Association of the Pro12Ala polymorphism in peroxisome proliferator-activated receptor 2 (PPARgamma2) with decreased Basic Metabolic Rate (BMR) in women with polycystic ovary syndrome (PCOS).

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Objective: The peroxisome proliferator-activated receptor (PPAR)gamma is a transcription factor involved in glucose homeostasis and energy metabolism. A missense mutation at codon 12 in the PPARgamma2 has been associated with increased body mass index and attenuated Insulin Resistance (IR) in PCOS. We have recently shown a decreased Basic Metabolic Rate (BMR) in PCOS. The aim of the present study was to determine the prevalence of the polymorphism Pro12Ala of the PPAR gamma2 gene and its associations with indices of IR, and BMR in lean and slightly overweight PCOS women.

Design: Case-control association study involving 156 PCOS women with biochemical hyperandrogenism and chronic anovulation and polycystic ovarian morphology on ultrasound and 56 unrelated healthy controls.

Methods: Hormonal determinations were performed by electrochemiluminescence quantitation or RIA. BMR was measured by indirect calorimetry. All subjects were genotyped by a PCR – RLFP assay.

Results: Genotype frequencies of the Pro12Ala polymorphism in (PPAR)gamma2 did not differ among PCOS women and control subjects. The presence of Pro12Ala polymorphism of PPARgamma2 was associated with lower BMR (p =0.04). This finding was valid in our subgroup of lean PCOS (BMI <25 Kg/m²), in which the Ala variant was also associated with higher total testosterone values.

Conclusion: The Pro12Ala polymorphism in the (PPAR)gamma2 gene is associated with decreased Basic Metabolic Rate (BMR) in women with polycystic ovary syndrome (PCOS) and biochemical hyperandrogenemia.
These young women are therefore at risk to increase their body weight and should restrict their energy intake by diet and enhance their energy expenditure by exercise.
Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder with prevalence in reproductive-aged women of approximately 6-10% (1). PCOS can be viewed as a heterogeneous androgen excess disorder with varying degrees of hormonal and metabolic abnormalities (2). Development of PCOS may require the interaction of multiple inherited and environmental factors; thereby PCOS appears to be a common and complex trait since the exact pattern of inheritance is yet to be fully explained.

It is well known that hyperinsulinemia and insulin resistance (IR) are common features of PCOS (3), while a substantial proportion of women with PCOS are overweight or obese. Obesity acts as an aggravating parameter of PCOS phenotype and weight loss is a usual manipulation in PCOS management. Reduction in body weight is known to restore ovulation and reduce androgen levels (3). Thus, metabolic features that make PCOS patients susceptible at gaining weight should be further investigated. Basal metabolic rate (BMR) represents the resting energy expenditure of an individual and a low BMR is considered a factor predisposing to obesity (4). Due to this fact, the role of many genes involved in insulin action and secretion, energy metabolism and adipogenesis has been investigated in order to elucidate the pathogenesis of PCOS.

The nuclear receptor peroxisome proliferator-activated receptor (PPAR)gamma is a transcription factor which modulates the expression of many genes involved in glucose and lipid homeostasis and regulates the adipocyte differentiation (5). Therefore, it is a strong candidate gene
predisposing to obesity via increased adiposity. PPARgamma functions are mediated by two major protein isoforms produced by alternative splicing: PPARgamma1, which is widely expressed, and PPARgamma2, which is most abundantly expressed in adipose tissue. A missense mutation in the PPARgamma2 specific domain, yielding the alanine-to-proline substitution at codon 12 (PPARgamma2 Pro12Ala SNP, refSNP ID: rs1801282), has been identified and related to functional consequences of PPARgamma2 (6,7). This genotype has been associated with increased body mass index (BMI) and attenuated IR in PCOS (8,9). Moreover, we have recently shown that women with PCOS, particularly those with IR, present a significantly decreased Basic Metabolic Rate (BMR) (10).

The aim of the present study was to determine the prevalence of the PPARgamma2 Pro12Ala SNP and its associations with indices of IR and BMR in a well characterized cohort of lean and slightly overweight PCOS women with biochemical hyperandrogenemia.

