Associations of Total, Dairy, and Meat Protein with Markers for Bone Turnover in Healthy, Prepubertal Boys

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Abstract

We previously reported that high intake of milk, but not meat, equal in protein content, increased serum insulin-like growth factor-I (sIGF-I) in prepubertal boys. sIGF-I plays a key role in bone metabolism. Therefore, the aim of this cross-sectional study was to investigate associations of total, dairy, and meat protein intake with markers for bone turnover and sIGF-I in prepubertal, healthy boys (n = 81). We measured bone turnover (enzyme-linked immunoassay) in serum osteocalcin (sOC), bone-specific alkaline phosphatase (sBAP), and C-terminal telopeptide of collagen type-I (sCTX); dietary intake was estimated from a 3-d weighed food record. sIGF-I and its binding protein-3 were assessed (immunoassay) in a subgroup of 56 boys. All statistical models included effects of age, BMI, and energy intake. Dairy protein was negatively associated with sOC (P = 0.05) but not significantly associated with sBAP and sCTX. Further analyses showed that dairy protein decreased (P = 0.05) sOC at a high meat protein intake (>0.8 g/kg), whereas meat protein increased (P = 0.03) sOC at a low dairy protein intake (<0.4 g/kg). Total and meat protein intake was positively associated with sBAP (P ≤ 0.04) but not significantly associated with sOC and sCTX. Free sIGF-I was positively associated with total (P < 0.01) and dairy (P = 0.06) protein but not with meat protein. Our results indicate that dairy and meat protein may exhibit a distinct regulatory effect on different markers for bone turnover. Future studies should focus on differential effects of dairy and meat protein on bone health during growth.


Introduction

Osteoporosis is a disease characterized by decreased bone mass and deterioration of bone tissue (1). It is estimated to cause 9 million fractures worldwide per year and the prevalence is increasing (2). The risk for osteoporosis later in life can be reduced by maximizing peak bone mass and optimizing bone geometry during growth (3). Around 20% of the variation in peak bone mass is affected by lifestyle, including nutrition (1). Therefore, it is important to identify nutritional factors that could be used for prevention of osteoporosis.

Little is known about the role of dietary protein on bone health. Although many studies have focused on its potential bone-detrimental effect, recent data indicate that dietary protein is necessary for bone health in elderly populations (4). Yet, the understanding of its action on bone growth early in life is limited. Several observational studies conducted in children reported a positive correlation between total protein intake and size-adjusted bone area (5), total bone mineral content (6), cortical bone mineral density (BMD)2 (7), and bone geometry (7) (all P < 0.05). Furthermore, as reviewed by Rizzoli et al. (8), the bone-enhancing effect of total protein intake seemed to be evident in prepubertal, but not in peri- or postpubertal children, indicating that dietary protein could be particularly important for bone growth during early stages of life.

Serum insulin-like growth factor-I (sIGF-I) is a key regulator of bone metabolism (9) and a major determinant of bone growth and mineral content (10). The biological activity of sIGF-I depends on the molar ratio of sIGF-I to its main binding protein, insulin-like growth factor binding protein-3 (sIGFBP-3) (11). Increasing protein intake increases circulating level of sIGF-I. Protein-induced increase in sIGF-I concentrations is believed to be the most likely explanation for the bone-anabolic effect of dietary protein (4). We previously reported that habitual total protein intake and milk consumption, but not meat consumption, were positively correlated with sIGF-I in 2.5-y-old boys (12). Correspondingly, a high intake of milk, but not meat, equal in protein content, increased sIGF-I by 19% in 8-y-old boys (13). These studies indicated that milk protein may exhibit a differential effect on bone metabolism compared with meat protein intake.

The aim of this cross-sectional study was to investigate associations of total, dairy, and meat protein intake with serum
markers for bone turnover and sIGF-1 concentrations in healthy, prepubertal boys.

