Minimally Invasive Sampling of Transdermal Body Fluid for the Purpose of Measuring Insulin-Like Growth Factor-I During Exercise Training

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ABSTRACT

Insulin-like growth factor-I (IGF-I) is a ubiquitous hormone that is secreted in both an endocrine and an autocrine/paracrine manner. IGF-I has conventionally been measured in serum; however, transdermal body fluid (TDF) remains as an unexplored biocompartment in which IGF-I also resides and may be more biologically relevant because of its proximity to tissues and cells. The purpose of this study was to compare IGF-I in serum versus IGF-I in TDF before and after 8 weeks of physical training. Twenty-eight healthy men (28 ± 5 years old, 176 ± 8 cm tall, weighing 83 ± 11 kg) had TDF obtained by a novel, minimally invasive method that included the application of continuous vacuum pressure on forearm skin perforated with tiny micropores created by a focused beam from a laser system and also had blood obtained by venipuncture. An enzyme-linked immunosorbent assay measured total IGF-I concentrations. A repeated-measures analysis of variance (biocompartment × time) and Pearson Product Moment Correlation coefficients (P ≤ 0.05) were used for statistical analyses. Data are presented as mean ± SE. Total TDF IGF-I was significantly lower than serum IGF-I both before (TDF, 91 ± 6 ng/mL; serum, 375 ± 17 ng/mL) and after (TDF, 83 ± 5 ng/mL; serum, 363 ± 19 ng/mL) the exercise training. Serum and TDF IGF-I values were not significantly different pre- to post-training. Serum and TDF IGF-I levels were significantly correlated pre-training (r = 0.41), but not post-training (r = 0.34). The percent change between serum and TDF was not correlated (r = 0.09). This study has demonstrated that total IGF-I can be sampled and measured in TDF via a minimally invasive manner and is appreciably (~76%) less than total IGF-I measured in serum. Additionally, the IGF-I measurements in these two biocompartments were not closely associated, possibly indicating an uncoupled, rather than a linked, regulation of IGF-I among the body’s biocompartments.

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INTRODUCTION

INTERSTITIAL FLUID is an important, yet relatively unexplored, biocompartment from which to gather meaningful information about physiological processes. While biomedical clinicians and researchers have traditionally used blood sampled from the systemic circulation, in part because of its easy accessibility, for diagnostic purposes in health and disease states, there are limitations in terms of providing definitive insight into local metabolic processes occurring in organs, tissues, and cells. The systemic circulation provides a transportation network from which hormones secreted from distal endocrine glands can be trafficked to local sites of action (i.e., tissues and cells). These hormones must first pass through the interstitial fluid as they make their way from the bloodstream to cellular membranes. Additionally, much research over the last decade has demonstrated that many growth factors and cytokines can be secreted from cells that act in autocrine/paracrine manners, suggesting that the local biochemical milieu immediately surrounding tissues and cells may be of potentially greater interest and relevance than those same analytes measured in blood.

The challenge in gleaning information from the interstitial fluid has been one of obtaining this fluid in an unobtrusive and minimally invasive manner as possible. While recent work had demonstrated the efficacy of microdialysis for measuring analytes in interstitial fluid, the invasiveness of the microdialysis procedures warrants investigation into other more user-friendly methods. Recently, SpectRx, Inc. (Norcross, GA) has developed a patented and proprietary method for obtaining transdermal body fluid (TDF) that uses a minimally invasive vacuum pressure in combination with a laser microporation process (creating micropores <100 μm in diameter) in the stratum corneum. The TDF can be collected in a small reservoir and later measured for determination of selected analytes. In two separate studies using this methodology, Gebhart et al. reported correlations of 0.87 and 0.95 between blood and TDF glucose. This minimally invasive technology for sampling TDF remains promising and warrants investigation into other biochemical compounds present in TDF.

Because of the growing recognition of insulin-like growth factor-I (IGF-I) as an important metabolic biomarker reflective of health, nutritional, and fitness status, the purpose of this study was to compare IGF-I concentrations between serum and TDF, before and after 8 weeks of physical training. Our prior work has examined IGF-I as a biomarker during exercise and military operational stress paradigms and has shown changes coincident with physical and cognitive decrements. IGF-I exerts many metabolic and anabolic effects during altered energy and health states, and it is important to identify novel, minimally invasive methods of sampling this important biomarker.

