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The lactase rs4988235 is associated with obesity related variables and diabetes mellitus in menopausal obese females

D.A. DE LUIS, O. IZAOLA, D. PRIMO

Endocrinology and Nutrition Research Center, School of Medicine, Department of Endocrinology and Nutrition, Hospital Clinico Universitario, University of Valladolid, Valladolid, Spain

Abstract. – OBJECTIVE: Some studies showed specific associations of the Lactase persistence (LP) genotype (CT/TT) with obesity and its related comorbidities. The aim of the present investigation was to describe the association of rs4988235 with metabolic parameters, diabetes mellitus type 2 (DM2), dairy product consumption in menopausal obese females.

PATIENTS AND METHODS: The study involved a population of 86 menopausal obese females. Measurements of anthropometric parameters, blood pressure, fasting blood glucose, insulin concentration, insulin resistance (HOMA-IR), lipid profile, bone metabolism biomarkers, and prevalence of (DM2) were recorded. The genotype of the Lactase gene polymorphism (rs48988235) was evaluated.

RESULTS: The distribution of the rs48988235 polymorphism was 16.3% (n=14) (CC), 38.4% (n=33) (CT) and 45.3% (n=39) (TT). The allele frequency was C (0.35) and T (0.65). In the recessive model, serum 25-OH Vitamin D, fasting glucose levels, insulin levels, and HOMA-IR were better in CC+CT genotype than TT genotype. In a dominant model, 25-OH Vitamin D, fasting glucose levels, insulin levels, and HOMA-IR were better in CC genotype than CT+TT genotype. In both genetic models, calcium, vitamin D, and milk intakes were higher in T allele carriers. In the dominant model (CT+TT genotype), logistic regression analysis showed an increased risk of hyperglycemia (OR=3.63, 95% CI=1.10-13.26, *p*=0.03) and prevalence of DM2 (OR=3.93, 95% CI=1.07-14.4, *p*=0.03), after adjusting by milk intake, BMI, and age. This association remained in recessive model (TT genotype); risk of hyperglycemia (OR=4.26, 95% CI=1.12-16.23, *p*=0.02) and prevalence of DM2 (OR=5.35, 95% CI=1.12-15.8, *p*=0.02).

CONCLUSIONS: T allele of rs48988235 variant in Lactase gene is associated with better glucose metabolism and lower risk of DM2 in menopausal obese females. In addition, dietary intakes of milk, calcium, and 25-OH vitamin D were higher too.

Key Words:
Rs4988235, Diabetes mellitus type 2, Lactase, Metabolic syndrome.

Introduction

In mammals, the possibility to digest lactose present in milk and dairy products declines with age, secondary to a decreased production of the enzyme lactase in the small intestine. Moreover, in human beings, the lactase used to digest this disaccharide continues to be expressed in adults, a phenotype known as lactase persistence (LP)[1]. LP individuals are lactose tolerant and maintain the ability to digest dairy products into adulthood; moreover, lactose non-persistence (LNP) individuals are unable to digest important amounts of lactose. LNP subjects can present adverse unspecific symptoms such as cramps, nausea, diarrhea, flatulence or abdominal pain after milk consumption[2].

Persistence of the lactase activity has been related to some single nucleotide polymorphisms (SNPs) in a region located about 14 kb upstream the lactase gene *(LCT)*[3-4]. In Caucasian populations, the rs4988235 genetic variant was associated with adulthood lactase activity: CC genotype was associated with hypolactasia and CT and TT genotypes with lactase persistence[5]. This allele acts as an enhancer for lactase expression, increasing transcription in cell lines[6].

Some scholars[7] have detected associations between dairy consumption and obesity. In addition, other studies[8-12] showed specific associations of the LP genotype (CT/TT) with body mass index and with fat mass and waist circumference[13]. Moreover, the intake of milk and dairy products has been reported to protect against cardiova-

Corresponding Author: Daniel A. de Luis, MD, Ph.D; e-mail: dadluis@yahoo.es

scular diseases with a blood-pressure lowering effect[14]. Nowadays, Metabolic Syndrome (MS) is an important clustering of several factors: abdominal obesity, glucose intolerance and/or insulin resistance, dyslipidemia, and increased blood pressure[15]. Therefore, this entity combines all the factors previously discussed, and observational studies have been reported that dairy product consumption was inversely associated with the occurrence of one or more components of MS[16]. Menopausal females are a high-risk metabolic group of patients and menopause nearly adversely affects all components of MS[17].

