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Changes of Plasma Lipids during Weight Reduction in Females Depends on APOA5 Variants

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Key Words

Dietary intervention · Apolipoprotein A5 · Plasma lipid · Glycemia

Abstract

Background: Apolipoprotein A5 (APOA5) is a determinant of plasma lipids, and its role in body mass index (BMI) determination is discussed. This study was aimed at the investigation of the relationship between common APOA5 gene variants and body weight/plasma lipid decrease in overweight females. ***Methods:*** We analyzed 98 unrelated overweight and obese nondiabetic Czech females (BMI >27.5). APOA5 T-1131→C and Ser19→Trp variants were genotyped. Before and after 9 weeks of lifestyle modification, biochemical and anthropometrical measurements and assessment of nutritional intake were performed. The lifestyle modification program consisted of a reduction in energy intake and an exercise program (aerobic exercise 4 times per week, 60 min each). ***Results:*** The mean age of the participants was 30.7 ± 3.7 years, the mean BMI before the intervention was 31.4 ± 3.8 and the weight loss was 5.9 ± 2.5 kg (7 ± 3%). There were 86 T-1131T homozygotes and 12 carriers of the C-1131 allele and 82 Ser19Ser homozygotes and 16 carriers of the Trp19 allele, respectively; 72 females had the commonest T-1131T/Ser19Ser haplotype. No significant association between BMI decrease and APOA5 variants was found, but T-1131T carriers have a significantly higher body weight both before and after the intervention ($p < 0.05$; p = not significant for BMI). The fasting glycemia was significantly higher in Trp19 carriers both before and after the intervention ($p < 0.01$). Further, plasma triglyceride levels decreased in Ser19Ser homozygotes but increased in Trp19 carriers (1.42 ± 0.62 to 1.28 ± 0.48 vs. 1.15 ± 0.47 to 1.41 ± 0.80 mmol/l; $p < 0.05$ for differences between the groups). Similarly, in carriers of at least 1 less common APOA5 allele (n = 26), plasma low-density lipoprotein cholesterol levels did not decrease as they did in T-1131T/Ser19Ser carriers (3.11 ± 0.70 to 3.27 ± 0.81 vs. 3.39 ± 0.81 to 3.16 ± 0.86 mmol/l; $p < 0.05$ for differences between the groups). ***Conclusions:*** APOA5 gene variants have effects on the decrease in plasma triglyceride and low-density lipoprotein cholesterol level in females in a model combining their dietary habits and physical activity changes.

Introduction

According to the latest surveys, the mean body mass index (BMI) in the Czech Caucasian population is approximately 27, and most of the inhabitants have elevated plasma lipids [1]. Their elevated body weight is a result of

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the imbalance between energy intake and energy expenditure. In addition, plasma lipid levels are affected by dietary habits and performed physical activity. In most individuals, the decrease in BMI and improvement in the lipid parameters can be achieved through dietary changes and exercise. Nevertheless, the individual responses to lifestyle modification vary [2], and it is clear that genetics play a partial role here.

Apolipoprotein A5 (APOA5) was described a couple of years ago [3]. The most commonly analyzed variants T-1131→C and Ser19→Trp are associated with differences in plasma triglyceride (TG) levels and are associated with a higher risk of myocardial infarction [4, 5]. In recent years, interesting studies have been published, pointing to the thus far undetected role of the APOA5 gene in lipid metabolism and energy homeostasis regulation in humans. Firstly, it was determined that male carriers of at least one C-1131 allele have a significantly higher BMI reduction upon short-time reduction in dietary fat intake [6]. Secondly, the Ser19→Trp variant in males was associated with significant differences in cholesterol decrease over an 8-year follow-up period [7, 8]. The aim of our study was to investigate the influence of APOA5 variants (T-1131→C and Ser19→Trp) on a response to lifestyle changes in obese females.

