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Interstitial fluid contains higher in vitro IGF bioactivity than serum: A study utilizing the suction blister technique☆

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ABSTRACT

Context: Circulating insulin-like growth factors (IGFs) are bound in complexes which affect their tissue-accessibility. Interstitial fluid is in close proximity to target cells, but the IGF-system is not well-described herein.
Objective: To perform a thorough comparison of the IGF-system in suction blister fluid (SBF) vs. in serum, with emphasis on bioactive IGF levels.
Design: Eight hour study including samples collected in the fasting state (20 h) and after a meal.
Setting: Clinical research facility.
Participants: Six healthy males (age 37.0 ± 8.8 years, BMI 22.5 ± 1.4 kg/m^2) (mean ± SD).
Main outcome measure: Serum and SBF concentrations of bioactive IGF (determined in vitro by specific IGF-I receptor (IGF-IR) phosphorylation assay), immunoreactive IGF and IGF binding protein (IGFBP) levels, Western ligand blotting (WLB) of IGFBPs and IGFBP-3 Western immunoblotting (WiB).
Results: The ability of SBF to phosphorylate the IGF-IR in vitro was 41 ± 27% higher than that of serum ($P = 0.007$ by repeated measures ANOVA). By contrast, immunoreactive IGF and IGFBP-concentrations were approximately 50% lower in SBF than in serum (all $P \leq 0.002$). A marked difference in the composition of IGFBPs between serum and SBF was observed, including 3-fold elevated amounts of IGFBP-3 fragments in SBF ($P < 0.001$). For both IGF-I, IGF-II and IGFBP-2, the effect of food intake differed between serum and SBF (all $P \leq 0.03$).
Conclusion: Despite lower concentrations, the in vitro IGF bioactivity was higher in SBF than in serum. This may relate to an increased enzymatic IGFBP-degradation and an altered IGFBP-composition in SBF, making more IGF-I and -II accessible to the IGF-IR. The impact of food intake on the IGF system differs between serum and interstitial fluid.

1. Introduction

In the circulation, the majority of IGF is bound in ternary complexes with IGFBP-3 and the acid-labile subunit (ALS). Ternary complex formation markedly extends the half-life of circulating IGF and reduces IGF action in vivo, most likely by reducing the ability of the IGFs to cross the endothelial barrier. This regulatory function of ALS has been elegantly demonstrated in rats, where ALS deficient animals were more susceptible to hypoglycemia following infusion of IGF-I:IGFBP-3 complexes than were normal rats [1]. Furthermore, the in vitro ability of IGF-I:IGFBP-3 complexes to cross cultured monolayers of human umbilical vein endothelial cells was diminished in the presence of ALS [2]. Thus, ALS appears to play a pivotal role in controlling the tissue accessibility of circulating IGF and consequently IGF action in vivo.

The ability of ALS to restrain circulating IGF within the vascular compartment indicates that extra-vascular IGF-levels are lower than their circulating counterparts. In accordance with this, early findings in dermal interstitial fluid evacuated by the suction blister technique have demonstrated that concentrations of IGFs and IGFBPs are 14–18% compared to their respective serum levels [3]. On the other hand, extra-vascular media, such as lymph, appear to be characterized by an increased proteolytic activity directed against IGFBP-3 [4]. An elevated proteolysis of IGFBP-3 has been reported to increase serum levels of free IGF-I as well as in vitro bioactive IGF [5,6], and accordingly, the extra-vascular bioactivity of the IGFs may be higher than what might be predicted from their concentrations. Supportive of this concept, we recently demonstrated that the ability to phosphorylate the IGF-I receptor (IGF-IR) in vitro was 4-fold higher in non-malignant ascites than in the corresponding serum [7]. Furthermore, the IGFBP

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composition and degree of IGFBP-3 proteolysis differed between ascites and serum. Accordingly, we speculated that the increased IGF bioactivity in ascites was secondary to an altered ratio between the IGFs and their IGFBPs combined with an increased IGFBP-proteolysis.

There are only sparse data on extra-vascular IGF and IGFBP levels, and therefore we undertook a thorough investigation of the IGF system within the interstitial compartment, obtaining corresponding measurements of IGF and IGFBP concentrations, IGFBP distribution by Western ligand blotting (WLB), IGFBP-3 proteolysis by Western immunoblotting (WiB) and, importantly, IGF bioactivity. Samples from the interstitial fluid were obtained using the suction blister method, and matched serum samples were drawn for comparison.

2. Materials and methods

2.1. Suction blister technique

This study utilized custom-made plastic containers with 28 (4 by 7) circular openings, each with a diameter of 5 mm. The 2 containers were fixed to the abdominal skin at the level just below the umbilicus and connected to a pressure regulated vacuum pump generating a suction of 300 mm Hg. After 90–120 min of suction, skin blisters were present corresponding to the 28 openings in each of the two containers. From these blisters a total volume of 1.5–2 mL of suction blister fluid (SBF) was harvested by a sterile syringe. A second collection site was contra-lateral to the first, i.e. at the same level on the abdomen. The first suction period lasted 103 ± 12 (mean $\pm$ SD) min, the second period 95 ± 19 min. The blister fluid was transparent with a faint yellow color and no visible traces of blood. The apparatus is shown in Fig. 1.

