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Evaluation of the influence of pre-analytical factors on total testosterone levels in healthy young men

Avaliação da influência de fatores pré-analíticos nas dosagens de testosterona total em homens jovens saudáveis

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ABSTRACT

Introduction: Testosterone is the main hormone that regulates male reproductive functions, directly participating in spermatogenesis and increasing sexual activity. The blood measurement of this hormone is essential for the diagnosis of neuroendocrine disorders, such as hypogonadism. However, there is lack of standardization regarding patient preparation for the hormone collection in clinical laboratories. **Objective:** Evaluate the effect of pre-analytical variables, including fasting, circadian and seasonal variation on testosterone levels in healthy young males. **Material and methods:** Forty-two volunteers were selected for the study, in the city of Barbacena, Minas Gerais. Four blood samples were collected from each of the participants, three in the winter: the first one in the morning after fasting; the second in the afternoon, without fasting; the third, in the next day morning, without fasting; and the last one taken in the summer, in the morning, after fasting. **Results:** The analyses showed that there was a significant decrease in total testosterone levels when there was no fasting for eight hours prior to collection and in the afternoon compared to the morning, both with $p < 0.001$. There was no significant difference in the results obtained in winter and summer. **Conclusion:** It is recommended that clinical laboratories standardize the collection of total testosterone by performing the test in the morning and after an eight-hour fast, in order to reduce variability and ensure reliability in the results.

Key words: total testosterone; pre-analytical factors; fasting; circadian rhythm; seasonal variation.

RESUMO

Introdução: *A testosterona é o principal hormônio regulador das funções reprodutivas masculinas, participando diretamente da espermatogênese e do aumento da atividade sexual. A dosagem sanguínea desse hormônio é fundamental no diagnóstico de distúrbios neuroendócrinos, como o hipogonadismo. Entretanto, há pouca padronização no preparo adequado do paciente para a coleta do hormônio em laboratórios clínicos.* ***Objetivo:*** *Avaliar o efeito de variáveis pré-analíticas, incluindo realização de jejum, variação circadiana e sazonal nas dosagens de testosterona em jovens saudáveis do sexo masculino.* ***Material e métodos:*** *Foram selecionados 42 voluntários para o estudo, na cidade de Barbacena, Minas Gerais. Quatro amostras de sangue de cada um dos participantes foram coletadas, sendo três no inverno: a primeira de manhã, em jejum; a segunda à tarde, sem jejum; a terceira no dia seguinte, de manhã, sem jejum. A última foi coletada no verão, na parte da manhã, em jejum.* ***Resultados:*** *As análises demonstraram que houve diminuição significativa dos níveis de testosterona total quando não foi realizado jejum de 8 horas antes da coleta e no período da tarde em comparação ao período da manhã, ambos com valor de* $p < 0,001$. *Não houve diferença significativa nos resultados obtidos no inverno e no verão.* ***Conclusão:*** *Recomendamos que os laboratórios clínicos padronizem a coleta de testosterona total com a realização do exame no período da manhã e em jejum de 8 horas, a fim de reduzir a variabilidade e garantir a confiabilidade nos resultados.*

Unitermos: *testosterona total; fatores pré-analíticos; jejum; ritmo circadiano; variação sazonal.*

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RESUMEN

Introducción: *La testosterona es la principal hormona reguladora de funciones reproductoras masculinas, participando directamente en la espermatogénesis y en el aumento de la actividad sexual. La medición sanguínea de esa hormona es fundamental en el diagnóstico de trastornos neuroendocrinos, como el hipogonadismo. Sin embargo, hay poca estandarización en la preparación adecuada del paciente para la recolección de la hormona en laboratorios clínicos.* ***Objetivo:*** *Evaluar el efecto de variables preanalíticas, incluyendo ayuno, variación circadiana y estacional en las mediciones de testosterona en hombres jóvenes sanos.* ***Material y métodos:*** *Se eligieron 42 voluntarios para el estudio, en la ciudad de Barbacena, Minas Gerais. Se tomaron cuatro muestras de sangre de cada uno de los participantes, de las cuales tres en invierno: la primera, matutina, en ayunas; la segunda, vespertina, sin ayunas; la tercera, el dia siguiente, matutina, sin ayunas; el última se recolectó en verano, por la mañana, en ayunas.* ***Resultados:*** *Los análisis demostraron que hubo reducción significativa en los niveles de testosterona total cuando no se realizó el ayuno de ocho horas antes de la recolección y en el período vespertino en comparación al matutino, ambos con valor de* $p < 0,001$. *No hubo diferencia significativa en los resultados obtenidos en invierno y en verano.* ***Conclusión:*** *Se recomienda que los laboratórios clínicos estandarizen la recolección de testosterona total con la realización de la prueba en el período matutino y en ayuno de ocho horas, para reducir la variación y garantizar la confiabilidad de los resultados.*

