Ascorbic acid supplementation and regular consumption of fresh orange juice increase the ascorbic acid content of human milk: studies in European and African lactating women[1–3]

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ABSTRACT

Background: Little is known about the influence of an increased intake of ascorbic acid (AA) on human milk AA output.

Objective: We aimed to compare human milk AA content in European and African women and to evaluate the influence of increased AA intake on human milk AA output.

Design: Apparently healthy lactating women were recruited. AA was analyzed by titration with 2,6-dichlorophenol-indophenol.

Results: Mean human milk AA was ≈50% lower ($P < 0.001$) in the African women (31 mg/kg; $n = 171$) than in the European women (63 mg/kg; $n = 142$). AA supplementation (1000 mg/d for 10 d) increased mean human milk AA from 19 to 60 mg/kg ($P < 0.001$) and from 60 to 70 mg/kg ($P = 0.03$) in 18 African and 10 European women, respectively. In 11 African women, mean human milk AA increased from 17 to 36 mg/kg ($P < 0.001$) after intake of 100 mg AA/d for 10 d. In African women, intake of 1 serving of orange juice per week had no significant effect, whereas 3 or 5 servings/wk (≈100 mg AA/serving) for 6 wk increased mean human milk AA from 16 to 32 mg/kg ($n = 13$) and from 21 to 46 mg/kg ($n = 13$), respectively ($P < 0.001$).

Conclusions: Human milk AA can be doubled or tripled by increased intake of AA in women with low human milk AA content at baseline. The response to a relatively high dose of AA was modest in European women in contrast with the 3-fold increase in mean human milk AA content in African women. These data indicate that human milk AA content is regulated. *Am J Clin Nutr* 2005;81:1088–93.

KEY WORDS Vitamin C, breastfeeding, infants, fruit juice, ascorbic acid

INTRODUCTION

Lactating women in developing countries have been reported to have significantly lower human milk ascorbic acid output than do women in industrialized countries (1–7). Human milk ascorbic acid content varies with maternal intake of ascorbic acid. Generally, lower maternal dietary intake of ascorbic acid and more pronounced seasonal variation in consumption of ascorbic acid–rich foods such as fruit and vegetables have been identified as major reasons for the low human milk ascorbic acid content of women living in resource-poor areas (4, 6, 8–10).

As a result of the large differences in the content of ascorbic acid in human milk between lactating women in different settings, the intake of ascorbic acid in exclusively breastfed infants varies widely. Although no cases of scurvy have been reported in breastfed infants, and the daily requirement for dietary ascorbic acid to prevent overt ascorbic acid deficiency in breastfed infants appears to be very low [7 mg/d; reviewed by the Institute of Medicine in 2000 (11)], the potentially negative influence of low ascorbic acid intake in early life related to the antioxidative properties of ascorbic acid has not been evaluated. Furthermore, human milk can be a major source of ascorbic acid in the overall diet of older infants consuming complementary foods, because traditional feeding practices in some settings limit the intake of ascorbic acid–rich foods during early life. For example, our recent study in Côte d'Ivoire clearly showed the importance of human milk as the major source of ascorbic acid in the diet of children aged 6–18 mo (12). Under these conditions, the child's dietary intake of ascorbic acid is directly dependent on his or her mother's human milk ascorbic acid output and therefore dependent on maternal intake of ascorbic acid. Ascorbic acid intake in the African infants and young children in our previous study varied widely and resulted in large variation in the molar ratios of ascorbic acid to iron in the overall diet (12).

Our findings in Côte d'Ivoire thus highlighted the need to evaluate sustainable approaches to increasing human milk ascorbic acid output in lactating women in resource-poor areas. Only limited information is available on the influence of increased intake of ascorbic acid during lactation, and we therefore initiated a series of studies to evaluate the effect of ascorbic acid supplementation and increased consumption of an ascorbic acid–rich food, orange juice.

The aims of the present study were to compare human milk ascorbic acid content of European and African lactating women and to evaluate the influence of an ascorbic acid supplement (1000 mg/d) in both study populations. In African women, we also evaluated the influence of a lower dose of ascorbic acid as a supplement (100 mg ascorbic acid/d) as well as the effect of

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regular consumption of fresh orange juice, served 1–5 times/wk (≈100 mg ascorbic acid/serving) for 6 wk. African women with relatively low human milk ascorbic acid content (<40 mg/kg) were enrolled into the longitudinal intervention studies.

