VITAMIN DEFICIENCIES IN THE AETIOLOGY OF PHRYNODERMA IN SRI LANKA

by

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SUMMARY. The present study was undertaken to elucidate the possible role of different nutrients in the aetiology of phrynoderma. Eleven women (ages 26 ± 7 years) from a poor socio-economic background in the outskirts of Colombo were examined.

The following results were obtained:

1. The plasma tocopherol was significantly lower in phrynoderma cases than in controls (3.3 ± 0.6 μM and 13.0 ± 2.3 μM respectively, P < 0.002).

2. There were clear indications of riboflavin deficiency in phrynoderma cases as well as in controls, but the deficiency was significantly more advanced in the phrynoderma cases.

3. The concentrations of the following in blood showed no significant differences between the groups, and the values, though low, were within the normal range:
   (a) Vitamin A and retinol binding protein
   (b) Plasma triglyceride and cholesterol
   (c) Serum lipoproteins and fatty acids
   (d) Thiamin, vitamin B₁₂, and folic acid
   (e) Plasma selenium, zinc and iron.

4. A dietary study indicated that the intake was marginal or low with respect to vitamin E, riboflavin and essential fatty acids.
INTRODUCTION

Phrynoderma (follicular hyperkeratosis) was first described in Sri Lanka\textsuperscript{24,25,26} and later in India\textsuperscript{1,22}.

Its causation has been ascribed to a vitamin deficiency, \textsuperscript{3,24} an essential fatty acid deficiency\textsuperscript{2}, a fat soluble factor \textsuperscript{30,31} and to vitamin E\textsuperscript{23}. In the treatment of phyrnoderma the vitamin B complex has also to be administered along with vitamin E\textsuperscript{23}.

The present study was undertaken to elucidate the possible role of different nutrients in the aetiology of phrynoderma. The results of biochemical estimations on samples of blood collected from subjects and controls are discussed in the light of information from a dietary survey carried out earlier in the Southern Province. A preliminary report of this study is in the press.\textsuperscript{7}

MATERIALS AND METHODS

Eleven women with phyrnoderma (age 26 ± 7 years) were identified in Kolonnawa, near Colombo. The controls (11 women, age 27 ± 9 years) were from the same community. Both groups were of low socio-economic level. None of them had any overt signs of intercurrent illness of potential significance in sampling.

Samples of blood were collected in heparinized vacutainer-tubes, the plasma separated and frozen. The erythrocytes were washed twice with saline and haemolysates prepared by adding a volume of distilled water equivalent to that of plasma removed. The frozen sample of plasma and haemolysates were analysed at the University of Oslo.

ANALYTICAL PROCEDURES

Vitamin E

Measurement of the plasma concentration of α-tocopherol was carried out by HPLC combined with fluorometry\textsuperscript{4,28}. Samples of 100 µl plasma were deproteinized with 99.5% ethanol and mixed on a cyclcemixer for 30 sec. The samples were extracted with 6 ml n-hexane, mixed for 2 min and centrifuged (10 min, 440 g.) Aliquots of 4 ml were drawn from the organic layer and evaporated to dryness. The residue was redissolved in 250 µl hexane. The equipment consisted of a LDC Constametric pump model 3, 100 µl Rheodyne injector (no. 7120), Chrompack Zorbax silica column (I.D. 3 mm, O.D. 1/4”, length 25 cm, particle size 5µm) and Shimadzu RF—530 fluorescence detector (excitation 292 nm, emission 324 nm.) The mobile phase consisted of n-hexane : diisopropylether (93 :7, v/v) with a flow rate of 3 ml/min.
Vitamin A

Retinol in plasma was determined according to Driskell et al.9 with minor modifications: To 100 μl plasma was added 400 μl ethanol containing 0.2 nmol retinyl acetate. After vigorous mixing for 20 sec, 1 ml of hexane containing butylated hydroxytoluene (5 μg/ml) was added, then 200 μl H2O. The mixture was centrifuged for 5 min and 750 μl of the hexane phase was transferred to another glass tube and evaporated to dryness under N2. The residue was dissolved in methanol and injected onto a HPLC Supelcosil 5 μm LC-8 column (25 cm × 4.6 mm, i.d.) from Supelco (Bellefonte, PA, U.S.A.). The column was eluted with water-methanol, 5:95 (v/v) at a flow rate of 1-2 ml/min. Quantitation was based on the peak ratio between the sample retinol and the internal standard of retinyl acetate added in known amounts.

The HPLC equipment consisted of a Model M45 pump from Waters Associated (Milford, MA, U.S.A.). The detector was a Model 481 Lambda Max from Waters Associates, monitoring at 328 nm. The injector was a Model 7120 from Rheodyne Incorporated (Cotati, CA, U.S.A.).

