Lack of Sex Differences in High Density Lipoproteins in Pima Indians
Studies of Obesity, Lipase Activities, and Steroid Hormones
Barbara V. Howard, Pan Xiaoren, Ingeborg Harper, Timo Kuusi, and Marja Riitta Taskinen

To investigate the reasons for the lack of sex differences in high density lipoproteins (HDL) observed in population studies of the Pima Indians, we selected 18 lean (9 men, 9 women, body mass index (BMI) < 27) and 22 obese (12 men, 10 women, BMI > 27) Pima Indians for an inpatient study of HDL composition. We measured lipase activities and steroid hormone concentrations, both of which have previously been implicated in the control of HDL. The lean women had higher concentrations of HDL and HDL₂ than did either the obese women or the lean or obese men. Lean women had significantly lower hepatic lipase activities and significantly higher concentrations of estradiol compared to obese women. Lean women also had different HDL₂ composition, as indicated by the molar ratio of HDL₂ cholesterol/A-I. Significant negative correlations between HDL and obesity measured by either BMI or percent body fat were observed in both sexes, but the slope of the relationship was steeper in women. Significant negative associations were observed between HDL or HDL₂ concentrations and hepatic lipase in both sexes, and there were significant positive associations between HDL₂ and plasma estradiol in women. The data suggest that obesity in this population has a stronger negative influence on HDL concentrations in women, possibly through changes in estradiol and hepatic lipase activities. Since there are so few lean women in the Pima population, the net result is that HDL levels in women in the population as a whole do not differ from those of men.

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Population studies have shown that women usually have a lower prevalence of coronary heart disease than do men.¹,² The difference has been attributed in part to their high density lipoprotein (HDL) concentrations, since in most populations HDL is higher in women than in men³-⁴ and since HDL concentrations have been shown to be inversely associated with the prevalence and incidence of coronary heart disease.⁵-⁷ Because of these observations, it is of interest to understand the mechanisms that control HDL and why HDL concentrations are influenced by sex.

There are some populations in which HDL appear to be similar in men and women.⁸-¹² One of these, the Pima Indian population, is especially interesting. Although there is a low prevalence of coronary heart disease (CHD) in the nondiabetics,¹³ the men appear to have a higher prevalence of CHD than do the women. The HDL concentrations in this population are low in both sexes, and no differences in HDL or HDL subfractions were observed between the men and the women when 1391 individuals were sampled in a population survey.¹⁰ The previous data on lipoproteins in this population were obtained on samples from outpatients. We, therefore, decided to examine more thoroughly the HDL composition in Pima subjects stabilized on the metabolic ward. We recruited groups of lean and obese men and women in order to examine the relationships between HDL and factors such as lipase activities and sex hormones, which have been found to be related to HDL concentrations in other populations.

Methods

Study Subjects

A group of 40 Pima Indians, 20 males and 20 females, were recruited from the Gila River Indian Community for this study (Table 1). Nine lean men and nine lean women (body mass index (BMI) of less than 27), 12 obese men and 10 obese women (BMI above 27) were selected for the study. Demographic data on virtually all adults in this community were available to identify subjects by BMI. Subjects who had BMIs considerably below or above 27 were especially sought, and no attempt was made to randomly select individuals. Subjects were nondiabetic according to the National Diabetes Data Group criteria.¹⁴ The subjects were admitted to the metabolic ward and written informed consent was obtained. The protocol was approved by the

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Subject was taking any medication at the time of the study. The samples were obtained for analyses of glucose and insulin at -15, 30, 60, 120, and 180 minutes after ingestion of glucose. Approximately 30% of the subjects smoked, but all smokers smoked less than 1 pack/day. All women were under 40 years old and, therefore, premenopausal. Many subjects were not able to give an accurate history of menarche. Concomitant measurements of plasma progesterone concentrations greater than 20 ng/ml indicated that three of the lean women and one obese woman were in the luteal phase of the menstrual cycle as measured by progesterone concentrations greater than 25 nmol/l, and one lean woman had a luteal peak concentration of lutelizinone hormone of 67 mIU/ml. No subject was taking any medication at the time of the study. The body composition was determined by underwater weighing. The residual lung volume was measured simultaneously by a helium dilution technique, and the fat-free mass was calculated according to the method of Siri.15

After at least 4 days on the diet, the subjects underwent an exercise of lipoprotein lipase activity. Another portion of fat was placed in saline for osmium fixation and measurement of adipose cell number.16

On the day after the biopsies, heparin (Riker Labs, Northridge, California; 60 IU/kg body weight) was injected intravenously as a bolus and blood was withdrawn 15 minutes later for measurements of lipoprotein lipase and hepatic lipase activities. For these assays, blood was obtained in heparinized tubes and plasma was immediately separated and stored at -70°C.

