ORIGINAL ARTICLE

ALANINE-AMINOTRANSFERASE: AN EARLY MARKER FOR INSULIN RESISTANCE?

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Abstract   In a population-based sample, after excluding alcohol consumption, hepatotoxic drugs and hepatitis B and C infected, we investigated if alanine-aminotransferase (ALT) was associated with metabolic syndrome and insulin resistance, and if this association was caused by non-alcoholic fatty liver disease (NAFLD). The sample (432 female and 119 male) was divided into two ALT thresholds corresponding to the 50th and 75th percentiles (P) (female $\geq$ 15 and $\geq$ 19 U/L; male $\geq$ 17 and $\geq$ 23 U/l, respectively). Blood pressure, body mass index, waist circumference, cholesterol, HDL cholesterol (HDLc), triglyceride (TG), TG/HDLc ratio, glycemia and homeostasis model assessment of insulin resistance (HOMA-IR) were compared between those above and below each ALT threshold. Female placed above the 50th P of ALT had higher levels of TG/HDLc ratio (p=0.029), glycemia (p=0.028), and homeostasis model assessment of insulin resistance, (p=0.045), and above the 75th P had higher SBP (p=0.036), DBP (p=0.018), TG (p=0.024), TG/HDLc ratio (p=0.028), glycemia (p=0.004) and HOMA-IR (p=0.0014). Male placed above the 50th P of ALT had higher BMI (p=0.017) and TG/HDLc ratio (p=0.048), and above the 75th P had lower values of HDLc (p=0.042). Only 16.5% of women and 14.5% of men, above the 75th P of ALT, showed an increase in liver brightness in the echography. This work shows in woman an early association of ALT with TG/HDLc ratio and HOMA-IR. Since the last two are independent predictors of cardiovascular risk, attention should be drawn to ALT values near the upper limit of the normal range even in the absence of NAFLD and obesity.

Key words: alanine-aminotransferase, insulin resistance, metabolic syndrome

Resumen   ***Alanino-aminotransferasa: ¿un marcador temprano de resistencia a la insulina?*** En una muestra poblacional, luego de excluir a quienes consumían alcohol y drogas hepatotóxicas y a los infectados con virus B y C de la hepatitis, investigamos si la alanino-aminotransferasa (ALT), o transaminasa glutámico pirúvica (TGP), se asociaba con el síndrome metabólico y con resistencia a la insulina y si esta asociación se explicaba por enfermedad hepática grasa no alcohólica (NAFLD). La muestra (432 mujeres y 119 varones) se dividió por los percentiles (P) 50 y 75 de la distribución de ALT (mujeres $\geq$ 15 y $\geq$ 19 U/l; varones $\geq$ 17 y $\geq$ 23 U/l, respectivamente). Las mujeres a partir del P50 de ALT tuvieron valores más altos de índice triglicéridos (TG)/HDLc (p=0.029), glucemia (p=0.028) y de la valoración del modelo homeostático de insulino-resistencia (HOMA-IR) (p=0.045); a partir del P75 tuvieron valores más altos de presión arterial sistólica (PAS) (p=0.036), presión arterial diastólica (PAD) (p=0.018), TG (p=0.024), índice TG/HDLc (p=0.028), glucemia (p=0.004) y HOMA-IR (p=0.001). Los varones a partir del P50 de ALT tuvieron valores más altos del índice de masa corporal (p=0.017) y del índice (TG/HDLc (p=0.048); a partir del P75 mostraron valores más bajos de HDLc (p=0,042). Sólo 16.5% de las mujeres y 14.5% de los varones, a partir del P75 de ALT, mostraron aumento del brillo hepático en la ecografía. Este trabajo muestra, en mujeres, asociación temprana de ALT con el índice TG/HDLc y el HOMA-IR. Dado que estos dos últimos son predictores independientes del riesgo cardiovascular se debería prestar atención a los valores de ALT cercanos al límite superior aun en ausencia de NAFLD y de obesidad.