Materials and Methods

Study population. In this cross-sectional study, we used baseline data obtained from 2 previous intervention trials conducted in 2000–2001 and 2004–2005 that investigated the effect of milk intake on growth factors and bone turnover in prepubertal boys. Selection of the participants included in the study conducted in 2000–2001 was described in details elsewhere (13). In brief, 313 boys, born between October and December 1992, were randomly recruited through the Central Personal Register in 2000. Thirty boys agreed to participate in the study, 28 were eligible, and 24 completed the trial. The participants included in the study conducted in 2004–2005 were recruited through the Central Personal Register in 2004. From 831 boys born between July and December 1996, 89 agreed to participate, 68 were eligible, and 57 completed the trial. In total, 96 boys were eligible and final baseline biochemical, dietary, and anthropometric data were obtained from 81 individuals. All participants were healthy, had normal growth, and did not take any medications known to affect bone metabolism and growth. The Ethics Committee of Copenhagen and Frederiksberg approved the protocols for both trials (J.No.KF01–097/00 and J.No.KF01–072/04).

Anthropometry and dietary assessment. Height was measured to the nearest millimeter with a wall-mounted stadiometer and weight was measured in light clothing with an electronic digital scale to the nearest 100 g. The mean dietary intake of selected nutrients and milk consumption were calculated for each subject from a 3-d weighed food record (2 weekdays and 1 weekend day) using the Danish food-composition database (DANKOST 2000 and 3000, Dansk Catering Center). Dairy protein intake (g/d) was estimated from the intake of dairy products (milk, yogurt, buttermilk, chocolate milk, cheese, cream, and ice cream). Meat protein intake (g/d) was estimated from the intake of red meat, poultry, and fish. In cases when dairy and/or meat proteins were part of a whole dish, the amount of respective protein was estimated based on the recipes used in the Danish food-composition database, DANKOST 2000 and 3000. Plant protein intake was estimated from the difference between total protein intake and dairy, meat, and egg protein intake.

Dietary assessment in children is difficult. However, in this study, the children’s dietary food record was kept both by the boys and by their parents and the importance of maintaining usual dietary intake was emphasized to the families. Furthermore, a 3-d food record was reported to yield the strongest agreement with actual dietary intake compared with 24-h recall and 5-d food frequency record in children (14).

Biochemical measurements. Fasting blood samples were drawn from a vein puncture between 0800 and 1000. Serum was separated and stored at −80°C until further analyses. Serum bone-specific alkaline phosphatase (sBAP) was measured using ELISA (Metra BAP Quidel). Before the analysis, 40 µL of each sample was diluted with 160 µL of assay buffer to achieve concentrations within the calibration curve (detection limit at 150 U/L). All samples were analyzed in duplicate. Maximum CV for each sBAP duplicate was 5.5%. Intra- and inter-assay precision CV of internal standards for sBAP were 1.3% (n = 6) and 2.4% (n = 20), respectively. Serum C-terminal telopeptide of collagen type I (sCTX) was measured by ELISA (Nordic Bioscience Diagnostics A/S). Before the analysis, 40 µL of each sample was diluted with 160 µL of standard-A to achieve concentrations within the calibration curve (detection limit at 3.26 µg/L). All samples were analyzed in duplicate. Maximum CV for each sCTX duplicate was 6.9%. Intra- and inter-assay precision CV of internal standards were 5.8% (n = 7) and 13.1% (n = 19), respectively. Serum osteocalcin (sOC) was analyzed using automated chemiluminescent immunoassay (IMMULITE 1000, DPC Biermann GmbH). Intra- and inter-assay precision CV of internal standards were 2.3% (n = 4) and 3.5% (n = 12), respectively. To reduce the variation of markers for bone turnover, their biochemical analyses were performed by the same person in standardized conditions (i.e. over the same time period, using kits with the same serial numbers for sBAP, sCTX, and sOC, respectively).

sIGF-I and sIGFBP-3 were measured by automated chemiluminescent immunoassay (IMMULITE 1000, DPC Biermann GmbH) in samples obtained during 2004–2005. The determination of molar ratio between sIGF-I and sIGFBP-3 (sIGF-I/IGFBP-3) was reported previously (13). Intra- and inter-assay precision CV of internal standards for sIGF-I were 2.8% (n = 11) and 7.8% (n = 6), respectively. Intra- and inter-assay precision CV of internal standards for sIGFBP-3 were 1.9% (n = 12) and 5.2% (n = 7), respectively.

