The influence of different types of fasting on lipid profile of volunteers from a city of Rio Grande do Sul, Brazil

ABSTRACT

Introduction: Determination of lipid profile includes triglycerides (TG), total cholesterol and fractions as high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c). These parameters are valuable in the risk assessment of developing cardiovascular disease. However, some pre-analytical factors, such as the fasting state, may interfere with the results of these tests. Objective: The aim of this study was to evaluate differences on lipid profile measurements in blood samples collected at different fasting periods in men and women with or without a diagnosis of hypercholesterolemia. Methods: Fifty volunteers of both sexes, aged between 22 and 86 years, were evaluated. Sociodemographic data and two blood samples were collected, one after 12 hours fast and another during postprandial period, with subsequent measurement of total cholesterol, HDL-c, LDL-c and TG. Results: Comparing the values of the lipid profile obtained in the two collections, it was observed that the total cholesterol and HDL-c did not present significant differences among the evaluated subjects. On the other hand, LDL-c and TG showed significant higher values on postprandial samples, preferably in male group. Conclusion: These data suggest that TG and LDL-c levels are the fractions with greater susceptibility to variations when they are collected without prior fasting.

Key words: fasting; postprandial period; lipoprotein.
RESUMEN

Introducción: La determinación del perfil lipídico incluye los triglicéridos (TG), colesterol total y fracciones del colesterol de la lipoproteína de alta densidad (HDL-c) y colesterol de la lipoproteína de baja densidad (LDL-c). Esos parámetros son muy valiosos en la evaluación del riesgo cardiovascular. Sin embargo, algunos factores preanalíticos, como el estado de ayuno, pueden interferir en los resultados de esos exámenes. **Objetivo:** Evaluar si hay diferencias significativas en la determinación del perfil lipídico en muestras de sangre recolectadas de hombres y mujeres con o sin diagnóstico de hipercolesterolemia. **Método:** Se evaluaron 50 voluntarios de ambos sexos, con edades comprendidas entre 22 y 86 años. Se recolectaron informaciones sociodemográficas y dos muestras de sangre, una con ayuno previo de 12 horas y otra posprandial, con determinación posterior de colesterol total, HDL-c, LDL-c, y TG. **Resultados:** Comparándose los valores obtenidos del perfil lipídico en las dos coletas, se observó que el colesterol total y el HDL-c no presentaron diferencias significativas en los sujetos evaluados. Al mismo tiempo, LDL-c y TG mostraron valores significativamente más elevados en la recoleta posprandial, preferencialmente en el grupo masculino. **Conclusión:** El conjunto de datos obtenidos sugiere que los niveles de TG y LDL-c son las fracciones con mayor susceptibilidad a variaciones cuando son recolectadas sin ayuno previo.

Palabras clave: ayuno; periodo posprandial; lipoproteínas.

INTRODUCTION

According to the World Health Organization (WHO), more than 20 million people will die from cardiovascular disease by the year 2030. Cardiovascular diseases, among chronic non-communicable diseases, account for about 47.2% of worldwide deaths and by 33% of deaths in Brazil[1].

In this context, screening for dyslipidemia as a risk factor for cardiovascular diseases and management of lipid-lowering drugs is a main part of primary care[2]. For many years, determination of the routine lipid profile, including triglycerides (TG), total cholesterol and fractions of low-density lipoprotein cholesterol (LDL-c) and cholesterol and high-density lipoprotein cholesterol (HDL-c), has been performed in the laboratory of clinical analyzes in samples of blood collected after fasting-fed or at least 8 hours fast[3, 4]. The set of these results added to the behavioral and nutritional genetic assessment of the subject can predict the risk for the development of chronic cardiovascular diseases[5-8]. Thus, there are several reasons for the fasting requirement, mainly because the postprandial state changes the lipoproteins composition, reflected in TG concentration increase, which is directly related to the lipid and carbohydrate contents of the diet. In addition, the TG increase impacts on the calculation of the LDL-c fraction when the Friedewald equation is used[9].