SUBJECTS AND METHODS

Subjects

Twenty-eight healthy men (28 ± 5 years old, 176 ± 8 cm tall, weighing 83 ± 11 kg, with 20 ± 8% body fat) volunteered for this study. All volunteers were briefed about the study both orally and in writing, and written informed consent was obtained before participation. Approval for this study was obtained by the U.S. Army Research Institute of Environmental Medicine and Medical Research and Materiel Command human use review boards. The investigators have adhered to the policies for protection of human volunteers as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 45 CFR Part 46. Subjects were randomly assigned to one of two physical training programs. Both programs were conducted 1.5 h/day (Monday–Friday) for 8 weeks. One training program emphasized resistance training, while the other training program emphasized more generalized callisthenic-type exercises. Both groups also conducted aerobic/running training. For the purposes of this report, all subjects have been pooled for the statistical analyses.

Collection of blood

Blood samples were collected before and after 8 weeks of physical training. Fasted, morning blood samples were collected in the labo-
ratory at ~0600–1100 h by traditional venipuncture. Investigators attempted to have the subjects return at the same time for the posttest as when they arrived for their initial blood draw. Approximately 7 mL per blood draw was collected after subjects were recumbent for ~15 min. Whole blood was allowed to remain on ice for ~30 min. After clotting, serum was separated from whole blood by centrifugation (2,500 g). Aliquots of ~2.5 mL of serum were obtained for analysis of IGF-I. Samples were then frozen in liquid nitrogen and stored at ~80°C until subsequent analysis was performed. TDF was collected simultaneously in order to compare IGF-I in different biocompartments at a similar time.

Collection of TDF

The instrumentation used to sample TDF was developed by SpectRx, Inc. and is a patented and proprietary methodology that uses minimally invasive vacuum pressure in combination with a laser microporation process (creating micropores <100 µm in diameter) in the stratum corneum. The TDF was then collected in a small reservoir.

The first step in this process is to porate the non-viable, superficial portion of the epidermis of the skin known as the stratum corneum. An alignment ring was placed on the skin (for this study, the volar portion of the forearm was used), and the laser porator was positioned inside the ring. This alignment ring served three purposes: (1) alignment of the laser porator to the skin, (2) disengagement of the safety on the laser porator, allowing it to fire, and (3) energy absorption by a dye it contained. Ablation of the stratum corneum was performed by coupling pulsed laser energy with an energy-absorbing dye in direct contact with the skin. The laser emitted a light source in the infrared...
range of 980 nm. The laser heated the energy-absorbing dye, thus creating four micropores (<100 µm in diameter) by thermal ablation (Fig. 1). Maximum power of the laser was 110–130 mW. Duration of this process was less than 3 s. Microporation was superficial and did not extend down into the dermis. This array of barely visible micropores acted as a channel for TDF to be drawn into the fluid harvesting unit.

Once this process was completed, a harvesting head (for collection of TDF) was placed over the alignment ring. Coupled with the harvesting head was a vacuum unit that applied a continuous vacuum pressure (undetectable by the subject) for ~2 h. The 2-h period was chosen in order to obtain an ample sample volume to perform IGF-I assays in duplicate. Under these conditions, TDF collection rates were ~5–15 µL/h. Previous literature suggests pores can remain viable to 2–4 days after stratum corneum ablation.6,14 TDF was aspirated from the harvesting head using a 30-gauge syringe (Becton-Dickinson, Franklin Lakes, NJ). For the collection of TDF, all products were manufactured and supplied by SpectRx, Inc. All material required and used in the collection of TDF are displayed in Figure 2. The sequential steps in this process are depicted in Figure 3.

