Impact of Insulin and Body Mass Index on Metabolic and Endocrine Variables in Polycystic Ovary Syndrome

Mario Ciampelli, Anna Maria Fulghesu, Francesco Cucinelli, Virginia Pavone, Elio Ronsisvalle, Maurizio Guido, Alessandro Caruso, and Antonio Lanzone

To assess the differential impact of the insulin secretory pattern and obesity on the endocrinometabolic features of the polycystic ovary syndrome (PCOS), we studied 110 PCOS women. Patients underwent a gonadotropin-releasing hormone (GnRH) test, an oral glucose tolerance test (OGTT), and basal evaluation of hormonal and biochemical parameters. Basal androgens and lipids, basal and stimulated gonadotropins, insulin, and glucose levels were measured. Patients were classified into four groups according to the body mass index (BMI) and insulin secretion: normoinsulinemic-lean ([NL] n = 24), normoinsulinemic obese ([NO] n = 24), hyperinsulinemic lean ([HL] n = 17), hyperinsulinemic obese ([HO] n = 45). HL patients showed a higher luteinizing hormone (LH) area under curve (AUC) after GnRH stimulus compared with NL patients (HL *v* NL, 4,285 ± 348 *v* 3,377 ± 314 IU/L · 120 min, $P < .05$), whereas we failed to find a statistically significant difference in a similar comparison among obese subjects (HO *v* NO, 3,606 ± 302 *v* 3,129 ± 602 IU/L · 120 min). A trend toward increased plasma testosterone and decreased sex hormone–binding globulin (SHBG) was found in relation to hyperinsulinemia and obesity, thus resulting in a higher free androgen index (FAI) in groups HL and NO versus NL (HL, 5.54 ± 0.51; NO, 5.64 ± 0.49; NL, 4.13 ± 0.33; $P < .05$ and $P < .01$, respectively). The presence of both exaggerated insulin secretion and obesity resulted in a synergistic additive effect on the FAI in the HO group (6.81 ± 0.34). Concerning the lipoprotein lipid profile, the NL group showed lower plasma triglyceride levels compared with the other three groups, whereas no significant differences were found for nonesterified fatty acid (NEFA) concentrations. Higher low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) and lower high-density lipoprotein cholesterol (HDL-C) levels were found in the obese groups compared with the lean counterparts, whereas the same parameters did not significantly differ in a comparison between normoinsulinemic and hyperinsulinemic groups. In conclusion, our data suggest an important role of hyperinsulinemia in the LH response to a GnRH stimulus and an independent and synergistic additive effect of obesity and hyperinsulinemia on the FAI in PCOS.

POLYCYSTIC OVARY SYNDROME (PCOS) is clinically characterized by anovulation and hyperandrogenism; it is one of the most common endocrine disorders, affecting approximately 5% to 10% of women of reproductive age.[1,2] The syndrome is also associated with a characteristic metabolic disturbance consisting of hyperinsulinemia and insulin resistance, which may have important implications for long-term health.[1] For example, recent data provide evidence that hyperinsulinemic women with PCOS have an increased risk to develop cardiovascular disease.[3] Moreover, insulin resistance in the general population is commonly associated with an abnormal lipoprotein pattern, particularly low serum levels of high-density lipoprotein cholesterol (HDL-C), hypercholesterolemia, and high triglyceride levels.[4] Obesity is frequently found in women with PCOS and may be involved as a significant factor in the pathogenesis of the syndrome.[5,6] The high prevalence of obesity in PCOS, with a range of 30% to 60%,[7] has profound clinical implications for these patients in terms of morbidity due to insulin resistance, diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disease.[6]

Considering the well-established relationship between insulin resistance and obesity, the relative contribution of these two parameters to the endocrinometabolic disturbances of PCOS has not been clearly elucidated. It was recognized that the body mass index (BMI) could be an important determinant[8,9]; in a similar fashion, other investigators have divided subjects into insulin-resistant or non–insulin-resistant groups based on comparable non-PCOS reference subjects of similar BMI.[10,11] Nevertheless, none of the studies have clearly highlighted the differential impact of insulin and obesity on the disorders of the syndrome.

In an attempt to better clarify the endocrinometabolic disorders of PCOS, we assessed hormonal variables and lipid patterns in lean and obese PCOS patients subdivided into hyperinsulinemic and normoinsulinemic groups.