However, most individuals’ live are known to occur in the postprandial metabolic state, and the collection of fasting samples to determine future cardiovascular risks has been strongly contested[10]. Recent evidence has shown that the TG concentration measured in periods with no previous fasting was considered a better predictor for future coronary events when compared to fasting TG tests results in both men and women[10]. However, there are still many controversies about this topic. The Danish Society of Clinical Biochemistry, 2009, and the National Institute of Health and Care Excellence in the United Kingdom (NICE), in 2014, recommended the use of blood sample with no previous fasting to determine the routine lipid profile[11]; the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine also follow this recommendation[12]. By contrast, guidelines issued in 2013 by the American College of Cardiology/American Heart Association (ACC/AHA) show a predilection for fasting samples collected for this purpose[8, 13].

In Brazil, according to the Brazilian Consensus for the Normalization of Laboratory Determination of Lipid Profile, in December 2016, the flexibilization of fasting for lipid profile evaluation follows the following motivations: a) patient is in fed state for a longer time, which makes their potential impact on cardiovascular risk more effective; b) convenience of sample collection for the patient; c) postprandial collection is safer in several situations, such as for diabetic patients; d) measures do not differ significantly if performed in the postprandial or fasted state; e) reduction of congestion in laboratories, especially in the morning; f) the main available tests mitigated the interference caused by higher turbidity in the samples due to high concentrations of TG, with technological advances in diagnostic methodologies[14].

Thus, due to remaining controversies regarding the measures of the lipid profile in postprandial and fasting state, this study aimed to evaluate if there are significant differences in the lipid...
profile levels of blood samples collected in different periods of fasting from men and women with or without diagnosis of hypercholesterolemia.

MATERIAL AND METHODS

This is a cross-sectional, exploratory and observational/descriptive study that was performed in 50 volunteers of both sexes, with or without diagnosis of hypercholesterolemia, inhabitants of the city Severiano de Almeida, Rio Grande do Sul, Brazil. This project was submitted to the Research Ethics Committee (REC) of the Universidade do Oeste de Santa Catarina (Unoesc) and approved under consult substantiated opinion no. 2.578.688.

As inclusion criteria in the study, we considered: to be an adult older than 18 years; did or did not receive treatment for hypercholesterolemia; and have time availability to the collections established. Volunteers younger than 18 years and those who did not attend the pre-collection procedures were considered as exclusion criteria. The volunteers enrolled signed a free and informed consent form (ICF) and completed a sociodemographic questionnaire that addressed questions that involved data related to gender, age, physical activity – days per week –, family history of cardiovascular disease, if has already been diagnosed with cardiovascular disease, if is taking any medication – which medications and the treatment time – and if has any dietary restriction.

The sample was obtained through two blood samples for each patient, performed at the Laboratório de Análises Clínicas Burlab S/c Ltda, by the pharmacist Claudia Regina Colla. The first collection was performed under 12 hours fasting and the second, on the same day, under postprandial conditions, that is, two hours after lunch. The tubes for blood collection were identified, and then the collection was performed by venipuncture with prior asepsis, obtaining a quantity of 5 ml of blood per sample, using a 25 × 7 mm needle with a syringe with a total volume of 5 ml. The collected blood was then transferred to a clot activator tube, already identified at the beginning of collection. At the end of the procedure, syringe and needle were discarded in a sharps disposal container; a stopper was placed at the puncture site. After the collection, the volunteers were informed about the date of report release, but only the lipidogram results corresponding to the fasting values were released.

Tubes containing the blood samples were centrifuged for 5 minutes at 3,000 revolutions per minute (RPM). After obtaining the serum, the biochemical analyzes were started. Two internal quality controls were measured before beginning the analysis for quality control evaluation of all the procedures and parameters analyzed. The equipment used for analyzes was the SX-160 from Sinnowa Brasil® automatic biochemical analyzer, which uses the primary tube. Measurements of total cholesterol and TG were determined by the colorimetric enzymatic method; the HDL-c, by the direct method, using Biotécnica® reagents; the LDL-c, in turn, using the formula recommended by Friedewald et al. (1972)(15).

All the results obtained were compiled and presented in graphs. Qualitative variables were presented in a descriptive way. Quantitative biochemical variables were analyzed by paired samples t-test for using GraphPad Prism® software. The statistical difference was considered significant when \( p < 0.05 \).

