Serum concentrations of sex hormone binding globulin are elevated in kwashiorkor and anorexia nervosa but not in marasmus\textsuperscript{1–3}

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ABSTRACT

**Background:** Customary blood protein markers for malnutrition are of limited value in the diagnosis of protein-energy malnutrition or anorexia nervosa in children and in the follow-up to refeeding in such children.

**Objectives:** For these diseases, we compared the diagnostic value of sex hormone binding globulin (SHBG) with that of albumin, transferrin, transthyretin, and retinol binding protein and determined the relations between concentrations of insulin, insulin-like growth factor I, and SHBG.

**Design:** SHBG was assayed in children with protein-energy malnutrition (29 children with kwashiorkor and 28 with marasmus), in 29 anorectic girls (before and after refeeding), and in age- and sex-matched control subjects.

**Results:** Mean (±SE) serum SHBG concentrations were higher in the children with kwashiorkor (0.18 ± 0.07 μmol/L) than in the children with marasmus (0.11 ± 0.05 μmol/L, \( P < 0.0001 \)) or the control subjects (0.11 ± 0.03 μmol/L, \( P < 0.0005 \)). In the children with anorexia nervosa before weight gain, serum SHBG concentrations were significantly higher (0.10 ± 0.04 μmol/L) than in the age-matched control subjects (0.06 ± 0.03 μmol/L, \( P < 0.001 \)) and decreased significantly after 30 d of refeeding (0.04 ± 0.01 μmol/L, \( P < 0.0001 \)). This decrease was negatively correlated with insulin-like growth factor I but not with insulin. Mean serum SHBG concentrations were influenced neither by inflammation, as indicated when C-reactive protein was used as a marker (0.27 ± 0.27, 0.34 ± 0.42, and <0.04 μmol/L in the children with marasmus, kwashiorkor, and anorexia nervosa, respectively), nor by glomerular filtration, as indicated when cystatin-C was used as a marker (68.46 ± 23.08, 66.90 ± 43.08, and 49.23 ± 7.69 μmol/L, respectively).

**Conclusions:** The high SHBG concentration observed in anorexia nervosa and kwashiorkor seems to be of multifactorial origin. For these 2 diseases, SHBG is a reliable marker of nutritional status, is unrelated to either C-reactive protein or cystatin-C, and may be helpful in distinguishing kwashiorkor from marasmus and as a follow-up marker after refeeding. *Am J Clin Nutr* 2002;76:239–44.

**KEY WORDS** Sex hormone binding globulin, anorexia nervosa, kwashiorkor, marasmus, malnutrition, inflammation, renal failure, insulin-like growth factor I, insulin

**INTRODUCTION**

The term *protein-energy malnutrition* (PEM) is widely used to describe a group of diseases (2 extreme forms, kwashiorkor and marasmus, and many mixed forms) that often affect young children in most developing countries. Inflammation and changes in hormonal patterns are usually associated with kwashiorkor and marasmus. For a long time, kwashiorkor and marasmus have been considered to be the clinical translation of either inadequate protein intake or deficient energy intake (1). However, it has been observed that kwashiorkor and marasmus may coexist in the same infantile population having the same diet (2). More recently, kwashiorkor has been considered a metabolic misadaptation of the organism to malnutrition, explaining the development of edema. According to Ingenbleek and Bernstein (3), the dramatically decreased liver synthesis of transthyretin and albumin seen in persons with kwashiorkor is mainly because of the hepatic damage observed in severe malnutrition. The serum concentrations of these 2 proteins are lower in kwashiorkor, which is associated with liver steatosis, than in marasmus, which is usually associated with a limited fatty infiltration of the liver (3). Anorexia nervosa is another kind of energetic privation, which affects 1.3% of adolescent girls in developed countries (4). There are striking clinical and biological resemblances between anorexia nervosa and marasmus, despite the psychological component and absence of inflammatory syndrome in anorexia nervosa. In concrete terms, the slight disturbance in the concentrations of albumin, transferrin, transthyretin, and retinol binding protein in anorectic (5) and

