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Chagas´disease, BMI, ACEI/D polymorphism interactions and some considerations on the "Obesity Paradox"

Sadí Cossy Isasi[1], Julio César Cosiansi[2], Susana Keim[2], Guillermo Giordano[1] and Juan Carlos Muiño[2]

[1]Cátedra de Bioquímica y Biología Molecular, [2]Cátedra de Medicina II, Medicina, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina

Abstract: *Chagas´ disease (ChD), a parasitic disease widely distributed in Argentina, courses mainly with myocardiopathy due to parasympathetic denervation evolving to right hypertrophy, dilatation, or left insufficiency and dilatation. Chagas´ disease comorbidities include hypertension, cardiac remodeling and obesity. The D allele of angiotensin-converting enzyme (ACE I) gene is associated with similar cardio-metabolic imbalances. Though obesity is a risk factor of disease, some studies, have suggested that reduces mortality due to infectious diseases. We discuss whether ChD high BMI may be attributed merely to parasitic damage or to a hypothetic interaction between parasite and ACE I/D polymorphism. DNA was obtained from leukocytes of 79 patients including control and infected individuals of closed and open communities to detect ACE I/D polymorphism, the rest of the sample underwent ELISA for Chagas´disease and routines for lipids and glucose. Chagas´disease strongly associated with D allele, ≥30 BMI, high LDL cholesterol, creatinine and triglycerides but lower glucose. ≥30 BMI was evenly distributed among DD, I/D and II genotypes. No II patient was infected. ≥30 BMI was not determined by ACE genotype. Since evolution was benign except for one morbid obese and two low BMI patients, overweight, not obesity seems to be protective*.

Keywords:*ACE I/D polymorphism, BMI, Chagas´ disease.*

I. Introduction

Chagas´ disease is an illness widely distributed in our country. The predominant form of Argentina presents a compromise of the myocardium with parasympathetic denervation, lesion of the conduction system, blockage of atrioventricular and of right and left branches which can evolve to right ventricle (RV) changes, including hypertrophy, dilatation[1], and dilated cardiomyopathy [2]. RV from ARVC was characterized by decreased PPARα pathway and a dramatic activation of the PPARγ pathway with altered mRNAs and proteins [3]. These alterations are usually treated with angiotensin II blockers or angiotensin converting enzyme 1 inhibitors (ACEi) which have additional therapeutic benefits beyond reduction in arterial blood pressure, including oxidative stress–lowering properties or effect on PPAR pathways [4]. The ACE gene presents an insertion/deletion polymorphism (I/D), the D (deletion)allele leads to the expression of a protein with greater activity, which turns into higher levels of angiotensin II and has been associated with hypertension, cardiovascular accidents, myocardial infarction, overweight, adiposity and obesity[5-9].Chagas Disease has also been associated with diabetes mellitus and thyroid disorders, overweight, adiposity and obesity which has led to the recommendation to monitor these comorbiditiesin Chagasic patients under treatment. The clinical-nutritional profile of individuals with chronic Chagas´ disease evaluated at the São Paulo State University, Brazil, revealed that 94%

of patients with Chagas disease were overweight or obese [10]. At the onset of obesity adipose tissue cell types, comprising adipocytes, fibroblasts, endothelial and smooth muscle cells, become enriched with macrophages and leukocytes and increase local angiotensin II a process called sterile inflammation [11, 12]. In experimental Chagas inflammation, macrophages were recruited into adipose tissue due to apoptosis/necrosis, tissue hypoxia and, lipolysis mediated cell damage/death [13]. Adipose tissue has also endocrine functions secreting adipsin, adiponectin [14], leptin [15], resistin[16], SAA3 [17], omentin [18], visfatin[19] and RBP4 [20]. Adiponectin and resistin, have opposing effects on whole-body glucose homeostasis [21, 22]. Adiponectin exerts insulin-sensitizing effectsin part, through the inhibition of hepatic glucose output. Lower levels of circulating adiponectin are associated with metabolic syndrome, diabetes, hypertension, obesity and an increase in the expression of endothelin-1 [23].Adiponectin levels are reduced under angiotensin II signaling[12; 24]. Adiponectin nRNA and protein levels are decreased during experimental infection with T. cruzi [25]. From the above we suspect that there is a mechanistic link between Chagas´ disease and the presence of ACE D allele comorbidities resulting in diabetes, unpaired lipid metabolism and overweight/obesity. Obesity is a rapidly growing public health problem, associated with an increased risk of poor outcome from many diseases but some studies, however, have suggested reduced mortality in obese individuals from a variety of diseases, a phenomenon known as the "obesity survival paradox" [26]. In this regard, Beleigoli AM et al, 2012[27], found that high BMI levels are associated with higher survival regardless of HD status in an elderly population with a high prevalence of Chagas disease, and Singanayagam A et al, 2012, [28] found that obesity was associated with reduced 30-day mortality in patients hospitalized with CAP (community acquired pneumonia). Brima W. et al, 2015,[29] reported lower mortalities due to T. cruzi experimental infection in obese mice compared to normal ones. These observations pose antagonistic interpretations when considering genetic background, obesity and T. cruzi infection. On one side the unfavorable comorbidities of obesity could determine a bad prognostic for Chagas disease patients and on the other side obesity could be the frame for a stronger inflammatory response with more energy resources to sustain a prolonged battle against the parasite.

