



## Establishing a blood fructosamine reference range for the Brazilian population based on data from ELSA – Brasil

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### ABSTRACT

**Objectives:** The fructosamine test is used in the monitoring of diabetes mellitus, particularly in cases with restrictions on the use of glycated hemoglobin (mainly in the setting of altered red blood cell lifespan and interference by hemoglobin variants). It could also provide additional information on shorter-term glycemic control. The objective of the study is to establish the reference range of the fructosamine in the Brazilian population.

**Design and methods:** The reference interval was defined as suggested by the Clinical and Laboratory Standards Institute (CLSI). The study participants were from a Brazilian cohort (The Longitudinal Study of Adult Health – ELSA-Brasil) with baseline data collected between 2008 and 2010. A total of 466 subjects were selected after exclusion of diabetic individuals, and those with altered glycemic markers and renal function tests.

**Results:** The reference interval was 186–248  $\mu\text{mol/L}$  for women and 196–269  $\mu\text{mol/L}$  for men. Fructosamine levels were higher in men than in women ( $p=0.006$ ) and in the non-white population ( $p=0.034$ ) and had a negative correlation with the body mass index ( $r=-0.117$ ;  $p=0.011$ ).

**Conclusions:** The reference intervals for fructosamine were affected by sex. Reference intervals stratified by sex would be more adequate in the interpretation of the fructosamine test.

### 1. Introduction

The International Diabetes Federation (IDF) estimates that more than 600 million people will be diabetic by 2040, making diabetes a leading cause of mortality and morbidity. In Brazil, the number of individuals with diabetes increased by 62% over the past ten years, and the disease now affects approximately 9% of the population [1,2].

Traditional markers used in diabetes diagnosis and monitoring include fasting plasma glucose (FPG), two-hour plasma glucose (2-hPG) measured during the oral glucose tolerance test (OGTT), and glycated hemoglobin (A1c) levels [3]. More recently,

**Abbreviations:** IDF, International diabetes federation; 1, 5 AG, 1,5-anhydroglucitol; A1c, Glycate hemoglobin; FPG, Fasting plasma glucose; 2-h PG, 2 h post glucose load; OGTT, Oral glucose tolerance test; NBT, Nitroblue tetrazolium method; CLSI, Clinical and laboratory standard institute; BMI, Body mass index

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healthcare professionals have increasingly used other markers of hyperglycemia, such as 1,5-anhydroglucitol (1,5-AG), glycated albumin and fructosamine levels [4].

The 1,5-AG and glycated albumin tests are rarely used in Brazil, in contrast to fructosamine test. Fructosamine levels provide information about an individual's glycemic levels over a period of two to three weeks [5]. Our work aimed at defining the first reference range for the Brazilian population.

## 2. Materials and methods

### 2.1. Population

The Longitudinal Study of Adult Health (ELSA-Brasil) aims to identify risk factors, and to assess the incidence and progression of cardiovascular diseases and diabetes mellitus in multicenter cohort of Brazilians. The baseline data were collected from 2008 to 2010 from a cohort and included 15,105 active and retired public servants aged 35–74 years of age, who worked in research institutions or universities in six Brazilian capitals. The following conditions were considered exclusion criteria: current pregnancy, pregnancy in the previous four months, severe cognitive or communication deficits, intention to leave the job in the near future, or, if retired, intention to move from the current metropolitan area.

The present study was conducted in participants of ELSA-Brasil residing in Belo Horizonte, state of Minas Gerais. The total number of samples collected in the city was 3115, from which 2288 were available for fructosamine testing. We excluded from the study individuals with a previous history of diabetes ( $n = 161$ ), with FPG  $\geq 5.6$  mmol/L (100 mg/dL) and/or 2-hPG  $\geq 7.8$  mmol/L (140 mg/dL) and/or A1c  $\geq (38.8$  mmol/mol) (5.7%) ( $n = 1804$ ). In addition, we excluded individuals with microalbuminuria  $\geq 20$   $\mu\text{g}/\text{min}$  ( $n = 2$ ) and creatinine  $> 114.9$   $\mu\text{mol}/\text{L}$  (1.3 mg/dL) ( $n = 57$ ). Overlap of exclusion criteria occurred for some individuals. The body mass index (BMI) was calculated as the weight in kg divided by height in meters raised to the power of two. Study participants were asked to identify their racial background and/or skin color as black, brown, Asian, native Brazilian, or white. All participants signed an informed consent form and the research protocol was approved by the Ethics Committee of each institution, as well as by the National Commission on Research Ethics [6,7].