The aim of the present investigation was to describe the association of rs4988235 genetic variant with metabolic parameters, components of MS, diabetes mellitus type2, calcium, vitamin D intake, and dairy product consumptions in menopausal obese females.

Patients and Methods

Subjects and Clinical Investigation

The population studied was selected from menopausal obese females sent by the primary care physicians to our Nutrition Unit. Obesity is defined by a body mass index (BMI) ≥30 kg/m^2 and menopause as a period of 6-12 months with amenorrhea without pregnancy and follicle-stimulating hormone above 30 UI/L. Eighty-six obese Caucasian females were enrolled in a non-probabilistic consecutive method of sampling in our Health Area. The enrolled subjects fulfilled the following inclusion criteria: age over 50 years, amenorrhea ≥ 6 months, body mass index ≥ 30 kg/m^2, had no history of cardiovascular disease, thyroid disease, chronic renal or hepatic disorders, had no history of active alcoholism, malignant tumor. Finally, within the 6 months before the study, they had not received medications known to influence lipid levels (for example, hormonal therapy, glucocorticoids, and anti-inflammatory drugs) or glucose levels (antidiabetic drugs or insulin). The exclusion criteria were ages under 50 years, BMI over 45 kg/m^2 years old, multivitamin complexes and a dietary treatment 3 months prior to the current study. The Ethics Committee of the HCUVA approved the study protocol and was in accordance with the guidelines laid down in the Declaration of Helsinki. All participants of the study had to read the informed consent form and gave written informed consent.

After signed consent was realized, all subjects underwent a medical evaluation, including physical examination and complete medical history. Blood samples were collected after 12 h fasting to determine glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, calcium, phosphorus, 25-OH vitamin D, parathormone (PTH), osteocalcin, the amino terminal propeptide of human type I procollagen (P1NP) and the β-CrossLaps. Data on blood pressure, anthropometric parameters (weight, height, body mass index (BMI), fat mass by impedance and waist circumference) were collected at 9:00 am. To estimate the prevalence of diabetes mellitus type 2 was considered American Diabetes Association criteria[18]. In this investigation, the definition of the ATPIII[1] for the MS was employed. Patients need to meet at least three of the next five criteria to be diagnosed of MS: 1) elevated triglycerides (>150 mg/dl) or treatment for dyslipidemia, 2) elevated fasting glucose or treatment for diabetes, 3) low HDL cholesterol < 40 mg/dl (males) or <50 mg/dl (females), 4) elevated systolic or diastolic blood pressure (>130/85 mmHg or antihypertensive treatment) and 5) increased waist circumference [>94 cm (males) or >80 cm (females)].

Anthropometric Parameters and Blood Pressure

Mean systolic and diastolic blood pressures were calculated by averaging three consecutive measurements (Omrom, Los Angeles, CA, USA). Body weight was determined while the subjects were unclothed and not wearing shoes. Subjects were measured using scales (Omrom, Los Angeles, CA, USA) and recorded to the nearest 10 g. Height was determined with a tape measure (Omrom, Los Angeles, CA, USA) while patients were standing with shoulders in normal alignment and no wearing shoes. Body mass index (BMI) was calculated with the next equation: [body weight (in kg) divided by height (in m^2)]. Waist circumferences (WC) were measured at the umbilical level with the use of an upstretched tape measure (Omrom, Los Angeles, CA, USA) while the subjects were standing after a normal expiration. Bioimpedance was used to determine fat mass with an accuracy of 25 g[19] (EFG, Akern, Pisa, Italy).

Biochemical Procedures

Total cholesterol, triglyceride, calcium, phosphorus, and glucose concentrations were deter-

mined by conventional enzymatic methods Cobas Hitachi platform (Roche Diagnostics GmbH, Mannheim, Germany), while HDL cholesterol was measured in the supernatant after precipitation of other lipoproteins by enzymatic methods. LDL cholesterol was calculated using the Friedewald equation (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/5)[20]. Insulin was determined by radioimmunoassay (RIA) (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L)[21], and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values[22].

