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ORIGINAL ARTICLE

Increased Triglyceride Levels in a Japanese Population Living in Southern Brazil

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Background. In the present study we investigated 96 individuals of Japanese descent living in southern Brazil (Cascavel-PR) in terms of triglyceride (TG) levels (> or ≤200 mg/dL) and compared them to non-Japanese control individuals.

Methods. We analyzed TG and total cholesterol (TC) levels by an enzymatic method and apolipoprotein A-I and B (apo A-I and apo B) by a turbidimetric method. We also determined the lipoproteins HDL and LDL by a direct method and by electrophoresis. All these determinations were performed in plasma.

Results. TG levels were above 200 mg/dL in 18.7% of the individuals of Japanese descent and in 8.4% of the controls. Mean TC levels were 259 mg/dL for Japanese descendants and 225 mg/dL for the control group. We observed that individuals of Japanese descent with TG levels above 200 mg/dL had the highest TC, LDL-c, and VLDL-c levels and the lowest HDL-c and apo A-I levels. Body mass index (BMI) was also higher in individuals of Japanese descent with TG above 200 mg/dL.

Conclusions. This Japanese population has high TG levels compared to control individuals, and diet did not influence these levels.

Key Words: Triglyceride, Lipoprotein, Apolipoprotein, Japanese.

Introduction

Altered plasma lipid levels are considered important risk factors for the development of ischemic heart disease (IHD). The relation between elevated total cholesterol (TC) concentrations and IHD has been well documented (1,2). However, the effect of triglyceride (TG) levels has been the subject of controversy for more than three decades and has been referred to as a "forgotten" risk factor (3,4). Despite this, there is strong epidemiological evidence suggesting that high TG levels are associated with an increase in cardiovascular disease, independent of HDL-cholesterol (HDL-c) levels (5).

Hypertriglyceridemia has been described as an increase in plasma TG levels above 200 mg/dL (6,7), being a common characteristic of the dyslipidemic profile among patients with IHD (8). Some authors suggest that the TG-IHD association is due to the relation between high TG levels and low plasma HDL-c concentration (9). Plasma HDL-c and TG concentrations are usually inversely correlated (1,9,10).

Among lipoproteins, the most atherogenic is LDL-cholesterol (LDL-c) (11). The risk factors for cardiovascular disease related to lipids are, in decreasing order of importance, LDL-c, TC, HDL-c and TG (12).

The levels of apolipoprotein (apo) A-I and B, major HDL-c and LDL-c proteins have been described as better biochemical markers for IHD risk factors (13,14).

According to Vartiainen et al. (15), changes in life style have a great influence on atherosclerosis risk factors. Itoh et al. (16) showed that a diet composed of fat and simple carbohydrates had significant effects on TC levels and body mass index (BMI) in young Japanese women.

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Data related to Japanese persons living in Japan showed low risk for IHD (11). Cullen (12) reported a significant increase in plasma lipid levels from 1960 to 1980 in the Japanese population, followed by a decrease during the last decade, with the disappearance of this significant difference. In addition to consuming a different type of diet, Japanese people who live in Japan are more physically active due to various factors such as limited use of automobiles and more commuting by public transportation and thus more walking in metropolitan areas (13).

The TG levels of Japanese descendants born in the U.S. are slightly higher than those of the American population (14,15). Burchfiel et al. (16) observed that diet composition differs considerably between American Japanese and those living in Japan, with the latter ingesting less protein and fat and more carbohydrates.

The objective of the present study was to determine the lipid profile of Japanese descendants living in the south of Brazil. After the subjects were divided into two groups according to plasma TG concentrations (above and below 200 mg/dL), we observed the relation between lipoprotein and apolipoprotein levels and the diet consumed by the study population.

Materials and Methods

Population

A total of 192 individuals living in the south of Brazil (Cascavel-PR) with no pre-existing diseases including diabetes, atherosclerosis or heart disease, participated in this study. We excluded subjects who presented blood glucose above 110 mg/dL.

The individuals were divided into two groups: 96 Japanese descendants and 96 non-Japanese controls (53.1% females and 46.9% males).