Palabras clave: alanino-aminotransferasa, resistencia a la insulina, síndrome metabólico

Non-alcoholic fatty liver disease (NAFLD) is clearly associated with the metabolic syndrome and with insulin resistance[1-3]. However, the scope of the liver disease in metabolic syndrome could exceed these morphological alterations. In this sense it has been observed that γ-glu-

tamyltransferase (GGT) is associated with arterial hypertension and its incidence[4], metabolic syndrome components and development of diabetes type 2[5-9]. While GGT is a sensitive but unspecific marker of liver injury since it also increases by oxidative stress and alcohol intake[10, 11], alanine aminotransferase (ALT) is the most specific marker of liver disease in clinical practice. Recently, high levels of ALT have been reported associated with obesity, hyperglycemia and the development of both diabetes and metabolic syndrome[5, 9, 12-17].

The explanation for this relationship between hepatic enzymes and metabolic syndrome has been centered in the existence of non-alcoholic fatty liver disease (NAFLD), and insulin resistance has been proposed as a link between metabolic syndrome and liver steatosis[18-21].

HOMA-IR (homeostasis model assessment insulin resistance) has been utilized and validated in population-based samples as an indicator of insulin resistance[22]. Recently Reaven[23] has also emphasized the usefulness of triglyceride (TG)/HDLc ratio as an insulin resistance indicator.

For the diagnosis of liver steatosis, echography is a non-invasive, reasonably sensitive and specific procedure[2, 24, 25]. It is based on the detection of an increase in liver brightness in comparison with that of spleen and kidney, and it has been estimated that 96% of the asymptomatic non-drinkers with altered hepatic enzymes and bright liver have NAFLD[26].

The aim of the present work was to determine in a population sample if ALT was associated with components of the metabolic syndrome and insulin resistance and if this association is explained by NAFLD.

Materials and Methods

A cross-sectional study was performed in a population-based sample of the city of Rauch. The universe was the inhabitants of Rauch between 15 and 75 years of age. This city lies in the centre-Southeast region of the province of Buenos Aires, 36° 45' 00'' south latitude and 59° 04' 00'' west longitude. It is 270 km far from Buenos Aires city. According to the 1991 National Census, there were 13 909 inhabitants in Rauch, 8 246 between 15 and 75 years of age (4 166 men and 4 080 women). No abnormalities had been shown since 1991 to assume changes in the population. In 1997, randomly chosen blocks were considered as units for the sampling. 1 526 inhabitants constituted the initial sample; their composition was previously described[27]. The initial survey was performed on subjects living in the randomly chosen blocks. Since the socio-economic features and the number of inhabitants were similar, a proportional probability was not taken into consideration. In that opportunity, we found a high prevalence of hypertension (43.20% in men and 28.50% in women) and obesity-overweight (54.81% in men and 44.65% in women). Smoking was present in 34.61% of men (14.80 ± 0.74 cigarettes per day) and in 20.83% of women (12.63 ± 0.74 cigarettes per day). Average alcohol intake was 163.02 ± 10.24 and 25.32 ± 2.42 g per week for men and women, respectively. A 1.2% of the population was illiterate while the percentages with a level of education of incomplete primary, complete primary, incomplete secondary, complete secondary, tertiary or incomplete university and complete university level were 16.5%, 37.4%, 17.5%, 9.4%, 5.2% and 12.7%, respectively. Other characteristics were previously published[27]. After six years (2003) of the initial survey on prevalence, a cohort study was performed as a second step[28]. 855 women and 452 men were re-interviewed (n=1307) by previously trained nurses from Rauch Hospital. Some subjects were not re-interviewed: 71 had died (4.65%) and 148 (9.70%) were not found due to several causes (moved to another city, out of home). Systolic blood pressure (SBP) and diastolic blood pressure (DBP), weight, height and waist circumference (WC) were measured in the domicile of each re-interviewed subject; and personal information and al-

cohol and drugs intakes were recorded. Alcohol intake was registered by specifically asking about the consumption of wine, beer, distilled beverages, and aperitifs and other beverages. In order to calculate alcohol intake in grams per week (g/week), weekly intake reported in centilitres was multiplied by 0.12, 0.05, 0.40 and 0.18 in the case of wine, beer, distilled beverages, and aperitifs and other beverages, respectively.