A total of 25-OH vitamin D was obtained with a measurement range between 3.00 and 70.0 ng/mL. The PTH reference level was 15-65 pg/mL with a functional sensitivity of 6.0 pg/mL. The osteocalcin reference level was 13-48 ng/mL. The aminoterminal propeptide of human type I procollagen (P1NP) was <76.3 ng/ml. The reference level for the β-CrossLaps was 0.556-1.008 ng/mL with a functional sensitivity of 0.07 ng/mL. All these molecules were measured with a chemiluminescence immunoassay using an autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Dietary Intakes

At the time of the analytical and anthropometric evaluation, records of daily dietary intake for 3 previous days (2 days of the week and one of the weekends) were evaluated with a computer-based data evaluation system (Dietosource®, Lausanne, Switzerland) and national composition food tables were used as reference[23]. A frequency questionnaire of the consumption of milk, cheese, and fermented milk was also carried out. The questions were the daily intake of these products, such as dichotomous variable (yes/no) and the number of servings per day (milk serving: 250 ml, cheese serving: 20 grams and serving of fermented dairy: a yogurt or similar product). All subjects with a self-reported questionnaire recorded the exercise activity.

Genotyping Lactase Gene

DNA was isolated from buccal swabs using QIAamp® Genotyping (rs48988235) was performed by using customized assays with the TaqMan® OpenArray™ Genotyping platform (Thermofisher, Pittsburg, PA, USA). Samples were loaded using the AccuFill system, and amplification realized on the QuantStudio 12K Flex Real-Time qPCR instrument (Thermofisher, Pittsburg, PA, USA). A total volume of 10 µl with 2.5 µl TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, CA, USA) and 2.5 µl human DNA samples were loaded and amplified on arrays following the manufacturer's instructions. The Polymerase Chain Reaction (PCR) was carried out with 0.5 uL of each oligonucleotide primer (primer forward: 5'-TGGCAATACAGATAAGATAATGTAG-3' and reverse 5'-CCCTGGCCTCAAAGGAACTCTCCTC-3' in a 2 uL final volume (Termocicler Life Technologies, Los Angeles, CA, USA). Genotype calling and sample clustering for Open Array assays were performed in TaqMan Genotyper (Life Technologies, Carlsbad, CA, USA).

Statistical Analysis

All the data were analyzed using SPSS for Windows, version 23.0 software package (IBM Corp., Armonk, NY, USA). Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed counts. The sample size was calculated to detect differences over 10% in DM2 prevalence between genotype groups with 90% power and 5% significance. The analyses were performed under a recessive genetic model with rs48988235 T-allele as the risk allele (CC+CT *vs*. TT) and under the dominant model (CC *vs*. CT+TT). The results were showed as average ± standard deviation (SD). Variables were analyzed with the Student *t*-test (for normally distributed variable) or the Kruskal-Wallis test (for non-normally distributed variable). Logistic regression analyses adjusted by milk intake, age, and BMI were used to calculated odds ratio (OR) and 95% confidence interval (CI) to estimate the association of the rs48988235 SNP with the risk of Metabolic syndrome, components of MS and diabetes mellitus type 2. A *p*-value under 0.05 was considered statistically significant.

Results

The sample comprised of 86 menopausal obese females. The mean age was 61.9±6.4 years (range: 55-68) and the mean body mass index (BMI) 40.1±3.1 kg/m^2 (range: 36.4-43.2). The distribution of the rs48988235 polymorphism in this adult population was 16.3% (n=14) (CC), 38.4% (n=33) (CT) and 45.3% (n=39) (TT). The allele frequency was C (0.35) and T (0.65). The variant was in Har-

Table I. Anthropometric variables and blood pressure.