SUBJECTS AND METHODS

Subjects

Apparently healthy, lactating women with apparently healthy infants older than 1 mo were recruited in Zurich, Switzerland, and in Abidjan, Côte d'Ivoire. Individual data on age, parity, date of birth, birth weight, and duration of breastfeeding were collected from each woman on the basis of recall or, in Abidjan, partly by using information from documents used for antenatal, prenatal, and infant health care ("carnet de santé pour la mère et l'enfant"). European women were recruited at meetings attended by lactating mothers. In Abidjan, lactating mothers were recruited at an information center that focused on infant nutrition at the National Institute of Public Health (Institut de Santé Publique). In addition, women were recruited by personal contacts in 2 low-socioeconomic areas (Atécoubé and Koumassi). To evaluate the effect of increased intake of ascorbic acid in women with different baseline ascorbic acid milk content, African women were recruited on the basis of an initial screening study. African women with milk ascorbic acid content <40 mg/kg were enrolled into studies 2, 4, and 5. All women enrolled into studies 2–5 were asked to not change their dietary habits or general lifestyle and to not consume ascorbic acid supplements (unless provided by the investigators) throughout the study.

The study protocol was reviewed and approved by the ethical committee at the University Hospital of Zurich, Switzerland, and by Institut National de Santé Publique in Abidjan. In addition, permission to implement study 5 was obtained from local authorities in Koumassi and Atécoubé. Women were informed about the aims and procedures of the study orally, and oral consent was obtained from each woman before the start of the study.

Study design

Five separate studies were implemented. Study 1 was a screening study to evaluate ascorbic acid in human milk collected from European and African women. One sample of human milk was collected from each woman. Study 2 was designed to evaluate the influence of an additional intake of 1000 mg ascorbic acid/d for 10 consecutive days in European and African women. One sample of human milk was collected every day from each woman. In study 3, a group of European women consumed 1000 mg ascorbic acid/d for 5 consecutive days and were followed for 35 d after discontinuation of the supplement. Three samples of human milk were collected during the 5-d supplementation. Additional samples were collected twice per week during the next 35 d. The influence of a smaller dose of ascorbic acid (100 mg ascorbic acid/d) for 10 consecutive days was evaluated in African women in study 4. One sample of human milk was collected every day from each woman. Finally, in study 5, a dietary intervention was implemented to evaluate the influence of 1, 3, or 5 servings of fresh orange juice (≈100 mg ascorbic acid/serving) per week for 6 consecutive weeks in African women. Samples of human milk were collected weekly during the supplementation (1 sample/wk from the women receiving 1 serving of orange juice per week and 2 samples/wk from the women served 3 or 5 servings of orange juice per week).

Ascorbic acid supplements

Effervescent tablets (1000 mg ascorbic acid; Redoxon, Roche Pharma AG, Reinach, Switzerland) were dissolved in water immediately before consumption. For preparation of the lower dose of ascorbic acid, individual doses of ascorbic acid (100 mg food-grade ascorbic acid; Merck, Dietikon Switzerland) were prepared and dissolved in water immediately before consumption. European women prepared and consumed their supplements at home daily, whereas in Abidjan, all supplements were administered by one of the investigators. All supplements were consumed between 0700 and 1200.

Dietary intervention

Fresh orange juice was used to increase dietary intake of ascorbic acid during study 5. Oranges were purchased in Abidjan, and each serving of orange juice (180 g) was prepared immediately before consumption by one of the investigators. It was estimated that 180 g orange juice would provide ≈70–100 mg ascorbic acid (37–54 mg/100 g orange juice) (13). Ascorbic acid in orange juice was analyzed by titration with 2,6-dichlorophenol-indophenol (14).

Human milk sampling and ascorbic acid analysis

The study protocol used during sampling and analysis of human milk samples was developed during a preliminary study (12). Human milk sampling could not be standardized, in particular during the fieldwork in Abidjan. Thus, milk samples were collected at different times after the last breastfeeding, and the amount of milk expressed at each sampling varied. Furthermore, because of the lack of access to sophisticated analytic equipment such as HPLC in the laboratory in Abidjan and concern over potential losses of ascorbic acid during transport of frozen samples to Zurich, we used a simple analytic technique based on titration with 2,6-dichlorophenol-indophenol (14) to measure ascorbic acid in the present study. A limitation of this method is that it measures only reduced ascorbic acid and not total ascorbic acid. According to information from previous studies, the content of dehydroascorbic acid compared with reduced ascorbic is low acid in human and cow milk (15, 16). In addition, because a major aim of the present study was to compare data before and after interventions to increase ascorbic acid intake, the methodologic limitations of the technique based on titration with 2,6-dichlorophenol-indophenol are considered to be of limited importance.

