Phosphorylation of the JAK2-STAT5 Pathway in Response to Acute Aerobic Exercise

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Abstract:

Growth hormone (GH) is a powerful stimulator of the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) pathway. Acute exercise is a known stimulus for GH secretion.

Purpose: The purpose of this study was to determine the phosphorylation of the JAK2-STAT5 pathway in human skeletal muscle in response to acute aerobic exercise.

Methods: Eleven young (22.5 ± 0.6, mean ± SE), healthy, aerobically trained males performed 30 min of cycling at 70% $V \cdot O_{2max}$. Blood samples were collected at 10- to 15-min intervals and analyzed for human GH, immunofunctional (IF) GH, GH binding protein, and insulin-like growth factor I (IGF-I). Muscle biopsies were taken from the vastus lateralis before exercise, immediately after exercise, as well as, 30 and 60 min postexercise. Muscle samples were analyzed for changes in JAK2 and STAT5 tyrosine phosphorylation, as well as changes in JAK2 and STAT5 protein content.

Results: Multivariate ANOVA with post hoc comparisons demonstrated that GH and IF GH were significantly elevated immediately after exercise compared with preexercise ($P < 0.001$). Exercise significantly increased the phosphorylation of JAK2 immediately after exercise ($P = 0.004$). A trend toward increasing levels of STAT5 phosphorylation was observed immediately after exercise ($P = 0.08$) and was significantly elevated 30 min after exercise ($P = 0.002$), compared with preexercise levels. Muscle JAK2 and STAT5 protein content did not change.

Conclusion: The results demonstrate that the JAK2-STAT5 pathway is activated in response to acute aerobic exercise in human skeletal muscle and suggests that the exercise-induced release of GH may play a role in the activation of this pathway.

Article:

It is well established that exercise is a powerful stimulant for the release of growth hormone (GH). Research has consistently documented increases in circulating GH in response to acute aerobic and resistance exercise in both genders (14,30,31). However, the cellular mechanisms by which GH may promote exercise-induced adaptations have yet to be elucidated.

GH has been linked to several physiological properties such as protein synthesis (7) and lipid metabolism (1). The GH receptor (GHR) has been identified in skeletal muscle (5,20), and exogenous GH administration has been reported to increase lean body mass in GH-deficient individuals (10). Also supporting the contention that GH may induce a physiological response in skeletal muscle, Sotiropoulos et al. (23) recently reported that the cross-sectional area of muscle fibers from GHR null mice was significantly smaller than their wild-type counterparts. Although most research linking GH to lipid metabolism has been conducted in adipocytes, preliminary research has suggested that this hormone may also play a role in skeletal muscle metabolism, perhaps through the regulation of oxidative enzymes (15).

Based on this knowledge, it is conceivable that GH may play a role in skeletal muscle hypertrophy and/or metabolic alterations traditionally observed with exercise training. It is clear that any biological response that occurs in skeletal muscle in response to exercise-induced GH is mediated through postreceptor signaling. To date, no known research has attempted to measure a GH-mediated pathway in human skeletal muscle in response to exercise. Therefore, to address this, preliminary research is necessary to identify the postreceptor pathways that are activated in response to exercise-induced GH.

It has been reported that the pulsatile release of GH is responsible for stimulating postreceptor pathway(s), and this ultimately results in the expression of GH-mediated properties (26). Exercise is considered a potent stimulator of GH release in humans and, therefore, provides an attractive model to investigate the effects of a natural physiological release of GH in vivo. The GH-mediated Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) pathway has received most of the attention because it has been reported to be activated in response to GH stimulation in a number of tissue cells, including skeletal muscle (6,12,20,26). In addition, the STAT5 protein is thought to be the major transcription factor responsible for GH-induced gene transcription (6,32).

