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Polymorphism *PLIN1* 11482 G>A interacts with dietary intake to modulate anthropometric measures and lipid profile in adults with normal-weight obesity syndrome

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Abstract

Evidence shows that genetic polymorphisms in perilipin 1 gene (*PLIN1*) are associated with excessive accumulation of body fat and disturbances in cardiometabolic markers. Therefore, the aim of this study was to verify whether the SNP *PLIN1* 11482 G>A (rs894160) interacts with nutrient intake, anthropometric, body composition and cardiometabolic markers in adults with normal-weight obesity (NWO) syndrome. A cross-sectional study was carried out with 116 individuals aged 20–59 years, with normal BMI and high percentage of body fat. Anthropometric and body composition measures, glycaemic control and serum lipid markers, SNP *PLIN1* 11482 G>A and nutrient intake were evaluated. Interactions between nutrient intake and the SNP were determined by regression models and adjusted for potential confounders. The SNP frequency was 56·0 % GG, 38·8 % GA and 5·2 % AA. Anthropometric measures and biochemical markers were not different according to genotype, except for total cholesterol (TC), LDL-cholesterol and non-HDL-cholesterol concentrations. However, important interactions between the SNP and dietary intake were observed. Carbohydrate intake interacted with the SNP *PLIN1* 11482 G>A to modulate waist circumference (WC) and the homeostatic model assessment of insulin resistance index. Interaction of lipid intake and the SNP modulated TC and LDL-cholesterol concentrations, and the interaction between protein intake and the SNP tended to modulate weight, WC and BMI. The SNP *PLIN1* 11482 G>A seems to modulate responses in anthropometric and lipid profile biomarkers of subjects with NWO depending on the dietary macronutrient composition, which may have long-term impact on cardiometabolic markers.

Key words: Dyslipidaemias: Food habits: Genetic polymorphism: Insulin resistance: Nutrigenetics

Despite the systemic and metabolic damages associated with excess of fat mass, the diagnostic of obesity is usually defined only considering the weight to height squared ratio (BMI)[1]. The BMI is the most widely used index for the classification of obesity, and it is considered an independent risk factor for CVD due to its strong association with fat mass. However, at individual level BMI presents limitations in the distinction between fat and lean mass and body fat distribution, which are very important parameters in the prediction of metabolic complications[2].

De Lorenzo *et al.*[3] described the normal-weight obesity (NWO) syndrome, characterised by normal BMI and a high percentage of body fat. However, there is no consensus regarding the cut-offs to classify obesity based on the percentage of body fat and these values range from 20 to 25·5 % for men and 30 to 38·9 % for women[4–7]. The prevalence of NWO in different populations is not well established, probably due to differences in the cut-off points[8]. Nevertheless, there is evidence associating high body fat, and particularly the NWO syndrome, with increased cardiovascular risks, high blood pressure, low concentrations of HDL-cholesterol, increased abdominal obesity and higher risk of the metabolic syndrome[6,9].

Among the aspects involved in the accumulation of body fat, genetic factors stand out. Studies have shown that polymorphisms in the perilipin 1 gene (*PLIN1*) may be related to excessive accumulation of body fat and to disturbances in lipid metabolism. As a response to the body's energy levels, perilipin has a specific action

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; IQR, interquartile range; NWO, normal-weight obesity; TC, total cholesterol; WC, waist circumference.

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on the lipid droplets in adipocytes, protecting them from the constant access of the hormone-sensitive lipase and therefore preventing TG broken into NEFA, especially in situations in which there is no need to activate lipolysis[10–12].

In humans, *PLIN1* is located at 15q26·1 chromosomal region, near one of the loci related to increased susceptibility to obesity, type 2 diabetes mellitus and hypertriacylglycerolaemia[11,13]. Among the polymorphisms described in *PLIN1*, the 11482 G>A (rs894160) is associated with lower expression of PLIN in adipocytes and with an increased noradrenaline-induced lipolysis process in individuals carrying the AA genotype compared with those carrying the GG genotype[14].

The interaction between perilipin and diet was tested for the first time in an animal model, in which transgenic mice overexpressing perilipin and fed a high-fat diet had lower body weight, fat mass and adipocyte size, as well as better glucose tolerance than wild mice under the same intervention[15]. In humans, the SNP *PLIN* rs894160 interacted with diet to modulate cardiovascular and glycaemic indexes. Individuals carrying the minor allele had higher insulin concentrations and homeostatic model assessment of insulin resistance (HOMA-IR) index when the saturated fat:carbohydrate ratio was above the group median[16]. As for complex carbohydrates, A allele carriers had higher waist circumference (WC) and BMI when the intake was below the median of the group. The authors suggested that the A allele may be protective against obesity in the presence of an increased intake of complex carbohydrates[17].

In obese women, these specific mechanisms when associated with energy restriction demonstrated that carriers of A allele had lower reduction in WC and higher decrease in lipid oxidation than non-carriers, confirming that the SNP rs894160 can interact with diet affecting energy metabolism[18]. Another SNP of *PLIN1* (rs2289487) was also associated with metabolic and anthropometric changes in individuals fed a very low energy diet; men carrying C allele had lower body weight, BMI, WC, body fat and leptin concentrations and women carrying C allele showed greater weight and fat loss[19]. The regulation of lipolysis and adipocyte energy homeostasis by perilipin may explain the influence of SNP *PLIN1* 11482 G>A on obesity[16,17].