Statistical analysis. The baseline data obtained from the study conducted in 2000–2001 and the study conducted in 2004–2005 were considered simultaneously, because selected dietary and biochemical variables between these 2 data sets (unpaired, 2-tailed Student’s t test) did not differ. All dietary variables were preadjusted for total energy intake using the residual method (15). Brieﬂy, the residuals from separate regression models, including an absolute intake of each nutrient as a dependent variable and total energy intake as an independent variable, were calculated and added to the mean value of respective dietary variable. We used energy preadjusted dietary variables for further statistical analyses.

The effects of total protein, plant protein, calcium, and milk intake (x1) on selected dependent variables (y) (sOC, sBAP, sCTX, sIGF-I, sIGFBP-3, and sIGF/I/IGFBP-3) were estimated from separate multiple linear regression models, adjusted for age and BMI (Model 1: y = β0 + β1(x1) + β2(age) + β3(BMI) + e). To study the relation between dairy (x2) and meat (x3) protein intake on selected dependent variables (y), both dairy and meat protein intake were included into regression models simultaneously [Model 2: y = β0 + β1(x2) + β2(x3) + β3(age) + β4(BMI) + e]. To further study whether there were interactions between dairy and meat protein intake with respect to their effect on selected dependent variables, the effect of interaction [i.e. (x2) × (x3)] was included in the Model 2. As there was a significant interaction between dairy and meat protein intake with respect to their effect on sOC, the intake of dairy and meat protein were divided into 3 levels: low (<0.4 g/kg; −11–23 g/d), medium (0.4–0.8 g/kg; −11–23 g/d), and high (>0.8 g/kg; −23–53 g/d). For each of these 6 levels, we constructed separate multiple linear regression models to estimate the effect of meat or dairy protein intake (as a continuous explanatory variable) on sOC (as a continuous dependent variable). The bivariate associations between sIGF-I status and markers for bone turnover were tested by Pearson’s correlation coefficients. SAS (version 8.2; SAS Institute) was used for data analyses. The results are means ± SD and the significance was P < 0.05.

Results

Anthropometric and biochemical characteristics of the participants are presented in Table 1. The mean age was 8.1 ± 0.1, which is ~3 yr before pubertal spurt in boys (16). Therefore, although the pubertal development was not assessed, we assumed that all the boys were at Tanner stage I. The mean weight, height,

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>8.1 ± 0.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>28.6 ± 4.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>132.6 ± 6.0</td>
</tr>
<tr>
<td>sOC, µg/L</td>
<td>82.4 ± 16.9</td>
</tr>
<tr>
<td>sBAP, U/L</td>
<td>141.8 ± 28.5</td>
</tr>
<tr>
<td>sCTX, µg/L</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>sIGF-I, µg/L</td>
<td>105.4 ± 39.1</td>
</tr>
<tr>
<td>sIGFBP-3, µg/L</td>
<td>4217 ± 615</td>
</tr>
<tr>
<td>sIGF-I/IGFBP-3, µmol/L</td>
<td>0.1 ± 0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 81 unless otherwise noted.
2 n = 56.
and BMI were between the 50th and 75th percentile of weight and height (17) and BMI (18) for 8-yr-old Danish boys. The mean serum levels of the analyzed markers for bone turnover, sIGF-I, and sIGFBP-3 were within the range observed in healthy, age- and sex-matched, European populations (19–21). Total protein intake (Table 2) varied from 1.4 to 3.3 g/kg, with the mean value (2.7 g/kg) ~200% higher than the recommended dietary intake (RDI) for this age (22). The mean intake of dairy and meat protein (Table 2) was equally distributed (i.e. 19.0 g/d and 18.4 g/d, respectively) and each comprised ~30% of the total protein. A total of 80% of the population had dairy and meat protein intake higher than 50% of RDI for total protein intake (22). The daily intake of calcium (Table 2) varied from 350 mg to 1960 mg, with the mean value (960 mg) modestly above RDI (22). The mean intake of vitamin D (2.5 µg) was 33% of RDI (Table 2) (22).