The use of the immunofunctional (IF) GH assay allows researchers to measure GH that contains the sequence of amino acids needed for the GH-GHR complex to be formed, a step that is critical for cellular transduction of the GH signal (18). Examination of the IF GH profile in response to exercise is important as this quantifies the amount of GH that is theoretically capable of binding to the GHR. It is believed that IF GH binds to and dimerizes the GHR on the skeletal muscle cell membrane, forming a 1:2 GH-GHR complex responsible for recruiting and phosphorylating the tyrosine kinase, JAK2 on tyrosine residues 1007/1008. Upon binding to the GHR, JAK2 is responsible for phosphorylating the receptor and consequently creating a number of docking sites for intracellular proteins, such as STAT5 (25). As a result of GH stimulation, STAT5 has been reported to be phosphorylated on tyrosine residues 694 (STAT5a) and 699 (STAT5b), and, through an unknown mechanism, dissociates from the GHR, undergoes hetero- and/or homodimerization with other STAT proteins, and rapidly translocates to the nucleus to mediate gene transcription (16,17).

Preliminary research suggesting that GH mediates the JAK2-STAT5 pathway have almost exclusively been in vitro or through the administration of supraphysiological doses of exogenous GH (6,12,20). To date, no known research has been published investigating the JAK2-STAT5 pathway in human skeletal muscle in response to a naturally occurring GH pulse in vivo. The

purpose of the present study was to examine the activation of this pathway in response to an acute bout of aerobic exercise in human skeletal muscle. We hypothesized that both JAK2 and STAT5 would be expressed in human skeletal muscle and that these intracellular proteins would undergo rapid tyrosine phosphorylation in response to acute exercise. In addition, it was hypothesized that the increased phosphorylation of these cellular proteins would be related to the exercise-induced increases in circulating GH.

METHODOLOGY

Subjects.

Eleven healthy endurance-trained men (aged 18-26 yr) were recruited to participate in the study. All subjects completed a detailed medical history and provided written informed consent; as approved by the institutional review board at Colorado State University. Subjects were nonsmokers, were not taking any medications that would alter endocrine function, and were habitual aerobic exercisers (>=30 min per session and >=3 sessions·wk^{-1}) for at least the previous 6 months.

Preliminary testing.

Subjects completed a maximal cycle ergometer graded exercise test on a Lode Excalibur Sport cycle ergometer (Lode, Excaliber Sport, Groningen, the Netherlands) using open-circuit spirometry (True Max 2400; Parvo Medics, Salt Lake City, UT) to determine peak oxygen consumption ($V \cdot O_{2max}$). Heart rate was determined using a Polar a5 heart rate monitor (Polar Electro Inc, Woodbury, NY). After a 2-min warm-up, subjects pedaled between 70 and 100 rpm at a starting workload of 100 W. The workload was increased by 25 W every 2 min until volitional fatigue (rpm's fell below 50), medical concerns, or their heart rate reached the predicted maximum (220-age). The highest mean 1-min $V \cdot O_2$ value obtained during testing was used to calculate workload during the submaximal aerobic exercise protocol. Percent body fat was determined by dual energy x-ray absorptiometry on a GE Lunar (DPX-IQ) at medium speed. At least 48 h after the aerobic capacity test, subjects underwent a 20-min cycling bout to determine a workload that would elicit 70% of their $V \cdot O_{2max}$. Subjects completed this session on a Monark cycle ergometer (Varberg, Sweden), similar to the one used during the experimental session. Oxygen consumption was measured throughout this session to verify the appropriate intensity was achieved. The workload was adjusted as necessary to correspond to 70% of their $V \cdot O_{2max}$. Once the subject had reached a steady state at the appropriate intensity (70% $V \cdot O_{2max}$), the workload and heart rate was recorded and used to ensure proper exercise intensity during the experimental session.

Experimental session.

Subjects completed a 12-h fast and were instructed not to exercise 48 h before the experimental session. All subjects arrived at the laboratory at 0800 hours, and an indwelling catheter was inserted into an antecubital vein for serial blood sampling. During the initial 75 min of the session, subjects rested in the supine position.

