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Standardization of collection requirements for fasting samples For the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

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ABSTRACT

Standardized protocols for patient preparation for laboratory testing are currently lacking. Moreover, a great heterogeneity exists in the definitions of "fasting" currently being used among healthcare workers and in the literature. Marked metabolic and hormonal changes occur after food ingestion, mainly due to the absorption of fluids, lipids, proteins, carbohydrates and other food constituents. This postprandial response varies markedly in response to numerous factors, such as eating behavior, food composition, fasting duration, time of the day, chronic and acute smoking, coffee and alcohol consumption. It is therefore crucial to minimize the total variability by controlling as many of these modifying factors as possible. Control of the abovementioned effects on postprandial response can only be achieved by standardizing the way patients are prepared for laboratory testing, i.e. by defining the fasting duration, as well as what is and what is not allowed (e.g., coffee, tea, smoking, water) during the period of fasting prior to sample collection. The aim of this article is to describe the range of effects of different approaches to fasting on laboratory tests, and to provide a framework for the harmonization of definitions for fasting requirements for laboratory tests.

1. Introduction

The pre-analytical phase is the major source of various factors potentially influencing the results of laboratory testing [1]. Pre-analytical errors can occur due to inappropriate test ordering, errors in patient preparation and identification, sample collection, transport and delivery to the laboratory, as well as in sample handling and storage. Most of these activities are performed outside the laboratory environment and out of the direct supervision of laboratory staff. Therefore, laboratory personnel and clinicians are quite often unaware of such variability with possible detriment to the quality of test results. If they go unrecognized, pre-analytical errors can increase healthcare costs and affect the quality of patient care by causing unnecessary delays and diagnostic errors.

To reduce the frequency of error, it is essential to standardize procedures, implement evidence-based policies and introduce continuous quality improvement. Unfortunately, pre-analytical procedures are neither fully standardized, nor harmonized worldwide.

Several standards exist for blood sample collection and handling procedures [2–4]. Nevertheless, the estimated degree of adherence to these guidelines is unacceptable [5–8]. Furthermore, several recent reports have shown a clear need for revision of the guidelines in terms of redefinition of the time needed for alcohol to dry after cleaning the venipuncture site [9], patient identification procedures [10,11], application of tourniquet [12,13] and sample mixing [14]. The Croatian Society of Medical Biochemistry and Laboratory Medicine has recently published the partially revised CLSI (Clinical and Laboratory Standards Institute) recommendations as a national standard for venous blood sampling, where several of these factors have been addressed [15]. Due to the abovementioned considerations, the pre-analytical phase is currently among the greatest challenges for laboratory professionals and for the healthcare system as a whole [16].

Although it has long been known that various 'controllable' factors such as diet, physical activity, smoking and alcohol consumption may affect laboratory test results, there is still a lack of standardization of patient preparation for laboratory testing. One of these controllable factors is the fasting of patients prior to sample collection for selected testing. However, a great heterogeneity exists in the definition of "fasting"

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currently being used by different healthcare facilities, as well as in the scientific literature [17]. Due to the recognized influence of the effect of fasting on the concentration of many laboratory parameters, it is absolutely essential to standardize and harmonize the fasting requirements for laboratory testing.

The aim of this article is to describe the wide range of effects of different approaches to fasting on laboratory tests, and to provide a framework for harmonization of the definitions of fasting requirements for laboratory tests.

2. Postprandial state

In response to the ingestion of food, marked metabolic and hormonal changes occur, principally due to the absorption of fluids (water and/or alcohol), lipids, proteins, carbohydrates and other food constituents. The period immediately after the meal is called the "postprandial state" and is characterized by oxidative stress, inflammation and endothelial dysfunction [18]. Unfortunately, because of eating habits and easy access to food, most individuals in developed countries spend much of their day in a postprandial state, a state of abrupt homeostatic imbalance.

Marked changes of numerous biochemical and hematological markers in response to the postprandial state have been widely documented. Most pronounced and clinically significant biochemical changes are observed up to 4 h after a meal for triglycerides, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, sodium, magnesium, potassium, C-reactive protein (CRP), uric acid and total bilirubin [19].