Materials and methods

Subjects
The study included 156 Caucasian Greek women with PCOS with a mean age of 22.46±4.41 years and a mean BMI of 25.7±0.64 kg/m² and 56 regularly menstruating, ovulatory women with a mean age of 22.91±1.5 years and a mean BMI 21.19±2.5 kg/m² as controls. The diagnosis of PCOS was based on the presence of biochemical hyperandrogenism, and chronic anovulation (fewer than six cycles in 12 months and serum Progesterone less than 5
ng/ml 20-24 days after a spontaneous bleeding) and polycystic ovarian
morphology on ultrasound, thus meeting both the criteria of the 1990 National
Institute of Child Health and Human Development Conference on PCOS (12)
and the experts meeting in Rotterdam in 2003, sponsored by the European
Society of Human Reproduction and Embryology (ESHRE) and the American
Society for Reproductive Medicine (ASRM) (13). Before recruitment, PCOS
women were followed up, until a spontaneous bleeding was achieved.
Biochemical hyperandrogenism was defined as increased serum testosterone
and/or increased free androgen index. We considered a woman as having
hyperandrogenemia when she had serum T levels and/or free androgen index
higher than two standard deviations above the mean T level of the controls.
Blood samples were collected between the 3rd and 4th days of a menstrual
cycle of healthy controls and on the 3rd and 4th day after a spontaneous
bleeding episode in women with PCOS, after an overnight fast. On the same
day, transvaginal ultrasound examination was performed.
All controls had normal ovulating cycles with serum Progesterone>10 ng/ml in
the luteal phase of the menstrual cycle (days 18th to 21st) and no signs of
clinical hyperandrogenism.
Exclusion criteria were congenital adrenal hyperplasia, androgen secreting
tumors and Cushing syndrome. All subjects had normal thyroid, kidney and
liver function.
All subjects gave written informed consent, and the study was performed
according to the guidelines of the Institutional Review Boards of the University
of Patras Medical School.
Methods

Physical measurements included weight, height, fat-free mass, fat mass. Biochemical parameters included serum testosterone, free testosterone, androstenedione, total cholesterol, triglycerides, HDL and LDL cholesterol, and glucose levels. A standard Oral Glucose Tolerance Test (OGTT) with 75g glucose was carried out. All PCOS women with Type 2 Diabetes or Impaired Glucose Tolerance were excluded from the study.

Free androgen index (FAI) was calculated according to the equation:

\[
\text{testosterone (nmol/l) \times 100/SHBG (nmol/l)}
\]

Insulin resistance was assessed by determining fasting insulin levels, fasting glucose levels, the fasting glucose/insulin ratio, as well as the HOMA and QUICKI indexes. Calculation of HOMA and QUICKI indices was made according to the following formulas:

\[
\text{HOMA-IR = Fasting Insuline (µIU/ml) \times Fasting glucose(mmol/l)} (14, 15),
\]

\[
\text{QUICKI =1 / log (Fasting insuline) + log (Fasting glucose)} (15).
\]

All assays for hormonal determinations were performed by electrochemiluminescence quantitation (Elecsys 2010, Roche Diagnostics, Laval, Quebec) with the exception of serum androstenedione and 17-OH progesterone which were determined by RIA using commercially available kits (BioSource, B-1400 Nivelles-Belgium).

BMR was measured by indirect calorimetry (Pulmolab EX505, Morgan Medical Ltd, Kent, U.K.) as previously described by Ferrannini (16) and expressed as kilocalories per day. Each subject’s BMR was adjusted for fat-free mass, fat mass, sex and age as previously described (16), using the equation: adjusted BMR = (group mean BMR) + (measured BMR - predicted...
BMR). For each subject the predicted BMR was obtained by substituting the individual lean body mass, fat mass, sex and age in the linear regression equation generated by the data of all patients. The study complied with the principles of the Helsinki Declaration; all subjects gave their informed consent.

Genotyping:
Genomic DNA was extracted from whole peripheral blood by the standard method of phenol/chloroform. All DNA samples were genotyping by a PCR - restriction fragment length polymorphism (RLFP) as previously described (5).

