Leptin gene, leptin gene receptor polymorphisms and body weight in pregnant women with type 1 diabetes mellitus

Polimorfizmy genu leptyny i jej receptora oraz masa ciała u kobiet ciężarnych chorujących na cukrzycę typu 1

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Summary

Leptin, as well as many other hormones, may play an important role in the pathogenesis of obesity. Several genetic variants of both leptin and its receptor genes may influence human body weight.

Aim: *To investigate the role of leptin gene polymorphism promotion region (-2548G/A) and leptin gene receptor polymorphism (668 A/G) in regulation of body weight in the group of women with type 1 diabetes (PGDM-1).*

Methods: *78 PGDM-1 first trimester pregnant women were qualified for the study group (SG). They were divided into normal and overweight subgroups, based on pre-pregnancy BMI. Control group (CG) consisted of first trimester healthy pregnant women with normal pre-pregnancy body weight. Genetic variants of leptin gene and its receptor were analyzed with the help of PCR-RFLP assays. In the SG, the following metabolic parameters were estimated: MBG, HbA$_{1C}$, insulin dose, LDL, HDL, T-CHOL, triglycerids, creatinine, creatinine clearance and blood pressure.*

Results: *A tendency for the majority of homozygous A and G variants in LEP -2548 G/A and LEPR 668 A/G was found in overweight and obese patients, in comparison to normal-weight subjects. No specific differences in selected first trimester metabolic parameters were found in relation to patients' genotypes in the diabetic group.*

Key words: **leptin** / **gene** / **gene leptin receptor** / **diabetes mellitus** /
/ **Body Mass Index (BMI)** / **obesity** /

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Streszczenie

Leptyna, podobnie jak wiele innych hormonów może odgrywać rolę w patogenezie otyłości. Odmienne warianty genu leptyny oraz jej receptora mogą wpływać na masę ciała u ludzi.

Cel pracy: *Określenie roli polimorfizmu regionu promotorowego genu leptyny (-2548 G/A) oraz polimorfizmu genu receptora leptyny (668 G/A) w regulacji masy ciała kobiet chorujących na cukrzycę typu 1 (PGDM-1).*

Metodyka: *78 kobiet z PGDM-1, w pierwszym trymestrze ciąży, podzielono na podgrupy w zależności od przed-ciążowej masy ciała wg współczynnika BMI.*

Grupę kontrolną stanowiły zdrowe cieżarne z prawidłową przed- ciążową masą ciała. Genotypy określono za pomocą PCR-RFLP. Dodatkowo u pacjentek z cukrzycą oznaczono: średni poziom glikemii (MBG), hemoglobinę glikowaną (HbA$_{1C}$), dawkę insuliny, LDL, HDL, T-CHOL, triglicerydy, kreatyninę, klirens kreatyniny, ciśnienie tętnicze.

Wyniki: *Zaobserwowano tendencję do wyższej częstości występowania genotypów homozygotycznych A i G zarówno w zakresie LEP -2548 G/A oraz LEPR 668A/G w grupie cieżarnych z nadwagą i otyłością w porównaniu do grupy kobiet z prawidłową masą ciała. Nie znaleziono istotnych statystycznie różnic w stężeniach parametrów metabolicznych w zależności od występującego genotypu w grupie badanej.*

Słowa kluczowe: **gen** / **leptyna** / **receptor leptyny** / **cukrzyca** / **współczynnik masy ciała** / / **otyłość** /

Introduction

Obesity is a major health problem in the developed countries and may have a significant impact on pregnancy, because is related to several complications of pregnancy. It has been shown that the risk for development of gestational diabetes is substantially higher among obese and overweight subjects [1]. Several studies were establish to answer the question, whether obesity among pregnant women modifies the risk of selected adverse pregnancy outcomes. Some of them revealed that the risk for fetal malformations is higher among obese mothers. Fetal malformations like spina bifida, anencephaly, hydrocephaly, neural tube defects occurred more frequently in obese diabetic mother in comparison to normal-weight subjects [2].

To assess the association between pre-gestational obesity and weight gain with cesarean delivery and labor complications, a few studies were established. Increased weight at the beginning of pregnancy was associated with a significantly higher risk of perinatal death and infections in women submitted to cesarean section. Also greater weight gain during pregnancy increased the risk for prematurity and for hemorrhage in women submitted to vaginal delivery and with cesarean section [3].

Leptin (gr. *Leptos – lean*) is 16 kDa polipeptide hormone and product of obesity gene (*ob* gene), first described by Zhang in 1994 [4]. This hormone is secreted mostly by adipose tissue. Leptin acts as a satiety factor– by inhibiting neuropeptide Y in hypothalamus, provides a satiety signal with subsequent increase of energy expenditure and intensification of metabolic processes. It also regulates the amount of fat tissue in organism, takes part in fertility processes and regulates blood pressure [5, 6]. Leptin gene is located on a long arm of chromosome 7 (7q31.3) and contains 3 exons and 2 introns. Several polymorphisms of leptin gene were described, like functional polymorphism V110M, promoting region polymorphism -188 C/A and -2548 G/A.

As we know from many publications differences in leptin genotype may be associated with overweight in humans [7, 8, 9]. Several genotypic variants of investigated genes may affect leptin blood concentration and biologic function of its receptor, influencing body weight.

Leptin acts actively via specific receptors located in adipose tissue, stomach, endometrium, liver, spleen, lungs, heart, ovaries and placenta. High representation of leptin receptor was discovered in hypothalamus, where leptin acts via negative loop feedback between its concentration in blood serum and its receptor expression on cell surface [10, 11].

There are several isoforms of leptin receptor, known as long and short isoforms. In humans four isoforms were identified: 1165 amino-acid long isoform, responsible for leptin signaling and 3 alternative splicing short isoforms [12, 13]. Leptin receptor it is translation product of leptin receptor gene (LEPR gene) located on chromosome 1 (1p31) which contains 20 exons [14]. Both leptin gene and its receptor gene are highly polymorphic. The most frequent genetic variants of leptin receptor gene are functional polymorphisms: Gln223Arg (668A/G), Lys109Arg, Ser343Ser and Pro1019Pro.

So the aim of the study was to investigate the frequency occurrence of both leptin gene and its receptor polymorphisms in pregnant women with type 1 diabetes mellitus (PGDM-1) and to estimate the association of several genotypes with normal and overweight subjects. Moreover we aimed at to evaluate the frequency of selected alleles in studied groups. Additionally relationship between metabolic parameters describing glycemic control and presented genotypes was evaluated. Obesity among diabetic pregnant women may cause several additional complications during pregnancy, so we wanted to find out if it has genetic background or it is only the consequence of behavioral habits.

