

Utility of Measuring Insulin-Like Growth Factor-I for Assessing Military Operational Stress: Supporting Future Force Warrior from the Bench Top to the Battlefield

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ABSTRACT

Military training and operational stress can compromise health and performance of Soldiers. The availability of a sensitive biomarker of nutritional and physiological strain would have tremendous utility for monitoring soldier readiness as well as assessing the effectiveness of intervention and recovery strategies. Insulin-like growth factor-I (IGF-I) is sensitive to underfeeding and malnutrition; falling 50-60% during operational stress. It is a stable marker, minimally affected by circadian rhythms. IGF-I can be measured rapidly using minimally invasive techniques. IGF-I monitoring offers the potential to reduce injury incidence and improve training effectiveness.

1. MILITARY RELEVANCE OF MONITORING INSULIN-LIKE GROWTH FACTOR-I

Mission success in military tactical environments dictate that the warfighter be able to perform prolonged physical exertion in the face of food and sleep restriction (i.e., military operational stress). The physiological strain produced by these operational stressors can have deleterious effects on muscle mass, endocrine and metabolic function, as well as physical and mental performance (Friedl et al., 1994; Friedl, 1999; Friedl et al., 2000; Nindl et al., 1997; Nindl et al., 2002; Nindl et al., 2003a; Nindl et al., 2003b) (see Figure 1). A goal of the Army Medical Research and Materiel Command's (MRMC) biomedical research program is to identify useful biomarkers that are indicative of nutritional and physiological status that can be assessed rapidly, with minimally or non-invasive collection methods. Once identified, these biomarkers will be used to sustain warfighter readiness and aid in assessing the effectiveness of intervention and recovery strategies.

The growth hormone/insulin-like axis is a central endocrine axis and is thought to mediate many of the somatotropic changes that are observed when warfighters are exposed to harsh field environments (Florini et al., 1996; Friedl et al., 2000; Nindl et al. 2003a; Rosen, 1999; Rosendal et al., 2002). For this reason, periodic assessment of the growth hormone/insulin-like growth factor axis may have utility for sustaining warfighter health and performance. In direct support of Objective Force Warrior's (OFW)

vision of revolutionizing soldier performance by aggressively employing science and technology efforts that enhance the warfighter's survivability, lethality, sustainment, and mobility on the modern battlefield, The Military Performance and Military Nutrition Division U.S. Army Research of Institute of Medicine have been evaluating IGF-I as a candidate biomarker for assessing nutritional stress. Our research has focused on 1) characterizing temporal response patterns of IGF-I and its family of binding proteins during military operational stress, 2) the influence of macronutrient and energy intake on the circulating IGF-I system responses to stress, and 3) assessment of minimally invasive and field expedient collections methods for determination of IGF-I.



Figure 1. Military operations place multiple stressors on the Warfighter. These stressors typically occur simultaneously. The magnitude of the resulting strain is dependent on the severity of the stressors. The resulting physiological strain can result in deleterious outcomes on lean body mass, soldier physical performance and can compromise warfighter readiness.

The purpose of this short review paper is to summarize why IGF-I has been of interest as a potential biomarker and our experimental strategies for evaluating the merits of IGF-I as a biomarker of nutritional and operational stress. The paper initially describes the complex nature of IGF-I regulation and relevance for the military and then describes initial work characterizing the IGF-I response to military operational stress. The

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experimental outcomes suggest that IGF-I has potential value as a biomarker of nutritional strain during operational stress.

2. INSULIN-LIKE GROWTH FACTOR-I PHYSIOLOGY AND REGULATORY COMPLEXITY

The primary source of circulating IGF-I is the liver, but local release from tissues that secrete IGF-I in an autocrine/paracrine manner also contribute. IGF-I itself is a 7.6-kDa polypeptide consisting of 70 amino acids with three intrachain disulfide bonds. Only a small amount (<2%) of IGF-I, however, circulates in free form. Most circulates in either a binary (~20-25%) or ternary complex (~75%). When circulating in the binary form, IGF-I is complexed with one of six binding proteins (BPs; BPs- 1-6) ranging in size from 22.8 to 31.4 kDa. The ternary complex consists of IGF-I, IGF BP-3, and an 80-86 kDa protein called the acid labile subunit (Baxter, 2000; Jones and Clemmons, 1995; Rajaram 1997; Sara and Hall, 1990). An IGF-I specific protease is responsible for breaking the bonds holding the ternary complex together, and making the IGF-I available for receptor binding. The IGF-I complexes are thought to regulate the availability of IGF-I to target tissues as only the free and binary complexes can pass from the vascular compartment into the interstitial space. The different forms of BPs are also thought to play a role in transporting the IGF-I to the target tissue (Baxter, 2000; Sara and Hall, 1990).