Retinol binding protein (RBP)

Plasma RBP was determined by quantitative radial immunodiffusion (LC-Partigen-RBP new, OTUM 04/05 with standard human serum from Behringwerke AG, Marburg, F.R.G.)

Triglycerides and cholesterol

These analysis were performed by automated analysis19,20 at the Institute of Clinical Biochemistry, University of Oslo.

Fatty acid pattern

Plasma lipids were extracted with chloroform-methanol (2:1) after the method by Folch et al.15. Lipid extracts were separated by thin layer chromatography using 0.4 mm silica gel H. The solvent system was petroleum ether (60—70°C) - diethylether-acetic acid (113:20:1, v/v/v). A zone corresponding to the phospholipid fraction was scraped into glass-stoppered tubes and prepared for gas chromatography. Fatty acids in the phospholipid fraction were methylated by using a mixture of benzene, methanolic HCl and dimethoxypropane at room temperature, overnight.21

The methyl esters were separated on a non-polar (CP. SIL SCB) vitreous silica capillary column in a Carlo Erba Fractovap 2150 GC equipped with a flame-ionization detector. Carrier gas flow was 0.75 ml/min split ratio 1:100. Injector and detector temperature was 275°C. Splitless injection was performed at 50°C, and column temperature was programmed (LT programmer model 232) to rise rapidly to 220° (40°C/min) and then by a slow gradient (0.5°C/min) to 260°C. Peak areas were measured by a Hewlett-Packard 3390 reporting integrator. Identification of major peaks was made by comparing the retention times with those of fatty acid methyl ester standards obtained from Supelco Inc., Bellefonte, PA, U.S.A.32.
Vitamin B

For assessment of riboflavin and thiamin status, haemolysates were prepared after the method of Sauberlich et al.\textsuperscript{28} and measured for glutathione reductase and transketolase activities.\textsuperscript{6,28} Glutathione reductase activity for riboflavin assessment was measured as NADPH oxidation at 37°C for 10 min in a recording spectrophotometer at 340 nm. The activity coefficient (AC) was determined in the following way:\textsuperscript{28}

$$AC = \frac{\text{glutathione reductase activity with added FAD}}{\text{glutathione reductase activity without FAD}}$$

Transketolase activity for thiamin assessment was measured by glucose formation at 38°C for 60 min using ribose 5–phosphate as substrate. Glucose was determined spectrophotometrically with the anthrone reagent. The activity coefficient was determined in the following way:\textsuperscript{6}

$$AC = \frac{\text{transketolase activity with added TPP}}{\text{transketolase activity without TPP}}$$

For assessment of normal and deficiency ranges of riboflavin and thiamin, see refs. 6, 8, 17 and 29 and table IV.

Vitamin B\textsubscript{12} and folic acid were determined by commercial kit from Diagnostic Prod. Cooperation, Los Angeles, CAL, U.S.A.

Trace metals

Fe, Se and Zn were determined by instrumental neutron activation analysis. About 0.5 ml serum were freeze-dried in Quartz ampoules. Sealed ampoules containing samples and appropriate standards were irradiated for 3 days in the reactor JEEP II, Kjeller, Norway, at a flux of $1.3 \times 10^{13}$ n.s\textsuperscript{-1} cm\textsuperscript{-2}. After a decay time of 50 days the samples were dissolved in 2 ml conc. H\textsubscript{2}SO\textsubscript{4} : HNO\textsubscript{3} (1:1) transferred to counting bottles and subjected to Ge (Li) \textgamma–spectrometry\textsuperscript{5}.

Dietary Survey

In the dietary study, the food consumed by all household members at meals and in between meals was recorded over two seasonal periods (high and low food availability) of 3 days each. Twenty-seven households belonging to different socio-economic groups in a community in the Hambantota district were selected for this study.

The method used for collection of dietary data was a combination of weighing and measuring, performed by one of the investigators in collaboration with a field assistant and the woman responsible for cooking in each household. The investigator made
several visits a day to each household during the period of recording. Intake of nutrients and energy were calculated on the basis of Tables of Food Composition for use in Sri Lanka.

Statistical analysis

The significance of differences between groups were calculated by the Mann-Whitney test, the correlation coefficients by Spearman's test. Probabilities of differences at the level of $P<0.05$ were regarded statistically significant. The results are given as mean values ±sd.

RESULTS.

Phrynoderma cases were identified in a village of low socio-economic level close to Colombo. Prior to this investigation preliminary analyses were carried out on blood samples from phrynoderma cases, living in a community in the Hambantota district, Southern Sri Lanka, where the dietary study was carried out.

