BODY COMPOSITION CHANGES IN ADULT FEMALES AFTER LIFESTYLE INTERVENTION ARE INFLUENCED BY THE *NYD-SP18* VARIANT

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SUMMARY

Aim: The study focuses on the analysis of the possible relationship between a common *NYD-SP18* (rs6971091, G>A) gene polymorphism and weight loss after lifestyle intervention (combined dietary intake and physical activity) in overweight/obese females.

Methods: We genotyped 139 unrelated non-diabetic Czech females (49.5 ± 13.3 years, average BMI at baseline 32.2 ± 4.6 kg/m^2). Biochemical and anthropometrical measurements were performed before and after ten weeks of lifestyle intervention.

Results: The mean weight loss achieved was 4.7 ± 3.1 kg ($p < 0.01$). Carriers of the *NYD-SP18* GG (N = 75) genotype lost significantly more body fat mass ($p = 0.04$) and gained more active muscle mass ($p = 0.037$) than the carriers of the A allele (N = 64). After adjustment of baseline values, both differences remained significant ($p = 0.03$ and $p = 0.016$).

Conclusion: Overweight/obese female carriers of the *NYD-SP18* rs6971091 GG genotype exhibited a more beneficial response to the intensive lifestyle intervention than others.

Key words: females, intervention, *NYD-SP18*, obesity, polymorphism

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INTRODUCTION

Obesity is one of the most common preventable risk factors for cardiovascular diseases, cancer and type 2 diabetes. Obesity can negatively affect self-perception and physical fitness (1). Overweight/obesity results from a positive energy balance, which is caused predominantly by a combination of low levels of physical activity and high energy intake as well as other less discussed but important factors (e.g. sleeping deficit) (2, 3).

Body weight is significantly influenced by genetic factors (both common polymorphisms as well as rare mutations), and twin studies estimate that up to 60% of BMI variability is attributable to genetics. Similarly, susceptibility to weight loss interventions seems to be under genetic control (4).

Variants within the *NYD-SP18* gene have been identified using fine mapping of a region on chromosome 7 that is well known for its high obesity LOD score. In the National Heart, Lung and Blood Institute (NHLBI) Family Heart Study, the effect of *NYD-SP18* variants on obesity was even greater (5) than that of one of the FTO variants with a well-established impact on body weight (6). However, no studies to date have examined the effect of *NYD-SP18* variants on the efficacy of weight loss interventions.

We present the results of a short-term intervention study on female volunteers, which focuses on the possible effect of the *NYD-SP18* genetic variant on body weight loss.

MATERIALS AND METHODS

Subjects

A population of 139 unrelated overweight and obese Czech Caucasian sedentary adult females, recruited via an advertisement on a lifestyle website and in a women's journal, was analysed. All volunteers were examined in a medical research centre. Females (with abdominal-type obesity) were selected according to the criteria of having a BMI over 28.6 kg/m^2 and aged between 25 and 65 years (mean age 49.5 ± 13.3 years). Exclusion criteria applied to known inflammatory or metabolic diseases (diabetes, thyroid gland disease, any other endocrine disorders, autoimmune diseases, and any chronic inflammation or neoplastic disease).

Procedures and Dietary Habits

Study participants underwent a ten-week lifestyle modification programme (7). Dietary intervention (comprised of a weekly, supervised dietary record) was aimed at adjusting energy intake to the amount recommended for the relevant age (max. 7,500 kJ/day, achieved mean was 7,186 kJ/day), and decreasing animal fat and dietary cholesterol intake. Eating more fruits and vegetables was also encouraged. Dietary intervention (comprised of a weekly, supervised dietary record) was aimed at lowering energy intake (to age-adjusted recommended values) as well as decreasing animal fat intake. Additionally, the volunteers participated 3 times per

week in a supervised 1-hour training session at a fitness centre, and 2 more sessions per week (cycling, jogging or brisk walking) were recommended (at least one session was performed by all individuals). All these activities included an aerobic exercise component – the participants were supervised (and advised) to maintain their heart rate between 115 and 145 beats (according to age) per minute during the 60-minute exercise session. Heart rates were continuously recorded telemetrically (Sport Tester S 410, RS 400, Polar Electro, Oy, Kempele, Finland). Probands had their lipid and anthropometrical parameters and blood pressure determined at baseline and at the end of the study.

Changes in diet were calculated from dietary records before intervention and at the last week of intervention.

