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The ATP-binding cassette transporter a1 R230C variant does not modify the weight loss response to hypocaloric diet

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ABSTRACT

Objective: To investigate if the R230C variant of the ATP-binding cassette transporter A1 (known to be associated with obesity) modifies the response to a hypocaloric diet. **Subjects**: The study sample consisted of 49 women, 21 R230C cases and 28 with the R230R genotype. Patients followed a hypocaloric diet (500 calories below their average daily consumption) for 12 weeks. The main outcome was weight loss. **Measurements**: At baseline and every four weeks, weight, waist, and hip circumference were measured and glucose, insulin, and plasma lipids were determined. At baseline and at the end of the study, leptin and adiponectin concentrations were measured and insulin sensibility (using the minimal model approach) along with body composition was estimated. Eating behavior was registered. Multiple linear regression analysis was applied to evaluate the primary outcome, adjusting for age, smoking, physical activity, and diet adherence. **Results**: At baseline, R230C patients had lower HDL-cholesterol and apolipoprotein AI values. Reduction of weight, waist, hip circumference, body mass index, glucose,

RESUMEN

Objetivo: Investigar si la variante R230C del transportador ABCA1 (asociado a la obesidad) modifica la respuesta a una dieta hipocalórica. **Sujetos:** La muestra del estudio estuvo compuesta por 49 mujeres, 21 con el alelo de riesgo R230C y 28 con el genotipo R230R. Los pacientes siguieron una dieta hipocalórica (500 calorías menos que su consumo diario promedio) durante 12 semanas. El desenlace principal fue la pérdida ponderal. **Mediciones:** En la visita inicial y cada 4 semanas, se midió el peso, la cintura, la cadera y se determinó la concentración de glucosa, insulina y lípidos en plasma. En la visita inicial y final se midieron las concentraciones de leptina y adiponectina y se midió la acción de la insulina (mediante el modelo mínimo) y la composición corporal. La conducta alimentaria fue registrada. La regresión lineal múltiple fue usada para identificar los determinantes del desenlace primario, ajustando por la edad, tabaquismo, actividad física y adherencia a la modificación dietaria. **Resultados:** En la visita inicial, los casos con el alelo de riesgo R230C tenían concentraciones menores de colesterol HDL y

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insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, homeostatic model assessment-insulin resistance and apolipoprotein A1 occurred in both groups. The presence of the C230 variant did not affect the response. No differences between groups were observed in the minimal model parameters and in the body composition variables. Cases with the R230C genotype had a significantly lower disinhibition score at the final visit (p = 0.006). **Conclusions**: The presence of the C230 allele in the ABCA1 gene has no effect on weight loss in response to hypocaloric diet. The association between obesity and the R230C variant of *ABCA1* cannot be explained by a different response to a hypocaloric diet. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:128-37)

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Key words: Obesity. ATP-binding cassette protein. Weight loss. Diet.

apolipoproteína AI. La reducción del peso, cintura, cadera, índice de masa corporal, glucosa, insulina, colesterol, colesterol HDL, colesterol LDL, HOMA-IR y apolipoproteína A1 ocurrieron en ambos grupos. La variante C230 no afectó la respuesta. No existieron diferencias en los parámetros del modelo mínimo y de la composición corporal. Los casos R230C tuvieron una mayor desinhibición del apetito en la visita final (p = 0.006). **Conclusiones:** La presencia del alelo C230 en el gen ABCA1 no tiene efecto en la respuesta ponderal a una dieta hipocalórica. La asociación entre la obesidad y la variante R230C no puede ser explicada por una respuesta distinta a la restricción calórica.

Palabras clave: Obesidad. ABC-A1. Pérdida ponderal. Dieta.

