A novel, noninvasive transdermal fluid sampling methodology: IGF-I measurement following exercise


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Submitted 12 May 2010; accepted in final form 28 February 2011

Scofield DE, McClung HL, McClung JP, Kraemer WJ, Rarick KR, Pierce JR, Cloutier GJ, Fielding RA, Matheny RW Jr, Young AJ, Nindl BC. A novel, noninvasive transdermal fluid sampling methodology: IGF-I measurement following exercise. Am J Physiol Regul Integr Comp Physiol 300: R1326–R1332, 2011. First published March 9, 2011; doi:10.1152/ajpregu.00313.2010.—This study tested the hypothesis that transdermal fluid (TDF) provides a more sensitive and accurate measure of exercise-induced increases in insulin-like growth factor-I (IGF-I) than serum, and that these increases are detectable proximal, but not distal, to the exercising muscle. A novel, noninvasive methodology was used to collect TDF, followed by sampling of total IGF-I (tIGF-I) and free IGF-I (fIGF-I) in TDF and serum following an acute bout of exercise. Experiment 1: eight men (23 ± 3 yrs, 79 ± 7 kg) underwent two conditions (resting and 60 min of cycling exercise at 60% VO2peak) in which serum and forearm TDF were collected for comparison. There were no significant changes in tIGF-I or fIGF-I in TDF obtained from the forearm or from serum following exercise (P > 0.05); however, the proportion of fIGF-I to tIGF-I in TDF was approximately fourfold greater than that of serum (P ≤ 0.05). These data suggest that changes in TDF IGF-I are not evident when TDF is sampled distal from the working tissue. To determine whether exercise-induced increases in local IGF-I could be detected when TDF was sampled directly over the active muscle group, we performed a second experiment. Experiment 2: fourteen subjects (22 ± 4 yr, 68 ± 11 kg) underwent an acute plyometric exercise condition consisting of 10 sets of 10 plyometric jumps with 2-min rest between sets. We observed a significant increase in TDF tIGF-I following exercise (P ≤ 0.05) but no change in serum tIGF-I (P > 0.05). Overall, these data suggest that TDF may provide a noninvasive means of monitoring acute exercise-induced changes in local IGF-I when sampled in proximity to exercising muscles. Moreover, our finding that the proportion of free to tIGF-I was greater in local IGF-I when sampled in proximity to exercising muscles. More-

THE PHYSIOLOGICAL SIGNIFICANCE of circulating hormones and growth factors is well studied. Insulin-like growth factor (IGF-I) is a 7.6-kDa pleiotropic and ubiquituous polypeptide secreted by the liver in response to growth hormone (GH), and is also expressed in multiple nonhepatic tissues. Approximately 1% of the circulating total IGF-I (tIGF-I) is in the form of an unbound, free IGF-I (fIGF-I) peptide, with the remaining ~99% bound to one of the six IGF binding proteins (IGFBPs 1–6) (3, 12, 22). Free IGF-I binds to its cognate receptor, which results in the activation of a number of intracellular signaling cascades that promote growth, cell division, and survival (20). Although IGF-I concentrations are not under diurnal control and typically remain stable over a 24-h period, factors including nutritional status, energy flux, and physical training have been reported to influence circulating IGF-I concentrations (7, 25). Consequently, the circulating IGF-I system may provide both an assessment and prognostic value in metabolic and body composition monitoring (15, 20).

However, the local biochemical milieu may provide better information about cellular metabolic events regulated by those growth factors than measurements of circulating concentrations because many growth factors, including IGF-I, are also secreted from cells and act in an autocrine/paracrine manner (6, 11, 12, 21).

