A Single Nucleotide Polymorphism in *STK11* Influences Insulin Sensitivity and Metformin Efficacy in Hyperinsulinemic Girls With Androgen Excess

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OBJECTIVE — Serine-threonine kinase STK11 catalyzes the AMP-activated protein kinase complex. We tested the hypothesis that a gene variant in *STK11* contributes to variation in insulin sensitivity and metformin efficacy.

RESEARCH DESIGN AND METHODS — We studied the effects of a single nucleotide polymorphism (SNP) (rs8111699) in *STK11* on endocrine-metabolic and body composition indexes before and after 1 year of metformin in 85 hyperinsulinemic girls with androgen excess, representing a continuum from prepuberal girls with a combined history of low birth weight and precocious pubarche over to postmenarchial girls with hyperinsulinemic ovarian hyperandrogenism. Metformin was dosed at 425 mg/day in younger girls and 850 mg/day in older girls. *STK11* rs8111699 was genotyped. Endocrine-metabolic features were assessed in the fasting state; body composition was estimated by absorptiometry.

RESULTS — Genotype effects were similar in younger and older girls. At baseline, the mutated G allele in *STK11* rs8111699 was associated with higher insulin and IGF-I levels (both $P < 0.005$). The response to metformin differed by *STK11* genotype: GG homozygotes ($n = 24$) had robust metabolic improvements, GC heterozygotes ($n = 38$) had intermediate responses, and CC homozygotes ($n = 23$) had almost no response. Such differences were found for 1-year changes in body composition, circulating insulin, IGF-I, free androgen index, and lipids (all $P < 0.005$).

CONCLUSIONS — In hyperinsulinemic girls with androgen excess, the *STK11* rs8111699 SNP influences insulin sensitivity and metformin efficacy, so that the girls with the least favorable endocrine-metabolic profile improve most with metformin therapy.

Diabetes Care 33:1544–1548, 2010

Genetic variation in enzymes and transporters mediating the actions and metabolism of medications contribute to interindividual variation in therapeutic response, on the efficacy as well as on the safety side (1).

Polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects ~5–10% of young women; PCOS is characterized by androgen excess plus either anovulation or polycystic ovaries (2,3). A majority of patients with PCOS are insulin resistant, and, accordingly, metformin is often prescribed for this condition, also in adolescents (4,5). In selected girls at high risk for developing hyperinsulinemic ovarian androgen excess, metformin is even under exploration as a potentially preventive treatment; among these high-risk girls are those with a combined history of low birth weight (LBW) and precocious pubarche (6–9).

The actions of metformin seem to be largely exerted through activation of AMP-activated protein kinase (AMPK), a conserved regulator of the cellular response to low energy, in many organs, including liver and skeletal muscle (10,11). The activation of AMPK in the liver is catalyzed by serine-threonine kinase (STK11, formerly known as LKB1), a tumor suppressor gene defective in Peutz-Jeghers syndrome (12); deletion of hepatic *STK11* in mice results in a nearly complete loss of AMPK activity, leading to adipogenesis and lipogenic gene expression (13). STK11 serves as a mediator of metformin effects, rather than as a direct target of metformin (14).

Recently, the C allele of a single nucleotide polymorphism (SNP) (rs8111699) in *STK11* has been associated with a reduced ovulatory response to metformin in women with PCOS (15). In a pilot study, we have tested the hypothesis that the same SNP in *STK11* also influences the endocrine-metabolic and body composition changes after metformin therapy in girls with hyperinsulinemic androgen excess.

RESEARCH DESIGN AND METHODS

The study population consisted of 85 Caucasian, Northern Spanish girls representing a continuum of female subjects with hyperinsulinemic androgen excess that commences in prepuberty in those girls with a combined history of LBW and precocious pubarche, is followed by a preclinical phase starting around menarche of biochemical ovarian hyperandrogenism and yields to an overt clinical period of ovarian hyperandrogenism 2–3 years after menarche. All subjects were included in similar clinical trials investigating the safety and efficacy of metformin treatment on their hyperinsulinemic androgen excess.