Assuming that individuals with NWO present increased risks of developing high body fat-related diseases, including type 2 diabetes mellitus and dyslipidaemia, this condition has to be investigated. In addition, considering the lack of studies addressing this population, mainly concerning genetic variations and diet, the aim of this study was to evaluate the interactions between the SNP *PLIN1* 11482 G>A (rs894160), nutrient intake, anthropometric and body composition measures, and cardiometabolic markers in adults with NWO. The hypothesis was that the SNP *PLIN1* 11482 G>A interacts with dietary intake to modulate anthropometric, body composition and cardiometabolic parameters.

Materials and methods

Study design, participants and ethics

This was a cross-sectional study, with adult (20–59 years) men and women, with normal BMI (between 18·50 and 24·99 kg/m^2)[20] and high percentage of body fat (20 % for men and 30 % for women)[7], recruited at the Federal University of Goiás, from May to September 2015. Considering that studies of *PLIN1* genetic variants do not exist in the Brazilian adult population, the sampling was by convenience. Individuals who were taking medicines, hormones, vitamin and mineral supplements, were smokers and under nutritional treatment, had acute or severe diseases, were pregnant or lactating, had intense physical activity levels, were dietitians or nutrition students were not included in the study. The final sample was composed by 116 individuals (Fig. 1). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Ethics Committee of the Clinical Hospital of the Federal University of Goiás (protocol number 834·649). Written informed consent was obtained from all subjects.

Anthropometry, lifestyle and nutrient intake measurements

Participants answered a questionnaire with socio-economic and lifestyle data addressing information such as age, sex, education level, household income, per capita income, living conditions and lifestyle habits. Weight (Líder® P150 M weighing scale, capacity of 200 kg; Líder Balanças), height (Welmy® mobile stadiometer, measuring range of 2·20 m; Welmy) and WC (body measure tape, measuring range of 205 cm and graduation of 1 mm; Seca® Deutschland) were measured. BMI was calculated and dual-energy X-ray absorptiometry (Lunar DPX NT – General Electric Medical Systems Lunar®; Madison, EUA) was used to determine body fat percentage.

Three 24-h food records were collected in non-consecutive days, including one weekend day, using the multiple-pass method in order to evaluate usual nutrient intake[21]. Information was collected by a trained nutritionist. The first 24-h food record was collected face-to-face and the second and third ones, by phone call. Information was obtained in household measures, transformed into g or ml and analysed by the software Avanutri®.

Biochemical markers

Blood samples (10 ml) were collected from the median cubital vein for the quantification of fasting glucose and lipid (enzymatic colorimetric method), fasting insulin (electrochemiluminescence method) and glycated HbA_{1c} (immunoturbidimetric method). HOMA-IR, homoeostasis model assessment of β-cell function and quantitative insulin sensitivity check indexes were calculated. Samples of 1 ml of whole blood were frozen at −80°C until DNA extraction and genotyping.

Fasting glucose values higher than 100 mg/dl, fasting insulin values outside the range of 2·6 and 24·9 mU/l and HbA_{1c} higher than 5·7 % (39 mmol/mol) were considered as altered results[22]. Values above percentile 90th for HOMA-IR and homoeostasis model assessment of β-cell function indexes (2·61 and 201·08, respectively) and under percentile 10th for quantitative insulin sensitivity check index (0·33) were also considered altered. The lipid profile determination and classification followed the methods described previously[7].

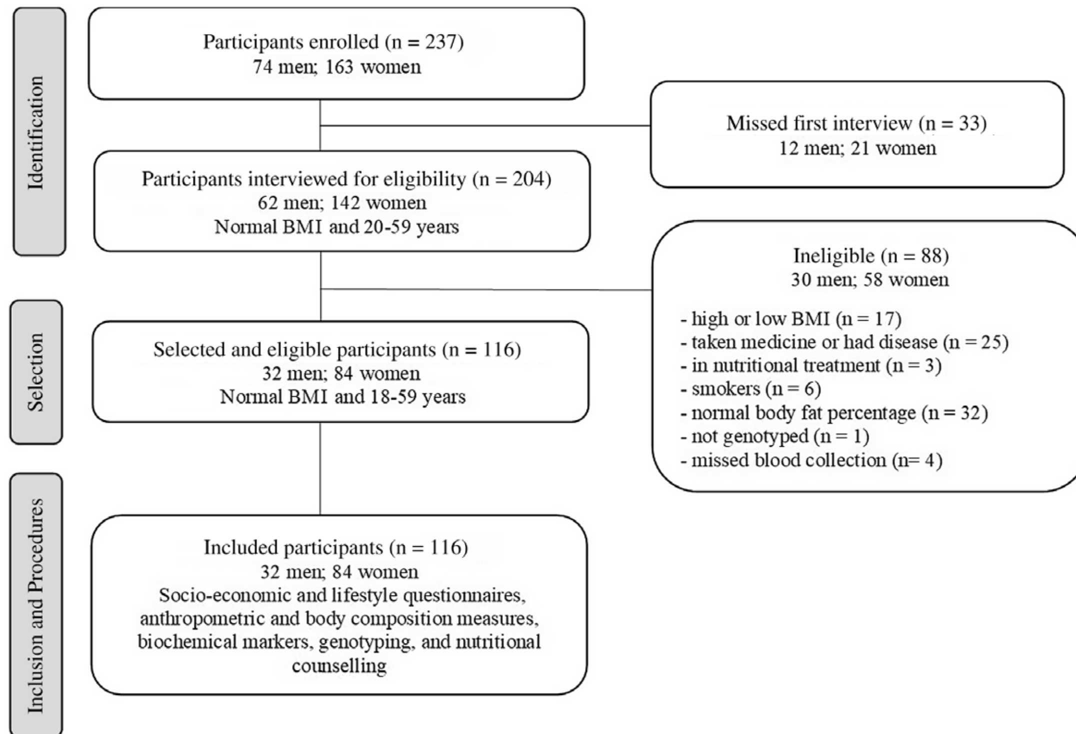

Fig. 1. STROBE flow chart of participants' recruitment.