The reported consequences of physical training on the IGF-I system have thus far been equivocal. Rosendal et al. (26) reported decreased levels of circulating fIGF-I and tIGF-I after 11 wk of intense physical training. Additionally, two studies examining 5 wk of aerobic training in adolescents (9, 27) reported a significant decrease in circulating IGF-I from baseline after the training intervention. Conversely, other studies have reported increases in indices of physical fitness (i.e., muscle strength and VO2max), as well as IGF-I peptide in overloaded, hypertrophied muscle, without a concomitant increase in circulating IGF-I (1, 10, 17, 20). However, several studies have reported acute increases in circulating IGF-I in response to both aerobic and resistance exercise. Previous work by Kraemer et al. (13, 14) reported significant increases in the temporal response of IGF-I to heavy resistance exercise with no increase in GH. In another study conducted by Cappon et al. (7), 10 min above lactate threshold cycle ergometer exercise elicited a significant increase in IGF-I that peaked by the 10th min of exercise and remained elevated through the first 20 min of recovery; this increase was not associated with elevated circulating GH. Likewise, it was also reported that intense running elicited an acute increase in tIGF-I (14). Together, these latter studies suggest that acute exercise-induced increases in circulating IGF-I may be GH independent. Therefore, it is possible that these increases in circulating IGF-I may not be liver derived, but rather may reflect the release of IGF-I from extracellular storage. Moreover, bioocompartments [i.e., transdermal fluid (TDF)] may be more sensitive than serum in capturing perturbations in IGF-I.

Recently, researchers have employed a moderately invasive microdialysis method to obtain extracellular fluid adjacent to muscle and tendon tissue to examine the response of local...
IGF-I concentration to exercise and some, but not all have reported a significantly greater concentration of fIGF-I in interstitial fluid vs. serum (4, 5, 8, 23). For example, Berg, et al. (4) reported large gradient differences of fIGF-I between muscle interstitial fluid and the circulation, evidence suggesting that monitoring the IGF-I system at the local level vs. circulation may provide more insight into the intrinsic mechanisms of muscle adaptation to physical training.

Recent advances in biotechnology have led to the development of noninvasive technologies for the purpose of continuous monitoring of glucose in TDF, and correlations ranging from 0.87 to 0.95 between blood and TDF glucose have been reported (6, 11). In addition to glucose, growth factors useful for physiological monitoring have been observed in TDF as well (e.g., IGF-I, IGF-II, IGFBP), but their representation of physiological status is unclear (29). Nindl, et al. (21) demonstrated that after 8 wk of physical training, circulating tIGF-I and TDF tIGF-I were not correlated, suggesting an uncoupled, rather than a linked regulation of tIGF-I among the body’s biocompartments. If information representative of autocrine/paracrine expression or other local release reservoirs can potentially be gathered from easily accessible biocompartments (i.e., TDF) in a noninvasive manner, then TDF biomolecules such as IGF-I may provide valuable insight into the homeostatic mechanisms adaptations to exercise and physical training.

The purpose of this study was to test the hypothesis that TDF is more sensitive than serum to exercise-induced increases in IGF-I concentration, and that these increases would be evident proximal, but not distal, to the working muscle. We further hypothesized that in response to acute exercise, the concentration of TDF IGF-I, but not serum IGF-I, would significantly increase to proximal, but not distal, to the working muscle. We further demonstrated that after 8 wk of physical training, circulating tIGF-I and TDF tIGF-I were not correlated, suggesting an uncoupled, rather than a linked regulation of tIGF-I among the body’s biocompartments. If information representative of autocrine/paracrine expression or other local release reservoirs can potentially be gathered from easily accessible biocompartments (i.e., TDF) in a noninvasive manner, then TDF biomolecules such as IGF-I may provide valuable insight into the homeostatic mechanisms adaptations to exercise and physical training.

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METHODS

Two experiments described in this report were both approved by the Human Use Review and Scientific Review Committees at the U.S. Army Research Institute of Environmental Medicine (Natick, MA) and by the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command (Pt. Detrick, MD). All participants gave their written permission for the study. The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70 –25, and the research was conducted in adherence with the provisions of 45 CFR Part 46. In experiment 1, aerobic exercise was employed and transdermal body fluid was sampled at a proximal site (i.e., the forearm), while in experiment 2, plyometric exercise was employed and transdermal body fluid was sampled at a proximal site (i.e., the thigh). By sampling at distal vs. proximal sites to the active musculature during exercise, it was our attempt to gain further insight into this novel methodology with regard to detecting the IGF-I response to exercise.