Blood pressure was measured on the right arm relaxed and at the heart level using a mercury-scale sphygmomanometer after five minutes of rest. SBP was the reading coinciding with the first arterial sound and DBP with the last one. This procedure was repeated three times and SBP and DBP were defined as the means of the three measurements. Weight was registered with light clothes using a personal scale calibrated before each measurement. Height was determined barefoot and with a metal tape measure. WC was measured with a metal tape measure over the iliac crest parallel to the floor and with a relaxed abdomen. Blood extractions were performed, to whom attended voluntarily, with no less than eight hour fasting, in Rauch Hospital. Samples were processed to obtain serum for determinations of glycemia, ALT, cholesterol, HDL cholesterol (HDLc), TG and the remaining serum was put in the fridge and then frozen within twelve hours at -18 °C for determi-nations of insulinemia and serology for hepatitis B and C.

ALT (GPT UV AA, Wiener Lab) (normal rank: 6-40 U/l for men and 5-31 U/l for women) (laboratory variability < 10%), glycemia, cholesterolemia, HDLc and TG, were determined (colorimetric method) using an autoanalyzer Technicon RA 1000. Insulinemia was measured by radio-immunometric method. Antibody against hepatitis C virus (anti-HCV) and antibody anti-core of hepatitis B virus (anti-HBc) were determined by enzyme-immunoassay, while surface antigen of hepatitis B virus (anti HBS) was determined by ELISA. Body mass index (BMI) (weight/height^2) and HOMA-IR (insulinemia (µUml) x [glycemia (mg/dl)/18]/22.5)[19] were calculated. Any amount of alcohol intake (> 0.00 g/week), drug intake with acknowledged hepatotoxicity[29], presence of anti-HCV and of anti-HBc with negative anti-HBs were the exclusion criteria.

A bidimensional liver echography was performed with equipment Sonoace 6 000 c digital color and multifrequence convex transductor, and liver fat was defined by two independent observers, comparing liver brightness with that of kidney and spleen. An interobserver variation of < 10% was accepted.

Two ALT thresholds were separately defined in men and women, corresponding to the 50th and 75th percentiles (P) of ALT distribution. BP, BMI, WC, cholesterol, HDLc, TG, TG/HDLc ratio, glycemia and HOMA-IR were compared between those above and below each ALT threshold. The percentage of subjects with increased liver brightness was compared among quartiles of ALT.

Statistics: continuous variables were expressed as mean ± SE and were compared with t test for independent samples. Discrete variables were expressed as percentage and compared using chi^2 test. P value < 0.05 was considered significant. Data were processed with the statistical program SPSS 11.0.

Results

394 alcohol-consuming subjects, 13 subjects consuming drugs with acknowledged hepatotoxicity and 5 subjects with anti-HCV were excluded. Subjects with anti-HBc had anti-HBs positive and consequently were not excluded. The definitive sample was constituted by 895 subjects (690 women and 205 men); 551 attended voluntarily to have blood extractions (432 women and 119 men). There were no significant differences in age, BP,

BMI and WC among those having blood extractions and those not having them (Table 1). The average value of ALT was 17.28 U/l (CI 95% 16.42-18.14) in women and 20.78 U/L (CI 95% 18.64-22.92) in men.

ALT thresholds for the 50th P and 75th P were ≥ 15 and ≥ 19 U/l in women and ≥ 17 and ≥ 23 U/l in men, respectively.

