The lactase persistence genotype is a protective factor for the metabolic syndrome

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Abstract

The Metabolic Syndrome (MetS) is defined as a pattern of metabolic disturbances, which include central obesity, insulin resistance and hyperglycemia, dyslipidemia, and hypertension. Milk has been promoted as a healthy beverage that can improve the management of MetS. Most human adults, however, down-regulate the production of intestinal lactase after weaning. Lactase encoded by the LCT gene is necessary for lactose digestion. The -13910C > T SNP (rs4988235) is responsible for the lactase persistence phenotype in European populations. We herein investigated whether the lactase persistence genotype is also associated with the MetS in subjects from a Brazilian population of European descent. This study consisted of 334 individuals (average age of 41 years) genotyped by PCR-based methods for the -13910C > T SNP. Clinical data were assessed and the genotypes were tested for their independent contribution to the MetS using chi-square tests and multiple logistic regression analysis. Univariate analyses showed that hypertension and MetS prevalence were higher in individuals with the lactase non-persistence genotype than in lactase persistence subjects. Furthermore, lactase persistence was associated with a lower risk for MetS (OR = 0.467; 95% CI 0.264-0.824; p = 0.009). These results suggest that LCT genotypes can be a valuable tool for the management of MetS treatment.

Keywords: metabolic syndrome, lactase persistence, hypolactasia, lactose.

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Introduction

The metabolic syndrome (MetS) has been defined as a cluster of metabolic risk factors, which include central obesity, hypertension, dyslipidemia, insulin resistance and hyperglycemia. It is predictive of an increased risk of type-2 diabetes and cardiovascular disease (CVD) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001; Després and Pérusse, 2008).

In observational studies, dairy product consumption was inversely associated with the occurrence of one or more individual components of the MetS, thus representing a protective effect of milk against MetS (Elwood et al., 2007; Pfeuffer and Schrezenmeir, 2007) and also against high blood pressure (FitzGerald et al., 2004). A possible explanation for these results was that dairy product intake may be associated with other healthy dietary habits or health promoting lifestyles (Pfeuffer and Schrezenmeir, 2007). Only one study reported that women who never drank milk had lower insulin resistance scores, lower triglyceride levels and body mass index (BMI), higher high-density lipoprotein (HDL) and cholesterol levels, and they also suffered less frequently from diabetes than those who drank milk (Pfeuffer and Schrezenmeir, 2007).

Most human adults down-regulate the production of intestinal lactase after weaning. Lactase is necessary for the digestion of lactose, the main carbohydrate in milk, and without it, milk consumption can lead to bloating, flatulence, cramps and nausea (Swallow, 2003). Lactase, secreted by small-intestinal enterocytes, is encoded by the LCT gene (OMIM #603202) which maps in 2q21 (Kruse et al., 1988). A C to T transition SNP (rs4988235) located within an intronic sequence of the MCM6, which is the upstream adjacent gene, acts as an enhancer for lactase expression in vitro and is responsible for the lactase persistence (LP) phenotype in European populations. The CC genotype determines the lactase non-persistence phe-
notype (LNP), also known as adult-type hypolactasia (Enattah et al., 2002). Other polymorphisms in the same enhancer region or in its vicinity were also related to the LP phenotype in populations from East Africa and the Middle East (Ingram et al., 2007; Tishkoff et al., 2007; Torniainen et al., 2009). Lactase persistent individuals are usually lactose tolerant and, hence, they can consume milk and dairy products without symptoms.

The LP predicted phenotype (-13910 CT and TT genotypes) was detected in 51% of southern Brazilian individuals of European ancestry. No other variant in the enhancer region was identified in this population by sequencing (Friedrich et al., 2012). In an attempt to further explore the complex genetic component of the metabolic syndrome, we evaluated whether the LP allele of the LCT SNP (rs4988235) would be associated with MetS.

**Subjects and Methods**

**Study subjects**

A total of 334 subjects of European ancestry, as ascertained by skin color and morphological characteristics, most of them of low socio-economic status, were included in this investigation. Lactase persistence allele frequency, clinical, demographic and laboratory variables from this sample have been fully described elsewhere (Mattevi et al., 2002; Fiegenbaum et al., 2007; Friedrich et al., 2012). Pregnant women, as well as those individuals with secondary hyperlipidemia due to renal, liver and/or thyroid disease were not invited to participate in the study. For all patients enrolled, a questionnaire was completed by an interviewer that included details on medicine intake and lifestyle variables such as smoking, physical activity, alcohol consumption, oral contraceptive usage, and menopause status. The Ethics Committee of the Federal University of Rio Grande do Sul approved the study protocol. All participants of the study were able to read the informed consent form and gave written informed consent.