Statistical Analysis:
The genotype frequency distributions were tested for Hardy-Weinberg equilibrium and compared to the genotype frequencies of a group of normal subjects by chi-square tests. The Independent sample t-test, with two-tailed test of statistical significance, and the Levene’s Test for Equality of Variances were used to assess the statistical significance of the difference of the means between variables that were found to be normally distributed according to Kolmogorov-Smirnov test. Values that were normally distributed were BMR, Androstenedione, QUICKI and Free Testosterone. For all other non-Gaussian variables such as HOMA-IR, fasting insulin, Testosterone, Fasting glucose/Fasting insulin ratio, Age, BMI, 17-OH Prog and Free Androgen Index we used the Mann-Whitney test. A critical value of p<0.05 was considered significant. All statistical procedures were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA).
Results

Genotype frequencies of the Pro12Ala in the nuclear receptor peroxisome proliferator-activated receptor 2 (PPAR)gamma2 are presented in Table 1. Genotype frequencies were compared between PCOS women and a group of regularly menstruating, ovulatory women used as control group. No association of the polymorphisms with PCOS phenotype was detected. Within PCOS women, the presence of the polymorphism was related to the clinical and biochemical features of the PCOS phenotype which are presented in Table 2. All statistical procedures related to PPARgamma2 polymorphism were performed between the Pro/Pro and Pro/Ala plus the Ala/Ala subgroups (X/Ala), as the Ala/Ala group was too small to provide statistical power.

The presence of Pro12Ala polymorphism of PPARgamma2 was associated with BMR, since the Ala allele carriers had lower BMR compared with the Pro/Pro women (1475.7±678.6 Kcal/day versus 893.2±312.3 Kcal/day, p =0.04) (Table 2) (Figure 1).

Subsequently, in order to further evaluate the relation of the Pro12Ala polymorphism of PPARgamma2 with Insulin Resistance (IR), we divided all PCOS women according to Insulin Resistance (IR) indices into 2 subgroups: those with IR (n=39) (Fast insulin>12mU/mL, Glu/Ins <6.4, HOMA>2.16, QUICKI<0.333), and those without IR (n=77) (Fast insulin<12mU/mL, Glu/Ins >6.4, HOMA<2.16, QUICKI>0.333). Forty (40) out of 156 PCOS women did not fit all the applied criteria for Insulin Resistance and therefore were not included in these two subgroups. Among PCOS women with and without IR, concerning the gene polymorphism studied, no statistically significant
differences were detected between groups with the normal or the polymorphic allele in all investigated clinical and biochemical features (p=ns).

However, when PCOS women were further divided in two groups according to BMI, PCOS women with BMI<25 Kg/m² (BMI=21.08±2.15 Kg/m²) carrying the Pro12Ala polymorphism had a BMR significantly lower than those without the polymorphic change (BMR=1481±594 Kcal/day, versus BMR=716±222 Kcal/day, respectively, p=0.035). In these lean PCOS women the Pro12Ala polymorphism of PPARgamma2 was also associated with higher total testosterone values but not to indices of Insulin Resistance. On the contrary, in PCOS women with BMI>25 Kg/m² (BMI=31.18±4.54Kg/m²) the association of the Ala variant was not replicated either with BMR or with total testosterone values.

Discussion

In the present study, we addressed the question whether PPARgamma2 Pro12Ala SNP is related to PCOS phenotype and quantitative traits of the syndrome such as obesity, Insulin Resistance (IR) and Basic Metabolic Rate (BMR) in a distinct phenotypic group of Caucasian young PCOS patients characterized by biochemical hyperandrogenemia, chronic anovulation and polycystic ovaries on ultrasound. Our PCOS women met both the diagnostic criteria of the 1990 NIH conference (12) and the revised consensus criteria of Rotterdam ESHRE/ASRM 2003 (13), hence bearing the form of the most severe PCOS with hyperandrogenemia and chronic anovulation (17), a suitable population for genotypic analysis.
In order to detect causative genetic variants for PCOS, confounding aggravating parameters of the syndrome's phenotypic feature such as obesity should be excluded from the study cohort. Therefore, ideally, lean and obese PCOS women should be studied separately (3) and compared to BMI-matched general population. However, there are few studies in the literature in which lean PCOS women have been separately studied (3) and no genetic association study, evaluating the effect of PPARgamma2 and BMR in lean PCOS women, has been reported to date.