INTRODUCTION

In Mexico, obesity is a mayor health problem and its incidence has increased notably during the last decades. In 2006, 34.5% of women and 24.4% of men were obese. The combined prevalence of overweight and obesity in men and women > 20 years old is 66.7 and 71.9% respectively[1]. Genetic factors are important determinants of body weight. The variation in the prevalence of obesity among ethnic groups may be explained by differences in the prevalence of susceptibility alleles. Hispanics are particularly susceptible to develop obesity and obesity-related comorbidities. The R230C variant of the ATP-binding cassette transporter A1 (ABCA1) is frequently found in the Mexican population and has been associated with obesity, hypoalphalipoproteinemia, and early type 2 diabetes mellitus[2,3]. This variant diminishes the transporter activity by 30%. Villarreal-Molina, et al. showed that individuals with the C230 genotype are at an increased risk of obesity (OR: 2.527; 95% CI: 1.667-3.819; p = 0.005). This association remained significant after adjusting for admixture (OR: 2.428; 1.548-3.706; p = 0.011). This polymorphism is specific for American native people and has a frequency of 12% in Mexican mestizos[4]. Acuña-Alonzo, et al. suggest that this variant results in a selective advantage, allowing storage of cholesterol during abundance and thus survival during long periods of starvation. Natural selection may have led to an increased frequency of this "saving phenotype" in populations who intermittently endure long periods of severe hunger. However, this advantage is lost when individuals with this variant are exposed to an affluent environment.

The ABCA1 transporter is a membrane protein that transports cholesterol, phospholipids, and other lipids across plasma membranes where they are removed by nascent HDL particles[5]. The ABCA1 mutations cause Tangier's disease[6-9]. Cholesterol efflux is carried out by this protein and in all cells including pancreatic β-cells and adipocytes. Defects in ABCA1 cause cholesterol accumulation in the cell membrane and the emergence of several possible phenotypes. *ABCA1* knockout mice have diminished insulin secretion; this defect is reversible with the correction of the intracellular cholesterol levels[10]. Recent data have shown that HDL particles and the ABCA1 transporter are involved in the regulation of adipocyte differentiation and function[11,12]. The cholesterol content of the cell membrane is associated with the size of the fat cells. Since large adipocytes are dysfunctional and a source of proinflammatory cytokines, it has been proposed that cholesterol accumulation may have a deleterious effect on adipocyte function[13]. The ABCA1 functions as a defense mechanism against cholesterol accumulation in fat cells. Diminished ABCA1 activity contributes to adipocyte enlargement, with potential functional and

secretory consequences[14]. Furthermore, ABCA1 is expressed abundantly in the intestine and participates in intestinal cholesterol absorption. Certain ABCA1 variants modify postprandial lipemia[15]. These observations may explain how an *ABCA1* allele with reduced activity is associated with obesity.

The aim of this study was to compare the weight loss response to a hypocaloric diet between subjects with the *ABCA1* R230C genotype and age- and sex-matched controls carrying the wild-type allele (R230R) to see if this variant, known to be a risk allele for obesity and metabolic syndrome, has a modulatory effect on weight response.

SUBJECTS AND METHODS

This is a comparative, longitudinal, and prospective 12-week study. This work was carried out in an out-patient clinic of a tertiary referral center. The study staff was blinded to the *ABCA1* genotypes. The study protocol was approved by the Ethics Committee of the Institution.

The inclusion criteria were: Mexican mestizos aged 20-60 years old (defined as having parents and grandparents born in Mexico), body mass index (BMI) 25.0-39.9 kg/m^2, who signed the informed consent form. Cases and controls were matched by age (± 5 years), gender, and BMI (± 3 kg/m^2). Exclusion criteria were: type 2 diabetes mellitus, weight loss > 3 kg during the previous three months, a cardiovascular event in the six months prior to inclusion, catabolic diseases, pregnancy, and any drug that may alter the weight or the metabolic variables of this study. All participants were unrelated. Any subject who did not complete an adherence of at least 70% to the diet or who failed to answer 30% or more of the 24-hour diet assessment instrument or who initiated prohibited medication as described in the exclusion criteria were removed from the study.