We measured blood pressure in all subjects. The mean blood pressure in the control group was 118/78 mmHg and the mean in the Japanese descendants group was 119.5/79 mmHg.

Mean age was 51 ± 14 years for the Japanese descendants and 48 ± 16 for the controls. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre and all subjects gave informed consent to participate.

All individuals answered a questionnaire about alcohol ingestion, smoking habit, use of drugs, pre-existent diseases, diabetes, heart problems and blood pressure before their participation in this study. We eliminated all subjects who presented any factors that could influence our study.

A blood sample (10 mL) was collected by venipuncture from each participant after a 12-h fast. The individuals answered a nutritional questionnaire concerning per capita intake of fiber, saturated fat, fish, oil, and sugar. Samples were centrifuged at 5000 × g for 10 min and plasma was frozen at −40°C for further analysis.

Groups

The individuals were divided into four groups. Groups 1 and 2 consisted of individuals with Caucasian ascendency (controls) with TG plasma concentrations below and above the cut-off point, respectively, and groups 3 and 4 consisted of individuals of Japanese descent similarly divided according to TG plasma concentrations. The normal values considered were those established by the World Health Organization: TC ≤200 mg/dL, LDL-c ≤130 mg/dL, HDL-c ≥35 mg/dL, VLDL-c ≤40 mg/dL, and TG ≤200 mg/dL.

Lipid Assays

TC and TG were determined by an enzymatic method using the Labtest Diagnostic Kit (Lagoa Santa, Minas Gerais, Brazil). HDL-c and LDL-c were determined by a direct method using the Labtest Diagnostic Kit (Lagoa Santa) and DiaSys Diagnostic System International kit (Holzheim, bei Lemburg, Germany), respectively. VLDL-c values were calculated by the triglyceride/5 formula. Apo A-I and B were determined by turbidimetry using the Spinreact Kit (Sant Esteve d Bas, Spain). BMI was calculated as weight (kg)/height (m^2). The lipoproteins of individuals of Japanese descent were visualized by electrophoresis using the Sebia Kit (Norcross, GA, USA). These determinations were performed with Cobas Mira (Roche) and Hydrasis System (Sebia) equipment.

Statistical Analysis

Data are reported as mean ± SD. To determine significant differences between groups (p <0.05), the following statistical tests were used: one-way ANOVA followed by Tukey test when necessary and Student's t test to compare two groups. All tests were performed using SPSS-PC software version 8.0.

Results

Plasma Lipids

Table 1 reports the concentrations (mean ± SD) of TG, TC, HDL-c, LDL-c, VLDL-c in plasma and the TG/HDL, TC/HDL and LDL/HDL ratios, with TG levels (> or ≤200 mg/dL) being considered as the basic parameter for all groups evaluated. We detected 8.4% of individuals without Japanese ascendancy and 18.7% of individuals of Japanese descent with TG >200 mg/dL. The TG levels of individuals of Japanese descent (group 4) were significantly higher than those of groups 1, 2, and 3 (Table 1).

Individuals of Japanese descent with TG levels lower than 200 mg/dL (group 3) showed mean TC levels of 215 mg/dL. Considering the normal upper limit of 200 mg/dL for TC, the TC levels of these individuals were higher than

Table 1. TC, LDL-c HDL-c and VLDL-c levels and ratios of individuals of Japanese descent and controls based on TG level

	Controls		Japanese descendants	
	Group 1 (TG ≤200)	Group 2 (TG >200)	Group 3 (TG ≤200)	Group 4 (TG >200)
n (96)	88	8	78	18
Age (years)	45 ± 19	58 ± 8	45 ± 19	52 ± 13
Triglyceride (mg/dL)	144 ± 28	250 ± 34[a]	130 ± 28	399 ± 206[b]
Total cholesterol (mg/dL)	182 ± 38[d]	225 ± 37	215 ± 46	259 ± 42[c]
HDL-cholesterol (mg/dL)	49 ± 11	42 ± 6	52 ± 10	40 ± 10[c]
LDL-cholesterol (mg/dL)	109 ± 33[d]	143 ± 28	139 ± 37	154 ± 35
VLDL-cholesterol (mg/dL)	28 ± 5	50 ± 6[a]	26 ± 5	79 ± 41[b]
TG/HDL	3.1 ± 1.1	6.1 ± 1	2.6 ± 0.9	13.1 ± 7.7
TC/HDL	3.8 ± 1.2	5.3 ± 0.9	4.2 ± 1.1	6.9 ± 2.5
LDL/HDL	2.3 ± 1	3.4 ± 0.7	2.7 ± 0.8	3.9 ± 0.7