SUBJECTS AND METHODS

The study population consisted of 110 consecutive recruitable women with PCOS who presented to our ambulatory clinic. All women were native Italians aged 17 to 33 years. All were healthy and euthyroid and had normal renal function as demonstrated by normal creatinine clearance. All patients had spontaneous onset of puberty and normal sexual development, and all had oligomenorrhea with chronic anovulation since puberty. PCOS was diagnosed by clinical findings (presence of amenorrhea or oligomenorrhea and hirsutism), plasma androgen levels at the upper limit or above the normal range (androstenedione, 0.57 to 1.6 ng/mL; testosterone, 0.17 to 0.58 ng/mL), and bilaterally normal or enlarged ovaries with the presence of at least seven to 10 microcysts (<5 mm diameter) at the time of ultrasonography. All diagnoses were confirmed by laparoscopy. A normal luteinizing hormone (LH) to follicle-stimulating hormone ratio (FSH) was not considered an exclusion criterion.[12] No patient received any medication known to affect carbohydrate metabolism for at least 3 months before the study. Obesity was defined as a body mass index (BMI) greater than 25 (normal range, 19 to 25), calculated as the body weight in kilograms divided by the height in meters squared. Informed consent was obtained

From the Department of Obstetrics and Gynecology, Catholic University of Sacred Heart, Rome; and the OASI Institute for Research, Troina, Italy.

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Address reprint requests to Antonio Lanzone, MD, Department of Obstetrics and Gynecology, L.go A. Gemelli 8, 00168 Rome, Italy.

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from each patient, and the study protocol was previously approved by our Institutional Review Board.

All studies were performed in the follicular phase 5 to 8 days after spontaneous or progestin-induced (medroxyprogesterone acetate 10 mg/d orally for 5 days) menses. The patients were hospitalized; after fasting overnight for 10 to 12 hours, blood samples were collected for basal hormone and lipoprotein assay. Then, the patients underwent a gonadotropin-releasing hormone (GnRH) test performed by insertion of indwelling catheters into the antecubital veins of both arms, one for blood sampling and the other for GnRH stimulus (bolus 100 μg). Blood samples were collected basally and 15, 30, 60, 90, and 120 minutes after GnRH administration. Samples for hormone assay were promptly centrifuged and the plasma was stored at −20°C until assay, whereas samples for biochemical assay were assayed immediately.

Plasma levels of estradiol, estrone, testosterone, dehydroepiandrosterone sulfate (DHEAS), androstenedione, 17-hydroxyprogesterone (17-OHP), cortisol, LH, FSH, progesterone, prolactin, sex hormone–binding globulin (SHBG), triglycerides, HDL-C, very–low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), cholesterol, and nonesterified fatty acid (NEFA) were determined in basal conditions. Moreover, LH and FSH were assayed during GnRH stimulus.

After a standard carbohydrate diet (300 g/d) for 3 days and an overnight fast for 10 to 12 hours, patients underwent an oral glucose tolerance test (OGTT) performed as follows. At 8 AM, an indwelling catheter was inserted into the antecubital vein of one arm. Blood samples were collected basally and, after ingestion of 75 g glucose in 150 mL water within 5 minutes, at 30, 60, 90, 120, 180, and 240 minutes. Insulin and glucose were assayed in all samples. Glucose concentrations were determined immediately, whereas samples for insulin assay were promptly centrifuged and the plasma was stored at −20°C until assay.

All hormone concentrations were determined by commercial radioimmunoassay kits (Radim, Pomezia, Italy). Gonadotropins and insulin were assayed by a double-antibody method, and all steroids were assayed by the dextran-charcoal separation technique. Plasma glucose was determined by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Palo Alto, CA). For each determination, all samples from the same patient were assayed simultaneously. The intraassay and interassay coefficients of variation were less than 8% and 15%, respectively, for all determinations.

Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). HDL-C concentrations were determined after precipitation of chylomicrons, VLDL-C, and LDL-C (Boehringer, Mannheim, Germany). VLDL-C was separated (as the supernatant) from LDL-C and HDL-C by lipoprotein ultracentrifugation. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL-C from the bottom fraction after ultracentrifugation. NEFAs were determined by an acylcoenzyme A oxidase–based colorimetric method.