Dietary intervention and clinical assessment

During the first visit, a complete clinical history was taken. The patient's routine food ingestion was estimated using 24-hour food records of the last three days (two week days and one weekend day). In addition, patients answered a one-week food frequency recall. Trained personnel applied the questionnaires. A dietary plan was prescribed based on the patient's preferences; the caloric content was decreased by 500 calories from the estimated caloric intake. The composition of the diet was: 50% carbohydrates, 20% proteins, and 30% lipids (of which 7% were saturated, 10% polyunsaturated, and 13% monounsaturated) and cholesterol 200 mg/day. Patients were seen each month for a clinical and anthropometric evaluation and contacted by phone every two weeks to reinforce adherence to the treatment. Three-day food records were filled out prospectively in each visit. Physical activity was registered using the Laval physical activity questionnaire previously validated in the Mexican population[16] at the beginning and later on, every visit. Patients were asked to maintain their usual physical activity without modifications. Trained personnel carried out anthropometric measurements. Daily calibrated equipment was used[17]. Patients were asked to wear light clothing for the measurements. A three-factor questionnaire assessing hunger, satiety, and eating behaviors was applied initially and at the end of the study[18]. Bioelectric impedance (Quantum X BIA Analyzer, RJL systems) was carried out at the beginning and at the end of the study.

Insulin sensitivity (S_I) and insulin secretion (AIRg) were measured using the minimal model at the beginning and at the end of the study[19].

Laboratory methods

All laboratory measurements were carried out at the Endocrinology and Metabolism Department (certified by the American College of Pathologists) in the INCMNSZ. Standardized procedures were used to measure glucose, total cholesterol, triglycerides, and HDL cholesterol concentrations using commercial reagents (Beckmann Co). Plasma insulin was determined with an enzyme immunoassay (EIA). Apolipoproteins A1 and B were measured by nephelometry. The HbA1c levels were estimated using high-performance liquid chromatography (Bio-Rad, Hercules, CA). Leptin and adiponectin concentrations were

estimated using an ELISA assay (Linco Research, St Charles, Missouri, USA).

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from peripheral blood leukocytes. The R230C variant was genotyped using TaqMan® assays (ABI Prism 7900HT Sequence Detection System, Applied Biosystems). Samples for the three genotypes obtained by sequencing were used as controls for genotyping. Deviation from the Hardy-Weinberg equilibrium was not observed.

Statistical analysis

The sample size (n = 21 participants per group) was estimated assuming that the risk allele would be associated with a 3 kg difference in the weight loss response to caloric restriction, a standard deviation of the effect size of ± 3 kg, an alpha error < 0.05, and a beta error < 0.1. Data are presented as either mean (± standard deviation) or percentages. One-way analysis of variance (ANOVA) was used for the comparison of continuous variables between genotypes. Non-parametric statistics were utilized (Mann-Whitney) when applicable. The effect of the variant R230C of *ABCA1* on the metabolic outcomes was assessed using multiple lineal regression models, the dependent variable being the difference between the initial and the final measurement. The main outcome variable was weight loss. Age, smoking, physical activity (mean kcal/day during the study), and caloric intake (mean caloric content of the diet during the study) were included in the model as covariates. Data were analyzed using SPSS for Windows (version 15.0, SPSS Inc, Chicago, Ill).

RESULTS

Baseline characteristics

The study sample consisted of 49 women, of which 28 had the wild genotype (R230R) and 21 had the risk allele (R230C). The majority of the participants were obese. Their mean caloric intake was 1965.31 kcal/day. At baseline, the mean diet composition was carbohydrates 54.8 ± 7.79%, fat 30.48 ± 5.68%, and proteins 14.71 ± 3.75%. No differences were observed in the caloric content or the composition of the diet between the R230R and the R230C groups. There was no significant difference in any of the clinical (Table 1) and laboratory parameters at baseline (Table 2), except for the lower levels of HDL cholesterol (p = 0.014) and apolipoprotein AI (p = 0.011) in patients with the R230C variant.