Table 1. Study Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean</th>
<th>Obese</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
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<tr>
<td>Number</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>22 ± 1</td>
<td>30 ± 3</td>
<td>22 ± 2</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 ± 4</td>
<td>96 ± 5</td>
<td>59 ± 3</td>
<td>97 ± 8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 1</td>
<td>33 ± 2</td>
<td>23 ± 1</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Percent fat</td>
<td>18 ± 2</td>
<td>29 ± 2</td>
<td>27 ± 2</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>93 ± 3</td>
<td>93 ± 2</td>
<td>95 ± 2</td>
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</tr>
<tr>
<td>2-hour</td>
<td>126 ± 7</td>
<td>117 ± 5</td>
<td>110 ± 7</td>
<td>131 ± 10</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>16 ± 1</td>
<td>38 ± 7</td>
<td>27 ± 4</td>
<td>43 ± 7</td>
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The values are the means ± SEM with the range in parentheses. The p values for obese vs lean: *p<0.05; **p<0.01; ***p<0.001. The p values for men vs women: $p<0.05; §p<0.001. The characteristics of the study groups are presented using Student’s t test. For all subsequent group comparisons, Duncan’s multiple range test is also presented.

BMI = body mass index.

HDL in Pima Indians

Lipid and Lipoprotein Measurements

Plasma was separated from ethylene diamine tetraacetic acid (EDTA) containing venous blood for measurement of total cholesterol, triglyceride, and apolipoprotein A-I concentrations. Thereafter, lipoprotein fractions were isolated by sequential ultracentrifugation as described previously.19 Briefly, 5 ml of plasma were overlaid with 2 ml of 0.16 M NaCl, 1 mM EDTA-saline, 1.006 g/ml, and very low density lipoproteins (VLDL) were isolated by ultracentrifugation for 16 hours at 40,000 rpm in a Beckman ultracentrifuge with a type 40 rotor. The top 2.5 ml from each tube was removed and, thereafter, low density lipoproteins (LDL) were isolated by centrifugation at density 1.063 g/ml for 20 hours at 40,000 rpm (15°C). The total HDL fraction consisted of the infranatant after the removal of the LDL fraction (approximately 1.5 ml) by tube slicing. The recovery of lipoprotein fractions after ultracentrifugation averaged 93%.

HDL subfractions were isolated from HDL as described by Anderson et al.17 Four ml of the HDL fraction was adjusted to d = 1.130 g/ml and was overlaid with EDTA saline (d = 1.125 g/ml); HDL₂ was isolated by centrifugation for 24 hours at 50,000 rpm (15°C). After 1.5 ml of the supernatant had been removed, the infranatant was adjusted to d = 1.210 g/ml and was overlaid with EDTA safefly 

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Human Studies Committees of the National Institutes of Health, the Phoenix Area Indian Health Service, and the Gila River Indian Community. The subjects were placed on a weight maintenance diet composed of 45% carbohydrate, 40% fat, and 15% protein (P/S 0.33, 500 mg cholesterol per day).

The subjects had normal physical examinations. Their liver, kidney, and thyroid functions were evaluated by routine blood chemistry tests and urinalyses and were within the normal range for this population. No accurate methods are available for ascertaining alcohol consumption, but no subject had any physical signs of alcohol abuse or dependence. Approximately 30% of the subjects smoked, but all smokers smoked less than 1 pack/day. All women were under 40 years old and, therefore, premenopausal. Many subjects were not able to give an accurate history of menstrual cycles. Concomitant measurements of plasma progesterone indicated that three of the lean women and one obese woman were in the luteal phase of the menstrual cycle as measured by progesterone concentrations greater than 25 nmol/l, and one lean woman had a luteal peak concentration of lutelizinone hormone of 67 mIU/ml. No subject was taking any medication at the time of the study. The body composition was determined by underwater weighing. The residual lung volume was measured simultaneously by a helium dilution technique, and the fat-free mass was calculated according to the method of Siri.15

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saline (d = 1.21 g/ml); HDL₃ was isolated after centrifugation for 48 hours at 50,000 rpm. The recovery of cholesterol in the HDL subfractions averaged 96%.