Experiment 1

Subjects. Eight men (23 ± 3 yr, 79.2 ± 6.6 kg, and V\textsuperscript{O2peak} of 44.2 ± 2.6 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}, means ± SD), participating in a larger study (24) examining skeletal muscle protein turnover during aerobic exercise, participated in this research experiment. All subjects were recreationally active based on assessment of physical activity history (2–4 days per week endurance exercise, 30–60 min per session, V\textsuperscript{O2peak} range of 40–50 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) and no significant weight change (≤ 2.2 kg) for at least 2 mo prior to the beginning of the study.

Overview of study design. This repeated-measures study design began with subjects completing a control condition (7 h of quiet supine rest) separated by 4 days from the subsequent exercise condition (EX). Subjects were housed at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University (Boscon, MA) where they received a standardized evening meal and stayed overnight in the Metabolic Research Unit. Subjects were instructed to avoid exercise and physical activity 48 h prior to each testing day and to maintain a 12-h fast leading up to the test day. The morning of the test day, subjects lay supine in a hospital bed. At time point −120 min preexercise, the TDF collection heads were placed on the subject’s forearm, and TDF collection began. At time point −15 min preexercise, baseline TDF (2 h of collection) was harvested from the TDF collection heads and blood was collected from a retrograde hand catheter. The subjects remained supine in a hospital bed until time point 0 min preexercise, at which time they either moved to a cycle ergometer and cycled upright for 60 min at 60 ± 5% V\textsuperscript{O2peak} for the exercise condition or remained lying supine for the control condition. Immediately following the exercise bout, the subjects moved back to the hospital bed and returned to a supine position. Subsequent blood and TDF samples were collected at time points +40, +105, and +225 min postexercise, respectively during both control and EX conditions.

Transdermal fluid sampling. The instrumentation used to sample TDF was developed by Guided Therapeutics (Norcross, GA) (Fig. 1), and applies vacuum pressure to the skin surface at the sampling site in combination with a laser microporation process (creating micropores < 100 μm in diameter) in the stratum corneum. The TDF is then collected in a small reservoir.

The first step in this process (Fig. 2A) is to porate (make pores) the nonviable, superficial portion of the epidermis of the skin, the stratum corneum. An alignment ring is placed on the skin and the laser porator is positioned inside the ring. This alignment ring serves three purposes: 1) alignment of the laser porator to the skin; 2) disengagement of the safety mechanism on the laser porator, allowing it to fire; and 3) energy absorption through the use of a dye. Ablation of the stratum corneum is performed by coupled pulsed laser energy with an energy absorbing dye in direct contact with the skin. The laser emits a light source in the infrared range of 980 nm. The laser heats the energy absorbing dye, creating four micropores (< 100 μm in diameter) by thermal ablation. Maximum power of the laser is 110–130 mW. Duration of this process is < 3 s. Microporation is superficial and does not extend into the dermis; therefore it provides minimal discomfort for the volunteer. This array of micropores acts as a channel for TDF to be drawn into the fluid-harvesting head.

Once this process is completed, a harvesting head (for collection of TDF) is placed over the alignment ring. Coupled with the harvesting head is a vacuum unit that applies a continuous vacuum pressure (150–400 mmHg) that is undetectable by the subject during the collection period (Fig. 2B). Under these conditions, TDF collection rates are ~5–15 μl/h per collection head. TDF is aspirated from the harvesting head using a 30-gauge syringe (Becton-Dickinson, Franklin Lakes, NJ) (Fig. 2C). For the collection of TDF, all products were manufactured and purchased from Guided Therapeutics (Norcross, GA).