As expected, dairy protein and milk intake, but not total, plant, and meat protein intake, were highly correlated with calcium intake (all r = 0.9; P < 0.0001). Total and meat protein intake were positively associated with sBAP (P ≤ 0.04) but not with sOC or sCTX (Table 3). Dairy protein intake was negatively associated with sOC (P = 0.05) but not with sBAP or sCTX. Analyses of the relations between dairy and meat protein intake, with respect to their effect on markers for bone turnover, showed a significant interaction between dairy and meat protein with regard to their effect on sOC (P < 0.02). Dairy protein decreased (P = 0.05) sOC at a high meat protein intake (>0.8 g/kg) (Fig. 1), whereas meat protein increased (P = 0.03) sOC at a low dairy protein intake (<0.4 g/kg) (Fig. 2). At an intermediate dairy (or meat) protein intake (i.e. 0.4–0.8 g/kg), no significant effect of meat (or dairy) protein intake on sOC was observed (data not shown). Dietary intake of calcium and plant protein was not significantly associated with any of the analyzed markers for bone turnover, but there was a tendency toward a positive association between milk intake and sBAP (P = 0.07).

Total protein, milk, and calcium intake were positively associated (P ≤ 0.03) with sIGF-I concentrations and sIGF/IGFBP-3 (Table 3). Furthermore, dairy protein tended to be positively associated (P = 0.06) with free sIGF-I (Table 3). There were no significant correlations between meat and plant protein intake and sIGF-I status. From all analyzed markers for bone turnover, only sBAP was positively correlated with sIGF-I and sIGF/IGFBP-3 (both r = 0.2; P < 0.0005).

**Discussion**

This was a cross-sectional study investigating associations of total, dairy, and meat protein intake on bone turnover in healthy, prepubertal boys. We showed that dairy protein was negatively associated with sOC (P = 0.05), whereas meat protein was positively associated with sBAP (P = 0.01). This indicates that dairy and meat proteins may exhibit distinct regulatory effects on different markers for bone turnover. The study also showed a significant interaction between dairy and meat protein intake (P = 0.02) with respect to their effect on sOC. Dairy protein decreased (P = 0.05) sOC at a high (>0.8 g/kg) intake of meat protein, whereas meat protein increased (P = 0.03) sOC at a low (<0.4 g/kg) dairy protein intake. Milk and calcium intake was not significantly associated with sOC, indicating that the negative correlation between dairy protein and sOC may be unrelated to dietary calcium. Furthermore, we demonstrated that total protein intake was not significantly associated with sOC but was positively associated with sBAP and sIGF-I (P < 0.01).

Previous studies investigating the effect of dietary protein on bone turnover are scarce and limited to adults and elderly people. In agreement with our observations, 2 cross-sectional studies did not show any significant associations between total protein intake and sOC in postmenopausal women (23), and healthy and men women aged ≥65 y (24). Unfortunately, these studies did not estimate daily intake of dairy and meat protein and, thus, could not determine their effect on sOC. However, Dawson-Hughes et al. (25) reported that meat supplementation at a high (0.75 g/kg) or at a low (0.04 g/kg) protein content did not significantly affect sOC in elderly men and women with a

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy, MJ/d</td>
<td>8.2 ± 1.5</td>
</tr>
<tr>
<td>Total protein, energy intake %</td>
<td>13.4 ± 2.0</td>
</tr>
<tr>
<td>Total protein, g/d</td>
<td>64.2 ± 9.6</td>
</tr>
<tr>
<td>Total protein, g/kg</td>
<td>2.67 ± 0.4</td>
</tr>
<tr>
<td>Dairy protein, g/d</td>
<td>19.0 ± 9.2</td>
</tr>
<tr>
<td>Dairy protein, g/kg</td>
<td>0.67 ± 0.3</td>
</tr>
<tr>
<td>Meat protein, g/kg</td>
<td>18.4 ± 8.0</td>
</tr>
<tr>
<td>Meat protein, g/kg</td>
<td>0.66 ± 0.3</td>
</tr>
<tr>
<td>Plant protein, g/d</td>
<td>25.4 ± 7.7</td>
</tr>
<tr>
<td>Plant protein, µg/L</td>
<td>0.89 ± 0.3</td>
</tr>
<tr>
<td>Milk intake, g/d</td>
<td>0.41 ± 0.2</td>
</tr>
<tr>
<td>Calcium, g/d</td>
<td>0.96 ± 0.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 81 unless otherwise noted.
2 Preadjusted for energy intake by the residual method (15).
3 Number for the model used in the analyses (see “Statistical analysis”).
4 Regression coefficients (β₁) and P-values (P), adjusted for age and BMI, are given.