Table I. Characteristics of the study group.

Parameter	Value	Parameter	Value
Mean age(years)	28 ± 4	Number of normal-weight patients N (%)	35 (45)
Mean BMI (kg/m^2)	25,6 ± 6	Number of overweight patients N (%)	27 (34)
Mean diabetes duration (years)	13 ± 6	Number of obese patients N (%)	13 (16)
Mean diabetes onset (years)	16 ± 7	Mean systolic BP (mmHg)	110 ± 15
Number of underweight patients N (%)	3 (5)	Mean diastolic BP (mmHg)	65 ± 10

Material and methods

78 Caucasian pregnant women in single pregnancy with type 1 diabetes mellitus were qualified to the study group. All women were hospitalized in Department of Obstetrics and Women Diseases. Study group was divided into 4 subgroups depending on their pre- pregnancy body mass index (BMI) [12]. First subgroup consisted of 3 underweight subjects - BMI≤18,5kg/m^2), second of 35 normal weight subjects with BMI between 18,5-24,9kg/m^2, the third subgroup consisted of 27 subjects with BMI between 25-29,9kg/m^2 (overweight) and fourth subgroup consisted of 13 subjects with BMI≥30kg/m^2 (obesity). (Table I).

The study group with overweight and obesity was additionally divided into two subgroups depending on the presence of metabolic syndrome features: hypertension, lipid disturbances, obesity. Lipid disturbances were defined according to International Diabetes Federation 2005 as follows: triglycerides serum concentration over 1,7mmol (150mg/dl), HDL serum concentration below 1,3 mmol/l (<50 mg/dl), total cholesterol serum level (T-CHOL) over 5,2mmo/l (>200mg/dl) and LDL over 3,0mmol/l (>115mg/dl). Moreover patients' age, height (cm), systolic and diastolic blood pressure in first trimester of pregnancy (mmHg) were measured. Control group consisted of 34 healthy subjects with normal pre- pregnancy body weight (BMI≤24,9kg/m^2), mean age 28 years.

Serum concentration of following metabolic parameters like: mean blood glucose (estimated from daily profile, i.e. 9 measurements during the day), HbA$_{1C}$, insulin dosage/day, creatinine level, creatinine clearance and daily urine protein loss were also analyzed. The glucose level in serum of venous blood was determined by means of the enzymatic (heksokinase) method with the Roche Diagnostics laboratory reagents on Hitachi 912 analyzer. The percentage of glycated hemoglobin (HbA$_{1c}$) in capillary blood was estimated using the Roche Diagnostics Tina-quant® Hemoglobin A1C II test.

The total serum cholesterol, HDL cholesterol and triglycerides levels were measured with the appropriate Roche Diagnostics reagents (Cholesterol CHOD-PAP, HDL-C plus and Triglycerides GPO-PAP respectively) on Hitachi 912 analyzer, and LDL cholesterol level was calculated form the formula: LDL cholesterol = total cholesterol – HDL cholesterol – TG/5.

Daily urine protein loss (g/24h), serum creatinine level (μmol/l), creatinine clearance (ml/min) were estimated by using Jaffe modified test. All these tests were performed in the Central Analytical Laboratory in Poznań, University Hospital, Polna Street 33.

Genetic analysis

In all subjects polymorphisms of leptin (LEP -2548G/A) and leptin receptor (668A/G) genes were determined using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. DNA was extracted from leucocytes using QIAamp DNA Blood Mini Kit (Qiagen Inc., Germany).

PCR assay was performed in PTC 200 Programmable Thermal Controller (MJ Research INC USA). Amplification of both polymorphisms was carried out in a volume of 25μl (25ng genomic DNA, 2,5μl 10x buffer with (NH$_4$)$_2$SO$_4$ (Fermentas, Lithuania), 1,5mM MgCl$_2$, 0,25 mM dNTP (GeneCraft GmbH, Germany), 0,45μM primers specific for each sequence variant (Tib Molbiol, Poland), 1U Taq polymerase (Fermentas, Lithuania). The PCR protocol was initial denaturation at 94°C for 5 minutes, following by 35 cycles: denaturation 94°C for 1 min., annealing 50°C for 1 min., extension at 72°C for 1,5 min., and after them one final elongation cycle at 72°C for 10 minutes.

As a next step, PCR product was digested with specific restriction enzymes: *Cfo*I 5'GCG/C3' (Fermentas, Lithuania) to identify genotypic variants within promoting region polymorphism of leptin gene and *Msp*I 5'C/CGG3' (EURx, Poland) to estimate polymorphisms of leptin receptor gene. All procedures were performed according to manufacturer recommendations: digestion temperature 37°C (16 hours), enzyme inactivation temperature 65°C (20 min.). After digestion PCR product was transferred on 2% agarose gel (90V, 120 min) with TBE buffer (Sigma Aldrich, USA), stained with ethidium bromide and visualized in ultraviolet light. The results were documented using a gel documentation system UVI-KS4000/Image PC (Syngen Biotech Molecular Biology Instruments, USA).

The following genotypes were found, including -2548G/A: heterozygote GA 242bp, 181bp, 61bp, homozygote GG 181bp, 61bp, mutated homozygote AA 242bp. Including LEPR 668A/G: heterozygote AG 216pz, 134pz, 82pz, homozygote AA 216bp, mutated homozygote GG 134bp, 82bp.

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Table II. Characteristics of the study group. White classification in relation to BMI.

Class of diabetes acc. to White	N	%	Underweight N (%)	Normal N (%)	Overweight N (%)	Obese N (%)
White B	22	28	0 (0)	7 (9)	6 (7)	9 (11)
White C	35	45	3 (4)	16 (21)	13 (16)	3 (4)
White D	9	11	0 (0)	4 (5)	4 (5)	1 (1)
White R	6	8	0 (0)	3 (4)	3 (4)	0 (0)
White RF	6	8	0 (0)	5 (6)	1 (1)	0 (0)
Total	78	100	3 (4)	35 (45)	27 (35)	13 (16)

Table III. Metabolic parameters in the study group according to patients' BMI value.

