Risk of glycemic disorder in elderly women adjusted by anthropometric parameters and cytokine genotypes

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Summary

Objective: The objective of the present study was to examine the association of glucose intolerance and type-2 *diabetes mellitus* with the -174 G > C and -308 G > A allelic variations of IL-6 and TNF-α, respectively, through anthropometric measurements and age strata. **Methods:** This is a cross-sectional study using data from 285 community dwelling elderly women who underwent physical, biochemical, and genetic examinations. **Results:** Genotype-unadjusted analysis revealed that the risk of glucose intolerance and diabetes in elderly women with elevated BMI was 1.71 and 2.82 times higher, respectively, whereas age and conicity index did not show predictive value. Prevalence ratios for glucose intolerance and diabetes across allelic variants of IL-6 and TNF-α did not associate IL-6 with unbalanced glucose levels, despite adjustment for BMI, age, and conicity index. Conversely, carriers of the TNF-α A allele were 2.06 and 5.58 times more likely to exhibit glucose intolerance and diabetes, respectively, compared to GG homozygotes. **Conclusion:** Our results suggest that bearing the A allele of the -308 G > A polymorphism of TNF-α predisposes to anthropometric measure-sensitive impaired glucose metabolism in older women.

Keywords: Elderly health; insulin resistance; cytokines; polymorphism, genetic; anthropometry.

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Introduction

The increase in the number of people over 60 years of age is a visible phenomenon in almost every country. In Brazil, the last census indicates the possibility that the number of elderly people is above 30 million[1], representing almost 13% of the population[2]. It is known that aging exposes people to higher prevalence of chronic diseases, such as type 2 *diabetes mellitus*, which aαects 17.4% of the population from 60 to 69 years of age, according to the census of 1986 and 1988[3]. Its high incidence rate, added to the diαerent clinical complications resulting from macro- and microvascular disruption, make this disease a public health problem.

Among anthropometric variables that commonly predict endocrine-metabolic disorders, the body mass index (BMI) and conicity index (C index) should be emphasized[4,5]. Despite the imminent relationship between age and deregulation of glycemic levels, primary and contributing causes for changes in this aspect of human metabolism are still debatable. Etiologic possibilities currently discussed involve aspects related to lifestyle due to inadequate diet and physical inactivity, factors closely related to obesity[6], as well as endocrine deαciency secondary to exhaustion of α cells and/or idiopathic humoral interferences in glucose uptake by cells[7,8], with possible allele-speciαc contributions from important inαammatory mediators[9-12].

The objective of the present study was to analyze the association between BMI, C index, and age with glucose intolerance phenotypes and type 2 *diabetes mellitus* in elderly women, considering the inαuence of classical allelic variations of IL-6 and TNF-α genes.

Methods

This is an analytical transversal study with data from 285 elderly females (α 60 years) recruited consecutively from the community to participate in the study conducted from 2005 to 2008, whose protocol was approved by the institutional ethics on research committee. Every participant signed an informed consent. Participants were not on nutritional follow-up nor did they practice regular exercises before laboratorial tests and anthropometric measurements were performed.

Physical and biochemical exams

Patients were wearing light clothes during the physical exam. Height was measured using a manual stadiometer placed on the wall, with patients barefoot and erect on a αat and rigid surface with arms along their body. Body mass index (BMI) was obtained by dividing body mass (in kg) by the square height (in m^2), while the C index was estimated to be 9.17 times the ratio between waist circumference (m) and the square root of the ratio between body mass (kg) and height (m).

Biochemical evaluation consisted of 12-hour fasting glucose serum levels using Roche® (USA) reagents. The reference value (α 126 mg/dL) recommended by the American Diabetes Association[13] or current use of oral hypoglycemic agent or insulin was used to classify type 2 diabetes. Glucose intolerance was deαned as glucose levels α 100 $mg.dL^{-1}$ or presence of diabetes, according to recommendations of the International Diabetes Federation[14,15].

Laboratorial analysis

To determine IL-6 alleles, a region of 628 bp of the gene containing a single nucleotide polymorphism (SNP) -174 G > C of the promoter region (rs1800795) was ampliαed using a pair of oligonucleotides: 5'-GAA CAC AGA AGA ACT CAG ATG ACT GG-3' (forward) and 5'- AGG AGT TCA TAG CTG GGC TCC TGG AG-3' (reverse). Variability of the TNF-α gene was investigated by amplifying a fragment of 327 bp containing the -308 SNP G > A (rs1800629) of the promoter region of the gene, using oligonucleotides A (5'-CCT CAA GCC TGC CAC CAA GC-3'; forward) and B (5'-TCC TCC CTG CTC CGA TTC CG-3'; reverse). Each individual reaction was composed of: 100 ng DNA. 10 mM Tris-HCL pH 9.2; 25 mM KCL; 1.5 mM $MgCl_2$; 0.2 mM dNTP; 20 pmol of each oligonucleotide; 0.5 µg ovalbumin; and 1 unit of *Taq* DNA polymerase (Phoneutria, Minas Gerais, Brazil) in a αnal volume of 50 µL. Aαer a hot start at 80°C for 1 minute and a initial denaturation step at 94°C for 2 minutes, each ampliαcation cycle consisted of 40 seconds at 94°C, 45 s at 64°C, and 50 s at 72°C repeated 36 times and αnalized by an extension step at 72°C for 5 minutes. Each PCR product was sequenced in the ABI PRISM 3130 DNA analyzer system (Applied Biosystems, Foster City, USA) using the oligonucleotide 5'-GCC TCA GAG ACA TCT CCA GTC C-3', for IL-6 products, and oligonucleotide A, for TNF-α products. Each sequence obtained was examined using the biostatistics soαware package Staden (MRC, Cambridge, United Kingdom) and conαrmed by visual inspection.