A normal glycemic response to the OGTT was defined according to the criteria of the National Diabetes Data Group.[13]

All results are expressed as the mean ± SEM. Insulin, glucose, LH, and FSH plasma concentrations are also expressed as the area under the curve (AUC) after glucose ingestion or GnRH bolus, as calculated by the trapezoidal rule. The patients were classified as normoinsulinemic and hyperinsulinemic according to the insulin response to the OGTT, using a cutoff value of 15,000 μIU/mL · 240 min for the AUC as previously described.[14] The free androgen index (FAI) was estimated by the testosterone and SHBG plasma concentration according to the formula, [testosterone] · (6.11 − 2.38 · $\log_{10}$ [SHBG]).[15]

Data were stored and analyzed using the SPSS program (Statistical Package for Social Science, release 5.0; SPSS, Chicago, IL) on an IBM-compatible computer. The Kolmogorov-Smirnov test was performed to assess differences in the general shape of the distribution. Data were analyzed by the nonparametric Kruskal-Wallis test for multiple comparisons. The analysis of contingency was made with Fisher's exact test. A P value less than .05 was considered significant.

RESULTS

Based on the plasma insulin response to the OGTT, these PCOS women were divided into four groups: 69 patients (63%) were classified as hyperinsulinemic (45 obese [HO] and 24 lean [HL]), and the remaining 41 (37%) were normoinsulinemic (17 obese, [NO] and 24 lean [NL]).

Table 1 shows endocrine, metabolic, and clinical parameters of the study groups. No differences were found for age in the four groups or for the BMI within the lean and obese PCOS women divided into normoinsulinemic and hyperinsulinemic groups. The HO group showed a higher insulin AUC compared with the HL group ($P < .01$); to verify whether this difference could influence our results, we also reanalyzed all data while matching these two groups for the insulin AUC. We then decided to consider the entire HO group, since we obtained similar results before and after matching for insulin levels.

The HL group showed the lowest value for fasting glucose, which was significantly different compared with all other groups. For the glycemic response to the OGTT, only HO patients showed impaired glucose tolerance, with an incidence of 15.5%. This condition is responsible for the higher glycemic AUC values found in this group compared with the lean groups. Fasting plasma insulin levels were higher in both hyperinsulinemic groups versus the NL group, whereas the difference between NL and NO groups did not reach statistical significance. For the steroids, we found lower 17-OHP levels in HL women compared with both obese groups and higher DHEAS values in HO patients compared with both lean groups.

Figure 1 shows other endocrine variables of the study groups. No differences were found in basal gonadotropin levels or the LH/FSH ratio among the patients when divided according to the BMI and insulin levels. However, HL subjects showed a higher LH response to GnRH stimulus compared with NL subjects, whereas we failed to find a statistically significant difference in a similar comparison within obese subjects.

Figure 2 shows testosterone and SHBG concentrations and FAI values in the four groups. A trend for increased plasma testosterone and decreased SHBG was found in the study patients in relation to hyperinsulinemia and obesity, thus resulting in a higher FAI in the HL and NO groups versus the NL group. The HO group showed a further increase in FAI values.

The lipoprotein lipid profile of the four groups is presented in Table 2. NL subjects had lower triglyceride concentrations versus the other three groups, whereas no significant differences were found among the study subjects for NEFA or total cholesterol plasma levels, except for the comparison of total cholesterol concentrations between the NL and HO groups. For the other analyzed variables, similar values were found for HDL-C, LDL-C, and VLDL-C within lean or obese women divided as a function of the insulin AUC. By contrast, the obese groups showed higher LDL-C and VLDL-C and lower HDL-C compared with their lean counterparts.