Parameter	BMI<18,5 (kg/m^2) N=3	BMI 18,5-24,9 (kg/m^2) N=35	BMI 25-29,9 (kg/m^2) N=27	BMI>30 (kg/m^2) N=13	p-value*
MBG (mmol/l)	7.5 ± 0.3	6.1 ± 1.0	6.1 ± 1.9	6.1 ± 2.0	NS
HbA$_{1C}$ (%)	7.5 ± 2.3	7.7 ± 1.5	8.1 ± 1.9	7.4 ± 1.6	NS
LDL (mmol/l)	2.0 ±0.3	2.1 ± 0.5	2.2 ± 0.7	2.3 ± 0.7	NS
HDL (mmol/l)	2.5 ± 0.0	1.9 ± 0.5	1.8 ± 0.5	1.3 ± 0.3	0.0000
T-CHOL (mmol/l)	4.8 ± 0.7	4.5 ± 0.8	4.5 ± 1.2	4.5 ± 0.7	NS
Triglycerides (mmol/l)	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.4	2.0 ± 1.2	0.0000
Insulin dose (Units/24h)	37.0 ± 21.0	44.0 ± 19.6	48.2 ± 18.3	37.1 ± 11.2	NS
Systolic BP (mmHg)	96 ± 5	100 ± 10	110 ±10	115 ± 20	NS
Diastolic BP (mmHg)	56 ± 5	60 ± 10	70 ± 10	70 ± 10	NS
Creatinine (µmol/l)	48.9 ± 13.7	55.1 ± 24.2	53.6 ± 29.1	42.5 ± 19.8	NS
Creatinine clearence (ml/min)	110 ± 1.4	55.1 ± 24.2	130 ± 40	210 ± 90	0.05

* - One-way Anova, post-hoc tests – Levene, Scheffe, Tukey

The genetic analysis was performed in Molecular Biology Laboratory in Department of Perinatology and Women Diseases, Karol Marcinkowski Poznań University of Medical Sciences.

Statistical analysis

Frequency of observed genotypes and alleles are shown as percentage of the whole group. The statistic significance between genotypes in the study subgroups was estimated by Chi-squared test. Differences between biochemical parameters in relation to genotypes were estimated by one-way Anova and post-hoc tests. Differences were acknowledged as statistically significant with p<0.05. SPSS 16.0 for Windows was used for statistical analyses.

To conduct the study, Local Ethics Committee of the University agreement was achieved.

Results

Table I presents characteristics of analyzed patients. Looking at these results we have to pay attention that mean age of analyzed patients was below 30 years and majority of them had diabetes without vascular complications. 34% of studied subjects presented overweight and 16% presented obesity. (Table I). Diabetes classes analysis acc. to White revealed that obesity was observed in 16% of whole group of patients, mostly in the class B diabetes. (Table II).

Table III presents metabolic parameters in relation to patients' BMI. Mean blood glucose level as well as, level of glycated hemoglobin did not differ between these subgroups. We observed tendency to increased atherogenic fraction of lipoproteins with the increasing value of BMI, however significant differences was found only in TG and HDL levels.

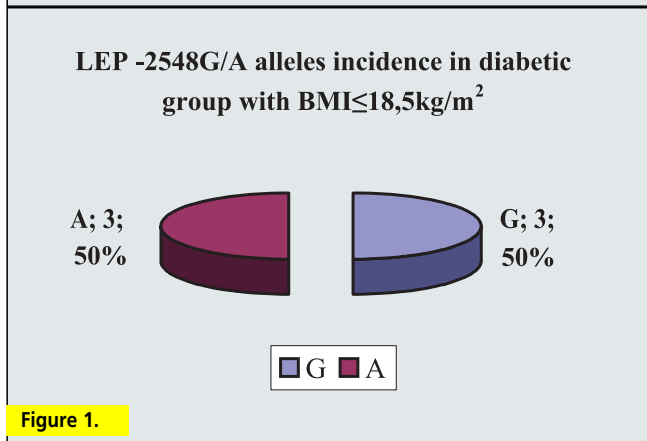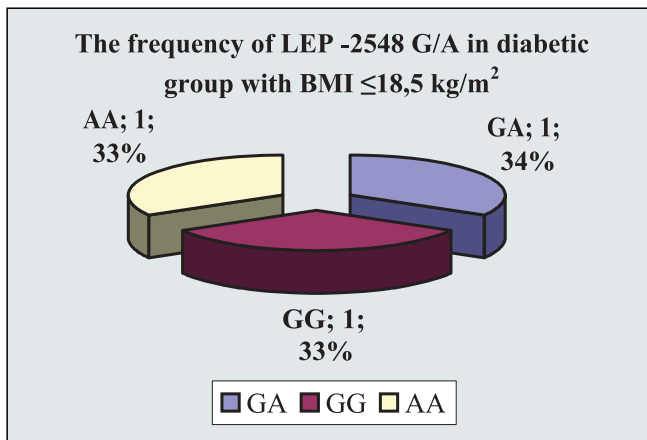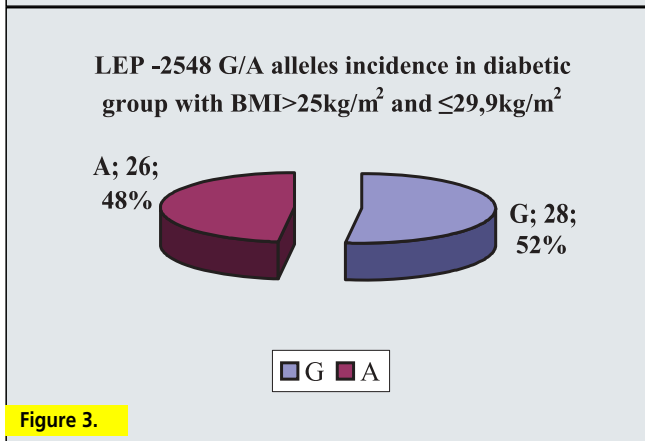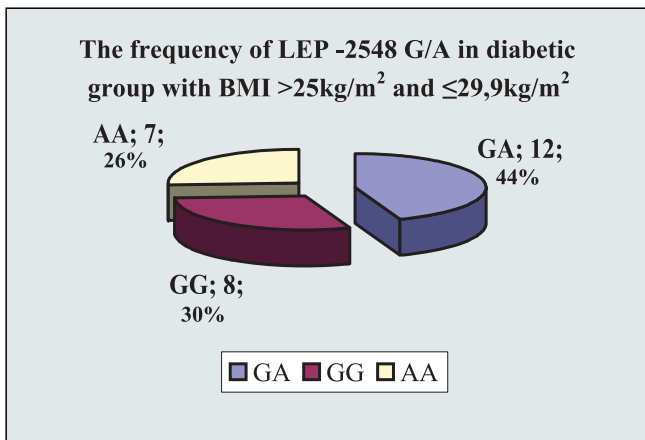Leptin gene, leptin gene receptor polymorphisms and body weight...

