DETERMINATION OF IODINE IN HUMAN MILK AND URINE

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Abstract

Human milk and urine samples were collected from 84 healthy volunteer nursing mothers living in Kano, Nigeria. The samples were analyzed for their iodine content using the Iodine-catalyzed reduction of Ceric ion by Arsenous acid. Separating the iodine by solvent extraction eliminated interferences. Physiological concentrations of iodine were determined in milk and urine. Recovery studies were reported along with results for the analysis of milk and urine samples. Iodine contents ranged from 10 - 110 (mean 52.88 ± 22.60 μg/l) and 10 - 90 (mean 27.64 ± 16.70) μg/l in milk and urine respectively. A significant difference is indicated between the mean iodine in milk and urine. Iodine in milk and urine show a progressive decrease from 60±19.35 μg/l in colostrums to 45±21.21 μg/l in the mature milk.

Keywords: Iodine, human milk, urine, catalytic determination.

1. Introduction

Iodine is an essential component of the thyroid hormones in man and animals (Darrell, 1991). The total body content of iodine is estimated at 10 - 50 mg for an adult (Pennington, 1988). It is present in all body tissues and fluids but 70 - 90% is located in the thyroid gland which has iodine concentration of about 0.4 - 1.0 μg/kg net weight. Iodine exists in blood in inorganic and organic forms. The normal plasma concentrations of inorganic iodine range from 0.08 - 0.6 μg/l with values less than 0.08 μg/l suggesting iodine deficiency (Hetzel and Maberly, 1986). Iodine concentrations of other tissues do not exceed 0.2 mg/kg. The daily requirement for iodine is 1 - 2 μg/kg of body weight. The daily intake of 50 -1000 μg/kg is considered safe (Food and Nutrition Board, 1970).

Children of mothers consuming less than 25 μg/day of iodine are often afflicted with cretinism as a result of in-utero iodine deficiency, which may result in neurologic cretinism; characterized by mental deficiency, deaf mutism and the myxadematous type characterized by hypothyroidism and dwarfism (Hetzel and Dann, 1989). Iodine deficiency may also result in miscarriages, stillbirths and congenital abnormalities (Hetzel and Mano, 1989). While iodine is of biological interest, it occurs as a trace element in human milk and urine and is difficult to determine by the conventional laboratory techniques. Catalytic methods are sensitive for trace analysis, though hampered by selectivity. By judicious coupling with separation procedures it is possible to apply the technique to the analysis of complex biological samples (Ayodele, 1996; Ayodele and Ogunlesi, 1998).

Byrne (1984) described a radiochemical separation technique followed by the extraction of iodine into carbon tetrachloride with a clean up based on a selective redox- stripping cycle using nitric and sulphuric acids. Gvrdjiancic et al. (1988) described a neutron activation procedure for the determination of iodine in milk in which a pre-irradiation separation procedure was employed. The iodine catalyzed reaction between Ce (IV) and As (III) has been used in the determination of iodine at the trace level in biological materials (Sandell and Kolthoff, 1934; Kambhapati et al., 1989). Rogina and Dubrovic (1953) described a catalytic procedure for the determination of iodine, which possesses the sensitivity required for its determination in biological fluids. The method was adapted for the analysis of iodine in milk and urine. In our desire to exploit these differences and to obtain selectivity for the determination of iodine in biological fluids, this paper reports the level of iodine in human milk and urine samples from Kano –Nigeria.

2. Materials and Methods

Analytical reagent grade chemicals were used whenever possible. Glass distilled water was used throughout. Absorbance measurements were made with Cecil Model CE 373 Linear grating Spectrophotometer at 525nm in a 100mm cell.

Milk and urine samples were collected from 84 healthy volunteers 17-37 years old from Kano-metropolis in Kano State under standardized conditions. Since trace element concentration is a function of the stage of the feed, (Sanner and Dubrovic, 1984) donors were requested to express their milk (10cm³) manually during feeding alternating between right and left breast. The sample from each donor represented the accumulated milk collection per feed. The samples were collected in collection vials and stored as reported earlier (Ayodele and Na’abba, 1993; Ayodele et al., 1999).