The prepubertal girls ($n = 18$; Tanner stage I; aged 7.9 ± 0.2 years; BMI 19.3 ± 0.6 kg/m^2) had a combined a history of LBW (2.3 ± 0.1 kg at term

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Received 18 September 2009 and accepted 18 March 2010. Published ahead of print at http://care.diabetesjournals.org on 31 March 2010. DOI: 10.2337/dc09-1750.
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birth) and precocious pubarche (appearance of pubic hair before age 8 years) attributable to an exaggerated adrenarche, based on high serum levels of dehydroepiandrosterone sulfate or androstenedione at diagnosis of precocious pubarche (8). The girls were, in addition, hyperinsulinemic, as judged by the insulin response after an oral glucose tolerance test (16). All of these subjects participated in a controlled long-term study exploring the effects of early metformin treatment (ISRCTN84749320); more specifically, they form together the study subgroup that was initially randomly assigned to receive metformin for at least 1 year (425 mg, daily at dinner time) (9).

The early postmenarcheal girls (n = 31; aged 12.2 ± 0.1 years; BMI 21.5 ± 0.5 kg/m^2) manifested biochemical androgen excess characterized as the ensemble of *1*) absence of clinical signs or symptoms of androgen excess (17) and *2*) hyperinsulinemia (oral glucose tolerance test) and *3*) ovarian androgen excess suggested by increased testosterone levels and confirmed by 17-hydroxyprogesterone hyperresponse to gonadotropin-releasing hormone agonist (17,18). The girls in the present analysis were enrolled in clinical studies that were initiated before 2003, thus before compulsory trial registration; as described, all these girls had a history of LBW-precocious pubarche and were already in the early postmenarche phase (6–12 months beyond menarche) at start of metformin intervention (8,19).

The adolescent girls (n = 36; aged 15.5 ± 0.3 years; BMI 21.9 ± 0.5 kg/m^2) were consecutive patients with PCOS who were at least 2 years beyond menarche and who were followed in the outpatient clinic according to standard procedures, including yearly blood sampling in the fasting state (once with DNA extraction) and yearly assessment of body composition. The inclusion criteria were the same as those for the early postmenarcheal girls, except that the presence of clinical markers of androgen excess was now required: hirsutism (Ferriman-Gallwey score >8) plus either amenorrhea (menses absent for >3 months) or oligomenorrhea (menstrual cycle >45 days). In all postmenarcheal girls, baseline assessments were performed in the follicular phase (day 3–7) or after 2 months of amenorrhea.

All postmenarcheal girls received metformin for at least 1 year in monotherapy (850 mg, daily at dinner time). At the start of metformin treatment, none of the 85 girls presented evidence for thyroid dysfunction, Cushing syndrome, hyperprolactinemia, glucose intolerance, or late-onset adrenal hyperplasia; for at least 6 months before the start of metformin start, none of the girls received an estro-progestagen or other medication known to affect gonadal function or carbohydrate metabolism.

Clinical, endocrine, and metabolic assessments

Height and weight were measured, and BMI was calculated (weight in kilograms divided by the square of height in meters). BMI standard deviation scores (SDSs) were derived from regional normative data (20).

Fasting blood glucose, serum insulin, IGF-I, sex hormone–binding globulin (SHBG), and testosterone were measured as described previously (9). Free androgen index was calculated as testosterone × 100/SHBG. Serum lipids were measured by routine laboratory methods.