Triglyceride and cholesterol were measured in total plasma and in isolated lipoprotein fractions using the Autoanalyzer II (Technicon, Tarrytown, New York). Cholesterol was measured by the method of Rush et al.¹⁸ and triglyceride, by the enzymatic method of Buccolo and David.¹⁹ For quantification of cholesterol in HDL subfractions, the sensitivity of the assay was increased by diluting the samples 1:10, rather than 1:20, in isopropanol, and the cholesterol standards were prepared in 90%, rather than 95%, isopropanol. The triglyceride and cholesterol assays and the HDL isolation procedure were standardized with control plasma calibration pools supplied by the Lipid Standardization Laboratory, Centers for Disease Control, Atlanta, Georgia. The use of these standards has been described previously.¹⁰

Phospholipids were measured in chloroform/methanol extracts of the HDL₂ and HDL₃ subfractions by the method of Fiske and Subbarow.²⁰ Proteins were measured in the HDL subfractions using the method of Markwell et al.²¹ Apolipoprotein A-I (apo A-I) was measured in plasma and HDL subfractions using the radioimmunodiffusion assay of Albers et al.²² Anti-A-I antibody was obtained from International Immunology Corporation (Murrietta, California) and the apo A-I standard was prepared by centrifugation and ion exchange chromatography as described by Albers and Cheung.²³

**Assay of Lipolytic Enzymes**

Heparin releasable lipoprotein lipase (LPL) activity was measured from needle biopsy specimens of subcutaneous adipose tissue aspirated from the gluteal region. The enzyme activity was measured using labelled triolein as the substrate.²⁴ The enzyme activity was calculated as the μmoles of FFA released from the triglyceride substrate in 1 hour by 1 g of tissue. The LPL activity was also expressed per cell; the cell number was measured by use of a Coulter Counter as described previously.²⁸

Postheparin plasma lipoprotein lipase and hepatic lipase activities were measured by use of an immunochromatographic method as described by Hutton et al.²⁵ Lipoprotein lipase was measured after inactivating hepatic lipase with a specific antisera. Hepatic lipase was measured concurrently at a 1 M NaCl concentration, which totally inactivates lipoprotein lipase. All adipose LPL and postheparin plasma LPL and hepatic lipase activities were measured in the laboratories of the Third Department of Medicine, University of Helsinki. The tissue specimens and postheparin plasma were mailed in dry ice and remained frozen on arrival. We had previously confirmed that lipase activities were intact under these conditions.²⁶

**Hormone Assays**

Serum lutetinizing hormone (LH) was measured with an immunoassay kit (Sorin/International CIS, St-Quentin-Yvelines, Cedex, France), and serum follicle stimulating hormone (FSH) was measured by use of an immunoassay kit (Amerlex, Radiochemical Centre, Amersham, England). Serum prolactin was measured with a radioimmunoassay (RIA) kit (Sorin/International, CIS). Serum testosterone was assayed as described by Ismail et al.²⁷ with a radioimmunoassay kit (Farmos Diagnostica, Oulunsalo, Finland). Serum estradiol concentration was measured with a direct radioimmunoassay kit (Eidg. Institut fur Reaktorforschung, Swurenlingen, Switzerland) as described by Cameron et al.²⁸ Steroid hormone binding globulin (SHBG) was analyzed with a liquid phase immunoradiometric assay²⁹ (Farmos Diagnostica, Oulunsalo, Finland). Progesterone was measured with a radioimmunoassay kit obtained from Farmos Diagnostica (Oulunsalo, Finland). All analyses were carried out as duplicate determinations with high and low value quality control samples included in each assay. The precision of the commercial RIA methods were within the limits published by each manufacturer. Hormone measurements for all subjects in the study groups were made in the same assay.