As little as 1 h after a meal, the lymphocyte count shows significant decreases and this effect is even more pronounced 2 h afterwards. The largest clinically significant variations in neutrophils, eosinophils, red blood cells, hematocrit and mean corpuscular hemoglobin (MCH) occur up to 4 h after ingestion of food [20].

The postprandial response varies markedly in response to numerous factors. It has been demonstrated that eating behavior and food composition can modify the degree of postprandial metabolic changes and level of oxidative stress. For example, regular consumption of phenol-rich vegetables may be beneficial in reducing postprandial oxidative stress [21]. Moreover, fasting duration and time of day also affect biochemical measurements [22]. It is therefore crucial to standardize not only the total fasting time, but also the exact time of day when blood sampling is performed, although this may be unfeasible when STAT/emergency testing is required for diagnosing urgent conditions [23]. Various pathological states, conditions such as prolonged starvation and various comorbidities like diabetes may modify the individual response to food ingestion. Blood groups B and O have been reported to alter the postprandial response of alkaline phosphatase (ALP) to a high-fat meal in Japanese individuals [24]. Marked differences in postprandial triglyceride response have been described in individuals with APOA5-1131T/C and hepatic lipase (HL)-250G/A polymorphic variants [25,26].

The effects on postprandial glycemic reactions of adding a glass of water (300 mL) to a meal after a 12 h fast were found to increase the peak blood glucose and serum insulin levels in healthy subjects, and the blood glucose concentration in well-controlled diabetic patients. Altering the physical property of a meal by dilution with water can thus affect the physiological responses [27]. Although we concur that recommendations should include a definition of the maximum allowed volume of water which an individual may drink on the day preceding the phlebotomy, it is difficult to make some general recommendation of the exact volume. We therefore believe that the ingested volume of water should mirror the usual daily ingested water volume of each individual.

Chronic and acute smoking, along with alcohol consumption are associated with changes in postprandial response. A marked increase in the triglyceride-rich lipoprotein metabolic rate has been observed immediately after smoking a single cigarette [28]. This mechanism is thought to account for increased susceptibility to atherogenesis in smokers. Even the stress associated with hospitalization of patients can lead to higher fasting glucose results. Coffee has also been shown to acutely increase glucose concentration. Fasting blood glucose increases by almost 12% within 1 h after the consumption of one 12 oz (about 350 mL) café latte [29,30]. This effect is more evident among females and overweight individuals [31].

Given the large heterogeneity of individual responses to food ingestion, it is crucial to minimize the total variability by controlling as many modifying factors as possible. Control of the abovementioned effects on postprandial response can only be achieved by standardizing the way patients are prepared for laboratory testing, i.e., by defining the fasting duration as well as what is and what is not allowed (e.g. coffee, tea, smoking, water) during the period of fasting prior to sample collection.

Some might dispute the need for fasting to be overnight with blood samples collected between 7 and 9 a.m. Moreover, if there is no diurnal variation, can the collection be performed at any time, as long as the patient fasts the recommended time period (e.g. 06:00–18:00 with collection at 18:01)? In theory at least, fasting need not be overnight if there is no diurnal variation, as long as the fasting time recommendation is followed. It is however inconvenient to fast during daytime and to draw blood at other times as other analytes are often included with the blood request. Many analytes with diurnal variation have their reference values set at 7–9 a.m. and their reference change values need to be calculated for samples drawn at the same time of day.

3. Existing recommendations

Although the Clinical and Laboratory Standards Institute (CLSI) H21-A5 Guideline does provide detailed recommendations for collection, transport and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays, there is no mention whatsoever about patient preparation in terms of fasting requirements [3]. CLSI H3-A6 guideline for collection of diagnostic blood specimens by venipuncture does state that patient diet restrictions should be verified, due to the fact that some tests may require a patient to fast [2]. Moreover, the guideline states that time and diet restrictions may vary depending on the test. However, the document does not specify the exact requirements for specified tests or groups of analytes, assigning the responsibility to the healthcare institution to define fasting requirements.