Blood sampling. Following an overnight fast, blood samples were obtained from a retrograde hand catheter. Blood samples were collected at baseline, +40, +105, and +225 min postexercise. Blood samples were allowed to clot at room temperature and then centrifuged at 5,000 rpm; the serum was frozen at −80°C for future analysis.

Analytical procedures. To ensure adequate sample volume for analysis, TDF aliquots for time points +40, +105, and +225 min postexercise were pooled (TP) for each condition. This was done by pipetting 50 μl of TDF from each time point aliquot into one pooled aliquot that was subsequently used for analysis. To make IGF-I comparisons between biocompartments, serum aliquots were also pooled at these same time points following the same pooling procedure. All assays were performed in duplicate, and all analyses for each subject were performed within the same assay batch to eliminate interassay variance. fIGF-I was measured by a commercially available

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ELISA (Diagnostic Systems Laboratories, Webster, TX). The sensitivity of the assay was 0.015 ng/ml and the intra-assay coefficients of variation was 9.8%. tIGF-I was measured using a commercially available immunoradiometric assay also from Diagnostic Systems Laboratories. The sensitivity of the assay was 2.06 ng/ml and the intra-assay coefficients of variation was 6.6%.

Experiment 2

Subjects. Nine men and five women (22 ± 4 yr, 68 ± 11 kg, means ± SD) participating in a larger study examining the osteogenic properties of plyometric exercise participated in this research experiment.

Overview of the acute exercise test. All subjects performed an acute exercise test (AET) consisting of performing 10 sets of 10 repetitions of Plyo-Jumps on a Plyopress (Athletic Republic, Park City, UT) at a load of 40% of their 1-RM with 2-min interset rest periods. Participants placed their feet slightly wider than shoulder width at the appropriate height on the platform so their knees did not drift over the toes when performing the exercise. The participants bent their knees to the appropriate depth and then jumped as forcefully as possible.

Transdermal fluid sampling. The method used for the collection of TDF is the same as experiment 1, except the TDF collection heads were placed on the vastus lateralis of the right thigh. TDF was collected at three time points: preexercise (T₀), +60, and +120 min postexercise.

Blood sampling. On the AET day, a fasting blood sample was collected from all volunteers 30 min prior to acute testing and at immediate postexercise, +60, and +120 min postexercise using the following procedure: ~15 ml of blood were drawn from an antecubital vein using an indwelling catheter with saline lock. Blood samples were centrifuged for 15 min at 3,000 rpm at 4°C, and serum aliquots were flash frozen in liquid nitrogen and stored at −80°C until analysis.

Analytical procedures. To make IGF-I comparisons between bio-compartments, the postexercise serum (immediate postexercise, +60, and +120 min postexercise) aliquots were pooled. This was performed by pipetting 20 µl from each time point aliquot into one pooled aliquot (Tₚ) that was subsequently used for analysis. All assays were performed in duplicate, and all analyses for each subject were performed within the same assay batch to eliminate interassay variance. The tIGF-I assay procedure is described in experiment 1.

Statistical Analysis

Statistical analysis was performed using Statistica version 9 (Statsoft, Tulsa, OK). For experiment 1, a three-way, repeated-measures ANOVA (three factor: bio compartment (TDF and serum) × condi-
tion (exercise and control) × time [T₀ (pre-) and Tₚ (postexercise)] was used to evaluate tIGF-I and fIGF-I concentration in TDF and serum. For experiment 2, a repeated-measures ANOVA (two factor: biocompartment (TDF and serum) × time [T₀ (pre-) and Tₚ (postexercise)]) was used to examine tIGF-I concentration in TDF and serum. A Tukey’s honestly significant difference post hoc test was performed when appropriate. A product moment correlation was used to examine relationships between TDF and serum. A Tukey’s honestly significant difference post hoc test was used to examine tIGF-I concentration in TDF and serum. A Tukey’s honestly significant difference post hoc test was used to examine tIGF-I concentration in TDF and serum at T₀ (TDF, 126 ± 19 ng/ml; serum: 206 ± 15 ng/ml, P < 0.01) and Tₚ (TDF, 165 ± 19 ng/ml; serum: 206 ± 15 ng/ml, P < 0.01). There was a significant increase (P = 0.02) in TDF tIGF-I from T₀ to Tₚ, with no change (P = 0.96) in serum tIGF-I from T₀ to Tₚ (Fig. 5). The two biocompartments were significantly correlated preexercise (r = 0.68, P < 0.01) and postexercise (r = 0.68, P < 0.01).