Briefly, data from the preliminary study (12) showed no statistically significant influence of the meta-phosphoric acid (MPA) concentration in human milk samples [2%, 4%, or 6% MPA; $n = 9$; $P = 0.245$ (multivariate analysis with repeated measures)] or on the amount of milk used during the analysis (1, 2, 3, 5, or 10 g/sample; $n = 9$; $P = 0.455$, multivariate analysis with repeated measures) on ascorbic acid content. In addition, ascorbic acid content was not significantly different between foremilk and hindmilk ($n = 25$; $P = 0.95$, paired Student's t test). Human milk samples (MPA concentration of 2%) were shown to be stable during storage at 4 °C for 24 h ($n = 13$; $P = 0.926$, multivariate analysis with repeated measures) and at −20 °C for 6 mo ($P = 0.384$, multivariate analysis with repeated measures)

(12). We also compared ascorbic acid content in human milk analyzed by titration with 2,6-dichlorophenol-indophenol and by HPLC (12). The results were significantly different ($P < 0.001$, paired Student's t test; $n = 26$): 32.5 ± 15.9 compared with 26.3 ± 15.1 mg/kg, respectively. The correlation between data based on the 2 analytic methods was significant (Pearson correlation coefficient = 0.99, $P < 0.001$). Significantly larger differences were found at higher human milk ascorbic acid contents than at lower ascorbic acid contents (Pearson correlation coefficient = 0.47, $P < 0.02$). The CV based on human milk samples analyzed by titration with 2,6-dichlorophenol-indophenol in triplicate ($n = 100$) was 1.3 ± 1.3%. Analysis of human milk samples collected on 3–7 consecutive days showed no significant difference (multivariate analysis, repeated measures) in ascorbic acid content (titration with 2,6-dichlorophenol-indophenol): 3 consecutive days ($n = 63$; $P = 0.334$), 5 consecutive days ($n = 22$; $P = 0.260$), and 7 consecutive days ($n = 7$; $P = 0.878$).

In the present study, all samples were collected between 0700 and 1200, before intake of ascorbic acid supplements or orange juice. In studies 1–4, women used battery operated or electric breast pumps (Medela AG, Baar, Switzerland). In study 5, women expressed milk manually. All milk samples were collected directly into preweighed, opaque polypropylene containers (35 mL) containing a preweighed amount of 10% MPA solution (Merck) to prevent autooxidation of ascorbic acid during storage and analysis (14). Fresh solutions of MPA were prepared weekly, stored refrigerated, and protected against light. MPA and human milk were mixed by gently shaking the container. The amount of human milk collected was 14 ± 5 g/sample (studies 1–5), and the MPA concentration was in the range of 2.4–5.2% before freezing. Immediately before analysis, the MPA concentration was adjusted to 2% in all samples. Samples were kept cool during transport to the local laboratory and were stored at −20 °C until analyzed.

In studies 2–5, two samples of human milk were collected on separate days before the intervention started, and the mean ascorbic acid content of these two samples is referred to as the baseline value. At other samplings, one spot sample was collected (*see* the information about sampling frequency in each study in the "Study design" section).

All samples were analyzed by one of the investigators. Samples collected in Abidjan were analyzed at Centre Suisse de Recherches Scientifiques, and samples collected in Zurich were analyzed at the Laboratory for Human Nutrition in Rüschlikon. Milk samples were thawed at room temperature immediately before analysis. All samples were analyzed within 2 wk, in duplicate. Results are presented as mg ascorbic acid/kg human milk.

Statistical analysis

Maternal age and duration of lactation were compared in European and African women by use of unpaired Student's t tests. Parity was compared in the 2 study populations by use of the Mann-Whitney U test (nonparametric test). The distribution of human milk ascorbic acid content was evaluated by the Kolmogorov-Smirnov test and the QQ-Normal-Distribution-Plot.

