The Relationship Between Anthropometry and Serum Concentrations of Alkaline Phosphatase Isoenzymes, Liver Enzymes, Albumin, and Bilirubin

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Abstract

Alkaline phosphatase (ALP) is present in human preadipocytes. The aim of this study was to determine the relationship between anthropometry and serum levels of ALP isoenzymes, liver enzymes, albumin, and bilirubin. Anthropometric variables; serum total, bone, liver, and intestinal ALP levels; and alanine aminotransferase (ALT), albumin, total protein, total bilirubin, and γ-glutamyltransferase serum levels were measured in 100 volunteers.

The levels (given as median [interquartile range]) for total (74.0 U/L [30.0 U/L] vs 62.0 U/L [22.0 U/L]; P < .05) and liver ALP (37.3 U/L [14.6 U/L] vs 26.1 U/L [12.0 U/L]; P < .05) were higher in obese than in lean subjects. The levels of the other ALP isoenzymes and blood analytes were not significantly different between these groups. Albumin and ALT were the only blood proteins studied with serum levels that correlated significantly with waist circumference.

This present study demonstrates a relationship between abdominal obesity and serum ALT levels and between body mass index and ALP levels. These findings suggest that serum ALP, particularly liver ALP, is derived from adipose and hepatic tissue.

Besides its well-known association with chronic diseases of lifestyle, obesity has been identified as an important contributing factor to raised serum levels of hepatic enzymes. It is thought that this relationship is due to the high release of free fatty acids from the visceral fat depot into the portal circulation, leading to nonalcoholic steatohepatitis (NASH). NASH is considered a characteristic of fat distribution that is associated strongly with the metabolic syndrome in obese and nonobese subjects.

Alkaline phosphatase (ALP) is a membrane-bound enzyme found in a wide variety of tissues, including liver. There are 4 ALP isoenzymes in humans, each coded by a separate gene: tissue nonspecific (TNALP; also known as liver-bone-kidney ALP), intestinal, placental, and germ cell. As its name implies, ALP works in an alkaline environment, suggesting that the enzyme, although present in blood, is inactive in this environment. The enzyme is known to have phosphoprotein phosphatase and transphosphorylation activity and might have an important role in bone mineralization. However, its function in other tissues is not known. The serum levels of liver and bone ALP are used widely in the diagnosis of hepatobiliary disease and various bone disorders, respectively. It recently was reported that the TNALP isoenzyme is present in human and murine preadipocytes and might have a role in the intracellular accumulation of triglycerides that characterizes the process of adipogenesis.

The existence of ALP in human preadipocytes is of interest because it is conceivable that adipose tissue might be a source of serum ALP. Furthermore, the positive relationship between measures of abdominal obesity and serum liver enzyme levels demonstrates that adipose tissue mass also can influence the release of liver products into the circulation.
the level of TNALP in serum might be influenced by total and abdominal adipose tissue mass. The purpose of the present study was to analyze the relationships between ALP isoenzyme, liver enzyme, albumin, and bilirubin serum concentrations and measures of total and abdominal adipose tissue mass.

Materials and Methods

Food Intake and Total Serum ALP Concentration

The effect of food intake on serum total ALP levels was studied to determine whether nonfasting blood samples could be used for the measurement of ALP. A total of 12 volunteers (10 women) were used, and ALP levels were measured in venous serum samples after an overnight fast and 60 minutes after breakfast.

The Use of Levamisole in Estimating Serum Intestinal ALP Concentration

Levamisole is a specific inhibitor of the TNALP isoenzyme. Therefore, this inhibitor was used to block activity of this enzyme in serum samples, the remaining activity being attributable to intestinal ALP. The level of levamisole required for maximal inhibition of TNALP activity was assessed by adding levamisole to serum samples obtained from 4 healthy male volunteers to give final concentrations of 0, 8, 16, 32, and 64 mmol/L.