DISCUSSION

Sampling of TDF offers a noninvasive method to allow the harvesting of fluid that is proximal to tissues and cells of interest compared with that of blood/serum. Consequently, TDF sampling may potentially provide information more reflective of local biochemistry than that obtained from measuring circulating IGF-I. Some advantages of the TDF collection methodology used in our study include its feasibility of continuous monitoring with little infection risk to the subject, coupled with the noninvasiveness of the procedure compared with venipuncture (6, 11). The noninvasive nature of this methodology is in stark contrast to other methods used in investigating biochemical changes at the local level such as

![Graphs A, B, C, D](http://ajpregu.physiology.org/)
microdialysis, which harvests interstitial fluid by way of invasive catheterization adjacent to tissues of interest (e.g., muscle, tendon, and adipose tissue).

Previous research reports an approximate 10- to 20-fold greater concentration of fIGF-I in muscle interstitial fluid collected by microdialysis than observed in circulation (4, 8), whereas our aerobic exercise experiment observed similar concentrations of fIGF-I in TDF and serum. The fIGF-I values we measured in TDF were similar to concentrations in serum, but much lower than values measured in muscle interstitial fluid by others (8). This observation suggests that TDF measurements of IGF-I sampled distally from exercising muscles may better reflect circulating IGF-I levels than local, extracellular IGF-I concentrations. On the other hand, TDF sampled directly over the exercising muscles of the thigh during the lower body plyometric exercise experiment demonstrated a significant increase in TDF tIGF-I concentration. Taken together, these findings suggest that the IGF-I concentration in TDF sampled proximally to exercising muscles may better reflect alterations in extracellular IGF-I than IGF-I concentrations sampled from serum.

Underscoring our rationale that fIGF-I is the most biologically meaningful component of the IGF-I system, two previous studies demonstrated that circulating fIGF-I explained a larger proportion of the variance when compared with other conventional nutritional biomarkers in examining changes in body composition (15, 20). The utility of monitoring fIGF-I in the TDF biocompartment is supported not only by our ability to measure fIGF-I in TDF, but also by our observation of larger ratios of fIGF-I/tIGF-I in TDF vs. serum. IGF-I circulates either in its free peptide form or as part of a multimeric complex (binary and ternary-bound moieties); and therefore its biological activity is dependent upon the proportion of the various IGF-I complexes residing in a given biocompartment. The higher concentrations for IGF-I in the circulation can be largely attributed to the propensity for the binary and ternary

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**Fig. 4.** Group means and individual values for the free IGF-I (fIGF-I) concentration during T0 (preexercise) and Tp (postexercise) for experiment 1: serum concentration of fIGF-I during control (A), TDF concentration of fIGF-I during control (B), serum concentration of fIGF-I during exercise (C), TDF concentration of fIGF-I during exercise (D).

**Fig. 5.** Group means and individual values for the tIGF-I concentration during T0 (preexercise) and Tp (postexercise) for experiment 2: serum concentration of tIGF-I (A), TDF concentration of tIGF-I (B). *Significantly different from T0, P < 0.05.
IGF-I complexes to be sequestered and confined within the vasculature. We observed that the mean proportion fIGF-I/tIGF-I was approximately fourfold greater in TDF than the proportion found in serum, possibly due to the inability of larger multimeric complexes to pass from the vascular network into the transdermal biocompartment.