Table 1. Clinical, Endocrine, and Metabolic Characteristics of the Subjects

Characteristic	NL	HL	NO	HO
No. of subjects	24	24	17	45
Age (yr)	25.59 ± 1.09	25.29 ± 1.34	25.67 ± 1.24	26.62 ± 0.81
BMI (kg/m²)	21.63 ± 0.42	22.28 ± 0.31‖#	30.27 ± 1.88	30.62 ± 0.63
Fasting glucose (mg/dL)	81.05 ± 2.23	77.55 ± 1.08¶	83.67 ± 2.77†	85.15 ± 1.66‡
Glycemic AUC (mg/dL · 240 min)	14,948 ± 325	14,871 ± 377‖	14,751 ± 644	17,002 ± 630¶
Fasting insulin (μIU/mL)	9.64 ± 1.05	14.35 ± 1.72¶	15.20 ± 3.00	18.89 ± 1.75#
Insulin AUC (μIU/mL · 240 min)	11,306 ± 480	22,314 ± 1,297*#	11,206 ± 686‖	26,726 ± 1,485‡#
IGT (n)	0/24	0/24	0/17	7/45 (15.5%)
Estrone (pg/mL)	23.67 ± 4.62	35.29 ± 3.14¶	44.33 ± 15.07	45.73 ± 2.92†#
Estradiol (pg/mL)	32.90 ± 4.67	49.33 ± 4.11#	54.01 ± 8.39¶	40.56 ± 2.99
Androstenedione (ng/mL)	1.63 ± 0.11	1.70 ± 0.13	1.91 ± 0.15	1.97 ± 0.13
DHEAS (ng/mL)	1,623 ± 136.60	1,614 ± 142.27§	1,699 ± 232.80	2,014 ± 95.23¶
17-OHP (ng/mL)	0.63 ± 0.05	0.54 ± 0.04‖	1.10 ± 0.25‡	0.72 ± 0.04
Cortisol (ng/mL)	125.27 ± 8.09	118.05 ± 6.91	158.55 ± 17.85	135.80 ± 6.92
Progesterone (ng/mL)	0.45 ± 0.06	0.46 ± 0.08	0.50 ± 0.12	0 43 ± 0.04
Prolactin (ng/mL)	12.54 ± 1.64	18.37 ± 3.36	12.80 ± 2.09	16.95 ± 1.35¶

Abbreviation: IGT, impaired glucose tolerance.
*$P < .01$ *v* NO.
†$P < .05$ *v* HL.
‡$P < .01$ *v* HL.
§$P < .05$ *v* HO.
‖$P < .01$ *v* HO.
¶$P < .05$ *v* NL.
#$P < .01$ *v* NL.

DISCUSSION

The prevalence of obesity in PCOS is higher than in the normal population.[16] Most women with PCOS become overweight just before or during puberty, and several lines of evidence suggest that the onset of obesity in this period of life could represent a specific factor for subsequent development of PCOS.[17] Moreover, the excess body fat in patients with this syndrome is associated with hyperandrogenism and other metabolic disturbances. In particular, the relationship between the BMI and insulin resistance is well established in normal subjects[18] and PCOS patients.[19]

Considering the endocrinometabolic effects of hyperinsulinemia in PCOS,[3,20,21] it is difficult to assess the relative contribution of elevated plasma insulin levels or obesity to the hormonal and metabolic variables in such patients. Many attempts have been targeted at this specific point; nevertheless, all of the investigators tried to separate the study population according to the BMI[22,23] or the insulin response to an OGTT,[20,24] eventually analyzing the impact of other variables within defined groups with correlation coefficients.[20]

On the other hand, there is controversy as to whether the insulin resistance of PCOS results from PCOS itself or from the obesity that is frequently present.[19,25-27] In a recent study,[14] we showed that there may be a discrepancy between insulin resistance and hyperinsulinemia. In fact, in our population, there was a group of lean hyperinsulinemic subjects who showed an exaggerated insulin response to a glucose load without peripheral insulin resistance. These patients could constitute an interesting study group to assess the effects of hyperinsulinemia on the endocrinometabolic features of PCOS without confounding effects such as obesity. A similar reasoning is possible for the evaluation of the impact of obesity on the above-mentioned features in the NO group. For this reason, we studied PCOS patients in relation to the BMI and insulin secretion; to our knowledge, this is the first study to address this specific point.

First, we showed that obesity and hyperinsulinemia have a synergistic effect on glucose tolerance in PCOS patients. In fact, only the HO group showed impaired glucose tolerance, with a prevalence of about 15% (the difference versus the other groups was not significant), thus confirming previous data from the literature.[19,28] Furthermore, HL subjects showed lower fasting glucose compared with NL subjects, although the glucose response to the OGTT was similar in the two groups. The different fasting glycemic levels could be explained by the different circulating insulin levels. Nevertheless, the similar response to the oral glucose load in lean subjects despite different insulin secretory patterns is surprising. These data confirm our previous observations[14] and could be explained, in part, by an increased β-cell mass or enhanced sensitivity to glucose in PCOS.