Pathological histories like age of the donor, period of lactation, etc. were noted. Anamnestic data were collected with regards to the smoking of tobacco, drinking of alcohol,
nutritional habits, medication and use of hormonal contraceptives (Muller, 1987).

Casual urine samples (25 cm³) of donors were collected in separate vials. Based on the interference studies of several authors (Sandell and Kolthoff, 1934; Chaney, 1940 and 1950; Moran, 1952) a separation procedure for the iodine was carried out. The separation employed was a combination of Fang et al. (1944) and Garvin et al. (1994). To 5 cm³ milk and 10 cm³ urine was added 0.1 cm³ of 2.8M KOH with heating at 100°C for 1 hr or at 55°C for 2 hr. The ashed sample on cooling was mixed with 5 cm³ water sonicated for 30 min and then centrifuged for 10 min at 900 rpm. The supernatant was mixed with 0.5 cm³ of 1% ascorbic acid and 2 g of Type 732 cation exchange sulphonic resin. After 2 hr, the supernatant was neutralized with 0.1M NaOH and the resulting solution reduced to 2 cm³ was assayed for iodine.

Standard iodine solution 100 μg/l was prepared by dissolving 261.60 mg of the iodine crystals and 1.0 g of potassium iodide in water in a 250 cm³ volumetric flask. By successive dilution, standard iodine solution with an iodide concentration of 1 μg/cm³ was prepared.

3. Procedure

Into one arm of a two-limbed reaction vessel was pipetted 10 cm³ of the sample solution. 1 cm³ of a solution of sodium chloride (200 mg/cm³) was added followed by 5.0 cm³ of the arsenious solution and 1 cm³ of 5M sulphuric acids. Into the other limb of the reaction vessel was pipetted 5 cm³ of 0.02M ceric ammonium sulphate. Enough water was added to both arms of the vessel to bring the total volume to 50 cm³. The vessel was placed in a 25°C water bath. After 30 mins the vessel was removed and the solutions in the two limbs were allowed to mix by inversion while at the same time starting the stopwatch. On mixing the tube was returned into the bath. After 15 mins, 1 cm³ of 0.04 M ferrous ammonium sulphate was added with mixing followed by 1 cm³ of 0.4% potassium thioionate solution. The absorbance was immediately read at 525 nm. The amount of the iodide present in each sample was established from the calibration curve.

4. Results

Table 1 summarizes the recovery of 0.2 g of iodine added to 2.0 and 10.0 cm³ milk and urine using the recommended procedure. Recoveries were quantitative within experimental error. These results illustrate that interferences were negligible and that iodine could be determined in whole milk and urine. Figs. 1 and 2 summarise iodine distribution in the milk and urine samples investigated. The iodine levels fall within a range 10 - 110 and 10 - 90 μg/litre for milk and urine respectively. The mean values of 52.88 ± 22.60 and 27.62 ± 16.70 μg/l for milk and urine are in close agreement with the dietary allowance reported by WHO (1973), NAS (1980), Food and Nutrition Board (1970), Joerin (1975), Hetzel and Maberly (1986) but in contrast to other authors who reported higher values (Bruhn and Franke, 1983; Gushurst, 1983; Muramatsu et al., 1983; Kosta et al., 1988) (Table 2). A significant difference was indicated between the mean iodine concentrations in milk and urine. Kosta et al. (1983) reported a mean of 88 ± 83 μg/l in mature milk from Yugoslav subjects. Muramatsu et al. (1983) reported much higher levels range 80 - 700 μg/l in human milk specimens from Japanese subjects who consumed dietary algae. Studies conducted under the auspices of IAEA (Iyengar, 1982) using similar analytical quality control procedures support the reported values in our study. Fig. 3 summarizes iodine distribution in milk and urine. With respect to the period of lactation, longitudinal studies extending up to several months following delivery provided evidence of a progressive decrease of iodine in milk and urine as iodine levels showed a progressive decrease from
### Table 1: Recovery of 0.2 μg iodine added to milk and urine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Iodine added (μg)</th>
<th>Iodine Found (μg)</th>
<th>Iodine Recovered (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2 cm² milk</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>2 cm² milk</td>
<td>0.2</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>2. 5 cm² urine</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
</tr>
<tr>
<td>5 cm² urine</td>
<td>0.2</td>
<td>0.36</td>
<td>0.15</td>
</tr>
<tr>
<td>3. 2 cm² milk</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>2 cm² milk</td>
<td>0.3</td>
<td>0.39</td>
<td>0.09</td>
</tr>
<tr>
<td>4. 5 cm² urine</td>
<td>-</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>5 cm² urine</td>
<td>0.3</td>
<td>0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>5. 2 cm² milk</td>
<td>-</td>
<td>0.11</td>
<td>-</td>
</tr>
<tr>
<td>2 cm² milk</td>
<td>0.4</td>
<td>0.52</td>
<td>0.12</td>
</tr>
<tr>
<td>6. 5 cm² urine</td>
<td>-</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>5 cm² urine</td>
<td>0.4</td>
<td>0.54</td>
<td>0.14</td>
</tr>
</tbody>
</table>