Different statistical methods were used to evaluate data in the separate studies. In study 1, the data were evaluated by unpaired Student's t tests. In study 2, changes in human milk ascorbic acid content (day to day) during the intervention were evaluated by using a general linear model (repeated measures) including repeated contrasts. Paired Student's t test was used to compare human milk ascorbic acid content at baseline with human milk ascorbic acid content at the end of the study (day 11). In study 3, human milk ascorbic acid content during the follow-up period was evaluated for each woman through visual inspection of the scatter plot. A general linear model (repeated measures) with simple contrast and ascorbic acid content at baseline as the reference category was used to compare milk ascorbic acid content at 3 time points: at baseline, after the completion of supplementation (day 6), and at the time when milk ascorbic acid content had returned to baseline. In study 4, paired Student's t tests were used to compare human milk ascorbic acid content at baseline with human milk ascorbic acid content at the end of the study (day 11). Paired Student's t tests were used to compare human milk ascorbic acid content at baseline with human milk ascorbic acid content at the end of study 5 and to evaluate whether changes in human milk ascorbic acid content were different from zero. Baseline values were compared by one-way ANOVA with post hoc Tukey's test. Analysis of covariance (with baseline as a covariate) was used to analyze the data from the 3 intervention groups in study 5.

P values < 0.05 are referred to as indicating significance. A commercial statistical software package (SPSS 12.0 for WINDOWS; SPSS Inc, Chicago, IL) was used to evaluate the data.

RESULTS

The African women were significantly younger (25 ± 5.5 y; range: 16–41 y), had higher parity (2.4 ± 3.3; range: 1–9), and had been breastfeeding for longer periods of time (8.2 ± 3.3 mo; range: 1.0–18.2 mo) than the European women [age, 33 ± 3.2 y (range: 22–43 y); parity, 1.5 ± 0.5 (range: 1–4); and breastfeeding duration, 4.5 ± 1.9 mo (range: 1.0–21.8 mo); $P < 0.01$; studies 1–5].

Human milk ascorbic acid values were normally distributed, and the results are presented as means ± SDs. No significant difference in human milk ascorbic acid content was observed between samples collected during the screening study and samples collected at baseline in 65 African women ($P = 0.451$, paired Student's t test; studies 2–5).

Study 1

Human milk ascorbic acid content was significantly lower in the African women (31 ± 15 mg/kg; $n = 171$) than in the European women (63 ± 14 mg/kg; $n = 142$; $P < 0.001$, unpaired Student's t test).

Study 2

Intake of 1000 mg ascorbic acid/d for 10 d resulted in significantly increased human milk ascorbic acid content in both the European and the African women. In 10 European women, human milk ascorbic acid increased from 60 ± 12 mg/kg at baseline to 70 ± 16 mg/kg after intake of a cumulative dose of 10 000 mg ascorbic acid ($P = 0.03$, paired Student's t test; **Figure 1**). Corresponding values for the 18 African women in this study were 19 ± 16 and 60 ± 11 mg/kg ($P < 0.001$, paired Student's t test; Figure 1). In the African women, ascorbic acid content increased significantly from day to day during the first few days of the intervention (general linear model, repeated measures). However, after intake of a cumulative dose of 4000 mg ascorbic