IGF-I has several metabolic effects. It is known to promote amino acid uptake, enhance protein synthesis, and attenuate protein degradation (Florini et al., 1996; Rosen, 1999; Thissen et al., 1999). Additionally, IGF-I plays a role in stimulating cell growth and differentiation (Baxter, 2000; Florini et al., 1996).

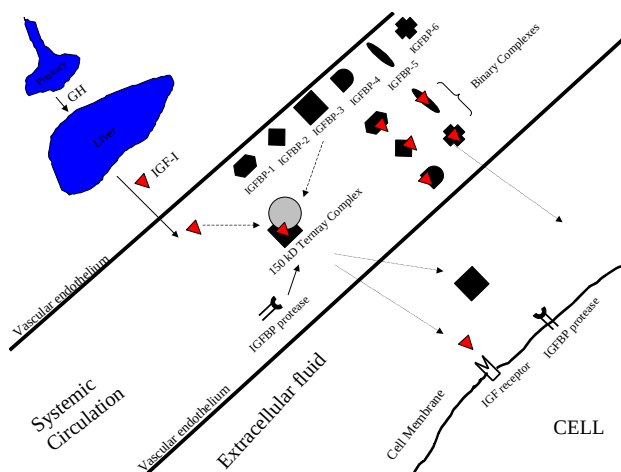


Figure 2. Schematic of the IGF-I system showing the origin of release (i.e. liver), its family of binding proteins (BPs 1-6), ternary and binary complexes, and IGF-I trafficking from the circulation to the receptor.

The appeal of IGF-I as a biomarker is the dynamic nature in which circulating concentrations respond to nutritional stress. Underfeeding and protein-calorie malnutrition result in substantial reductions in IGF-I concentrations and the response persists until the nutritional stress is removed (Friedl et al., 2000; Frystyk et al., 1999; Nindl et al., 2003a; Rand et al., 2003; Thissen et al., 1992; Thissen et al., 1999). Additionally, IGF-I concentrations are relatively stable. Unlike hormones such as growth hormone, IGF-I displays little in the way of circadian variability, thus single time point samples are indicative of IGF-I status.

3. EFFECTS OF MILITARY OPERATIONAL STRESS ON THE CIRCULATING IGF-I SYSTEM

3.1 US Army Ranger Training

Friedl and colleagues performed experimental studies characterizing the physiological responses of soldiers participating in the U.S. Army Ranger Training Course (Friedl et al., 1994; Friedl et al., 2000; Nindl et al., 1997). The data provide insight into the adaptive process that occurs as soldiers cope with sustained physical work, energy restriction and sleep disruption. The U.S. Army Ranger training course is 62-days in length and is designed to teach and evaluate individual leadership and small unit tactics under physically and mentally challenging conditions. The course includes multi-day periods consisting of near-continuous physical activity, energy restriction and sleep deprivation. In the first investigation, energy intake was restricted to 1,300 kcal per day during the field training portion of the course and the periods of underfeeding produced average energy deficits of ~1000 kcal/day over the entire course (Friedl et al., 2000). Average energy expenditures were ~4000 kcal/day. At the end of the course, the participants had lost 13-16% of their initial body mass, ~65% of their fat mass, and had lost 7% of their initial lean body mass. IGF-I, measured every 2 weeks during the Ranger Course, progressively declined through the first 6 weeks, with no further reduction over the final 2 weeks of the course (Figure 3). At the end of the course, IGF-I values had fallen 62% (pre: $198 \pm 54 \text{ ng} \cdot \text{ml}^{-1}$ vs. post: $75 \pm 25 \text{ ng} \cdot \text{ml}^{-1}$). As Figure 3 illustrates, most of the decrease in serum IGF-I occurred during the initial 2 weeks of the course. The potential of IGF-I as a discriminating variable for assessing nutritional and/or metabolic stress was the separate observation that the

soldiers who had the greatest decline in IGF-I were the soldiers that lost the most weight ($r = -0.38$, $P < 0.01$).