Concerning the gonadotropin patterns in our population, we did not find any difference in basal LH or FSH plasma levels or the LH/FSH ratio. However, the hyperinsulinemic groups showed an increased LH response to GnRH stimulus compared with the normoinsulinemic counterparts, although the difference was significant only within lean subjects. It could be hypothesized that insulin has an important role in determining the hypersensitivity of gonadotroph cells to GnRH stimulus at the pituitary level, in keeping with previous data from the literature.[2,12] Most[2,29,30] but not all[31] studies using metformin or troglitazone have also shown reduced LH levels in conjunction with suppression of insulin levels in PCOS women, suggesting an enhancing influence of insulin on LH secretion. By contrast, other investigators[21] showed that obesity in PCOS is associated with an attenuation of the LH pulse amplitude and LH response

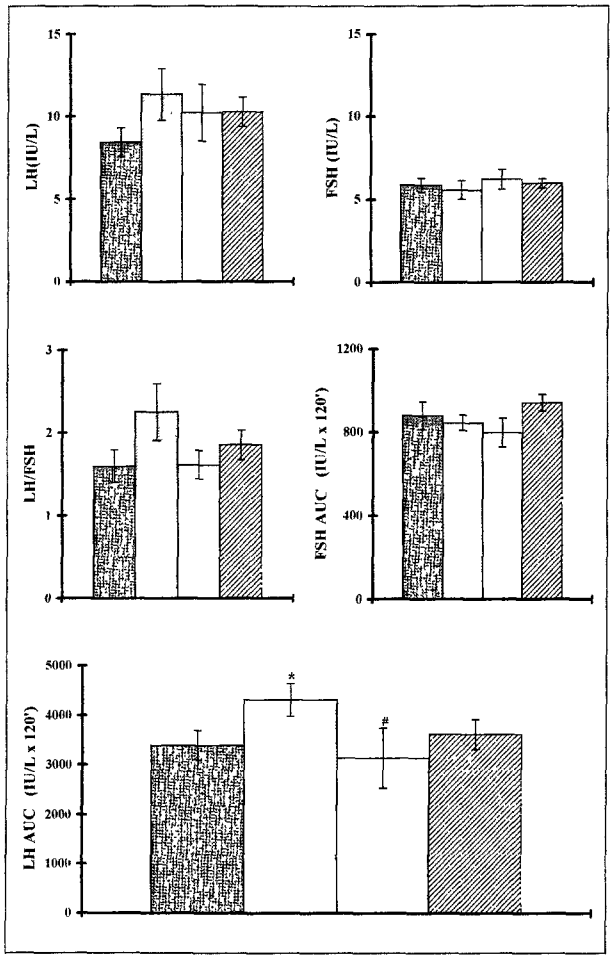

Fig 1. Gonadotropin (LH and FSH) levels, the LH/FSH ratio, and gonadotropin response to GnRH bolus evaluated as the AUC, in NL (□), HL (▨), NO (▩), and HO (▧) women with PCOS. Data are the mean ± SEM. *$P < .05$ *v* NL; #$P < .05$ *v* HL.

to GnRH not accounted for obesity per se but also for insulin resistance, thus suggesting a blunting effect of hyperinsulinemia and reduced insulin sensitivity on LH levels in PCOS.[32] A possible explanation for this discrepancy could be a different impact of hyperinsulinemia in lean and obese patients; in fact, in the latter, the main cause of hyperinsulinemia could be reduced peripheral insulin sensitivity, hypothetically present also at the pituitary level. By contrast, in HL patients, the increased circulating insulin levels could act directly or indirectly by sensitizing gonadotroph cells. This seems to be confirmed by the fact that within the hyperinsulinemic subjects, the obese group showed a slightly but not statistically significant lower LH response to GnRH versus the lean group, despite a greater insulin-secretory response by β cells. On the other hand, these results do not seem to be ascribable to possibly different dosages per kilogram of body weight, since we used a maximal GnRH stimulus (100 μg), greater than the stimulus reported in other studies (10 μg).[21,32]

Conflicting reports exist about the insulin influence on adrenal steroidogenesis. We have previously shown that insulin can influence the responsiveness of the adrenal to its trophic hormones.[24] On the other hand, in women, improving insulin sensitivity has been associated with no change,[33] a decrease,[29] or an increase[34] in DHEAS levels. In the present study, only HO patients showed increased DHEAS with respect to the other groups, thus supporting a synergistic effect of body fat and hyperinsulinemia on adrenal steroidogenesis.