Data are expressed as mean ± SD.
[a]Significant difference between groups 1, 3, and 4.
[b]Significant difference between groups 1, 2, and 3.
[c]Significant difference between groups 1 and 3.
[d]Significant difference between groups 2, 3, and 4.
p <0.05.

normal. When the four groups were compared in terms of TC levels, Japanese descendants (group 4) were found to have significantly higher TC levels than groups 1 and 3. Individuals without Japanese ascendancy with TG below 200 mg/dL (group 1) showed significantly lower TC levels than groups 2, 3, and 4 (Table 1). For TC, there was no significant difference between groups 2 and 4.

LDL-c levels (Table 1) were significantly lower in group 1 compared to all other groups (2, 3, and 4).

We observed that individuals with high TG levels had low HDL-c levels, with these two parameters being inversely correlated. Group 4 individuals had the lowest HDL-c levels, with a significant difference compared to groups 1 and 3.

VLDL-c, a major endogenous TG carrier, is positively correlated with plasma TG levels. The mean VLDL-c levels of group 4 individuals were significantly higher than those of the other three groups.

Relation between TG and Body Mass Index (BMI)

Figure 1 shows the BMI values of two groups studied: group 3, individuals of Japanese descent with TG ≤200 mg/dL, and group 4, individuals of Japanese descent but with TG levels >200 mg/dL. BMI values differed significantly between groups (Figure 1) with group 4 individuals (TG levels >200 mg/dL) having higher BMI (BMI = 24.8 kg/m^2) than group 3 individuals (BMI = 22.3 kg/m^2).

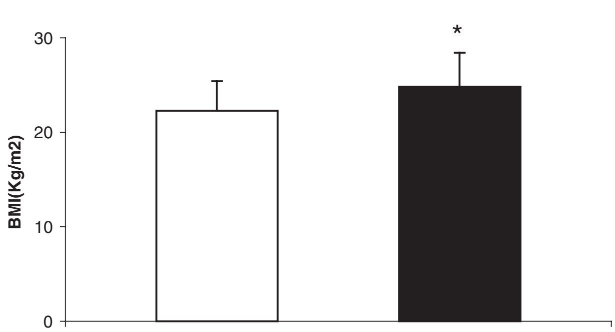

Figure 1. Comparison of body mass index (BMI) between individuals of Japanese descent according to plasma triglyceride concentration. Group 3 (Japanese descendants with TG <200 mg/dL), group 4 (Japanese descendants with TG >200 mg/dL).
*Significant difference between groups; p <0.05.

Relation between TG, apo A-I and B

Table 2 shows the apo A-I and B values of individuals of Japanese descent with TG levels equal to or lower than 200 mg/dL (group 3) or higher than 200 mg/dL (group 4). Individuals with TG values >200 mg/dL showed significantly lower plasma apo A-I levels (Table 2) than group 3. However, the levels of apo B, a major LDL-c apolipoprotein, tended to be higher in plasma of group 4 individuals, although the difference was not significant.

Relation between TG and Diet

To obtain information about the diet of individuals of Japanese descent, they were submitted to a nutritional inquiry. In this inquiry we obtained information about fiber, saturated fat and fish intake, and oil and sugar intake. As shown in

Table 2. Comparison of apolipoprotein A-I and B levels between individuals of Japanese descent with TG ≤200 and >200

	Japanese volunteers	
	TG ≤200	TG >200
Apolipoprotein A-I (mg/dL)	138 ± 19	122 ± 18*
Apolipoprotein B (mg/dL)	89 ± 22	99 ± 23
Apo B/A-I	0.6 ± 0.1	0.8 ± 0.1*

*Significant difference between groups; p <0.05.