After 79 min of resting, subjects began a 6-min warm-up period on a Monark cycle ergometer with 2-min workloads calculated at 40%, 50%, and 60% of their predetermined $V \cdot O_{2max}$. At 85 min, the workload corresponding to 70% of the subject's predetermined $V \cdot O_{2max}$ was set (as determined during the previous workload verification session), and the subject cycled at this

relative intensity for 30 min. Heart rate and workload were recorded every minute, and RPE was recorded every 5 min. The workload was adjusted as needed to keep the subject's heart rate within the target heart rate zone determined during the workload verification session. After completion of 30 min of cycling, subjects rested in the supine position for the remaining 60 min of the session.

Blood collection and analysis.

A total of 16 blood samples were collected through an indwelling venous catheter at 10- to 15-min intervals throughout the 175-min experimental session to ensure an accurate GH profile was documented. Blood samples used for serum analysis were allowed to clot at room temperature for 30 min. Blood samples used for plasma analysis were collected in EDTA tubes. All samples were centrifuged at 3000 rpm for 15 min at 4°C, and then serum and plasma were stored at -80°C for future analysis. Diagnostic Systems Laboratory ELISA were performed to determine serum human GH and IF GH at all 16 time points, as well as GH binding protein (GHBP) and plasma total IGF-I at six selected time points.

Muscle biopsy.

Four percutaneous muscle biopsies were taken from the vastus lateralis with a Bergström needle with suction as previously described (9). Muscle biopsies were taken 30 min before exercise, immediately after exercise, as well as 30 and 60 min postexercise. Each consecutive muscle biopsy was taken from the alternating leg through a separate incision. Muscle samples were quickly dissected free from the surrounding fat and connective tissue and immediately placed in liquid nitrogen and stored at -80°C.

Muscle processing.

Muscle samples were homogenized in ice-cold lysis buffer containing 1.0 M Tris (pH 7.4), 150 mM sodium chloride, 1% triton X-100, 0.5% nonidet P-40, 1 mM EDTA, and 1 mM EGTA. In addition, appropriate protease (0.3 µM aprotinin, 20 µM leupeptin, 1 µM pepstatin A, and 0.5 µM phenylmethylsulfonyl fluoride) and phosphatase inhibitors (2 µM sodium vanadate, 10 mM sodium fluoride, and 20 mM [beta]-glycerophosphate) were added. Homogenates were centrifuged at 13,000g for 10 min at 4°C to remove insoluble material. The supernatant was then removed and protein concentration determined by BCA method (Pierce Chemical Company, Rockford, IL).

NuPAGE 4× LDS sample buffer (Invitrogen, Carlsbad, CA) and NuPAGE 10× reducing agent (Invitrogen) were added to each sample and heated at 80°C for 5 min. Twenty micrograms of total protein was loaded and resolved onto a NuPAGE Novex 4-12% Bis-Tris PAGE (Invitrogen). After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). The PDVF membranes were blocked with 4% nonfat dry milk in phosphate-buffered saline/Tween and incubated overnight with anti-STAT5a (Upstate, Chicago, IL), anti-STAT5b (Upstate), anti-JAK2 (Cell Signaling Technology, Beverly, MA), anti-phosphospecific STAT5 (Upstate), or anti-phosphospecific JAK2 (Cell Signaling Technology). The antibodies used to detect phosphorylation were specific to the tyrosine 694/699 and tyrosine 1007/1008 residues on the STAT5 and JAK2 proteins, respectively. These are residues previously shown to be phosphorylated in response to GH. Membranes were washed and exposed to appropriate horseradish peroxidase conjugated secondary for 1 h. An enhanced

chemiluminescence reagent (Pierce Chemical Company) was used to detect antibody binding and was visualized when exposed to Kodak autoradiographic film. Kodak densitometry software was used to quantify results based on band pixel intensity.

Statistical analysis.

Values are presented as mean ± SE. Serum human GH, IF GH, GHBP, and plasma IGF-I were analyzed for the effects of time with a multivariate ANOVA (MANOVA). Six selected post hoc comparisons were performed using the least square difference (LSD) method with appropriate Bonferroni adjusted level of significance (P = 0.0083). The two GH assays (human GH and IF GH) were compared by MANOVA for integrated AUC, peak concentration, and time-to-peak GH concentration.