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marasmic (6) patients indicates that these 4 proteins are of little value in the follow-up to therapy for these diseases. A marker adapted for the diagnosis of either anorexia nervosa or marasmus and for the follow-up to treatment must not only be specific, i.e., unaffected by inflammation or renal failure, but also be sensitive (with a short half-life and a dramatic change in blood concentration). The specific binding protein of sex steroid hormones, sex hormone binding globulin (SHBG), seems to be a good candidate. SHBG is a 90-kilodalton homodimeric glycoprotein that is synthesized in the liver (5). Kiddy et al (7) reported that SHBG concentrations were higher in patients who consumed a very-low-energy diet (300 kcal/d) than in those who consumed a higher energy diet. In addition, the biological half-life of SHBG is short enough (4 d) that it can be used as a follow-up marker in refeeding programs (8). However, the mechanisms of SHBG synthesis regulation, especially the putative role of insulin and insulin-like growth factor I (IGF-I), are not well known. The results of previous studies conflict with regard to serum SHBG concentrations in anorexia nervosa: Estour et al (9) and Barbe et al (5) found higher SHBG concentrations in subjects with anorexia nervosa than in healthy subjects, whereas Baranowska and Zgliczynski (10) found lower concentrations. These differences were related to differences in serum testosterone concentrations in the studied groups (9). SHBG has never been studied in kwashiorkor and marasmus.

In the present study, we evaluated SHBG as a diagnostic marker in kwashiorkor and marasmus and as a refeeding follow-up marker in anorexia nervosa. Particular attention was paid to the effect of IGF-I, insulin, inflammation, and renal failure on serum SHBG concentrations in these 3 diseases.

SUBJECTS AND METHODS

Patients

Patients with kwashiorkor or marasmus and the related control group

The first patient group consisted of 29 young children [16 boys and 13 girls with a mean (±SD) age of 20 ± 8 mo] who lived in Togo or Benin, suffered from kwashiorkor, and had a mean weight-for-height z score of −2.61 (kwashiorkor patients, KP group). The second patient group consisted of 28 fellow citizens [15 boys and 13 girls with a mean (±SD) age of 18 ± 7 mo] who suffered from marasmus and had a mean weight-for-height z score of −2.83 (marasmus patients, MP group). The diagnosis of malnutrition type (kwashiorkor or marasmus) was made by using the Wellcome classification (11). Additional criteria for the diagnosis of kwashiorkor were the existence of edema, thin and discolored hair, skin lesions, and low weight-for-height. The control group was composed of 30 healthy young African children admitted to the health center for vaccination [15 boys and 15 girls with a mean (±SD) age of 16 ± 8 mo] (kwashiorkor and marasmus African control subjects, KMAC group). All patients and control subjects were recruited in accordance with the Helsinki Declaration and provided informed consent.

Patients with anorexia nervosa and the related control group

The patient group consisted of 25 girls who suffered from anorexia nervosa (anorexia nervosa patients, ANP group) and had a mean (±SD) age of 15.6 ± 2.3 y and a mean (±SD) body mass index (BMI; in kg/m²) of 14.6 ± 2. The control group consisted of 23 healthy age-matched girls (anorexia nervosa control subjects, ANC group) who had a mean BMI of 22.0 ± 4.3.

Blood sampling

For the subjects in the ANP group, a blood sample was taken on the first day of the study (day 0) and another after 30 d (day 30) of isolation, psychotherapy, and refeeding, whereas for the subjects in the other 4 groups, a single blood sample was taken. The effect of refeeding on SHBG concentrations in kwashiorkor and marasmus could not be studied because no systematic refeeding program was performed in the health center where the patients were recruited. All blood samples were taken in the morning after the subjects had fasted overnight, and the samples were centrifuged at 1500 × g for 15 min at 4°C. The sera were frozen at −20°C until analyzed.

