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The effects of sex steroid replacement therapy on an expanded panel of IGF-related peptides

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Abstract

Background: Oral estrogen alone (EA) decreases concentrations of total IGF-I while increasing IGFBP-1, but data on other IGF-related peptides are inconsistent and/or sparse. Combined oral estrogen and progestin (EP) may have differential effects on IGF-related peptides dependent on its progestin-associated androgenic activity. The aim of this study was to clarify these relationships, as circulating IGF-related peptides are potential surrogates of predisposition to common chronic diseases.
Design: Using an open-labelled cross-sectional design within a bowel cancer screening trial (aged 55–64 years), we determined total IGF-I, IGF-II, IGFBP-2 and IGFBP-3 in fasted serum from 210 healthy women and free IGF-I (by ultrafiltration), insulin, IGFBP-1 and IGFBP-1:IGF-I binary complex in a selected subset of 92 women. Unadjusted and adjusted (using generalized linear models) means were compared.
Results: Among EA users, mean concentrations for total IGF-I (adjusted $P = 0.004$) and free IGF-I ($P < 0.001$) were reduced, whereas mean concentrations of IGFBP-1 ($P = 0.001$) and binary complex ($P = 0.01$) were increased compared with non-users. Taken as a whole group, EP use was not associated with differences in concentrations of IGF-related peptides, but on sub-group analyses, mean concentrations associated with the use of progestins with reduced androgenic activity reflected the use of EA. By contrast, mean IGFBP-2 concentrations were significantly reduced among both EA ($P = 0.008$) and EP ($P = 0.002$) users, irrespective of androgenic activity. Neither EA nor EP influenced mean concentrations of IGF-II, insulin and IGFBP-3.
Conclusions: The uses of oral sex steroid replacements are associated with significant changes in several IGF-related analytes in a preparation-specific manner, suggesting different regulatory mechanisms. However, the directions of these changes do not fit simple correlative models of predisposition to common diseases.

Keywords: Insulin-like growth factors; Binding proteins; Free IGF-I; Estrogen; Progestin; Cancer risk

1. Introduction

A number of studies have shown that hormonal replacement therapy (HRT) usage in women influences the growth hormone insulin-like growth factor (GH-IGF) axis [1]. Specifically, oral estrogen alone (EA) decreases concentrations of total IGF-I in both small-sized interventional designs [2–10] and larger cross-sectional settings [11–15], while also increasing IGF binding protein (IGFBP)-1 concentrations in interventional studies [4,8,9]. However, relationships with IGFBP-3 are inconsistent, being reported as either decreased [7,9,13],

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increased [16] or absent [3,4,8,14,15,17]. Studies determining serum levels of other IGF-related peptides – free IGF-I [8,9], IGF-II [4,9,12] and IGFBP-2 [16,18] – are few. On the other hand, reports of the relationships between combined oral estrogen and progestin (EP) and circulating IGF-related peptides are inconclusive, perhaps reflecting differential effects depending on the androgenic activity of the progestin used [9,10,19].

Accumulating evidence implicates circulating IGF-related peptides in the development of several common chronic diseases. Specifically, concentrations of total IGF-I in the upper physiological ranges are associated with increased risk of non-smoking related cancer (pre-menopausal breast, colorectal, prostate) [20–22], whereas low circulating levels are associated with increased cardiovascular risk [23] and osteoporosis [24]. On the other hand, high levels of circulating IGFBP-3 may be positively associated with risks of pre-menopausal breast cancer [20] and cardiovascular disease [23]. IGFBP-1, an insulin sensitive IGF binding protein, and an acute regulator of IGF-I bio-availability, is inversely related to the development of type 2 diabetes mellitus, particularly, in the presence of low IGF-I concentrations [25]. In turn, HRT usage impacts upon risk of several common diseases, and whilst HRT may modify disease predisposition directly through sex hormone-related pathways, interactions with the GH-IGF axis may offer alterative mechanisms of disease association.

Against this background, it seems timely to explore the inter-relationships between circulating IGF-related peptides, HRT usage, and predisposition to common chronic diseases. The aim of this study was thus to investigate the relationships between HRT preparation types and serum concentrations of an expanded panel of IGF-related peptides; the latter as potential surrogates of disease predisposition.

2. Materials and methods

2.1. Subjects and study design

An open-labelled cross-sectional design within a colorectal cancer screening trial was used. Blood samples were collected from 210 fasted post-menopausal women attending one centre (Christie Hospital NHS Trust, Manchester, UK) within the Flexi-Scope Trial between 1999 and 2001: a multicentre randomized trial to evaluate the effect of a "once only" flexible sigmoidoscopy in the prevention of colorectal cancer morbidity and mortality [26]. By trial protocol, all participants were ambulatory individuals aged 55 to 64 years invited by open invitation from general medical practitioner registries (from an original 350 000 people invited, 55% responded positively; and of those subsequently assigned screening, 71% attended) [26]. With Ethics Committee approval (ERP/97/065) and after obtaining informed consent, a trained researcher interviewed participants; demographic details, medical history and lifestyle factors were documented, weight and height were measured and body mass index (BMI) calculated.

The use of HRT was recorded in each female participant, and individuals categorized as never, former or current user (see footnote to Appendix 1). In current users, the type of preparation used, the mode of administration and the dose of medication were recorded (although doses were too heterogeneous to analyze). The specific constituents and formulations of each proprietary preparation of HRT were obtained from the British National Formulary (<www.bnf.org>). This information was used to categorize HRT usage in a similar manner to that used in the Million Women Study [27] as (i) non-users; (ii) oral estrogen alone (EA); (iii) combined oral estrogen and progestin (EP); (iv) oral gonadomimetics (GM); and (v) transdermal estradiol (TD). The numbers in the latter two groups were too small for statistical comparisons and are thus only presented in the baseline tabulation. The EP group was further sub-classified into two groups based on the androgenic activity of the progestin used: norgestrel and levonorgestrel as non-androgenic; medroxyprogesterone acetate (MPA) and norethisterone as androgenic [9,10].

Blood was obtained in clotted tubes and immediately transported to the laboratory. Serum was isolated by centrifugation at 3000 rpm for 10 min at room temperature and stored at −80 °C before analyte determination. Within the study, several quality control tests were performed, which demonstrated: (i) repeated analyte sampling over short periods in healthy individuals showed minimal variation; (ii) time from venepuncture to processing had little impact; and (iii) long-term stability at −80 °C storage (see *IGFs and Cancer* <www.christie.man.ac.uk/proinfo/departments/surgery/default.htm>).

2.2. Assays

Total IGF-I, IGF-II, IGFBP-2 and IGFBP-3 were measured in all 210 samples. IGF-I was measured, following acid–alcohol extraction, by an established radioimmunoassay (RIA) using a polyclonal rabbit antiserum (R557A) raised against purified human IGF-I as described elsewhere [28]. Serum IGF-II was determined using a commercially available immunoradiometric assay (IRMA) kit (DSL, Webster, Texas, USA). IGFBP-2 and IGFBP-3 were measured using a RIA and IRMA, respectively (DSL, Webster, Texas, USA) [29]. All samples were determined blind to HRT status,