The reference values for the hormone concentrations in men were as follows: LH = 3 to 14 mIU/ml, FSH = 2 to 10 mIU/ml, prolactin = 0.090 to 0.259 mIU/ml, serum testosterone = 14 to 38 nmol/l, SHBG = 12 to 40 nmol/l, estradiol <130 pmol/l, and progesterone = 0.22 to 1.7 nmol/l. The reference values for testosterone, SHBG, and prolactin in women were: 0.85 to 3.5 nmol/l, 50 to 100 nmol/l, and 120 to 500 mIU/l, respectively; the values for LH, FSH, estradiol, and progesterone in the follicular phase were 5 to 20 mIU/ml, 3 to 12 mIU/ml, and 110 to 440 pmol/l, respectively; the values for LH, FSH, estradiol, and progesterone in the luteal phase were: 30 to 100 mIU/ml, 10 to 25 mIU/ml, and 550 to 1290 pmol/l, respectively. The reference values for testosterone, SHBG, and prolactin in the follicular phase were: 5 to 20 mIU/ml, 2 to 10 mIU/ml, 370 to 770 pmol/l, and 20 to 90 nmol/l for LH, FSH, estradiol, and progesterone, respectively.

**Statistics**

Simple assessments of differences between the two sexes or between lean and obese subjects were made using Student’s unpaired t test. In addition, all four groups were compared using Duncan’s multiple range test with alpha = 0.05. When lean and obese groups were combined for each sex to evaluate relationships between HDL composition, lipase activities and hormones, multiple regression analysis was performed adjusting for percent fat. To normalize their distributions, the logarithms of fasting insulin, total triglyceride, HDL cholesterol, all components of HDL₃ (cholesterol, phospholipid, protein, apo A-I, and triglyceride), HDL₂ cholesterol/apo A-I ratio, adipose tissue lipoprotein lipase, SHBG, and estradiol were used for computing the significance of differences in the t test, in correlation and multiple regression analyses, and for Duncan’s multiple range test. For clarity of presentation in the tables and figures, however, the arithmetic means and the SEM are presented for these variables. All statistical procedures used the Statistical Analysis System (Cary, North Carolina).

**Results**

The data for age, weight, plasma glucose, and insulin for the four groups of subjects are shown in Table 1. The
obese men were slightly older than the lean men, and lean and obese men had a significantly lower proportion of body fat than did lean or obese women. (Comparisons of the relationship between BMI and percent fat for men and women in a large number of the Pima population have shown that women of any BMI have approximately 9% more body fat. Data are not shown).

**Plasma Lipids and High Density Lipoprotein Subfractions**

Lean and obese men had higher LDL cholesterol than did lean women (Table 2). Lean men also tended to have higher total cholesterol and triglyceride levels, but these differences did not reach statistical significance. HDL cholesterol was significantly higher in the group of lean women compared to lean men, obese men, or obese women. There was no difference in HDL cholesterol levels between the obese men and the obese women. HDL cholesterol was slightly, but not significantly, higher in lean men compared to obese men. The total apo A-I values had the same pattern as HDL cholesterol, with higher values in lean women, no difference between obese men and obese women. Lean and obese men had higher LDL cholesterol than did lean women (Table 2). Lean men also tended to have higher total cholesterol and triglyceride levels, but these differences did not reach statistical significance.

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**Table 2. Plasma Lipids and High Density Lipoprotein Subfractions**

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Men</th>
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<tr>
<td></td>
<td>Lean</td>
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</tr>
<tr>
<td>LDL</td>
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<td>119±8</td>
</tr>
<tr>
<td>HDL</td>
<td>40±3</td>
<td>35±2</td>
</tr>
<tr>
<td>Total triglyceride</td>
<td>121±20</td>
<td>95±10</td>
</tr>
<tr>
<td>Total apo A-I</td>
<td>135±5</td>
<td>126±5</td>
</tr>
<tr>
<td>HDL subfractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL_2 Cholesterol</td>
<td>13±2</td>
<td>11±1</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>25±5</td>
<td>18±1</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>4±0</td>
<td>4±0</td>
</tr>
<tr>
<td>Protein</td>
<td>37±1</td>
<td>27±2</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>19±1</td>
<td>15±3</td>
</tr>
<tr>
<td>HDL_3 Cholesterol</td>
<td>27±1</td>
<td>24±1</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>51±3</td>
<td>43±3</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>7±1</td>
<td>6±0</td>
</tr>
<tr>
<td>Protein</td>
<td>124±4</td>
<td>103±5</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>93±4</td>
<td>83±5</td>
</tr>
</tbody>
</table>

The values are given in mg/dl and are the means ± SEM. The p values for f test of obese vs lean: *p < 0.05, †p < 0.01, ‡p < 0.001. The p values for f test of men vs women: §p < 0.05, ‡p < 0.01, ††p < 0.001.

