Trace elements and their distribution in protein fractions of camel milk in comparison to other commonly consumed milks

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SUMMARY. Studies on camels' milk, whether with respect to concentration or bioavailability of trace elements from this milk, are limited and warrant further investigation. The object of this study was to analyse the concentration and distribution of zinc, copper, selenium, manganese and iron in camel milk compared to those in human milk, cows' milk and infant formula under similar experimental conditions. Camels' milk and cows' milk were collected from local farms, human milk samples were obtained from healthy donors in Kuwait and infant formula was purchased locally. Milk fractionation was performed by ultra-centrifugation and gel-column chromatography. The concentration of trace elements was analysed by atomic absorption spectrometry and that of protein was determined spectrophotometrically. The concentration of manganese and iron in camels' milk was remarkably higher (7–20-fold and 4–10-fold, respectively) than in human milk, cows' milk and infant formula. The zinc content of camels' milk was higher than that of human milk but slightly lower than in cows' milk and infant formula. The concentration of copper in camels' milk was similar to that of cows' milk but lower than in human milk and infant formula. The selenium content of camels' milk was comparable to those of other types of milk. Approximately 50–80% of zinc, copper and manganese in camels' milk were associated with the casein fraction, similar to that of cows' milk. The majority of selenium and iron in camels' milk was in association with the low molecular weight fraction. It is recommended that camels' milk be considered as a potential source of manganese, selenium and iron, perhaps not only for infants, but also for other groups suspected of mild deficiency of these elements. Further investigations are required to confirm this proposal.

KEYWORDS: Trace elements, milk proteins, camel milk, cow milk, human milk.

Deficiency of trace elements such as those of zinc, copper, manganese, selenium and iron might occur due to their low intake and impaired bioavailability. Since human milk or cows' milk-based formula is the only food of infants, it is important to confirm that they provide an adequate amount of essential elements. It has been reported that the daily intake of zinc and iron from human milk is below the recommended level (Garry *et al.* 1981; Gross *et al.* 1998). Furthermore, zinc intake of infants from breast milk was inadequate during the weaning period, especially if the weaning foods were introduced at an early stage (Dewey *et al.* 1992). The

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concentration of selenium in the milk of lactating women living in low selenium areas was found to be much lower than those in other areas (Kumpulainen *et al.* 1987; Litov & Combs, 1991). Thus, in view of the possible risk of sub-optimal supply of such elements from human milk, several studies have focused on analysing the concentration of these elements in breast milk substitutes, such as cows' milk and various formulas. It has been suggested that bioavailability of elements from different foods including milk varies with their chemical forms. For example, the absorption of several elements from human milk has been shown to be better than from cows' milk (Franson & Lönerdal 1983). In a previous study, we reported that selenomethionine is a major selenium-containing protein located in the whey fraction of human milk, but cows' milk did not appear to contain this compound (Al-Awadi & Srikumar, 2001). It is probable that this may be the reason for a higher absorption of selenium from human milk than from cows' milk. At present, there is substantial information available regarding the distribution of minerals and trace elements in human and cows' milk (Lönnerdal, 1997; Fransson & Lönnerdal, 1983). Such data on camels' milk are very limited in spite of the fact that camels' milk is a major source of protein and energy for desert inhabitants especially for those in the Middle East. Recently camels' milk has been introduced in local markets in countries of the Gulf region. Few investigations have focused on studying the chemical composition and nutritional quality of camels' milk (Sawaya *et al.* 1984; Farah, 1993; Gorban & Izzeldin, 1997). However, the trace element concentration in relation to the different type of protein in camels' milk has not been unequivocally demonstrated. The present study is designed to analyse the concentration and distribution of trace elements in camels' milk in comparison with that of human milk, cows' milk and infant formula under similar experimental conditions.

Materials and methods

Collection of milk samples

The human milk samples analysed in the present study were obtained from a parallel investigation where trace element status in the milk of Kuwaiti mothers was compared with that of non-Kuwaitis ($n = 17$, each; Al-Awadi & Srikumar, 2001). Nationalities of the latter group were American, Egyptian, Indian, Czech and Taiwanese, representing the major groups of expatriates living in Kuwait. All the donors had been living in Kuwait for at least 2 years prior to the present study. The lactation period extended from 0 to 18 months after parturition and the minimum lactation period included was 2 weeks. All donors and their infants were healthy, did not have a history of medical problems and did not use any kind of mineral or trace element supplementation. Cows' milk ($n = 8$) and camels' milk ($n = 5$) samples (2–5 months lactation) were obtained from local farms in Kuwait. Animals were traditionally fed on *alfa-alfa* and wheat grains. All milk samples were collected in metal-free tubes and were stored at -80 °C until analysed. Infant formula (Similac) was purchased from the local market in Kuwait. The study protocol was in accordance with the guidelines of the Ethical committee, Faculty of Medicine, Kuwait University.