Palabras clave: *testosterona total; factores preanalíticos; ayuno; ritmo circadiano; variación estacional.*

INTRODUCTION

Testosterone is a steroid hormone formed in interstitial Leydig cells and secreted by the testicles, being the major regulator of male reproductive functions[1], such as development and maintenance of secondary sex characters in the beginning of puberty, maintenance of spermatogenesis and increase in sexual activity[2]. It also acts as an anabolic hormone, promoting secondary sexual characteristics in men, such as growth of body hair, muscle development, libido, penis growth, sexual differentiation and spermatogenesis, besides having effects upon behavior. Its release is dependent on hypothalamic gonadotropin releasing hormone (GnRH), which stimulates anterior hypophysis to synthetize and release luteinizing hormones (LH) and follicle stimulant hormone (FSH), responsible for stimulation of pulsatile secretion of testosterone by the testicle[3].

The serum determination of testosterone is used for detection of abnormal levels in male and female individuals, aiding in the diagnosis of hormonal disorders, in the monitoring of gonadal status and in the hormone replacement therapy[4].

Aiming at minimizing the possible associated variations and ensure a higher reliability level of results, it is necessary to identify the pre-analytical factors involved in the measurement of total testosterone[5]. As a rule, these factors can correspond to the greater part of "errors" in laboratory measurements[6]. The influences of circadian rhythm[1, 7, 8], fasting and seasonal change have been demonstrated in the levels of testosterone[9, 10], however, references that corroborate the actual effect and the magnitude of these pre-analytical factors in the testosterone results are still limited[2].

OBJECTIVE

The objective of this study was to evaluate the effect of pre-analytical variables such as fasting or not prior to collection, the influence of circadian rhythm and the impact of seasonal variation in the measurements of total testosterone in young, healthy, male individuals.

MATERIAL AND METHODS

Experimental design

This is an experimental prospective study to assess pre-analytical effects in the measurements of total testosterone. The protocol was approved by the Ethics Committee of Faculdade de Medicina de Barbacena, under number 3.059.834.

Sample

The calculation of sample size demonstrated that a sample formed of 30 individuals, in each measurement, would permit a 95% significance level and a sample power of 97.2%. Aware of the possibility of losing volunteers during the study, we applied

the correction factor to ensure safety to sample power, justifying a larger initial number of volunteers. We invited 138 healthy male volunteer students aged between 20 and 30 years for the research; among them, 42 agreed to participate. People with diseases affecting testosterone production and/or metabolism, users of medications altering the synthesis of this hormone, and users of exogenous testosterone and derivatives, or any synthetic anabolic steroids in the last year were excluded from the study. Two volunteers met one or more exclusion criteria, and the sample was composed of 40 subjects.

Material collection

Sample collection was performed at the laboratory of Faculdade de Medicina de Barbacena, in Barbacena-MG. The volunteers underwent four blood collections: the first three in the winter, the fourth in the summer. The first collection was done in winter, in the morning period, between 7 a.m. and 8:30 a.m, with a previous eight-hour fast. In the afternoon of the same day, the second collection was conducted, between 6 p.m. and 7 p.m., after the volunteers have fed all day long. The third collection was held on the next day, in the morning, between 7 a.m. and 8:30 a.m., with no previous eight-hour fast. Finally, the fourth collection was carried out in the summer, approximately six months after the first collections, in the morning period, between 7 a.m. and 8:30 a.m., with a previous eight-hour fast.

After collections, the samples were kept under refrigeration between 2°C and 8°C, and then, transported to Laboratório São José de Barbacena, responsible for test conduction. Total testosterone was measured by a commercial quantitative chemiluminescent immunoassay at the instrument Access II®, Beckman Coulter®[11, 12].