Extraction of DNA was performed using a commercial kit (Roche Diagnostics GmbH). Genotyping was performed by real-time polymerase chain reaction using an inventoried assay (TaqMan SNP Genotyping Assays; Thermo Fisher Scientific) for the SNP *PLIN1* 11482 G>A in a 7500HT Fast Real-Time PCR (Thermo Fisher Scientific) at Centro de Genomas® Laboratory.

Statistical analyses and justification of sample size

The database was double entered to check the consistency of the results. Data distribution was verified by the Shapiro–Wilk's W test and otherwise stated, all reported values are mean and standard deviation or median (interquartile range (IQR)), according to the distribution. Outliers were defined as values outside 1·5 times the IQR above the upper quartile and below the lower quartile[23] and were excluded of the analyses. The statistical power was estimated using the linear multiple regression approach, with an effect size of 0·15, considered to be medium, a type I error probability of 0·05 and a sample size of 116 observations. The estimated power was 0·98566.

Adherence of the SNP *PLIN1* 11482 G>A to the Hardy–Weinberg equilibrium was checked using the χ^2 square test, according to the allele frequencies of the individuals included in the study. To verify whether the results of biochemical and anthropometric markers differed among the genotypes, the additive model for the SNP *PLIN*1 11482 G>A was applied (GG × GA × AA). In this approach, the results were evaluated using one-way ANOVA test followed by Tukey test or Kruskal–Wallis test followed by Dunn's test using the false discovery rate. In addition, the dominant model (GG × GA + AA) was also applied, and statistical analysis was performed using the Student's *t* test or Mann–Whitney test. Biochemical markers independence through the dominant model was tested using Pearson's χ^2 test or Fisher's test, and the OR was calculated when a significant association was found.

The interaction between macronutrients intake and the SNP *PLIN1* 11482 G>A was calculated using linear regression models (y ~ a + b + a:b), where a:b represents the interaction between the two independent variables. Thereafter, models were adjusted for potential confounders such as sex, weight, height, BMI, percentage of body fat and percentage of android and gynoid fat. For lipid profile biomarkers, the models were adjusted for sex, age, energy intake, physical activity level, saturated fat and dietary cholesterol intake. To minimise divergent results due to age, this variable was considered as a confounder, as well as its interaction with nutrient intake (age:macronutrient) and with genotype (age:genotype) in all adjusted models. A *P* value < 0·05 was adopted to indicate statistical significance. When a *P* value between 0·05 and 0·10 was observed in the regression, the model was unfolded. All analyses were performed in R software version 4.0.3[24].

Results

Total sample consisted of 116 individuals and women represented 72·6 % (*n* 84) of the total sample. The median age was 22·5 (IQR 21·4–24·9) years. Anthropometric measures, body composition, biochemical markers and nutrient intake data according to genotypes are described in Table 1. The median body fat percentage was 35·9 % (IQR 30·3–40·8 %) and the median BMI was 21·9 kg/m^2 (IQR 20·6–23·5 kg/m^2).

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Table 1. Markers of anthropometry, body composition, cardiometabolic health and nutrient intake for the total sample and according to the SNP *PLIN1* 11482 G>A genotypes (dominant model)
(Means; medians; standard deviations; interquartile ranges)