Parameters	Recessive model		Dominant model	
	CC+CT n = 47	TT n = 39	CC n = 14	CT+TT n = 72
Age (years)	63.1 ± 5.9	62.8 ± 5.0	61.5 ± 5.8	62.9 ± 4.7
BMI (kg/m^2)	39.1 ± 3.6	40.2 ± 5.0	40.2 ± 4.1	39.4 ± 4.1
Weight (kg)	96.5 ± 12.0	97.3 ± 10.7	97.6 ± 15.1	96.7 ± 12.3
Fat mass (kg)	45.9 ± 3.3	46.5 ± 4.1	46.6 ± 5.2	45.8 ± 3.1
WC (cm)	117.3 ± 7.0	115.6 ± 6.0	119.1 ± 7.2	115.9 ± 6.1
SBP (mmHg)	131.0 ± 6.1	131.2 ± 7.0	129.0 ± 8.8	130.5 ± 6.1
DBP (mmHg)	79.3 ± 5.5	79.4 ± 4.9	77.4 ± 5.2	79.3 ± 4.1

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference. NO statistical differences between genotype groups.

dy Weinberg equilibrium (p=0.28). The subjects were analyzed into two different models: dominant model as genotype groups (CC *vs*. CT+TT) and recessive model (CC+CT *vs.* TT), too.

Table I shows anthropometric parameters and blood pressure. Age was similar in all genotype groups. Applying a dominant genetic model (CC *vs.* CT+TT) and a recessive genetic model (CC+CT *vs.* TT), we did not detect a significant association between rs48988235 and fat mass, weight, waist circumference, blood pressure, and BMI in all groups.

Biochemical characteristics according to genotype are shown in Table II. Fasting glucose levels (CC+CT *vs.* TT: delta: 13.3±4.2 mg/dL; p=0.02) insulin levels (CC+CT *vs.* TT: delta: 5.2±2.1 UI/L; p=0.03) and HOMA-IR (CC+CT *vs.* TT delta: 1.8±0.3 units; p=0.02) were higher in CC+CT genotype than TT genotype (recessive model). In a dominant model, fasting glucose levels (CC *vs.* CT+TT: delta: 10.2±4.1 mg/dL; p=0.02), insulin levels (CC *vs.* CT+TT: delta: 7.6±2.9 UI/L; p=0.03) and HOMA-IR (CC *vs.* CT+TT delta: 2.7±0.4 units; p=0.01) were higher in CC genotype than CT+TT genotype. In both models, levels of vitamin-D were higher in T allele carriers, in dominant model (CC *vs.* CT+TT: delta: 7.3±3.1 ng/dL; p=0.02) and in recessive model (CC+CT *vs.* TT: delta: 7.8±3.0 ng/dL; p=0.01). No differences were detected in the levels of serum bone metabolism biomarkers such as osteocalcin, PTH, P1NP and β-CrossLaps.

Table III shows the dietary parameters. In the recessive model, calcium intake (CC+CT *vs.* TT: delta: 550.8±187.2 mg/day; p=0.02) and vi-

Table II. Biochemical parameters (mean ± SD).

Parameters	Recessive model		Dominant model	
	CC+CT n = 47	TT n = 39	CC n = 14	CT+TT n = 72
Fasting glucose (mg/dl)	115.2 ± 4.9	101.9 ± 3.1$^{\$}$	116.0 ± 5.1	105.8 ± 4.1*
Total cholesterol (mg/dl)	204.3 ± 11.8	197.6 ± 18.7	192.5 ± 18.1	201.7 ± 11.1
LDL-cholesterol (mg/dl)	118.0 ± 9.9	113.6 ± 8.1	111.2 ± 12.3	116.3 ± 10.1
HDL-cholesterol (mg/dl)	53.1 ± 7.1	60.7 ± 5.2	51.1 ± 8.4	57.5 ± 7.2
Triglycerides (mg/dl)	138.7 ± 27.1	128.6 ± 21.7	149.1 ± 34.9	130.9 ± 32.9
Insulin (mUI/l)	21.0 ± 5.0	15.8 ± 3.9$^{\$}$	24.4 ± 5.0	16.8 ± 4.2*
HOMA-IR	6.1 ± 1.0	4.3 ± 0-9$^{\$}$	7.2 ± 3.1	4.5 ± 2.2*
Calcium (mg/dl)	9.9 ± 1.1	9.6 ± 0.9	9.8 ± 1.1	9.7 ± 0.8
Phosphorus (mg/dl)	2.9 ± 0.1	2.7 ± 0.2	2.8 ± 0.1	2.9 ± 0.3
25-OH Vitamin D ng/ml	16.7 ± 3.1	24.5 ± 3.4$^{\$}$	14.7 ± 3.1	22.4 ± 3.8*
PTH pg/ml	54.1 ± 7.0	57.7 ± 5.8	56.8 ± .1	56.7 ± 7.8
Osteocalcin ng/ml	21.4 ± 6.1	24.7 ± 6.1	18.8 ± 4.1	24.4 ± 5.8
P1NP ng/ml	49.7 ± 18.1	53.3 ± 17.8	48.8 ± 18.1	52.1 ± 13.8
Beta cross lap ng/ml	0.35 ± 0.14	0.36 ± 0.12	0.34 ± 0.13	0.36 ± 0.15

