The combined effect of dietary supplement “Leptin Manager” and power fitness exercises on weight loss in women with different LEPR (rs1137101) genotypes

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Summary

The aim of this study was to establish the effect of combined action of the dietary supplement “Leptin Manager™” and the power fitness program on weight loss in women with different genotypes of the 1st and the 2nd period of mature age. The study involved 62 overweight women (BMI≥25). The experimental group consisted of 21 women, who participated in the power fitness training program while simultaneously administering the drug “Leptin Manager” (manufactured by Xymogen, USA). The control group consisted of 17 women, who were engaged in power fitness and did not take the drug, and 24 women, who were not engaged in power fitness. The duration of the study was three months. The training method was based on the CrossFit system, functional training, included using machines (block and lever devices) according to the Full-body system in each training. The Q223R polymorphism of the leptin receptor gene (LEPR) was determined by the polymerase chain reaction in real time. Leptin indicators in Q allele carriers were twice higher than in R/R-genotype carriers (p = 0.045). Combined effect of the training program of power fitness and the use of the drug “Leptin Manager” resulted in more significant changes of anthropometric indices of the body composition of overweight women when compared with the control group. The most drastic changes in the body composition occurred in women with the R/R genotype of the LEPR gene. The usage of the drug “Leptin Manager” reduced leptin levels: in the experimental group by 33.4% (p < 0.05), meanwhile in the control group by 6.1%.

Conclusion. The Q223R polymorphism of the LEPR gene can be a molecular genetic marker of leptin resistance. Q allele Q223R polymorphism of the LEPR gene facilitates the development of obesity. R alleles and the R/R-genotype of the LEPR gene help reducing leptin levels after exercise. The usage of “Leptin Manager” combined with physical activity reliably decreases leptin levels when compared with the control group.

Keywords: leptin, power fitness, leptin receptor gene polymorphisms, “Leptin Manager”, overweight.

Introduction

Today, one of the most pressing problems is overweight and obesity. Worldwide there is a trend toward increased prevalence of this disease. 39% of the world’s population over age of 18 have excess body weight and 13% are obese. According to the report, made by the World Health Organization in 2017, the number of people, suffering from obesity, increased in many European countries. Obesity is becoming an epidemic and leads to a significant deterioration of human health. In industrialized countries, almost 50% of the population are overweight with 30% of them suffering from obesity. In Ukraine, every fourth woman and every sixth man are overweight. In total, about 15–20% of our country’s population are obese.

It is believed that 77% of the intensity of metabolism and the predisposition to overweight are determined genetically with only 23% dependent on the environment and individual lifestyle (Wardle, 2008). Today, genetic factors are considered to be significant contributors to the pathogenesis of obesity (from 30 to 70%) (Rankinen, Bouchard, 2007; Bouchard, 2008). Such indicator as waist circumference depends on genetic factors by 60%, since the body mass index – by 40%.

Obesity can be conditioned both monogenetically and poly-genetically, that is, obesity can be caused by one or several genes. The latest map of genes that contributes to obesity – “The Human Obesity Gene Map: The 2005 Update” (published in the journal “OBESITY”) contains a list of 11 genes, whose mutation leads to obesity, 50 loci, which are inherited, according to Mendel’s laws. In addition, this map contains 253 loci that affect obesity (Rankinen, Bouchard, 2007).

Studies, conducted in the UK, aiming to assess the contribution of each allele, which increases...
the body mass index to the probability of obesity, obtained data, suggesting that even obesity, largely inherited genetically, can be reduced by 40% due to physical activity (Li et al., 2010). The researchers found each allele that increases the body mass index to increase obesity 1.158 times in physically inactive people, and 1.166 times – in physically active people. The development of obesity is also influenced by epigenetic factors that determine the efficiency of gene expression (Dunstan et al., 2017). Most genes are inactive; it is the state, controlled by methyl groups.

According to modern scientific data, one of the causes of obesity is resistance to leptin, which is a hormone of saturation. As an adipocyte-dependent hormone leptin plays a key role in appetite regulation by limiting food consumption and stimulating metabolism to support energy balance (Lenard, Berthoud, 2008). Leptin acts through a leptin receptor, belonging to the first class of the family of cytokine receptors.