	GG		GA + AA			Total sample	
	n 65 (56·0 %)		*n* 51 (44·0 %)			*n* 116	
Markers	Mean or median	SD or IQR	Mean or median	SD or IQR	*P*	Mean or median	SD or IQR
Body weight (kg)	60·93	8·99	59·80	55·85–66·60	0·506	59·90	54·85–67·85
Height (m)	1·65	1·59–1·73	1·65	1·60–1·75	0·600	1·64	1·59–1·74
WC (cm)	74·15	5·96	73·72	5·20	0·676	73·96	5·62
BMI (kg/m²)	22·17	20·54–23·49	21·79	20·98–23·60	0·533	21·88	20·59–23·52
% BF	35·67	7·84	36·00	30·55–40·65	0·654	35·90	30·27–40·75
% android BF	39·08	7·76	38·09	8·29	0·514	38·64	7·98
% gynoid BF	47·20	42·20–51·80	47·30	39·10–51·15	0·732	47·20	39·15–51·48
% A/G ratio	0·87	0·12	0·86	0·11	0·876	0·86	0·12
Glucose (mg/dl)	84·95	7·62	85·12	7·10	0·905	85·03	7·36
HbA_{1c} (%)	5·33	0·69	5·22	0·67	0·379	5·28	0·68
Insulin (μU/ml)	6·20	4·60–9·50	6·40	4·42–9·40	0·986	7·04	3·25
HOMA-IR index	1·32	0·92–2·10	1·27	0·88–2·16	0·946	1·31	0·90–2·13
HOMA-β index	110·16	42·55	109·18	41·92	0·906	109·73	42·08
QUICKI index	0·37	0·03	0·36	0·34–0·39	0·965	0·37	0·34–0·39
TC (mg/dl)	201·28	51·60	178·24	41·36	**0·008**	191·15	48·55
Non-HDL-cholesterol (mg/dl)	137·46	48·08	118·53	36·62	**0·017**	129·14	44·26
HDL-cholesterol (mg/dl)	63·82	13·56	58·00	48·00–70·00	0·096	61·00	51·75–71·25
LDL-cholesterol (mg/dl)	117·55	44·98	95·93	33·85	**0·010**	106·50	77·00–135·50
VLDL-cholesterol (mg/dl)	18·00	12·00–26·00	18·00	14·00–25·00	0·682	18·00	13·00–25·25
TG (mg/dl)	88·00	62·00–128·00	91·00	69·5–123·50	0·642	88·00	66·75–127·25
Energy (kJ/d)	7763·20	2142·21	8878·95	2208·57	**0·007**	8003·15	6714·48–9889·30
Carbohydrate (g)	225·80	69·07	261·80	219·10–310·00	< **0·001**	235·77	194·98–293·77
Protein (g)	83·65	64·79–109·74	64·26	28·58	0·159	86·97	67·33–111·33
Protein (g/kg per d)	1·33	1·12–1·75	1·52	0·40	0·166	1·40	1·15–1·81
Total fat (g)	67·32	23·44	70·03	20·70	0·513	68·50	22·23
Cholesterol (mg)	233·75	148·92–325·81	275·19	127·43	0·216	242·67	166·60–345·55
Dietary fibre (g/d)	11·22	4·00	12·06	3·88	0·270	11·58	3·96
Alcohol intake (g/d)	0·00	0·00–0·95	0·00	0·00–1·11	0·815	0·00	0·00–0·95

SD, standard deviation; IQR, interquartile range; WC, waist circumference; BMI, body mass index; % BF, percentage of body fat; % android BF, percentage of android body fat; % gynoid BF, percentage of gynoid body fat; % A/G ratio, ratio between android body fat and gynoid body fat; HbA_{1c}, glycated Hb; HOMA-IR index, homeostatic model assessment of insulin resistance; HOMA-β index, homoeostasis model assessment of β-cell function; QUICKI index, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triacylglycerol.

Considering the whole sample (*n* 116), the mean intake of pantothenic acid, folate, calcium, magnesium and potassium was lower, while sodium and phosphorus were higher than that recommended by the dietary reference intakes[25] (data not shown). The mean intake of alcohol was under the maximum daily limit proposed by the WHO[26].

When the dominant model was applied to the SNP *PLIN1* 11482 G>A analysis, there were no differences in anthropometric and body composition markers between genotypes. For nutrient intake, total energy ($P = 0·007$) and carbohydrates ($P < 0·001$) were higher in individuals carrying the A allele. For biochemical markers, differences were found in total cholesterol (TC) ($P = 0·008$), non-HDL-cholesterol ($P = 0·017$) and LDL-cholesterol ($P = 0·010$) concentrations, all higher in individuals carrying the GG genotype compared with those carrying the GA + AA genotype (Table 1).

SNP *PLIN1* 11482 G>A frequencies are described in Table 2. Genotype frequency did not departure from Hardy–Weinberg equilibrium ($\chi^2 = 0·252$; $P = 0·615$).

The prevalence of disturbances in markers of glycaemic and lipid profiles is presented in Figs. 2 and 3. It is worth noting that at least one disturbance in glycaemic control and lipid profile markers was found in 31·0 and 54·3 % of participants, respectively. Disturbances in TC and non-HDL-cholesterol concentrations as well as the occurrence of more than one disturbance were more prevalent in individuals carrying the GG genotype ($P < 0·001$, $< 0·001$ and 0·004, respectively). The prevalence of glycaemic disturbances did not differ between GG genotype and A allele carriers (GA + AA).

Associations between lipid profile markers with GA + AA genotype were found, in which this genotype seemed to be a protective factor for TC and non-HDL disturbances (OR = 0·221, $P < 0·001$ and OR = 0·185, $P < 0·001$, respectively), while it increased the odds of disturbances in HDL-cholesterol

Table 2. Distribution of the SNP *PLIN1* 11482 G>A genotypes and allele frequency for the total sample and according to sex
(Numbers and percentages)

	Genotypes						Allele frequency	
	GG		GA		AA		G	A
	n	%	*n*	%	*n*	%		
Total	65	56·0	45	38·8	6	5·2	0·750	0·250
Female	48	57·1	31	36·9	5	6·0	0·760	0·240
Male	17	53·1	14	43·8	1	3·1	0·750	0·250

