**Research Article**

**Haptoglobin 2-2 Genotype Is Associated with TNF-α and IL-6 Levels in Subjects with Obesity**

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Received 7 February 2014; Revised 4 April 2014; Accepted 18 April 2014; Published 29 April 2014

Academic Editor: Giuseppe Murdaca

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**Objective.** To evaluate the association between Haptoglobin (HP) gene polymorphisms with inflammatory status in obese subjects.

**Materials and Methods.** A cross-sectional study was carried out. A total of 276 apparently healthy men and nonpregnant obese women were enrolled and allocated according to the HP genotype into the $HP^1/HP^1$, $HP^2/HP^1$, and $HP^2/HP^2$ groups. Distribution of HP genotypes was 49, 87, and 140 for the $HP^1/HP^1$, $HP^2/HP^1$, and $HP^2/HP^2$, respectively. The HP genotype was determined using the polymerase chain reaction method. A multiple linear regression analysis adjusted by age, sex, waist circumference, and total body fat was used to determine the association between HP genotypes with TNF-α, IL-6, and high-sensitivity C-reactive protein (hsCRP) levels.

**Results.** A multiple linear regression analysis adjusted by sex, waist circumference, and total body fat was performed showing a significant association between the $HP^2/HP^2$ genotype and TNF-α ($\beta = 0.180; 95\%$ CI 14.41–159.64, $P = 0.01$) and IL-6 ($\beta = 0.188; 95\%$ CI 1.53–12.72, $P = 0.01$) levels, but not with hsCRP ($\beta = -0.008; 95\%$ CI -1.64–1.47, $P = 0.914$) levels, whereas the $HP^2/HP^1$ genotype showed no association compared with the $HP^1/HP^1$ genotype (control group). **Conclusion.** Results of our study show that the $HP^2/HP^2$ genotype is associated with elevated TNF-α and IL-6, but not with hsCRP, levels in obese subjects.

**1. Introduction**

Obesity is associated with chronic low-grade inflammation through expansion of white adipose tissue (WAT), a lipid storage organ that secretes leptin, adiponectin, and adipokines such as interleukin (IL)-6, IL-1β, and TNF-α [1, 2]; in addition, hypertrophic adipocytes secrete chemoattractants such as monocyte chemoattractant protein (MCP-1) that promotes immune cells infiltration into WAT. Both events, chronic low-grade inflammation and infiltration of adipose tissue by macrophages, contribute to development of metabolic disorders [3, 4]. In this regard, although IL-6 and C-reactive protein (CRP) are well correlated with body mass index (BMI), TNF-α shows no correlation with neither BMI nor weight reduction [5].

The adipokine haptoglobin (HP), a plasmatic glycoprotein with tetrameric structure of 2 alpha and 2 beta polypeptides that are covalently binding by disulfide bonds [6], is a positive acute phase protein synthesized in the liver and expressed by WAT, exhibiting capacity to recruit monocytes and macrophages and thus playing an important role in the link between obesity and chronic systemic inflammation [7, 8].

In human, there are 3 common phenotypes of HP, Hp 1-1, Hp 1-2, and the heterozygous phenotype Hp 2-1. These phenotypes are controlled by two autosomal codominant alleles identified as $HP^1$ and $HP^2$ [9]. The $HP^2$ allele contains 1.7-kb intragenic duplication that arose after a unique nonhomologous DNA crossing-over within different introns of two $HP^2$ genes [10].

The HP protein, resulting from HP polymorphisms, has distinct biochemical and biophysical properties; it has been hypothesized that there is a greater expression of markers of activation in the macrophages of individuals with the HP 2-2 isoform as compared with individuals with HP 1-1 [11] that results in higher inflammatory status [8].
Thus, the aim of this study was to evaluate the association between HP gene polymorphisms with inflammatory status in obese subjects.

2. Materials and Methods

After protocol approval by the Mexican Social Security Institute Research Committee and after obtaining the written informed consent to participate, a cross-sectional study was carried out. Eligible subjects, apparently healthy obese men and nonpregnant obese women aged 18 to 65 years, were recruited from the general population of Durango, city in northern Mexico. According to the National Institute of Anthropology classification, all individuals were Mexican mestizos born in Mexico, had last name of Spanish origin, and had a family history of Mexican ancestry [12].