In the aerobic exercise experiment, we observed no change in tIGF-I or fIGF-I in either biocompartment during rest or after acute aerobic exercise. Conversely, in the plyometric exercise experiment, we observed a significant increase in TDF tIGF-I T0 to Tp vs. no change in circulating tIGF-I. Previous research investigating the circulating IGF-I response to exercising muscle has revealed that circulating IGF-I remains stable (19) unless physical activity is superimposed on a short-term energy deficit (18). Moreover, Nindl et al. (16) observed no effects of acute aerobic exercise on the circulating IGF-I ternary complex (tIGF-I-IGFBP-3-ALS). The mechanism whereby the circulating IGF-I system is influenced by stressors such as exercise and metabolic perturbations may not only be dependent on the partitioning of IGF-I among its binding proteins, but also on the secretion of IGF-I from the tissue into circulation (2, 19, 25, 28). Furthermore, the ability to monitor an exercise-induced change in TDF IGF-I is likely dependent on the anatomical proximity of the harvested TDF to the exercising muscle. We were unable to determine whether acute exercise influenced TDF concentrations of IGF-I or IGFBPs when collected in proximity to exercising muscles due to insufficient TDF sample volume. However, work is presently underway in our laboratory to improve sample volume acquisition, enabling our ability to sample more time points as well as measure a greater number of analytes in future experiments.

In summary, during the cycling exercise experiment where TDF was sampled from the forearm, we observed significant differences in the tIGF-I concentration and the proportion of fIGF-I/tIGF-I between biocompartments. However, we observed no change in fIGF-I or tIGF-I concentration in TDF or serum in response to aerobic exercise, which may be due to the distal TDF sampling procedure. During the plyometric exercise experiment, where TDF was collected directly over the working muscle, we observed a significant increase in the concentration of TDF tIGF-I from pre- to postexercise, but observed no change in circulating tIGF-I. Thus, our ability to observe a local increase in tIGF-I concentration was achieved when TDF was collected in an anatomical region proximal to the working muscles (i.e., thigh region). These findings underscore the necessity of collecting TDF from an anatomical location proximal to the tissue/cells undergoing the perturbation. Nonetheless, these studies had the following limitations: 1) experiment 1: we were limited to analyzing only postexercise pooled time points, and 2) experiment 2: the TDF was collected for 2 h during postexercise period before being harvested, thus limiting resolution. Future research will pursue improving the TDF sampling methodology such that greater TDF sample volume can be collected per unit of time to provide more resolute serial analysis.

**Perspectives and Significance**

This technology may potentiate the ability of clinicians to monitor in real time the physiological status of target populations (e.g., diabetic, burn and/or wounded patients) by not only providing a noninvasive surrogate measure for serum biomolecules but also providing a more sensitive method of capturing autocrine/paracrine activity or other local releasable reservoirs that otherwise may not be reflected in the circulation. The evolution of this technology should not only address the functionality of TDF sampling but also the operability and tolerability of this methodology by the patients who may be wearing these systems for hours or even days on end. Optimally, future generations of this system will have the capability to monitor physiological status via noninvasive, tetherless sensors integrated into personal items such as clothing or small medical devices worn next to the body. By providing critical, real time physiological information (e.g., metabolic and hormonal biomarkers, blood glucose, heat/cold stress, fatigue, dehydration, etc.), these technologies will provide a convenient means of monitoring target populations.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge the technical assistance of Jeffery S. Staab and Jeremy K. Miller as well as the efforts of the staff and volunteers that made the data collection possible.

Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Dept. of Agriculture, the Army or the Department of Defense. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

**GRANTS**

This material is based upon work supported by the U.S. Department of Agriculture, under agreement no. 58-1950-7-707 and by funding from the U.S. Army Medical Research and Materiel Command to Task Area 5: Physiological Mechanisms of Musculoskeletal Injury.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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