Genetic analysis

Genomic DNA was purified from whole blood samples using commercial reagents (Gentra Puragene Cell Kit; Qiagen Iberia, Madrid, Spain). The *STK11* rs8111699 SNP was genotyped by a fluorescent genotyping system (KASPar SNP Genotyping System; KBiosciences, Hoddesdon, U.K.). In brief, 10 pg genomic DNA was incubated in a 8-μl solution of a combined mix containing 100 mol/l allele specific primer 1 (FAM, *x*-axis, allele G), 5′-GAAGGTGACCAAGTTCATGCTCCACTGACTGTTCAGGAAAGCAC-3′, 100 mol/l allele-specific primer 2 (VIC, *y*-axis, allele C), 5′-GAAGGTCGGAGTCAACGGATTCCACTGACTGTTCAGGAAAGCAG-3′, 100 mol/l common reverse primer 5′-GGTGGAAGCCTGACTGTGAGAGT-3′, 10 mol/l Tris-HCl, pH 8.3, KTaq polymerase, 1.8 mmol/l $MgCl_2$, and ROX as a passive reference. The PCR mix was incubated at 94°C for 15 min, followed by 20 cycles of 94°C for 10 s, 57°C for 5 s, and 72°C for 10 s. Thereafter, the PCR mix was incubated for 18 cycles at 94°C for 10 s, 57°C for 20 s, and 72°C for 40 s. Samples were then read in a fluorescence plate reader. The KASPar system uses the fluors FAM and VIC for distinguishing among genotypes and ROX as a passive reference. The corresponding genotypes for this SNP (CC vs. CG vs. GG) did not differ among prepuberty (33% vs. 44% vs. 22%), early postmenarche (26% vs. 49% vs. 26%), and PCOS (25% vs. 42% vs. 33%) subjects in our study (P = 0.89). The distributions were also tested for Hardy-Weinberg equilibrium, and none deviated significantly.

Body composition

Body composition was assessed by dual-energy X-ray absorptiometry with a Lunar Prodigy and Lunar software (version 3.4/3.5; Lunar, Madison, WI) (9). Total irradiation dose per assessment was 0.1 mSevert; coefficients of variation for scanning precision are 2.2 and 2.6% for fat and lean mass (8,9,19).

Ethics and statistics

The study protocol was approved by the Institutional Review Board of Hospital of Sant Joan de Déu. Informed consent was obtained from the patients and/or from parents, and assent was obtained from girls, as appropriate. The clinical, biochemical, and body composition data from prepubertal and early postmenarcheal subjects have been reported separately (8,9,19). Statistical analyses were performed using SPSS (version 12.0; SPSS, Chicago, IL). Quantitative phenotypic data were compared across genotypes by a repeated-measures general linear model, computing the genotype as the between-subjects effect and both baseline and 1-year values as the within-subjects effect. Differences in 1-year changes across genotypes were tested by the interaction term among the between- and within-subjects effects. $P < 0.05$ was considered statistically significant.

RESULTS — Clinical and laboratory characteristics of the study subjects are shown in Table 1. Genotype effects were similar in prepubertal, early postmenarcheal, and PCOS subjects, and therefore genotype-phenotype associations are presented for all 85 girls studied as a single group (adjusted for group allocation) to maximize the statistical power.

At diagnosis, the G allele in *STK11* rs8111699 was associated with a poorer metabolic profile, specifically with higher insulin and IGF-I (both $P < 0.005$) (Table 2). The presence of this allele was at baseline unrelated to age, weight, body composition parameters, and serum androgens and lipids (Table 2).

Table 1—*Clinical, biochemical, and body composition variables before and after 1 year of metformin in the study subjects*

