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Review

Measurement of testosterone and its sub-fractions in Canada

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Abstract

Interest in measuring bioactive testosterone in aging males has increased considerably in the last 5 years in Canada. Emerging andropause clinics have submerged our laboratories with requests for bioavailable testosterone (BAT) testing in replacement or addition to the traditional total testosterone (TT) and direct free testosterone (FT) assays. Beginning with a brief explanation of the bioavailability concept of Pardridge, this review examines the technical characteristics of various approaches currently available to measure TT and its sub-fractions. First, limitations in the measurement of TT, SHBG, and particularly direct (analog) FT assays are extracted from the scientific literature and recent external and internal QC reports. It is concluded that the free direct T assay is useless in the clinical context of andropause. The impact of the observed limitations of TT and SHBG measurements on calculated FT and BAT or BAT obtained by precipitation with ammonium sulfate is then discussed. A comparative evaluation of the advantages and disadvantages of calculated FT or BAT vs. precipitated BAT is presented before concluding that doing a TT as a first line test remains overall the most cost-effective measurement in the diagnosis of hypogonadism in males, and that this sole determination will be sufficient in over 75% of the cases.

Keywords: Total testosterone; Free testosterone; Bioavailable testosterone; SHBG; Andropause

Contents

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Introduction

Since the last 5 years, the interest of measuring circulating bioactive testosterone in aging males has increased considerably. This renewed interest has come almost exclusively from emerging andropause clinics largely supported by the pharmaceutical industry. Canadian laboratories have been submerged with a large increase of requests for bioavailable testosterone (BAT) testing in replacement (or addition) to the traditional total testosterone (TT) and direct (analog) free testosterone (FT) measurements commonly offered. This new trend in testing has created difficulties for many laboratories confronted with the development of a non-automated high volume homebrew test for which proper calibrators, internal and external quality control material are not available. In many provinces, health authorities are still waiting for more input before approving this test for reimbursement. Reference scientific societies like the AACC (NACB) or the CSCC have not yet produced guidelines on the subject. In this presentation, I will try to review some analytical aspects of testosterone measurement as used in Canada in 2005 and propose some ideas for eventual guidelines.

Physiological aspects of circulating testosterone

Even though discussions are still going on concerning the exact physiological significance of each circulating testosterone sub-fraction, there is at least a general agreement on their relative proportions. Fig. 1 shows a typical distribution diagram that can be found in many endocrinology textbooks. Circulating testosterone is distributed in three principal sub-fractions: in males, approximately 50% of circulating testosterone is strongly bound to SHBG (sex hormone binding globulin), a specific sex steroid binding protein synthesized in the liver. Another 50% is loosely and non-specifically bound to albumin, thus leaving a very small 2% of free hormone [1]. In females, a higher level of SHBG is responsible for the tight binding of roughly 80% of circulating testosterone to this carrier protein leaving about 20% bound to albumin and, again, a small 2% unbound or free testosterone. Historically, the full bioactivity of circulating testosterone has been attributed in males and females to the small free fraction, directly or through its intracellular transformation into dihydrotestosterone.

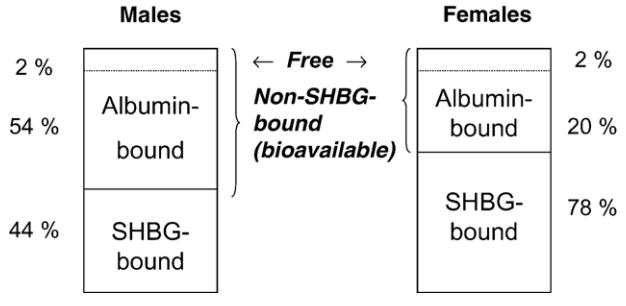

Fig. 1. Distribution of circulating testosterone in males and females.

Bioavailable testosterone: the Pardridge hypothesis

The association between the free fraction and bioactivity has constituted a dogma in endocrinology since the first evidence gathered over 50 years ago [2]. For testosterone, this hypothesis was already questioned in the early 60s when it was realized that the dissociation half-time of albumin-bound testosterone was of the order of 1 s compared to 20 s for SHBG-bound testosterone. It was proposed that such a short dissociation time could allow part of the albumin-bound fraction to be available for tissue uptake [3]. In the early 1980s, Pardridge and Manni [4–6] indicated, based on kinetic studies, that many albumin-bound and even some SHBG-bound steroids were available to tissues. The Pardridge hypothesis was largely based on first pass kinetic experiments involving rapid injection of various tritium-labeled steroids with or without carrier proteins through the carotid artery of rats or rabbits. These injections were followed by autoradiographic evaluation of the extra-vascular distribution of the injected substances. Animals were sacrificed within 15 s of the injection in order to eliminate contamination of the tracer via peripheral circulation.

Based on this unidirectional extraction model, Pardridge demonstrated that in the absence of carrier proteins, brain extraction of testosterone was very high, as expected, and attained nearly 80% of the injected dose. Brain extraction fell to zero with the addition of large amounts of SHBG to the steroid mixture. However, Pardridge showed that in the presence of large amounts of albumin, in conditions where the free testosterone fraction was theoretically negligible, the