Assay of serum proteins and hormones

Serum SHBG concentrations were assayed with the use of electroimmunodiffusion (Hydragel-SBP; SÉBIA, Issy-les-Moulineaux, France). The intraassay and interassay CVs were 5% and 9%, respectively. Serum albumin, transferrin, and cystatin-C concentrations were assayed with the use of immunonephelometry (Array; Beckman Instruments, Galway, Ireland). Both the intra-assay and inter-assay CVs were ≤5%. Serum retinol binding protein (RBP) and cystatin-C concentrations were assayed with the use of immunonephelometry (BN II nephelometer; Dade Behring, París la Défense, France). Both the intra-assay and inter-assay CVs were ≤5% (12). Serum C-reactive protein (CRP) concentrations were measured with the use of immunoturbidimetry (Hitachi 911; Roche Diagnostics, Meylan, France). The intra-assay and inter-assay CVs were ≤1.5% and 6%, respectively (13).

Serum insulin and IGF-I concentrations were determined with the use of an immunoradiometric assay (IMUNOTECH, Marseille, France). Before the IGF-I assay, IGF-I binding proteins were extracted with the use of HC1:ethanol (15:85, by vol) according to the manufacturer’s instructions.

Determination of z scores

We used the EPINUT computer program (version 4; Société Micro6, Nancy, France), which calculates z scores from weight, height, sex, and age. In developing countries, children who are below the customary weight-for-height z score cutoff of −2 may have a higher risk of death (14).

Statistical analysis

STATVIEW software (version 4.02; Abacus Concepts, Inc, Berkeley, CA) was used for statistical analyses. Data are expressed as means ± SEs and as ranges. In the case of skewed data distributions, logarithmic transformations were carried out to normalize the distributions before the statistical analyses (Student’s t test for unpaired data and Bonferroni adjustment). Differences were considered significant at P < 0.05. Linear regression correlation coefficients were used to assess the relations between nutritional proteins.

RESULTS

Kwashiorkor and marasmus

The SHBG concentration in the KP group was higher than that in the MP and KMAC groups (Table 1). Albumin and transferrin
concentrations in the KP group were significantly lower than those in the MP and KMAC groups. The z scores of the 2 patient groups were not significantly different, and no correlation was found between the z score and SHBG concentration. The mean insulin concentrations in the KP and MP groups were not significantly different (Table 2). The mean IGF-I concentration was significantly lower in the KP group than in the MP group. No correlation was found between IGF-I and SHBG in either the KP or the MP groups. Serum cystatin-C concentrations in the KP, MP, and KMAC groups were not significantly different. Mean serum CRP concentrations in the KP and MP groups were not significantly different. No correlation was found between SHBG and either cystatin-C or CRP. In addition, there was no significant influence of either age or sex on the blood concentrations of these protein markers.

Anorexia nervosa before (day 0) and after weight gain (day 30)

The mean SHBG concentration in the ANP group before weight gain was significantly higher than that in the ANC group (Table 3). The mean serum albumin concentration in the ANP group before weight gain was also significantly higher than that in the ANC group. We found lower serum transferrin and higher serum transthyretin concentrations in the ANP group than in the ANC group. The concentration of these protein markers was not influenced by age or sex.

The mean IGF-I concentration in the ANP group at day 0 was significantly lower than that in the ANC group, whereas the mean insulin concentrations were not significantly different (Table 2). We found a positive correlation between insulin and IGF-I ($r = 0.84, P < 0.001$) in the ANP group at day 0. However, no correlation was found between either insulin or IGF-I and SHBG. SHBG was negatively correlated with BMI ($r = -0.48, P = 0.045$), RBP ($r = -0.51, P = 0.009$; Figure 1), and transthyretin ($r = -0.50, P = 0.01$; Figure 1) in the ANP group at day 0. Finally, transthyretin and RBP were positively correlated ($r = 0.64, P < 0.001$) in the ANP group at day 0. None of the anorectic patients had a serum CRP concentration >5 mg/L. There was no marked alteration of glomerular filtration in the ANP group because the mean cystatin-C concentration was close to that of the ANC group (Table 2).