PTH (parathormone) HOMA-IR (homeostasis model assessment of insulin resistance). P1NP amino terminal propeptide of type I procollagen; $^{\$}$ p<0.05 in CC+CT *vs*. TT genotypes. *p<0.05, in CC *vs*. CT+TT genotypes.

Table III. Dietary Intakes and physical activity (mean ± SD).

Parameters	Recessive model		Dominant model	
	CC+CT n = 47	TT n = 39	CC n = 14	CT+TT n = 72
Calories (cal/day)	1615.5 ± 343.2	1667.2 ± 611.2	1709.3 ± 604.2	1631.5 ± 450.5
Carbohydrates (g/day)	174.7 ± 62.0	173.2 ± 35.5	170.3 ± 43.1	174.2 ± 85.1
Proteins (g/day)	81.1 ± 25.9	78.6 ± 22.3	80.5 ± 11.2	79.5 ± 24.4
Lipids (g/day)	62.2 ± 29.4	69.5 ± 21.5	37.6 ± 8.9	30.7 ± 7.8
Fiber (g/day)	15.9 ± 3.4	14.8 ± 2.5	15.8 ± 3.9	14.9 ± 3.8
Calcium (mg/day)	735.6 ± 250.9	1285.7 ± 153.8$	821.6 ± 250.9	1115.7 ± 177.8*
Phosphorus (µg/day)	1246.8 ± 251.9	1244.7 ± 232.1	1235.6 ± 351.2	1239.7 ± 372.1
Vitamin D (ug/day)	16.3 ± 4.1	31.4 ± 4.2$	12.6 ± 4.6	28.4 ± 3.8*
Consumption of milk (yes/no)	40.2%/59.9%	87.2%/12.8%	42.9%/57.1%	76.4%/23.6%*
Servings per day milk	0.21 ± 0.18	1.71 ± 0.22$	0.24 ± 0.21	1.64 ± 0.32*
Consumption of cheese (yes/no)	19.1%/80.9%	28.2%/71.8%	21.4%/78.6%	23.6%/76.4%
Servings per day dairy products	0.25 ± 0.12	0.39 ± 0.11	0.28 ± 0.30	0.32 ± 0.19
Consumption of fermented Milk (yes/no)	70.2%/29.8%	76.9%/23.1%	64.3%/35.7%	61.1%/38.9%
Servings per day fermented milk	0.76 ± 0.11	0.71 ± 0.12	0.82 ± 0.13	0.71 ± 0.21
Physical activity (hours/week)	1.34 ± 0.91	1.41 ± 0.88	1.32 ± 0.87	1.415 ± 0.93

1 ug = 40 UI vitamin D. $p<0.05 in CC+CT *vs*. TT genotypes. *$p<0.05$, in CC *vs*. CT+TT genotypes.

tamin D intake (CC+CT *vs*. TT: delta: 15.1±1.9 ug/day; $p=0.03$) were higher in TT genotype group. In the dominant model, calcium intake (CC *vs*. CT+TT: delta: 310.8±123.9 mg/day; $p=0.02$) and vitamin D intake (CC *vs*. CT+TT: delta: 15.8±2.1 ug/day; $p=0.04$) were higher in T allele carriers. The dairy derivatives consumption frequency questionnaire only showed significant differences in the frequency of milk consumption, being superior in the dominant model in the genotype (CT + TT) (OR 4.3, 95% CI; 1.3-13.13; $p = 0.01$) and in the recessive model in the TT genotype (OR 10.1, 95% CI; 3.3-30.1; $p = 0.02$). This fact produced a greater number of milk servings per day in the CT + TT genotype in the dominant model and the TT genotype in the recessive model, too. No statistical differences were found in the intake of cheese or fermented dairy products.