Study Subjects, Anthropometric Measurements, and Blood Sampling

The study subjects comprised 100 black Africans (26 men) of varying body mass index (BMI) values. Ethical approval for the collection of serum samples was obtained from the University of Witwatersrand (Johannesburg, South Africa), Faculty of Health Sciences Human Ethics Committee. Informed consent was obtained from each volunteer. Only subjects who had no known liver pathology or bone fractures within the last 2 years were included in the study.

Weight and height were measured by using a stadiometer (Modern Scale, Johannesburg). The waist/hip ratio (WHR) was measured by taking waist circumference as the midpoint between the lower rib margin and the iliac crest and hip circumference as the widest circumference of the buttock. The subjects were split into 3 groups according to BMI, ie, lean (BMI, <25), overweight (BMI, ≥24.9 and <30), and obese (BMI, ≥29.9). Women also were divided into 2 groups based on age, using a cutoff age of 50 years to denote premenopausal and postmenopausal status.

A 5-mL, nonfasting blood sample was obtained from the antecubital vein of the forearm from each subject. The sample was centrifuged immediately and the serum removed and stored at −20°C until assayed.

Estimation of Serum ALP Isoenzyme Concentrations

Bone ALP was measured by using a bone-specific ALP enzyme immunoassay (Quidel, San Diego, CA). Serum levels of intestinal ALP were estimated by measuring ALP activity in serum treated with 32 mmol/L of levamisole. Liver ALP levels were calculated by subtracting the sum of the bone and intestinal ALP concentrations from the total serum ALP activity measured using a colorimetric assay (ALP IFCC liquid assay, Roche Diagnostics, Mannheim, Germany) performed on a Modular autoanalyzer (Roche Diagnostics).

Measurement of Serum Concentrations of Liver-Derived Proteins

Serum levels of albumin, total bilirubin, alanine aminotransferase (ALT), total protein, and γ-glutamyltransferase were measured using commercial kits (Roche Diagnostics) performed on the Modular autoanalyzer using standard procedures. The reference ranges for these analytes were obtained from the kit manufacturers.

Statistical Analyses

All study variables displayed skewed distributions and, therefore, were transformed to normality by taking log, reciprocal, or squared values. Data in tables and the text are expressed as median (interquartile range) unless otherwise stated. Comparisons between sexes were performed by analysis of covariance (ANCOVA) with adjustment for age, BMI, and WHR or waist circumference. Subjects were split into 3 groups according to BMI and data compared across groups using ANCOVA adjusted for age, sex, and WHR or waist circumference. The analysis of specific differences between 2 BMI groups was performed by using the Tukey post hoc test for unequal numbers. The relationship between age and serum ALP isoenzyme activity was analyzed separately in men and women by using Pearson regression. Differences in means between premenopausal and postmenopausal women were analyzed by using analysis of variance. Multiple regression analysis was used to determine the relationship between serum peptide levels and measures of abdominal obesity, ie, WHR and waist circumference.

Results

The Effect of Food Intake on Serum Total ALP Concentration

The serum total ALP level (mean ± SD) was 61.4 ± 17.6 U/L before breakfast and 60.8 ± 17.1 U/L after breakfast.
(P = .43). Thus, food intake had no effect on serum ALP levels, and, therefore, in the larger study, nonfasting blood samples were used to determine serum levels of ALP.

The Use of Levamisole for Estimating Serum Intestinal ALP Concentrations

Maximal inhibition of ALP activity occurred at 32 mmol/L of levamisole, producing an inhibition of 85%. Therefore, the estimation of intestinal ALP activity was performed using serum samples treated with 32 mmol/L of levamisole.