In this paper our contribution consists on some cases of Chagas' disease patients from a close and open communities, considering the individual course of the disease and discussing whether the clinical manifestations may be attributed to the presence of the parasite solely or to a specific yet not well understood interaction between the parasite and ACE I/D polymorphism associated metabolic alterations.

II. Methodology

2.1Subjects of study: patients with heart diseases of non-Chagasic etiology, patients with chagasic heart disease, patients with eczema, bronchial asthma or parasitic diseases and healthy individuals from the localities of Rosario del Saladillo and Argüello neighborhood, city of Córdoba.

2.2 Inclusion and selection criteria: Participants of both sexes, from 21 to 100 years of age, who wished to participate in the study. Only those patients who did not wish to participate in the study were excluded.

2.3 Recruitment of subjects or estimation of the institutional origin of the patients*:* The patients attending the medical examination within the framework of the Campaign for the Detection of Prevalent Diseases (University Extension Work) of the cities of Rosario del Saladillo and of the city of Córdoba, Argüello neighborhood, were informed of the study and asked for consent to be included. These steps were carried out in the presence of a witness. The personal history of each patient was received. A clinical history and complete physical examination in addition to anthropometric measurements, and family tree that includes at least the last three generations taking into account the age of the members and the ethnic group to which they belong were made. Each patient was assigned a code number so that the data obtained by the biochemical or genetic procedures and the identity of the patient can only be linked by the principal investigator. The rest of the researchers or technical personnel participating in the project can only link the data with the code number without being able to identify which identity corresponds

2.4 Monitoring of patients. Patients participating in this study were monitored twice, at the beginning and six month later by clinical and laboratory routines described below.

2.4.1 Clinical and biochemical exam

2.4.1.1Clinical diagnosis: The symptoms and signs of physical examination in Chagas heart disease are those of cardiomyopathies in general and none of them is pathognomonic of this disease. Heart murmurs of valvular dysfunction may appear as a result of dilation of the cavities. In more advanced stages, the usual signs of congestion and peripheral hypoperfusion are detected. Cardiac cachexia is also a manifestation of more advanced stages of Chagas heart disease and has an important prognostic value. The clinical examination included electrocardiogram and arterial tension control.

2.4.1.2 Techniques and information collection instruments

2.4.1.2.1 Specimen collection: Peripheral blood (10 cc) was extracted by intravenous puncture in the ulnar vein of the right arm after antisepsis with alcohol 96 degrees. The sample was placed in two tubes with a) 5 cc. with EDTA anticoagulant, to obtain genetic material to perform molecular techniques b) 5 cc. with anticoagulant, to control blood glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, creatinine level s and control of serology for Chagas.

2.4.1.2.2 Obtaining DNA: The DNA will be extracted through the use of conventional techniques [3].

2.4.1.2.3 Study of mutations: Genomic DNA will be extracted from blood leukocytes, and in ACE gene, intron 16 of the gene located in 17q23 will be amplified by PCR. Two fragments of 497 and 190 bp, normal and deletion will be obtained. The frequency of each genotype in each sample lot will be analyzed and the association between Chagas´ disease and each polymorphism was determined. Mutations were detected by the PCR technique using specific primers that surround the area of the genes where the mutations are located obtaining DNA fragments of different sizes. Then they were separated in agarose gels to 1.2 - 4% or acrylamide and stained with ethidium bromide to be finally observed in a UV transilluminator.

