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ORIGINAL ARTICLE

Observational Study

Hypolactasia is associated with insulin resistance in nonalcoholic steatohepatitis

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Abstract

AIM

To assess lactase gene (*LCT*)-13910C>T polymorphisms in Brazilian non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) patients in comparison with healthy controls.

METHODS

This was a transverse observational clinical study with NAFLD patients who were followed at the Hepatology Outpatient Unit of the Hospital das Clínicas, São Paulo, Brazil. The polymorphism of lactase non-persistence/lactase persistence (*LCT*-13910C>T) was examined by PCR-restriction fragment length polymorphism technique in 102 liver biopsy-proven NAFLD patients (steatosis in 9 and NASH in 93) and compared to those of 501 unrelated healthy volunteers. Anthropometric, clinical, biochemical and liver histology data were analyzed. Continuous variables were compared using the *t* or Mann-Whitney tests, and categorical data were compared with the Fisher's exact test. Univariate logistic regression and

multivariate logistic regression adjusted for gender and age were performed.

RESULTS

No differences in the *LCT*-13910 genotype frequencies were noted between the NAFLD patients (66.67% of the patients with steatosis were CC, 33.33% were CT, and none were TT; 55.91% of the patients with NASH were CC, 39.78% were CT, and 4.3% were TT; $P = 0.941$) and the healthy controls (59.12% were CC, 35.67% were CT, and 5.21% were TT) or between the steatosis and NASH patients. That is, the distribution of the lactase non-persistence/lactase persistence polymorphism (*LCT*-13910C>T) in the patients with NAFLD was equal to that in the general population. In the NASH patients, the univariate analysis revealed that the lactase non-persistence (low lactase activity or hypolactasia) phenotype was associated with higher insulin levels (23.47 ± 15.94 μU/mL *vs* 15.8 ± 8.33 μU/mL, $P = 0.027$) and a higher frequency of insulin resistance (91.84% *vs* 72.22%, $P = 0.02$) compared with the lactase persistence phenotype. There were no associations between the *LCT* genotypes and diabetes ($P = 0.651$), dyslipidaemia ($P = 0.328$), hypertension ($P = 0.507$) or liver histology in these patients. Moreover, in the NASH patients, hypolactasia was an independent risk factor for insulin resistance even after adjusting for gender and age [OR = 5.0 (95%CI: 1.35-20; $P = 0.017$)].

CONCLUSION

The *LCT*-13910 genotype distribution in Brazilian NAFLD patients was the same as that of the general population, but hypolactasia increased the risk of insulin resistance in the NASH patients.

Key words: Lactose intolerance; Genetic polymorphism; Insulin resistance; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis

Core tip: Non-alcoholic fatty liver disease (NAFLD) exhibits a close relationship with metabolic syndrome (MetS), but the associations of the lactase non-persistence/lactase persistence genotypes with MetS components are controversial. Therefore, we assessed hypolactasia (*LCT*-13910CC) and lactase persistence genotypes in 102 Brazilian NAFLD patients in comparison with 501 healthy controls, the associations of these polymorphisms were verified with the results of biochemical tests, MetS and severity of liver histology in nonalcoholic steatohepatitis (NASH) patients. No differences in the *LCT*-13910C>T polymorphisms were noted between the NAFLD and controls, but hypolactasia increased the risk of insulin resistance in the NASH patients.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage that ranges from steatosis to nonalcoholic steatohepatitis (NASH) and advanced fibrosis/cirrhosis in persons without significant alcohol consumption[1,2]. NAFLD is currently considered the most common liver disease and is associated to metabolic syndrome (MetS) components, such as obesity and diabetes[3-5]. Several studies have correlated the severity of liver injury with increased frequencies of such components, thus making these components important targets in the management of this condition[1,6-8]. However, while specific pharmacological therapy are still far from solving all of the issues related to fatty liver disease, the pursuit of high-risk individuals can be a strategy for concentrating efforts on its diagnosis and management.