### Table 2: Typical reported values of iodine in human milk.

<table>
<thead>
<tr>
<th>Country</th>
<th>Units</th>
<th>No. of samples</th>
<th>Mean</th>
<th>Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand</td>
<td>μg/day</td>
<td>-</td>
<td>0-0.04</td>
<td></td>
<td>Joerin [1975]</td>
</tr>
<tr>
<td>U.S.A</td>
<td>μg/day</td>
<td>40</td>
<td>142±81</td>
<td>68-396</td>
<td>NAS [1980]</td>
</tr>
<tr>
<td>U.S.A</td>
<td>μg/litre</td>
<td>8</td>
<td>178</td>
<td>29-490</td>
<td>Bruhn &amp; Franke [1983]</td>
</tr>
<tr>
<td>U.S.A</td>
<td>μg/litre</td>
<td>61</td>
<td>29-490</td>
<td></td>
<td>Gusthurt [1983]</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>μg/kg</td>
<td>-</td>
<td>132-510</td>
<td></td>
<td>Kosta et al [1983]</td>
</tr>
<tr>
<td>U.S.S.R</td>
<td>μg/°C</td>
<td>16</td>
<td>7.71±0.5</td>
<td></td>
<td>Boriev [1982]</td>
</tr>
<tr>
<td>Turkey</td>
<td>μg/cm²</td>
<td>26</td>
<td>109±0.5</td>
<td>45-208</td>
<td>Gokmen &amp; Dagh [1995]</td>
</tr>
<tr>
<td>Europe</td>
<td>μg/cm²</td>
<td>80</td>
<td>38</td>
<td>10-74</td>
<td>Delange et al [1978]</td>
</tr>
<tr>
<td>Guatemala</td>
<td>μg/cm²</td>
<td>17</td>
<td>38</td>
<td>17-74</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Hungary</td>
<td>μg/cm²</td>
<td>40</td>
<td>17-66</td>
<td>10-73</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Nigeria</td>
<td>μg/cm²</td>
<td>32</td>
<td>14</td>
<td>8-17</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Philippines</td>
<td>μg/cm²</td>
<td>36</td>
<td>75</td>
<td>33-104</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Sweden</td>
<td>μg/cm²</td>
<td>36</td>
<td>14</td>
<td>8-17</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Zaire</td>
<td>μg/cm²</td>
<td>60</td>
<td>3.2</td>
<td>3.5</td>
<td>Iyengar [1982]</td>
</tr>
<tr>
<td>Japan</td>
<td>μg/litre</td>
<td>-</td>
<td>80-700</td>
<td></td>
<td>Muramatsu et al [1983]</td>
</tr>
<tr>
<td>Finland</td>
<td>μg/litre</td>
<td>40</td>
<td>219</td>
<td></td>
<td>Koiranen &amp; Stabel-Taucher [1976]</td>
</tr>
<tr>
<td>WHO</td>
<td>μg/day</td>
<td>84</td>
<td>52.9</td>
<td>150-200</td>
<td>WHO [1983]</td>
</tr>
<tr>
<td>Nigeria</td>
<td>μg/litre</td>
<td>84</td>
<td>52.9</td>
<td>10-110</td>
<td>This study</td>
</tr>
</tbody>
</table>
60±19.35 μg/l in colostrums to 45± 21.21μg/l in the matured milk such that a significant difference was indicated between the two stages.