Response to caloric restriction: effect of the R230C allele of ABCA1

Six patients were excluded because they declined to complete the follow-up period. No subject was excluded due of lack of adherence to the diet. Consequently, 43 patients were enrolled (24 women with the R230R genotype and 19 with the R230C genotype). All participants completed the study. The mean caloric content of the diet during the intervention period was 1405.76 ± 240.5 kcal/day; this represents 500 kilocalories less than their basal caloric input. The weight-loss intervention had the expected results. The average weight difference was –3.16 ± 2.41 kg (p = 0.001). The waist and hip circumference were reduced in direct proportion to the weight change. Weight loss was associated with significantly lower insulin and HOMA-IR values in both groups.

The changes that occurred in the clinical and biochemical parameters in each genotype group are shown in table 3. The presence of the R230C allele did not affect the weight loss response due to the intervention. Significantly lower apolipoprotein AI and total HDL and LDL cholesterol were found following caloric restriction in the R230R group. The same trend was observed in the R230C group, but the differences did not achieve statistical significance. The intervention resulted in lower triglyceride concentrations in the R230C patients, but not in the wild-type allele group. Despite these within-group differences, none of the between-group differences reached statistical significance.

Table 1. Baseline characteristics by genotype

Parameter	R230R (n = 28)	R230C (n = 21)	P value
Age (years)	41.36 (± 9.44)	43.76 (± 6.81)	0.328
Weight (kg)	77.18 (± 9.33)	75.58 (± 8.32)	0.538
BMI (kg/m^2)	31.46 (± 3.66)	30.65 (± 2.53)	0.387
Waist circumference (cm)	94.5 (90-103)	95 (94-100.95)	0.693
Hip circumference (cm)	108 (105-117)	108 (104.5-115.25)	0.297
Waist/hip ratio	0.88 (± 0.09)	0.89 (± 0.05)	0.462
Average blood pressure (mmHg)	87.54 (± 10.58)	83.51 (± 10.87)	0.219
Physical activity (calories)	3619.2 (± 532.84)	3461.33 (± 386.37)	0.282
Caloric content of the diet	1960.51 (± 405.9)	1954.71 (± 301.18)	0.958
DM2 family history [n(%)]	16 (57.1)	11 (52.3)	0.740
Smoking [n(%)]	1 (3.6)	3 (14.3)	0.193
Medication use [n(%)]			
– Statins	3 (10.7)	0	NS (0.250)
– Fibrates	2 (7.1)	0	
– Oral anticonceptives	2 (7.1)	0	
– Tiazides	0	1 (4.8)	
Hypertension [n(%)]	3 (10.7)	3 (14.3)	0.518

Data are n (%) or means ± SD, or median ± interquartilar range when there is an non normal distribution.

Insulin secretion and action: effect of the R230C allele of ABCA1 (Table 3)

In the baseline evaluation there were no differences between the two groups regarding the insulin sensitivity index (SI) or the acute insulin response (AIRg). Weight loss resulted in a decrement of the AIRg ($p = 0.039$) with a significant modification of the S_I. The risk allele did not alter the response induced by weight loss on AIRg and S_I.

Body composition (Table 3)

Two bioelectric impedance measurements (baseline and post-diet) were completed in 20 patients with the R230R genotype and 16 patients with R230C genotype. This method distinguishes whether weight loss is caused by changes in water content or decreased fat or lean mass. In both groups, the weight loss was mainly due to fat loss ($p = 0.002$) and fat-free body mass ($p = 0.003$). The water content was not altered ($p = 0.109$). Body composition was no different between allele groups at baseline or after weight loss.

Eating behavior (Table 4)

Cognitive restriction, disinhibition, and hunger scores (assessed using the three factors questionnaire) were similar between groups at baseline. The questionnaire was completed in 32 patients (20 R230R cases and 12 R230C subjects) at the end of the study. Good correlations were observed between the scores obtained at the beginning and at the end of the study (disinhibition $r = 0.566$, hunger $r = 0.538$, and cognitive restriction $r = 0.382$, with $p < 0.001$ for all variables). Cases with the R230C genotype had a significantly lower disinhibition score at the final visit ($p = 0.006$); this change was not observed for the wild-type allele group. Weight loss resulted in higher cognitive restriction scores and lower hunger marks; these changes were not different between allele groups.