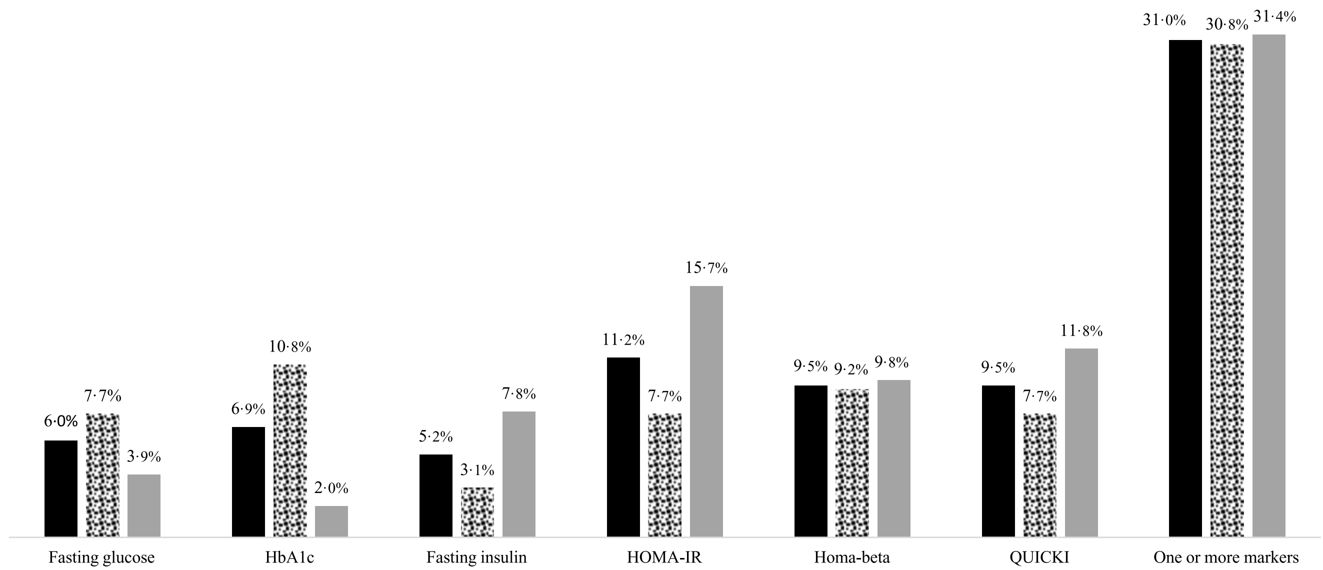

Fig. 2. Prevalence of disturbances in glycaemic control markers of adults with NWO syndrome according to the SNP *PLIN1* 11482 G>A genotypes. NWO, normal-weight obesity; HbA_{1c}, glycated Hb; HOMA-IR, homeostatic model assessment of insulin resistance index; HOMA-beta, homoeostasis model assessment of β-cell function index; QUICKI, quantitative insulin sensitivity check index. ■, total; ▦, GG; ■, GA + AA.

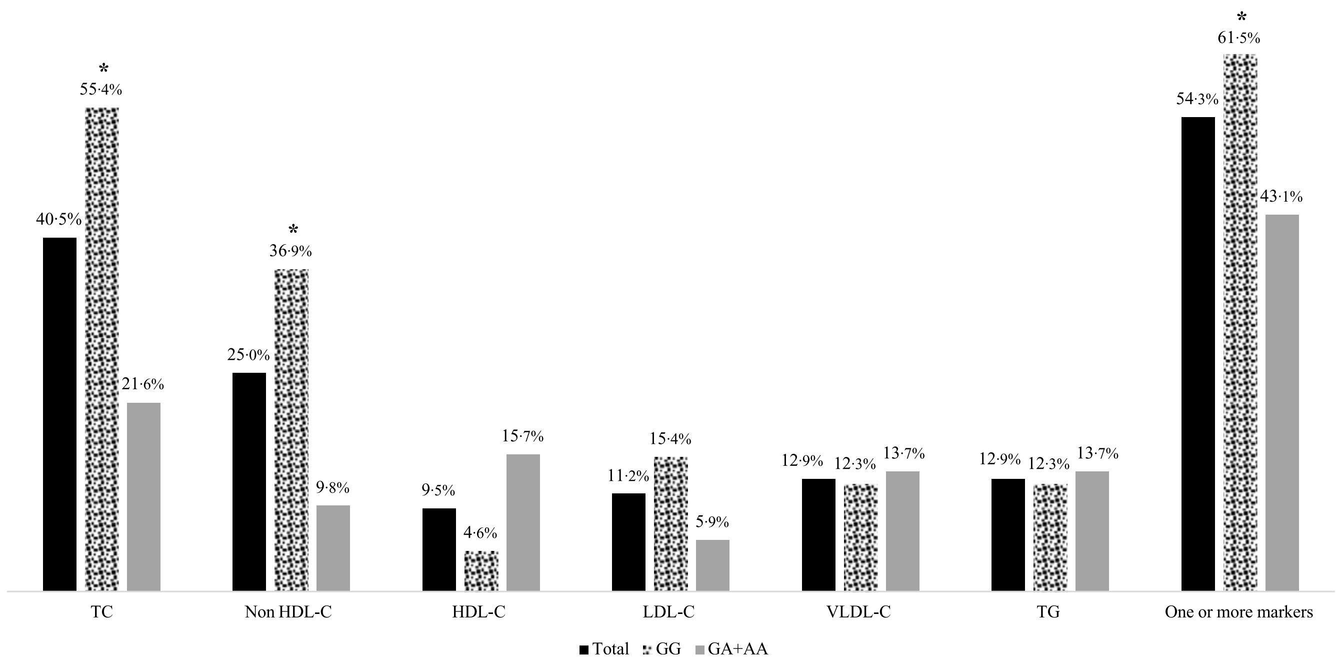

Fig. 3. Prevalence of disturbances in lipidaemic control markers of adults with NWO syndrome according to the SNP *PLIN1* 11482 G>A genotypes. * Significant differences between GG and GA + AA genotypes (Pearson's χ^2 test or Fisher's test) NWO, normal-weight obesity; TC, total cholesterol; TG, triacylglycerol. ■, total; ▦, GG; ■, GA + AA.

concentrations (OR = 3·844, P = 0·044) compared with the GG genotype (data not shown).