In our series, the FAI seems to be independently influenced by both insulin and body fat; this effect seems related to a trend for lower SHBG values and higher testosterone levels in relation to hyperinsulinemia and obesity. Furthermore, the coexistence of exaggerated insulin secretion and obesity resulted in a synergistic additive effect on unbound testosterone.

Concerning the lipid features of our patients, we have found that insulin plays an important role in the regulation of triglyceride levels, in accordance with other reports.[35-37] Moreover, we found similar HDL-C values within lean and obese PCOS women divided as a function of the insulin secretory pattern, thus showing that body fat, rather than insulin, is a main determinant of HDL-C levels in PCOS.

In conclusion, the design of this study, for the first time including a substantial number of two new classes, ie, HL and NO PCOS patients, was used to assess the relative contribution of obesity and insulin levels in determining the endocrinometabolic features of PCOS by analyzing the involvement of one of these two variables in the absence of the confounding influence of the other. From the bulk of our data, it can be hypothesized that insulin has a main role in influencing the LH response to GnRH stimulus. Furthermore, obesity alone seems able to determine a change in lipid patterns, whereas insulin and body fat seem to play a synergistic role in influencing adrenal steroidogenesis, glucose tolerance, androgen levels, and FAI values.

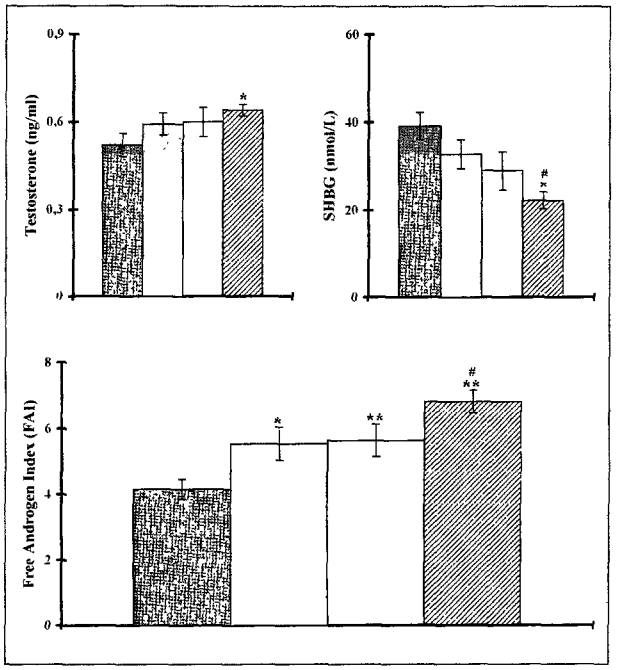

Fig 2. Testosterone SHBG, and FAI in NL (□), HL (▨), NO (▩), and HO (▧) women with PCOS. Data are the mean ± SEM. *$P < .05$ *v* NL; **$P < .01$ *v* NL; #$P < .05$ *v* HL.

Table 2. Lipid Profiles in the Study Subjects

Parameter	NL	HL	NO	HO
Triglycerides (mg/dL)	65.40 ± 8.59	94.50 ± 12.34‡	108.40 ± 16.49§	135.69 ± 11.69‡¶
NEFA (mEq/L)	0.54 ± 0.14	0.58 ± 0.13	0.53 ± 0.05	0.52 ± 0.03
Cholesterol (mg/dL)	173.09 ± 9.05	184.91 ± 6.91	189.10 ± 12.90	204.58 ± 10.14†
HDL-C (mg/dL)	59.39 ± 3.28	63.70 ± 4.68*	41.70 ± 2.95‡¶	48.44 ± 2.77†
LDL-C (mg/dL)	90.78 ± 8.95	106.61 ± 5.70*	121.38 ± 9.70†	133.36 ± 9.63‡
VLDL-C (mg/dL)	16.11 ± 3.14	17.22 ± 2.37*	26.63 ± 6.99†	26.16 ± 2.20‡

*$P < .01$ *v* HO.
†$P < .05$ *v* NL.
‡$P < .01$ *v* NL.
§$P < .05$ *v* NL.
¶$P < .01$ *v* HL.

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