DISCUSSION

The ABCA1 is a transmembrane protein responsible for the efflux of cholesterol, phospholipids, and other lipid molecules across the cell membrane. Mutations

Table 2. Baseline biochemical characteristics by genotype

Parameter	R230R (n = 28)	R230C (n = 21)	P value
Glucose (mg/dl)	88.07 (± 8.86)	88.9 (± 8.96)	0.747
Insulin (UI/dl)	11.1 (8.2-17.6)	12.9 (10.55-17.2)	0.645
Triglycerides (mg/dl)	162 (97.8-203)	162 (96-206.5)	0.832
Total cholesterol (mg/dl)	195.5 (± 38.01)	185.52 (± 58.82)	0.475
HDL-C (mg/dl)	44.96 (± 10.5)	38.19 (± 6.97)	0.014
LDL-C (mg/dl)	118.92 (± 29.32)	119.25 (± 29.8)	0.970
HbA1c (%)	5.77 (± 0.44)	5.72 (± 0.35)	0.670
Apo A-I (mg/dl)	145.11 (± 19.05)	129.7 (± 20.31)	0.011
Apo B (mg/dl)	99.76 (± 24.67)	104.78 (± 28.71)	0.515
Leptin (ng/ml)	27.36 (± 7.83)	29.36 (± 9.36)	0.486
Adiponectin (ng/ml)	9.4 (7.33-11.6)	10 (8-18.3)	0.557
Disposition Index ($S_1 \times AIR$)	3.94 (± 2.48)	3.99 (± 3.01)	0.947
Acute insulin response ($\mu U/ml \times min$)	625.5 (421.4-1010)	521.4 (382.4-757.15)	0.334
HOMA-IR	2.37 (1.77-4.27)	2.93 (2.2-3.81)	0.513

Data are means ± SD, or median ± interquartilar range when there is an non normal distribution.

of ABCA1 are associated with low HDL levels and premature atherosclerosis. Recent data demonstrated that the function of ABCA1 is not limited to the transport of transmembrane lipids and HDL synthesis. It participates in inflammatory and immunologic processes[20] and intervenes in the regulation of specific functions such as cellular differentiation[21] and insulin secretion[22]. Thus, genetic variations that alter the activity of this protein may participate in phenotypes other than hypoalphalipoproteinemia. The R230C variant is associated with low HDL and apolipoprotein A1 levels, obesity, and early onset type 2 diabetes[2,3]. In this report, we study the effect of *ABCA1* R230C variant on the weight loss response to caloric restriction (e.g. a hypocaloric diet). We specifically search for changes over time in response to modifications in fat mass, while evaluating the potential role of the R230C variant as a predictor of the response to treatment. The intervention was successful; patients lost –3.16 ± 2.41 kg, as expected. However, the weight loss response was not influenced by the R230C allele. No differences in the baseline parameters (including AIRg, S_I, adiponectin, leptin, and body composition) were found between genotype groups. After weight loss, cases with the R230C genotype had a significantly lower disinhibition score at the final visit (p = 0.006). However, the risk allele was not associated with differences in any other parameter following weight loss. Thus, the R230C variant of *ABCA1* does not alter the weight loss response to caloric restriction and was not associated with differences in body composition or in insulin secretion and action. Therefore, other mechanisms besides energy expenditure during caloric restriction may explain the association between obesity and the R230C variant.