All muscle protein content and phosphorylation levels were quantified based on band pixel intensity and reported relative to preexercise values (fold change from preexercise). Relative protein content and phosphorylation levels were analyzed by MANOVA for changes with respect to time, and three selected post hoc comparisons were performed using the LSD method with appropriate Bonferroni adjusted level of significance (P = 0.017).

To evaluate possible relationships between hormone and JAK2-STAT5 signaling events, we calculated Pearson correlation coefficients between selected variables. In all instances, with the exemption of post hoc analysis using the adjusted Bonferroni method, the level of significance was set at $P < 0.05$. All statistical analysis was performed using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Table 1 presents the subject characteristics. All 11 subjects completed 30 min of cycling and obtained a mean heart rate (164 ± 3 bpm) within 10% of their target heart rate (range, 93.1% to 109% of target heart rate) to attain an exercise intensity of 70% $V \cdot O_{2max}$. The mean RPE recorded during cycling was 14.5 ± 0.4, and the mean external work completed during the 30 min of cycling was 337 ± 14 kJ (range, 244-419 kJ).

Variable	Mean ± SE
Age	22.5 ± 0.6
Height (cm)	182.6 ± 2.1
Weight (kg)	72.9 ± 2.7
BMI ($kg \cdot m^{-2}$)	21.8 ± 0.6
Percent body fat	8.9 ± 0.7
Aerobic exercise per week (h)	7.4 ± 1.1
Cycling exercise per week (h)	1.9 ± 0.5
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	54.7 ± 1.2

Values are mean ± SE.

Table 1. Subject demographics (n = 11).

Hormonal parameters.

Hormone analyses were based on data from 10 subjects because of an incomplete blood profile from one subject. The mean pattern of human GH and IF GH release is presented in Figure 1. GH and IF GH increased immediately after exercise (approximately 26-fold, $P < 0.001$, and approximately 15-fold, $P < 0.001$, respectively, mean-fold change over preexercise). Also, GH and IF GH were elevated at preexercise (30 min) compared with baseline (0 min) levels ($P = 0.002$ and $P = 0.005$, respectively). The mean integrated hGH AUC was significantly greater than the mean integrated IF GH AUC (1028 ± 135 $\mu g \cdot L^{-1}$, 175 min, vs 765 ± 111 $\mu g \cdot L^{-1}$, 175 min, respectively, $P < 0.001$). Peak hGH concentrations were significantly higher then peak IF GH concentrations (17.21 ± 1.80 vs 11.84 ± 1.45 $\mu g \cdot L^{-1}$, respectively, $P < 0.001$); however, no differences were observed between the two assays with respect to the time-to-peak GH concentrations ($P = 0.47$).