RESULTS

From the 50 volunteers who participated in the study, 23 (46%) were male and 27 (54%) were female, age ranging from 22 to 86 years and median of 32 years.

According to the sociodemographic questionnaire filled out by the volunteer participants, it was observed that 15 (30%) individuals performed physical activities, including walks one to three times a week; 35 (70%) volunteers did not engage in any type of physical activity. Regarding cardiovascular diseases, it was found that 14 (28%) participants had no history of this disease and 36 (72%) had relatives with some cardiovascular disease, with a close relationship (father, mother and siblings). From these 36 (72%), 10 (27.7%) were already diagnosed with any cardiovascular disease; among them, four (40%) use medication: atorvastatin 90 mg and simvastatin 10 mg.

Regarding the diet, 40 (80%) volunteers admitted not to make dietary restriction; only 10 (20%) make restriction, avoiding fries and fats.

The results obtained from the lipid profile were analyzed according to the data found in the Brazilian Guideline for Dyslipidemia and Atherosclerosis (2017), under metabolic conditions of fasting and postprandial, comparing them in relation to gender, with or without diagnosis of hypercholesterolemia and use of drugs.

Figure 1 shows the values of serum TG (A and B), total cholesterol (C and D), LDL-c (E and F) and HDL-c (G e H) of all subjects evaluated in fasting and postprandial conditions, as well as when they were grouped according to sex. It can be observed that there was a significant increase in TG concentrations.
when the postprandial value was measured, both in the total population ($t_{(49)} = 7.653; p < 0.001$; Figure 1A) and in the men group ($t_{(20)} = 5.751; p < 0.001$) and in the women group ($t_{(28)} = 5.457; p < 0.001$) (Figure 1B). On the other hand, a significant decrease in LDL-c concentration was observed when evaluated in all volunteers ($t_{(47)} = 4.499; p < 0.001$; Figure 1E) and in the men group ($t_{(19)} = 6.151; p < 0.001$) and in the women group ($t_{(27)} = 2.342; p < 0.05$) (Figure 1F).

In addition to these values, a significant decrease in HDL-c concentration was also observed when fasting and postprandial results were compared, only in the women group ($t_{(28)} = 2.057; p < 0.05$) (Figure 1H). The concentrations of total cholesterol did not present significant difference between the groups.

Figure 2 shows the values obtained for the measurement of serum TG in men and women, with or without hypercholesterolemia. It was possible to observe that the increase of this lipid fraction in the postprandial period remained significantly in all groups evaluated.

There was a significant TG increase in men group during the postprandial period, both in the individuals with ($t_{(8)} = 4.880; p < 0.01$) and without ($t_{(5)} = 3.701; p < 0.01$) hypercholesterolemia (Figure 2A) and those who underwent treatment ($t_{(5)} = 3.459; p < 0.05$) or not ($t_{(8)} = 7.835; p < 0.05$) for this metabolic condition (Figure 2B). The same pattern of results was observed in women: significant TG increase in the postprandial period in those with ($t_{(13)} = 3.89; p < 0.01$) and without ($t_{(15)} = 5.151; p < 0.001$) hypercholesterolemia (Figure 2C), as well as those who underwent ($t_{(8)} = 3.889; p < 0.01$) or not ($t_{(6)} = 3.310; p < 0.05$) treatment for this metabolic condition (Figure 2D).

Regarding total cholesterol, there were no significant differences in the results of serum concentrations, either in women or in men with or without hypercholesterolemia, as well as in those who did or did not use medication (Figure 3).
Figure 4 shows the results obtained from the measurement of serum LDL-c in men and women with and without hypercholesterolemia. In the men group, it was possible to observe a significant decrease in this lipid fraction when measured in the postprandial period of the individuals with \( t(7) = 45.827; p < 0.001 \) and without \( t(11) = 3.758; p < 0.01 \) hypercholesterolemia (Figure 4A), as well as in those who underwent \( t(4) = 4.384; p < 0.05 \) or not \( t(2) = 11.59; p < 0.01 \) treatment for this metabolic condition (Figure 4B).

Regarding the women group, only a significant decrease in postprandial LDL-c concentration was observed in volunteers without hypercholesterolemia \( t(14) = 3.172; p < 0.05 \) and in those women which do not receive any treatment for this metabolic condition \( t(2) = 4.856; p < 0.05 \) (Figure 4C). The data represent the mean ± standard error of mean (paired samples t-test).