TABLE I. Content of vitamin E ($\alpha$—Tocopherol) and Vitamin A (retinol) in blood plasma of subjects with phrynoderma and of controls

<table>
<thead>
<tr>
<th>Case</th>
<th>$\alpha$—Tocopherol</th>
<th>Retinol (ROH)</th>
<th>Retinol Binding Protein</th>
<th>ROH/RBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$M</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
<td>$\mu$M</td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
<td>1.2</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
<td>1.1</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>3.8</td>
<td>1.2</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>3.0</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>2.3</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>1.3</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>3.7</td>
<td>1.8</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>4.4</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean 3.3 1.1 1.2 1.0
S.D. 0.6 0.4 0.4 0.1

Controls

Mean 13.0* 1.3 1.3 1.0
S.D. 2.3 0.2 0.2 0.1
n 11 10 9 9

*Significantly different from controls $P < 0.002$.

As presented in Table I the plasma content of $\alpha$—tocopherol was significantly lower in phrynoderma cases than controls, 3.3 ± 0.6$\mu$M and 13.0±2.3$\mu$M respectively($P<0.002$). The normal range of $\alpha$—tocopherol is considered to be 14-15 $\mu$M in Western countries.
Thus the phrynoderma cases were clearly deficient in vitamin E, and the controls were also low. The levels of vitamin A and retinol binding protein are outlined in Table I. There was no significant difference between the groups and the values were low but within the normal range.

TABLE 2. Triglyceride and cholesterol contents in blood-plasma of phrynoderma cases and of controls

<table>
<thead>
<tr>
<th></th>
<th>Phrynoderma</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>0.73±0.42</td>
<td>0.78±0.27</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>3.96±0.34</td>
<td>4.19±0.91</td>
</tr>
</tbody>
</table>

Plasma triglyceride and cholesterol levels were within normal ranges, and there was no significant difference between the groups (Table 2). Furthermore, the electrophoretic pattern of the lipoproteins gave no indication of any abnormal pattern (not shown).

There was no significant difference in the fatty acid composition in the phospholipid fraction of plasma between the groups (Table 3). The ratio 20:3 ω9/20:4 ω6 is commonly used as an indicator of essential fatty acid deficiency. A ratio higher than 0.4 would indicate an essential fatty acid deficiency. In our samples all the values were below 0.4. The cases from Hambantota also had fatty acid pattern as well as 20:3/20:4 ratios within the normal range (not shown). Although being within the normal range, the 20:3/20:4 ratios of patients as well as controls from Sri Lanka were higher than commonly found in industrialized societies.

TABLE 3. Composition of phospholipid fatty acids in blood-plasma of 11 subjects with phrynoderma and of 12 controls

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Phrynoderma (n=11) (μg/ml)</th>
<th>Controls (n=12) (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>12.8 ± 4.5</td>
<td>14.3 ± 5.1</td>
</tr>
<tr>
<td>16:0</td>
<td>454.8 ± 32.3</td>
<td>469.1 ± 25.4</td>
</tr>
<tr>
<td>16:1 ω7</td>
<td>18.6 ± 8.6</td>
<td>15.3 ± 2.7</td>
</tr>
<tr>
<td>18:0</td>
<td>218.7 ± 14.8</td>
<td>230.1 ± 22.3</td>
</tr>
<tr>
<td>18:1 ω7</td>
<td>33.4 ± 7.2</td>
<td>25.9 ± 8.3</td>
</tr>
<tr>
<td>ω9</td>
<td>118.7 ± 22.0</td>
<td>120.8 ± 14.5</td>
</tr>
<tr>
<td>18:2 ω6</td>
<td>269.8 ± 34.2</td>
<td>256.3 ± 34.5</td>
</tr>
<tr>
<td>20:3 ω3</td>
<td>2.0 ± 0.8</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>ω6</td>
<td>51.1 ± 8.3</td>
<td>50.2 ± 8.7</td>
</tr>
<tr>
<td>ω6</td>
<td>6.0 ± 3.5</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td>20:4 ω6</td>
<td>130.4 ± 19.6</td>
<td>131.5 ± 16.7</td>
</tr>
<tr>
<td>20:5 ω3</td>
<td>20.0 ± 6.3</td>
<td>19.1 ± 8.7</td>
</tr>
<tr>
<td>22:5 ω3</td>
<td>5.7 ± 2.0</td>
<td>6.0 ± 2.9</td>
</tr>
<tr>
<td>22:6 ω3</td>
<td>97.3 ± 26.8</td>
<td>102.4 ± 24.3</td>
</tr>
<tr>
<td>20:3 ω9/20:4 ω6</td>
<td>0.046 ± 0.020</td>
<td>0.035 ± 0.014</td>
</tr>
</tbody>
</table>
VITAMIN E DEFICIENCY IN PHRYNODERMA