Milk is the primary energy source for newborns and is rich in lactose. Lactase phlorizin hydrolase in the microvillus membrane of the small intestinal cells digests lactose. However, after 2-12 years of age, a physiological genetically programmed reduction in lactase activity occurs, hypolactasia or lactase non-persistence, which, when accompanied by symptoms, defines lactose intolerance[9]. In contrast some populations mainly from Northern Europe present lactase persistence during adulthood[10]. The most interesting report published in 2002[11] found that the polymorphisms in intron 13 [lactase gene (*LCT*)-13910C>T] and in intron 9 (*LCT*-22018G>A) of the *MCM6* gene conferred lactase persistence in several populations[9,12-14]. These genotypes render a person a lactose digester. The lactase-persistence phenotype has a prevalence of 43.4% in Caucasian Brazilians, and there is no difference between genders[12].

Recent studies have raised concerns regarding the possible associations of lactase persistence with the components of MetS. In Europeans those with hypolactasia genotype (*LCT*-13910CC) had lower body mass indices and waist circumferences than those with lactase persistence genotypes[15,16]. Likewise, in the Canary Islands, those with lactase persistence genotypes exhibit higher odds ratios for MetS than do subjects with the *LCT*-13910CC genotype[17].

However, other studies have demonstrated that dairy food consumption showed lower susceptibility to type 2 diabetes or worsening of glucose homeostasis indices[18-20]. Nicklas *et al*[21] applied a questionnaire to a sample of 3452 American adults and reported that diagnosis of diabetes and hypertension were higher in individuals that considered themselves lactose intolerant with lower ingestion of calcium from dairies. Additionally, Samara *et al*[22] assessed a French population and

reported that better metabolic profiles in men was associated with more dairies intake.

As noted, the role of milk in MetS is not clearly defined at this moment, and the literature is controversial[23]. Moreover, publications regarding the *LCT*-13910C>T polymorphism in patients with NAFLD are scarce. Therefore, the purpose was to assess expression profiles of the *LCT*-13910 genotypes in Brazilians with NAFLD compared to those of healthy individuals to investigate whether the *LCT*-13910C>T variant could be a predictor of NASH. An additional goal was to analyze the associations of the lactase-persistence genotype with biochemical tests, components of MetS and the severity of liver histology in NASH patients.

MATERIALS AND METHODS

Ethical considerations

The Ethics Committee of the Hospital das Clínicas (number 448520) approved this study that was conducted following the ethical guidelines of the 1975 Declaration of Helsinki.

Patients and clinical design

This was a transverse study with NAFLD patients who were followed at the Hepatology Outpatient Unit of the Hospital das Clínicas, São Paulo, Brazil. *LCT*-13910C>T polymorphism was investigated in 102 liver biopsy-proven NAFLD patients and 501 unrelated healthy volunteers. All NAFLD patients were previously evaluated for other liver diseases, being excluded viral hepatitis, autoimmune hepatitis, hemochromatosis, Wilson disease and alpha 1-antitrypsin deficiency. MetS components identification followed the recommendations of the Adult Treatment Panel Ⅲ Report as follows: Triglycerides $\geqslant$ 150 mg/dL, high-density lipoproteins (HDL) < 40 mg/dL in men and < 50 mg/dL in women, fasting glucose $\geqslant$ 110 mg/dL, $\geqslant$ 130 mmHg systolic or $\geqslant$ 85 mmHg diastolic pressure, and abdominal obesity[24]. The study inclusion criteria were patients 18-75 years old with NAFLD diagnoses based on liver histology. Exclusion criteria were any other liver disease, significant alcohol intake (> 100 g/wk), previous exposure to drugs associated with liver steatosis or not accepting to participate in the study.

Liver histology were scored according to the macro- and micro-vacuolar steatosis, the inflammation and the hepatocyte ballooning. Fibrosis pattern and zonal distributions of the analysed variables were also recorded. The slides were classified according to the NASH Clinical Research Network[25]. The biochemical investigations included the following: Fasting glucose, plasma insulin, total cholesterol and fractions, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT), which were collected after a 12-h overnight fast and evaluated at the time of the liver biopsy. Homeostatic Model of Assessment (HOMA-IR) was used to evaluate insulin resistance [22.5 × fasting insulin (mU/mL) × glucose (mmol/L)][26]. A HOMA-IR $\geqslant$ 2.5 was used as the cutoff point to define insulin resistance[27,28]. Retrospective information regarding co-morbidities was also collected.

Genotyping

Leukocytes were used for genomic DNA extraction (Miller *et al*[29] 1988). The technique for *LCT*-13910 genotyping was described elsewhere[11,30-32].