Finally, no significant differences were observed in fasting and postprandial HDL-c serum concentrations results in patients with or without hypercholesterolemia of both sexes evaluated and in those who did or did not take medication for this pathology (Figure 5).
In the present study, the sociodemographic characteristics and the lipid profile were evaluated in two metabolic periods: 12-hours fasting and postprandial period, from 50 volunteers of both sexes, with or without diagnosis of hypercholesterolemia. Discrepancy was observed mainly when compared the biochemical findings in the serum concentrations of LDL-c and TG, which were higher in the postprandial period. Moreover, total cholesterol and HDL-c showed little variation when comparing the fasting and postprandial states results.

In Brazil, atherosclerotic cardiovascular disease is considered a serious public health problem due to the large number of individuals affected and the lack of knowledge on this pathology, or even the lack of guidelines and appropriate treatment, increasing the risks for heart diseases development\(^{(16)}\). In this context, dyslipidemia is considered a risk factor for the development of cardiovascular diseases due to the alteration in lipoproteins amount, represented by the increase and/or decrease of the particles, besides currently being recognized as a common metabolic dysregulation\(^{(17)}\). More accurately, the biochemical determinations of TG, total cholesterol and HDL-c and LDL-c fractions constitute the lipid profile broadly, which is important for the analysis of possible acute coronary syndrome in individuals\(^{(18)}\).

Regarding the measurement of serum TG, an increase was observed in our study when evaluated during the postprandial period that is 2 hours after food intake. According to a consensus, because of this circumstance, 10 to 12-hours fasting is suggested for the sample collection and subsequent measurement, since there may be possible variations between 25% and 50%\(^{(18)}\).

According to Pereira et al. (2014)\(^{(19)}\), the variation of the fatty acids consumed and the relation regarding the increased risk of cardiovascular diseases, as well as the plasma concentrations of lipids and lipoproteins have been widely demonstrated in several experimental and population studies. Furthermore, some studies have shown hypertriglyceridemia as an independent risk factor for coronary heart disease, since it directly contributes to the atherogenesis of TG-rich lipoproteins, particularly very low-density lipoproteins (VLDL)\(^{(20)}\). However, according to the Brazilian Society of Cardiology (Sociedade Brasileira de Cardiologia, 2017), hypertriglyceridemia may be an easier to control disorder because it depends on lifestyle, diet (intake of simple carbohydrate in general) and frequency of any physical activity. TG levels elevation is hardly found isolated; it is always accompanied by increased total cholesterol and LDL-c and decreased HDL-c. On the other hand, the increase in TG and cholesterol may be associated with a genetic disease of lipoprotein metabolism, which is does not dependent on diet. Familial hypercholesterolemia (FH) is an autosomal dominant hereditary disease; the diagnosis is based on clinical and laboratory criteria. This condition is characterized by the increase of plasma LDL-c particles, which are susceptible to oxidation and contribute to the formation of atherosclerotic plaque\(^{(18)}\).

Corroborating our study, Schiavo et al. (2005)\(^{(21)}\) measured TG in subjects of both sexes on different days of the week and showed that the measures on Monday were higher than those on Thursday, due to the weekend diet, with intakes of more greasy
foods, usually differs from that during the week. This finding allows us to conclude that only the 12-hour fasting is not sufficient for a reliable analysis of the patient’s actual conditions in relation to TG measurement. Likewise, it was possible to observe that TG changes in the present study do not show the actual condition of the patient in the postprandial collection, highlighting that this collection should not be performed in patient without prior fasting state. As a non-drug alternative, studies have shown that reducing dietary carbohydrate intake leads to lower total caloric intake and that carbohydrates could be substituted for protein, which would assist in weight loss, resulting in metabolic changes, improving lipid variables and reducing plasma levels of TG, VLDL, total cholesterol and LDL-c\(^2(22)\).