2.2. Study design

We recruited 6 healthy male subjects (age 37.0 ± 8.8 years, BMI 22.5 ± 1.4 kg/m^2). Participants fasted for nearly 24 h from noon until noon. In the morning (after approximately 20 h of fasting) the participants were admitted to the department and placed in the supine position while suction blister fluid (SBF) was collected. Hereafter, an ad libitum lunch was provided. A second suction was commenced 3 h after the end of the first SBF collection period. Blood samples were drawn at the beginning and at the end of each SBF collection.

The study was approved by the regional ethics committee. Written informed consent was obtained from each participant, and the study was conducted in accordance with the guidelines in The Declaration of Helsinki.

2.3. Analytical methods

Assays developed and validated by our laboratory were used for measurement of IGF-I and IGF-II, IGFBP-1 and IGFBP-2 [7]. Bioactive IGF was determined by an IGF kinase receptor activation (KIRA) assay [8]. In brief, cells transfected with the IGF-IR were stimulated with serum or rhIGF-I standards under physiological conditions (i.e. temperature 37 °C, pH 7.4). Subsequently, cells were lysed, whereafter the amount of phosphorylated IGF-IR was quantified in a time-resolved immunofluorometric assay. The assay integrates stimuli from IGF-I, IGF-II, intact IGFBPs and proteolysed IGFBPs at the receptor level. IGFBP-3 and ALS were determined by commercial assays from DiaSource (Nivelle, Belgium) and Mediagnost (Reutlingen, Germany), respectively. WLB and WiB were performed as previously described [9]. Glucose was measured in plasma using YSI 2300 STAT Plus (YSI, Yellow Springs, OH, USA). Insulin was measured by ELISA kit (Dako, Copenhagen, Denmark). All samples were completed in one assay-run. Intra-assay CVs were below 4% for IGF-I, IGF-II, IGFBP-1 and −2, below 7.5% for insulin (according to manufacturer) and below 10% for bioactive IGF.

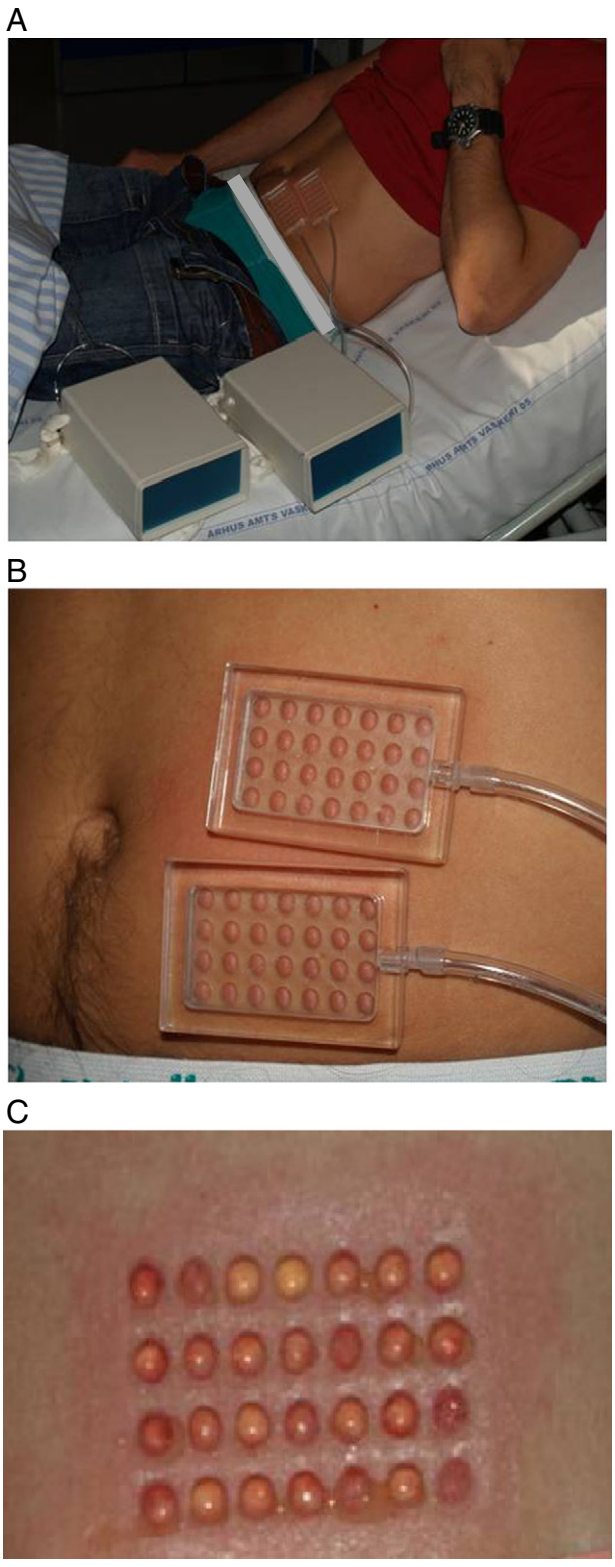

Fig. 1. A. Vacuum pumps generate a suction of 300 mm Hg. B. Suction in progress with the reservoirs placed on the abdominal skin at the level of the umbilicus. C. After the suction period, blisters are present on the skin. The blister fluid is harvested with a syringe. A white box has been added in panel A to mask the underwear brand name.

2.4. Statistical analysis

Data from the 2 blood samples corresponding to each SBF were averaged prior to analysis. A two-way repeated measures ANOVA was applied (two intra-subject repeated factors, *meal* and *compartment*, as