Total IGF-I assays

Total IGF-I was quantified using assays developed and modified to measure TDF by Diagnostic Systems Laboratories (Webster, TX; and Toronto, ON, Canada). Assays for total IGF-I were performed using a non-competitive enzyme-linked immunosorbent assay for both the serum and TDF IGF-I. Procedures for the assay have been described elsewhere. The sensitivity of the assay was 0.03 ng/mL. All samples were assayed in duplicate, and all samples were assayed in the same batch in order to eliminate inter-assay variance. Intra-assay variance was less than 8%.

Statistics

A repeated-measures analysis of variance (biocompartment × time) was used to evaluate IGF-I concentrations before and after training from serum and TDF. Pearson Product Moment Correlations (r values) were used to evaluate the relationship between serum and TDF at both before and after training as well as percent changes between variables after training. Statistical analysis was performed using Statistica version 7 (Statsoft, Tulsa, OK). An alpha level of less than 0.05 was used as the criterion of statistically significant differences. All data are presented as mean ± SE.

RESULTS

Circulating IGF-I versus IGF-I in TDF

Total TDF IGF-I was significantly lower than serum IGF-I both before (TDF, 91 ± 6 ng/mL; serum, 375 ± 17 ng/mL) and after (TDF, 83 ± 5 ng/mL; serum, 363 ± 19 ng/mL) the 8 weeks of exercise training. Serum and TDF IGF-I were not significantly different pre- to post-training (Fig. 4). TDF IGF-I was ~24% of the concentration observed in serum.

Association between IGF-I in the serum versus TDF

Figure 5 shows the relationship between IGF-I in serum versus in TDF. There was a modest, positive correlation at baseline for IGF-I between serum and TDF (Fig. 5A; r = 0.414). However, at the post-training time point there was no longer a significant relationship (Fig. 5B; r = 0.336). The percent changes in serum and TDF were moderately, but significantly, correlated (r = 0.60) primarily because of one outlier. When this outlier was removed, the correlation for percent change between serum and TDF IGF-I was no longer significant (Fig. 5C; r = 0.09). A Bland-Altman plot (Fig. 5D) indicated that there was no bias in serum versus TDF IGF-I across the range of concentrations measured in this study.

DISCUSSION

This study sampled TDF with the use of a novel, minimally invasive method that included the application of continuous vacuum pressure on forearm skin perforated with tiny micropores created by a focused beam from a
laser system and subsequently measured total IGF-I concentrations. On average, total IGF-I concentrations measured in TDF were only 24% of those measured in the serum. Additionally, the total IGF-I measurements in these two biocompartments were not closely associated, possibly indicating an uncoupled, rather than a linked, regulation of IGF-I among the body’s biocompartments. The procedure used in the present study to sample TDF and measure IGF-I has applicability to many other analytes found in TDF and can be performed with minimal inconvenience/discomfort to subjects. Whereas other studies have examined IGF-I physiology through invasive tissue biopsy or microdialysis procedures, our results demonstrate that TDF can be obtained minimally invasively and could potentially be used in large-scale population studies (e.g., studies of military trainees, astronauts on extended space exploration missions, and diabetes populations with a need for continuous monitoring), where information about local growth factors or other metabolic biomarkers is desired.

Total IGF-I measured in TDF was only 24% of that measured in serum. This finding is in accordance with Xu et al., who had previously reported that in blister fluid artificially raised by negative pressure in 10 healthy volunteers, IGF-I was ~18% of that measured in serum. It is important to note that while the assay used in the current study detected both free and bound IGF-I (i.e., total IGF-I), IGF-I biological activity is determined by the relative proportions in which IGF-I circulates in a free, binary-bound, or ternary-bound molecular com-
plex. For example, using fast protein liquid chromatography, Xu et al. demonstrated that IGF-I found in the binary and ternary complexes of blister fluid was 37% and 13%, respectively, of the corresponding peak found in matched serum, thereby illustrating that although IGF concentrations were lower in the blister fluid than in the circulation, a greater proportion of the IGF-I was in the form (i.e., binary) more readily available for interaction with tissue receptors. Furthermore, Desvigne et al. recently employed microdialysis to measure free (i.e., unbound of IGF binding proteins) IGF-I in human skeletal muscle and reported a mean free IGF-I concentration in interstitial fluid 17 times higher than that observed in plasma (6.8 ± 3.2 ng/mL vs. 0.4 ± 0.2 ng/mL) and attributed this large gradient to the local release/action of IGF-I. Although the various forms of IGF-I and its family of binding proteins were not measured in the current study, the findings of Xu et al. and Desvigne et al. make it apparent that evaluating IGF-I in the body’s various biocompartments is important in developing a comprehensive understanding of IGF-I regulatory complexity, especially within the context of bioavailable IGF-I. We contend that monitoring IGF-I in biocompartments proximal to tissue receptors rather than the distal systemic circulation should provide greater and more meaningful information about the physiological and metabolic effects that IGF-I exerts at the cellular level.