The frequency of LEP -2548 G/A in diabetic group with BMI ≤18,5 kg/m²

AA; 1; 33%
GA; 1; 34%
GG; 1; 33%

□ GA ■ GG □ AA

LEP -2548G/A alleles incidence in diabetic group with BMI≤18,5kg/m²

A; 3; 50%
G; 3; 50%

□ G ■ A

Figure 1.

The frequency of LEP -2548 G/A in diabetic group with BMI >18,5 and ≤24,9kg/m²

AA; 11; 31%
GA; 18; 52%
17%
GG; 6;

□ GA ■ GG □ AA

LEP -2548 G/A alleles incidence in diabetic group with >18,5 and ≤24,9kg/m²

G; 30; 43%
A; 40; 57%

□ G ■ A

Figure 2.

The frequency of LEP -2548 G/A in diabetic group with BMI >25kg/m² and ≤29,9kg/m²

AA; 7; 26%
GA; 12; 44%
GG; 8; 30%

□ GA ■ GG □ AA

LEP -2548 G/A alleles incidence in diabetic group with BMI>25kg/m² and ≤29,9kg/m²

A; 26; 48%
G; 28; 52%

□ G ■ A

Figure 3.

The frequency of LEP -2540 G/A in diabetic group with BMI >30kg/m²

GA; 2; 15%
AA; 7; 54%
GG; 4; 31%

□ GA ■ GG □ AA

LEP -2548 G/A alleles incidence in diabetic group with BMI>30kg/m²
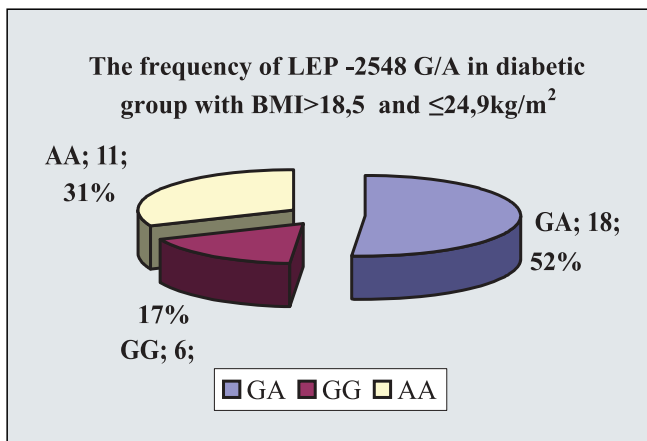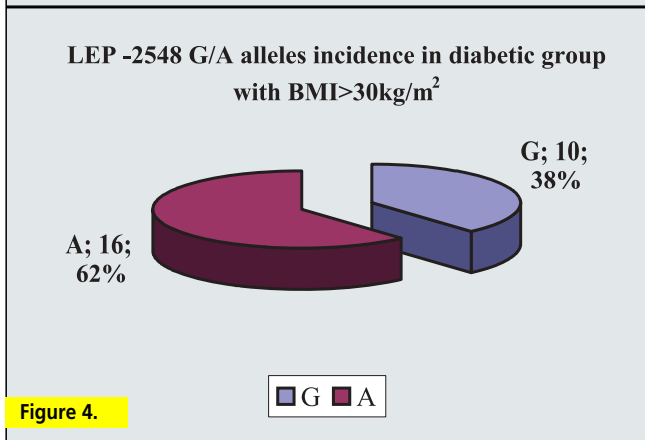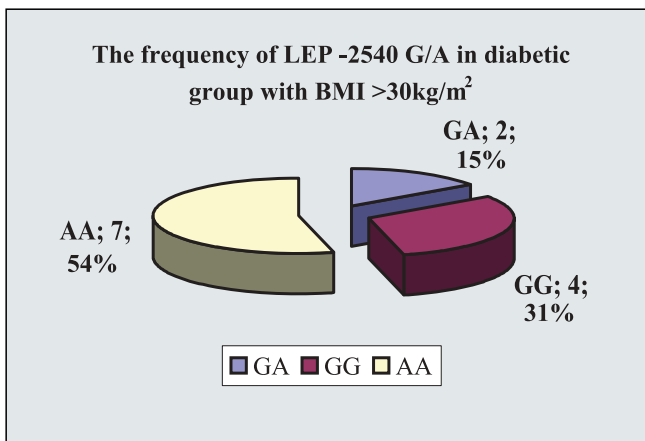
G; 10; 38%
A; 16; 62%

□ G ■ A

Figure 4.

Figures 1-4. Frequency of LEP -2548 G/A and its alleles in the study group in relation to pre-pregnancy BMI.
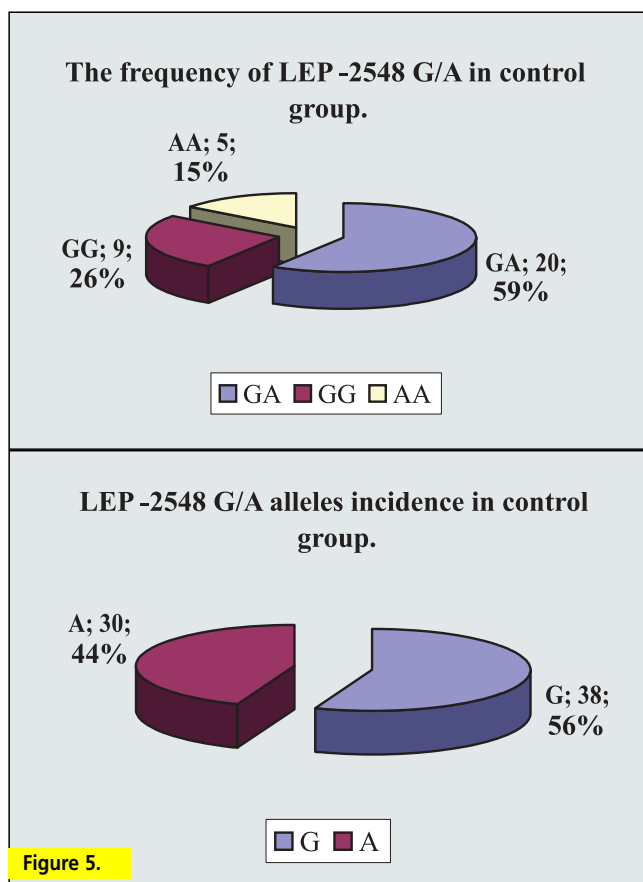*(Chi² test– comparison between subgroups, not significant).*

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Figures 5. Frequency of LEP -2548 G/A and its alleles in the control group with BMI<25 kg/m². *(Chi² test).*

In the diabetic group the relationship between observed genotypes and biochemical parameters describing lipid and glycemic control was analyzed. We did not find statistically significant differences between these parameters and observed genotypes of leptin gene and leptin gene receptor polymorphisms.