When markers of anthropometry, body composition or cardiometabolic profile were evaluated according to genotypes in the additive model, no significant differences were found, except for TC concentrations, which were higher in individuals carrying the GG genotype compared with those carrying the GA genotype (GG = 201·3 mg/dl^{a}, GA = 178·2 mg/dl^{b} and AA = 178·5 mg/dl^{ab}, P = 0·038) (data not shown).

For total energy intake, individual carrying the GA genotype had the highest values, followed by AA and GG individuals (8929·3^{a}, 8501·0^{ab} and 7763·2^{b} kJ/d; P = 0·024). The same results were observed for carbohydrates intake, with the highest values seen in GA, followed by AA and then GG carriers (70·2^{a}, 67·8^{ab} and 48·8^{b} g/d; P = 0·003) (data not shown).

Significant interactions between nutrient intake, genotype, anthropometry and biochemical markers were found. Carbohydrate intake (in g) interacted with genotype to modulate

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Table 3. Interactions between anthropometry, biochemical and nutrient intake markers according to the SNP *PLIN1* 11482 G>A genotypes (dominant model) (*β*-coefficients and 95 % confidence intervals)

		Model 1	Effect				Model 2	Effect			
Marker	Genotype	$P_{interaction}$	β	95 % CI	P	P_{global}	$P_{interaction}$	β	95 % CI	P	P_{global}
Carbohydrate (g)											
WC	GG	0·018	0·033	0·013, 0·052	0·001	0·012	0·031*	0·012	0·003, 0·022	0·008	< 0·001
	GA/AA		–0·002	–0·023, 0·019	0·841			0·001	–0·008, 0·011	0·796	
Carbohydrate (%)											
Insulin	GG	0·069	–0·074	–0·183, 0·034	0·180	0·303	0·678†				
	GA/AA		0·092	–0·050, 0·234	0·203						
HOMA-IR	GG	0·027	–0·014	–0·039, 0·010	0·263	0·162	0·021‡	0·000	–0·002, 0·001	0·773	< 0·001
	GA/AA		0·032	–0·000, 0·064	0·053			0·003	0·000, 0·005	0·009	
Protein (%)			–		–	–	–	–		–	–
Weight	GG	0·060	0·249	–0·123, 0·622	0·187	0·009	0·155§	–		–	–
	GA/AA		0·902	0·332, 1·471	0·002			–		–	
WC	GG	0·084	0·106	–0·145, 0·358	0·404	0·056	0·453*	–		–	–
	GA/AA		0·510	0·125, 0·895	0·009			–		–	
BMI	GG	0·080	0·081	0·008, 0·154	0·029	0·001	0·542‖	–		–	–
	GA/AA		0·199	0·088, 0·311	0·001			–		–	
Lipid (%)											
Non-HDL-cholesterol	GG	0·073	–2·096	–4·316, 0·125	0·064	0·025	0·122¶	–		–	–
	GA/AA		1·005	–1·562, 3·573	0·439			–		–	
LDL-cholesterol	GG	0·031	–2·410	–4·462, –0·358	0·021	0·006	0·059**	–2·410	–4·462, –0·358	0·021	0·006
	GA/AA		1·027	–1·344, 3·399	0·390			1·027	–1·344, 3·399	0·392	
TC	GG	0·043	–2·467	–4·880, –0·153	0·045	0·009	0·078††	–1·795	–4·006, 0·926	0·150	0·003
	GA/AA		1·341	–1·449, 4·131	0·343			1·856	–0·686, 4·888	0·190	
Dietary fibre (g)											
HbA_{1c}	GG	0·076	–0·023	–0·066, 0·019	0·280	0·307	0·354‡‡	–		–	–
	GA/AA		0·036	–0·014, 0·087	0·159			–		–	

WC, waist circumference; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; HbA_{1c}, glycated Hb; β, β-coefficient of regression. Model 1: Simple linear regression between SNP:intake variable. Model 2: Adjusted linear regression.
* Adjusted for body fat percentage, android fat percentage, weight and height.
† Adjusted for age:carbohydrate (g) interaction and blood glucose.
‡ Adjusted for age:genotype interaction, blood glucose and insulin.
§ Adjusted for sex, body fat percentage and height.
‖ Adjusted for sex, weight and height.
¶ Adjusted for age:genotype interaction and genotype.
** Adjusted for genotype.
†† Adjusted for genotype, age:genotype interaction and energy.
‡‡ Adjusted for insulin and age.

WC ($P_{interaction}$ = 0·018) so that for each 1-g increase in intake, there was an increase of 0·03 cm in WC of GG genotype carriers (β = 0·033, P = 0·001). The interaction remained significant after adjustments (P = 0·031) (Table 3). WC also tended to be modulated by the interaction between protein as percentage of total energy intake and genotype ($P_{interaction}$ = 0·084), so that the unfolded model revealed a direct association (β = 0·510, P = 0·009) for A allele carriers, but the trend disappeared after adjustment for potential confounders (P = 0·453). Protein as percentage of total energy intake also tended to interact with the SNP to modulate body weight ($P_{interaction}$ = 0·060) and BMI ($P_{interaction}$ = 0·080). In both cases, the anthropometric markers increased more in individuals carrying the A allele (β for weight = 0·902, P = 0·002 and β for BMI = 0·199, P = 0·001) than in individuals with GG genotype (β for weight=0·249, P = 0·187 and β for BMI = 0·081, P = 0·029), but the trends observed in the raw models disappeared after adjustments (P = 0·155 and P = 0·542, respectively for weight and BMI) (Table 3).