Regarding total cholesterol measures, hypercholesterolemia, which is an independent factor for atherosclerosis, would be an explanation for the fact that this molecule did not present its value significantly changed when compared to both fasting and postprandial collections, since, although it is also associated with caloric food intake, it is mainly related to non-controllable factors, such as age, race and heredity\(^2(23)\). Thus, the early recognition of patients with familial hypercholesterolemia assists in the reduction of morbidity and mortality using adequate guidelines and necessary therapeutic measures\(^1(19)\).

Furthermore, the increase in total cholesterol combined with LDL-c represents a close correlation with increased cardiovascular risk. Therefore, it is safe to say that the increase in LDL-c does not follow the fall or elevation of TG levels, since these two parameters are independent\(^1(19)\).

In this study, when evaluating the LDL-c fraction – cholesterol particles that, when in excess in the bloodstream, oxidize and enter the innermost layer of the artery wall, causing an inflammatory process (which favors vessel obstruction) and relatively increasing the risk for the progression of cardiovascular disease\(^2(24)\) – there was a significant variation after the second collection (postprandial). According to Poleancky (2005)\(^2(23)\), high concentrations of LDL-c are considered an important risk factor for cardiovascular diseases. In most cardiovascular events, with a few exceptions, the concentration of serum lipoproteins increases with the presence of smooth muscle cells highlighting the atherosclerotic lesion. The evolution of this process modifies the natural structure of the smooth muscle, which leads to tone and resistance loss. As the atherosclerotic plaque increases in size within the arteries, the lumen decreases, reaching a critical level, making blood flow difficult and leading to angina and other complications, until death\(^2(26)\).

According to Oliveira et al. (2010)\(^3(27)\), LDL-c levels are higher in males compared to females. We can associate this factor with abdominal fat and body mass index (BMI). However, the correlation between anthropometric indices, lipid profile and life habits in both genders result in risk factors for cardiovascular disease.

In this study, LDL-c levels did not show significant statistical variation after the second collection. According to Kelley et al. (2012)\(^2(25)\), LDL-c is the most sensitive indicator of the profile, as it responds quickly to diet and physical activity. Therefore, there is an inversely relation between TG and HDL-c levels, so that high TG levels tend to be associated with low HDL-c levels\(^1(23)\). Furthermore, serum levels of HDL-c represent a protective factor against atherosclerosis by removing oxidized lipids from LDL-c, inhibiting the attachment of adhesion molecules and monocytes to the endothelium, and stimulating the release of nitric oxide. Therefore, low levels of HDL-c are known as risk factors for cardiovascular disease\(^2(28)\).

HDL-c particles are formed in the liver, intestine and blood circulation and their main protein content is represented by the Apo AI and Apo AII proteins\(^2(29)\). As this lipoprotein is not present in the diet, this fact could explain the reason why it did not suffer alteration of the two collections performed in this study, regardless of fasting.

Studies have shown that the early diagnosis of changes in the lipid profile is beneficial, both for the identification of those individuals at greater risk, and for improving treatment and avoiding future cardiovascular diseases\(^2(26)\). For this purpose, the minimum fasting requirement was established in the literature and in the current norms, used for the majority of the laboratory tests, varying according to the particularities of each test. Thus, fasting was defined as a period of 8 to 12-hours with no food intake, only water, because it is understood that the consumption of food hours before the collection of laboratory tests can alter its results, particularly those whose methodology is sensitive to turbidity\(^1(14)\).

As we can observe in this study, some fractions underwent changes when the patient was not fasting adequately, which resulted in higher values and did not show the real condition of the organism, which could lead to inadequate or even unnecessary treatment. Although the data presented refer to a single locality and to a particular socioeconomic group, the present study is important for the contribution to the national data, mainly due to the lack of literature on studies focusing on the lipid profile related to fasting, as well as on possible variations in the fractions, which were also demonstrated.
CONCLUSION

Considering the results of this study, it was observed that total cholesterol and HDL-c are the components of the lipid profile that could be measured with no previous fasting, since they did not presented significant alterations among the individuals evaluated. However, studies that discuss these criteria are still scarce, so further studies on a larger and more heterogeneous population are needed, including socioeconomic, dietary and lifestyle differences. In addition, it was verified the need for health promotion actions to improve the quality of life of the study population.

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ACKNOWLEDGEMENTS

The authors declare no conflicts of interest and claim to be responsible for the content and writing of this manuscript.

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