Figures 1-5 describes the frequency of observed genotypes and alleles of leptin gene polymorphism (-2548 G/A) in the study subgroups and in the control group in relation to their BMI. With the increasing value of BMI in studied subgroups, the trend to GG and AA homozygous genotypes majority was observed, however it did not reach significant differences. In the control group that consisted of healthy lean individuals with BMI<25kg/m², majority of GA heterozygous genotypes was observed, what corresponds with the frequency of heterozygous variants in normal weight diabetic subjects. The trend to majority of allel A was observed in the studied diabetic subgroups. Similar results were observed in relation to frequency of leptin receptor gene polymorphism (668A/G). With the increasing value of BMI, the trend to GG and AA homozygous genotypes majority was noticed, however again without significant differences. In the control group, the majority of GA heterozygous genotypes were observed, what also corresponds with the frequency of this variant in the lean diabetic women. The above results are shown in figures 6-10.

We also estimated if there is any difference in presence of studied metabolic parameters and combination of possible genotypes including both leptin gene and leptin gene receptor polymorphisms. Since there are 3 possible genotypes including LEP -2548G/A (GA, GG and AA) and 3 possible genotypes including LEPR 668A/G (AG, AA, GG), we established 9 groups of patients in the diabetic group (e.g. LEP GA heterozygote and LEPR AA homozygote or LEP GA heterozygote and LEPR GG homozygote etc.), depending on genetic configuration. The same analysis was performed in the control group. In the study group, no specific genotype was significantly related to obesity. We also did not find statistically significant differences in any of the studied biochemical parameters between these subgroups. In the control group no specific genotype was dominantly observed.

In the study group, we observed the occurrence of lipid disturbances, mostly in the group of subjects with overweight. Therefore we divided the over-weighted group of women into 2 groups: one with the features of metabolic syndrome (overweight, lipid disturbances, hypertension), second without the above features. After the analysis of leptin gene and its receptor polymorphisms, we found that among the patients with lipid disturbances no specific differences were found in relation to the frequency of LEP -2548 G/A. The trend to majority of allel A in the group with metabolic disturbances was found. While analyzing the frequency of LEPR 668 A/G among the same subgroups of patients, we discovered that in the overweight subjects without lipid disturbances the majority of AA and GG homozygous genotypes was found in comparison to the subgroup with the features of metabolic syndrome. The difference in the frequency of alleles was also observed: in the group without lipid disturbances allel G was dominant whereas in the subject with lipid disturbances the majority of allel A was found.

The above is shown in figures 11-14.

Discussion

Mutations in leptin and leptin receptor genes may lead to extreme obesity what was found in mice by Chen and co. and in humans by Clement and co. They found, that presence of homozygous mutations in leptin gene (homozygotes ob/ob) and also homozygous mutations in leptin gene receptor (homozygotes db/db) were associated with early onset of extreme obesity due to hyperphagia, poor energy expenditure and severe insulin-resistance [16, 17]. These studies gave rise to the idea that several genotypes of leptin gene and its receptor may lead to obesity.

Leptin gene and leptin gene receptor are polymorphic, which means that in different subjects, differences in genetic variants might be found. Investigations involving these polymorphisms were conducted in relations to obesity in different populations. Wang et all performed a study on 200 Taiwanese subjects with extreme obesity, where relation between obesity and several polymorphism in promoting region of leptin gene -2548G/A and leptin gene receptor 668A/G was analyzed. They found, that homozygous variant of LEP -2548G/G was strongly associated with extreme obesity development, whereas no specific association between obesity and leptin gene receptor polymorphism was discovered [7].

Leptin gene, leptin gene receptor polymorphisms and body weight...
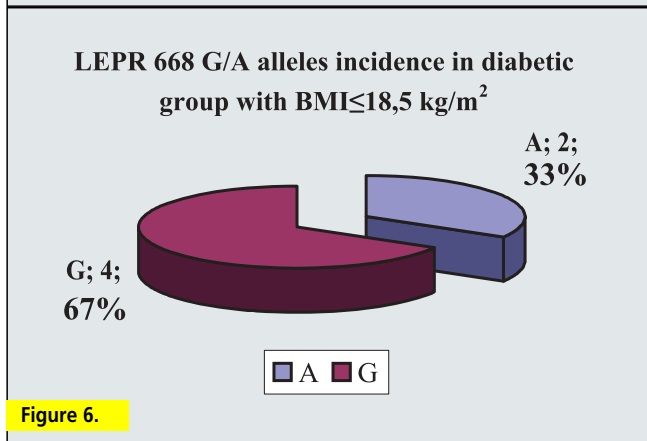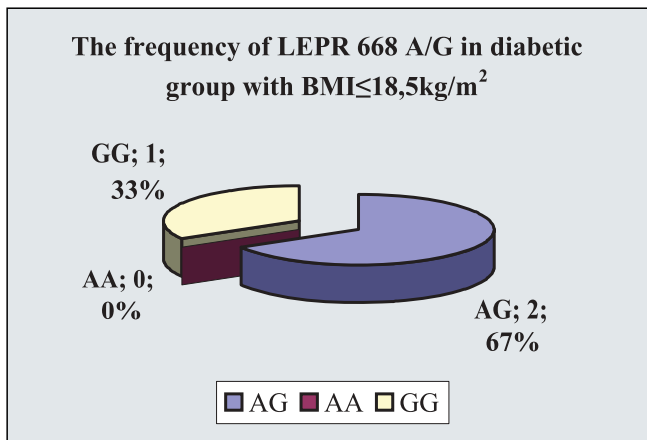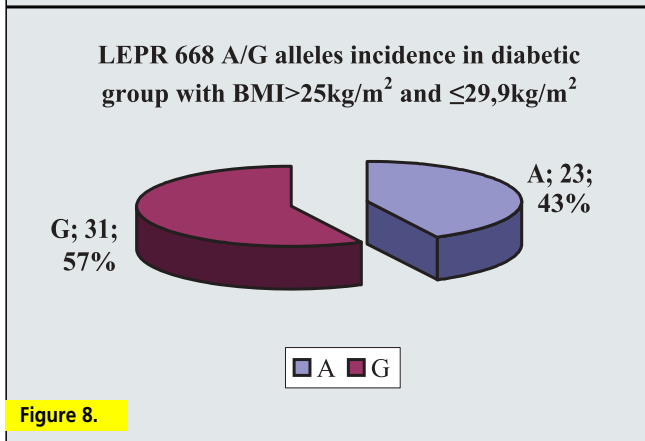
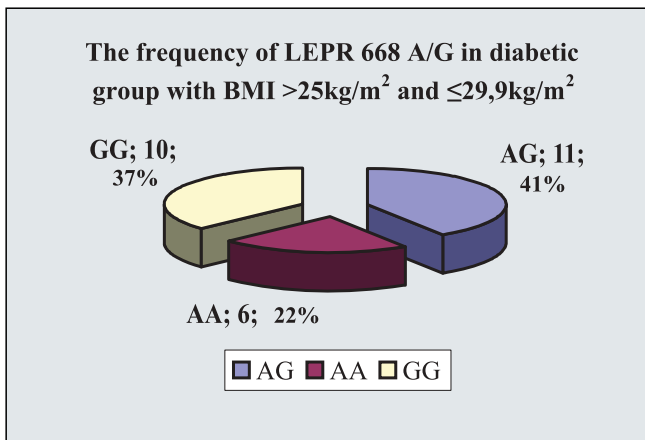**The frequency of LEPR 668 A/G in diabetic group with BMI≤18,5kg/m^2**