Statistical analysis

The continuous variables are presented as the means ± the standard deviations and were compared using the *t* test (the assumption of normality was verified using the Anderson-Darling test). When appropriate, the Mann-Whitney test was used. The categorical variables are expressed as the percentages (frequencies) of affected individuals and were compared using Fisher's exact test. Univariate logistic regression was performed to evaluate the odds ratios with the respective 95%CIs. Multivariate logistic regression adjusted for gender and age was performed. The best predictive cut-offs for the continuous variables were determined using conditional trees when the traditional cut-offs did not provide interesting information[33]. *P* values below 0.05 were considered statistically significant. The R Project for Statistical Computing ver. 3.1.1 (R Core Team, Vienna, Austria, 2014) software package was used for the statistical analyses[34]. A statistical review of the study was performed by a biomedical statistician (Márcio Augusto Diniz).

RESULTS

The anthropometric, clinical, and biochemical characteristics of the patients are provided in Table 1. We evaluated 102 NAFLD patients, including 9 steatosis and 93 with NASH. All of the steatosis patients were women, whereas in the NASH group, 32 patients (34.41%) were men (P = 0.04). The NASH patients had higher fasting glucose levels than did the patients with steatosis only (123.14 ± 48.28 *vs* 91.71 ± 9.2, respectively, P = 0.033). There were no differences between the groups in terms of age, MetS components, BMI, insulin, HOMA-IR values $\geqslant$ 2.5, AST, ALT, GGT, total cholesterol, HDL, LDL or triglycerides (Table 1).

The distributions of alleles and genotypes are presented in Table 2. No differences in *LCT*-13910 genotype frequencies were noted between the NAFLD patients (66.67% patients with steatosis were CC, 33.33% were CT and none were TT; 55.91% of those with NASH were CC, 39.78% were CT and 4.3% were TT; P = 0.941) and the healthy controls (59.12% were CC, 35.67% CT, 5.21% TT). Likewise, no differences in the *LCT*-13910C>T allele frequencies were noted between the groups (76.95% of the controls, 83.33% of those with steatosis and 75.81% of the NASH patients had the *LCT*-13910C allele; P = 0.764). That is, the distribution of the *LCT*-13910C>T polymorphism in the patients with NAFLD was equal to that in the general population.

Table 1 Demographic, clinical and biochemical characteristics of the non-alcoholic fatty liver disease patients

	Steatosis (n = 9)	NASH (n = 93)	P value
Age	55.11 ± 10.3	56.51 ± 10.13	0.692
Men/women (n)	0% (0)/100% (9)	34.41% (32)/65.59% (61)	0.04[a]
Type 2 diabetes (n)	33.33% (2)	60.67% (54)	0.224
Dyslipidaemia (n)	83.33% (5)	79.78% (71)	1
High-blood pressure (n)	66.67% (4)	64.04% (89)	1
BMI	31.28 ± 5.79	31.25 ± 5.93	0.969
Fasting glucose (mg/dL)	91.71 ± 9.2	123.14 ± 48.28	0.033[a]
Insulin (μU/mL)	12.44 ± 4.2	19.92 ± 13.29	0.102
HOMA-IR value ⩾ 2.5	57.14%	83.53%	0.115
AST (U/L)	25.14 ± 6.89	38.8 ± 37.99	0.159
ALT (U/L)	40 ± 16.74	50.65 ± 54.99	0.934
GGT (U/L)	56.57 ± 59.9	87.36 ± 96.33	0.185
Total cholesterol (mg/dL)	203.29 ± 54.39	195.31 ± 45.71	0.863
HDL (mg/dL)	53 ± 6.58	46.15 ± 13.42	0.067
LDL (mg/dL)	124.14 ± 51.87	114.89 ± 39.74	0.72
Triglycerides (mg/dL)	130.43 ± 59.19	167.25 ± 82.02	0.258

[a]P value < 0.05. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; HDL: High-density lipoprotein; HOMA-IR: Homeostatic Model of Assessment; LDL: Low-density lipoprotein; GGT: Gamma glutamyl transferase; NASH: Nonalcoholic steatohepatitis.