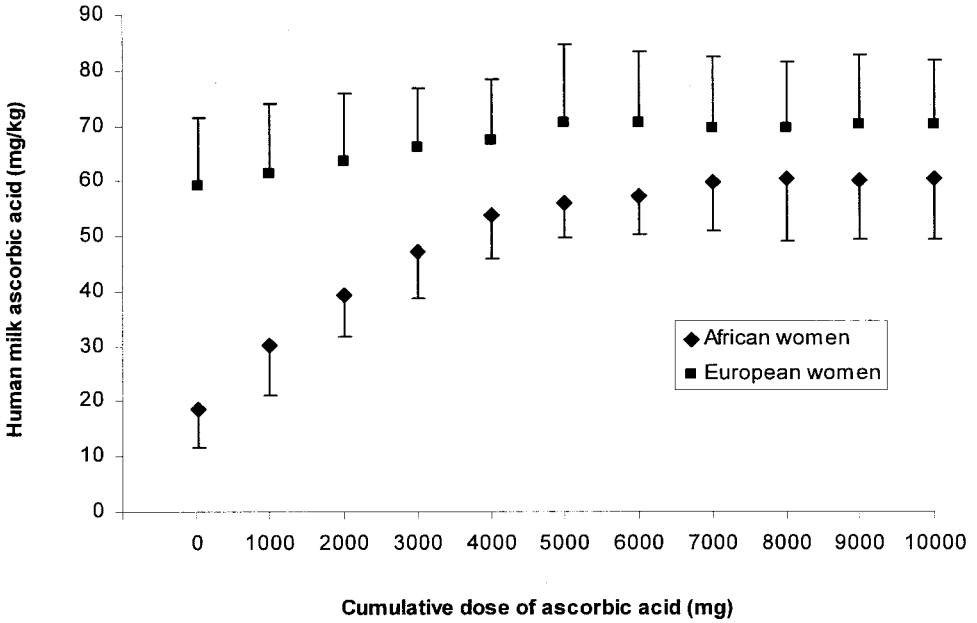

FIGURE 1. Mean (±SD) human milk ascorbic acid content during supplementation with 1000 mg ascorbic acid/d for 10 consecutive days in European and African women (study 2). In 10 European women, human milk ascorbic acid increased from 60 ± 12 mg/kg at baseline to 70 ± 16 mg/kg after intake of a cumulative dose of 10 000 mg ascorbic acid ($P = 0.03$, paired Student's t test). Corresponding values for 18 African women were 19 ± 16 and 60 ± 11 mg/kg ($P < 0.001$, paired Student's t test).

acid, no further increase in ascorbic acid content was observed. In the European women, no significant day-to-day increase was observed.

Study 3

Human milk ascorbic acid content increased from 70 ± 11 to 82 ± 11 mg/kg after intake of 1000 mg ascorbic acid/d for 5 d ($P < 0.001$, paired Student's t test) in 17 European women. After discontinuation of the supplementation, human milk ascorbic acid content was not significantly different from baseline for the first time (68 ± 12 mg/kg; $P = 0.303$, general linear model, repeated measures) after 21 d.

Study 4

Human milk ascorbic acid content increased from 17 ± 6.5 to 36 ± 8.0 mg/kg ($P < 0.001$, paired Student's t test) in 11 African women after intake of 100 mg ascorbic acid/d for 10 d.

Study 5

Mean ascorbic acid content per serving of orange juice (180 g) was 105 mg (range: 72 to 159 mg; $n = 15$). Baseline milk ascorbic acid content was significantly higher in the African women served one glass of orange juice per week (23 ± 5.3 mg/kg) than in the women consuming 3 servings of orange juice per week (16 ± 6.0 mg/kg; $P = 0.02$, one-way ANOVA with post hoc Tukey's test). Baseline milk ascorbic acid was not significantly different in the African women consuming 5 servings of orange juice/wk (21 ± 4.3 mg/kg) than in the women consuming 1 or 3 servings/wk. At the end of the study, human milk ascorbic acid content was 26 ± 7.1 mg/kg (1 serving/wk), 32 ± 6.9 mg/kg (3 servings/wk), and 46 ± 6.2 mg/kg (5 servings/wk). Changes in human milk ascorbic acid content were not significantly different from zero in the women consuming one serving of orange juice per week ($P = 0.75$, paired Student's t test), whereas human milk ascorbic acid content was significantly different from zero in the other 2 intervention groups at the end of the study ($P < 0.001$, paired Student's t test).

Analysis of covariance (with baseline as a covariate) showed a significant influence of baseline values ($P = 0.0041$) and study group ($P < 0.001$), whereas their interaction was not significant. The main effect model with polynomial contrast showed the linear term to be significant for the study group ($P < 0.001$); the estimated effect was 11.8 mg/kg per 2 servings of orange juice/wk. The parameter estimate for baseline was −0.5 mg/kg. The change in human milk ascorbic acid content during the 6-wk dietary intervention period (adjusted for baseline ascorbic acid content) is presented in **Figure 2**.

DISCUSSION

Mean human milk ascorbic acid content was ≈50% lower in the African women (31 mg/kg; $n = 171$) than in the European women (63 mg/kg; $n = 142$) in the present study. These results provide additional information on the significant difference in human milk ascorbic acid output in women living in different settings. A striking difference between the 2 study populations was noted when evaluating the distribution of the data: 29% of the African women had very low human milk ascorbic acid output, 20–29 mg/kg, whereas none of the milk samples expressed by European women had such low ascorbic acid content. Although we did not collect data on dietary intake in all women participating in the study, preliminary data based on a subsample of subjects indicate a significant difference in ascorbic acid intake between African women living in Abidjan (mean intake: 22 mg/d) and European women living in Zurich (mean intake: 106 mg/d) (12). Furthermore, the importance of ascorbic acid intake on human milk ascorbic acid content was clearly shown in the subsequent intervention studies in both study populations.