According HP genotypes, individuals were allocated into the HP\(^1/\text{HP}^1\), HP\(^1/\text{HP}^2\), and HP\(^2/\text{HP}^2\) groups.

A body mass index (BMI) < 30 kg/m\(^2\), smoking, alcohol intake, acute or chronic inflammatory disease, acute or chronic infections, glomerulopathies, renal or hepatic disease, malignancy, and cardiovascular disease as well as intake of statins, fibrates, or anti-inflammatory drugs were exclusion criteria. A standardized interview, clinical examination, and laboratory tests were performed to carefully determine the presence of the inclusion and exclusion criteria.

2.1. Measurements. In the standing position and fasting conditions, weight, height, and waist circumference (WC) were measured with the subjects in light clothing and without shoes. Weight and height were measured using a fixed scale with stadiometer (Tanita TBF-215, Tokyo, Japan). The BMI was calculated as weight (kilograms) divided by height (meters) squared. Obesity was defined by BMI ≥ 30 kg/m\(^2\). The WC was measured to the nearest centimeter with a flexible steel tape; the anatomical landmarks used were midway between the lowest portion of the rib cage and the superior border of the iliac crest. Total body fat was measured by bioelectric impedance (Tanita TBF-215, Tokyo, Japan).

Blood pressure was measured according to recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [13].

2.2. Assays. Whole blood sample was collected from antecubital venous under minimal tourniquet pressure, after 8–10 h overnight fasting. Serum biochemical determinations included plasma fasting glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, TNF-α, and IL-6.

Serum glucose was measured using the glucose-oxidase method; the intra- and interassay coefficients of variation were 1.1 and 1.5%. HDL-c fraction was obtained after precipitation by phosphotungstic reagent. Total cholesterol and triglycerides levels were enzymatically measured using spectrophotometric methods. The intra- and interassay coefficients of variation were 1.3% and 2.6% for HDL-c, 3.0% and 2.5% for total cholesterol, and 1.7% and 3.1% for triglycerides. All measurements were performed in an automatic chemical autoanalyzer (Data Pro Plus, Arlington Tx, USA).

TNF-α and IL-6 were measured using high sensitivity ELISA assays (Thermo Scientific kits, Rockford, IL), with range of 15.6–1000 pg/mL and 10.24–400 pg/mL, respectively, according to instructions provided by the manufacturer and evaluated using an iMark Microplate Absorbance Reader (Bio-Rad Laboratories Hercules, CA, USA).

Analysis of high-sensitivity C-reactive protein (hsCRP) was performed in a VITROS 5.1 FS Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ, USA) based on particle-enhanced turbidimetry using the VITROS Chemistry Products (Johnson and Johnson Clinical Diagnostics Inc., Rochester, NY) with a detection limit of 0.32 mg/L and an extended measuring range of 0.32 mg/L to 42.83 mg/L (with auto-rerun), according to the manufacturer.

2.3. DNA Extraction and HP Genotyping. Genomic DNA was extracted from whole blood using DNAzol BD Reagent (Invitrogen, Carlsbad, CA). The HP genotype was determined using the polymerase chain reaction (PCR) method as previously described [14].

2.4. Statistical Analysis. The normality of the data and the homogeneity of variances were tested using Shapiro-Wilk and Levene tests. When the data were normally distributed, the groups were compared using one-way ANOVA with post hoc Bonferroni test or a Chi-square test. When the distribution was not normal, particularly when homogeneity of variances was not observed, median values were compared using the Kruskal-Wallis and Mann-Whitney U test.

Given the nonparametric distribution of hsCRP levels, relationship between age and hsCRP levels was evaluated using the Spearman rank correlation test.

A multiple linear regression analysis was used to determine the association between HP genotypes (independent variable) with TNF-α and IL-6 levels (dependent variables). An additional multiple linear regression analysis, adjusted by age, sex, WC, and total body fat, was performed in order to control the influence on dependent variables.