Samples were collected after a 12-h fasting period. The OGTT was conducted in all participants included in the present study. Samples were stored at  $-80$  °C. Serum fructosamine levels were determined by the colorimetric method (NBT – BioSystems S.A. Costa Brava, Barcelona, Spain) in an automatic analyzer (AU 5800, Beckman Coulter, USA) with a variation coefficient  $\leq 5.3\%$ .

### 2.2. Statistics

The reference range was defined as per the Clinical and Laboratory Standards Institute C28-A3c standard (CLSI), with 2.5 and 97.5 percentiles as the lower and upper limits, respectively. The sample was evaluated according to the presence of Dixon (with criteria of Reed) and Tukey outliers. The generation of the reference interval was performed by non-parametric technique. The percentiles were calculated as the observations corresponding to rank  $r = p * (n + 1)$ . For the 90% confidence intervals of the reference limits we followed the CLSI guidelines and conservative confidence intervals were calculated using integer ranks. The evaluation for the data partition was performed by the Harris & Boyd method. [8,9]. Continuous variables with a normal distribution (Shapiro-Wilk Test) were expressed as mean and standard deviation; otherwise, variables were expressed as median and interquartile range. The T-test, Mann-Whitney and chi-squared tests were used for comparisons between two groups. Kruskal-Wallis was used in comparisons among three or more groups. Multiple linear regression analysis was used to assess the influence of different variables on blood fructosamine levels. Spearman's correlation was adopted for quantitative variables. Tests were run on MedCalc for Windows, version 18 (MedCalc, Ostend, Belgium).

## 3. Results

The number of study participants was 466, and Table 1 shows the characteristics of this group. Women had higher mean age than men, but no differences existed in the distribution between genders of self-indicated race/skin color and BMI. No differences in fructosamine levels were found among self-indicated race/skin color groups ( $p = 0.185$ ). However, when the sample was divided into whites and non-whites, fructosamine levels were higher among non-whites ( $p = 0.034$ ). Men had higher blood fructosamine than women ( $p < 0.001$ ). Overall, fructosamine levels were negatively correlated with BMI ( $r = -0.117$ ;  $p = 0.011$ ). Although age showed no correlation to fructosamine concentration, it was included in the multiple regression model, which was significant ( $p < 0.001$ , adjusted correlation coefficient 0.108). Significant variables included gender, self-indicated race/skin color and BMI; whereas age showed no significance in the model. Gender, BMI ( $< 25$  kg/m<sup>2</sup> versus  $\geq 25$  kg/m<sup>2</sup>) and self-indicated race/skin color (whites versus non-whites) were separately evaluated according to Harris-Boyd criteria (CLSI) for the stratification of reference ranges. Only gender warranted stratification of the range. No outlier was observed in the sample before and after stratification by sex. After stratification by sex, BMI and self-indicated race/skin color were evaluated within each gender group. However, differences were not significant and did not warrant another level of stratification. Table 2 displays the reference ranges we defined, as well as other ranges established in previous studies using the colorimetric method (second generation NBT) [10–16].

**Table 1**

Laboratorial and clinical characteristics of the study sample, ELSA-Brasil (n = 466).