Analysis *via* simple logistic regressions of the associations of the *LCT*-13910C>T polymorphisms with the results of the biochemical tests, components of MetS and severity of liver histology in the NAFLD patients (steatosis and NASH groups) did not reveal any associations (data not shown). Subsequently, we evaluated the patients with NASH (Table 3). In this group (n = 93), univariate analysis revealed that the hypolactasia phenotype was associated with higher insulin levels (P = 0.027) and greater insulin resistance (P = 0.02). No associations were noted between the liver histology parameters (*i.e.*, steatosis, inflammation and fibrosis) and the *LCT*-13910 genotype or phenotype. Moreover, no associations were found between the components of MetS or MetS diagnosis (P = 1.0) and the *LCT*-13910 genotype or phenotype.

Table 4 illustrates the logistic regression analysis that was adjusted for gender and age and assessed the independent associations of the *LCT*-13910C>T polymorphism with HOMA-IR, BMI ⩾ 30, insulin value and MetS in the NASH patients. Hypolactasia phenotype was associated with a 5-fold increase in insulin resistance (95%CI: 1.35-20; P = 0.017). The *LCT*-13910CT genotype conferred a 6.25-fold decrease in insulin resistance (95%CI: 0.04-0.64; P = 0.009). In this multivariate regression analysis, we no longer observed an association between hypolactasia and insulin level (even when using the cut-off of > 29.8 μU/mL, P = 0.197) after adjusting for gender and age. Similarly, the MetS diagnosis and a BMI ⩾ 30 were not associated with the *LCT*-13910C>T polymorphism.

DISCUSSION

Key findings

In this transverse clinical study, we were unable to find any differences in the *LCT*-13910C>T polymorphism expression profile between Brazilian NAFLD patients and healthy controls (P = 0.941). Moreover, the presence of the T allele was not able to differentiate steatosis from NASH in NAFLD patients (P = 0.764). However, in NASH patients, the hypolactasia phenotype (*i.e.*, the *LCT*-13910CC genotype) was associated with insulin resistance, and conversely, the *LCT*-13910CT genotype conferred protection against its occurrence.

The *LCT*-13910C>T polymorphism prevalence varies among different populations across the globe. The lactase-persistence phenotype (*i.e.*, the *LCT*-13910-CT and *LCT*-13910-TT genotypes) can occur at rates as high as 72% and 73.7% in New Zealand and Sweden, respectively[13,35]. In Hungary, the prevalence is 35.9%, and in Caucasian Brazilians, the prevalence is 43.4%[12,36]. In contrast, in Chinese and Japanese Brazilians, the lactase-persistence phenotype was not found at all in some published studies[12,37]. The *LCT* genotype distribution was also the same in NAFLD patients regardless of the presence of NASH or steatosis only.

In a recent European meta-analysis with 31720 individuals, Kettunen *et al*[16] found that the *LCT*-13910CC genotype was associated with a decreased body mass index (BMI), when compared to *LCT*-13910CT/TT. In an analysis of 17374 Finns, it was observed that when the lactase persistent allele was present, BMI was 0.3 kg/m^2 higher, which corresponds to approximately 1 kg[16]. These findings were reproduced by Corella *et al*[15] in a Mediterranean population in which *LCT*-13910CC individuals exhibited a lower risk of obesity, lower body weights, lower BMIs and smaller waist circumferences than *LCT*-13910T-allele carriers. Although the association between the *LCT*-13910C>T genotypes and the diagnosis of full-blown MetS was not significant in the overall analysis in the study, a subgroup analysis revealed a significant association in the subjects with a lactose intake higher than 8 g/d[15]. In a cross-sectional

Table 2 Allele and genotype frequencies of the lactase-13910C>T polymorphisms

		Allele frequency % (*n*)[a]		Total (%)	Genotype frequency % (*n*)[b]			Total (%)
		C	T		CC	CT	TT	
LCT-13910	Control (*n* = 501)	76.95 (768)	23.05 (230)	100	59.12 (295)	35.67 (178)	5.21 (26)	100
	Steatosis (*n* = 9)	83.33 (15)	16.67 (3)	100	66.67 (6)	33.33 (3)	0 (0)	100
	NASH (*n* = 93)	75.81 (141)	24.19 (43)	100	55.91 (52)	39.78 (37)	4.3 (3)	100

[a]*P* = 0.764; [b]*P* = 0.941. NASH: Nonalcoholic steatohepatitis; *LCT*: Lactase gene.