***2.5Data analysis*:**

For genetic studies, the frequency of the different genotypes (II-I/D-DD) was obtained for each polymorphism in the population. The frequency data of each polymorphism was analyzed in Case-Control Contingency Tables for the estimation of ODDS ratio, in which the polymorphisms are the exposure factor. In addition, measures of central tendency and dispersion, and graphs for the variables of interest were used and all the crossings of variables that could arise after obtaining the data were carried out.

III. Results

3.1

In the sample studied (n = 79) the genotypes conserved the Hardy-Weinberg equilibrium. In both samples, distribution of BMI categories amongst the DD, I/D, II genotypes, was statistically significant when polymorphism was aleatory factor. In patients from Rosario del Saladillo BMI≥30 was prevalent in DD genotype, whilst in the community of Argüello neighborhood, BMI ≥30 prevailed in I/D genotype and to a lesser extent in II genotype. Serum total cholesterol, triacylglycerides could be grouped according to genotype with the same trend as BMI in each sample. Blood glucose presented similar values in both groups and same trend with the highest values for II genotype, intermediate for DD individuals and the lowest for heterozygotes.

When Chagas ´disease was considered, we found in Rosario del Saladillo positive significant correlation ($p < 0.04$) for Chagas´ Disease ELISA test positive result (n = 19) with serum creatinine, total cholesterol, triacylglycerides and ≥30 BMI ($p<0.04$). On the other hand, HDL cholesterol negatively correlated with positive Chagas diagnostic while LDL cholesterol showed positive correlation (Fig. 1a). Average blood glucose was lower in Chagas´ disease patients, but differences were not significant (Fig. 1b).

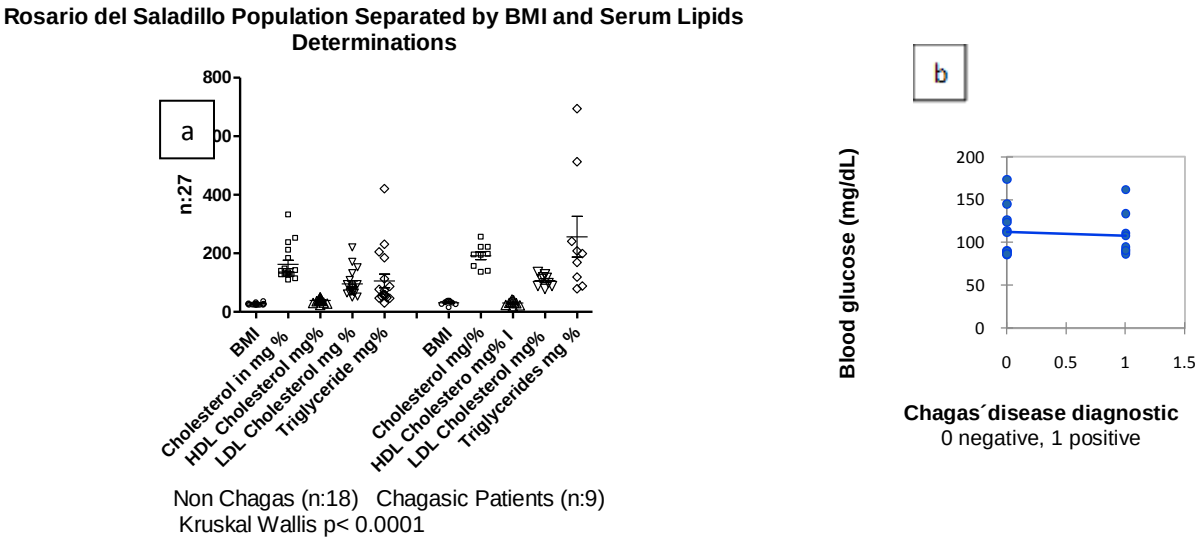
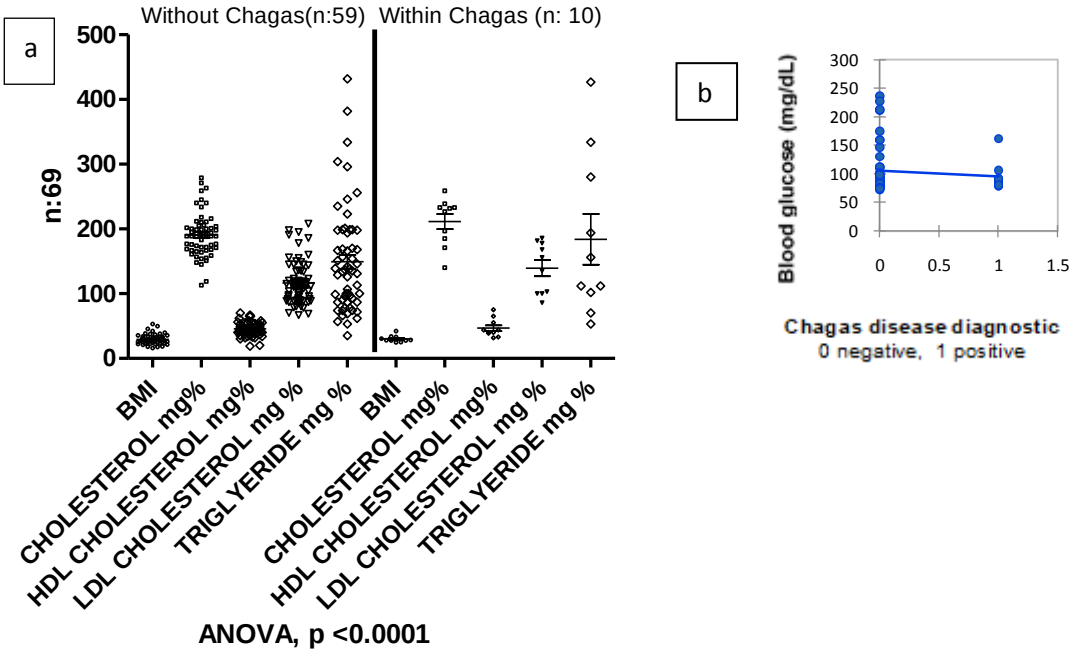