The World Health Organization (WHO) Guidelines on Drawing Blood give thorough recommendations on best practices in phlebotomy [4]. However this document does not contain any indication about the necessary requirements for the fasting state of patients. The only mention is a generic question, viz "*Have you eaten or drunk anything in the past two hours?*", but no indications are then provided should the patient reply "*yes*".

Obviously, according to the existing guidelines and recommendations issued by competent international authorities, the exact definition of fasting requirements is therefore left within the jurisdiction of every single institution.

By searching the Internet for available instructions, one can find a great heterogeneity of stated fasting requirements. Table 1 illustrates different recommendations available on the several national Lab Tests Online (LTO) web pages, which are the most commonly visited health sites on the Internet, used regularly by patients, laboratory professionals, physicians, and other healthcare workers who need to confirm their understanding of the various characteristics of the laboratory tests [32].

It is clear that definitions of fasting requirement differ from one LTO site to another, in terms of:

- definition of fasting time
- whether fasting is required or not for a certain analyte
- whether water is allowed during the fasting period.

Table 1
Recommendations available at several national LTO Internet sites for fasting requirements for some serum/plasma blood tests.

	Glucose	ALP	Triglycerides	Bilirubin	Cortisol	Homocysteine	C-peptide
USA	8 h fast is recommended (nothing to eat or drink except water)	Fasting is preferred but not required for this test.	9–12 h fasting is recommended (only water is permitted, alcohol should not be consumed for 24 h before the test)	Fasting requirements vary by laboratory. Ask your lab or health care provider for instructions. You may need to fast (nothing but water) for several hours before the test.	No requirements	Fasting for 10 to 12 h may be required prior to blood testing.	Fasting for 8 to 10 h
UK	8 h fast is recommended	Fasting is preferred but not required for this test	9–12 h fasting is recommended (only water is permitted, alcohol should not be consumed for 24 h before the test)	No requirements	No requirements	9–12 h fasting is recommended classical homocysteinuria	Varies according to local laboratory clinical practice
Australia	8–10 h fast is recommended	Fasting overnight is recommended[a]	10–16 h fasting is recommended (only water is permitted, alcohol should not be consumed for 24 h before the test)	No requirements	No requirements	Fasting for 10 to 12 h	You may need to fast
Germany	12 h fast is recommended	Fasting overnight is recommended[a]	12–14 h fasting is recommended (only water is permitted, alcohol should not be consumed for 24 h before the test)	No requirements	No requirements	No requirements	No requirements
Czech Republic	8–10 h fast is recommended	Fasting is recommended	12–14 h fasting is recommended (only water is permitted, alcohol should not be consumed for 24 h before the test)	No requirements	No requirements	10–12 h overnight fast prior to collection	No requirements
Italy	8 h fast is recommended (nothing to eat or drink except water)	No requirements	8 h fast is recommended.	No requirements	No requirements	No requirements	No requirements

[a] Eating a meal can increase alkaline phosphatase (ALP) slightly for a few hours in some people.

Moreover, LTO instructions provide no information on restrictions for smoking and caffeine consumption although it is well known that many laboratory tests are affected by either caffeine or cigarette smoke (e.g., platelet count and function, some hemostasis assays) [33].

Many other web resources are available where patients can seek and obtain information on how to prepare for laboratory testing. Health institutions usually have their own instructions for patient preparation. As in the case of the LTO example, those institutional instructions also vary greatly even within the same country. The observed lack of harmonized requirements for fasting is a growing problem due to the increasing patient mobility.

4. Patient adherence to fasting recommendations

Another hurdle for successful standardization of fasting requirements is poor patient knowledge and awareness about the need to prepare for laboratory testing and the potential effect of diet, physical exercise, stress, smoking, alcohol and other modifiable factors on results of laboratory testing.

Kackov et al. have recently published the results of their anonymous survey study on consecutive samples of 150 outpatients older than 18 years, who were admitted to the laboratory in the morning between 7 and 9 am for routine blood testing to one medical laboratory in Zagreb, Croatia [34]. Their principle aim was to assess whether patients know how to prepare properly for laboratory tests and their level of compliance with existing institutional recommendations. They also investigated the preferred means by which patients were informed about preparing for laboratory testing. Interestingly, the authors found an unsatisfactory level of patient knowledge about fasting requirements and poor adherence to available recommendations. Those patients who self-declared as being informed about fasting requirements, have most often been informed by the requesting physician or a nurse, but without any explanation about what the fasting state actually means. Only a few patients obtained further instructions via the Internet or other written instructions.