Table 3. Comparison of fiber, saturated fat and fish (%) intake and per capita oil and sugar (mL/day) intake between individuals of Japanese descent with TG ≤200 and >200

	Japanese volunteers	
	TG ≤200	TG >200
Fiber (%)	47 ± 15	55 ± 23
Saturated fat (%)	22 ± 12	20 ± 10
Fish (%)	4.7 ± 3.2	4.9 ± 1.8
Per capita oil intake	19.7 ± 11.4	19.8 ± 9.9
Per capita sugar intake	23.8 ± 19.1	23.5 ± 18.3

Table 3, the fiber (%) intake by individuals of Japanese descent with plasma TG levels >200 mg/dL, although tending to be higher than that consumed by group 3, did not differ significantly. The other items analyzed also did not differ between groups.

Lipoprotein Electrophoresis

Lipoprotein electrophoresis was performed for all individuals of Japanese descent in order to detect possible alterations in lipoprotein bands. Two distinct electrophoretic patterns were obtained (Figure 2). Some individuals (group 4) showed alterations in both chylomicrons and VLDL-c lipoproteins while others in the same group showed alterations only in VLDL-c lipoprotein. Group 3 did not show any alteration in electrophoretic pattern.

Figure 2 shows the results of some of the individuals analyzed. Positions 1 and 2 on the gel correspond to individuals without TG level alterations, showing a normal electrophoretic pattern. At positions 3, 4, and 5, which are samples of individuals with altered TG levels (group 4), we can observe an alteration in the pre-beta band that corresponds to VLDL-c. However, positions 6 to 11, again corresponding to individuals from the same group, showed an altered band corresponding to chylomicrons (origin) and VLDL (pre-beta).

Densitometric analysis concerning this gel can be visualized in Figure 3.

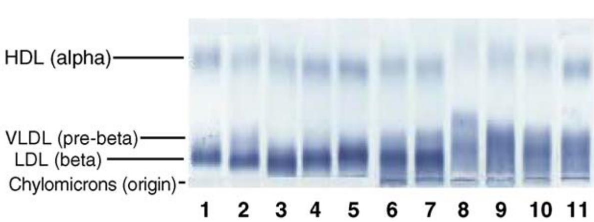

Figure 2. Lipoprotein electrophoresis in individuals of Japanese descent and controls. 1- Japanese descendant with normal triglyceride levels; 2- control individual with normal triglyceride levels; 3-11 Japanese descendants with abnormal triglyceride levels.

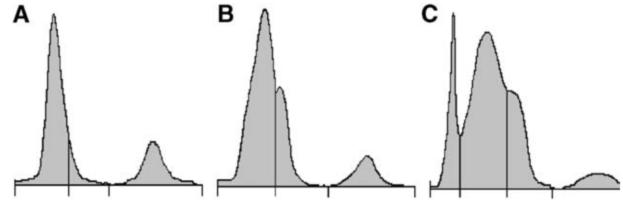

Figure 3. Electrophoretogram obtained with a densitometric scanner. The graph represents (A) normal standard (line 1 and 2 in Figure 2), (B) abnormal pre-beta band, related to VLDL lipoprotein (lines 3−5 in Figure 2), (C) abnormal origin and pre-beta band, related to chylomicrons and VLDL lipoprotein (lines 6−11 in Figure 2).

Discussion

The risk factor for IHD analyzed in this study was plasma TG concentration, which was used as a reference point for the other assays. It is well known that TG is a risk factor for heart disease. TG values accompanied by high TC and LDL-c levels, low HDL-c concentrations and insulin resistance increase the risk factors for IHD (10,12,17).

In this study we compared two groups of individuals, i.e., subjects of Japanese descent living in Brazil and subjects without this ascendancy. We divided these individuals into four groups according to plasma TG levels. Other biochemical parameters analyzed were based on these groups.