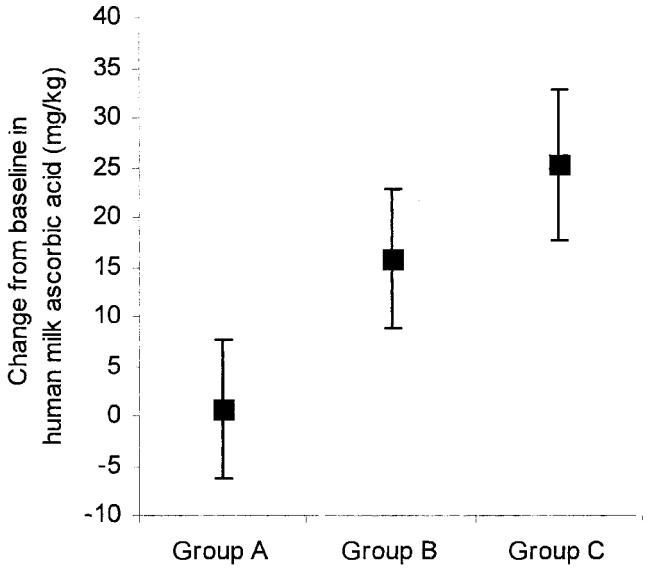

FIGURE 2. Mean (±SD) changes in human milk ascorbic acid content, adjusted for baseline ascorbic acid content, after 6 wk of dietary intervention in African women (study 5). Study group A consumed 1 serving of orange juice per week ($n = 10$), study group B consumed 3 servings of orange juice per week ($n = 13$), and study group C consumed 5 servings of orange juice per week ($n = 13$). Each serving contained ≈100 mg ascorbic acid. Analysis of covariance (with baseline as a covariate) showed a significant influence of baseline values ($P = 0.0041$) and study group ($P < 0.001$) but no significant interaction.

Although earlier studies reported on the influence of increased intake of ascorbic acid in women living in developing countries, limited information is available on the effect of increased intake of ascorbic acid on human milk output in Western women. Because previous intervention studies in lactating women living in industrialized countries reported no effect of smaller doses of ascorbic acid (20–200 mg/d; 2, 3, 17) on human milk ascorbic acid content, we investigated the effect of a relatively high dose of ascorbic acid (1000 mg/d) in the present study. Intake of supplements providing 1000 mg per dose is not uncommon in industrialized countries, and the dietary supplement used in the present study is available over the counter in Switzerland.

To our knowledge, only one previous study included supplementation of lactating women with 1000 mg ascorbic acid/d; this study reported no significant influence on human milk ascorbic acid output (18). These results are not surprising because the intervention was short-term (2 d) and only 5 women participated in the study. Our data show that supplementation with 1000 mg ascorbic acid/d for 10 d increases mean human milk ascorbic acid significantly—from 60 to 70 mg/kg—in European women ($P = 0.02$). However, although the effect on human milk ascorbic acid output was significant, the overall increase was modest compared with the effect observed in the African women. Mean human milk ascorbic acid increased 3-fold in the 18 African women participating in the supplementation study: from 19 mg/kg at baseline to 60 mg/kg on day 11 ($P < 0.001$). In addition to the pronounced overall effect of ascorbic acid supplementation on human milk ascorbic acid content, a significant day-to-day effect was observed during the early phase of the intervention in the African women; no such effect was found in the European women. Clearly, the response to ascorbic acid supplementation differed in the 2 study groups and, although the results from the present study do not provide any information on the mechanisms involved, the data indicate that human milk ascorbic acid content is regulated.

Earlier studies suggested that there is an upper limit to the ascorbic acid secretion by the mammary gland, presumably related to the saturation of the mammary tissue with the vitamin (18–20). The considerable interindividual variability of the upper limit of human milk ascorbic acid secretion by the mammary gland—and therefore the presumably large individual variation in the degree of saturation of mammary tissue—was emphasized in the previous study by Pratt et al (19) and also indicated in the present study (Figure 1). The mechanism or mechanisms regulating ascorbic acid saturation of the mammary tissue and secretion of the vitamin by the mammary gland are not known. We did not monitor urinary excretion of ascorbic acid in the present study, but it can be assumed that a large proportion of absorbed ascorbic acid was lost in urine (at intakes that raised plasma concentrations above the renal threshold). Fluctuations in human milk ascorbic acid content have been shown to be slower and less pronounced than the response in urinary excretion (18, 19). The potential influence of differences in the fractional absorption of ascorbic acid on human milk ascorbic acid output is not known, and we are not aware of any data evaluating differences in the fractional absorption of ascorbic acid related to habitual intake of ascorbic acid (and thus differences in body stores of ascorbic acid). Fractional absorption of ascorbic acid is dose dependent and has been reported to fall from 70–90% to ≈50% or less with doses >1000 mg/d (21).