Analytical methods

Milk fractionation and gel-column chromatography. Milk fractionation was performed as described previously (Al-Awadi & Srikumar, 2001). Briefly, fat content of milk samples from humans, cows and camels were first separated by

centrifugation at 4000 ***g*** for 30 min using a refrigerated Beckman centrifuge. The supernatant (skim milk) was then subjected to ultra-centrifugation (150000 ***g***, 4 °C, 60 min) to obtain casein pellets. Of the resultant supernatant containing whey and low-molecular weight fractions, 1 ml was applied on a Sephacryl S200 Superfine column (2·6 × 80 cm) equilibrated in 10 mM Tris HCl (pH 7·8). The column was developed at a flow rate of 20 ml/h and 80 fractions of 10 ml each were collected. The eluate as well as fat and casein fractions were analysed for trace elements and for protein absorbance at 280 nm (using a Beckman DU7500 spectrophotometre). Gel-column fractions containing the whey and low molecular weight compounds obtained from the above chromatographic procedure were pooled separately, lyophilized and used for the analysis of trace elements. By applying commercially available pure whey and casein protein standards (Sigma Chemical Company Ltd., UK) to the gel filtration column under the same conditions, the accuracy of the chromatographic procedures was checked.

Determination of casein, whey and low molecular weight proteins. Milk protein concentrations were estimated as described previously (Al-Awadi & Srikumar, 2001). Commercially available pure casein and whey protein standards were suspended in the above Tris-HCl buffer and used to construct standard curves by measuring the absorbance at 280 nm. The absorbance of the casein (obtained from the ultra-centrifugation) and whey fractions (collected from the gel-column chromatography) of the unknown samples was also measured and the contents were computed using the above standard curve. Fractions eluted after the whey protein were treated as low molecular weight protein. The sum of the content of casein, whey and low molecular weight proteins was considered to be the total protein concentration. The accuracy and precision of this method was checked and found to be acceptable. The accuracy of the above procedure was tested on the basis of the recovery of compounds added to some milk samples. The recoveries of casein and whey protein varied from 91% to 97% and from 89% to 96%, respectively. The precision was expressed as coefficient of variation, which was 6%, of data obtained after analysing these components in six samples of the same milk sample.

Determination of trace elements in whole milk and in different milk fractions. Concentrations of zinc, copper, manganese, selenium and iron in whole milk samples, and in fat, casein, whey and low molecular weight fractions were analysed by atomic absorption spectrophotometer (Srikumar, 1993; Al-Awadi & Srikumar, 2001). Whole milk samples and milk fractions were first digested using a mixture of nitric and perchloric acid (4:1). The concentrations of manganese, selenium and iron were estimated using a graphite furnace, while zinc and copper were estimated using flame atomic absorption spectrophotometer (Varian, Spectra AA, Australia). The accuracy of the trace element analytical procedures has been reported previously (Srikumar *et al.* 1992). Seronorm 103 serum standards (Nycomed, Oslo, Norway) were used for standardization of trace element analysis. The mean ($n = 7$) concentration of zinc, copper, iron, manganese and selenium deviated −4%, −5%, −9%, −2% and −4%, respectively. As reference material, Bovine liver standard 1577a (National Institute of Standards and Technology, Gaithersberg, MD, USA) was used. The analytical values ($n = 7$) for zinc, copper, iron, manganese and selenium deviated −0·8%, −1·3%, −1·5%, −3% and −2%, respectively from the certified values.

Statistical Analysis

Results are presented as mean ± SD values. Data were analysed using Student's t test. A value of $P < 0{\cdot}05$ was accepted as statistically significant.

Table 1. *Concentration of zinc, copper, manganese, selenium and iron in camels' milk, cows' milk, human milk and infant formula*

Data presented as means ± SD

Milk source	Zinc (mg/l)	Copper (mg/l)	Manganese (μg/l)	Selenium (μg/l)	Iron (mg/l)
Camel ($n = 5$)	4·9 ± 0·5	0·36 ± 0·02	79·6 ± 7·4	13·9 ± 2·4	3·16 ± 0·03
Cow ($n = 6$)	6·2 ± 0·3	0·27 ± 0·04	27·8 ± 5·2	12·6 ± 3·6	0·29 ± 0·02
Human ($n = 11$)	2·9 ± 0·4	0·6 ± 0·1	4·4 ± 0·4	14·3 ± 2·1	0·26 ± 0·05
Infant formula ($n = 7$)	5·7 ± 0·3	0·53 ± 0·03	36·9 ± 6·2	14·1 ± 3·6	0·71 ± 0·1