TABLE 4. Riboflavin and thiamin status of phrynoderma cases and of controls

<table>
<thead>
<tr>
<th></th>
<th>Glutathione reductase AC</th>
<th>Trans-ketolase AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phrynoderma cases</td>
<td>1.92 ± 0.22*</td>
<td>1.10 ± 0.15</td>
</tr>
<tr>
<td>Controls</td>
<td>1.60 ± 0.23</td>
<td>1.14 ± 0.10</td>
</tr>
<tr>
<td>Normal range</td>
<td>0.9 — 1.2</td>
<td>1.00 — 1.15</td>
</tr>
<tr>
<td>Marginal range</td>
<td>1.2 — 1.3</td>
<td>1.15 — 1.25</td>
</tr>
<tr>
<td>Deficient range</td>
<td>&gt; 1.3</td>
<td>&gt; 1.25</td>
</tr>
</tbody>
</table>

*Significantly different from controls at p < 0.005

Riboflavin and thiamin levels in the erythrocytes were estimated by enzymatic tests (activity coefficients) on haemolysates (Table 4). There were clear indications of riboflavin deficiency in the phrynoderma cases as well as in the controls, but the deficiency was significantly more advanced in the phrynoderma cases. These analyses were verified by microbiological analysis of riboflavin levels (not shown). The thiamin levels were within the normal range for both groups (Table 4). The plasma levels of vitamin B₁₂ and folic acid were estimated as well, and were within the normal range (not shown).

As shown in Table 5 the plasma selenium values were within the normal range. The zinc and iron values as well were within the normal range (Table 5).

TABLE 5. Plasma concentrations of selenium, zinc and iron. The number of subjects is given within brackets

<table>
<thead>
<tr>
<th></th>
<th>Selenium μg/ml</th>
<th>Zinc μg/ml</th>
<th>Iron μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phrynoderma cases</td>
<td>0.15±0.02 (11)</td>
<td>0.91±0.02 (11)</td>
<td>1.5±0.5 (9)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.14±0.03 (10)</td>
<td>0.88±0.01 (10)</td>
<td>1.5±0.4 (8)</td>
</tr>
</tbody>
</table>

The dietary study indicated that the diet of the lower socio-economic groups in the study area may be marginal or low with respect to vitamin E and riboflavin as well as the essential fatty acids.

The diet in this area, like the rest of Sri Lanka, is based on rice and coconut. The more well-to-do families generally consume a large variety of foods such as vegetables, fruits, milk, fish and meat in addition to these two basic foods. However, in a considerable proportion of families belonging to the lower socio-economic groups, where also the phrynoderma cases were found, the diet included only small amounts of these additional foods. These families also experienced periods when the supply of rice and coconut was not enough to satisfy their hunger,
Table 6 shows the intake of energy and some nutrients as a percentage of the recommendations by the FAO/WHO\(^{10,11,12,13}\) for the different socio-economic groups. An intake of riboflavin in the low socio-economic groups of about 60—70% of that recommended, may in part be explained by low energy or food intake in general. The composition of the diet may also be a contributing factor. Coconut is a poor source of riboflavin. Rice has a low content of this vitamin compared to other grains. The intake may be particularly low when the polished type is used, which was the case in a number of the households in the low socio-economic groups.

Table 6 also indicates a very low intake of vitamin A. However, the intake of this vitamin is, in contrast to the other nutrients evaluated, subject to large seasonal variations. The intake of carotenes may be very high when certain fruits and vegetables, such as mangoes and green leaves, are in season. It should be pointed out, however, that green leaves are available at any time of the year. The mango season was not covered in this study. Therefore, these data should be judged with caution.

### TABLE 6. Intake of energy and nutrients as a percentage of recommendations by FAO/WHO (refs. 10—13) of a rural population in District of Hambantota, Southern Province

<table>
<thead>
<tr>
<th></th>
<th>Poor peasant rural worker</th>
<th>Rich farmer, landlord, teacher, trader</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household, no.</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Energy</td>
<td>79</td>
<td>94</td>
</tr>
<tr>
<td>Protein</td>
<td>103</td>
<td>125</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>37</td>
<td>58</td>
</tr>
<tr>
<td>Thiamin</td>
<td>92</td>
<td>116</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>65</td>
<td>91</td>
</tr>
<tr>
<td>Niacin</td>
<td>96</td>
<td>122</td>
</tr>
</tbody>
</table>

About 20% of the energy in the diet of the low socioeconomic groups was provided by fat. About 70% of the this fat came from coconut, 8% from rice and 5% from milk products. The rest of the fat was provided by small amounts of other grains and nuts, fish, vegetables and fruits.