We showed that, in women with PCOS, the presence of Pro12Ala polymorphism in the PPARgamma2 gene was associated with lower Basic Metabolic Rate (BMR). To our knowledge, this is the first study to demonstrate an association of the PPARgamma2 Pro12Ala SNP with lower BMR in PCOS.

The association of the Pro12Ala polymorphism with BMR was investigated only once in a cohort of very obese women but never in women with PCOS (8). In this study by Valve et al, no association of the Pro12Ala polymorphism with BMR was detected. It should be noted that PCOS women included in our study were normal weight or slightly overweight. This discrepancy might be due to the presence of elevated expression of PPARgamma by excessive fat mass which inhibits the suppression of insulin induced lipolysis through transcriptional alterations due to the Pro12Ala polymorphism. Therefore, the influence of the PPARgamma2 Pro12Ala SNP on BMR might be different among lean or obese subjects. Nevertheless, low BMR does not necessary mean high BMI, as BMR values in obesity are reported within normal range (18), or increased (19), while we have recently shown decreased BMR in
normal weight PCOS women (10). Nevertheless, the association of the Pro12Ala polymorphism with BMR was valid and in our subgroup of lean PCOS with BMI <25 Kg/m², excluding the confounding effect of obesity.

Thanks to the dual role of peroxisome proliferator –activated receptor gamma (PPARgamma) in adipogenic regulation and in modulation of intracellular insulin –signaling events, this molecule is in a prominent position in the list of various candidate genes involved in multi – genetic traits such as type 2 diabetes mellitus and PCOS (17). Concerning Pro12Ala allelic frequencies, our findings are in agreement with the majority of studies that did not detect any difference between PCOS women and the general population (20-29). On the contrary, in a Finnish population of PCOS women (30), the frequency of Ala allele was found to be reduced with a slight significance, suggesting a protective role against the development of the syndrome. Despite this general agreement concerning allelic frequencies, several studies reported different associations of the of Pro12Ala polymorphism in the PPARgamma2 gene with several phenotypic traits of hyperandrogenism and insulin resistance (IR) in PCOS women. Hara et al (9), in a multi ethnic study of obese PCOS women, reported that the Ala allele carriers were more insulin sensitive and presented a tendency to lower total and free testosterone levels. Hahn et al (27) reported lower hyperinsulinaemia and all parameters of IR in obese but not in lean PCOS subjects carrying the Ala allele. Tok et al (31) reported that lean women with PPARgamma2 Pro12Ala SNP had significant higher BMI, while, on the contrary, Yılmaz et al, in a population of similar ethnic origin lean PCOS women reported an association of the Pro12Ala with lower BMI and
lower waist-to-hip ratio, reduced androgen levels, less hirsutism, and lower insulin resistance index (32).

Although, concerning BMI, a statistically significant difference was noted between PCOS women and the control group (p< 0.001), still in our studied cohort no association between BMI and the presence of the Ala allele was detected neither in PCOS women nor in the control group. Nevertheless, after dividing our PCOS women according to BMI, PCOS women with BMI <25 Kg/m$^2$ (BMI=21.08±2.15 Kg/m$^2$) did not present any statistically significant difference in allelic frequencies with the matched for BMI control group.

In our study, we did not detect any association of the PPARgamma2 Pro12Ala SNP with neither BMI nor glucose homeostasis. Nevertheless, the PPARgamma2 12Ala allele was associated with higher testosterone levels in PCOS women of normal weight (BMI<25 Kg/m$^2$). These results are apparently contradictory with the findings of Hara et al (9) and Yilmaz et al (32) but it should be noted that these two studies refer to obese PCOS women. It is well known that testosterone induces lipolysis and therefore it is reasonable to find an association of the Ala variant with higher testosterone levels in these lean women with PCOS and biochemical hyperandrogenemia.