Subjects and Methods

Subjects

A population of 98 unrelated overweight and obese premenopausal Czech Caucasian sedentary females, recruited via an advertisement on a lifestyle website and a women's journal, was analyzed. All volunteers were studied in a medical research center and signed informed consent and agreement with the participation in the study, which was approved by the institutional ethics committee. Females (with abdominal type of obesity) were selected with the criteria of a BMI >27.5 and age between 25 and 55 years. The exclusion criteria were known inflammatory or metabolic diseases (diabetes, thyroid gland disease, any other endocrine disorders, autoimmune diseases, any chronic inflammation, or neoplastic disease).

Procedures and Dietary Habits

The 9-week lifestyle modification program consisted of an equilibrium to the recommended dietary energy intake for the appropriate age and controlled physical activity [for more details, see ref. 9].

Dietary intervention (comprising a weekly, supervised dietary record) was aimed at adjustment of energy intake to the amount recommended for the age, together with a decrease in animal fat and dietary cholesterol intake. Eating more fruits and vegetables was also encouraged. Additionally, the volunteers participated in a supervised 1-hour training session at a fitness center 3 times weekly, and 3 more sessions per week (cycling, jogging or brisk walking) were recommended (at least 1 session was performed by all individuals). All these activities included an aerobic exercise component – the participants were supervised (and advised) to sustain a heart rate of 115–145 beats (according to age) per minute within 60 min of exercise. The heart rate was continuously recorded telemetrically (Sport Tester S 410, RS 400, Polar Electro, Oy, Kempele, Finland). The probands had their lipid and anthropometrical parameters and blood pressure determined at baseline and at the end of the study.

Genotyping and Biochemical Assays

DNA was isolated from frozen EDTA blood by the standard method [10]. Genotyping of the APOA5 variants has been performed as described in more detail before [5]. Plasma triacylglycerol, total cholesterol and cholesterol in high-density lipoprotein cholesterol and low-density lipoprotein cholesterol fractions were measured enzymatically by a standardized procedure (Centers for Disease Control and Prevention external quality control system), using the Cobas Mira analyzer (Hoffmann-La Roche).

Anthropometric Measurements

Body weight was measured with an electronic weight scale (scaled to the nearest 100 g), which was placed horizontally and calibrated before each weighting session. Height was measured with a stadiometer to the nearest 0.5 cm. Waist (defined as narrowest diameter between xiphoid process and iliac crest) and hip (defined as widest diameter over the greater trochanters) circumferences were measured with the accuracy of 0.5 cm. The waist-to-hip ratio and BMI were calculated from obtained measurements. Diastolic and systolic blood pressures were measured after 10 min in a sitting position as an average of 3 readings on the right arm with an automated blood pressure unit (automated sphygmomanometer BP-203 NA, Nippon Colin Co., Ltd.). A trained nurse performed all measurements.

Statistical Analyses

ANOVA for repeated measures was used in order to evaluate the statistical significance of the differences between before and after study tests. TGs and glycemia were logarithmically transformed before the analysis to obtain the normal distribution of the values. All data are presented as the mean ± SD. Differences are considered to be statistically significant if $p < 0.05$.

Results

All 98 subjects completed the follow-up during 9 weeks, with an average weight loss of 5.9 ± 2.5 kg (7 ± 3%), with a minimum loss of 1.8 kg and a maximum loss of 15.5 kg. There were significant positive differences in most, but not in all analyzed parameters (for more details, see table 1). Changes in daily energy, macronutrient and cholesterol intakes at the beginning and at the end of the study are given in table 2. Energy intake decreased from approximately 3,000 kcal/day to approximately 2,000 kcal/day ($p < 0.001$), total fat intake decreased by about 30% ($p < 0.001$), and additionally, plant fat intake

Table 1. Characteristics of the study participants at the beginning and at the end of the study (mean ± SD)