Another survey study by Kljakovic [35] was performed on 135 participants attending two hospital blood collection centers in Canberra, Australia, to evaluate patient understanding of diagnostic tests. The results of that study also demonstrate a lack of knowledge about how to prepare for the blood test (e.g. the need for fasting) in a substantial proportion of participants. Out of those who self-reported to have received information on proper preparation, the majority had received instructions from their doctor. One quarter of study participants claimed that they knew themselves how to prepare for lab testing, possibly even more concerning because most patients were not competent to define what is right or wrong for their health. Patients were more likely to be aware of preparation requirements if they knew the general practitioner who ordered the test.

Patient awareness about the importance of the proper preparation for laboratory testing is not adequate and should be improved. With their recently published study, Miler and coworkers have shown that patients are mostly unaware of the importance of proper preanalytical procedure for urine collection [36]. Moreover, even when they were informed by their physicians or laboratory staff, patients were not collecting their samples according to obtained instructions. This clearly shows that patients are not well informed about the importance of the proper preparation for laboratory testing, an issue that needs to be considered seriously. Ways to improve patient knowledge and awareness should therefore be urgently identified.

5. Conclusions

The postprandial response to food and drink is an interplay of numerous factors such as age, gender, type of diet, genetics, blood group, chronic and acute smoking, alcohol consumption, body mass and

many others. Each of those factors has a moderate but additive effect on laboratory test results. These factors have previously not been included in publications estimating the contribution of pre-analytical uncertainty to variability of laboratory test results, except for glucose measurements [37]. Some analytes can be affected by metabolic and hormonal effects occurring in the postprandial state whereas others can be influenced by the interfering effect of postprandial lipemia due to increased triglyceride concentration up to 9–10 h after a fatty meal.

The question of standardization of fasting protocols also includes collection of salivary cortisol, where rigorous sampling protocols are needed to correctly interpret test results. There is an emerging role for the use of salivary cortisol in diagnosing adrenal insufficiency [38], particularly in conditions associated with low cortisol-binding globulin levels, and in monitoring of glucocorticoid replacement [39].

The fasting state is not the only pre-analytical variable which has potential effects on the results of laboratory testing. Every step within the total testing process is prone to error and those errors have an additive effect in contributing to the total uncertainty of the laboratory result. The critical steps of the pre-analytical phase need to be identified and standardized [7]. To minimize total pre-analytical variability, standardization of patient preparation, prior to blood sampling is therefore essential for routine laboratory testing. Without such precautions, patient longitudinal data cannot be used neither for comparison nor for medical decision making.

6. Proposed recommendations

1. Existing guidelines for phlebotomy need revision. Revised recommendations should include the exact definition of requirements for patient preparation for laboratory testing. Blood for all blood tests should be drawn preferably in the morning from 7 to 9 a.m. [30]. Fasting should last for 12 h, during which water consumption is permitted. Alcohol should be avoided for 24 h before blood sampling. In the morning before blood sampling, patients should refrain from cigarette smoking and caffeine containing drinks (tea, coffee, etc.).
2. Professional associations (IFCC, EFLM and other) should support harmonization efforts by disseminating standardized recommendations for fasting.
3. Laboratories worldwide should implement standardized procedures for blood sampling and patient preparation.
4. Laboratories should have policies for sample acceptance criteria related to fasting samples. Blood samples for routine testing should not be taken if a patient has not been appropriately prepared for sample collection. 'No sample is better than a bad sample' should always be the leading principle.
5. Laboratory professionals are responsible for disseminating information about fasting requirements to patients as well as to clinicians and general practitioners who are the preferred source of information for patients.

By implementing standardized policies, harmonization is possible within the healthcare sector. Moreover, such standardization would allow harmonized reporting of scientific data in the field of laboratory diagnostics.

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