After the initial supplementation study in European women, we also evaluated the effect of a shorter intervention (study 3) and monitored the decrease in human milk ascorbic acid output after discontinuation of the supplement. In this study group of European women, mean ascorbic acid milk output increased from 70 mg/kg at baseline to 82 mg/kg ($P < 0.001$) after intake of 1000 mg ascorbic acid/d for 5 d, and human milk ascorbic acid content had returned to values not significantly different from baseline values after 21 d. These results indicate that the body pool of ascorbic acid increased during the 5-d supplementation and that storage ascorbic acid was excreted in human milk after the supplementation was discontinued.

Clearly, high-dose ascorbic acid supplements are effective in increasing human milk ascorbic acid output in both European and African lactating women. However, the usefulness of this approach is obviously limited from a public health perspective. A more sustainable approach would be to encourage increased dietary intake of ascorbic acid by consumption of locally available ascorbic acid–rich foods. We therefore evaluated the influence of increased intake of fresh orange juice on human milk ascorbic acid output in African women. One serving of orange juice per week had no significant effect on human milk ascorbic acid output, but the beneficial effect of regular consumption of fresh orange juice 3 or 5 times/wk (≈100 mg ascorbic acid/serving) for 6 wk was clearly shown. Mean human milk ascorbic acid output increased ≈2-fold: from 16 to 32 mg/kg and from 21 to 46 mg/kg in the 2 study groups ($P < 0.001$ compared with zero difference, paired Student's t test). The results from this dietary intervention also showed the dose effect of ascorbic acid on human milk ascorbic acid content ($P < 0.001$, analysis of covariance with baseline as a covariate; Figure 2). For comparison, we also evaluated the effect of a smaller dose of ascorbic acid as a supplement (100 mg ascorbic acid/d) for 10 d in 11 African women. At the

end of the study, mean human milk ascorbic acid content had increased ≈2-fold: from 17 to 36 mg/kg ($P < 0.001$). Thus, a doubling of human milk ascorbic acid content can be achieved by intake of relatively low doses of the vitamin, either as low-dose supplements or as ascorbic acid–rich fruit juice.

In conclusion, ascorbic acid supplementation with a relatively high dose of ascorbic acid (1000 mg/d for 10 d) increased human milk ascorbic acid output in both European and African women. Although significant, however, the overall effect was modest in well-nourished European women in contrast with the 3-fold increase in mean human milk ascorbic acid content observed in African women. These results indicate that human milk ascorbic acid content is regulated and are in agreement with previous observations (5, 18–20).

With lower doses of ascorbic acid, in the form of dietary supplements or as ascorbic acid–rich fruit juice, human milk output can be doubled in African women with relatively low human milk ascorbic acid content at baseline. The results from this study highlight the importance of encouraging regular consumption of ascorbic acid–rich foods by lactating mothers to ensure adequate human milk ascorbic acid output. Apart from the obvious importance of human milk as the sole source of ascorbic acid in exclusively breastfed infants, human milk can be an important source of ascorbic acid in the diet of breastfed older infants and young children consuming complementary foods. Although our recent study in Bangladesh did not show any significant difference in iron bioavailability from a traditional complementary food consumed with water or with human milk (22), further studies are needed to evaluate the effects of human milk as a source of ascorbic acid on iron bioavailability from less inhibitory meals.

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All authors contributed to the study design. SD-O was responsible for the implementation of the study and for data collection and analysis. LD was responsible for data analysis and for the preparation of the manuscript. SD-O and RH contributed to the preparation of the final manuscript. None of the authors had any conflicts of interest.