	Basal	After 9 weeks	p
Age, years	30.7 ± 3.7	–	
BMI	31.4 ± 3.8	29.3 ± 3.7	<0.001
Weight, kg	87.7 ± 13.9	81.8 ± 13.5	<0.001
Total cholesterol, mmol/l	5.42 ± 0.86	5.35 ± 0.89	n.s.
LDL cholesterol, mmol/l	3.31 ± 0.79	3.19 ± 0.84	n.s.
HDL cholesterol, mmol/l	1.44 ± 0.31	1.50 ± 0.35	n.s.
TGs, mmol/l	1.37 ± 0.61	1.30 ± 0.55	n.s.
Waist-to-hip ratio	0.825 ± 0.069	0.810 ± 0.065	<0.001
Systolic BP, mm Hg	131.5 ± 11.9	122.3 ± 10.5	<0.001
Diastolic BP, mm Hg	82.6 ± 7.7	76.2 ± 7.0	<0.001
Glycemia, mmol/l	5.50 ± 0.66	5.35 ± 0.55	<0.05

The ANOVA for repeated measures was used in order to evaluate the statistical significance of differences between baseline and postintervention. No significant differences (n.s.) were observed when basal and postintervention results were compared ($p > 0.05$). LDL = Low-density lipoprotein; HDL = high-density lipoprotein; BP = blood pressure.

Table 2. Changes in daily energy, macronutrient and cholesterol intakes from 1-day food records at the beginning and at the end of the study (mean ± SD)

	Basal	At 9 weeks	p
Energy intake, kcal/day	2,973 ± 838	1,986 ± 518	<0.001
Energy from proteins, %	12.2 ± 5.2	18.5 ± 5.4	n.s.
Energy from fats, %	34.6 ± 8.9	29.7 ± 9.4	<0.001
Energy from carbohydrates, %	53.2 ± 11.20	51.8 ± 8.9	<0.01
Animal proteins, g/day	55.4 ± 29.3	55.1 ± 22.3	n.s.
Animal fats, g/day	87.3 ± 65.7	24.4 ± 28.4	<0.001
Cholesterol, mg/day	393 ± 223	220 ± 136	<0.001
Plant proteins, g/day	25.3 ± 11.9	37.1 ± 11.3	<0.05
Plant fats, g/day	22.1 ± 19.5	39.3 ± 16.7	<0.001

The ANOVA for repeated measures was used in order to evaluate the statistical significance of differences between baseline and postintervention. No significant differences (n.s.) were observed when basal and postintervention results were compared ($p > 0.05$).

Table 3. Significant responses to the intervention (mean ± SD)

	APOA5	n	Before intervention	After intervention	p_1	p_2
BMI	T-1131T	86	31.6 ± 3.9	29.5 ± 3.7	0.08	0.83
	+ C-1131	12	29.7 ± 2.2	27.5 ± 2.4		
Body weight, kg	T-1131T	86	88.9 ± 14.2	82.9 ± 13.7	0.02	0.94
	+ C-1131	12	79.2 ± 7.6	73.3 ± 7.2		
Glycemia, mmol/l	Ser19Ser	82	5.45 ± 0.58	5.27 ± 0.49	<0.01	0.27
	+ Trp19	16	5.74 ± 0.95	5.76 ± 0.66		
TGs, mmol/l	Ser19Ser	82	1.42 ± 0.62	1.28 ± 0.48	0.62	0.02
	+ Trp19	16	1.15 ± 0.47	1.41 ± 0.80		
LDL cholesterol, mmol/l	T-1131T/Ser19Ser	72	3.39 ± 0.81	3.16 ± 0.86	0.62	0.02
	Others	26	3.11 ± 0.70	3.27 ± 0.81		

p_1 for differences between genotypes; p_2 for interaction between time and genotypes. LDL = Low-density lipoprotein.

increased by over 50% ($p < 0.001$). Dietary cholesterol intake also decreased by more than 40%/day.

The APOA5 genotype distributions among the volunteers were similar to the Czech population [5]. We did not confirm the results obtained by Aberle et al. [6], who found an elevated frequency of C-1131 allele carriers in overweight males (25%, usual frequency in Caucasians is about 15%).