Fig. 1 Lipids and glucose determinations in Rosario del Saladillo population. a) Lipids, b) glucose

In the community of Argüello neighborhood, statistical analysis presented similar trends but, in these patients, ≥ 30 BMI association with Chagas´ disease was not statistically significant considered independently (Fig. 2).



. Fig 2: Lipids and glucose determinations in Argüello: a) lipids b) glucose, separated by Chagas´ positive and negative disease.

3.2

When both groups were fused and analyzed, discriminant analysis revealed two well defined categories, Chagas´s disease positive or negative patients, the former with lower blood glucose and higher BMI, cholesterol, triglyceride and creatinine levels ($p< 0.03$- χ^2, Table I and II).

Table I: mean values of BMI and the biochemical variables (mg/dl) after discriminant test.

Class \ Variable	B.M.I.	GLUCOSE	CREATININE	CHOLESTEROL	TRIGLYCERIDES
NEG	33,578	106,650	0,556	188,183	139,183
POS	**37,974**	**101,316**	**0,665**	**199,789**	**211,421**

Table II: χ^2 test for the discriminant test results of Table I

-2Log(M)	41,969
χ^2 (Observedvalue)	37,529
χ^2 (Criticalvalue)	24,996
FD	15
p-value	**0,001**
alpha	**0,05**

Evolution of patients shown in Fig. 3 was measured as the difference between initial and second recorded values after six-month period (ECG, ATH, BMI). It can be appreciated that hyperlipidemia appears to protect against bad evolution. A similar paradox occurred with ATH which shows that hypertensive patients improve to a normal tension while those with better indicator evolve to hypotension.

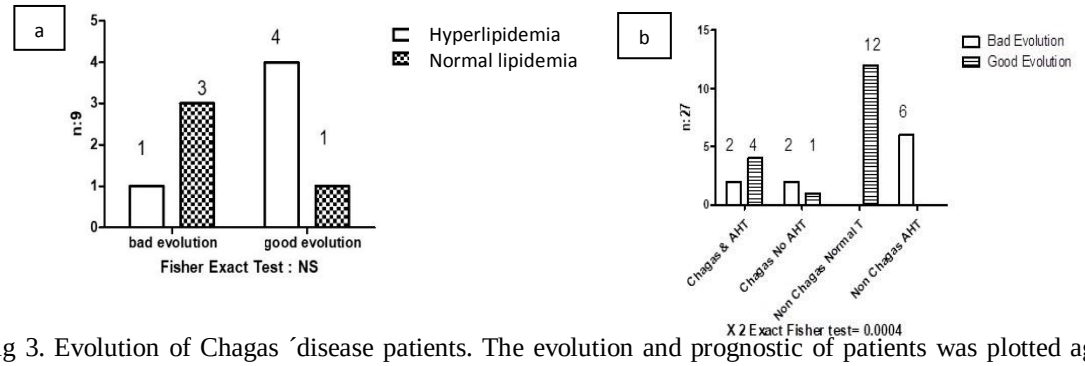


Fig 3. Evolution of Chagas ´disease patients. The evolution and prognostic of patients was plotted against: a) blood lipidic profile and b) the presence or absence of ATH.