The frequency of subjects with metabolic syndrome, different components of MS (central obesity, hypertriglyceridemia, hypertension or hyperglycemia), and diabetes mellitus type 2 have been reported in Table IV. According to the results of metabolic characteristics, the percentage of individuals who had metabolic syndrome (MS) was lower in TT group (recessive model) and (CT+TT) (dominant model) but without statistically significant differences. In the dominant model (CT+TT genotype), the logistic regression analysis showed an increased risk of hyperglycemia (OR=3.63, 95% CI=1.10-13.26, $p=0.03$) and

Table IV. Metabolic syndrome (MS), components of MS and diabetes mellitus.

Parameters	Recessive model		Dominant model	
	CC+CT n = 47	TT n = 39	CC n = 14	CT+TT n = 72
Percentage of MS	40.4% (19)	30.7% (12)	57.1% (8)	31.9% (23)
Percentage of central obesity	97.8%	94.8%	92.8%	94.4%
Percentage of hypertriglyceridemia	38.3%	26.3%	50.0%	29.6%
Low HDL cholesterol	19.1%	13.1%	21.4%	14.2%
Percentage of hypertension	78.7%	64.1%	73.6%	64.3%
Percentage of hyperglycemia	29.8%	7.6%$	42.8%	15.7%*
Diabetes mellitus	23.4% 11	5.1%$ 2	35.7%	11.1%*

The cutoff points for the criteria of; central obesity (waist circumference >88 cm in female and >102 in male), hypertension (systolic BP>130 mmHg or diastolic BP>85 mmHg or specific treatment), hypertriglyceridemia (triglycerides >150 mg/dl or specific treatment) or hyperglycemia (fasting plasma glucose >110 mg/dl or drug treatment for elevated blood glucose). *$p<0.05$, in CC *vs*. CT+TT genotypes. $p<0.05 in CC+CT *vs*. TT genotypes

prevalence of diabetes mellitus type 2 (OR=3.93, 95% CI=1.07-14.4, p=0.03), after adjusting by milk intake, BMI and age. This association remained in recessive model (TT genotype); risk of hyperglycemia (OR=4.26, 95% CI=1.12-16.23, p=0.02) and prevalence of diabetes mellitus type 2 (OR=5.35, 95% CI=1.12-15.8, p=0.02).

Discussion

The main finding of this cross-sectional study was the fact that menopausal obese females with the T allele of SNP (rs48988235) of the *Lactase* gene showed low values of fasting glucose, insulin levels, HOMA-IR, and low risk of diabetes mellitus type 2. In addition, we detected better intakes of milk servings, calcium, and 25 OH-vitamin D in both models, dominant and recessive.

To better understand the genetic basis of metabolic syndrome, it is important to replicate association evaluations in independent studies worldwide. Indeed, previous studies[24-25] showed that T allele frequency is not uniform across the literature, varying from 0.269 until 0.402. Our results are slightly higher than those reported in these studies. In the literature, it has been demonstrated an association between LP genotype and obesity risk. For example, Corella et al[26] reported in high cardiovascular risk Spanish subjects a significant association of CT/TT genotype with higher BMI, weight, and waist circumference. Moreover, they reported that this association was modulated by dairy lactose intake. Other scholars[27] have reported this relationship without controlled the daily milk intake. However, Fumeron et al[28] reported that LP genotype was associated with lower BMI. In our work, there is no association between this genetic variant and body weight.