Blood samples were collected after 12 h fasting. Total cholesterol (TC), triglyceride (TG) and glucose concentrations were determined by conventional enzymatic methods, on a Mega Merck Analyzer (Merck Darmstadt, Germany). HDL cholesterol was determined with a selective immunoseparation-based homogenous assay, followed by colorimetric quantification. LDL cholesterol was calculated according to Friedewald et al. (1972). Waist circumference was measured at mid-concentration between the lower rib margin and the iliac crest (World Health Organization, 1997). Body mass index (BMI) was calculated as weight in kg divided by square height in meters (kg/m²). Genomic DNA extraction and genotyping procedures were as previously described (Friedrich et al., 2012)

**Definition of the metabolic syndrome**

In this investigation the International Diabetes Federation (IDF) definition for the MetS was employed (International Diabetes Federation, 2005). Inclusion criteria were: waist circumference ≥ 94 cm for men and 80 cm for women, plus two of the following factors: 1) HDL-cholesterol < 40 mg/dL for men and < 50 mg/dL for women, or specific treatment for this lipid abnormality; 2) triglycerides ≥ 150 mg/dL or specific treatment for this lipid abnormality; 3) fasting glycaemia ≥ 100 mg/dL, or previously diagnosed type 2 diabetes; 4) blood pressure ≥ 130/85 mmHg, or treatment of previously diagnosed hypertension.

**Statistical analysis**

Differences between means were estimated by Student’s t-test and Mann-Whitney U-test when there was a deviation from normal distribution. Chi-square analysis was performed for categorical variables. Logistic regression was used to assess the effect of the LCT polymorphism on the occurrence of MetS. Gender, age, BMI, and physical activity (dichotomous variable, sedentary or not) were included in the model as confounders, as they are biologically involved in MetS development. All analyses were performed using SPSS version 18 software. Two sided p-values < 0.05 were considered as statistically significant.

**Results**

Demographic and clinical characteristics of the investigated sample are presented in Table 1, stratified by LCT -13910C > T genotypes. Univariate analyses for the lactase persistence genotype in a dominant model are shown in Table 1. The non-persistence genotype (CC) was associated with a higher prevalence of hypertension (22.7%) when compared to the lactase persistence genotypes (CT+TT) (13.4%; p = 0.032). The other components of MetS did not differ among groups. The prevalence of MetS was higher in individuals with the lactase non-persistence genotype (34.3%) than in lactase persistence subjects (21.6%; p = 0.01)

Multiple logistic regression analysis was performed for the association between the LCT polymorphism and MetS presence, controlling for gender, age, BMI and physical activity. Age and BMI remained in the final model as MetS risk factors (p < 0.001 for both), whereas lactase persistence was associated with a lower risk (OR = 0.467; 95% CI 0.264-0.824; p = 0.009, Table 2).

**Discussion**

The metabolic syndrome plays an important role as a predictor for increased risk of type-2 diabetes and cardiovascular disease. Any tools that potentially help the management of the MetS provide important benefits for health.
In this study we reported that lactase persistent individuals presented a lower risk to develop MetS.

Association studies between LP genotypes and MetS or its single components are scarce. Most investigations focused on BMI. Lamri et al. (2013) observed that the LP genotype was associated with a lower risk of MetS in a low dairy product consumer group, even after adjusting for BMI. MetS was more prevalent in the higher consumer group, but after adjusting for BMI the statistical significance was lost. These investigators suggested that the negative association between LP genotype and MetS in the low consumer group seemed more consistent than the positive one. Fumeron et al. (2011) reported that the LP genotype was associated with lower BMI and lower frequency of BMI-related metabolic diseases. However, the LP genotype was also associated with higher BMI in cross-sectional studies in European populations (Almon et al., 2010; Kettunen et al., 2010; Corella et al., 2011), and with higher risk of developing MetS (OR = 1.56; Almon et al., 2010). Malek et al. (2013) reported that the LP allele was significantly associated with BMI, fat mass, and waist circumference in children. The inconsistencies among these studies could be explained by different genetic backgrounds or environmental conditions of the populations studied, given that all investigations were performed with at least moderate-sized samples, and thus probably are not caused by chance alone. Lifestyle components such as differences in smoking habits, physical activity, diet or alcohol consumption may act as modifiers of the effect of gene variants in determining the risk of obesity.

There are plenty of evidences that certain milk components could have a direct benefit for insulin sensitivity, weight, blood pressure, and cholesterol level (Pfeuffer and Schrezenmeir, 2007). The higher intake of calcium could be one of the many possible mechanisms because: a) it could play a critical role in the regulation of energy metabolism; b) it could promote an increased faecal fat excretion by formation of insoluble calcium-fatty acid soaps or by binding of bile acids that impair the formation of micelles (Pfeuffer and Schrezenmeir, 2007); c) it may promote blood pressure reduction (Hajjar et al., 2003); and d) it may decrease oxidative stress and the expression of inflammatory cytokines typically associated with MetS (Zemel and Sun, 2008).