Furthermore, association studies of the Pro12Ala polymorphism in populations of different BMI demonstrated conflicting results. Among normal weight or slightly overweight subjects, the Ala12 variant was associated with lower than average BMI (7). Beamer et al, in two independent Caucasian populations found that the Pro12Ala was associated with higher BMI in subjects with morbid obesity, while in lean to moderately obese subjects a tendency towards a higher BMI was reported, which did not reach statistical
significance (33). Furthermore, Valve et al, reported an association of PPARgamma2 Pro12Ala SNP with severe overweight and increased fat mass among obese women (8). These results suggest that genetic variation at the PPARgamma - locus may influence susceptibility to obesity in humans in different ways.

The Ala allele has been shown to present lower affinity and trans-activation capacity than the Pro allele in pre-adipocytes cell lines, while both insulin sensitivity of glucose disposal and insulin sensitivity of lipolysis were greater in the subjects carrying the Ala allele (34). Indeed, in our study a tendency, which did not reach statistical significance probably due to small number of Ala carriers detected, was noted for lower fasting insulin levels in subjects carrying the Ala allele. Giving the fact that the messenger ribonucleic acid expression of PPARgamma2 is increased in adipose tissue of obese subjects, and is down regulated by a low calorie diet, the functional significance of the Pro12Ala substitution may be dependent on energy reserves, been different among normal weight, obese or morbid obese subjects (34). This observation could, at least partly, explain the contradictory associations of the Ala allele in different populations.

The finding that the presence of the Ala allele in adipocytes, through altered transcription activity of the receptor, enhances the suppressing insulin’s action on lipolysis, indicates a shift of the lipolysis-lipogenesis balance towards lipogenesis. Insulin-sensitive subjects are more likely to gain weight than insulin-resistant ones (35), therefore the subjects carrying the Ala allele in our cohort are prone to gain weight.
A major concern in our study is that in the control group used for allelic frequencies determinations no assessment of BMR was performed, therefore further studies are needed to determine the influence of the PPARgamma2 Pro12Ala SNP on BMR in distinct populations of lean, overweight and obese regularly menstruating, ovulatory women as well as in overweight and obese women with PCOS.

In conclusion, the results of this study demonstrate that the PPARgamma2 Pro12Ala SNP is associated with decreased Basic Metabolic Rate (BMR) in women with polycystic ovary syndrome (PCOS) and biochemical hyperandrogenemia when compared to Pro/ProPCOS women, still, an eventual similar association in regularly menstruated women can not be excluded. These young women are therefore at risk to increase their body weight and should restrict their energy intake by diet and enhance their energy expenditure by exercise.

Disclosure:

This study did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector, so there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure I: Basic Metabolism Rate (BMR) in women with PCOS according to the presence of the Pro12Ala PPARγ2 Genotype. Values are mean±SEM * are P<0.05
**Table I**: Genotype frequencies of the Pro12Ala PPARgamma2 gene polymorphism in women with PCOS and in the control group.