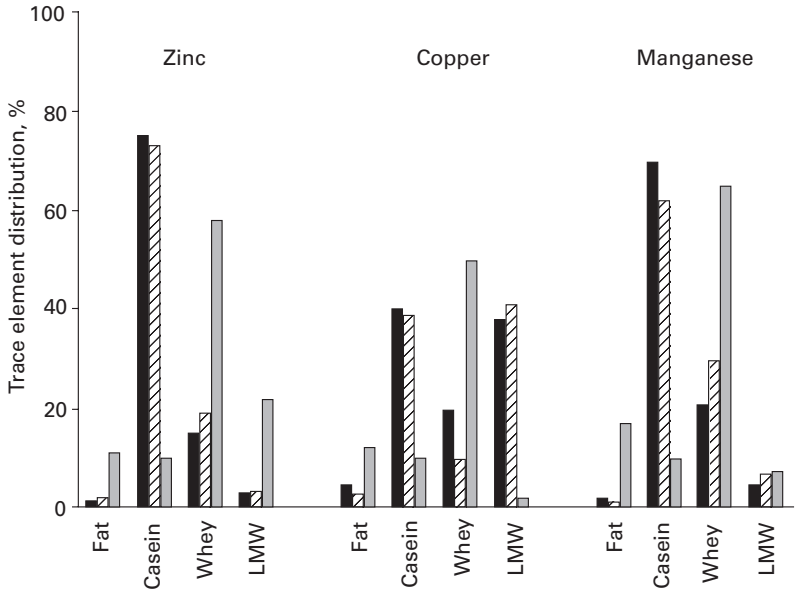

Fig. 1. Percentage distribution of zinc, copper and manganese in fat, casein, whey and low molecular weight fractions (LMW) of camels' milk (black shading), cows' milk (hatching) and human milk (grey shading).

RESULTS

Concentration of trace elements in camels' milk in comparison with that of cows' milk, human milk and infant formula

Camels' milk was remarkably higher in manganese and iron concentrations (7–20-fold and 4–10-fold, respectively) compared to cows' milk, human milk and infant formula. Human milk contained a lower concentration of zinc but a higher concentration of copper than in the camels' and cows' milk and infant formula (Table 1). The concentration of selenium was similar in all milk samples analysed.

Distribution of trace elements in different milk fractions

The percentage distribution of elements in different milk protein fractions is presented in Figs 1 and 2. Approximately 50–80 % of zinc, (Fig. 1), copper (Fig. 1) and manganese (Fig. 1) in camels' milk was associated with the casein fraction, similar to that of cows' milk. The majority of selenium (Fig. 2) and iron (Fig. 2) both in camel and cows' milk was in association with the low molecular weight fraction. However, in the human milk the major distribution of zinc, copper,

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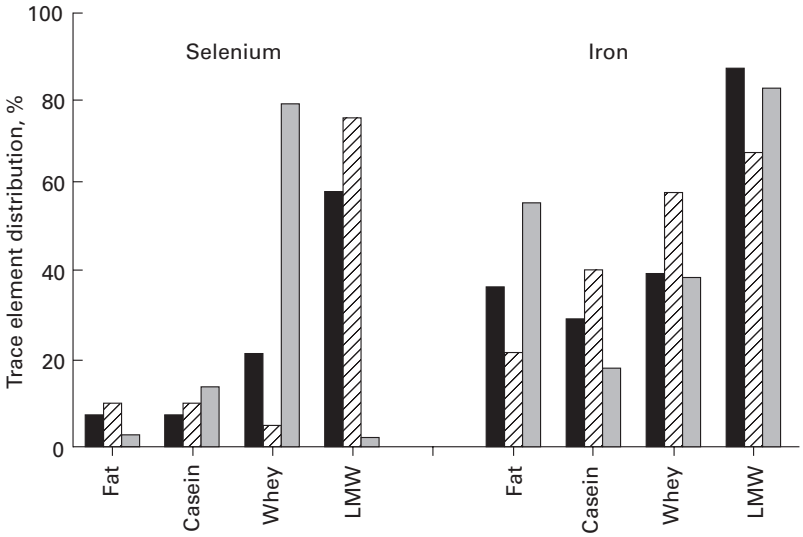

Fig. 2. Percentage distribution of selenium and iron in fat, casein, whey and low molecular weight fractions (LMW) of camels' milk (black shading), cows' milk (hatching) and human milk (grey shading).

manganese and selenium was in association with the whey fraction (Figs 1 and 2). Except for selenium, the fat fraction of human milk contained higher concentration of trace elements than in the same fraction of animal milks.