**TABLE 3**

<table>
<thead>
<tr>
<th>sOC, µg/L</th>
<th>sBAP, µg/L</th>
<th>sCTX, µg/L</th>
<th>sIGF-I, µg/L</th>
<th>sIGFBP-3, µg/L</th>
<th>sIGF-II/IGFBP-3, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.09</td>
<td>0.68</td>
<td>0.89</td>
<td>0.01</td>
<td>&lt;−0.01</td>
</tr>
<tr>
<td>Plant protein</td>
<td>0.24</td>
<td>0.36</td>
<td>−0.16</td>
<td>0.72</td>
<td>&lt;−0.01</td>
</tr>
<tr>
<td>Dairy protein</td>
<td>−0.45</td>
<td>0.05</td>
<td>0.53</td>
<td>0.16</td>
<td>−0.01</td>
</tr>
<tr>
<td>Meat protein</td>
<td>0.44</td>
<td>0.11</td>
<td>0.86</td>
<td>0.04</td>
<td>−0.01</td>
</tr>
<tr>
<td>Milk intake</td>
<td>−0.01</td>
<td>0.35</td>
<td>0.03</td>
<td>0.07</td>
<td>&lt;−0.01</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>−0.01</td>
<td>0.79</td>
<td>0.02</td>
<td>0.11</td>
<td>&lt;−0.01</td>
</tr>
</tbody>
</table>

1 Preadjusted for energy intake by the residual method (15).
2 n = 81 for analyses including markers for bone turnover and n = 56 for analyses including sIGF-I and sIGFBP-3.
3 Number for the model used in the analyses (see “Statistical analysis”).
4 Regression coefficients (β₁) and P-values (P), adjusted for age and BMI, are given.
the present positive association between free sIGF-I, total pro-
ancies between the present and previous (26,28) results. Finally,
women) and prepubertal boys could account for the discrep-
bone modeling and remodeling. Therefore, different physiology
values of BMI (kg/m²) and age (y). Regression coefficients (b) and P-value for
the effect of dairy protein are given.

habitual protein intake <0.85 g/kg. Similarly, sOC did not differ
between postmenopausal women consuming diets based on
either high (1.6 g/kg) or low (0.8 g/kg) meat protein intake (26).
Furthermore, in our previous study, high intake of milk, but not
meat, equal in protein content, decreased sOC in healthy,
prepubertal boys (27). In contrast, the observed positive
correlation between meat protein intake and sBAP contradicts
previously published studies. Roughhead et al. (26) did not find
any significant influence of high or low meat protein intake on
sBAP in postmenopausal women over 8 wk. Similarly, sBAP
correlations did not differ between young women consuming
diets either low (0.7 g/kg) or high (2.1 g/kg) in total protein for
4 d (28). In children, serum levels of markers for bone turnover
are ~3 times higher compared with adults (29) and reflect both
bone modeling and remodeling. Therefore, different physiology
of bone turnover between adults (especially postmenopausal
women) and prepubertal boys could account for the discrep-
ancies between the present and previous (26,28) results. Finally,
the present positive association between free sIGF-I, total pro-
tein intake, and milk consumption (P < 0.01), but not meat
protein intake, agrees with our previous observations (12). In a
cross-sectional study of 2.5-y-old children, total protein and
milk intake were positively associated with sIGF-I (P < 0.05),
whereas meat consumption did not show any significant associ-
ations with this marker. Similarly, high intake of milk, but not
meat, equal in protein content, increased sIGF-I in prepubertal
boys after 7 d (13).