Statistical analysis

Data on sample characterization are presented as mean and standard deviation or relative frequency. General prevalence of glucose intolerance and diabetes was calculated for each stratum (age α 65 years and > 65 years, BMI α 27 kg/m^2 and > 27 kg/m^2, conicity index α 1.25 and > 1.25), as well as the prevalence of IL-6 and TNF-α genotypes in each stratum. The Chi-square test was used to identify frequency diαerences among strata. Prevalence ratios (PR) of glucose intolerance and diabetes were calculated as dichotomies in elected strata (lower stratum = 0 and upper stratum = 1). Additionally, dummy variables were created for comparison among strata, assuming the lower stratum (= 0) as the reference, of each study variable for IL-6 and TNF polymorphisms, for IL-6 (CG = 0 and

GC and CC = 1), for TNF (GA and AA = 0 and GG = 1) and, when the genes were analyzed together (GG, for IL-6, and GA and AA, for TNF = 0 and the remaining combinations = 1). Differences were analyzed by the Mantel-Hanzel test. The confidence interval of 95% was used. Data were analyzed by the statistical software Stata, version 7.0.

Results

Table 1 shows the characteristics of the study population, including age profile, anthropometric data, and representative variables of glycemic metabolism in elderly women who participated in the study.

Table 1 – Characteristics of the study population

Variable	Mean ± Standard deviation
n (elderly)	285
Age (years)	67.8 ± 6.1
Weight (kg)	93.9 ± 10.8
Height (m)	1.52 ± 0.10
BMI (kg/m²)	27.5 ± 4.3
C index (m)	1.29 ± 0.10
Glycemia (mg/dL)	106.3 ± 29.4
Glucose intolerance (%)	27.6
Diabetes (%)	16.8

BMI, body mass index; C index, conicity index.

Considering the mean values of age, BMI, and C index, we decided to use dichotomization of the sample (age ≥ 65 years or > 65 years, BMI ≥ 27 kg/m² or > 27 kg/m², conicity index ≥ 1.25 or > 1.25) in subsequent analysis. Thus, statistics comparing the prevalence of glucose intolerance and type 2 diabetes among groups are presented in Table 2. In this analysis, age and C index were not predictive of glucose intolerance or diabetes. However, the group of elderly women with BMI > 27 kg/m² was 1.71 and 2.82 times more likely to present glucose intolerance and diabetes, respectively.

Table 2 – Prevalence ratio for glucose intolerance and type 2 diabetes among elderly women stratified by BMI, age, and CoI

Variable (numerator)	Prevalence ratio (95% CI)	
	Glucose intolerance	Diabetes
BMI (> 27 kg/m²)	1.71 (1.14-2.56) *	2.82 (1.50-5.30) *
Age (> 65 years)	1.05 (0.70-1.59)	1.30 (0.71-2.37)
C index (> 1,25)	1.26 (0.84-1.89)	1.03 (0.61-1.76)

BMI, body mass index; C index, conicity index; CI, confidence interval; * p < 0.05 compared to the group with BMI ≥ 27 kg/m².

When prevalence ratios of glucose intolerance and diabetes were analyzed, according to variants of IL-6 and TNF-α genes, adjusted for BMI, age, and C index, it was noticed that neither IL-6 genotype was a risk or protection factor for glycemic deregulation (Table 3). On the other hand, carriers of the TNF-α allele A were 2.06 and 5.58 times more likely to have glucose intolerance and diabetes, respectively, when compared to GG homozygous in the strata of people with BMI ≥ 27 kg/m² (Table 4).