HDL cholesterol in both lean and obese men was significantly higher than in lean women with Duncan's multiple range test. HDL cholesterol was significantly higher in lean men compared to women and HDL cholesterol with this test. When this test was used for subtraction data, HDL cholesterol, phospholipid, triglyceride, protein, and A-I were all significantly higher in lean women than in the other three groups. HDL cholesterol and protein were also significantly higher in obese women compared with obese men. HDL cholesterol and A-I were significantly higher in lean men compared with obese men, and HDL_3 protein was higher in lean men compared with all three groups.

![Figure 1. HDL subfractions and obesity. The height of the bar in each group indicates the total HDL level; HDL_2 concentrations are shown in the open areas and HDL_3 in the hatched areas. The significance of differences by f test between lean and obese groups: *p < 0.05; **p < 0.01. The significance of differences in f test between men and women: ††p < 0.001. HDL_2 cholesterol was higher in lean women compared with all three groups by Duncan's multiple range test. HDL_3 cholesterol was significantly higher in lean men compared with obese women by this test.](http://jabiournals.org/)

**Lipase Activities**

Because levels of HDL may be controlled by lipoprotein lipase and hepatic lipase activities, these were measured in all subjects. Lean women (Figure 3) had significantly higher heparin releasable adipose tissue lipoprotein lipase activity than did lean men. This difference was significant whether expressed per gram of adipose tissue or per cell. Postheparin plasma hepatic lipase activity was significantly lower in lean women than in lean men, obese men, and obese women; the value in obese women did not differ significantly from that of lean men and obese men. Post-
Figure 2. Molar ratios of cholesterol/A-I in HDL₂. The values shown are the means ± SEM. The significance of differences by t test between lean and obese: **p < 0.01. The significance of differences by t test between men and women: \( t_t < 0.01 \). By Duncan's multiple range test, the molar ratio of cholesterol/A-I was significantly different in lean women compared with both groups of men.

Steroid Hormones

A profile of serum steroid hormones and steroid hormone binding globulin concentrations was obtained for all subjects (Table 3). Obese men had lower total testosterone concentrations than did lean men; the free testosterone concentration in obese men was 86% of that in lean men; this difference was not significant by \( t \) test, but the difference between the groups of lean men and obese men was significant by Duncan's multiple range test (Table 3). There was no difference in total or free testosterone between the two groups of women. Obese women had dramatically lower estradiol concentrations than did lean women, and the concentration in obese women was similar to that in both groups of men (Table 3). There was no difference in estradiol between the groups of lean men and obese men. There were significant correlations between HDL₂ concentrations and steroid binding globulin in both men and women and a significant correlation between HDL₂ and estradiol in women (Table 4).

Obesity

Since women appeared to show a more pronounced influence of obesity than did men, regression lines were computed between HDL and percent fat or BMI for both men and women (Figure 4). Women had a greater decrease in HDL cholesterol with increasing obesity than did men as measured by percent fat (slopes different at \( p = 0.023 \)) or BMI (slopes different at \( p = 0.016 \)). These relationships explain why the mean HDL cholesterol differed between lean men and lean women but not between obese men and obese women (Table 2).

The relationships between obesity and HDL, lipase activities, and hormone concentration are shown in Table 5. Percent fat was significantly associated with hepatic lipase in women but not in men, and negatively with estradiol in women but not in men.

Figure 3. Adipose lipoprotein lipase (A) and hepatic lipase (B) activities in lean and obese Pima men and women. The values are the means ± SEM. The significance of differences by \( t \) test between lean and obese: **\( p < 0.01 \). The significance of differences by \( t \) test between men and women: \( t_t < 0.05 \); \( t_t < 0.01 \). By Duncan's multiple range test, hepatic lipase was lower in lean women compared with all three groups, and also lower in obese women compared with lean men. Adipose lipoprotein lipase was significantly higher in lean women than in lean men.
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Discussion

This study was undertaken to investigate the possible
reasons for the lack of sex differences in HDL concentra-
tions observed in our previous population study of the
Pima Indians. We found, as in our previous study, that HDL
concentrations were lower than those of Caucasians, 4
in the leanest subjects. However, a difference in HDL
cholesterol was observed between the lean Pima men and
the lean women in this inpatient study. In both sexes, obe-
sity was associated with decreasing HDL, but the de-
crease was more pronounced in women than in men. In
addition, obese women compared to lean women had low-
er serum estradiol, higher hepatic lipase, lower lipoprotein
lipase, and altered HDL₂ composition. Since there is a
stronger negative influence of increasing body fat on HDL
in the women, and since there are so few lean women in
this population, the net result is that no sex differences in
HDL are observed in the population as a whole.