Coconut and milk thus contribute about 75% of the fat intake. Coconut is almost devoid of and milk low in essential fatty acids. These foods, as well as rice, are also poor sources of vitamin E. Furthermore, vitamin E in these foods is predominantly in the form of tocopherols of low biological activity, such as \( \beta \) and \( \gamma \) - tocopherol. Thus, this diet is likely to provide a marginal or low supply of alpha tocopherol as well as of essential fatty acids.
Phrynoderma is common in Sri Lanka, especially in the lower socio-economic communities. Investigations by Nicholls\textsuperscript{25,26} in 1934—5 revealed a frequency of 10—20\% among school children and up to 80\% in institutions such as boarding schools, prisons, hospitals. More recent studies by Wijayapala\textsuperscript{34} in 1979 confirms that this condition is still frequent in rural areas in Sri Lanka; in the Hambantota district the frequency was 14\% among women.

Since the dietary survey was performed in a rural area of the Hambantota district, the findings may not strictly apply to the Kolonnawa population. However, these findings may shed some light on the characteristics of the diet in a community afflicted by phrynoderma.

It is clear from the present study that phrynoderma cases from the Colombo area coincides with \(\alpha\)-tocopherol and riboflavin deficiencies as judged by the plasma analysis of these vitamins (Table I and 4). This is in accordance with results from India\textsuperscript{23}, where E—vitamin as well as B—vitamin complex had to be administered to phrynoderma cases to obtain a cure.

No symptoms other than those characteristic of phrynoderma were found during the interview and examintion.

The diet of the lowest socio-economic groups in Sri Lanka is characterized by a low food intake in addition to the less varied diet. This is mostly due to poverty and inability to provide the desired foods. The traditional diet in Sri Lanka includes a large variety of foods, which can supply enough nutrients when eaten in sufficient amounts.

The dietary survey indicates that phrynoderma is present in a community where there is a combination of marginal intakes of essential fatty acids, vitamin E and riboflavin. Primary malnutrition may partly explain the reduced plasma levels of \(\alpha\)– tocopherol and riboflavin.

The low plasma level of \(\alpha\)– tocopherol could also be due to malabsorption of the vitamin. This is, however, unlikely since the cholesterol and triglyceride levels as well as the lipoprotein patterns were normal (Table 4). Neither could the low \(\alpha\)– tocopherol level be explained by a low selenium supply. The plasma levels of selenium did not indicate any deficiency (Table 5). This is in agreement with the finding that the staple rice contained fairly large amounts of Se (86—130 ng/g rice from local markets in Sri Lanka).
The plasma level of vitamin A was low, but did not indicate any deficiency. It is known, however, that the plasma level is well regulated even in slight deficiency states. Therefore, to get a more correct picture of the state of deficiency, a measurement of the liver store should have been performed.

Even if the essential fatty acid intake was low (Table 6) there was only a slight indication of marginal essential-fatty-acid deficiency as judged from the 20:3/20:4 ratio (Table 3).

Phrynodema cases in Sri Lanka have been cured by treatment with cod liver oil or sesame oil. The vitamin E supply in these foods could well have been an active ingredient in the treatment. Even though vitamin E in sesame oil is predominantly in the form of \( \beta \)-tocopherols with low physiological activity, this may be offset by a high content of this form of the vitamin.

In conclusion, phrynodema appears to be a condition derived from a combined deficiency of \( \alpha \)-tocopherol and vitamin \( B_2 \). The relative importance of the vitamins in the aetiology of phrynodema remains to be elucidated. To achieve information on the possible beneficial effect of nutritional therapy in phrynodema further investigations have to be made.

ACKNOWLEDGEMENTS

We thank Arne Oshang and Dr. Davy Perera for initiating the preliminary investigation of phrynodema in the Hambantota district. Our sincere thanks to Dr. U. T. Vitharana, Director of the Medical Research Institute, Colombo, for support given to this investigation. Assistance given during field work by J. S. Dean., W. A. T. M. Wijayaratne, and by Dr. S. Sinnathamby of the Medical Research Institute, Colombo, is acknowledged.

We thank Dr. S. Skrede and K. T. Stokke of the Institute of Clinical Biochemistry, University of Oslo and Dr. Lie of the Institute of Nutrition, Fish College, Bergen, Norway, for analytical help, and T. Rortveit and E. Kjekshus for technical assistance.

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