SpectRx, Inc. originally developed the technology for obtaining TDF under continuous vacuum pressure via micropores in the stratum corneum for the purpose of real-time glucose monitoring. In the initial clinical studies by Gebhart et al., they reported that TDF glucose and blood glucose had correlations ranging from 0.87 to 0.95 and concluded that TDF glucose could serve as a surrogate measure for blood glucose. These results are in contrast to the low to moderate correlations observed between TDF and blood IGF-I (0.09–0.41) in the current study. The fact that TDF and blood measures are more congruent for glucose than IGF-I could be attributed to the difference in size as larger molecules (e.g., total IGF-I, ~150 kDa) are found at lower relative concentrations in interstitial fluid than in blood in comparison with smaller molecules (i.e., glucose). Because the SpectRx technology draws fluid from the stratum corneum and does not probe below the epidermis, Gebhart et al. pointed out that the measured analytes in TDF may be more reflective of capillary levels and are at higher concentrations than in true interstitial fluid. We conclude that the lack of congruence between TDF and blood IGF-I illustrates that IGF-I is not homogeneous among the body’s biocompartments and is subject to differential regulation. Therefore, our data clearly indicate that blood and TDF IGF-I measures are not interchangeable.

Neither TDF nor blood IGF-I concentrations were significantly altered by longitudinal training. As the IGF-I system has an integral role in mediating many of the beneficial effects of exercise particularly via tissue remodeling, the IGF-I response to exercise training has drawn much interest. Some current literature has suggested that IGF-I may exhibit a two-phased response pattern (an initial decrease followed by an increase after 5 weeks) to short-term physical training. We had previously reported that in end-stage renal disease patients undergoing 12 weeks of resistance training, a 15% decline in circulating IGF-I was observed. That there was not a significant decline in IGF-I could be related to initial fitness level as Rosendal et al. have demonstrated that when exposed to a physical training regimen, untrained subjects experience a greater disruption response in the IGF-I system than do well-trained subjects. According to the American College of Sports Medicine guidelines, the baseline mean values for maximum oxygen consumption (48 mL/kg/min) for the subjects participating in this study classifies them in the good to high range of aerobic fitness. Future studies evaluating the IGF-I system using TDF during longitudinal exercise training studies should also evaluate free IGF-I as well as the family of IGF binding proteins.

In summary, this study describes a minimally invasive technology that involves the application of continuous vacuum pressure on forearm skin perforated with tiny micropores created by a focused beam from a laser system that allows collection of TDF. This procedure is safe, painless, reasonable in cost, and well
tolerated by subjects, and does not involve needles or lancets. There have been no reports of infection from this procedure, and the device may be worn for 3–4 days. This minimally invasive procedure provides advantages over blister and microdialysis studies in that it avoids local irritation and subsequent inflammatory responses that may influence the composition of the harvested fluid. In addition to its ease of use, this procedure can potentially advance the field of physiology as it is now feasible to collect biological fluids from a biocompartment other than blood. Our results differ from previous reports of a close association between TDF and blood glucose as the IGF-I measurements in these two biocompartments were not closely associated, possibly indicating an uncoupled, rather than a linked, regulation of IGF-I among the body’s biocompartments. Future efforts toward minimally invasive sampling of the biochemical milieu surrounding muscle in conjunction with continuous, real-time measurements should provide diagnostic and clinical value above what is currently available.

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