Milk protein-derived peptides are also beneficial. They show hypotensive effects and improve the bioavailability of other minerals and trace elements like magnesium, manganese, zinc, selenium and iron (FitzGerald et al., 2004). In the present study, the prevalence of hypertensive individuals was higher among individuals with the lactase non-persistence genotype.

Almon et al. (2010) showed that high milk consumption is not linked to higher measures of body fat mass in children and adolescents. In the French D.E.S.I.R. cohort

Table 1 - Demographic, laboratory and clinical characteristics of the investigated sample stratified by genotype.

| Variables                        | Genotypes     | p-value  |
|----------------------------------|---------------|----------|
|                                 | CC            | CT + TT  |
| Total number                     | 163           | 171      |
| Age (years)                      | 40.6 ± 14.6   | 42.3 ± 15.6 | 0.444b |
| Gender (% of male)               | 47.9          | 46.8     | 0.913b  |
| Waist circumference (cm)         | 92.1 ± 13.6   | 89.9 ± 11.9 | 0.127a |
| Total cholesterol (mg/dL)        | 199.1 ± 45.2  | 198.2 ± 42.9 | 0.902b |
| HDL- cholesterol (mg/dL)         | 44.1 ± 10.9   | 44.1 ± 12.4 | 0.796b  |
| LDL- cholesterol (mg/dL)         | 125 ± 37.9    | 127.3 ± 38 | 0.711b  |
| Triglycerides (mg/dL)            | 146 ± 111.1   | 131.2 ± 85.2 | 0.418b |
| Glycaemia (mg/dL)                | 97.7 ± 30.7   | 92.7 ± 25.3 | 0.081b  |
| Hypertension (% of hypertensive individuals) | 22.7   | 13.4     | 0.032ad |
| Physical activity (% of sedentary individuals) | 66     | 61.2     | 0.356d  |
| Body mass index (kg/m²)          | 26.4 ± 5.13   | 26 ± 4.34 | 0.333b  |
| Metabolic syndrome (%)           | 34.3          | 21.6     | 0.014d  |

*Statistically significant.
^CT and TT genotypes were grouped because carriers of the -13910*T allele have the predicted lactase persistence phenotype.
\[Mann-Whitney U-test.\]
\[Student’s t-test.\]
\[Chi-square test.\]

Table 2 - Logistic regression analysis predicting the Metabolic Syndrome.

| OR     | 95% CI      | p-value |
|--------|-------------|---------|
| LP genotype | 0.467      | 0.26-0.82 | 0.009   |
| Age    | 1.049       | 1.03-1.07 | < 0.001 |
| Body mass index | 1.247     | 1.16-1.33 | < 0.001 |
study, a high consumption of milk and dairy product was associated with lower body weight gain in a 9-year follow-up, and with lower incidence of impaired fasting glycaemia or type 2 diabetes and/or the metabolic syndrome (Fumeron et al., 2011).

The causal relation between LP and MetS could not be determined in the present study. Probably the tolerant individuals consume higher amounts of milk than the intolerant ones, and consequently they have more benefits from this beverage. But, we did not have information about the dietary pattern of the participants of this study, and thus we could not test whether the LP group has higher milk intake than the LNP group.

The genetic approach used is similar to the Mendelian randomisation in which the genetic variant acts as a proxy for the risk factor, in this case, the MetS (Burgess et al., 2012). In this study, the presence of the risk factor (LCT genotypes) is likely a causal factor for the MetS outcome.

Therapeutic lifestyle changes, including healthy eating habits and weight loss significantly improve most MetS abnormalities (Levesque and Lamarche, 2008). It has been suggested that milk consumption, especially the low-fat milk, as recommended by the DASH (Dietary Approaches to Stop Hypertension) diet is desirable. Moreover, people who consume higher amounts of dairy products also consume higher amount of fiber, fruit, vegetables and whole grains (Azadbakht et al., 2005). Since no dietary data was available for the investigated sample, it is not possible to decide if the inverse relation between dairy consumption and MetS observed herein could be attributed to a healthier lifestyle associated with higher intake of dairy or to genotype itself. Also, lactose can modify the intestinal microbiota increasing the total faecal number of bifidobacterium and lactobacilli that are health-promoting bacteria (Francavilla et al., 2012).

The overall results presented in this study should be viewed in the context of certain limitations. The present results were based on predicted phenotypes as we have only genotypes, although the phenotype-genotype correlation for the -13910 C/T polymorphism is well established.

Lactase phenotypes can be a valuable tool for the management of MetS during nutrition counseling. However more studies are warranted to confirm and refine the present results as well as to disclose the causal mechanisms of this association.

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