Comparison of Male and Female Study Subjects

The study comprised 26 male and 74 female black African volunteers. The women were older (39 [15] vs 32.5 [6] years; P = .002) and had a higher BMI (30.6 [11.1] vs 21.9 [4.4]; P < .0001, corrected for age) and a lower WHR (0.81 [0.13] vs 0.88 [0.05]; P = .002, corrected for age and BMI) than the men. The men had higher serum albumin levels (46 g/L [3 g/L] vs 42 g/L [3 g/L]; P < .0001, corrected for age, BMI, and WHR) and lower total protein levels (76 g/L [4 g/L] vs 79 g/L [10 g/L]; P = .004 corrected for age, BMI, and WHR) than the women. (Albumin and protein values are given in Système International units; to convert to conventional units [g/dL], divide by 10.0.) Similar trends were observed when ANCOVAs were performed with waist circumference replacing WHR as an independent variable. The ALT levels were significantly higher in men (15.5 U/L [5 U/L]) than in women (12.5 U/L [6 U/L]; P = .04, corrected for age and BMI), but this difference became nonsignificant (P = .35) when WHR or waist circumference was included as an independent variable in the ANCOVA.

The Effect of BMI on Serum ALP Activity and Liver Enzyme, Albumin, and Bilirubin Concentrations

The data in Table I demonstrate that obese subjects were significantly older than lean subjects and had a higher waist circumference than all the other subject groups. Furthermore, the obese group had significantly higher total and liver ALP levels than the lean group after adjustments for age, sex, and WHR or waist circumference.

Multivariate regression analysis using sex, age, and WHR as independent variables Table II demonstrated that ALP levels were determined predominantly by WHR. The main determinant of albumin levels was found to be sex, with a very weak input from WHR. Total and liver ALP concentrations correlated positively with age but not with WHR. This also was the case for bone (β coefficient = .34; P = .001 with age) and intestinal (β coefficient = .26; P = .015 with age) ALP. None of the other ALP isoenzyme or liver enzyme, albumin, or bilirubin serum levels showed significant correlations with WHR. When waist circumference was used as an independent variable in place of WHR, relationships similar to those shown in Table 2 were observed.

Effect of Age on Serum ALP Isoenzyme Activity

Serum levels of total (r = 0.44; P < .0001), bone (r = 0.42; P < .0001), liver (r = 0.31; P = .008), and intestinal (r = 0.28; P = .014) ALP correlated positively with age in women, whereas only liver ALP (r = 0.48; P = .013) correlated with age in men.

Table I

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean (n = 37)</th>
<th>Overweight (n = 22)</th>
<th>Obese (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33.0 (10.0)</td>
<td>37.5 (14.0)</td>
<td>42.0 (16.0)†</td>
</tr>
<tr>
<td>BMI</td>
<td>21.9 (3.6)</td>
<td>278 (10.6)‡</td>
<td>35.5 (8.6)†‡</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>74.0 (8.0)</td>
<td>86.7 (10.5)‡</td>
<td>98.5 (14.5)†‡</td>
</tr>
<tr>
<td>WHR</td>
<td>0.82 (0.10)</td>
<td>0.85 (0.08)</td>
<td>0.82 (0.16)</td>
</tr>
<tr>
<td>Total ALP (U/L)</td>
<td>62.0 (22.0)</td>
<td>66.5 (21.0)</td>
<td>74.0 (30.0)</td>
</tr>
<tr>
<td>Liver ALP (U/L)</td>
<td>26.1 (12.0)</td>
<td>29.3 (15.2)</td>
<td>373 (14.6)†</td>
</tr>
<tr>
<td>Bone ALP (U/L)</td>
<td>28.2 (12.0)</td>
<td>30.9 (11.1)</td>
<td>32.6 (21.9)</td>
</tr>
<tr>
<td>Intestinal ALP (U/L)</td>
<td>4.0 (3.0)</td>
<td>4.0 (5.0)</td>
<td>5.0 (4.0)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>14.0 (6.0)</td>
<td>14.5 (6.0)</td>
<td>12.0 (6.0)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>22.0 (17.0)</td>
<td>24.5 (48.0)</td>
<td>25.0 (34.0)</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>76.0 (9.0)</td>
<td>79.0 (10.0)</td>
<td>76.0 (6.0)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43.0 (5.0)</td>
<td>43.0 (4.0)</td>
<td>42.0 (3.0)</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>7.0 (4.0)</td>
<td>6.5 (4.0)</td>
<td>6.0 (4.0)</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; WHR, waist/hip ratio.