The potential cause of leptin resistance is thought to be leptin receptor gene polymorphisms (LEPR). The gene was found to contain 23 thousand polymorphisms. Four of these cause a pathogenic effect and shortage of leptin receptors. The Q223R polymorphism as leptin resistance has been established in most studies. Even though, changes in leptin levels during exercise have been previously researched, changes in its concentration in blood after power fitness workouts under the influence of the drug “Leptin Manager” have not been studied yet. If the effectiveness of weight loss in women with different genotypes in terms of Q223R polymorphism as leptin resistance is established, it will help us personalize and customize the training process in the case of obesity.

The effect of exercises on changes of hormone levels, involved in the regulation of energy metabolism, has been demonstrated in studies of leptin, nestinophil-1, and irisin levels (Bostrom et al., 2012). But the results of studies on the effect of exercises on leptin are unconvincing, since some researchers have determined the decrease of leptin levels (Voss, 2016), others have established the increasing of leptin levels (Uysal et al., 2017), and some others have found no change (Ozcelik et al., 2005).

30-minute-long aerobic exercises twice a day (in the morning and evening) for three consecutive days caused changes in leptin levels both in trained and untrained persons. But post-workout individual fluctuations in leptin levels were not related to the direct stress effect of workouts, but rather to changes in the energy balance of the people, who exercised. Therefore, after the morning training, the level of leptin increased in 13% of subjects and decreased in 16% of subjects. After the evening workout, it increased in 30% of subjects and decreased in 20% of subjects (Algul et al., 2017). The study casts doubt on the assertion that leptin is a hormone, induced by physical exercises.

According to Estonian researchers’ data, leptin and insulin levels in the group of overweight individuals are significantly higher than in people with normal body weight. The leptin level was inversely correlated with VO2 max in both groups: the higher leptin level, the lower the VO2 max. The level of leptin correlates with physical activity. The higher physical activity, the lower leptin level. Low physical activity in the group of overweight individuals is related to the leptin level in their blood (Remmel et al., 2017).

Year-long aerobic exercises in untrained persons without excess body weight caused unreliable fluctuations in both men and women due to the fact that the adipose tissue in these individuals changed only slightly (Salehzadeh, Agaziyev, 2011).

Several polymorphisms of leptin gene (LEP) and leptin receptor (LEPR) are associated with the development of obesity (Rojano-Rodriguez et al., 2016). These variants can modify the effect of regular physical exercise on various characteristics, connected with obesity, such as glucose homeostasis (Lakka et al., 2004).

Some scientific studies assert that the polymorphisms of this gene are related to the most informative genetic markers of metabolic pathways of maintaining the energy balance and body composition changes in response to training programs, along with such markers as polymorphisms of FTO, MC4R, ACE, PPARG, LEP, ADRB2, and ADRB3 genes (Leońska-Duniec et al., 2016). Some of these polymorphisms have been thoroughly studied. The variants of the LEPR gene were found to influence the activity of the leptin receptor.

Q223R (rs1137101) is characterized by the substitute of adenine with guanine in position 668 in exon 6 and results in the replacement of
glycine with arginine in position 223 in a protein (Gln223Arg). Another way of notating is c.668A > G (Q/R). It was established that the above-mentioned polymorphism affects the ability of the receptor to bind to leptin (Sook-Ha, Yee-How, 2014). The minor allele frequency (MAF) is varied in different studies. In addition, in different studies different alleles are called minor ones. Japanese scientists have shown that Q223R is associated with levels of physical activity. Thus, individuals with the RR-genotype demonstrated a shorter time of motor activity and longer inactive time (Murakami et al., 2014).

Q223R along with rs 1137100 (K109R) indicated an association with the body mass index (BMI) and the degree of obesity in Indonesian residents. G allele is associated with bigger percentage of muscle mass than in participants with the AA-genotype. In addition, G allele contributed to favourable changes in the percentage of hypodermic fat in response to power fitness trainings (Wardle et al., 2008).

Among those individuals, who have a higher body weight index, R-genotypes are more common. Other studies established that FTO rs9939609 and LEPR rs1137101 polymorphisms of parents affect the body weight and BMI of new born babies (Marginean et al., 2016). Thus, the Q223R can serve a molecular genetic marker of leptin resistance and can contribute to the development of obesity.