Statistical analysis

The obtained results were transcribed to a database processed at statistical software Minitab 17® for Windows®. Tables of the line by column type were produced with absolute and relative frequency, and the measures of central tendency and dispersion were calculated. Due to the non-normal distribution of values, a median was used in measurements for calculation of statistical differences, by means of non-parametrical Mann-Whitney test. A p-value < 0.01 was regarded as statistically significant.

RESULTS

The **Table** demonstrates the distribution of values obtained of total testosterone in each of the collections performed in the study.

TABLE – Testosterone levels obtained in the performed collections

Collection	Sample type	*n*	Total testosterone levels (ng/dl)				
			Minimum	Q1	Median	Q3	Maximum
1st	Winter (morning with fasting)	40	282	355	452	519	809
2nd	Winter (afternoon)	40	129	274	354	419	669
3rd	Winter (morning without fasting)	40	173	264	326	394	579
4th	Summer (morning with fasting)	38	287	393	484	591	692

After initial collection of the three first samples, two volunteers did not attend the last collection; therefore, the analysis of testosterone levels in the summer was conducted with 38 subjects.

The comparison among the samples collected in the morning period, with or without prior eight-hour fasting, demonstrated that the absence of fasting led to a decrease of 28% in the average levels of total testosterone, with p-value < 0.0001 (**Figure 1**).

The effect of circadian rhythm on total testosterone was determined by the comparison between median levels obtained in the morning period and those found in the afternoon. A decrease of 22% was verified in the afternoon levels regarding the morning levels, with p-value = 0.0002 (**Figure 2**).

Finally, the seasonal effect was determined by the comparison among total testosterone average levels of samples obtained in the morning period, with previous fasting, collected in the winter, and the ones collected in the summer. There was no statistical difference among the average levels of those samples, with p-value = 0.151 (**Figure 3**).

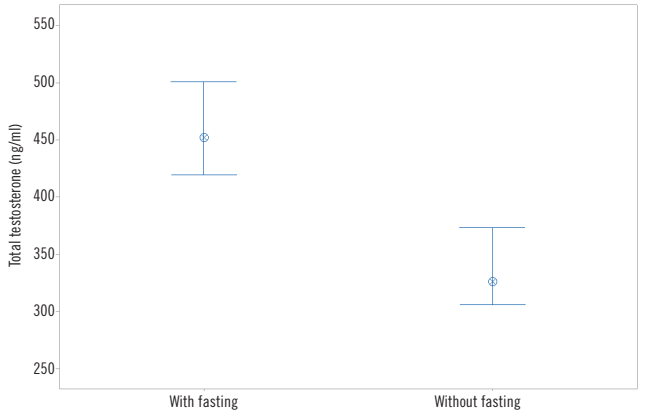

FIGURE 1 – *Distribution of median values of total testosterone with collection participants with or without previous eight-hour fasting*

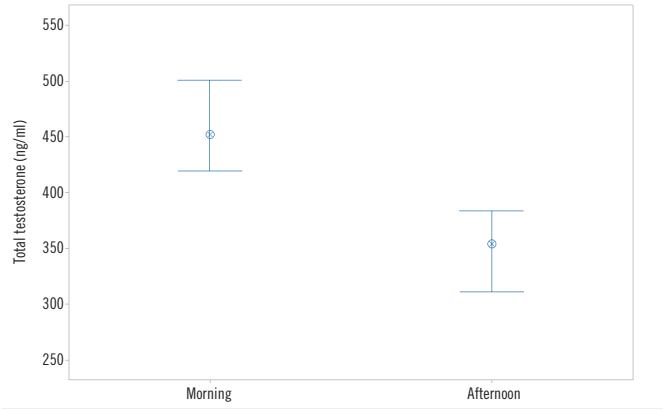

FIGURE 2 – *Distribution of median values of total testosterone in the morning and afternoon periods*