After the refeeding program (at day 30), the mean BMI (17.2 ± 1.4) in the ANP group had significantly increased ($P < 0.0001$) but was still lower than that in the control group. At day 30, the serum SHBG concentration in the ANP group had decreased significantly and was even lower than that in the control group (Table 3). In contrast, the mean IGF-I concentration increased 2.7-fold. The mean insulin concentration also tended to increase after weight gain (Table 2). The decrease in SHBG concentration was correlated with a significant increase in IGF-I concentration ($r = -0.68, P < 0.0001$; Figure 1) and also with a significant increase in BMI ($r = -0.52, P = 0.02$). No correlation was found between insulin and SHBG either at day 0 or at day 30. Between days 0 and 30, serum albumin concentrations decreased significantly, whereas serum transferrin, transthyretin, and RBP concentrations increased significantly. Because SHBG and RBP underwent an inverse variation after 1 mo of refeeding, we evaluated the SHBG-RBP ratio as a follow-up index for refeeding. In fact, this index seemed to be more sensitive than each marker considered separately. The ratio was estimated at 0.31 ± 0.19 and 0.08 ± 0.04 before and after refeeding, respectively, i.e., a 3.9-fold decrease ($P < 0.001$). This ratio was also lower in the ANP group at day 30 than in the control group (0.20 ± 0.12; $P = 0.0002$).
range after refeeding. Low transferrin concentrations are associated with a poor prognosis in children with kwashiorkor (16) and marasmus (17). However, the frequency of iron-deficiency anemia in malnourished children may limit the clinical value of this marker because a decrease in the transferrin concentration caused by malnutrition can be masked by an increase because of a lack of iron (18). Compared with their respective control groups, both the KP group and the ANP group at day 0 had significantly lower serum transferrin concentrations. We found that transthyretin concentrations were equally low in both kwashiorkor and marasmus, in agreement with the findings of Smith et al (19). Similar to the results of Golden (20), blood RBP concentrations in children with kwashiorkor and marasmus were not significantly different in the present study. Transferrin, transthyretin, and RBP concentrations in anorectic patients before weight gain were found to be close to those of the control range (5). We observed a higher transthyretin concentration in the patients with anorexia nervosa before refeeding than in the control subjects. After refeeding, the transthyretin concentration was even higher than before weight gain. This finding was also reported previously by Barbe et al (5). In addition, the RBP concentration also increased after refeeding, and both transthyretin and RBP were always positively correlated (r = 0.64, P < 0.001), suggesting that they remained complexed in a 1:1 molar ratio. These 2 variables are not specific to PEM because their concentration can be lowered by inflammation (3). In addition, hyperthyroidism is associated with a low transthyretin concentration (21), and vitamin A deficiency inhibits the release of RBP from the liver (1).

In the present study, we evaluated the clinical value of SHBG in PEM and anorexia nervosa because of the lack of specificity and sensitivity of the 4 other protein markers. SHBG is a potentially reliable candidate because its concentration depends, via the activities of insulin or IGF-I, on food intake. In the anorectic patients, we found high SHBG concentrations before refeeding and a considerable decrease in SHBG concentrations after weight gain, although the patients remained amenorrheic and still had a low BMI. Contrary to the results of Estour et al (9) but in agreement with those of Tomova et al (22), an inverse correlation between SHBG and BMI in anorexia nervosa was found in the present study. Unlike SHBG concentrations, albumin concentrations were altered in both kwashiorkor and marasmus, with the lowest values being observed in kwashiorkor. This variable was therefore valuable in distinguishing patients with kwashiorkor or marasmus from control subjects. In the present study, SHBG was the only marker whose concentration was abnormally high with malnutrition (anorexia nervosa and kwashiorkor) and decreased with refeeding (anorexia nervosa). Because of the short biological half-life of SHBG, this decrease was already significant after 30 d of therapy. Whereas high SHBG concentrations were reported in both anorexia nervosa and kwashiorkor, we observed no difference in SHBG concentrations between the marasmic patients and the control subjects. SHBG could therefore be useful in determining the major component in mixed forms of kwashiorkor and marasmus whose clinical diagnosis is often difficult.