* Values are expressed as median (interquartile range) in Système International units. Conversions to conventional units are as follows: total ALP, ALT, and GGT (U/L), divide by 1.0; total protein and albumin (g/dL), divide by 10.0; total bilirubin (mg/dL), divide by 17.1.

† P < .005 vs lean group.
‡ P < .0005 vs lean group.
§ P < .0005 vs overweight group.
∥ P < .05 vs lean group.
Women 50 years or older had significantly higher total (89 U/L [33 U/L] vs 66 U/L [25 U/L]; \( P = .0005 \)) and bone (43.1 U/L [15.5 U/L] vs 27.1 U/L [15.8 U/L]; \( P = .0002 \)) ALP activity than women younger than 50 years. Intestinal ALP activity was not different between these groups (4.0 U/L [4.0 U/L] vs 4.0 U/L [3.0 U/L]; \( P > .05 \)), whereas liver ALP activity was higher in the older group (39.5 U/L [15.8 U/L] vs 32.8 U/L [19.0 U/L]; \( P = .067 \)) but just failed to reach statistical significance.

Serum Levels of ALP Isoenzymes

The serum ranges for the different ALP isoenzymes based on the mean value ± 2 SD were as follows (units are U/L): total, 29 to 114; liver, 4 to 64; bone, 9 to 57; and intestinal, 0 to 11. The minimum and maximum values for each isoenzyme were as follows: total ALP, 33 and 142; liver ALP, 11 and 117; bone ALP, 15 and 66; and intestinal ALP, 1 and 16. When expressed as a percentage of the total ALP activity (mean ± SD), bone (47% ± 12%) and liver (46.5% ± 11%) contributed equally, whereas intestinal ALP contributed only 7.2% ± 3.4% of the total activity.

Discussion

The present study demonstrates that total and liver but not bone and intestinal ALP serum levels are higher in obese than in lean subjects. These data are supported by results from a study in which serum ALP levels were measured in 32,329 subjects,\(^{19}\) and it was observed that females who were more than 15% overweight had a 20% higher ALP activity than those who were not overweight. However, it also must be noted that another study detected no relationship between body weight and total serum ALP concentrations.\(^{20}\) It is possible that the higher level of liver ALP in obese than in lean subjects is a result of ALP release from adipose tissue. Liver ALP is a TNALP isoenzyme (as are bone and kidney ALP), and it is known that TNALP is present in human preadipocytes\(^{16}\) and in the murine preadipocyte cell, 3T3-L1.\(^{15}\) Therefore, the association of liver but not bone serum ALP levels with obesity suggests that the TNALP isoform in adipose tissue may be the liver form.

The function of ALP in adipose tissue has been studied. Thus, it has been demonstrated in human and 3T3-L1 preadipocytes that inhibition of ALP activity blocks intracellular lipid accumulation and that ALP is localized to the lipid-containing droplets of preadipocytes.\(^{15,16}\) It, therefore, has been hypothesized that ALP may be involved in the control of lipid accumulation during the maturation of preadipocytes into adipocytes.

Serum ALT but not ALP activity was associated with measures of abdominal obesity. Abdominal obesity is known to be associated with an increased risk of NASH,\(^{5,21}\) leading to elevated serum levels of liver enzymes, particularly ALT. Previous studies also have demonstrated that simple measures of abdominal obesity correlate positively with serum ALT levels.\(^{22,23}\) These data suggest that elevated ALT levels are due largely to the effect of abdominal obesity on liver function, whereas elevated ALP activity is not related to depot-specific adipose tissue mass but rather to whole body fat mass, which may be a source of serum ALP. This theory is supported further by ANCOVA, which demonstrated that serum liver and total ALP activities were significantly higher in obese than in lean subjects, even after correcting for waist circumference or WHR.