The *ABCA1* Arg230Cys variant is exclusively present in Amerindian and Amerindian derived populations[]. It that has been shown to be associated with low HDL cholesterol levels in three case-control studies[2-4] and in a genome-wide association study[14]. In a recent study, the authors found that the C230 allele is inherited as a single block and is not part of other haplotypes[3]. The R230C variant decreases the activity of the ABCA1 transporter *in vitro* by 30%. This allele may be a result of a survival effect. This variant may be preserved in Amerindian populations because it provided some advantage in the past (i.e. allowing the cells to preserve cholesterol

Table 3. Changes in the clinical and biochemical parameters during the study

Parameter (genotype)	Basal	Visit 1	Visit 2	Visit 3	P value	P between groups
Weight (kg)						
– R230R	76.5 (± 9.3)	74.7 (± 8.9)	74.0 (± 8.9)	73.7 (± 8.5)	< 0.001	0.184
– R230C	74.5 (± 7.6)	72.2 (± 7.2)	71.4 (± 7.4)	70.7 (± 7.7)	< 0.001	
Waist circumference (cm)						
– R230R	94.5 (90-103)	94 (89.6-102)	93.5 (88-100.8)	93.3 (86-103)	0.010	
– R230C	95 (94-100.95)	93 (91-99)	93 (84.6-98.5)	91 (87-96)	< 0.001	0.2
Hip circumference (cm)						
– R230R	108 (105-117)	107.5 (105-116)	107 (104-117)	108.5 (103.2-113.8)	< 0.001	
– R230C	108(104.5-115.3)	105 (102-112)	105.5 (101-111)	103.8 (99.8-109.3)	< 0.001	0.069
BMI (kg/m2)						
– R230R	31.5 (± 3.9)	30.8 (± 3.8)	30.5 (± 3.8)	30.4 (± 3.7)	< 0.01	0.165
– R230C	30.3 (± 2.5)	29.4 (± 2.4)	29.1 (± 2.5)	28.8 (± 2.6)	< 0.001	
Glucose (mg/dl)						
– R230R	88.3 (± 9.1)	88.2 (± 8.3)	91 (± 8.3)	87.1 (± 7.2)	0.287	0.708
– R230C	89.5 (± 9.1)	88.1 (± 6.8)	84.9 (± 7.0)	87.6 (± 7.9)	0.325	
Insulin (UI/dl)						
– R230R	11.1 (8.2-17.6)	7.9 (6.3-12)	9 (7.1-10.9)	8.8 (4.8-11.1)	0.001	0.414
– R230C	12.9 (10.6-17.2)	8.6 (8-13)	8.5 (5.9-11.7)	6.8 (4.6-9.6)	0.001	
Total cholesterol (mg/dl)						
– R230R	196.3(± 39.5)	197 (± 41.6)	197.8(± 37.4)	184.4(± 38.8)	0.001	0.660
– R230C	193.6 (± 47)	190.5 (± 32)	188.8(± 39.4)	176.1(± 38.9)	0.085	
HDL-C (mg/dl)						
– R230R	45.6 (± 10.6)	43.5(± 10.7)	44.3 (± 11.5)	41.6 (± 10.8)	0.004	0.895
– R230C	38.4 (± 7.3)	38.1 (± 5.8)	37.3 (± 6.2)	34.6 (± 6.7)	0.081	
LDL-C (mg/dl)						
– R230R	119.9(± 28.9)	124.6(± 35.3)	126 (± 33.7)	107.1(± 29.1)	0.035	0.234
– R230C	121.4(± 29.7)	124.8(± 25.1)	123.5 (± 29)	117.6(± 32.9)	0.375	
Triglycerides (mg/dl)						
– R230R	162 (97.8-203)	135.5(109-186.5)	137(105-193.8)	146 (92.5-214)	0.581	0.098
– R230C	162 (96-206.5)	142 (98-166)	126 (73-192)	125 (89-139)	0.028	
HOMA-IR						
– R230R	2.37 (1.77- 4.3)	1.7 (1.29-2.62)	1.99 (1.57-2.6)	1.78 (1.01-2.36)	0.003	0.428
– R230C	2.93(2.2– 3.81)	1.95 (1.6-2.95)	1.8 (1.28-2.51)	1.4 (0.97-2.07)	< 0.001	
Apo A-I (mg/dl)						
– R230R	149 (± 24.8)	139.3 (± 25.4)	144.1 (± 29.2)	134.9 (± 28.5)	< 0.001	0.086
– R230C	127.1 (± 18.5)	126.4 (± 17.3)	125.9 (± 19.8)	123.7 (± 19.9)	0.263	
Apo B (mg/dl)						
– R230R	99.1 (± 26.3)	98.9 (± 24.5)	100.6 (± 23.1)	94.4 (± 25.9)	0.119	0.678
– R230C	105.1 (± 30.2)	101.8 (± 21.4)	101.7 (± 24.4)	98.1 (± 23.3)	0.161	
Leptin(ng/ml)						
– R230R	27.36 (± 7.83)			34.58 (± 16.19)	0.092	0.121
– R230C	29.36 (± 9.36)			29.95 (± 10.45)	0.884	
Adiponectin (ng/ml)						
– R230R	9.4 (7.33-11.6)	NA	NA	11.5 (9.4-14.7)	0.135	0.827
– R230C	10 (8-18.3)			8.3 (6.3-11.9)	0.650	
HbA1c (%)						
– R230R	5.78 (± 0.46)	NA	NA	5.69 (± 0.44)	0.273	0.788
– R230C	5.74 (± 0.36)			5.68 (± 0.35)	0.401	