The 95% confidence intervals (CI 95%) were determined, and a P value < 0.05 defined statistical significance. Data were analyzed using the statistical package SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results

A total of 328 subjects were screened; 52 (15.8%) individuals were excluded because they did not fulfill the inclusion criteria or by the presence of exclusion criteria. Thus, 276 (84.1%) individuals, 180 (65.2%) women and 96 (34.8%) men, with average age of 36.2 ± 12.5 years, were enrolled and allocated according to HP genotype into the HP\(^1/\text{HP}^1\), HP\(^1/\text{HP}^2\), and HP\(^2/\text{HP}^2\) groups (Table 1).

Distribution of HP genotypes was 17.7%, 31.5%, and 50.7% for the HP\(^1/\text{HP}^1\), HP\(^1/\text{HP}^2\), and HP\(^2/\text{HP}^2\), respectively. The genotype frequencies observed in this population showed a significant deviation from HWE (P < 0.05).
Table 1: Characteristics of the study groups, n = 276.

|                      | HP1/HP1 | HP1/HP2 | HP2/HP2 |
|----------------------|---------|---------|---------|
|                      | n = 49  | n = 87  | n = 140 |
| Age, years           | 33.0 ± 11.7 | 37.1 ± 13.8 | 36.6 ± 11.6 |
| Women, n (%)         | 22 (44.8%) | 55 (63.2%) | 103 (73.5%) |
| Body mass index, kg/m² | 34.6 ± 3.1 | 35.0 ± 4.9 | 35.0 ± 5.1 |
| Waist circumference, cm | 111.6 ± 9.4 | 109.8 ± 13.4 | 106.6 ± 11.1 |
| Total body fat, %     | 39.5 ± 6.3 | 42.1 ± 6.5 | 42.3 ± 6.4 |
| Systolic blood pressure, mmHg | 118.4 ± 9.6 | 120.0 ± 14.6 | 117.5 ± 12.9 |
| Diastolic blood pressure, mmHg | 75.8 ± 8.4 | 76.9 ± 10.3 | 76.0 ± 8.7 |
| Fasting glucose, mg/dL | 104.7 ± 10.6 | 104.1 ± 14.8 | 106.0 ± 10.0 |
| Total cholesterol, mg/dL | 182.2 ± 39.9 | 187.6 ± 33.0 | 184.9 ± 42.4 |
| HDL-cholesterol, mg/dL | 38.5 ± 13.6 | 39.9 ± 13.9 | 38.3 ± 19.4 |
| LDL-cholesterol, mg/dL | 108.6 ± 40.2 | 111.4 ± 32.9 | 114.7 ± 47.8 |
| Triglycerides, mg/dL*   | 154 (66.5–205) | 163.0 (110–223) | 137 (108–197) |
| TNF-α, pg/mL*         | 115.5 (66.5–232.7) | 137.8 (91.3–202.2) | 174.0 (91.9–298.5) |
| IL-6, pg/mL*          | 3.4 (1.0–6.5) | 2.7 (1.0–8.2) | 4.3 (1.0–9.5) |
| hsCRP, mg/L*          | 4.0 (1.8–6.5) | 3.8 (1.6–8.4) | 3.7 (2.2–8.6) |

Values are mean ± standard deviation.
hsCRP: high-sensitivity C-reactive protein.
* P value estimated using one-way ANOVA with post hoc Bonferroni test.
* Median (25–75th percentile), P value estimated using Kruskal-Wallis test.
** Statistical significant difference between HP1/HP1 and HP2/HP2.
† Statistical significant difference between HP2/HP1 and HP2/HP2.