Table 3 Associations of the lactase-13910 phenotype in nonalcoholic steatohepatitis patients (*n* = 93)

	Hypolactasia	Lactase persistence	*P* value
Age	55.96 ± 10.91	57.61 ± 9.33	0.443
Gender: Female % (*n*)	70.83 (34)	60.98 (25)	0.51
Type 2 diabetes % (*n*)	66.67 (30)	57.5 (23)	0.664
Dyslipidaemia % (*n*)	82.22 (37)	77.5 (31)	0.792
High-blood pressure % (*n*)	68.89 (31)	57.5 (23)	0.273
BMI	31.39 ± 6.55	31.28 ± 5.37	0.714
BMI ⩾ 30 % (*n*)	58.14 (25)	65 (26)	0.388
Fasting glucose (mg/dL)	122.61 ± 50.14	123.83 ± 46.43	0.892
Insulin (μU/mL)	23.47 ± 15.94	15.8 ± 8.33	0.027[a]
HOMA-IR value ⩾ 2.5 (n)	91.84 (45)	72.22 (26)	0.02[a]
AST (U/L)	38.94 ± 37.66	42.67 ± 40.15	0.121
ALT (U/L)	51.47 ± 69.44	52.12 ± 35.02	0.072
GGT (U/L)	97.49 ± 118.27	80.51 ± 65.6	0.427
Total cholesterol (mg/dL)	196.62 ± 47.13	195.55 ± 44.06	0.965
HDL (mg/dL)	46.38 ± 12.26	46.05 ± 15.06	0.698
LDL (mg/dL)	117.19 ± 39.47	114.25 ± 39.9	0.893
Triglycerides (mg/dL)	169.11 ± 82.97	162.3 ± 83.11	0.477
Steatosis			
1	21.28 (10)	24.39 (10)	0.453
2	51.06 (24)	39.02 (16)	
3	27.66 (13)	36.59 (15)	
Inflammation			
0	2.13 (1)	7.32 (3)	0.133
1	61.7 (29)	46.34 (19)	
2	23.4 (11)	39.02 (16)	
3	12.77 (6)	7.32 (3)	
Fibrosis			
0	18.75 (9)	14.63 (6)	0.804
1	39.58 (19)	43.9 (18)	
2	16.67 (8)	17.07 (7)	
3	20.83 (10)	19.51 (8)	
4	4.17 (2)	4.88 (2)	
MetS	51.92 (27)	53.66 (19)	1

[a]*P* value < 0.05. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; HDL: High-density lipoprotein; HOMA-IR: Homeostatic Model of Assessment; LDL: Low-density lipoprotein; GGT: Gamma glutamyl transferase; MetS: Metabolic syndrome.

work conducted in the Canary Islands, Almon *et al*[17] demonstrated that subjects with the *LCT*-13910CT and *LCT*-13910TT genotypes exhibited higher odds ratio for MetS than subjects with the *LCT*-13910CC genotype. The authors concluded that the T allele might constitute a nutrigenetic factor that increases the susceptibility to MetS development, and this susceptibility was particularly noted in women[17].

Despite the aforementioned studies that have demonstrated correlations of the CC genotype with decreased BMI, a lower risk of obesity, a lower body weight, and smaller waist circumference compared with the CT and TT genotypes[15,16] and the even further increased higher odds ratio for MetS in individuals with the T allele[17], we could not corroborate these findings in our NAFLD population. Studying only the NASH patients in the univariate analysis, we did not find associations between the *LCT*-13910C>T polymorphism and BMI or MetS diagnoses even after adjusting for gender and age in the multivariate analysis. In fact, the patients with NASH and a genetic profile of persistent lactase activity exhibited less insulin resistance than the patients with hypolactasia. These divergences in our findings could be related to differences in the studied populations and possible positive effects of dairy ingestion on the metabolic profiles of these individuals.