(Continue)

Table 3. Changes in the clinical and biochemical parameters during the study *(continued)*

Parameter (genotype)	Basal	Visit 1	Visit 2	Visit 3	P value	P between groups
Disposition Index ($S_1 \times AIR$)						
– R230	4.26 (± 2.42)	NA	NA	5.19 (± 3.68)	0.299	0.978
– R230C	4.33 (± 3.25)			5.21 (± 2.97)	0.446	
AIRg (μU/ml × min)						
– R230	569.5 (± 339)	NA	NA	487.8 (± 252.4)	0.147	0.512
– R230C	625.3 (± 385)			472.2 (± 266.8)	0.140	
Fat (g)						
– R230	32.1± 8.3	NA	NA	30.2± 7.5 (41.1± 5.7)		0.720
– R230C	30.6± 3.2			28.8± 3.9 (41.5± 4.0)		
Fat free mass (g)						
– R230	43.9± 4.2	NA	NA	42.5± 4.9 (58.9± 5.7)		0.571
– R230C	42.6± 4.0			40.7± 4.7 (58.5± 4.0)		
Total corporal water (ml)						
– R230	31.3± 3.3	NA	NA	30.9± 3.7 (42.8± 4.2)		0.463
– R230C	31.3± 2.7			30.2± 3.4 (43.6± 3.6)		
Energy expenditure (cal/d)						
– R230	1900 (1800.3-2039.8)	NA	NA	1847.5(1743.8-1977.5		0.374
– R230C	1822.5 (1768.3-1957.5			1771 (1708-1921)		

Data are means ± SD, or median ± interquartilar range when there is a non normal distribution.

during times of famine). No similar phenomenon has been reported between an ethnic-specific variant and hypoalphalipoproteinemia. In addition, associations with early onset type 2 diabetes and obesity have been reported. Animal models and human studies provide support for the association of an abnormal ABCA1 function and diabetes. Brunham, et al. generated mice lacking *Abca1* specifically in beta cells[26]. They found that ß-cell ABC-AI was necessary to maintain a normal insulin secretion; animals had glucose intolerance at eight weeks of age. The islet content of insulin was normal. The absence of ABCA1 led to cholesterol accumulation within the ß-cell plasma membrane, suggesting that excessive cholesterol may alter the insulin secretory pathway. The association between diabetes and ABCA1 variants have been replicated in studies carried out in humans. The disposition index of heterozygous patients for ABCA1 variants is significantly reduced compared to that found in properly matched controls with the wild-type allele[30].