Table 2: Multiple linear regression analysis adjusted by age, sex, waist circumference, and total body fat that evaluates the association between HP2/HP2 and HP1/HP1 genotypes (independent variables) with TNF-α, IL-6, and C-reactive protein (dependent variables). The HP1/HP1 was the control group.

|                      | HP2/HP2 genotype | HP1/HP1 genotype |
|----------------------|------------------|------------------|
|                      | β                | 95% CI           | P   | β                | 95% CI           | P   |
| Model 1*             |                  |                  |     |                  |                  |     |
| TNF-α                | 0.154            | 6.26–142.16      | 0.03 | 0.026            | −32.67–44.47      | 0.763 |
| IL-6                 | 0.142            | 0.04–10.69       | 0.04 | 0.142            | −0.79–9.13        | 0.099 |
| hsCRP                | 0.030            | −1.25–1.93       | 0.676 | 0.044            | −1.70–2.88        | 0.610 |
| Model 2**            |                  |                  |     |                  |                  |     |
| TNF-α                | 0.180            | 14.41–159.6      | 0.01 | 0.048            | −28.01–49.57      | 0.583 |
| IL-6                 | 0.188            | 1.53–12.72       | 0.01 | 0.171            | −0.83–10.14       | 0.054 |
| hsCRP                | −0.008           | −1.64–1.47       | 0.914 | −0.018           | −2.46–1.99        | 0.834 |

hsCRP: high-sensitivity C-reactive protein.
* Unadjusted.
** Adjusted by age, sex, waist circumference, and total body fat.

Table 1 shows the clinical and biochemical characteristics of the population in study; although subjects with HP2/HP2 genotype had lower WC than individuals with the HP1/HP1 genotype, they showed higher TNF-α levels; however, there were no significant differences in IL-6 and hsCRP levels between the groups.

In the overall population, age and hsCRP levels showed a significant positive relationship (r = 0.150, P = 0.02).

The unadjusted multiple linear regression analysis showed that HP2/HP2 genotype is significantly associated with TNF-α and IL-6, but not with hsCRP levels, whereas the HP2/HP1 genotype showed no association. In a subsequent multiple linear regression analysis adjusted by age, sex, WC, and total body fat the HP2/HP2 genotype remained significantly associated with TNF-α and IL-6 levels (Table 2).

A subanalysis comparing women and men who had HP2/HP2 genotype showed that WC, total body fat, triglycerides, and hsCRP, but not IL6 or TNF-α levels, were significantly higher in women than men (Table 3).

4. Discussion

Results of our study indicate that the HP2/HP2 genotype is associated with elevated TNF-α and IL-6 levels in obese subjects. However, in contrast with our finding, a recent study among Saudi diabetics and healthy subjects showed no
association between HP genotypes and hsCRP levels, as the inconsistency in our results and those by Mohieldein et al. [15] could be related to the target population and/or sample size.

Subjects with the HP1/HP2 genotype exhibited lower WC and higher levels of TNF-α and IL-6 than individuals with the HP2/HP2 genotype, finding that strongly suggests an increased capacity of type 2-2 to recruit macrophages in WAT, which are involved in the synthesis and release of TNF-α and IL-6 [16].

In addition, in our study, hsCRP levels were similar in the different groups included, findings in accordance with previous reports showing that there are no significant statistical differences in the hsCRP levels of apparently healthy individuals [17], patients with type 2 diabetes [14], and patients with peripheral occlusive disease [18], who exhibit genotype Hp 2-2 compared with individuals exhibiting phenotypes Hp 1-1 and Hp 2-1. However, data are controversial with some studies showing that patients with chronic kidney disease [19] and those with essential hypertension [18] with phenotype Hp 2-2 have significantly higher levels of hsCRP than individuals with phenotypes Hp 1-1 and Hp 2-1. These inconsistencies have been explained based on genetic variations of C-reactive protein (CRP) gene, low levels of physical activity, and gradual increase of CRP with aging [14, 20]. Although in our population there was a positive relationship between hsCRP levels and age, we did not measure genetic variations of CRP gene nor physical activity.

Interestingly, among individuals with HP2/HP2 genotype, women showed significant higher hsCRP levels, but not differences between TNF-α and IL-6 levels than men; although differences could be related to the elevated total body fat and the high WC, further research is mandatory to elucidate the involved mechanisms in the association between HP genotypes and the triggering of low chronic systemic inflammation.