Regarding cardiometabolic profile markers, carbohydrate as the percentage of total energy intake interacted with genotype to modulate the HOMA-IR index ($P_{interaction}$ = 0·027). After unfolding the model, a positive marginal association of the GA + AA genotype with the index was observed (β = 0·032, P = 0·053), which remained significant after adjustments ($P_{interaction}$ < 0·001). The interaction of carbohydrate intake with genotype also tended to modulate insulin concentrations ($P_{interaction}$ = 0·069), but after unfolding and adjustments of the model, significance was lost.

Lipid as the percentage of total energy intake interacted with genotype to modulate LDL-cholesterol ($P_{interaction}$ = 0·031) and TC ($P_{interaction}$ = 0·043) concentrations, and a trend was observed for non-HDL-cholesterol concentrations ($P_{interaction}$ = 0·073). For LDL-cholesterol and TC, an indirect association of lipid intake with GG genotype was observed (β = –2·410, P = 0·021 and β = –2·467, P = 0·045, respectively). After correcting the models for potential confounders, the associations with LDL-cholesterol ($P_{interaction}$ = 0·059) and TC ($P_{interaction}$ = 0·078) concentrations became a trend and that with non-HDL-cholesterol concentrations disappeared ($P_{interaction}$ = 0·122).

Dietary fibre intake tended to interact with genotype to modulate HbA_{1c} concentrations ($P_{interaction}$ = 0·076), but after unfolding the model, no significant impact on β coefficients was found (β = –0·023, P = 0·280 and β = 0·036, P = 0·159, for GG *v*. GA + AA genotypes, respectively).

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There were no significant interactions of energy and saturated fat intake or the ratio between saturated fat and carbohydrate with anthropometric or cardiometabolic parameters. Furthermore, no association between macronutrients intake and body fat content or distribution (gynoid or android) was observed. Interactions between carbohydrate, lipid, protein and dietary fibre intake that did not show significant associations are presented in online Supplementary Table S1.

Discussion

It is important to highlight that a few studies analysing Brazilian individuals with NWO were published, and only one assessed genetic variation in this population[7,27]. In addition, there is also only one study addressing the SNP *PLIN1* 11482 G>A in Brazilian children[28]. Thus, this is the first study to assess interactions of the SNP *PLIN1* 11482 G>A with nutrient intake, anthropometric and body composition measures, and cardiometabolic markers in individuals with NWO syndrome. The SNP *PLIN1* 11482 G>A interacted with nutrient intake, anthropometric measures and cardiometabolic markers in adults with NWO syndrome.

Although presenting a normal BMI, individuals with NWO have high percentages of body fat and are at risk of developing metabolic conditions such as insulin resistance, type 2 diabetes mellitus and dyslipidaemia. In addition, normal BMI commonly leads health professionals to overlook metabolic parameters that could reveal disease development risks, which may cause delay in the detection of risks associated with the metabolic syndrome, insulin resistance and other diseases[27]. High percentages of body fat can affect glucose homoeostasis because adipokines from white adipose tissue and batokines from brown/beige adipose tissue regulate hepatic lipogenesis and glucose output and disposal. Brown/beige adipose tissue contributes to glucose disposal and oxidation of lipids, while white adipose tissue can generate fatty acid and glycerol overload by lipolysis, leading to hepatic enhancement of gluconeogenesis, glucose output and muscle insulin resistance[29].

In the present study, it was verified that 31·0 % of individuals with NWO showed at least one disturbance in glucose profile, especially in the HOMA-IR and homoeostasis model assessment of β-cell function indexes. A reduced sensitivity to insulin and an increase in pancreatic β cell function have been observed in individuals with NWO syndrome[27,30]. It is possible that the high insulin secretion is a compensatory response to the low insulin sensitivity found in these individuals. Disorders in biochemical markers of glycaemic control, such as HOMA-IR and homoeostasis model assessment of β-cell function point to an insulin resistant state and could indicate a risk for type 2 diabetes mellitus and CVD development in the near future[29]. Lipid profile markers such as HDL-cholesterol, LDL-cholesterol and TG seem to be independent factors to myocardial infarction, angina pectoris and heart failure[31]. This highlights the importance of the results found in the present study and indicates the need for early identification and intervention, aiming at controlling the body fat percentage and cardiometabolic markers of individuals with NWO syndrome.

Inappropriate dietary habits combined with disturbances in cardiometabolic markers result in higher risk of metabolic diseases for individuals with NWO syndrome. In the present study, the mean intake of all macronutrients was dissonant to the recommended values for healthy eating. Mean carbohydrate intake was below the minimum recommended value, while total fat and protein intake were higher than the maximum values[32]. Similar data of inadequate nutrient intake were observed in a study of 4786 Finnish subjects, of whom 697 had NWO, in which carbohydrate intake was low and protein intake was higher compared with the recommendations[33].