DISCUSSION

The present data on concentration of trace elements in camels' milk, cows' milk, human milk and infant formula were within the range reported in the literature (Gorban & Izzeldin, 1997; Lönnerdal, 1997; Krachler *et al.* 1998). Several previous reports including ours indicated that bioavailability of elements from the milk of humans and cows might depend on the chemical form of these elements present in these milks (Fransson & Lönnerdal, 1983; Al-Awadi *et al.* 2000, 2001). Although, various studies have reported on the distribution of elements in different protein fractions of human and cows' milk, such data on camels' milk are not available. The present study revealed that the majority of zinc and manganese, including a significant amount of copper in camels' milk, was located in the casein fraction, but most of the selenium and iron were associated with the low molecular weight fraction. This distribution pattern in camels' milk was identical to that observed in cows' milk. It has been reported that camels' milk has a broader casein micellar size (260–300 nm) than cow milk (110–140 nm; Farah, 1993). It seems that this variation did not affect the distribution of trace elements in casein fractions of camel and cows' milk, as our data showed a similar distribution pattern for all trace elements including iron. It can be suggested that bioavailability of these elements from camels' milk might be similar to that of cows' milk. However, this has to be confirmed. Furthermore, as in the case of cows' milk, since majority of trace elements in camels' milk are also associated with the casein fraction, this type of milk may not be considered as a substitute for human milk with respect to the bioavalabiltiy of

trace elements. However, the remarkably high content of iron in camels' milk suggests that this milk might be a better alternative to human milk under circumstances where iron supplementation is required. This suggestion is supported by the fact that the majority of iron in this milk is located in the low molecular weight fraction which is easily accessible for intestinal absorption and may facilitate the bioavalability of this element. It has been reported that although the daily intake of iron from breast milk is 0·3 mg, far below the recommended level of 6 mg (RDA 1989), normal term infants can maintain satisfactory haemoglobin levels by utilizing body iron stores. It seems that camel milk could be considered as a good source of iron not only for infants, but also for other groups whether healthy or with health problems due to anemia or mal-nourishment. In cases where camels' milk is to be provided for infants as a supplement or as a substitute, it is advisable to use the skimmed form. This suggestion is based on the present data that the content of fat in camels' milk was found to be slightly higher than that in cows' milk (39 ± 3 g/l and 28 ± 5 g/l, respectively). Therefore, skimmed milk would be easier to digest compared to the whole milk. Moreover, removal of fat from camels' milk should not considerably affect its content of iron, since fat fraction contains only less that 10% of total milk iron. Other reports from Saudia Arabia have shown similar fat content in both camels' and cows' milk (Gorban & Izzeldin, 1997). Present data showed that camels' milk is not only an exceptional source of iron, but also an excellent source of manganese, 7–20-fold that of other commonly used milks. Manganese is known as an essential trace element with various functions related to human development, growth and maintenance. Deficiency of manganese in humans has only recently been reported in relation to osteoporosis, congenital malfunctions, hip abnormalities, multiple sclerosis and some other diseases (Freeland-Graves & Turnlund, 1996). It has been reported that the daily output of manganese in human milk (approximately 30 nmol) is almost negligible compared to the daily intake of this element (70–90 μmol; Claire *et al.* 1989; RDA 1989). As a result, infant formulas were fortified with manganese to satisfy recommended intake. Under these circumstances, camels' milk can be recommended as a substitute to the milk formula fortified with manganese. Manganese is a natural component of plant foods, which is considered a major natural source compared to other sources such as commonly used milks. Our present data on manganese may introduce camels' milk as an additional important natural source, which in turn may be recommended for cases arising from manganese deficiency as stated above. It seems that the nutritional value of camels' milk does not only arise from the high content of iron and manganese, but the nature of the association of trace elements to the proteins. Similar to iron, selenium, another essential trace element, was unique with respect to its strong association with the low molecular weight proteins, as in cows' milk. Therefore, it could be assumed that the intake and bioavailbility of this trace element from camels' milk should be efficient. Although camels' milk is not widely recognized as an infant food in the Gulf region, it is an important source of protein and energy for the Bedouin community (an indigenous local population) in this region and recently it has been sold in supermarkets in Kuwait. More detailed investigation of various trace element binding compounds present in camels' milk is needed to evaluate the nutritional quality of this milk with respect to trace element absorption.

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