However, the association of these markers with obesity has ethnic implications. Thus, the informational value of these markers in connection with obesity was not confirmed among the Malaysians (Sook-Ha, Yee-How, 2014). Nonetheless, LEP A19G, G2548A, LEPR K109R, and Q223R were found to have a synergistic effect on obesity. Studies on the Mexican population (Rojano-Rodriguez et al., 2016) concluded that neither rs1137101 nor rs 1137100 are associated with obesity, but C allele of T/C polymorphism (rs 1805134) is characterized by such association.

The purpose of this study was to establish the efficiency of the combination of the diet supplement “Leptin Manager™” and the power fitness program on body weight loss in women with different genotypes of the 1st and 2nd period of mature age.

Material and methods
The study involved 62 people. The experimental group (EG) consisted of 21 women with excess body weight (BMI ≥ 25, age 36–55), who participated in the power fitness program while consuming the drug “Leptin Manager”. The control group (CG) consisted of 17 women, who were engaged in power fitness without taking the drug, and 24 women, who were not engaged in power fitness.

For the molecular-genetic analysis we used DNA samples, obtained by rinsing out the epithelial cells of the oral cavity. The DNA was sampled with the help of a universal probe. The oral cavity had been washed with 0.9% NaCl before collecting the material. DNA was isolated from the buccal epithelium using a set of reagents, DiatomTM DNA Prep (Biokom).

The Q223R polymorphism of the LEPR gene was determined in real-time by PCR method with the help of the device “7500 Fast Real-Time PCR” (Applied Biosystems, USA) using TaqMan® Master Mix (2x) (Thermo Fisher Scientific, USA) (assay C_8722581_10). Leptin was measured by the enzyme immunoassaying immunosorbent method based on the sandwich principle using Leptin Sandwich reagents, produced by the firm DRG Germany on the Tecan Sunrise immune enzyme analyser (Austria). Research material was received from blood samples, taken from the peripheral vein, taken in the morning at rest on empty stomach without prior physical activity.

The measurements of different body parts were taken with the help of a centimetre tape: breast circumference, shoulder circumference, forearm circumference, waist circumference, abdominal circumference, and hip circumferences. The body mass index (BMI) was calculated as well. Body composition was determined using “TANITA Body Composition Analyser BC-418” using the bioelectric impedance method. The following parameters were determined: fatty tissue (%), fat mass (kg), fat-free body mass (kg), total water content (kg).

The study lasted for three months (from November to December, 2017) in the fitness club “Interfit”, Kyiv. The research program included a preliminary examination of participants, questionnaires, measurement of anthropometric indicators, circumferences and body composition (ratio of body fat and muscle mass), genetic analysis, and the fitness classes program during the period of three months. All participants gave their informed consent to participate in the project and received recommendations for healthy diet as well as individualized training recommendations and the drug “Leptin Manager”, which was designed for a 12-week treatment course (weekly). Collection of blood
samples and buccal epithelium and determination of body composition by biompedansometry method with the help of the “Tanita” device were carried out on the basis of the research institute of the National University of Physical Education and Sports. DNA isolation and detection of genetic polymorphisms were occurred in the laboratory of the General and Molecular Physiology Department of O.O. Bohomolets Institute of Physiology of the National Academy of Sciences of Ukraine.

The whole training process was divided into three weeks: easy, medium, and intensive weeks. The training method was based on the CrossFit system, a functional training, and included simulators (block and weight accessories) under the Full-body system in each training. All participants followed the rules of a healthy diet. They had 5–6 meals (including snacks) per day.

The drug “Leptin Manager” (produced by Xymogen, USA) is a dietary supplement with one capsule consisting of 15 mg of ascorbic acid and 80 mg of ORALVISC® formula (registered trademark), submitted by a mixture of glucuronic acid and other glycosaminoglycans. The drug target is fat cells. It influences adipogenesis and expression of genes of adipogenic markers in multipotent cells. It affects the level of leptin and other cytotoxic chemokines in serum and synovial fluid as well as facilitates body weight loss.