Two analyzed parameters were associated with APOA5 variants per se, both before and after the intervention (table 3). At first, T-1131T carriers have a significantly higher body weight both before and after the intervention ($p < 0.05$). In addition, fasting glycemia was significantly higher in Trp19 carriers both before and after the intervention ($p < 0.01$).

No significant association between BMI ($p = 0.07$) or waist-to-hip ratio changes and APOA5 variants was found. The APOA5 gene had an influence on lipid changes after dietary intervention (table 3). Plasma TG levels decreased in Ser19Ser homozygotes, but increased in Trp19 carriers ($p < 0.05$). Similarly, in carriers of at least 1 less common APOA5 allele ($n = 26$), plasma levels of low-density lipoprotein cholesterol did not decrease as they did in T-1131T/Ser19Ser carriers ($p < 0.05$).

Discussion

After 9 weeks of lifestyle modification, consisting of at least 4 units of exercise per week and changing of dietary habits, we have observed a significant decrease in almost all of the analyzed anthropometric and biochemical parameters. Further, our results confirmed a large interindividual variability in response to the applied lifestyle modification. For example, the decrease in body weight varied between 1.8 and 15.5 kg. As all analyzed subjects were highly motivated volunteers and the exercise training was performed 3 times a week under supervision at the fitness center, we suppose that the differences most likely reflect the different genetic predispositions rather than failure to adhere to the dietary regimen and physical activity program.

Recently, it was reported that polymorphisms in the APOA5 gene influenced the decrease in plasma cholesterol in males in an 8-year prospective study [7, 8], as well as diet-induced weight loss in men [6].

Our study, for the first time, analyzed the role of APOA5 variations on lifestyle modification-associated changes on anthropometric and biochemical parameters in females. Our results did not absolutely confirm all the findings from male studies, suggesting that in the case of APOA5 variants, there could be significant sex differences in gene-environmental interaction. In addition, differences in intervention itself could play a very important role.

The gene for APOA5 could thus be included among the genes which could have an effect on lifestyle modification response. For example, genes for fatty acid binding protein 2 [11], cholesterol 7α hydroxylase [12], β_3-adrenergic receptor [13] and some others [14] were analyzed. In comparison with the association studies, intervention studies are much more difficult to compare. To replicate the study under absolutely the same conditions is virtually impossible, as not only differences like, for example, ethnicity, sex, age and smoking will influence the study results.

In intervention studies, also the differences in dietary changes (even the same dietary recommendation can be differently implemented leading to slightly varying dietary habits in different countries), the intensity of the exercise and the preselection of volunteers will have an important role in the final results of the studies. Nevertheless, some genes/genotypes could have a wide spectrum of positive effects, which can differ between the studies, but taken together, it will be possible to point out the generally beneficial genotype. For example, according to the majority of these studies, carriers of the APOE4 allele have a better response to dietary intervention [15].

In contrast, the analyzed APOA5 Ser19Trp variant could be a good example of the sex-specific effect of the gene. It was previously shown in an 8-year prospective study (on males) that the Trp19 allele could be beneficial in the case of lifestyle modification predominantly affecting dietary habits. Our study, on the other hand, has shown that Ser19Ser homozygotes will benefit more from lifestyle modification where primary intervention is a physical activity.

The molecular mechanism underlying the different responses of plasma lipids after a lifestyle modification according to the APOA5 variant remains unclear. It was shown by experiments with fusion proteins (apoA5 signal peptide-secretory alkaline phosphatase) [16] that substitution of Ser19 with Trp19 reduced the amount of secreted phosphatase from liver HePG2 cells by 49%. We can speculate that the dietary/lifestyle changes could further affect the production of this apolipoprotein (and also its biological effect).

In conclusion, in obese females, APOA5 variants affect body weight and fasting glycemia per se and plasma lipid changes over time. Carriers of the common Ser19Ser genotype received higher benefit from their lifestyle changes. Additional studies need to be performed to clarify the exact role and possible mechanism of APOA5 variants in lifestyle-induced changes in anthropometric and biochemical parameters.

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