Table 3 – Prevalence ratio for glucose intolerance and type 2 diabetes among elderly women dichotomized by -174 G > C IL-6 polymorphism in each age and anthropometric groups

Stratum	Prevalence ratio (95% CI)	
	Glucose intolerance	Diabetes
BMI ≥ 27 kg/m²	1.17 (0.59-2.34)	1.33 (0.43-4.13)
BMI > 27 kg/m²	0.71 (0.43-1.19)	0.64 (0.32-1.24)
Age ≥ 65 years	0.64 (0.29-1.39)	0.49 (0.14-1.66)
Age > 65 years	0.93 (0.58-1.50)	0.86 (0.45-1.65)
C index ≥ 1.25	0.86 (0.42-1.80)	0.97 (0.40-2.38)
C index > 1.25	0.81 (0.49-1.31)	0.62 (0.29-1.31)

BMI, body mass index; C index, conicity index; CI, confidence interval; differences were not observed when GG homozygous were compared to carriers of the C allele.

Table 4 – Prevalence ratio for glucose intolerance and type 2 diabetes among elderly women dichotomized by the -308 TNF-α G > A polymorphism in each age and anthropometric groups

Stratum	Prevalence ratio (95% CI)	
	Glucose intolerance	Diabetes
BMI ≥ 27 kg/m²	2.06 (1.03-4.17)*	5.58 (1.86-16.74)*
BMI > 27 kg/m²	0.72 (0.37-1.42)	0.57 (0.22-1.49)
Age ≥ 65 years	1.98 (0.96-4.07)	1.87 (0.58-6.00)
Age > 65 years	0.84 (0.45-1.55)	1.05 (0.50-2.22)
C index ≥ 1.25	0.62 (0.21-1.85)	0.59 (0.15-2.36)
C index > 1.25	1.37 (0.82-2.29)	1.70 (0.84-3.46)

BMI, body mass index; C index, conicity index; CI, confidence interval; * p < 0.05 whenever carriers of allele A are compared to genotype GG.

Discussion

In the present study, glucose intolerance and diabetes were observed in 27.6% and 16.8% of all cases investigated, respectively. The prevalence of glucose intolerance and diabetes did not differ among the different strata for age, C index, or IL-6 or TNF-α genotypes. However, it differed between BMI strata (p = 0.031), with glucose intolerance and diabetes significantly higher in the group with

BMI higher than 27 kg/m². Subsequent analyses were adjusted to cancel the influence of the BMI, being observed that prevalence of glucose intolerance or diabetes did not show significant variation according to IL-6 genotypic category. Similar analysis performed for TNF-α genotypic categories showed different results, in which carriers of the TNF-α A allele had more chances of presenting glucose intolerance and diabetes. However, this finding was observed only in the stratum below the threshold stratification established for the study (BMI < 27 kg/m²). This result was most likely due to the fact that excessive adiposity superseded the effects of an isolated gene on the pathophysiology of insulin resistance, masking the contribution of the TNF-α A allele on glycemic disorders, making it evident only in strata of body composition with a tendency to eutrophy[16].

However, one cannot deny the hypothesis of the TNF-α A allele being an explanation in cases of insulin resistance and diabetes. To this end, González-Sanches et al.[17], in a population-based epidemiologic study, analyzed the interaction among both genes, some cardiovascular risk factors, and biochemical disorders in more than 800 Caucasian patients ages 35 to 74 years. They observed that the -308 G > A SNP of the TNF-α gene can be related to a higher prevalence of insulin resistance and incidence of type 2 *diabetes mellitus*, regardless of insulin resistance, BMI, and waist-hip relationship.

Nicaud et al.[12] investigated the role of -308 G > A SNP of the TNF-α gene in insulin resistance and they observed the association between the A allele with type 2 diabetes. Studies have suggested that the TNF-α -308A/G gene SNP is related with serum levels of the respective mediator because the A allele seems to predispose higher transcriptional levels of the gene, producing increased serum concentrations compared to the allele G[18,19]. According to the evidence that different cardiovascular risk factors (including hypertension, dyslipidemias, and insulin resistance) are intensified by circulating levels of TNF-α, regardless of gender and age stratum[20-23], allele A is more common among patients with metabolic disorders common in the elderly[12,16,24].

It has been reported in the literature that TNF-α has an important role in insulin resistance, interfering with the insulin signaling pathway[12]. The mechanism of induction of insulin resistance by this cytokine is probably due to induction of phosphorylation of the insulin receptor substrate 1 (IRS-1), decreasing its affinity for the insulin receptor, attenuating signal transmission by compromising phosphorylation of the receptor to tyrosine[25-27]. The impossibility to determine circulating levels of the inflammatory mediators investigated represents a limitation of the present study. Besides, performing this study with elderly women implies that caution should be taken when extrapolating the results to other gender or age group.

Summarizing, non-adjusted analysis for genotype showed that elderly women with elevated BMI had 1.71 and 2.82 times greater risk of developing glucose intolerance and diabetes, respectively, while age group and C index did not show any predictive value. Apart from this association, our results suggest that the presence of the A allele of -308 G > A TNF-α gene predisposes to the development of glycemic disorders sensitive to the context, since glucose intolerance and type 2 diabetes phenotypes are not influenced by any of the genotypes being investigated among individuals with BMI compatible with obesity.

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