Table 4 Multivariate logistic regression analysis in nonalcoholic steatohepatitis patients

Factor	OR	95%CI	*P* value
HOMA-IR value ⩾ 2.5			
Hypolactasia phenotype	5	1.35-20	0.017[a]
CT genotype	0.16	0.04-0.64	0.009[a]
TT genotype	-	-	0.994
BMI ⩾ 30			
Hypolactasia phenotype	0.49	0.13-1.81	0.285
CT genotype	1.73	0.69-4.35	0.244
TT genotype	1.01	0.12-8.39	0.991
Insulin > 29.8			
Hypolactasia phenotype	2.04	0.68-6.25	0.197
CT genotype	0.52	0.17-1.56	0.25
TT genotype	-	-	0.991
MetS			
Hypolactasia phenotype	0.94	0.47-2.42	0.89
CT genotype	1.07	0.46-2.49	0.866
TT genotype	0.91	0.11-7.3	0.929

[a]*P* value < 0.05. HOMA-IR: Homeostatic Model of Assessment; OR: Odds ratio; BMI: Body mass index; MetS: Metabolic syndrome.

Our studied population consisted only of NAFLD patients, among which the prevalences of MetS components are expected to be higher than those of the overall population. Therefore, firm direct comparisons are precluded. However, a recently published Brazilian study demonstrated that in the general population, the lactase non-persistence genotype subjects exhibit higher prevalences of hypertension (P = 0.032) and MetS (P = 0.01) than lactase-persistence genotype individuals based on univariate analysis[38]. Furthermore, multivariate analyses revealed that lactase persistence was associated with a lower risk for MetS after adjusting for gender, age, BMI and physical activity (OR = 0.462; P = 0.009). These data are in line with our findings that demonstrated a favourable profile of MetS components and glucose homeostasis in the NASH patients with lactase persistence. Moreover, in a longitudinal French study encompassing 3575 subjects, Lamri *et al*[39] demonstrated that the C allele was associated with a higher frequency of impaired fasting glycaemia and type 2 diabetes. However, Enattah *et al*[40] were unable to demonstrate that lactase persistence polymorphisms were risk factors for type 1 or type 2 diabetes in the Finnish study. Similar to NASH, polycystic ovary syndrome is also frequently associated with metabolic disturbances, including dyslipidaemia, insulin resistance and central obesity, and NASH often coexists in these patients[41]. Lerchbaum *et al*[42] demonstrated a significantly higher prevalence of hypolactasia in polycystic ovary syndrome women, which also corroborates our findings.

Ultimately, we believe that dairy consumption appears to modulate the metabolic profiles of these different populations because of the strong association of the *LCT*-13910 genotype with dairies intake and lactose malabsorption[11,31,39,43]. Several studies have highlighted the benefits of dairy and dairy components on MetS components[18-22,44-46] and cardiovascular health[47]. The benefits of dairy products may be mediated through several mechanisms, including the following[23,48]: The insulinotropic role of whey and its beneficial effect on body weight and fat; the favorable effects of amino acids, medium chain fatty acids, calcium and other minerals found in milk and its derivatives; improvements in insulin sensitivity due to medium chain fatty acids; reductions in the absorption of cholesterol and other fats from fermented products; the probiotic bacteria present in these foods and the associated proteins and peptides; and improvements in weight control, blood pressure and plasma lipids due to lactose, citrate, proteins and peptides. Specifically addressing glucose homeostasis, a hypothetical explanation is that milk and dairy consumption may be associated with an enhanced insulinaemic response, decreased glycemic fluctuations, and increased secretion of glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide[49].

Experimental models also provide some mechanistic explanations that link dairy consumption with lower incidences of insulin resistance and diabetes[50]. Milk components such as rumenic acid, vaccenic acid, phytanic acid and its derivative pristanic acid have been demonstrated to improve insulin resistance though PPAR signalling activation in different rat models[51-54]. These findings suggest that dairy consumption could have a role in insulin resistance and NASH management.