In addition, Friedrich et al[29] reported that genotype CC was associated with a higher prevalence of hypertension (22.7%) when compared to CT+TT genotypes. In addition, the MS prevalence was higher in individuals with non-persistence genotype (CC) 34.3% than in lactase persistence subjects (CT+TT) (21.6%)[29]. Our results did not show this association with blood pressure, but we reported an association with hyperglycemia and type 2 diabetes mellitus. There is evidence that certain milk components could have a beneficial effect on insulin sensitivity, cholesterol levels, and blood pressure[30]. Milk protein-derived peptides show hypotensive effects[31], and the higher intake of calcium from milk could be a benefit mechanism because it may decrease oxidative stress and the expression of inflammatory cytokines typically associated with MS and diabetes mellitus[32-33]. Finally, in the D.E.S.I.R (Data from the Epidemiological Study on the Insulin Resistant Syndrome) cohort[28], a high consumption of milk was associated with a lower incidence of impaired fasting glycemia and diabetes mellitus type 2. It is necessary to remark that milk proteins are insulinotropic, too[34]. In the D.E.S.I.R cohort, the C allele was associated with a higher prevalence of impaired fasting glycemia and type 2 diabetes mellitus, too. Moreover, this association was still significant after adjustment for dairy product consumption. For all above-mentioned, lifestyle changes with weight loss, healthy eating habits improve MS, and it has been suggested that milk consumption, the low-fat milk, as recommended by Dietary Approaches to Stop Hypertension (DASH) diet is desirable. In our study, the genetic variant is associated with milk consumption, but not with the remaining dairy milk products, as it has been recently shown in another study[35]. In this investigation, the genetic variant rs48988235 was associated with a modestly lower HDL cholesterol; this data has not been found in our study. Moreover, in our logistic regression analysis adjusted for age, BMI, and milk intake, only the genetic variant remained in both genetic models (recessive and dominant). Perhaps it is a direct effect of genetic variation on the glycemic metabolism added to other small factors such as the increase in the intake of calcium, vitamin D, and other nutrients, but not a direct effect of milk intake[35].

We did not find differences in bone metabolism between the different genotypes to be analyzed using serum biomarkers such as osteocalcin, PTH, amino terminal propeptide of procollagen type I (P1NP), and β-CrossLaps. Yang et al[36] reported a lack of association of this genetic variant with bone health measured by densitometry in femoral neck and lumbar spine. Perhaps the slight effect of this genetic variant on total calcium and vitamin D intake is not enough to affect bone mass. Moreover, in this study, fasting insulin levels showed a direct association with the genetic variant rs48988235; these findings are in line with our findings. Koek et al[37] reported a lack of association of this genetic variant with bone mineral density. Moreover, calcium intake was significantly lower in C-homozygotes.

The inconsistencies among the literature results could be explained by environmental factors or different genetic backgrounds of the populations

studied. For example, other lifestyle components such as alcohol consumption, smoking habits, and so on may act as modifiers of the effect of genetic variants in obesity and its associated risk factors such as diabetes mellitus. A possible explanation for these contradictory results in the literature is due to the influence of other genes, which could be in linkage disequilibrium, too. Finally, diabetes mellitus type 2 has a multifactorial polygenic origin so that an abnormality in the Lactase gene may act or interact with other environmental or genetic factors and induce to development of diabetes mellitus.

Limitations

The limitations of our study are: one is that the study has been realized in menopausal obese females, so the data are not generalizable to the entire population. Second, the design as a cross-sectional design does not allow to extract causality. Third, the lack of assessing the combined effect of other genetic factors on glucose metabolism. The fourth is that dietary intake was determined with a diet questionnaire, which would lead to under or overestimated the intakes. Fifth, we have evaluated only Caucasian subjects; ethnic differences in genetic background and the environment they live in would play crucial roles in genetic effects. Sixth, the wide range of the BMI of included patients may be a factor that distorts our results by including some patients in the morbid obesity range. Finally, we have not determined the lactase persistence in our sample, and it is well-known that. In Europe, the distribution of the lactase persistence phenotype is clinal with a range of frequency from 15-54% in the south-east to 89-96% in the north-west[38] the women they studied.

Conclusions

TT genotype in the recessive model and TT+CT genotype in the dominant model of the rs48988235 variant in the *Lactase gene* are associated with better glucose metabolism, lower risk of diabetes mellitus type 2, and metabolic syndrome in menopausal obese females. Lactase phenotypes can be an important tool for the management of metabolism during nutrition counseling in this population, and they have been related to bone mineral density[39]. More studies are necessary to evaluate the role of these associations with possible therapeutic measures in obese patients at risk of diabetes mellitus. It has even been described how this polymorphism can modify weight loss after a hypocaloric diet related to the gut-microbiome[40].

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee (HVUVA Committee 2/2018) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Informed Consent

Informed consent was obtained from all individual participants included in the study.

Authors' Contribution

Daniel Antonio de Luis designed the study an wrote the article. Olatz Izaola, realized nutritional evaluation. David Primo realized biochemical evaluation..

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