SHBG is a glycoprotein of liver origin whose secretion is stimulated by thyroid hormones (23) and estradiol (24) and inhibited by testosterone (25). The elevated SHBG concentrations observed in anorexia nervosa cannot be explained by changes in sex steroid or thyroid hormones. Indeed, anorexia nervosa is characterized by a low triiodothyronine syndrome, which is reversed by weight gain (5). Estradiol concentrations are very low before weight gain and do not increase during hospitalization, explaining why menses does not return (5). Testosterone concentrations in anorexia

### TABLE 3

<table>
<thead>
<tr>
<th>Protein Marker</th>
<th>ANP group at day 0</th>
<th>ANP group at day 30</th>
<th>ANC group</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG (μmol/L)</td>
<td>0.10 ± 0.04±1,2</td>
<td>0.04 ± 0.012</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Albumin (nmol/L)</td>
<td>0.67 ± 0.06±1,4</td>
<td>0.63 ± 0.06</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>Transferrin (μmol/L)</td>
<td>28.50 ± 6.37±5</td>
<td>33.75 ± 7.37</td>
<td>34.50 ± 6.00</td>
</tr>
<tr>
<td>Transthyretin (μmol/L)</td>
<td>5.45 ± 1.09±5,6</td>
<td>6.36 ± 1.45±7</td>
<td>4.18 ± 0.73</td>
</tr>
<tr>
<td>RBP (μmol/L)</td>
<td>1.68 ± 0.48±1</td>
<td>2.23 ± 0.46±7</td>
<td>1.40 ± 0.38</td>
</tr>
</tbody>
</table>

\*Significantly different from the ANC group (Bonferroni adjustment): 1.5,7 P < 0.001, 2 P < 0.01, 3 P < 0.0001.

\*Significantly different from the ANP group at day 30 (Bonferroni adjustment): 4 P < 0.0001, 5 P < 0.05, 6 P < 0.001.
nervosa vary from study to study. Baranowska and Zgliczynski (10) found high testosterone concentrations, which may explain the lower SHBG concentrations observed in their anorexia nervosa group, whereas normal or lower testosterone concentrations with high SHBG concentrations were reported by Estour et al (9) and Tomova et al (22), respectively.

The respective roles of insulin and IGF-I in the regulation of SHBG concentration are also the subject of controversy. In vitro, insulin inhibits SHBG production by the human hepatocarcinoma cells Hep G2 (26). Kiddy et al (7) showed a negative correlation between SHBG and insulin concentrations in vivo in obese women with polycystic ovary syndrome. Such a correlation has never been found in anorexia nervosa, even when the fasting insulin concentration is low. IGF-I is another factor related to nutritional status that may also regulate SHBG concentrations (27, 28). Indeed, IGF-I inhibits SHBG production by Hep G2 cells and is negatively correlated with this binding protein during puberty in boys but not in healthy girls (29). The decrease in SHBG concentration during refeeding in anorexia nervosa is clearly related in part to the increase in IGF-I concentration because we found a significant correlation between the decrease in SHBG concentration and the increase in IGF-I concentration in treated anorectic patients. The IGF-I concentration in the patients with anorexia nervosa before refeeding was significantly lower than that in the control subjects, but the concentration increased to that of the control subjects after refeeding (Table 2). The increase in IGF-I concentration after refeeding was previously observed in children with protein-energy malnutrition (30).

In addition, we found a lower mean IGF-I concentration in the patients with kwashiorkor than in those with marasmus, and this finding was not related to a difference between sexes. Therefore, on the basis of the present study, IGF-I would seem to be a marker of interest for evaluating the nutritional status of patients with kwashiorkor and anorexia nervosa. In contrast, an earlier study found no significant difference in IGF-I concentrations between children with kwashiorkor and those with marasmus (30); however, another study found a higher IGF-I concentration in 9 subjects with kwashiorkor than in 13 subjects with marasmus (31). The number of patients with PEM recruited in our study was greater than that in the previous studies.

In conclusion, SHBG is a promising marker of nutritional status in anorexia nervosa and kwashiorkor. SHBG may be helpful in distinguishing kwashiorkor from marasmus and as a follow-up marker in refeeding programs.

REFERENCES