3.3

The introduction of genotype as grouping factor against Chagas's disease diagnostic showed that in Rosariodel Saladillo, positive individuals were of the DD genotype almost exclusively ($p<10^{-4}$) but in Argüello neighborhood, positive cases included heterozygotes, thus the association was with the presence of D allele, not DD genotype. No patients with II genotype and positive diagnostic were found (Fig. 4).

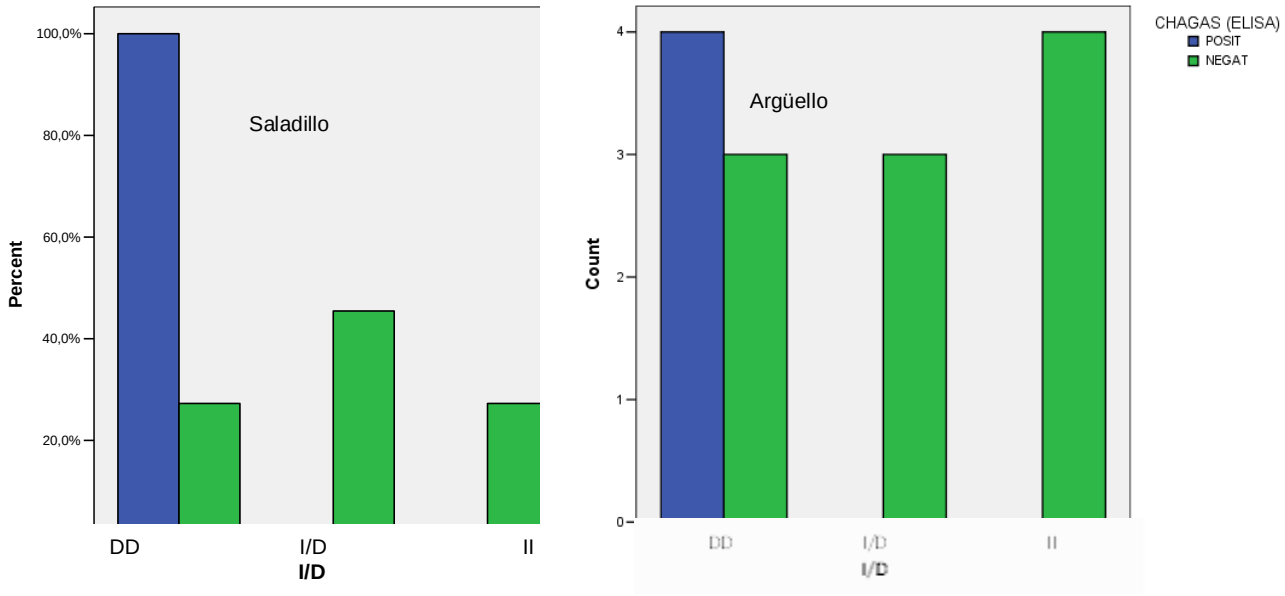


Fig 4. Proportion of positive and negative diagnostic for Chagas 'disease in Rosario del Saladillo and Argüello neighborhood populations. p= 0.00001

IV. DISCUSSION

4.1

Chagas 'disease is a parasitic disease caused by the unicellular parasite Trypanosoma cruzi which invades striated muscle originating the cardiac form characterized by arrythmia, cardiomegaly, cardiac insufficiency, or smooth muscle originating the digestive form with mega esophagus and megacolon. Important inflammatory and neurological disorders explain each form of the disease. Other clinical signals that are present in Chagas ´disease patients belong to a category yet undefined to be just comorbidities or misdiagnosed clinical manifestations of the evolution of the disease. High BMI and the biochemical characteristics of type 2 diabetes mellitus and metabolic syndrome are widely distributed among patients with Chagas 'disease. The molecular mechanisms that underlie these comorbidities may be related not only to immunological disorders but to the presence of genetic variants like the I/D polymorphism of the ACE gene. This gene has been associated with cardiac hypertrophy, left ventricle remodeling, is antagonized by adiponectin and promotes TGFβ1 secretion.

A natural question rises and is whether metabolic alterations are always the result of the genetic background of the patient, that would be triggered by different stimuli like Trypanosoma cruzi infection, or emerge as a natural defense mechanism against the infection or an undesired side effect independently of the genotype of the patient.