Laboratory Analysis

Genomic DNA was extracted from the patients' peripheral white blood cells. The *NYD-SP18* SNP rs6971091 polymorphism was genotyped using polymerase chain reaction-restriction fragment length analysis. The chemicals used were purchased from Fermentas International Inc., Burlington, Ontario, Canada, and the Polymerase Chain Reactions (PCR) were performed using the DYAD Disciple PCR machine from MJ Research (Waltham, MA, United States). DNA was amplified in a total volume of 25 μL using the oligonucleotides 5´ cct tgg tca tta gct gaa tga gaa gct and 5´ aag gcc tta acc tgg ttc tgc. The PCR product (105 bp) was cut with 5 units of the HindIII restriction enzyme. Restriction fragments of 79 bp and 26 bp represented the A allele, whereas the presence of an uncut product represented the more common G allele.

Plasma triacylglycerols, total cholesterol and cholesterol in high-density (HDL-C) and low-density (LDL-C) fractions were measured enzymatically by standardised procedure, using the Cobas Mira analyzer (Hoffman-LaRoche).

Anthropometric Measurements

Body weight was measured using an electronic weight scale (scaled to the nearest 100 g), which was placed horizontally and calibrated before each weighing session. Height was measured

Table 1. *Characteristics of study participants before and after intervention (mean ± SD)*

	NYD-SP18 rs6971091		
	Before	**After**	**p-value**
N	139		
Weight (kg)	91.3 ± 14.1	86.7 ± 14.7	< 0.001
BMI (kg/m^2)	32.2 ± 4.6	29.9 ± 6.0	< 0.001
Total fat mass (%)	42.1 ± 6.4	38.5 ± 5.4	< 0.001
Muscle mass	52.4 ± 5.9	53.5 ± 5.8	< 0.01
Hip (cm)	114.4 ± 11.2	107.5 ± 12.7	< 0.001
Waist (cm)	103.0 ± 13.4	94.3 ± 13.0	< 0.001
Total cholesterol (mmol/L)	5.3 ± 1.2	4.9 ± 1.0	< 0.01
Triglycerides (mmol/L)	1.51 ± 0.78	1.38 ± 0.82	ns
HDL-cholesterol (mmol/L)	1.49 ± 0.42	1.45 ± 0.39	ns
Glucose (mmol/L)	5.67 ± 1.27	5.48 ± 1.1	< 0.01

Table 2. *Characteristics of study participants before intervention (mean ± SD) using the NYD-SP18 rs6971091 genotype. No significant differences were detected*

	NYD-SP18 rs6971091		
	GG	**GA**	**AA**
N (%)	75 (54.0%)	57 (41.0%)	7 (5.0%)
Weight (kg)	89.6 ± 13.7	93.3 ± 12.7	87.2 ± 13.4
BMI (kg/m^2)	32.2 ± 4.6	32.2 ± 4.0	31.5 ± 3.8
Total fat mass (%)	41.4 ± 5.2	42.2 ± 4.6	39.8 ± 6.4
Muscle mass (%)	52.3 ± 6.4	53.7 ± 5.8	52.2 ± 7.0
Hip (cm)	114.0 ± 9.6	114.5 ± 7.2	111.3 ± 7.2
Waist (cm)	103.6 ± 13.1	104.6 ± 10.3	99.4 ± 12.0
Total cholesterol (mmol/L)	5.2 ± 1.1	5.3 ± 0.8	5.3 ± 0.7
Triglycerides (mmol/L)	1.53 ± 0.69	1.60 ± 0.96	1.35 ± 0.60
HDL-cholesterol (mmol/L)	1.45 ± 0.35	1.46 ± 0.38	1.51 ± 0.18
Glucose (mmol/L)	5.96 ± 1.99	5.85 ± 1.28	5.37 ± 0.55

with a stadiometer to the nearest 0.5 cm. Waist (defined as the narrowest diameter between the xiphoid process and iliac crest) and hip (defined as the widest diameter over the greater trochanters) circumferences were measured to an accuracy of 0.5 cm. The waist-to-hip ratio (WHR) and BMI were calculated from obtained measurements. Diastolic and systolic blood pressure were measured after 10 minutes in a sitting position with an average of 3 readings on the right arm using an automated blood pressure unit (Automated sphygmomanometer BP-203 NA, Nippon Colin Co., Ltd). Body composition, basal metabolic rate and estimated average requirements were determined by impedance analysis using a Bodystat analyzer (1500 MDD; Bodystat, Isle of Man, UK). A trained nurse performed all measurements.

Statistic Analysis

The differences in lipids, anthropometrical parameters, body composition, basal metabolic rate and estimated average requirements were evaluated by performing an ANOVA test. Because of the low number of AA homozygotes (N = 7), this group was joined to CA heterozygotes for the purpose of this study, and both groups were evaluated together for all parameters. All data are presented as a mean ± SD. Differences are considered to be statistically significant if $p < 0.05$.