This study shed new light on the understanding of the role of
dietary protein on bone turnover. It was reported that in in vitro
models, bone-specific alkaline phosphatase exhibited greater
activity in trabecular bone samples, whereas osteocalcin exhib-
ted greater activity in in cortical bone samples (30,31), indi-
cating that trabecular and cortical bone may be subjected to
distinct regulatory mechanisms. Furthermore, as hypothesized
by Mora et al. (32), sOC and sBAP can be expressed at different
stages of osteoblast development and therefore may be regulated
differently. Correspondingly, in a study by Yilmaz et al. (33),
sOC and sBAP did not show a similar pattern in boys and girls at
different pubertal stages. Based on these findings, the results
from this study indicate that higher intake of dairy protein
compared with meat protein (or higher intake of meat protein
compared with dairy protein) may affect bone composition
differently. However, this hypothesis should be verified in studies
designed to investigate effects of protein quality on trabecular
and cortical bone.

This study has some limitations. First, we did not assess BMD
of the participants; thus, serum markers for bone turnover were
used to evaluate the effect of dietary protein intake on bone
metabolism. In children, markers for bone turnover reflect both
bone modeling and remodeling, providing only a qualitative
measurement of bone formation and resorption. Therefore, they
cannot be directly interpreted in terms of BMD. Although it has
been hypothesized that reduced sOC concentrations in prepu-
bertal Caucasian children and in African American children may
be associated with greater BMD (34), the correlations between
markers for bone turnover and BMD remains unclear. Specific
markers for bone formation and resorption have been both
positively (35), negatively (33,36), and nonsignificantly (37,38)
correlated with BMD in children.

Second, it is difficult to separate the influence of dairy protein
and dairy calcium with respect to their effect on markers for
bone turnover. Dietary calcium intake was not included as the
covariate in the main statistical model (Model 2) due to a high
correlation between dairy protein and calcium intake (r = 0.9;
P < 0.0001). However, in additional analyses, when calcium
intake was included in the model together with dairy and meat
protein, the negative correlation between dairy protein and sOC,
and the positive correlation between meat protein and sBAP,
remained significant (P ≤ 0.04). Similarly, after replacing dairy
protein with calcium intake in Model 2, calcium intake was not
significantly correlated with markers for bone turnover. These
results suggest that the observed effect of dairy protein on sOC
was more likely to be related to protein rather than to calcium.

Another limitation may include the method we used for
estimation of dietary data, because 3-d weighed food record
provide information only about current dietary intake. How-
ever, 3-d food records were reported to yield the strongest
agreement with actual dietary intake compared with 24-h recall
and 5-d food frequency record in children (14).

The main strength of our study is a large number of subjects
who were homogenous in terms of chronological age (8 y old),
sex (boys), pubertal development (tanner stage 1), and race
(Caucasian), factors that all have a large influence on serum

Figure 1 Scatter diagrams of sOC and energy-preadjusted dairy protein (g/d) at
low (<0.4 g/kg) (A) and high (>0.8 g/kg) (B) intake of meat protein, including
partial regression lines of sOC on energy-preadjusted dairy protein at constant
values of BMI (kg/m²) and age (y). Regression coefficients (b) and P-value for
the effect of dairy protein are given.

Figure 2 Scatter diagrams of sOC and energy-preadjusted meat protein at
low (<0.4 g/kg) (A) and high (>0.8 g/kg) (B) intake of dairy protein, including
partial regression lines of sOC on energy-preadjusted meat protein at constant
values of BMI (kg/m²) and age (y). Regression coefficients (b) and P-value for
the effect of meat protein are given.
concentrations of markers for bone turnover. Furthermore, the variation of markers for bone turnover was further minimized by performing analyses in fasting serum samples by the same person and in standardized conditions.

To summarize, we showed that dairy protein was related to sOC, whereas meat protein was related to sBAP and that there was an important interaction between dairy and meat proteins with respect to their effect of sOC. How the observed discrepancy in bone turnover, related to the protein source, reflects bone density remains unclear and requires future studies.

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Literature Cited