<table>
<thead>
<tr>
<th></th>
<th>PPARgamma2 Pro/Pro</th>
<th>PPARgamma2 Pro/Ala</th>
<th>PPARgamma2 Ala/Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCOS women</strong></td>
<td>n=136 (87.73%)</td>
<td>n=19 (11.66%)</td>
<td>n=1 (0.61%)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>n=48 (85.72%)</td>
<td>n=6 (10.71%)</td>
<td>n=2 (3.57%)</td>
</tr>
</tbody>
</table>
Table II: Clinical characteristics in PCOS women according to different PPARgamma2 variants. Values are mean±SD, Range.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Pro/Pro</th>
<th>X/Ala</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=156</td>
<td>n=136</td>
<td>n=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>22.82±4.99</td>
<td>22.81±5.17</td>
<td>22.92±3.12</td>
<td>0.54</td>
</tr>
<tr>
<td>(15-42)</td>
<td>(15-42)</td>
<td>(18-30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>25.62±6.44</td>
<td>25.76±6.69</td>
<td>24.40±3.49</td>
<td>0.94</td>
</tr>
<tr>
<td>(17.10-47.3)</td>
<td>(17.10-47.30)</td>
<td>(19-29.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Insulin (µU/mL)</td>
<td>9.88±5.88</td>
<td>9.97±6.05</td>
<td>9.12±4.42</td>
<td>0.81</td>
</tr>
<tr>
<td>(1.0-37.0)</td>
<td>(1.0-37.0)</td>
<td>(3.19-19.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose/insulin</td>
<td>11.85±12.06</td>
<td>11.98±12.59</td>
<td>10.75±5.94</td>
<td>0.73</td>
</tr>
<tr>
<td>(0.78-86)</td>
<td>(0.78-86.00)</td>
<td>(4.37-25.39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.32±2.45</td>
<td>2.35±2.56</td>
<td>2.08±1.19</td>
<td>0.66</td>
</tr>
<tr>
<td>(0.15-18.26)</td>
<td>(0.15-18.6)</td>
<td>(0.94-5.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.357±0.044</td>
<td>0.357±0.05</td>
<td>0.358±0.028</td>
<td>0.97</td>
</tr>
<tr>
<td>(0.257-0.525)</td>
<td>(0.257-0.525)</td>
<td>(0.308-0.414)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>0.77±0.04</td>
<td>0.77±0.40</td>
<td>0.80±0.386</td>
<td>0.67</td>
</tr>
<tr>
<td>(0.15-2.30)</td>
<td>(0.15-2.30)</td>
<td>(0.30-1.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free Testosterone (pg/mL)</td>
<td>2.99±1.77</td>
<td>2.97±0.18</td>
<td>3.11±1.30</td>
<td>0.79</td>
</tr>
<tr>
<td>(0.61-14.0)</td>
<td>(0.61-14)</td>
<td>(1.60-5.60)</td>
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<tr>
<td>17-OH Prog (ng/mL)</td>
<td>1.23±0.75</td>
<td>1.24±0.78</td>
<td>1.09±0.47</td>
<td>0.70</td>
</tr>
<tr>
<td>(0.34-5.50)</td>
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<td>(0.50-2.10)</td>
<td></td>
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<tr>
<td>Androstenedione (ng/mL)</td>
<td>3.31±1.33</td>
<td>3.30±1.32</td>
<td>3.40±1.48</td>
<td>0.79</td>
</tr>
<tr>
<td>(1.20-8.00)</td>
<td>(1.25-8)</td>
<td>(1.20-6.00)</td>
<td></td>
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<tr>
<td>Free androgen index</td>
<td>0.70±1.43</td>
<td>0.73±0.15</td>
<td>0.47±0.39</td>
<td>0.56</td>
</tr>
<tr>
<td>(0.04-15.07)</td>
<td>(0.04-15.07)</td>
<td>(0.06-1.06)</td>
<td></td>
<td></td>
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<tr>
<td>Basic Metabolic rate</td>
<td>1415.7±672.9</td>
<td>1475.7±678.6</td>
<td>893.2±312.4</td>
<td>0.04</td>
</tr>
<tr>
<td>(Kcal/day)</td>
<td>(328.2-3969)</td>
<td>(328.2-3969.0)</td>
<td>(532.2-1299.7)</td>
<td></td>
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<tr>
<td><strong>Cholesterol</strong></td>
<td>192.80±46.78</td>
<td>193.32±47.21</td>
<td>188.33±44.54</td>
<td>0.87</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td>(111-384)</td>
<td>(114-384)</td>
<td>(111-252)</td>
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<tr>
<td><strong>Triglycerides</strong></td>
<td>92.28±48.86</td>
<td>94.49±49.74</td>
<td>73.75±37.32</td>
<td>0.15</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td>(27-295)</td>
<td>(27-295)</td>
<td>(31-144)</td>
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<tr>
<td><strong>HDL-C</strong></td>
<td>56.74±13.76</td>
<td>55.57±12.21</td>
<td>66.58±21.36</td>
<td>0.10</td>
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<tr>
<td>(mg/dL)</td>
<td>(26-109)</td>
<td>(26-92)</td>
<td>(39-109)</td>
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<tr>
<td><strong>LDL-C</strong></td>
<td>116.91±41.15</td>
<td>118.07±41.51</td>
<td>107.28±38.29</td>
<td>0.54</td>
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<tr>
<td>(mg/dL)</td>
<td>(54-301)</td>
<td>(63.8-301)</td>
<td>(54-174)</td>
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