A second study with the U.S. Ranger Training Course enabled investigators to study the effects of altering the energy content of the diet on the metabolic and hormonal responses to the course (Friedl et al., 2000). In the second study, the training conditions were nearly identical, but the participants received additional calories during the energy restriction periods embedded within the course (+400 kcal/day). Additionally, to gain information about short-term responses to refeeding (while other course stressors remained undiminished), a blood sample was obtained after a week of access to food that was preceded by multiple days of energy restriction (~1700 kcal/day) coupled with high-energy expenditures (>4500 kcal/day). As illustrated in Figure 3, the addition of 400 kcal/day significantly attenuated the decline in circulating IGF-I concentrations when compared to the group receiving fewer calories. Additionally, the investigators found that the brief period of refeeding was sufficient to temporarily restore IGF-I concentrations to baseline values. When food was again restricted after this brief refeeding period, IGF-I concentrations rapidly fell and remained low through the remainder of the course. These data demonstrate the sensitivity and responsiveness of IGF-I to energy and nutrient delivery. When energy is restricted, IGF-I values fall and remain low until energy restriction is removed. The provision of energy and the restoration of fuel stores are accompanied by an increase in IGF-I.

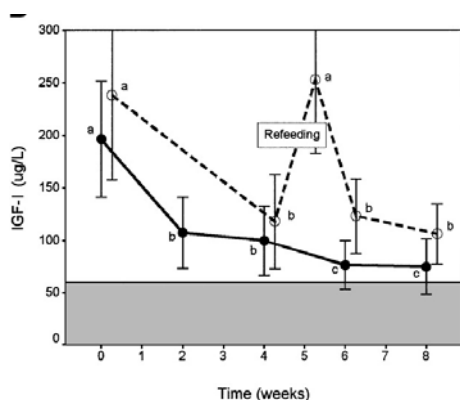


Figure 3. Serum IGF-I concentrations over the 8-wk U.S. Army Ranger Training Course. The solid lines represent the values from Study 1 and the dotted line represents the values from Study 2. Study 1 and Study 2 were conducted under identical conditions with the exception that during Study 2, the subjects received 400 more kcal/day than Study 1. Values are mean $\pm$ SD. Different letters represent mean values that are statistically different; shaded area represents values below normal for

young men. The figure is printed with permission. (See Friedl et al., 2000)

The traditional evaluation of nutritional status utilizes a global assessment of parameters that include anthropometric measures and the assay of serum proteins (Baxter et al., 1998). The proteins commonly measured include albumin, transferrin, prealbumin and retinol binding protein. Transferrin is indicative of iron binding capability; retinol binding protein is indicative of Vitamin A status and ability to transport Vitamin A; prealbumin (considered by some to be best single marker of malnutrition due to its short half-life) is sensitive to protein malnutrition and zinc deficiency. The strength of these markers is that they provide insight into the nutritional status of the individual. Unfortunately, a number of non-nutritional factors can affect serum levels independent of dietary adequacy. For example, prealbumin levels fall with inflammation, albumin levels are affected by hydration state and oral contraceptive use, transferrin levels decline in response to protein malnutrition but also chronic illness and inflammatory states and with liver disease. In contrast, IGF-I appears to be a more responsive and selective biomarker of energy status due to its rapid response to depletion and repletion (Baxter et al., 1998). The 2-4 hour half-life of IGF-I provides a distinct advantage vs. other traditional serum protein biomarkers (prealbumin ~ 2 d, albumin 20 d, transferrin 20 d).

In a study examining endocrine and metabolic recovery responses, Nindl et al. (1997) measured IGF-I, transferrin, ferritin, and prealbumin before, at the end of the U.S. Army Ranger Training Course, and after 5 weeks of recovery. The five week recovery period produced a rebound effect such that body mass was significantly higher than measured before starting the course. Body composition analysis revealed a 1.1 kg increase in fat free mass and a 4.1 kg increase in fat mass above pre-course values. IGF-I fell ~50% during the course and was 30% above baseline values after 5 weeks recovery. Transferrin levels did not significantly change during Ranger training or during recovery. Prealbumin levels declined 21% during the course (26.8 to 21.3 mg/dl) and returned to baseline levels during recovery period, but the levels at the end of the course (despite accruing an 11% body mass loss) were well above values indicative of malnutrition (< 15 mg/dl). Thus, in this study, IGF-I appeared more sensitive to changes in energy balance and body composition changes than the other markers of nutritional status.