REFERENCES

1. Squires BT. Ascorbic acid content of the milk in Tswana women. R Soc Trop Med Hyg 1950;53:170–4.
2. Thomas MR, Kawamoto J, Sneed SM, et al. The effects of vitamin C, vitamin B6, and vitamin B12 supplementation on the breast milk and maternal status of well-nourished women. Am J Clin Nutr 1979;32:1679–85.
3. Sneed SM, Zane C, Thomas R. The effect of ascorbic acid, vitamin B6, vitamin B12, and folic acid supplementation on the breast milk and maternal nutritional status of low socioeconomic lactating women. Am J Clin Nutr 1981;34:1338–46.
4. Bates CJ, Prentice AM, Prentice A, Paul AA, Whitehead RG. Seasonal variation in ascorbic acid status and breast milk ascorbic acid levels in rural Gambian women in relation to dietary intake. Trans R Soc Trop Med Hyg 1982;76:341–7.
5. Bates CJ, Prentice AM, Prentice A, Lamb WH, Whitehead RG. The effect of vitamin C supplementation on lactating women in Keneba, a West African rural community. Int J Vitam Nutr Res 1983;53:68–76.
6. Prentice AM, Roberts SB, Prentice A, et al. Dietary supplementation of lactating Gambian women I. Effect on breast-milk volume and quality. Hum Nutr Clin Nutr 1983;37:53–64.
7. Salmenperä L. Vitamin C nutrition during prolonged lactation: optimal in infants while marginal in some mothers. Am J Clin Nutr 1984;40:1050–6.
8. Bates CJ, Prentice AM, Paul AA. Seasonal variations in vitamin A, C, riboflavin and folate intakes and status of pregnant and lactating women in a rural Gambian community: some possible implications. Eur J Clin Nutr 1994;48:660–8.
9. Deodhar AD, Ramakrishnan CV. Studies on human lactation: effect of dietary supplementation on vitamin concentrations of breast milk. Acta Pediatrica 1964;53:43–8.
10. Tawfeek HI, Muhyaddin OM, al-Sanwi HI, al-Baety N. Effect of maternal dietary vitamin C intake on the level of vitamin C in breastmilk among nursing mothers in Baghdad, Iraq. Food Nutr Bull 2002;23:244–7.
11. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. Washington, DC: National Academy Press, 2000.
12. Daneel S. The ascorbic acid content of human milk in relation to iron nutrition. PhD thesis. SwissFederal Institute of Technology, Zurich, Switzerland, 2003 (Internet: http://e-collection.ethbib.ethz.ch/cgi-bin/show.pl?type=diss&nr=14921).
13. Food and Agriculture Organization of the United Nations. Food composition table for use in Africa. Rome, Italy: FAO, 1968.
14. Association of Analytical Communities International. Official methods of analysis. 16th ed. Method 967.21. Gaithersburg, MD: AOAC, 1997:16–7.
15. Chatranon W, Siddhikol C, Chavalittamrong B. Ascorbic acid and dehydroascorbic acid in breast milk of Thai mothers. J Med Assoc Thai 1979;62:315–8.
16. Weiss WP. Effect of dietary vitamin C on concentrations of ascorbic acid in plasma and milk. J Dairy Sci 2001;84:2302–7.
17. Thomas MR, Sneed SM, Wei CW, et al. The effects of vitamin C, vitamin B6, vitamin B12, folic acid, riboflavin, and thiamin on the breast milk and maternal status of well-nourished women at 6 months postpartum. Am J Clin Nutr 1980;33:2151–6.
18. Byerley LO, Kirksey A. Effects of different levels of vitamin C intake on the vitamin C concentration in human milk and the vitamin C intakes of breast-fed infants. Am J Clin Nutr 1985;41:665–71.
19. Pratt JP, Munks B, Macy IG. Metabolism of women during the reproductive cycle. XII. The effect of multi-vitamin supplements on the vitamin C utilization of nursing mothers. J Nutr 1947;33:621–32.
20. Ortega RM, Quintas ME, Andrés P, Martinez RM, Lopez-Sobaler AM. Ascorbic acid levels in maternal milk: differences with respect to ascorbic acid status during the third trimester of pregnancy. Br J Nutr 1998;79:431–7.
21. Kallner A, Hartmann D, Hornig D. Steady-state turnover and body pool of ascorbic acid in man. Am J Clin Nutr 1979;32:530–9.
22. Davidsson L, Jamil KA, Sarker SA, Zeder C, Fuchs G, Hurrell R. Human milk as a source of ascorbic acid: no enhancing effect on iron bioavailability from a traditional complementary food consumed by Bangladeshi infants and young children. Am J Clin Nutr 2004;79:1073–7.