Obesity

A negative correlation between HDL and obesity has
previously been reported in other groups, 20 31 including the
Lipid Research Clinics population studies. 32 In the latter,
regression coefficients between HDL and obesity as mea-
sured by the Quetlet index were higher in women than in
men over 12 years of age. 32 We have previously reported a
negative correlation between HDL and obesity in our popu-
lation study of the Pima Indians. 10 It must be asked why we
found that the regression lines between BMI and HDL were
identical in men and women in the population and there
were no sex differences in HDL cholesterol, even in the
thinnest quintiles of the population. 10 In comparing values
for HDL in the thinnest subjects in the present study with
those of the population, it appears that the lean men stud-
ed as inpatients had lower HDL (the mean value for Pima
men of BMI less than 27 for the population study was 46 ±
1 mg/dl). The most likely explanation for the difference
between HDL in the thin subjects in this compared to the
previous study lies in the environmental influences on
HDL. One possibility is that the differences can be attribut-
ed to alcohol consumption. Rapid decreases in HDL and
HDL₂ have been previously demonstrated within 2 days of
abstinence. 33 Thus, HDL levels in the lean Pima men stud-
ed in the population survey may be elevated due to alco-
hol consumption, whereas they were lower after admission
and stabilization on the metabolic ward. Alternately, the
differences in HDL between lean outpatient and inpatient
men could be due to differences in diet or to the decreased
activity levels on the metabolic ward. Finally, it must be

Table 3. Hormones

<table>
<thead>
<tr>
<th></th>
<th>Men Lean</th>
<th>Men Obese</th>
<th>Women Lean</th>
<th>Women Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/ml)</td>
<td>7.4 ± 0.2$t$</td>
<td>5.9 ± 0.6</td>
<td>14 ± 7$t$</td>
<td>7.3 ± 1.0 $</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>2.3 ± 0.4</td>
<td>3.0 ± 0.3</td>
<td>3.6 ± 1.1</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Progesterone (mIU/ml)</td>
<td>0.278 ± 0.021</td>
<td>0.285 ± 0.049</td>
<td>0.343 ± 0.044</td>
<td>0.311 ± 0.046</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>29 ± 2$</td>
<td></td>
<td></td>
<td>22 ± 2$</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>524 ± 26$</td>
<td>449 ± 33$</td>
<td>40 ± 5$</td>
<td>45 ± 6$</td>
</tr>
<tr>
<td>Testosterone index (%)</td>
<td>136 ± 11$</td>
<td>128 ± 21$</td>
<td>7.7 ± 1.9$</td>
<td>13.0 ± 3.1$</td>
</tr>
<tr>
<td>Steroid binding globulin (nmol/l)</td>
<td>23 ± 35$</td>
<td>20 ± 3</td>
<td>59 ± 16$</td>
<td>28 ± 5$</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>133 ± 7</td>
<td>125 ± 11</td>
<td>634 ± 108$</td>
<td>176 ± 29$</td>
</tr>
</tbody>
</table>

The values are shown for HDL₂ cholesterol. Similar results were found for total HDL cholesterol. The relation
between hepatic lipase and HDL₂ cholesterol was significant in men (p = 0.003) but not in women (p = 0.10) after
adjusting for percent fat. The relation between HDL₂ cholesterol and estradiol was significant in women (p = 0.04)
after adjusting for percent fat. The relation between HDL₂ cholesterol and SHBG was significant in women (p = 0.03)
and marginally significant in men (p = 0.07) after adjusting for percent fat.
emphasized that the individuals selected for the present inpatient study are not representative of the population as a whole, in that there was an equal distribution of lean and obese subjects in the present study compared to the general Pima population where almost all men and women have a BMI over 27.34

Lipase Activities

Pima women, especially the lean ones, had lower hepatic lipase activities than did men. This is consistent with the data from Caucasian populations.35-37 In both sexes there was a strong negative association between hepatic lipase and HDL2 concentrations. Thus, these results support the proposal that HDL2 levels may be controlled by hepatic lipase.36,37 The significant correlation between hepatic lipase activity and obesity in Pima women is consistent with previous studies. As in our previous study,38 we did not observe sex differences in LPL in obese Pima men and women, although adipose LPL was higher in lean women compared to lean men. Sex differences in adipose lipoprotein lipase have been observed in studies of lean subjects in other groups.39,41 We observed a trend toward higher LPL in obese men compared to lean; this was significant in a larger group of Pima men studied previously.36 However, obese Pima women did not have increasing LPL with obesity, a finding different from other studies.36,37