3.2 Short-Term Military Sustained Operations

To study the acute responses to energy restriction we recently measured the circulating IGF-I and IGF binding proteins pattern of response to 4 days of near-continuous

physical work, energy restriction, and sleep disruption (Nindl et al., 2003a). The participants had morning fasted blood drawn on days 1, 3, and 4 during a control week that contained physical performance testing, but no sustained physical activity, caloric restriction or sleep deprivation. They also had blood samples drawn on days 1, 3, and 4 of the experimental period that included the physical performance tests, near-continuous physical activity (energy expenditure $\sim 4,500$ kcal/day), energy restriction ($\sim 1,600$ kcal/d), and sleep deprivation (6.2 ± 1.1 h over 84 h course). Blood was assayed for concentrations of total IGF-I, free IGF-I, IGFBPs 1, 3, and 6 and the acid labile subunit. Additionally, in order to gain further insight into whether this type of stress altered the partitioning of IGF-I among its various molecular complexes, IGF-I and IGFBP-3 were measured before and after immunoaffinity depletion of acid-labile subunit complex (i.e., ternary complex removal), thus yielding estimates of ternary (high molecular weight complexes) vs. non-ternary (low molecular weight complexes) IGF-I (Khosravi et al., 2000). Two days of military operational stress significantly lowered circulating total and free IGF-I values and they remained low with continued operational stress (Nindl et al., 1997). Accompanying the IGF-I reductions were small reductions in IGFBP-3 and large increases in IGFBP-1. These changes in circulating IGFBP levels, however, were not associated with a measurable shift in the quantity of IGF-I circulating in ternary, binary or free forms (Nindl et al., 1997). The importance of these data for metabolic monitoring is that they show the speed with which the IGF-I system responds to energy and/or nutritional restriction. They also illustrate a potential method for investigating changes in the bioavailable IGF-I in response to nutritional stress.

3.3 Influence of Dietary Protein Content of Circulating IGF-I During Military Training

Both energy restriction and protein energy malnutrition are known to suppress circulating IGF-I. There are many logistical challenges to sustaining adequate nourishment for soldiers during military field training such as food preparation, storage, delivery, and meals that provide adequate levels of calories and macro- and micronutrients. With increased operational tempo of current military maneuvers, space allocation for food is often sacrificed for weapons, ammunition and other necessary field gear. It would therefore seem essential that the nutrients that are provided during military operational stress consist of an optimal macronutrient mix that may protect against the decline in circulating anabolic and growth factors (Friedl, 1999). The recommended daily allowance for protein is 0.8 g/kg body mass. Current recommendations for physically active populations are 1.2 to 1.5 g protein/kg body mass

(Fielding and Parkinton, 2002; Rand et al., 2003). It is common for infantry type units to subsist on one-to-two Meals Ready-to-Eat (MRE) per day during field operations. The MRE is a 1,300 kcal ration comprised of 24 menus. Protein content of the ration ranges from 26 to 60 grams with a mean value of 44 grams. Thus, if a soldier is limited to one-MRE per day the diet is low both in energy and protein content. Even consuming 2 MREs per day, the soldier may still not meet the minimal RDA for protein.

To examine the hypothesis that dietary protein supplementation during military operational stress would attenuate the decline in IGF-I observed when units were fed insufficient energy and protein, we recently conducted a study where dietary protein was manipulated, while controlling both carbohydrate and energy intake. Thirty-five Marines were randomly divided into either a group receiving a low energy-low protein diet (1,600 kcal/day and 0.5 g protein/kg body mass per day) or a group receiving a similar amount of energy but with sufficient added protein to receive approximately 1.0 g protein/kg body mass per day. The group was participating in an 8-day field exercise consisting of sustained physical activity (total daily energy expenditure measured in previous iterations has ranged from 17-25 MJ/d) and sleep deprivation. Morning fasted blood was obtained before, mid-way and at the end of the course. Preliminary results show trends suggesting that protein supplementation may have attenuated the decline in IGF-I during the course. If more thorough examination of the data supports this conclusion, these data would provide further support for the merit of monitoring IGF-I as a biomarker for metabolic status. Another observation from this study was that IGF-I displayed a different temporal pattern in response to the course than other conventional nutritional status indicators (e.g. ferritin, prealbumin, transferrin, and retinol binding protein). Transferrin and ferritin initially increased during the course but reversed towards baseline values during the latter half of the course. Retinol binding protein and prealbumin declined over the course but more abruptly during the latter half of the course. Thus, while both IGF-I and the conventional markers responded to the training stress their differential response suggests that they each provide a different index of nutritional status.