However, in our study, there was no association between *LCT*-13910 genotype and the severity of liver histology in the NASH patients. The reason for this finding may be that the pathogenesis of NASH involves a complex multiple parallel hits process in which a number of different events may contribute to liver injury[55]. Lifestyle and genetic predisposition remain relevant disease determinants. The consumption of high-calorie diets rich in lipids results in weight gain, obesity and insulin resistance. Moreover, a diet high in carbohydrates (mainly fructose) and saturated fatty acids contributes to the production of excess free fatty acids, whose safe disposal is impaired, which results in oxidative stress and NASH[56]. Recent data have also demonstrated a potential role of the microbiota in the induction of insulin resistance and the development of NAFLD/NASH[57-59]. The major components of the gut microbiota at the phylum level are *Bacteroidetes* and *Firmicutes*[60]. It has been demonstrated that *Firmicutes* levels are elevated in obesity and related diseases, whereas *Bacteroidetes* levels are decreased, which leads to an increase in the *Firmicutes*/*Bacteroidetes* ratio[61,62]. Interestingly, it has been shown that lysozyme-rich milk consumption results in a decline in *Firmicutes* levels (mainly *Clostridia* spp.) and in an increase in *Bacteroidetes* levels over time[63,64]. Despite the absence of high levels of lysozyme in the milk of dairy animals, these studies highlighted the potential role of milk and its components in the composition of the microbiome in health and disease.

The main limitations of our study are the lack of alimentary reports from the NAFLD patients to quantify the dairy intakes and the absence of ethnic data because

the prevalence of *LCT*-13910C>T polymorphisms may vary widely, as has been previously demonstrated[12].

In conclusion, we demonstrate that hypolactasia (*i.e.*, the *LCT*-13910CC genotype) is associated with a higher insulin resistance frequency in NASH patients. However, further studies that include dairy ingestion reports are needed to elucidate the associations of the lactase-persistence phenotype with MetS and NAFLD/NASH in different populations.

COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage that ranges from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis in persons without significant alcohol consumption and has a close relationship with metabolic syndrome (MetS). The lactase gene (*LCT*)-13910C>T polymorphism located upstream of the *LCT* is tightly associated with lactase persistence. The *LCT*-13910CT and *LCT*-13910TT genotypes are associated with the lactase-persistence phenotype, *i.e.*, they render a person a lactose digester, whereas the *LCT*-13910CC genotype is associated with lactose malabsorption.

Research frontiers

The role of milk in MetS is not currently clearly defined, and the literature is controversial. Moreover, to our knowledge, there are no published data regarding the *LCT*-13910C>T polymorphism in patients with NAFLD. Therefore, the authors assessed the expression profile of *LCT*-13910 genotypes in Brazilian patients with NAFLD in comparison with those of healthy controls to investigate whether the *LCT*-13910C>T variant could be a predictor of NASH. Furthermore, in NASH patients, the authors analyzed the associations of the lactase-persistence genotype with the results of biochemical tests, components of MetS and the severity of liver histology.

Innovations and breakthroughs

The authors were unable to find any differences in the *LCT*-13910C>T polymorphism expression profiles between Brazilian NAFLD patients and healthy controls. Moreover, the presence of the T allele was not able to discriminate steatosis from NASH in NAFLD patients. However, in NASH patients, the hypolactasia phenotype (*i.e.*, the *LCT*-13910CC genotype) was associated with insulin resistance, and conversely, the *LCT*-13910CT genotype conferred protection against its occurrence.

Applications

Specific pharmacological therapy for NASH is still lacking, so the pursuit of high-risk individuals can be a strategy for concentrating efforts on its diagnosis and management. Dairy consumption appears to modulate the metabolic profile because hypolactasia was found to be an independent risk factor for insulin resistance in NASH patients. Further studies that include dairy ingestion reports are needed to elucidate the associations of the lactase-persistence phenotype with MetS and NAFLD/NASH in different populations.

Terminology

NAFLD: Non-alcoholic fatty liver disease, which encompasses a wide spectrum of liver damage that ranges from steatosis to NASH and cirrhosis in persons without significant alcohol consumption. The MetS components include the following: Fasting glucose $\geqslant$ 110 mg/dL, triglyceride $\geqslant$ 150 mg/dL, high-density lipoprotein < 40 mg/dL in men or < 50 mg/dL in women, $\geqslant$ 130 mmHg systolic or $\geqslant$ 85 mmHg diastolic pressure and abdominal obesity. The *LCT*-13910CT and *LCT*-13910TT genotypes are associated with the lactase-persistence phenotype, *i.e.*, these genotypes render a person a lactose digester, whereas the *LCT*-13910CC genotype is associated with hypolactasia, *i.e.*, lactose malabsorption.

Peer-review

The paper indicated that among nonalcoholic steatohepatitis patients, hypolactasia is associated with insulin resistance in Brazil. It is a very interesting and well-written paper.

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