The age of women with ALT ≥ 15 (n=245) and with ALT < 15 U/l (n=187) was 52.48 ± 0.98 and 51.05 ± 1.21 years, respectively (p=0.353); while for those with ALT ≥ 19 (n=109) and < 19 U/l (n=323) it was 53.92 ± 1.38 and 51.17 ± 0.91 years, respectively (p=0.097). The age of men with ALT ≥ 17 (n=67) and with ALT < 17 U/l (n=52) was 51.13 ± 1.94 and 51.75 ± 2.29 years, respectively (p=0.837); while for those with ALT ≥ 23 (n=30) and < 23 U/l (n=89) it was 46.30 ± 2.73 and 53.12 ± 1.71 years, respectively (p=0.044).

Women above the 50th P of ALT (Table 2) had higher TG/HDLc ratio (p=0.029), glycemia (p=0.028), and HOMA-IR (p=0.045). Above the 75th P of ALT, they had higher SBP (p=0.036), DBP (p=0.018), TG (p=0.024), TG/HDLc ratio (p=0.028), glycemia (p=0.004) and HOMA-IR (p=0.001). Men above the 50th P of ALT (Table 3) had higher BMI (p=0.017) and TG/HDLc ratio (p=0.048). Above the 75th P of ALT, they had lower HDLc (p=0.042).

There were no significant differences in the percentage of subjects with an increase in liver brightness among ALT quartiles. Above the 75th P of ALT, 16.5% of women and 14.5% of men had an increase in liver brightness (Table 4).

Discussion

It has been observed that GGT is associated with arterial hypertension and its incidence[4], metabolic syndrome components and development of diabetes type 2[5-9]. While GGT is a sensitive but unspecific marker of liver injury since it also increases by oxidative stress and alcohol intake[10, 11], ALT is the most specific marker of liver disease in clinical practice.

This work shows that women in upper levels of the ALT distribution, in which viral hepatitis and alcohol and hepatotoxic drugs intake have been excluded, have higher levels of components of metabolic syndrome and insulin resistance. This association was early evidenced (above the 50th P of ALT) with indicators of dislipidemia (TG/HDLc ratio) and disglycemia (glycemia, HOMA-IR) and the association with BP became evident only above the 75th P of ALT distribution. In men, the scarce number of non-drinkers and the misbalance in ages limit the analysis above the 75th P. However, above 50th P of ALT, TG/HDLc ratio and BMI were higher.

Likewise other epidemiological studies that evaluate the association of ALT with the development of diabetes and with the components of the metabolic syndrome, such as the ones carried out on the Pima Indians[12], the West of Scotland Coronary Prevention Study[13] and the Insulin Resistance Atherosclerosis Study[15, 17], this work is based in only one determination of ALT.

The mechanisms of the association of ALT with metabolic syndrome and insulin resistance are unclear. NAFLD is clearly associated with metabolic syndrome[1, 2] and insulin resistance evaluated by HOMA-IR[21]. However, only a minority above the 75th P of ALT showed an increase of liver brightness in the echography. This could be caused by the fact that ALT was more sensitive than the echography to detect early fat deposits in the liver or by the existence of other mechanisms to account for the association of ALT to metabolic syndrome and insulin resistance. It has been postulated that inflammation[30]

TABLE 1.– *Features of subjects who had blood extractions and who had not*

| | | Blood extraction | | No blood extraction | | |
		Mean	SE	Mean	SE	p
Women	Age (years)	51.86	0.76	49.40	1.14	0.073
	SBP (mm Hg)	130.77	0.87	130.05	1.28	0.630
	DBP (mm Hg)	81.13	0.54	79.58	0.80	0.096
	BMI (Kg/m²)	25.72	0.24	25.36	0.56	0.501
	WC (cm)	92.63	0.67	91.40	0.78	0.248
Men	Age (years)	51.40	1.48	48.55	1.79	0.217
	SBP (mm Hg)	136.34	1.86	133.91	2.23	0.402
	DBP (mm Hg)	84.83	1.16	84.40	1.54	0.821
	BMI (Kg/m²)	26.72	0.45	25.98	0.47	0.261
	WC (cm)	96.70	1.15	96.64	1.95	0.975

SBP: systolic blood pressure
DBP: diastolic blood pressure
BMI: body mass index
WC: waist circumference.