It is well known that hypertriglyceridemia is associated with a B pattern (denser) of LDL-c subfractions due to TG enrichment from VLDL (18). The group of Japanese descent with elevated TG levels showed a higher increase of LDL-c and VLDL-c levels than group 3. These data show that individuals of Japanese descent with TG levels above normality living in the south of Brazil differ from individuals of Japanese descent with normal TG levels and from group 1 and 2 individuals.

The ratios determined in biochemical assays (TG/HDL, TC/HDL and LDL/HDL) give us information about IHD. The ratios have a numerator that is positively associated with coronary artery disease and a denominator of HDL-c, which is inversely associated with coronary artery disease (19). Thus, higher ratios are produced by an increase in the numerator (TG, TC or LDL-c) or a decrease in the denominator, or both. Higher ratios represent a higher risk for IHD. Table 1 shows that group 4 individuals had higher ratios differing from those of the other groups and possibly indicating a higher risk for IHD.

BMI is used to calculate body composition, and it has been used as a measure of overweight and obesity, although it is insufficient to estimate visceral obesity (20). Jeppensen (21) stated that subjects with high TG and low HDL-c levels tend to have higher BMI, a higher prevalence of hypertension and to be less physically active. In our study, the group who had higher TG levels and lower HDL-c levels (group 4) were not overweight or obese, according to the National Health and Nutrition Examination Survey, but subjects in that group had higher BMI values than group 3 (with normal TG and HDL-c values).

Tanaguchi et al. (17) observed that non-obese Japanese with type 2 diabetes had high TG levels, low HDL-c levels and high remnant lipoprotein concentrations (RLP-c). Abbasi et al. (22) identify insulin resistance in non-diabetic subjects with higher RLP-c concentration, hypertriglyceridemia and lower HDL-c level. These subjects had BMI values between 19 and 30 kg/m^2. In contrast, Cnop et al. (23) stated that insulin resistance is associated with obesity. Our sample had normal glucose blood levels, but we did not measure insulin resistance in order to compare this data with TG and HDL-c levels as done by those authors.

We noted that individuals with plasma lipid alterations had healthy and normal dietary habits, without exaggerated fat or carbohydrate intake. Shekelle et al. (24) showed that diet is not the only influence on TC levels. According to Burchfiel et al. (25), Japanese men living in Japan eat less protein and fat and more carbohydrate than Japanese men living in Hawaii and California. We observed that individuals of Japanese descent with alterations in plasma TG concentrations (group 4) seem to consume the same diet as individuals of the same ascendancy and with normal TG levels. These data show that diet probably is not responsible for the difference in plasma TG levels in this population.

Plasma apo A-I concentration was significantly lower in group 4 than in group 3. Apo A-1 is the major HDL component, being inversely correlated with coronary artery disease.

An increase in TG levels reflects accumulation of two lipoproteins: chylomicrons and VLDL-c, and an abnormal composition in the apolipoproteins belonging to these lipoproteins (26). Lipoprotein electrophoresis showed alterations only in group 4 individuals. Two types of electrophoretic patterns were observed. One of them, alteration in the pre-beta band, refers to VLDL-c, which is responsible for endogenous TG transport to the tissues. The other alteration in the electrophoretic profile observed referred to the origin (chylomicrons) and pre-beta band.

As we can see, in individuals of Japanese descent an increase in TG levels reflects an increase of these two lipoproteins, chylomicrons and VLDL.

In conclusion, individuals of Japanese descent living in the southern area of Brazil showed a different lipid profile from those without this ascendancy. We detected differences even between individuals of Japanese descent according to their different TG levels.

Some possible causes for increased plasma TG levels can be suggested such as (1) genetic inheritance, (2) alteration in plasma apo CII concentration, which is responsible for activation of lipoprotein lipase, (3) abnormal regulation of apo CIII, which is an inhibitor of lipoprotein lipase, and (4) lipoprotein lipase deficiency, which is responsible for TG hydrolysis inside the cell. Further studies are needed to elucidate these mechanisms.

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