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Determination of IGFs and their binding proteins

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The worldwide clinical and scientific interest in peptides belonging to the insulin-like growth factor (IGF) system has brought along a call for standardization of assays used to quantify the different IGF related proteins. This relates in particular to the measurement of IGF-I, which has stood the test of time as an important biochemical tool in the diagnosis and treatment of growth hormone (GH) related disorders. The first international consensus statement on the measurement of IGF-I in 2011 represents an important milestone and will undoubtedly improve commutability of reference ranges for IGF-I and clinically applicable cut-off values. By contrast, there is no consensus addressing the measurements of the other IGF-related peptides. Nevertheless, measurement of these peptides may be of interest, either as additional tools in GH disorders or as prognostic biomarkers of various diseases. Therefore, standardization of assays for the other IGF-related peptides is highly relevant. This chapter discusses the recent consensus on IGF-I measurements and how this approach may be applied to measurement of the other IGF-related peptides. In addition, assay pitfalls, pre- and post-analytical challenges, alternative methods for IGF-I measurements and potential assays of tomorrow will be discussed.

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Introduction to the insulin-like growth factor system

The established components of the IGF system include IGF-I and the structurally related IGF-II, six high-affinity IGF-binding proteins (IGFBPs; IGFBP-1 to -6) and the acid labile unit (ALS). Measurement of serum IGF-I levels has stood the test of time as an important tool in the diagnosis and treatment of growth hormone (GH) related disorders [1], making IGF-I by far the most clinically relevant peptide within the IGF system. This may explain why the consensus does not address the measurement of other IGF-related proteins [2]. Nevertheless, it appears that many of the other IGF-related peptides may also have some clinical relevance, either as additional tools in GH disorders (IGFBP-3 and ALS) [1], or as prognostic biomarkers of various diseases, for instance cancer (IGFBP-2) [3], type 2 diabetes (IGFBP-1) [4] and atherosclerosis (IGFBP-4 fragments) [5]. Therefore, standardization of assays for the other IGF-related peptides is highly relevant.

The IGFBPs bind close to 99% of the circulating IGFs with high affinity, hereby affecting the half-life of the circulating IGF-pool as well as its tissue accessibility [6,7]. Although the IGFBPs usually inhibit the IGFs from activating their receptors *in vitro*, numerous *in vivo* studies have demonstrated that the IGFBPs may also potentiate IGF-mediated actions. For this reason the IGFBPs are often referred to as modulators of IGF-action [8–11]. Additionally, *in vitro* experiments have demonstrated that the IGFBPs possess biological effects independent of the IGFs [12]. To further complicate the system, a number of IGFBP-specific proteases partake in the regulation of IGF-action. Following enzymatic cleavage the ligand affinity of the IGFBPs becomes markedly reduced, leading to dissociation of the IGFs, which hereby becomes accessible for receptor activation. Thus, enzymatic cleavage of the IGFBPs is considered to play a key role in controlling IGF-action [10,12].

IGF-I and IGF-II serve as ligands for the ubiquitously expressed, cell surface-associated IGF-I receptor (IGF-IR), albeit with different affinities [13,14]. Key downstream pathways following IGF-IR activation include the phosphatidylinositol 3-kinase (PI3K) pathway, which primarily favours metabolic, insulin-mimicking effects and the mitogen-activated protein kinase (MAPK) pathway, which primarily favours mitogenesis. Furthermore, IGF-IR activation prevents apoptosis. For further details the reader is referred to excellent reviews [15–17].

IGF-II also interacts with the insulin-like growth factor/mannose-6-phosphate receptor (IGF-IIR). This receptor is structurally and functionally distinct from the IGF-IR as it contains no intrinsic tyrosine kinase activity. One of the main functions of the IGF-IIR is to serve as an IGF-II scavenger, clearing and degrading IGF-II from the extracellular environment without activating intracellular signalling cascades. Thus, many, but not all, IGF-II actions may be explained by its interaction with the IGF-IR [18,19], and consequently, the IGF-IR may be considered as the primary target for both growth factors. For further information on IGF-II please see [20–23].

The clinical interest in IGF-I originates from its intimate association with GH, which is by far the most important regulator of IGF-I. Positive correlations between the integrated 24-h secretion of GH and serum IGF-I have been demonstrated in healthy prepubertal and pubertal children [24], healthy adults [25], GH deficient patients [25] as well as acromegalic patients before and after treatment [26]. These findings constitute the scientific rationale for using serum IGF-I as a marker of the spontaneous GH secretion as well as a marker of treatment, whether this involves replacement therapy with recombinant human GH or treatment with GH antagonists such as pegvisomant and somatostatin analogues. However, it is important to stress that only in selected patient cases serum IGF-I can serve as a standalone test in the diagnosis of GH deficiency [27], whereas serum IGF-I is performing better in the diagnosis of GH excess [1,28].

The relationship between IGF-I and GH is bidirectional, as IGF-I inhibits GH secretion at the level of hypothalamus and pituitary [29]. This bidirectional relationship has been clearly demonstrated in humans following administration of exogenous IGF-I, which leads to blunting of the GH secretion [30]. Insulin is the second most important regulator of IGF-I and stimulates IGF-I action via two different mechanisms. When the liver becomes exposed to insulin delivered via the portal vein, the hepatic synthesis of IGFBP-1 is rapidly suppressed. IGFBP-1 down regulates free and bioactive IGF-I *in vitro* and most likely this impacts IGF-I action *in vivo* [11]. Secondly, insulin stimulates the hepatic GH receptor density and consequently the hepatic sensitivity to GH [31]. The latter may explain why obese subjects, who are likely to be insulin resistant and hyperinsulinemic, are more sensitive to GH than lean subjects