RESULTS

Basic characteristics of volunteers before the intervention are summarised in Table 1. Analysed parameters were not associated with examined polymorphism at baseline (Table 2).

There was a significant positive change in the anthropometrical parameters of interest after the intervention. Average BMI decreased ($p < 0.01$) and fat mass also fell significantly. We documented a high inter-individual variability in response to the intervention; the most significant body weight decrease was – 15.5 kg, and the lowest was in fact a gain of + 2.0 kg.

The call rate of the *NYD-SP18* rs6971091 variant was 100%. The Hardy-Weinberg test confirmed the independent segregation of individual genotypes ($p = 0.37$); the frequency of the minor A allele (0.26) was similar to previously published results (5) and did not significantly differ from the general population (Hubáček, unpublished observation).

Baseline measurements showed that minor AA homozygotes had slightly lower BMIs, waist and plasma glucose, but the differences did not reach statistical significance.

The *NYD-SP18* rs6971091 variant was a significant determinant of the success of the intervention. Despite the fact that there were no differences in body weight (or BMI) reduction, carriers of the GG genotype reduced their fat mass significantly more (−5.0 ± 3.3 kg; $p = 0.037$) than carriers of at least one A allele (−3.7 ± 3.5 kg) (Table 3).

Vice versa, carriers of the GG genotype showed larger increases in total muscle mass (Table 3) than A allele carriers (1.6 ± 4.4 kg vs. 0.4 ± 1.1 kg; $p = 0.04$), and the difference remained statistically significant ($p = 0.016$) after adjustment for matching baseline values.

Changes in other anthropometrical and biochemical parameters were not dependent on *NYD-SP18* genotypes.

DISCUSSION

Our study is the first to document the important role of the common *NYD-SP18* gene variant in determining body composition changes after an intensive ten-week lifestyle intervention in overweight/obese females. Homozygotes for the rs6971091 *NYD-SP18* major G allele benefited significantly more from the intervention than carriers of the minor A allele.

NYD-SP18 is one of the genes that has been suggested as a candidate for determining obesity. The gene is located in a locus that is known for its high LOD score for obesity. So far, the putative association between rs6971091 *NYD-SP18* variants and BMI has been reported in one original study; (5) however, they have not been confirmed in any further studies. Importantly, no studies focusing on obesity treatment efficacy have been performed on either adults or on children/adolescents.

Intervention studies have tested dozens of genes in an attempt to identify a genetic background that could contribute to weight loss after intervention. Unfortunately, a number of factors make intervention studies difficult to compare: differences in dietary changes (caused both by recommendations and patient compliance); the intensity and frequency of physical activity; the pre-selection of participants; and the relatively low number of individuals included (often less than 100 participants). Therefore, it is not surprising that most of the studies published to date have recorded inconclusive results and are difficult to replicate (8–14).

On an identical group of individuals, we previously analysed the effect of some other candidate genes on the possible effectiveness of a short-term intensive lifestyle intervention on anthropometrical and biochemical parameters. We detected positive gene-intervention interaction in the case of angiotensin-converting enzyme (7), AHSG (9) and apolipoprotein A5 (14). However, variants within the genes, such as FTO (8), INSIG-2 (15) and preproghrelin, (16) were not associated with efficacy of the intensive lifestyle intervention.

Despite the strength of our study (on a homogenous group of participants), it has several limitations. First, our results have

Table 3. *Significant effects of the NYD-SP18 polymorphism on fat mass decrease and active muscle mass increase. Unadjusted p and p* adjusted for baseline values are given*

	NYD-SP18 genotype			
	GG	**+A**	**p-value**	**p-value***
N	**75**	**64**		
Total fat mass	Δ −5.0 ± 3.3	Δ −3.7 ± 3.5	0.037	0.040
Muscle mass	Δ +1.6 ± 4.4	Δ +0.4 ± 1.1	0.040	0.016

not been confirmed by another independent study, which seems to be of importance, especially given the relative low number of females who underwent intervention. Moreover, the functional importance of the gene variant's relationship to body weight is not clear. Because *NYD-SP18* has been associated with testosterone production, we speculate that it may predominantly influence exercise-based muscle development and formation.

Our results suggest that the rs6971091 *NYD-SP18* variant could be a genetic determinant of body composition changes after lifestyle intervention, at least in adult females. Determining whether these results hold true for other population groups and different types of interventions requires further study.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Adherence to Ethical Recommendations

All volunteers signed informed consent forms and agreed to participate in the study, which was approved by the Institutional Ethics Committee.

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