	All		Prepubertal		Early postmenarcheal		PCOS	
	Baseline	1 year of metformin	Baseline	1 year of metformin	Baseline	1 year of metformin	Baseline	1 year of metformin
n	85	85	18	18	31	31	36	36
Age (years)	12.6 ± 0.3	—	7.9 ± 0.2	—	12.2 ± 0.1	—	15.5 ± 0.3	—
BMI (kg/m^2)	21.2 ± 0.3	21.4 ± 0.3	19.3 ± 0.6	19.7 ± 0.6	21.5 ± 0.5	21.7 ± 0.5	21.9 ± 0.5	22.0 ± 0.5
BMI SDS	0.8 ± 0.1	0.9 ± 1.1	1.5 ± 0.3	1.5 ± 0.4	0.6 ± 0.3	0.7 ± 0.4	0.8 ± 0.3	0.8 ± 0.2
Glucose (mg/dl)	88 ± 1	88 ± 1	88 ± 2	91 ± 2	89 ± 1	90 ± 1	87 ± 1	86 ± 1
Insulin (mIU/l)	12.7 ± 0.5	9.9 ± 0.4†	9.6 ± 1.1	9.3 ± 0.9	13.2 ± 0.7	10.4 ± 0.7*	13.8 ± 0.9	9.8 ± 0.7†
IGF-I (ng/ml)	286 ± 10	254 ± 7†	200 ± 17	219 ± 12	342 ± 17	283 ± 13†	287 ± 10	245 ± 7*
SHBG (μg/dl)	1.1 ± 0.1	1.3 ± 0.1†	1.3 ± 0.1	1.5 ± 0.1*	1.1 ± 0.1	1.3 ± 0.1*	1.0 ± 0.1	1.2 ± 0.1†
Testosterone (ng/dl)	73 ± 5	52 ± 3†	35 ± 4	30 ± 4	71 ± 5	52 ± 3†	94 ± 8	62 ± 4†
Free androgen index	8.7 ± 0.8	4.6 ± 0.3†	3.3 ± 0.4	2.5 ± 0.4*	8.1 ± 0.9	4.7 ± 0.4†	12.0 ± 1.6	5.6 ± 0.6†
Triglycerides (mg/dl)	79 ± 4	64 ± 3†	77 ± 12	70 ± 11	75 ± 5	62 ± 3†	82 ± 6	62 ± 4*
LDL cholesterol (mg/dl)	99 ± 3	89 ± 2†	107 ± 8	94 ± 5	93 ± 4	87 ± 3	101 ± 4	89 ± 4*
HDL cholesterol (mg/dl)	56 ± 1	62 ± 1†	57 ± 3	60 ± 3	53 ± 2	59 ± 2*	57 ± 2	66 ± 2†
LDL cholesterol-to-HDL cholesterol ratio	1.9 ± 0.1	1.5 ± 0.1†	2.0 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.4 ± 0.1†
Fat mass (kg)	17.1 ± 0.8	16.8 ± 0.8	11.4 ± 0.9	12.2 ± 1.0	17.7 ± 1.2	17.5 ± 1.2	19.5 ± 1.3	18.9 ± 1.3*
Abdominal fat (kg)	5.1 ± 0.3	4.7 ± 0.3†	3.5 ± 0.4	3.5 ± 0.4	4.8 ± 0.4	4.5 ± 0.3	6.2 ± 0.5	5.5 ± 0.5†
Lean mass (kg)	31.2 ± 0.7	33.0 ± 0.7†	21.2 ± 0.8	23.9 ± 0.9†	33.2 ± 0.7	35.0 ± 0.6†	34.5 ± 0.7	35.9 ± 0.7†

Values are means ± SEM. Values in healthy girls matched for BMI: prepubertal (n = 24; aged 8.3 ± 0.3 years): insulin 6.5 ± 0.5 mIU/l, IGF-I 200 ± 15 ng/ml, SHBG 3.6 ± 0.1 μg/dl, testosterone 16 ± 2 ng/dl; pubertal (n = 24; age 15.3 ± 0.2 years): insulin 8.8 ± 0.4 mIU/l, IGF-I 384 ± 26 ng/ml, SHBG 1.9 ± 0.1 μg/dl, testosterone 31 ± 3 ng/dl. Lipid profile: triglycerides 55 ± 4 mg/dl; LDL 83 ± 3 mg/dl; HDL 65 ± 2 mg/dl (8,23 and Ibáñez et al.; JCEM 2004; 89:4,331–337). P values are from paired-samples Student t-test. *$P < 0.005$; †$P < 0.0001$.

After 1 year of therapy, there were pronounced differences in the response to metformin according to the *STK11* rs8111699 genotype, as reflected by a robust response in the main endocrine-metabolic and body composition parameters in GG homozygotes, with an intermediate response in CG heterozygotes and almost no response in CC homozygotes (Table 2). Such differences were found for 1-year changes in body composition, circulating insulin, IGF-I, free androgen index, and lipids (all $P <$ 0.005; Table 2). All of these comparisons were adjusted for differences in baseline insulin levels.

CONCLUSIONS— Our results indicate that the *STK11* rs8111699 SNP influences both insulin sensitivity and metformin efficacy in hyperinsulinemic girls with androgen excess. Despite the fact that neither STK11 nor AMPK is a direct target of metformin (14), both are necessary for metformin actions (14). It has been proposed that STK11 is constitutively active and that metformin exerts its hypoglycemic effects through a modification in AMPK, rendering it a better substrate for STK11 (10). Although the role of the studied intronic SNP in *STK11* has yet to be defined, it is known that intronic SNPs may regulate gene expression and alternative splicing (21). The *STK11* rs8111699 SNP may also be in linkage disequilibrium with an active SNP in the kinase gene region.