### Results and discussion

Analysis of the frequency of Q/R polymorphism in the leptin receptor gene (*LEPR*) (rs1137101) revealed that, in a group of women with excess body weight, the incidence of this polymorphism is higher than in the European population. Therefore, in our studies, the frequency of the Q/Q genotype was 25%, Q/R – 57%, and R/R – 19%. The frequency of Q allele was 0.53, whereas R was 0.46. According to the NCBI and ESEMBL databases, the incidence of the A (Q) allele worldwide is 0.415, however, different populations have different frequency of this allele. Thus, according to the Quebec Family Study, the frequency of the Q allele in the British population is 0.56, in the Danish – 0.56, in the American – 0.54, and in the French – 0.56. In more closed communities, this allele is less common: 0.15 for the Japanese and 0.25 for the Pima Indians. Therefore, the frequency of Q alleles in our sample was somewhat lower, compared to the European population, which can be due to the principle of enrolling women in our studies according to the criterion of excess body weight.

When measuring the level of leptin in venous blood by the ELISA method, we established that the concentration of leptin in the blood of women with excess body weight is characterized by wide variability of this indicator within the range from 3.55 to 41.86 ng × ml⁻¹ with an average value of 12.1 ± 7.8 ng × ml⁻¹. That is twice higher than the average percentage for women with normal body weight.

Since the reference values of normal blood leptin level in women is considered the range from 3.63 to 11.09, all women were divided into three groups: “normal leptin level”, “above-normal leptin level”, and “below-normal leptin level”. Assuming that the normal leptin content varies from 3.63 to 11.09, 4% of women had “below the norm” of leptin levels, and 36% – “above the norm”. Although, most researchers point out that obese women have a high leptin levels (Walsh, 2012), we have not received confirmation in our studies. The reasons for such high variability of leptin parameters can be unaccounted factors, namely, the effect of polymorphisms of the leptin gene itself and post-splicing processes.

The results of the study of leptin blood concentrations demonstrate a lower level of leptin in women with the genotype R/R (Fig.1). This peculiarity represents the tendency and is statistically unreliable. However, the comparison of leptin indexes in Q allele women-carriers (Q/Q- and Q/R-genotypes) with those of R/R-type carriers showed that leptin percentage in women-carriers of Q alleles is twice higher than in women-carriers of the R/R-genotype (p = 0.045). This pattern indicates that Q allele can contribute to the development of leptin resistance. The phenomenon we have established contradicts previously established postulates that R allele facilitates the development of leptin resistance (Sook-Ha, Yee How, 2014; Marginean et al., 2016). But these differences can be explained by ethnic characteristics, since most of these patterns were discovered on island populations.

In the group of women with genotype Q/Q, 50% of women had above-normal leptin levels; among women with Q/R genotype, 33.3% of them had above-normal leptin levels; among women with the R/R genotype, 11% of test subjects had above-normal leptin levels. In women with Q/Q genotype the average value of BMI was 30.78 ± 3.11, in
women with the genotype Q/R – 31.37 ± 5.8, and in women with the genotype R/R – 27.4 ± 2.72. In Q allele carriers (Q/Q- and Q/R-genotypes), BMI was significantly lower than in carriers of the R/R-genotype (p = 0.04).

Even though the level of leptin depends on the polymorphism of the LEPR gene, there is no direct dependence, since other genetic and metabolic factors can influence this indicator. Therefore, when discussing the role of leptin levels as a marker of genetic deficiency of leptin receptors, it should be mentioned that leptin levels in blood serum in obese individuals change disproportionately, indicating that it cannot be used as a marker of leptin receptor deficiency.

The Pearson’s pair correlation coefficient (r) between leptin levels and BMI is 0.702, whereas between leptin and body weight it is 0.648, and between leptin and adipose tissue it is 0.73. It indicates that there is a linear link between the anthropometric indexes and the level of leptin. More precisely, there is a strong connection between leptin, BMI, and the percentage of adipose tissue as well as close link between body weight and leptin levels.

Thus, this study established a close linear connection between leptin levels and indicators such as body mass index (r = 0.7) and adipose tissue content (r = 0.73); a moderate relationship between leptin levels and body weight (r = 0.65). Women, who are carriers of Q allele and Q/Q-genotype, have higher levels of leptin than R/R-genotype carriers. In women with the R/R-genotype, “above normal” leptin levels are 39% less common than in carriers of the Q/Q-genotype.

It was proven that, even though leptin levels change under the influence of physical activity, these changes are not proportional to the intensity or duration of physical activity, but rather reflect individual metabolic features. In our study, in all subjects of both the control and the experimental group, leptin levels changed in various ways. In total, leptin levels decreased by 2.92 ng/ml, which constituted 13% of the baseline level.