GG; 1; 33%
AA; 0; 0%
AG; 2; 67%

□ AG ■ AA □ GG

The frequency of LEPR 668 A/G in diabetic group with BMI >25kg/m^2 and ≤29,9kg/m^2

GG; 10; 37%
AG; 11; 41%
AA; 6; 22%

□ AG ■ AA □ GG

LEPR 668 G/A alleles incidence in diabetic group with BMI≤18,5 kg/m^2

A; 2; 33%
G; 4; 67%

□ A ■ G

Figure 6.

LEPR 668 A/G alleles incidence in diabetic group with BMI>25kg/m^2 and ≤29,9kg/m^2

A; 23; 43%
G; 31; 57%

□ A ■ G

Figure 8.

The frequency of LEPR 668 A/G in diabetic group with BMI>18,5 and ≤24,9kg/m^2

GG; 7; 20%
AA; 10; 29%
AG; 18; 51%

□ AG ■ AA □ GG

The frequency of LEPR 668 A/G in diabetic group with BMI >30kg/m^2

GG; 2; 15%
AG; 5; 38%
AA; 6; 47%

□ AG ■ AA □ GG

LEPR 668 G/A alleles incidence in diabetic group with BMI>18,5 and ≤24,9kg/m^2

A; 28; 47%
G; 32; 53%

□ A ■ G

Figure 7.

LEPR 668 A/G alleles incidence in diabetic group with BMI>30kg/m^2
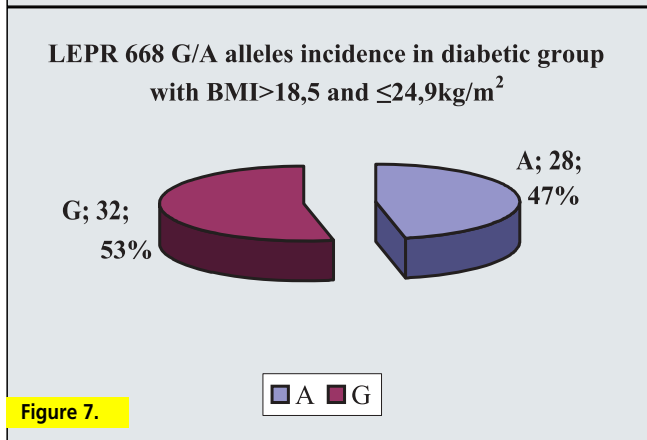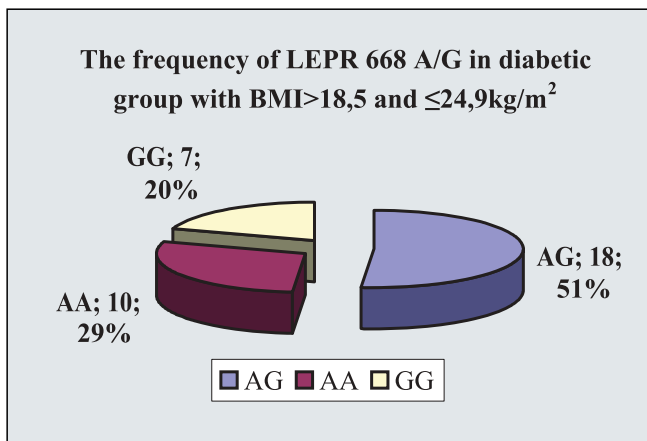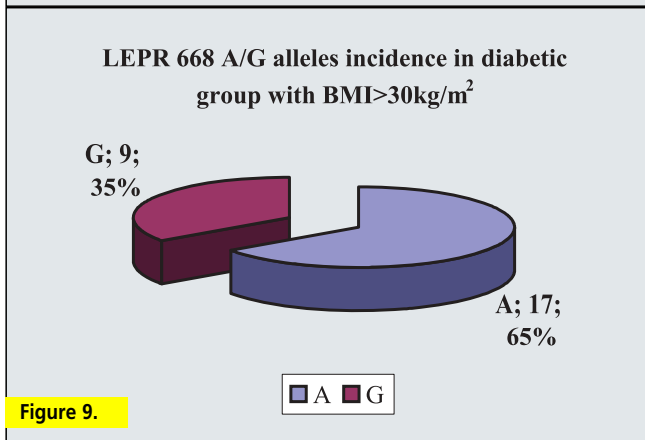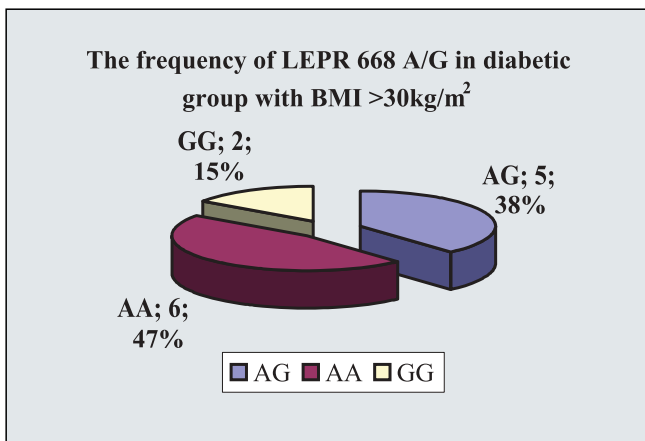
G; 9; 35%
A; 17; 65%

□ A ■ G

Figure 9.