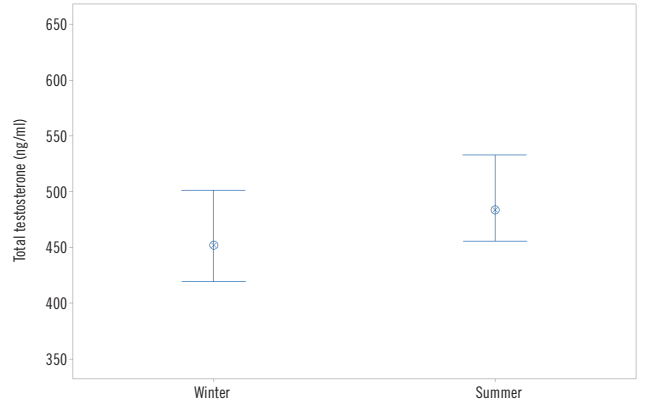

FIGURE 3 – *Distribution of median values of total testosterone in the morning period during winter and summer*

DISCUSSION

The medical laboratory literature presents numerous examples of significant alterations in laboratory measurements due to pre-analytical factors, these being the main factors responsible for inadequacies in laboratory results[6]. In the present study, we demonstrated the significant influence of fasting, previously to collection, and of circadian rhythm, in the concentration of total testosterone.

The determination of serum levels of this hormone is fundamental for the diagnosis of hypogonadism in men, and according to the Brazilian Society of Endocrinology and Metabology [Sociedade Brasileira de Endocrinologia (SBEM)] and the American Urology Association (AUA) (Guidelines of AUA – Evaluation and Management of Testosterone Deficiency), total testosterone below 300 ng/dl is considered a reasonable cutoff value in the diagnosis of this condition. To this effect, two values below this cutoff point are recommended, in two measurements performed in different occasions, both in the beginning of the morning. Such a recommendation is corroborated by the Brazilian Society of Clinical Pathology/Laboratory Medicine [Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML)][13].

Our study confirmed the absolute necessity of at least an eight-hour fasting, prior to collection, to standardize result interpretation, as its no conduction caused a decrease of 28% in levels, around 126 ng/dl in absolute numbers, enough to erroneously classify a healthy individual as a subject with hypogonadism. No scientific materials were found that demonstrated the influence of fast within a short time period, like the eight hours proposed by the present study, as a variable over testosterone levels.

A study designed by Rojdmark (1987)[14] associates intermittent fasting, of 48 hours, with the decrease of baseline hormone values. The author suggests that this alteration could arise from the reduction of GnRh hormone and, consequently, LH decrease, causing smaller activation of Leyding cells and smaller production of testosterone. The difference is evident between the evaluation proposed by the cited author and what was proposed in this study. However, we understand that further studies are necessary to better evaluate the impacts of fasting over testosterone levels.

Regarding the morning time, total testosterone levels were confirmed to be lower in around 22%. In absolute numbers, the difference of 98 ng/dl in the obtained medians was also enough to classify a healthy individual with hypogonadism. This circadian variation had been previously described[3, 8], especially in healthy young men[7, 10]. Lac and Chamoux (2006)[15] demonstrated a reduction in hormone concentration of 30%-40% in the afternoon period regarding the morning. A single study, by Kirschner *et al*. (1965), was found, which did not verify a circadian variation in testosterone levels, however, possibly due to a small total sample ($n = 8$).

Influence of season in levels of total testosterone was not found in this study, not agreeing with what was observed in other studies previously published[16-20]. A study carried out in Topson, Norway, demonstrated an inverse relation between sunlight hours and mean monthly temperature with the total level of testosterone. Thus, lower levels are observed in months with the highest temperatures and greater exposure to sunlight[21]. We believe the major factor that can have led to this discrepancy of results lies in the fact that Norway is a country located in the extreme North of the planet, with possible variations of temperature and sun exposure in year seasons. In our region, a small variation was observed in the mean monthly temperatures in the collection months, being in both periods, close to 20°C[22]. Also, we are subjected to lower variation in solar incidence, in comparison to Norway. Thus, we believe seasonal variation is really not a relevant pre-analytical factor in our region.

CONCLUSION

The present study made it possible to conclude about the importance of clinical laboratories to standardize testosterone collection, with at least an eight-hour fasting prior to test, and in the beginning of the morning, in order to minimize the impact of these pre-analytical variables upon the hormone result interpretation. The important observed reduction can lead to an incorrect diagnosis of gonadal disorder in a healthy individual.

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