Much less information exists to support the association between obesity and *ABCA1* variants. ABCA1 is expressed in pre-adipocytes and mature adipocytes. The ABCA1 mRNA levels increase several fold during the differentiation of pre-adipocyte into a mature adipocyte. However, only a moderate increase in ABCA1 protein levels occurs during adipocyte differentiation. This suggests a post-transcriptional regulation mechanism in the adipocyte for ABCA1 expression[32]. *In vitro* studies have shown that ABCA1 has a limited ability to transport cholesterol from the adipocyte to HDLs; however, it is an active pathway for phospholipids efflux. *ABCA1* expression decreases as adipocytes become dysfunctional[22]. In addition, decreased ABCA1 function has been reported in chronic inflammation states such as insulin resistance. Various authors have explored the association between obesity and different ABCA1 polymorphisms. Kitjaroentham, et al. investigated the hypothesis that I883M and R219K variants, frequent in Asian individuals, were associated with obesity. This study failed to confirm an association[23]. In a Japanese study, six ABCA1 polymorphisms were associated with obesity; however, only the variant was persistently significant after post hoc adjustment[24].

In the present study, the reduced sample size and/or a small allelic effect could explain a negative modulatory response of the R230C allele on weight loss.

Table 4. Three factor questionnaire analysis

	Basal Median (interquartilar range)	Final Median (interquartilar range)	Paired analysis	Between genotypes change comparition (p)
Cognitive restriction				
– R230	7.5 (6.25-10)	13 (10.25-17)	< 0.001	0.608
– R230C	11 (7.25-12.75)	15.5 (12-16.75)	0.014	
– Both	8 (7-12)	13 (11-17)	< 0.001	
Dysinhibition				
– R230	7 (5.25-11.75)	7.5 (4-9)	0.499	0.006
– R230C	9 (5.5-12)	5 (2.5-6.75)	0.007	
– Both	8 (5.25-12)	6.5 (4-9)	0.010	
Hunger				
– R230	7 (3.25-9.75)	4 (2-7.5)	0.019	0.376
– R230C	6.5 (4.25-10.75)	4.5 (2-6)	0.007	
– Both	7 (4-10)	4 (2-6)	0.001	
Total				
– R230	22.5 (14.5-29)	23.5 (19.25-29.25)	0.022	0.005
– R230C	26 (22.5-32.75)	25.5 (20.75-27.75)	0.185	
– Both	24.5 (18.25-29)	25 (20-27.75)	0.342	

In this regard, it is well known that different systems participate in the regulation of body weight. In addition, several homeostatic responses are activated or repressed during caloric restriction. Our main outcome variable integrates these factors. However, changes in systems or responses may have occurred, but assessment of these was not included in our study design. For example, we evaluate eating behavior patterns using the three factors inventory. Cases with the R230C genotype had a significantly lower disinhibition score at the final visit (p = 0.006). The significance of this finding remains to be clarified. ABCA1 is highly expressed in central nervous system cells, particularly in Purkinje cells and cortical pyramidal cells[34,35]. It is unclear how decreased ABCA1 function may affect eating behavior. Similar results have been published for other gene variants not clearly related with eating behavior[33]. On the other hand, the allele effect discernable with our sample size was estimated to detect the smallest weight difference between groups (i.e. three kilograms) that may have an impact on obesity-related metabolic variables[36-40]. Although the risk allele may influence body weight or the response to a hypocaloric diet, a sample size several times larger will be required to study this. If this is the case, the allele effect will be relevant for research purposes, but would have very little clinical significance.

This is the first study evaluating the potential role of the R230C variant of ABCA1 as a predictor of the response to weight loss. Standardized procedures were applied to study carefully selected cases and age-, gender and BMI-matched controls. The main limitation of our study is that the sample size was insufficient to detect differences in the variables that were considered secondary outcomes (i.e disposition index, acute insulin response, changes in the molecules related to lipid metabolism, leptin and adiponectin, as well as the parameters evaluated by electrical impedance). Finally, it is worth noticed that the present study included only women.

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