	Total	Males	Females	P-value
N	466	145 (31.1)	321 (68.9)	
Age (years) <sup>a</sup>	48 (43–56)	47 (42–54)	49 (44–56)	0.006
Race/skin color <sup>b,c</sup>				0.240
Black	40 (8.6)	9 (6.2)	31 (9.7)	
Brown	166 (35.6)	59 (40.7)	107 (33.3)	
White	243 (52.1)	73 (50.3)	170 (53.0)	
Others <sup>d</sup>	12 (2.6)	2 (1.4)	10 (3.1)	
BMI (kg/m <sup>2</sup> )				0.853
< 25	284 (60.9)	91 (62.8)	193 (60.1)	
25 to < 30	143 (30.7)	42 (29.0)	101 (31.5)	
≥ 30	39 (8.4)	12 (8.3)	27 (8.4)	
Fructosamine	218 (207–230)	226 (215–238)	215 (205–207)	< 0.001
FPG (mmol/L)	5.3 (5.1–5.4)	5.3 (5.2–5.4)	5.3 (5.1–5.4)	0.017
2-h PG (mmol/L)	5.9 (5.2–6.5)	5.8 (5.1–6.5)	5.9 (5.2–6.5)	0.542
A1c (mmol/mol)	31 (28–34)	31 (28–34)	31 (28–34)	0.763
Creatinine (μmol/L)	79.6 (70.7–88.4)	88.4 (79.6–97.2)	70.7 (61.9–79.6)	< 0.001
Microalbuminuria (μg/min)	0.093 (0.092–0.097)	0.094 (0.093–0.0102)	0.093 (0.092–0.095)	< 0.001

<sup>a</sup> Median and interquartile range.<sup>b</sup> Self-indicated race/skin color.<sup>c</sup> Absolute frequency (%).<sup>d</sup> Asians and native Brazilians; BMI= Body Mass Index; FPG= Fasting Plasma Glucose; 2-h PG= 2 h post glucose load; A1c = Glycated Hemoglobin.

#### 4. Discussion

The reference ranges proposed here include lower fructosamine concentrations when compared to those established in other previous studies. These reference ranges generated in different populations are available in Table 2, as well as information about the sample selection criteria, kit manufactures and statistical evaluation (when available). Several factors may account for the differences. Each study adopted very distinct inclusion and exclusion criteria, such as the use of FPG alone as a marker [12,14]; the use of FPG and 2-hPG as the only markers [10,13]; and the use of A1c alone as a marker [11]. Moreover, each study used different levels of these markers to determine the exclusion of participants. We used three tests of glycemic metabolism, and only individuals with normal levels in all three were included in the study, which could explain the lower fructosamine levels found in our study participants. Both Chen et al. [13] and Zhou et al. [14] applied other clinical and laboratorial variables as exclusion criteria. Selvin

**Table 2**

Blood fructosamine reference ranges estimated with second-generation nitroblue tetrazolium methodology available in the literature.

Reference	Sample	Range (μmol/L)	Kit and Analyzer	Comments
Baker et al. [10] – New Zealand	2211 non-diabetics (FPG < 7.8 mmol/L <sup>a</sup> ; 2-hPG < 11.1 mmol/L; age 33–78 yrs; median age 47 yrs)	202–296	F. Hoffmann-La Roche/Cobas-Fara	No need for stratification based on gender or age.
Cefalu et al. [11] – USA	230 non-diabetics (A1c < 53 mmol/mol <sup>b</sup> ; age 18–70 yrs)	≤ 289	Roche Reagents/Cobas-Mira	Did not evaluate the need for stratification.
Lin et al. [12] – USA	228 non-diabetics (FPG < 5.0 mmol/L)	202–282	Boehringer Mannheim/Hitachi 747–200	Did not evaluate the need for stratification.
Chen et al. [13] – China	1497 healthy individuals (FPG < 6.1 mmol/L; 2-hPG < 7.8 mmol/L; normal BMI; age 20–85 yrs; 73.3% females)	20–65 yrs 215–287 > 65 yrs 226–325	Roche/Cobas 8000	Evaluated the need for gender- and age-specific ranges.
Zhou et al. [14] – China	458 individuals; (FPG 3.9–6.1 mmol/L; normal creatinine; age 20–79 yrs; median age 43 yrs; 50.7% females)	220–298	Roche/Roche Modular DPP	Evaluated the need for gender- and age-specific ranges. Did not find the need for them.
Selvin et al. [15] – USA	1799 non-diabetics (age 47–68 yrs; 51.4% females)	195–258	Roche/Roche Modular P800	Evaluated the need for gender- age-, BMI- and race-specific ranges.
Pedrosa et al. 2018 – Brasil (present study)	466 individuals (normal FPG, A1c, 2-hPG; age 35–73 yrs; median age 48 yrs; 68.9% females)	Females <sup>c</sup> 186 – 248 Males 196–269	BioSystems S.A/AU 580 Beckman Coulter	Evaluated the need for gender- age-, BMI- and race-specific ranges.

Manufacturer's reference value interval for adults 205–285 μmol/L.