Steroid Hormones

Of interest is the significant negative association between obesity and estradiol in women. The concentrations of SHBG were parallel to those of estradiol, being low in obese women and higher in lean women. This is consistent with the concept that SHBG reflects estrogenic effects.42 Since the women in the study were not all sampled at the same phase of their menstrual cycles, it is difficult to directly evaluate the influence of menstrual cycle on the difference in estradiol between lean and obese women. Obese women are often amenorrheic or anovulatory and this may further confound the interpretation of the estradiol values. The difference in estradiol between the obese and lean women, however, was significant even if the subjects who had luteal phase LH and progesterone values were removed from the analysis. Irrespective of the cause, it is apparent that obese premenopausal women seem to have lower estradiol concentrations, which are not significantly different from men. The influence of obesity on estradiol concentration has been examined previously only in postmenopausal women where increases in estrone have in fact been shown; these are thought to be the result of adipose tissue conversion of androgens to estrogens.43,44 In this study we did not observe any significant relationships between serum-free or total testosterone and HDL. Some previous studies have reported positive correlations

Table 5. Pearson Simple Correlation Coefficients between Obesity (Percent Fat) and HDL, Lipase Activities, and Hormone Concentrations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>-0.37</td>
<td>-0.61</td>
</tr>
<tr>
<td>HDL2 Cholesterol</td>
<td>-0.21</td>
<td>-0.53</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>-0.35</td>
<td>-0.58</td>
</tr>
<tr>
<td>HDL3 Cholesterol</td>
<td>-0.32</td>
<td>-0.59</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>-0.43</td>
<td>-0.03</td>
</tr>
<tr>
<td>Adipose LPL</td>
<td>-0.10</td>
<td>-0.47</td>
</tr>
<tr>
<td>Postheparin LPL</td>
<td>-0.07</td>
<td>-0.51</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>0.13</td>
<td>0.50</td>
</tr>
<tr>
<td>Estradiol</td>
<td>-0.09</td>
<td>-0.47</td>
</tr>
<tr>
<td>SHBG</td>
<td>-0.45</td>
<td>-0.29</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>-0.43</td>
<td>-0.007</td>
</tr>
</tbody>
</table>

The values are r values with the p values in parentheses when p < 0.05.

LPL = lipoprotein lipase; SHBG = steroid hormone binding globulin.
between HDL cholesterol and testosterone, although one other study did not.

**High Density Lipoprotein Composition**

Our data are in agreement with previous observations that when HDL is elevated, as in our lean Pima women, this elevation represents an increase in the HDL2 subfraction. In addition, in lean women the HDL2 particles were relatively enriched in apo A-I compared to those from men or obese women. The protein content of the subfractions reflected that of A-I, whereas phospholipid and triglyceride appeared to remain in proportion to cholesterol. This relative enrichment of A-I with respect to cholesterol and phospholipids is consistent with the higher estradiol concentrations in lean women, since estrogens have been reported to increase the production of apo A-I. The relationship between HDL structure and HDL concentrations must be further examined using more detailed analyses of HDL composition. Changes in HDL2 composition with increased ratios of cholesterol/A-I have been previously observed in the Pima diabetics.

**Relationships between High Density Lipoprotein, Obesity, Lipase Activities, and Sex Hormones**

From the data in this study we can propose the following hypothesis: obesity in women is associated with decreased estradiol. This results in increased hepatic lipase, and thus decreased HDL2. This scheme is supported by recent studies showing decreased hepatic lipase after estrogen administration. The data on hormones, lipases, and HDL in men are consistent with this scheme, in that obesity in men had only minimal effects on sex hormone levels. SHBG concentrations and hepatic lipase were similar in obese and lean men, because estradiol and testosterone were not different. There is thus less influence of obesity on HDL concentrations in men, and the inverse relationship between HDL and obesity in men (which reaches significance when larger Pima groups are evaluated) may, in fact, be due to factors other than the hormonal effects which appear to predominate in women.

**Acknowledgments**

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Lack of sex differences in high density lipoproteins in Pima Indians. Studies of obesity, lipase activities, and steroid hormones.

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