TABLE 2.– *Indicators of metabolic syndrome and insulin resistance in women according to the 50th and 75th percentiles of ALT**

	50th Percentile of ALT				75th Percentile of ALT			
	ALT U/l	Mean	SE	p	ALT U/l	Mean	SE	p
SBP (mm Hg)	≥ 15	131.49	1.13		≥ 19	133.93	1.61	
	< 15	129.83	1.38	0.347	< 19	129.71	1.03	0.036
DBP (mm Hg)	≥ 15	81.52	0.67		≥ 19	83.30	1.12	
	< 15	80.62	0.87	0.406	< 19	80.40	0.60	0.018
BMI (Kg/m^2)	≥ 15	25.54	0.32		≥ 19	26.57	0.52	
	< 15	25.94	0.36	0.412	< 19	25.43	0.27	0.054
WC (cm)	≥ 15	91.97	0.74		≥ 19	94.54	1.20	
	< 15	93.49	1.21	0.260	< 19	91.98	0.79	0.096
Cholesterol (mg/dl)	≥ 15	232.96	3.40		≥ 19	229.46	4.77	
	< 15	228.11	4.28	0.369	< 19	231.25	3.20	0.772
HDLc (mg/dl)	≥ 15	61.13	0.82		≥ 19	60.52	1.28	
	< 15	62.93	3.03	0.533	< 19	62.39	1.84	0.575
TG (mg/dl)	≥ 15	141.07	5.68		≥ 19	153.72	9.64	
	< 15	127.49	7.59	0.153	< 19	128.77	5.22	0.024
TG/HDLc ratio	≥ 15	2.33	0.10		≥ 19	2.50	0.17	
	< 15	2.03	0.09	0.029	< 19	2.09	0.07	0.028
Glycemia (mg/dl)	≥ 15	92.45	1.49		≥ 19	97.70	3.10	
	< 15	88.44	1.04	0.028	< 19	88.37	0.71	0.004
HOMA-IR	≥ 15	1.93	0.10		≥ 19	2.30	0.18	
	< 15	1.69	0.07	0.045	< 19	1.67	0.06	0.001

*50th and 75th percentiles of ALT (alanine-aminotransferase) correspond to thresholds of 15 and 19 U/l, respectively.
SBP: systolic blood pressure
DBP: diastolic blood pressure
BMI: body mass index
WC: waist circumference
TG: triglycerides
HOMA-IR: homeostasis model assessment insulin resistance

could be the mechanism for explaining the elevation of hepatic enzymes in subjects with metabolic syndrome, but there has not been a consistent association between ALT and inflammation markers in population studies[9]. It is very important to point out that ALT levels in our study are within normal limits or slightly elevated. It is possible that the top extreme of the population´ALT distribution expresses a functional disorder, with an increase of hepatocyte turnover, more than a hepatic cytolysis. In animal models the decrease in the production of "hepatic insulin sensitizing substance" (HISS) produces insulin resistance[31] suggesting a bidirectional pathway between metabolic syndrome and hepatic injury.

Recently, in a consensus meeting of the International Diabetes Federation, it was proposed that central obesity is a necessary component for the diagnosis of metabolic syndrome[32]. However, Reaven[23, 33] sustains that an elevated TG/HDLc ratio indicates early metabolic syndrome and insulin resistance in subjects without obesity. In the case of women, our study showed that TG/HDLc ratio and HOMA-IR were higher in those with higher ALT despite no differences in BMI or WC. This is consistent

with the findings in elderly Caucasian men from the British Regional Heart Study, in which the association between hepatic enzymes with HOMA-IR, TG and HDLc remained significant after adjusting by BMI[9]. Similarly, in a multivariable regression analysis in the Mexico City Diabetes Study, ALT showed a significant correlation with TG and fasting insulinemia and no correlation with BMI or WC[5]. Likewise, in the Insulin Resistance Atherosclerosis Study, the upper quartile of ALT distribution predicted the development of diabetes mellitus and metabolic syndrome, independent of obesity markers[15, 17].