Fig. 4 summarizes iodine distribution in milk and urine with respect to the age of the lactating mothers. Young mothers’ milk and urine appear richer in iodine than their older counterparts. However, the iodine level in the older mothers was within the daily requirements considered safe (Food and Nutrition Board, 1970; Bruhn and Franke, 1983).

5. Discussion

There have been reports of socio-economic and seasonal differences in elemental concentrations in milk (WHO, 1985; WHO, 1989). In our studies there were no signs of systematic differences between samples collected from donors of different socio-economic status. However this study was not designed to intercept such variance and the existence of such difference cannot be excluded. The nutritional status of the mother as reflected by their socio economic status does not appear to influence the concentration of iodine in milk. However, the ranges obtained may be useful in determining the desirable concentration of iodine in milk substitutes following the recommendation of a WHO Expert Committee (1973) that milk formula products should contain all the minor and essential trace elements at least in those levels that are present in human milk.

The composition of human milk is by no means constant and several factors both physiological and non-physiological have been responsible for the observed variations (Lonnardal et al., 1976a and b; Hibber, 1982; Hartmann and Prosser, 1984; Helsing and King, 1985; Muller, 1987). Physiological factors include the stage of lactation, the time of day, the time of sampling and the nutritional status of the mother. In addition, there may be effects caused by disease, medication and the use of hormonal contraceptives. Non-physiological factors include geochemical and other environmental aspects as well as the impact of certain habits such as tobacco smoking and drinking of alcohol (Picciano and Guthrie, 1977; Underwood, 1977; Simes et al., 1979; Bobriev, 1982; WHO, 1985; Muller, 1987).

Urinary iodine excretion is often used as an indicator of iodine status. Excretion of 50mg of iodine/kg is marginal and excretion of less than 25mg/kg is indicative of serious iodine deficiency (Querido et al., 1974). There were no significant differences between the mean concentrations for the various age groups. Therefore concentrations of iodine in human milk and urine are probably controlled by homoeostatic mechanism, which accounts for the small variations. The recommended adult allowance of 150μg/day for both sexes provides a margin of safety (Food and Nutrition Board, 1970). An additional allowance of 25μg/day is recommended during pregnancy to meet the needs of the fetus and additional 50μg/day are recommended during lactation to meet the needs of the infants.

None of the volunteered mothers were smokers and only two were on medication/contraceptives. The results point out no differences between these sub-collectives. Also no final statement can be made about other possible correlations: such as duration of suckling, number of children, etc.

Although seasonal variations in the concentrations of some trace elements have been reported (Iyengar, 1982; WHO, 1985), neither a systematic effect nor correlation with a particular season was observed in this study as all samples were collected within a season. Differences in the concentrations of some minor and trace elements have been reported between urban well to do, urban poor and rural groups. However, no consistent pattern was identified in this study.
6. Conclusion

Determination of iodine in human milk and urine was achieved using separation and catalytic procedures. The current intake of iodine by infants fed on human milk in this study group are within the WHO recommended value. It may then be concluded that there is little difference in the iodine levels for the different age groups of the mothers. Concentrations of iodine in human milk and urine may probably be controlled by homeostatic mechanism, which accounts for the small variations.

REFERENCES