3.4 Measurement of IGF-I with a Filter Paper Blood Spot Assay

If IGF-I is to be used as a metabolic status indicator during military operational stress, field expedient methods for collection and measurement must be established. Field environments present unique logistical challenges compared to the laboratory. There is more likelihood of sample contamination, and since it is difficult and sometimes impossible to bring the

laboratory equipment to remote field environments, sample collection, processing, and transportation become significant logistical hurdles.

A technique that has been used successfully to study malnutrition in underdeveloped countries is chemical analysis of dried blood spots (Diamandi et al., 1998; Mitchell et al., 1987). The technique requires minimal amounts of blood, minimal field processing, and no refrigeration during shipping. Mitchell et al. (1987) originally described measurement of IGF-I from blood spots using a conventional RIA. More recently, Diamandi et al., (1998) described the extraction and measurement of IGF-I and IGFBP-3 from dried blood spots using an enzyme-linked immunoabsorbent assay.

To study whether the dried blood spot methodology could track IGF-I responses to military operational stress, both blood spots and conventional blood samples were collected in a recent field study that manipulated dietary protein intake (described above). We found that IGF-I measured from blood spots declined during the 8-day course and the magnitude of decline was similar to the decline measured using serum samples (Figure 4) (Nindl et al., 2003b). Overall, the blood spot IGF-I and serum IGF-I significantly ($p < 0.05$) correlated ($r = 0.92$), but the blood spot values were on average 61% lower than serum (Nindl et al., 2003b). Diamandi et al. (1998) also reported lower (20-25%) IGF-I values from blood spots when compared to plasma samples. Several possible factors could have contributed to the differences in IGF-I using the two sampling techniques. First, in order to reduce pre-analytical variance and ensure maximal extraction it is essential that complete dryness of the blood spot is maintained until the sample is analyzed. In both our study and that of Diamandi et al. (1998), the blood spots were stored in plastic bags without addition of desiccant. Work by others suggests that moisture can produce a glassing effect whereby hygroscopic blood proteins impede elution. Assaying dried blood spots also assumes an absolute and consistent blood volume is distributed onto each punch. If the volume of blood on filter paper was consistently overestimated, it may have contributed to the bias between the two sampling methods as IGF-I was purposefully measured using different assays. Regardless of the reason for the bias, the outcomes of this study reveal that the blood spot on filter paper technique can be applied for measurement of IGF-I responses to military operational stress. The technique requires minimal blood, and minimal equipment assets for sample collection, processing and shipment. The filter paper blood spot method for IGF-I detected reductions accompanying nutritional stress and may be of potential value for characterizing the IGF-I response when conventional blood sampling methods are not feasible.

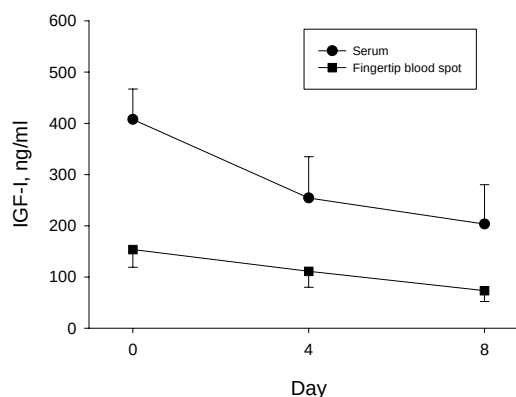


Figure 4. Comparison between serum and filter paper blood spot IGF-I concentration during Days 0, 4, and 8 of military operational stress. For both methods, a progressive decline over time was observed (Day 0 > Day 4 > Day 8). Serum IGF-I was greater than filter paper blood spot IGF-I at each respective timepoint. (See Nindl et al., 2003b)

4. Future Directions and Enablers for Objective Force Warrior

The data collected on the physiological responses to military operational stress support the potential utility of IGF-I as a metabolic sensor of energy status. IGF-I responds rapidly to energy restriction and remains a viable indicator of an altered energy state until the stressor is removed. IGF-I responds rapidly to dietary changes and can be used to evaluate adequacy of protein intake independent of energy intake. IGF-I monitoring may also be useful for optimizing training and injury prevention. Research is needed regarding the role of IGF-I on muscle repair and bone remodeling after the microtrauma of physical exertion. However, it remains to be determined whether tracking IGF-I during military training reduces injury incidence. In addition, sampling and processing techniques must be established that are safe, reliable, require minimal logistical support and most important, provide rapid feedback to personnel tracking physiological status.

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