Although there exist other causes of hepatic damage that were not investigated, their frequency is too low to modify the results of this study. Among them it is worth mentioning the Wilson's disease, with a prevalence in most populations of one in 30 000[34], and homozygous genetic hemochromatosis wich prevalence ranges from 0.2% to 0.4% in general populations from Australia[35], Europe[36] and North America[37].

As HOMA-IR[38, 39] and TG/HDLc ratio[40-42] are independent predictors of cardiovascular risk, attention should be paid to ALT levels near the upper limit of the normal range

TABLE 3.- *Indicators of metabolic syndrome and insulin resistance in men according to the 50th and 75th percentiles of ALT**

	50th Percentile of ALT				75th Percentile of ALT			
	ALT U/l	Mean	SE	p	ALT U/l	Mean	SE	p
SBP (mm Hg)	≥ 17	137.75	2.66		≥ 23	138.51	3.76	
	< 17	134.52	2.51	0.390	< 23	135.61	2.14	0.499
DBP (mm Hg)	≥ 17	86.32	1.55		≥ 23	87.86	2.26	
	< 17	82.91	1.72	0.145	< 23	83.81	1.34	0.130
IMC (Kg/m^2)	≥ 17	27.64	0.62		≥ 23	27.66	0.71	
	< 17	25.55	0.60	0.017	< 23	26.40	0.54	0.219
Waist (cm)	≥ 17	98.40	1.64		≥ 23	99.00	1.89	
	< 17	94.55	1.52	0.088	< 23	95.92	1.39	0.243
Cholesterol (mg/dl)	≥ 17	224.11	5.82		≥ 23	227.52	8.93	
	< 17	229.65	5.47	0.499	< 23	226.19	4.51	0.887
HDLc (mg/dl)	≥ 17	51.42	1.28		≥ 23	49.62	1.62	
	< 17	54.70	2.01	0.153	< 23	54.08	1.42	0.042
TG (mg/dl)	≥ 17	178.90	16.90		≥ 23	160.10	15.36	
	< 17	162.67	17.29	0.509	< 23	175.89	15.47	0.571
TG/HDLc ratio	≥ 17	3.34	0.29		≥ 23	3.43	0.36	
	< 17	2.60	0.19	0.048	< 23	2.87	0.21	0.180
Glycemia (mg/dl)	≥ 17	94.75	2.57		≥ 23	90.67	2.32	
	< 17	101.04	5.04	0.238	< 23	99.80	3.41	0.134
HOMA-IR	≥ 17	1.99	0.17		≥ 23	2.03	0.28	
	< 17	2.12	0.32	0.703	< 23	2.05	0.20	0.943

*50th and 75th percentiles of ALT (alanine-aminotransferase) correspond to thresholds of 17 and 23 U/l
SBP: systolic blood pressure
DBP: diastolic blood pressure
BMI: body mass index
WC: waist circumference
TG: triglycerides

TABLE 4.– *Percentage of subjects with an increase in liver brightness in echography among ALT quartiles*

	Quartiles of ALT				
	1	2	3	4	p*
Women n = 394	9.2	9.5	6.9	16.5	0.170
Men n = 119	7.1	12.9	19.2	14.3	0.627

*p between quartiles of ALT

even when they are not associated with obesity or echographic data of NAFLD. On the other hand, it would be of unquestionable interest to identify subjects with insulin resistance through simple and widely available clinical data. A recent work[43] indicates that a TG/HDLc ratio higher than 3 and a TG level higher than 130 mg/dL are adequate for that purpose and similar to the ATPIII definition in sensitivity and specificity. More research is necessary in order to elucidate whether ALT can contribute in this aspect.

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