Figures 6-9. Frequency of LEPR 668 A/G and its alleles in the study group in relation to pre- pregnancy BMI.
(Chi^2 test – comparison between subgroups, not significant).

The frequency of LEPR 668 A/G in control group.

GG; 9; 26%

AA; 4; 12%

AG; 21; 62%

☐ AG ☐ AA ☐ GG

LEPR 668 G/A alleles incidence in control group.

A; 29; 43%
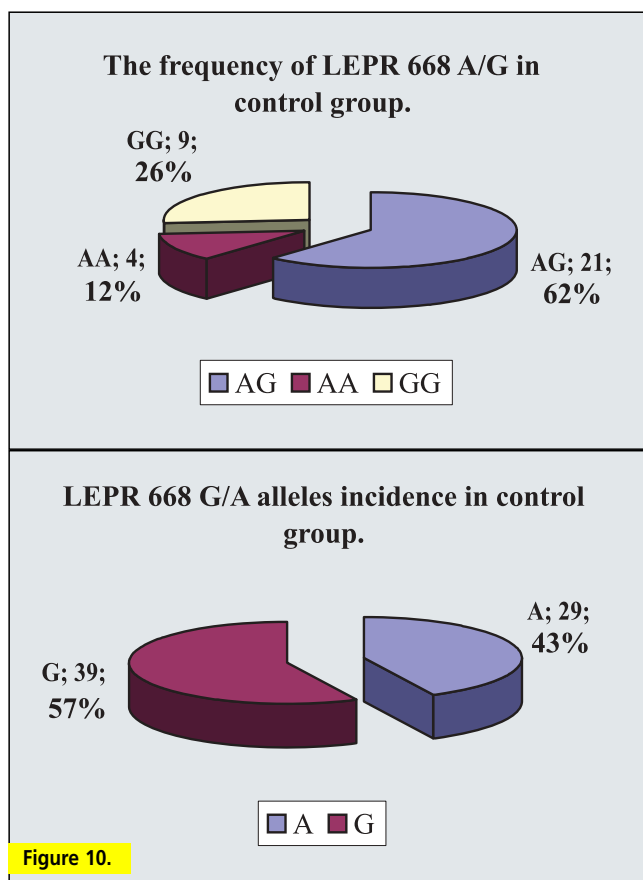
G; 39; 57%

☐ A ☐ G

Figure 10.

Figures 10. Frequency of LEPR 608A/G and its alleles in the control group with BMI<25 kg/m². *(Chi² test).*

Several studies conducted in Caucasian and Afro-American populations discovered that common leptin gene polymorphisms in the flanking region -2548G/A may affect the level of circulating leptin in humans, what was also associated with increase in birth weight, depending on gender. It was found that the majority of alleles A were related to an increased birth weight in females, while the G allele was associated with decreased male birth weight in Caucasian population. Namely, among African-Americans, the A allele was associated with a decrease in umbilical cord leptin in females and with an increase in cord leptin in males [18].

Obesity is the result of an imbalance between food intake and energy expenditure resulting in the storing of energy as fat, in most of the cases due to specific eating patterns. The study conducted on huge number population was focused on identification, whether carriers of common leptin receptor and cholecystokinine gene polymorphisms are genetically predisposed to obesity. It was found that common genetic variations of leptin gene receptor are related to specific snacking behavior, whereas the carriers of cholecystokinine polymorphisms had increased risk to eating enlarged meal sizes [19]. In Spanish population, leptin gene receptor variants (Q223R) are also associated with obesity, rather than -2548 G/A leptin gene polymorphism [8]. The same results were found in Brazilian population [9]

Studies including lipid profile were carried in normal and over-weight populations. Van der Vleuten discovered that presence of homozygous genotypes rather than heterozygous of leptin gene receptor may be associated with familiar combined hyperlipidemia [20]. In pregnancies complicated by preeclampsia the majority of leptin mutated AA homozygous genotype was found [21].

In our study, we found that with the increasing value of BMI in pregnancy complicated by diabetes mellitus type 1, the tendency to more frequent presence of homozygous A and G genotypes in both leptin gene and leptin gene receptor was observed, in comparison to the lean control group. Our data correspond with results reached by other investigators as mentioned above. In the group of lean diabetic women in comparison to lean healthy subjects, similar frequencies of observed genotypic variants were defined. In both groups the majority of heterozygous variants were found. When comparing the frequency of genetic variants in leptin gene between obese diabetic and lean healthy subjects, we found that the majority of homozygous GG (31%) and AA (54%) were present in the obese subgroup. Allel A was dominant also in the obese subgroup. Analogical situation was found in relation to leptin gene receptor. The difference is in the frequency in alleles, where allel G was dominant in obese subgroup. This may confirm the hypothesis that obesity is related to homozygotic variants of leptin gene and its receptor polymorphisms.

We also tried to find association between investigated polymorphisms and biochemical parameters describing glycemic control, lipid profile and blood pressure value in studied patients. We found that in the whole group of diabetic patients these parameters are not related to leptin gene and leptin gene receptor polymorphisms. What was very interesting, among obese patients lack of metabolic disturbances was correlated, however not significantly with homozygous GG variant of leptin gene and its receptor. In contrast, patients with obesity and presence of metabolic disturbances are more often homozygous AA leptin gene subjects.

So our study confirms that also in diabetic pregnant women tendency to obesity may be related to genetic background and further investigations should be performed to define if these patients with genetically related obesity are at higher risk of obstetrical complications.

Leptin gene, leptin gene receptor polymorphisms and body weight...