The present study confirms data from previous studies demonstrating that serum ALT concentrations increase with rising waist circumference in populations of apparently healthy subjects.\(^{22,23}\) This suggests that NASH is the endpoint of a process that begins with a nonpathologic level of hepatic steatosis. The majority of subjects in the present study had

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Multiple Regression Analyses*</th>
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<tbody>
<tr>
<td>Model No.</td>
<td>Dependent Variable</td>
</tr>
<tr>
<td>1</td>
<td>ALT (log)</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Albumin (square root)</td>
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<td></td>
<td></td>
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<tr>
<td>3</td>
<td>Total ALP (log)</td>
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<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Liver ALP (log)</td>
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<td></td>
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</tbody>
</table>

ALP, alkaline phosphatase; ALT, alanine aminotransferase; WHR, waist/hip ratio.

* ALT, total ALP, liver ALP, and age values were log transformed to normality, while square root and reciprocal values were used to transform albumin and WHR values, respectively, to normality.
serum ALT levels within the normal range and had no abnormal liver function test results. This demonstrates that if ALT is an accurate measure of steatohepatitis, liver function within this population has not been affected adversely by the current level of hepatic steatosis.

The activity of all the ALP isoenzymes measured in serum correlated positively with age in the women, whereas in men, only the liver ALP level increased with age. The lack of correlation in men may be related to the smaller number or to the effect of menopausal status on ALP levels. This is emphasized by the higher total, bone, and liver ALP levels in women 50 years or older compared with those younger than 50 years. Previous studies also have shown that postmenopausal women have higher total ALP levels than do premenopausal subjects and that this may be due to the increased bone loss observed after menopause. Thus, in the present study, bone ALP activity was 59% higher in women older than 50 years compared with an increase of only 20% for liver ALP.

Intestinal ALP was measured in the present study by inhibiting the TNALP isoenzymes with levamisole. Levamisole is known to specifically inhibit the TNALP isoenzymes. The drawback of using this method is that levamisole inhibited TNALP activity by 85%, and this may lead to a slight overestimation of intestinal ALP activity. Furthermore, levamisole does not inhibit placental ALP activity, and if the placental enzyme is present in the circulation, this will lead to an overestimate of intestinal ALP activity. However, placental ALP is present in blood only during pregnancy and close to term it constitutes up to 80% of the total serum ALP activity. The female volunteers for this study were not screened for pregnancy, and, therefore, it is possible that the serum intestinal ALP activity in some women might have been affected by pregnancy. It also is known that intestinal ALP blood levels increase after food intake, particularly after a high-fat-containing meal and in the present study, non-fasting blood samples were obtained. However, it was shown that food intake did not affect total serum ALP activity. Despite these drawbacks, the levels of intestinal ALP measured were similar to those observed in other studies, suggesting that the method used in the present study for the estimation of serum intestinal ALP activity is accurate and reliable. Furthermore, this method is easy to perform and has a short turnaround-time, features that are essential for a diagnostic assay.

The level of the liver ALP isoenzyme was estimated by subtracting the sum of bone and intestinal ALP from total serum ALP activity. Therefore, this method is reliant on the accuracy of 3 assay measurements and is susceptible to all of the interfering factors discussed for the intestinal ALP assay. Furthermore, the presence of kidney ALP might lead to an overestimation of liver ALP levels; however, this ALP isoform is found rarely in the circulation. Despite these limitations, reference ranges for the serum activity of liver ALP published from a number of studies show close agreement with the ranges observed in the present study.

Liver function is known to be affected by the hepatitis viral family. Subjects were not screened for the presence of the hepatitis A, B, or C virus, and, therefore, it cannot be ruled out that viral infection influenced the liver function test results in some subjects. However, for the key liver enzymes, ALT and total ALP, only 1 and 2 subjects, respectively, had serum values that were outside the normal range.

Total and liver serum ALP activities are higher in obese than in lean subjects and do not correlate with measures of abdominal fat. However, serum ALT activity rises with increasing abdominal fat mass but is not associated with BMI. The TNALP isoenzyme is present in human preadipocytes, and, therefore, these data suggest that overall but not abdominal fat mass influences serum ALP concentrations and might result from the release of ALP from adipose tissue.

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References


