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A determination of the pre-analytical storage conditions for insulin like growth factor-I and type III procollagen peptide

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Abstract

Objective: IGF-I and type III procollagen (P-III-P) have been proposed as markers to detect GH abuse. This study aims to determine whether the pre-analytical storage temperature or delayed centrifugation affect the measured IGF-I and P-III-P concentrations.
Design: Observational study.
Setting: Wellcome Trust Clinical Research Facility, Southampton.
Subjects: Nineteen healthy volunteers.
Intervention: Blood was collected into bottles containing a clotting agent, lithium heparin or EDTA. One sample from each group was centrifuged and stored at −80 °C (control sample). The remaining samples from each group were stored as either serum or whole blood at 4 °C or room temperature for up to five days prior to storage at −80 °C.
Outcome measures: IGF-I and P-III-P.
Results: The storage temperature or timing of centrifugation did not appear to affect IGF-I concentration. In contrast, the measured P-III-P concentration rose by 6.5–7% per day in clotted and lithium heparin samples when stored as whole blood ($p < 0.006$) or serum (6.2–6.5% per day) at room temperature ($p < 0.001$). P-III-P did not change when the samples were stored at 4 °C. Although collection into EDTA inhibited the rise in P-III-P, the baseline measured values were significantly higher than in other media and spiking experiments demonstrated that EDTA exerted a significant matrix effect on the assay.
Conclusion: While the optimum collection method is immediate centrifugation and storage at −80 °C, it would seem acceptable to store serum or clotted blood samples at 4 °C, but not ambient temperature, for up to five days. It is incumbent on the anti-doping authorities to provide facilities to allow this.
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1. Introduction

There is widespread anecdotal evidence that growth hormone (GH) is used by athletes for its anabolic and lipolytic properties to improve their performance. The

Abbreviations: IGF-I, insulin like growth factor-I; P-III-P, type III procollagen peptide; GH, growth hormone; EDTA, ethylenediamine tetraacetic acid; IRMA, immunoradiometric assay.

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GH-2000 project proposed a test to detect GH abuse based on the measurement of two GH-dependent markers, insulin like growth factor-I (IGF-I) and type III procollagen peptide (P-III-P) [1]. Prior to the introduction of testing for doping with GH with these markers, it is necessary to determine the stability of these markers over time and in various storage and transportation conditions in order to minimise pre-analytical variability of samples [2]. Pre-analytical handling conditions, such as the choice of collection medium, storage and transportation temperatures, are especially important when sampling is performed in a non-clinical setting and requires transportation to an off-site laboratory.

Post competition and out-of-competition drug testing of athletes is performed where the provision of a fridge or freezer, dry or wet ice or centrifuge at individual venues may not be routinely available. If storage and transportation conditions affect IGF-I and P-III-P concentrations, the anti-doping authorities will need to provide equipment and train personnel to guarantee the optimal storage and transportation of samples to minimise the risk of false positive results.

The established recommendation in clinical practice is that samples should be separated as soon as possible after sufficient time for clot formation with a maximum time limit of 2 h. This time limit prevents biological processes such as transmembrane diffusion of separated serum substances and enzymatic cleavage of the analyte and binding proteins [2].

As IGF-I and P-III-P are protein bound *in vivo* and may be subject to enzymatic degradation *in vitro*, the aim of our study was to determine whether the collection medium, pre-freezing storage temperature or delayed centrifugation affected the measured concentrations of IGF-I and P-III-P.

2. Methods and materials

2.1. Study 1 – Effect of storage temperature and centrifugation timing

2.1.1. Subjects

Blood samples were obtained from four male and six female healthy volunteers (mean age 27.9 ± 2.5 years, range 21–40 years). Exclusion criteria included endocrine disorders affecting GH or marker concentrations, use of exogenous GH or age above 45 years. The study was approved by the Southampton and South West Hampshire Research Ethics Committee and all subjects gave written informed consent.

2.1.2. Sample collection and analysis

Approximately, 50 mL of whole blood were obtained by venesection from the antecubital fossa of each subject. This sample was divided equally into 21 sample tubes containing a clotting agent and separating gel (Becton/Dickinson vacutainer bottles, Franklin Lakes, NJ, USA) (Fig. 1).

2.1.3. Control sample

One control sample was immediately centrifuged at 3000 rpm at room temperature for 15 min. The serum was separated and immediately frozen at −80 °C.

2.1.4. Effect of storage temperature on serum samples

Ten clotted blood samples were centrifuged within 1 h of collection. The serum was separated and placed in plain aliquot bottles. Half of the serum samples were placed in the fridge at 4 °C and the remainder were left to stand at room temperature. The ambient temperature in the laboratory was approximately 20 °C. At 24, 48, 72, 96 and 120 h post-venesection, one sample from both temperature groups was transferred to the freezer and stored at −80 °C until analysis was performed.

2.1.5. Effect of storage temperature on whole blood

The remaining whole blood samples were divided into two groups and one kept at room temperature while the other was stored at 4 °C. At 24, 48, 72, 90 and 120 h post-venesection, one clotted sample from both temperature groups was centrifuged according to the study protocol. The serum was separated and stored at −80 °C until analysis.

2.2. Study 2 – Assessment of storage medium

2.2.1. Subjects

A further six male and three female healthy volunteers (mean age 22.1 ± 0.1 years; range 21.5–22.9) were recruited using the same inclusion and exclusion criteria.

2.2.2. Sample collection and analysis

Approximately, 50 mL of blood was obtained by venesection from the antecubital fossa of each volunteer. The sample was divided equally into 1 mL aliquots, a third of which contained a clotting agent (Becton/Dickinson vacutainer bottles, Franklin Lakes, NJ, USA), another third EDTA and the remaining third Lithium Heparin (Fig. 1).

A control sample and the effects of storage temperature and delayed centrifugation were examined as per the first study. The timings, however, were limited to 24, 48 and 96 h post-venesection in order to ensure that we complied with the ethics committee approval to take a maximum of 50 mL of blood.

2.2.3. Assays

Samples were transferred frozen to the Drug Control Centre Laboratory in King's College London where they were analysed. All samples were assayed after a single freeze thaw cycle and within two months of collection. Laboratory personnel were blinded to the storage

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