kon and Mawson (15) have not reported as high variabilities in vitamin A content as were found in the present study. This may have been due to the random selection of the donors in this study but was most probably due to the diverse vitamin supplementation habits of the donors.

Despite the wide variation in vitamin A content of human milk found in this study, it did compare quite closely with the average value reported by Hartman and Dryden (11) in their review article on vitamins in milk. In comparing the results of this study to those of Kon and Mawson (15), it is necessary to convert I.U. to μg, using the assumption that 1 μg equals 2.93 I.U. On this basis, the mean vitamin A content of human milk in the present study was 55.5 μg per 100 ml, which compares quite closely with the 58.4 μg per 100 ml reported by Kon and Mawson (15).

Thiamine values obtained during this study are given in Table 2. The average value of 14.45 μg per 100 ml of milk agrees quite closely with previously reported values reviewed by Kon and Mawson (15) and also by Hartman and Dryden (11). There was not nearly as high a variation between donors or within individual donors as there was in vitamin A content. Thiamine, being water soluble, would not vary with drastic changes in fat percentage as would vitamin A. Thiamine would thus not be dependent on fat content as was the case with vitamin A.

There were no differences in the thiamine content of samples that had been stored for several months and those which had been frozen for only a few days. Thus, thiamine does not appear to deteriorate with long-term freezing.

Table 2. Mean Thiamine Content of Human Milk

Donor	Number of Samples	Mean Value (μg/100 ml)	Standard Deviation
		Fresh Milk	
R	3	10.06	0.71
G	6	15.17	3.35
D	2	12.04	2.87
A	4	18.23	3.97
B	3	12.32	0.19
E	1	15.56	---
C	1	17.42	---
Subtotal		14.41	3.82
		Stored Milk	
H	2	17.05	1.16
115	2	16.31	1.39
X	3	12.12	1.40
128	1	14.92	---
122	1	11.87	---
B.F.	1	15.64	---
Subtotal		14.55	2.52
Total		14.45	3.40

It should be pointed out that the thiochrome method (9) for the estimation of thiamine is not a very satisfactory procedure. The oxidation of thiamine to thiochrome with alkaline ferricyanide appears to be affected by influences difficult to control. Thus, erratic results are often obtained. It is hoped that a more satisfactory chemical procedure will be developed to gain a better estimate of thiamine content.

The riboflavin values obtained in this study are given in Table 3. The values obtained from this study are very similar to those of Hartman and Dryden (11). While there were no significant differences noted between long-term stored milk and fresh milk, there was a wider variation both between donors and with individual donors than was noted with the thiamine content. Kon and Mawson (15) have pointed out that diet does have a greater influence on riboflavin content of milk than it does on thiamine content. It is presumed that much of the variation in riboflavin in this study was due to variation in the dietary intake of this vitamin.

Table 3. Mean Riboflavin Content of Human Milk

Donor	Number of Samples	Mean Value (μg/100 ml)	Standard Deviation
		Fresh Milk	
R	3	26.63	4.13
G	6	20.59	9.64
D	2	49.67	9.05
A	4	36.19	13.09
B	3	24.65	1.03
E	1	34.00	---
C	1	54.76	---
K	1	53.13	---
Subtotal		32.37	14.10
		Stored Milk	
H	2	33.00	3.00
115	2	32.70	0.30
X	3	23.70	0.30
Subtotal		29.80	5.10
Total		31.84	12.73

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