A model for the role that the HP plays in inflammation has been proposed; according to this model, through the IL-6 activity, the stressed cells emit warning signals triggering HP expression. In this way, among subjects with HP2/HP2 genotype, the HP significantly decreases synthesis of reactive oxygen species through its potent antioxidant function, whereas in the individuals with the HP2/HP2 genotype, the antioxidant activity is weak favoring the persistence of inflammatory response [21]. However, the effect of HP genotype in the inflammatory process is unclear and requires further research.

There is a worldwide variation in the frequency of alleles HP1 and HP2; in this regard, it has been proposed that HP2 allele emerged in India and probably it was propagated due to a selective pressure, suggesting a selective advantage over the HP1 allele [22].

Frequency of the HP2 allele in our study was higher (66.4%) as compared with frequency previously reported in indigenous populations from Durango State (37.9%) [23], findings that could be explained in part because our study was focused on Mexican mestizos, from urban area of Durango city.

Several limitations of this study deserve to be mentioned. First, we did not measure serum levels of Hp; however, previously, no significant differences between HP genotypes and Hp phenotypes in healthy subjects with overweight or obesity were reported [15]; so, this limitation exerts minimal influence on our results and conclusion. Second, we did not include a control group of nonobese subjects; however, taking into account the aim of our study and the lack of consistency

Table 3: Characteristics of the HP2/HP2 genotype according to sex. n = 140.

|                     | Women (n = 103) | Men (n = 37) | P  |
|---------------------|----------------|--------------|----|
| Age, years          | 37.3 ± 11.3    | 34.5 ± 12.3  | 0.229 |
| Body mass index, kg/m² | 35.0 ± 5.5    | 35.0 ± 3.8   | 0.992 |
| Waist circumference, cm | 103.8 ± 10.2  | 114.5 ± 9.7  | <0.001 |
| Total body fat, %   | 44.6 ± 4.6     | 35.8 ± 6.5   | <0.001 |
| Systolic blood pressure, mmHg | 115.8 ± 13.1 | 122.0 ± 11.2 | 0.008 |
| Diastolic blood pressure, mmHg | 75.3 ± 8.3   | 77.8 ± 9.6   | 0.172 |
| Fasting glucose, mg/dL | 106.7 ± 10.2  | 104.0 ± 9.2  | 0.148 |
| Total cholesterol, mg/dL | 184.3 ± 43.1  | 186.5 ± 41.0 | 0.778 |
| HDL-cholesterol, mg/dL | 37.2 ± 8.9     | 41.2 ± 35.0  | 0.498 |
| LDL-cholesterol, mg/dL | 119.0 ± 41.0  | 102.8 ± 62.2 | 0.146 |
| Triglycerides, mg/dL | 37.2 ± 8.9     | 35.8 ± 6.5   | 0.307 |
| hsCRP, mg/L*        | 128 (100–168) | 192 (143–252) | <0.001 |
| TNF-α, pg/mL*       | 156.5 (90.1–301.8) | 216.4 (99.9–314.8) | 0.502 |
| IL-6, pg/mL*        | 4.3 (1.0–8.7)  | 4.8 (1.0–10.8) | 0.983 |
| hsCRP, mg/L*        | 4.2 (2.7–8.9)  | 2.2 (1.0–8.5) | 0.007 |

Values are mean ± standard deviation.
hsCRP: high-sensitivity C-reactive protein.
* Median (25–75th percentile), P value estimated using Mann-Whitney U test.
between TNF-α and BMI [5], which could introduce an analysis bias comparing individuals with different BMI, the noninclusion of a control group of normal weight individuals has no influence on our main conclusion. Third, population in study was not in HWE, which could be related to the target population that included only subjects with obesity.

5. Conclusion

In conclusion, our results show that the $H_{P^2}/H_{P^2}$ genotype is associated with elevated TNF-α and IL-6, but not with hsCRP, levels in obese subjects, suggesting that the functional differences of HP subtypes could be associated with different outcomes of obesity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by Grants from the Mexican Social Security Institute (Project FIS/IMSS/PROT/542), the National Council of Science and Technology (CONACYT) (Project FOMIX-DGO-2007-C01-66735), and Foundation IMSS A.C.

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