Under the influence of the drug “Leptin Manager”, there was a tendency toward normalization of leptin levels in subjects with higher-than-average leptin levels. In the experimental group, which took medicine and practiced workout, leptin levels decreased by 3.85 ± 0.25 ng/ml, which constituted 33.4% (p < 0.05). And in the control group leptin level was 0.49 ± 0.3 ng/ml lower, which constituted 6.1% of the baseline level (Fig. 2).

In the experimental group, in women with Q/Q-genotype leptin level increased by 0.14 ng/ml; in women with Q/R-genotype, it decreased by 5.47 ng/ml and, in women with the R/R-genotype, it decreased by 1.4 ng/ml, which constitutes 21.2%. That is, the R allele and the R/R-genotype contribute to a decline of leptin level after exercise. In all groups after three months of trainings, body composition indicators changed in the direction of normalization. A wide variability of individual anthropometric indicators was observed.

In the control group, body weight decreased by an average of 2 kg, BMI decreased by 4%, the percentage of fat decreased from 32 to 29% (i.e., by 3%), the percentage of visceral fat did not decrease. Whereas in the experimental group, body weight decreased by 5%, the percentage of fat decreased from 36 to 34% (i.e., 2%), the percentage of visceral fat decreased by 2% due to increasing the percentage of muscle mass.

The results of changes of the indicators under the influence of physical activity have shown that the most significant changes occurred in the experimental group.

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In subgroups of women, who were divided by the genotype in the experimental group, physical activity and drug intake, resulted in similar changes in direction, but different in amplitude. Body mass in women with Q/Q-genotype decreased by 5.8%, in women with Q/R genotype – by 4%, and in women with R/R genotype – by 6.1%. The BMI decreased in women with Q/Q-genotype the most (by 7%). The biggest changes in the percentage of fat mass occurred in women with the R/R-genotype. The biggest changes in the percentage of visceral fat were noticed in women with R/R-genotype (17%). Thus, the most drastic changes in body composition were observed in women with the R/R-genotype.

Conclusions

1. The causes for alimentary obesity include hypodynamia, genetic factors, incorrect nutrition, and bad eating habits. The contribution of genetic factors to the development of obesity is estimated to be from 30 to 70%. A number of leptin gene polymorphisms contribute to the expression of genes, which control fat and carbohydrate metabolism. That will make it possible to develop recommendations for the use of this drug by individuals with excess body weight and obese individuals, who have above-normal leptin level, and will help customize the medication for individuals with different genotypes.

2. Leptin level in women with excess body weight varies within a wide range that exceeds the norm both for low concentrations and for high concentrations. 4% of women had “below-normal leptin level”, and 36% had “above-normal level”.

3. The study revealed a close linear link between leptin levels and such indicators as body mass index (r = 0.7), adipose tissue content (r = 0.73) and a moderate connection between leptin levels and body weight (r = 0.65). Women-carriers of Q allele and Q/Q-genotype have higher leptin levels than R/R carriers. In women with R/R-genotype, “above-normal” leptin levels are 39% less common than in women with the Q/Q-genotype.

4. Physical activity resulted in a slight decrease in leptin levels: in the experimental group – by 33.4% (p < 0.05) and in the control group by 6.1%. R alleles and the R/R-genotype of the LEPR gene contribute to reduction of leptin levels after exercise. The use of the drug “Leptin Manager” leads to a possible decrease of leptin levels compared to those in the control group.

5. The combined effect of the power fitness program and the use of the drug “Leptin Manager” lead to more significant changes in anthropometric indicators and body composition of women with excess body weight in comparison with the control group. The most drastic changes in body composition occurred in women with the R/R-genotype of the LEPR gene.

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SANTRAUKA

Tyrimu buvo siekiami nustatyti maisto papildo „Leptin Manager“ ir svorio metimo jėgos treniruočių kompleksinį poveikį moterims, turinčioms skirtingą genotipą ir antsvorį. Tyrimo metu buvo analizuojama 40 moterų, kurios dalyvavo įvairiose treniruotėse. Įvairios kūno svorio metimo programos turėjo įtakos testų rezultatams. Didžiausia kūno svorio mažėjimo įtaka buvo įrodyta moterims, kurios turėjo skirtingą genotipą, o moterims, kurios turėjo skirtingą antsvorį, didelė įtaka buvo įrodyta įvairiose treniruotėse.