The *STK11* rs8111699 SNP was associated with baseline insulin sensitivity in girls with hyperinsulinemic androgen excess. Bearing in mind that STK11 may be constitutively active, we postulate that carrying the G allele in the *STK11* rs8111699 SNP can result in lower STK11 activity and therefore lower efficiency of AMPK phosphorylation by STK11.

The same G allele in the *STK11* rs8111699 SNP was associated with pronounced changes in endocrine-metabolic and body composition parameters upon metformin treatment, effects that were nearly absent in C homozygous subjects. We suggest that metformin restores the efficiency of AMPK phosphorylation by STK11 in carriers of the G allele and has less or no effect in the absence of such allele. Our observations concur with those of Legro et al. (15), who found a stepwise increase in ovulation rate from C/C to C/G to G/G genotype in women with PCOS treated with metformin.

AMPK is an indirect target of a number of drugs besides metformin. These include the commonly used thiazolidinediones and statins (22). The *STK11* rs8111699 SNP may thus play a broader role in metabolic and cardiovascular diseases by modulating the baseline condition as well as the response to treatment with the above-mentioned pharmacological agents.

A limitation of our study is the insufficient power to analyze the effect of the *STK11* SNP in each of the three subgroups within the total study population. Such an analysis would have been of interest because each of the consecutive subgroups is thought to represent a developmental stage of hyperinsulinemic androgen excess. It should be noted, however, that the results remained essentially the same with and without adjustment for subgroup allocation in the multivariate model.

Hyperinsulinemic androgen excess is a rather heterogeneous condition (3). The identification of genetic polymorphisms that contribute to predict the response to commonly prescribed medications should assist clinicians in their selection

of available drugs to individualize treatment, thereby improving the therapeutic response, both on the efficacy and on the safety side.

Acknowledgments— A.L.-B. is an Investigator of the Fund for Scientific Research I3 (Ministry of Science and Innovation, Spain). M.D. and L.I. are clinical investigators of Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (Instituto de Salud Carlos III, Madrid, Spain). F.d.Z. is a clinical investigator of the Clinical Research Fund of the University Hospital Leuven.

No potential conflicts of interest relevant to this article were reported.

Table 2—*Clinical, biochemical, and body composition variables before and after 1 year on metformin according to STK11 rs8111699 genotype in the study subjects*