In this work we try to discuss this point and present preliminary results of a project focused on the question.

4.2

The results were obtained from two populations with important bio-social differences. Rosario del Saladillo is a small isolated rural town with homogeneous genetic background, an undetermined degree of endogamy and located in an endemic area for Chagas 'disease. Argüello neighborhood is part of the second city of Argentina, a cosmopolitan city, and numerous individuals come from different parts of the country and neighbor countries like Bolivia, Paraguay and Peru, so more variability was observed, regarding genotype and biochemical or clinical characteristics. Patients from this location with DD genotype presented lowest BMI, in contrast to patients from Rosario del Saladillo whose DD genotype accounted exclusively for ≥ 30 and 30-25 BMI. Argüello II patients presented the higher average BMI while those of Rosario del Saladillo presented exclusively <25 BMI. Interestingly two common features were, (a), blood glucose levels were lower in the heterozygotes, with no correlation with BMI nor with the other biochemical variables measured,(b), that no II patient was positive for Chagas ´disease diagnostic.

4.3

Considering both samples independently two interpretations of the data seem to be equally acceptable. Data from Rosario del Saladillo show a clear correlation between highest BMI DD genotype and positive diagnostic for Chagas ´disease. Argüello neighborhood data are more in the line with studies that report BMI, blood glucose and other characteristics of metabolic syndrome to have sources of variation other than DD genotype or positive diagnostic for Chagas 'disease. We want to call attention to this point because if both populations are statistically treated without stratification -as if the samples were obtained from a hypothetical geographic location with average characteristics amongst those effectively sampled or categorized as patients from a hospital- it turns to a significant separation into two clusters: Chagas 'disease ELISA positives with higher BMI, triglycerides, LDL cholesterol and creatinine, lower blood glucose and HDL cholesterol vs Chagas 'disease ELISA negatives with lower BMI, triglycerides, LDL cholesterol and creatinine, normal or high blood glucose and normal HDL cholesterol ($p<0.024$). Though this final analysis seems to be statistically significant and from a conceptual scope reinforce the hypothesis of an association of DD genotype with high BMI and a positive diagnostic, it may not be a good clinical counsel for a clinician working in Argüello community.

4.4

Another point to be considered is whether a high BMI is previous to the infection or developed as a positive adaptation after infection. We cannot make a substantial contribution since our study was transversal, case control study, and a small sample, but it is outstanding the absence of chagasic patients with II genotype that present the lowest BMI in Rosario del Saladillo and the highest BMI in Argüello. The first concern is that the development of increased body mass seems not to be determined by the infection since there were positive individuals without or with mild overweight. A second observation is that DD genotype comprised patients with normal or low BMI in Argüello, so it seems unprovable that DD genotype determines a trend to high BMI. Third, the absence of positives amongst individuals of the II genotype, may be the result of just chance, death due to Chagas ´disease, selectivity of bug predation (not bitten not infected), an extremely good response with total elimination of the parasite or another unknown variable related to the I allele not to BMI.

4.5 Clinical considerations

In this study the expectation of evolution of chagasic patients was measured with the hypertension variable as function of BMI and hyperlipidemia. These two polar conditions, morbid obesity and extremely low BMI represented probably two clinical evolutions (Table 3).

Table III

Evolution of improvement in Chagas´ patients from Argüello and Rosario del Saladillo (RS)

Region	Morbid obese	Obese	Over weight	Normal weight	Low weight
RS Chagas (+) Good evolution	0	3	3	0	0
RS Chagas (+)Bad evolution	0	1	0	0	1
Argüello Chagas (+) Good evolution	0	2	4	0	0
Argüello Chagas (+) Bad evolution	1	1	0	2	0

X 2: p= 0.0187, with signification in relationship in good evolution in obesity and overweight, but probably bad response in morbid obese and low weight

One of them, morbid with high compromise with hypertension and hyperglycemia associated with hyperlipidemia and cardiac arrest triggered by bad adaptation or Hypothalamus-Pituitary-Adrenal axis unbalance. The other one with low BMI represented probably the cachectic expression of an unbalanced inflammatory expansion associated with cardiomegaly (not shown). Both patterns result in diminished kidney filtration due to reduced blood flow originated on progressive autonomic impairment, and muscle lysis, which could be the explanation for increased blood creatinine. The rest of the cases with obesity, overweight or normal weight presented relatively good evolution and hyperlipidemia.
These results would favor the interpretation that overweight but not morbid obesity in chagasic patients may be a relatively good prognostic.

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