<sup>d</sup>90% confidence interval is 178–199 μmol/L for the lower limit and 263–279 μmol/L for upper limit.<sup>a</sup> To convert glucose concentrations from milimoles per liter to milligrams per deciliter, multiply by 18.<sup>b</sup> To convert glycated hemoglobin from milimol per mol to %, multiply by 0.0915 and add 2.15%.<sup>c</sup> 90% confidence interval is 183–189 μmol/L for the lower limit and 243–255 μmol/L for upper limit.

et al. [15] had results similar to ours. These authors excluded diabetic individuals from their study, as well as those with elevated hepatic enzymes, thyroid dysfunction, hypertension, and smokers. The authors reported, however, that the exclusion of diabetics had the greatest impact on reported ranges.

Differences among studies may also emerge from the use of different kits. In the present work, we used the BioSystems kit and an automatic analyzer AU580 (Beckman Coulter). None of the other reports listed on Table 2 used this combination: all of them used the Roche Diagnostics kit in different Roche analyzers.

Finally, regarding the statistical evaluation, not all studies had a systematic approach to the establishment of ranges. However, all authors adopted the 2.5 and 97.5 percentiles as limits, except for Cefalu and colleagues [11], who set the limit at 95 percentile only. Two of the studies did not evaluate the need for range stratification [11,12]. Among the variables assessed here, only gender warranted a stratification of the range. Four other studies [10,13–15] evaluated but did not find the need for stratification. Chen et al. [13] found that different age groups required distinct ranges, in contrast to our findings. Much like observed in other work [10,15], we only evaluated individuals with  $\geq 35$  years-old, which limited our ability to identify ranges for other age groups. Zhou and colleagues worked with younger individuals. However, the median age in their study was similar to ours and they used a different tool (Lahti's algorithm) to evaluate the need for data stratification [14]. Selvin et al. used histograms and forest plots. We, in turn, stratified the range according to Harris-Boyd and the CLSI document [8,9].

Here, we assessed the need for specific ranges for different BMI groups. Chen et al. [13] only evaluated individuals with normal BMI. Much as observed by Selvin et al. [15], we found lower fructosamine concentrations in study participants with high BMI. Previous work found elevated levels of fructosamine among black individuals [15]. Evaluation of fructosamine concentration according to race (or skin color) is not usually reported. The reasons could be the lack of miscegenation in some populations, the unavailability of the information about the race. In Brazil, due to the great miscegenation, we find it is very difficult to evaluate individuals according to race. In the present study, individuals whose self-indicated race/skin color as non-white had higher fructosamine levels than white individuals.

The present work has limitations that include the sample size, which does not allow us to extrapolate results to the general population. Originally, our population was not selected for the construction of a reference interval. As a convenience sample, its applicability may be reduced for the general population. Moreover, albumin serum levels were not available, and low albumin levels are known to affect fructosamine concentration. In healthy individuals this effect on fructosamine is minor and has little impact on test results [15,16]. In addition, we only included in the study individuals with normal creatinine and microalbuminuria, which certainly restricted the inclusion of individuals with hypoalbuminemia. The tests currently used for the diagnosis and follow-up of DM had their association classically established with clinical outcomes. In addition, they have international standardization and international harmonization. More recently, high concentrations of fructosamine have been related to microvascular complications of DM [17]. However, more information is still needed to establish a better correlation between the levels of fructosamine and the clinical outcomes of DM. In addition, the test of fructosamine lacks standardization to more widespread use.

This study represents the first effort to establish a reference range for fructosamine levels in the Brazilian population. Our report, which used the ELSA-Brasil baseline as a source of data, might contribute to future work on the diagnosis and monitoring of DM, especially in situations when A1c and OGTT cannot be used.

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## Declaration of conflicts of interest

We, William Pedrosa; Maria de Fátima Hauesein Sander Diniz, Sandhi Barreto e Pedro Guatimosim Vidigal, authors of the manuscript entitled " Establishing a blood fructosamine reference range for the Brazilian population based on data from ELSA – Brasil" declare that we do not have conflict of interest that are financial, political, personal or others.

We also declare that financial support and (or) material received for the development of this work are clearly mentioned in the text.

Belo Horizonte, Monday, October 08, 2018. Brazil.

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