The frequency of LEP -2548 G/A in diabetic group with BMI >25kg/m^2 and lipid disturbances.
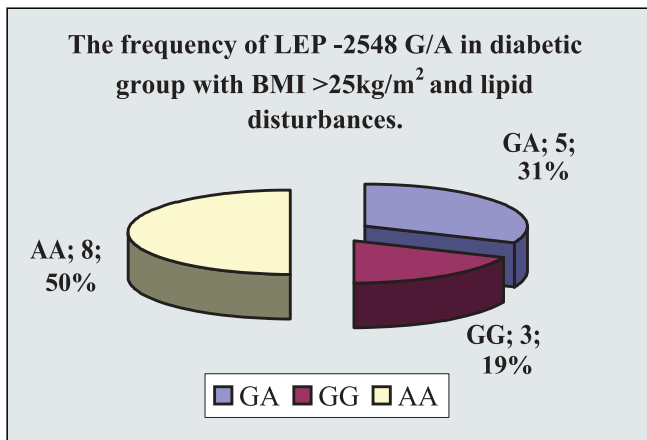
GA; 5; 31%
AA; 8; 50%
GG; 3; 19%

Legend: GA, GG, AA

LEP -2548 G/A alleles incidence in diabetic group with BMI>25kg/m^2 and lipid disturbances.

G; 11; 34%
A; 21; 66%

Legend: G, A

Figure 11.

The frequency of LEP -2548 G/A in diabetic group with BMI >25kg/m^2 without lipid disturbances.

AA; 6; 25%
GA; 9; 37%
GG; 9; 38%

Legend: GA, GG, AA

LEP -2548 G/A alleles incidence in diabetic group with BMI>25kg/m^2 without lipid disturbances.

A; 21; 44%
G; 27; 56%

Legend: G, A

Figure 12.

The frequency of LEPR 668 A/G in diabetic group with BMI >25kg/m^2 and lipid disturbances.

GG; 2; 13%
AA; 5; 31%
AG; 9; 56%

Legend: AG, AA, GG

LEPR 668 A/G alleles incidence in diabetic group with BMI>25kg/m^2 and lipid disturbances.

G; 13; 41%
A; 19; 59%

Legend: A, G

Figure 13.

The frequency of LEPR 668 A/G in diabetic group with BMI >25kg/m^2 without lipid disturbances.

GG; 10; 42%
AG; 7; 29%
AA; 7; 29%

Legend: AG, AA, GG

LEPR 668 A/G alleles incidence in diabetic group with BMI>25kg/m^2 without lipid disturbances.
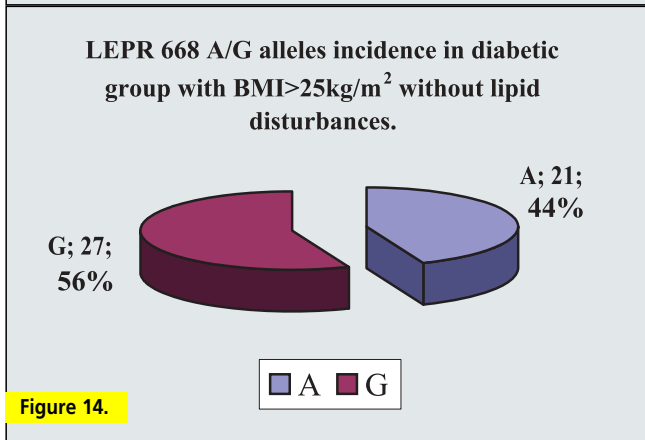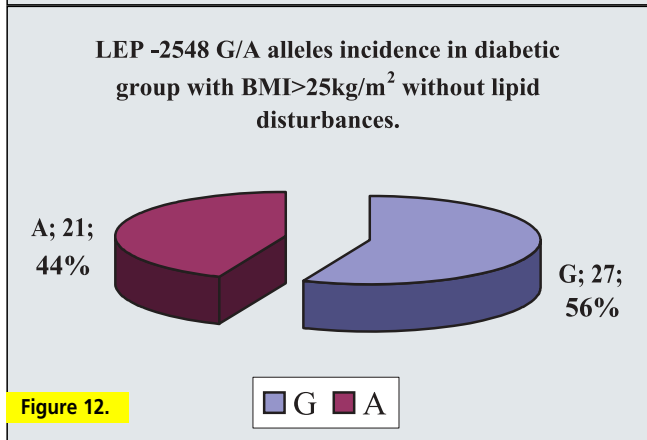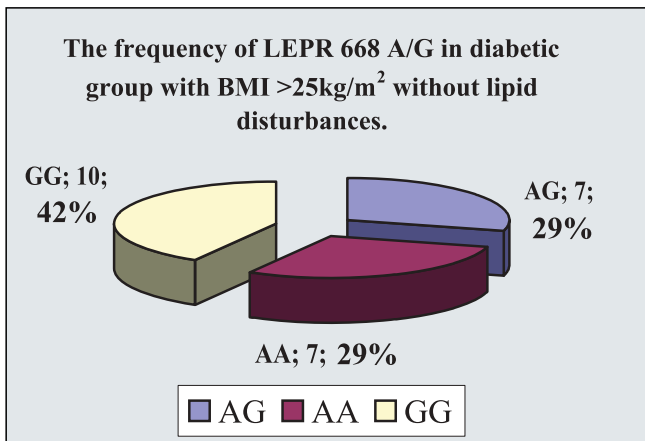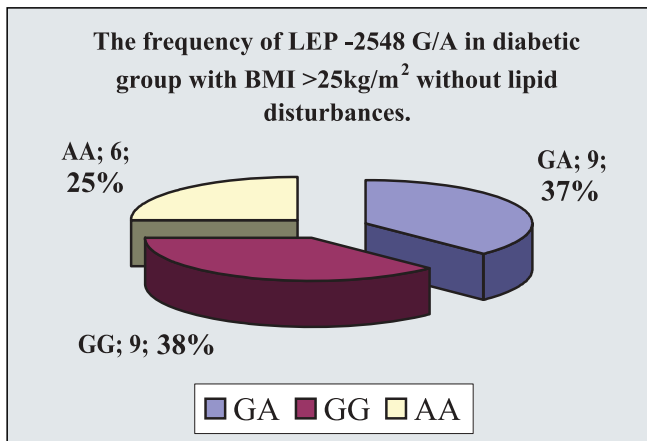
A; 21; 44%
G; 27; 56%

Legend: A, G

Figure 14.

Figures 11-12. Frequency of LEP -2548 G/A and its alleles in the study group with overweight in relation to the serum lipid disturbances. (*Chi^2 test*).

Figures 13-14. Frequency of LEPR 668 A/G and its alleles in the study group with overweight in relation to the lipid control. (*Chi^2 test*).

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