Although inadequate dietary habits, mainly high-fat diets, may be related to excessive accumulation of body fat in individuals with NWO syndrome[34], it is important to highlight the influence of genetic variability in the phenotypic response to eating habits. In the present study, the frequency of the minor allele of the SNP *PLIN1* 11482 G>A was 24·6 %, and ranged from 24·0 to 44·0 % in previous publications[35,36]. In a study of 1589 Spanish (781 women), lower BMI, weight, waist-to-hip ratio and fasting glucose concentrations were observed in women who carried the minor allele (GA + AA) compared with the homozygous major allele carriers (GG)[11]. Another study of 150 Spanish individuals found that carriers of the minor allele had lower weight and BMI[36]. In contrast, similar to the present study results, a study of 234 obese Brazilian children and adolescents did not associate the minor allele with disturbances in anthropometric or biochemical markers[28]. However, these studies did not evaluate nutrient intake. On the other hand, most studies that addressed nutrient intake did not consider genetic characteristics[17].

In the present study, important interactions between the SNP *PLIN1* 11482 G>A and intake of carbohydrates and lipids were found. These results suggest a detrimental effect of the GG genotype in relation to carbohydrates intake and WC. WC also appears to be negatively affected by protein intake in the presence of the A allele. In the present study, sex was considered a confounding factor, but it did not change the results of the associations. In contrast, other authors found association of the SNP *PLIN1* 11482 G>A with anthropometric markers only in women[11,17,35].

Nevertheless, our results did not show a significant interaction of the SNP with carbohydrates intake on the WC of A allele carriers, diverging from a study of 920 obese Caribbean in which individuals carrying the A allele and consuming high amounts of complex carbohydrates presented lower WC and hip circumference[17]. Another study of 4107 individuals with NWO, overweight or obesity from Singapore found that genetic variations in *PLIN1* are able to modulate the effects of saturated fat and carbohydrates intake on insulin resistance, as women carrying the minor allele of the SNP *PLIN1* 11482 G>A and consuming higher amounts of saturated fat and lower amounts of carbohydrate showed higher values of HOMA-IR[35]. Our results showed a trend of interaction between the SNP and carbohydrates intake in modulating insulin concentrations ($P = 0{\cdot}069$), but it disappeared after adjustments.

Our results also disagree with those from one with 970 overweight North American, which identified interaction between the minor allele of the SNP *PLIN1* 11482 G>A and the saturated fat to carbohydrates ratio on biochemical markers related to the

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development of insulin resistance[17]. However, those individuals were older (48·5 years) and had higher BMI values (28·3 kg/m^2) than the participants in the present study. This observation suggests that metabolic disturbances due to the interaction between genotype and diet may depend on the time of exposure to a particular dietary habit. To our knowledge, there are no studies addressing such characteristics in individuals with NWO syndrome.

As the protein encoded by *PLIN1* gene is directly associated with lipid storage in the adipocytes, this can interfere with lipid mobilisation and serum lipid concentrations, so that an important relationship with the development of dyslipidaemias can be expected. As previously reported in a study with obese children and adolescents, the minor allele of the SNP *PLIN1* 11482 G>A was associated with higher TG and lower HDL-cholesterol concentrations[28]. In the present study, no associations were found between the SNP *PLIN1* 11482 G>A genotypes and HDL-cholesterol and TG concentrations; however, it was observed that individuals carrying the GG genotype showed higher TC, LDL-cholesterol and non-HDL-cholesterol concentrations, and that lipid intake and genotype interacted to negatively modulate TC and LDL-cholesterol concentrations when compared with those carrying the GA + AA genotype. It was also observed that the A allele appeared to protect against disturbances in TC (OR = 0·221) and non-HDL-cholesterol (OR = 0·185) concentrations, but as a risk factor for disturbances in HDL-cholesterol concentrations (OR = 3·884).

In the present study, no interaction was observed between dietary fibre intake and the SNP in the modulation of cardiometabolic risk parameters, but only a trend of interaction in the modulation of HbA_{1c} concentrations. Dietary fibre intake is known to stimulate satiety and regulate total energy intake, reducing TC and/or LDL-cholesterol concentrations and attenuating postprandial glycaemia/insulinaemia[7,37]. Therefore, our results regarding dietary fibres and the SNP interaction in HbA_{1c} concentrations modulation could be significant if participants' intake was higher or closer to recommendations.

Limitations of our study include the homogeneity of anthropometry and age data, and the lack of standard cut-offs for body fat percentage and some biomarkers. The sample size can be seen as a limitation; however, the sample's estimated power was very high (0·985).

In conclusion, this study revealed that interactions between macronutrient composition of diet and the SNP *PLIN1* 11482 G>A can modulate responses in anthropometric parameters and lipid profile markers of adults with NWO syndrome. Similar to other nutrigenetic studies, the findings of the present study need to be cautiously generalised to other populations, as the results may be influenced by differences in racial and ethnic attitudes related to lifestyle. Certainly, studies including larger number of participants with NWO syndrome are necessary to endorse our results.

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The authors' contributions are as follows: L. C. H. participated in analysis, interpretation and discussion of data and wrote the manuscript; A. G. Z. S. and L. P. F. participated in data acquisition, analysis and interpretation; M. A. H. was the secondary supervisor, provided laboratory expertise and contributed to the study design and interpretation of the findings; C. C. was the primary supervisor responsible for the study conception, participated in data acquisition, analysis and interpretation and reviewed the manuscript. All authors read and approved the final version of the manuscript.

M. A. H. is a former consultant of Centro de Genomas® Laboratory. All other authors have no conflict of interest to declare.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521004396

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