	Baseline				1 year of metformin				Δ 0–1 year			
	CC	GC	GG	*P* value	CC	GC	GG	*P* value	CC	GC	GG	*P* value
n = 85	23	38	24		23	38	24		23	38	24	
Age (years)	12.6 ± 0.8	12.6 ± 0.5	12.8 ± 0.6	0.842	—	—	—		—	—	—	
BMI (kg/m^2)	21.3 ± 0.7	21.0 ± 0.5	21.4 ± 0.6	0.836	21.5 ± 0.6	21.3 ± 0.5	21.4 ± 0.5	0.226	0.21 ± 0.22	0.25 ± 0.15	0.02 ± 0.15	0.693
BMI SDS	1.0 ± 0.3	0.8 ± 0.2	0.7 ± 0.3	0.726	1.0 ± 0.3	0.9 ± 0.2	0.9 ± 0.3	0.382	−0.02 ± 0.15	0.05 ± 0.08	0.10 ± 0.11	0.827
Glucose (mg/dl)	89 ± 1	87 ± 1	88 ± 1	0.719	90 ± 2	87 ± 1	88 ± 1	0.176	1.8 ± 1.7	−0.3 ± 1.4	0.6 ± 1.7	0.706
Insulin (mIU/l)	10.8 ± 1.0	12.8 ± 0.8	14.3 ± 1	**0.0029**	11.2 ± 0.8	9.4 ± 0.7	9.4 ± 0.6	**0.0011**	0.4 ± 0.9	−3.5 ± 0.7	−4.9 ± 0.7	**0.0006**
IGF-I (ng/ml)	251 ± 16	283 ± 17	322 ± 13	**0.0015**	253 ± 10	253 ± 13	255 ± 8	0.553	1.8 ± 14.5	−32.4 ± 10.1	−66.8 ± 10.4	**0.0043**
SHBG (nmol/l)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.606	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.3	0.026	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.027
Testosterone (ng/dl)	77 ± 10	71 ± 7	73 ± 7	0.401	64 ± 6	48 ± 3	47 ± 4	**0.0004**	−13.9 ± 5.3	−22.8 ± 5.3	−26.3 ± 4.1	**0.0038**
Free androgen index	9.4 ± 1.2	8.0 ± 1.1	9.2 ± 1.2	0.935	6.3 ± 0.9	4.1 ± 0.4	3.9 ± 0.4	**0.0001**	−3.2 ± 1.5	−3.9 ± 0.8	−5.3 ± 1.1	**0.0006**
Triglycerides (mg/dl)	86 ± 10	70 ± 5	85 ± 7	0.903	78 ± 8	59 ± 4	58 ± 3	**0.0041**	−7.4 ± 6.1	−11.7 ± 3.5	−26.7 ± 6.1	0.068
LDL cholesterol (mg/dl)	105 ± 6	97 ± 4	97 ± 3	0.282	104 ± 4	85 ± 3	82 ± 3	**0.0004**	−1.8 ± 4.3	−12.0 ± 3.5	−14.7 ± 3.0	0.042
HDL cholesterol (mg/dl)	57 ± 3	57 ± 2	53 ± 2	0.311	58 ± 2	65 ± 2	62 ± 2	0.078	1.3 ± 1.8	8.3 ± 1.8	9.2 ± 1.6	0.011
LDL cholesterol-to-HDL cholesterol ratio	2.0 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	0.785	1.9 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	**0.0001**	−0.1 ± 0.1	−0.4 ± 0.1	−0.5 ± 0.1	**0.0037**
Fat mass (kg)	16.8 ± 1.6	16.4 ± 1.2	18.6 ± 1.5	0.537	17.7 ± 1.6	16.0 ± 1.2	17.3 ± 1.3	0.313	0.9 ± 0.4	−0.5 ± 0.3	−1.2 ± 0.4	**0.0034**
Abdominal fat (kg)	4.9 ± 0.6	4.8 ± 0.4	5.7 ± 0.5	0.331	5.2 ± 0.5	4.3 ± 0.4	4.8 ± 0.4	0.184	0.2 ± 0.1	−0.5 ± 0.1	−0.9 ± 0.2	**0.0032**
Lean mass (kg)	29.9 ± 1.6	31.6 ± 1.0	31.8 ± 1.2	0.516	30.9 ± 1.3	33.6 ± 1.0	34.1 ± 1.2	0.233	0.9 ± 0.4	2.1 ± 0.2	2.4 ± 0.2	**0.0031**

Values are means ± SEM. *P* values for comparisons of baseline, 1-year data, and 1 year changes across genotypes are from a repeated-measures general linear model. Results are adjusted for group allocation (i.e., prepubertal, early postmenarcheal, or PCOS); those for 1 year of metformin and for Δ 0–1 year are also adjusted for baseline insulin levels. $P \leq 0.005$ is considered the acceptable threshold of significance in these data because of the multiple tests performed; these values appear in bold in the table. Values in healthy girls matched for BMI: prepubertal (n = 24; aged 8.3 ± 0.3 years): insulin 6.5 ± 0.5 mIU/l; IGF-I 200 ± 15 ng/ml; SHBG 3.6 ± 0.1 μg/dl; testosterone 16 ± 2 ng/dl; pubertal (n = 24; aged 15.3 ± 0.2 years): insulin 8.8 ± 0.4 mIU/l; IGF-I 384 ± 26 ng/ml; SHBG 1.9 ± 0.1 μg/dl; testosterone 31 ± 3 ng/dl. Lipid profile: triglycerides 55 ± 4 mg/dl; LDL 83 ± 3 mg/dl; HDL 65 ± 2 mg/dl (8,23).

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