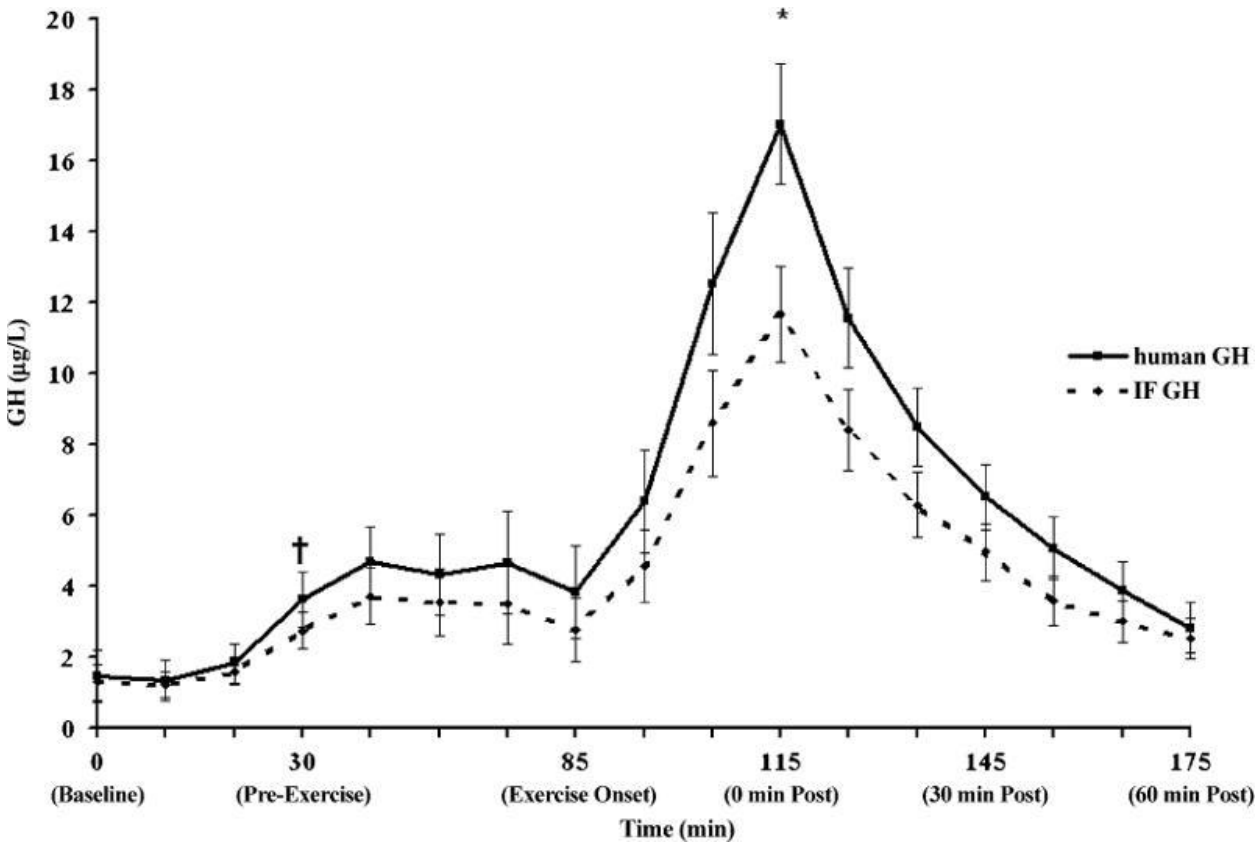

FIGURE 1-Human GH (solid line) and IF GH (dotted line) profiles during rest, exercise, and recovery. Values are mean ± SE for 10 subjects. *Different from preexercise, $P < 0.004$; †different from baseline, $P < 0.006$.

GHBP and IGF-I were measured at six selected time points and are presented in Table 2. GHBP and IGF-I were significantly increased ($P < 0.001$) immediately after exercise compared with preexercise concentrations. Increases were transient and returned to preexercise levels within 30 min of recovery.

	0 min (Baseline)	30 min (Preexercise)	115 min (Postexercise)	145 min (30-min Recovery)	175 min (60-min Recovery)
GHBP (pmol·L^{-1})	628.3 ± 55.4	619.3 ± 61.6	732.3 ± 49.2*	610.1 ± 53.5	633.8 ± 53.2
IGF-I (ng·mL^{-1})	350.6 ± 26.7	337.7 ± 21.7	390.0 ± 27.7*	343.1 ± 25.4	335.1 ± 20.4

Values are mean ± SE.
Different from preexercise, $P < 0.001$.

Table 2. GHBP and IGF-I concentrations at rest and in response to exercise (n = 10).

JAK2 and STAT5 isoform expression in human skeletal muscle.

JAK2, STAT5a, and STAT5b proteins were expressed in all 11 subjects at all four time points. No significant changes in protein content occurred in response to exercise (P > 0.20).

JAK2 and STAT5 phosphorylation in response to exercise.

The effects of acute aerobic exercise on JAK2 and STAT5 phosphorylation are shown in Figures 2 and 3, respectively. Acute exercise increased JAK2 phosphorylation approximately 1.9-fold immediately after exercise compared with the preexercise levels (P = 0.004). This increase was transient with phosphorylation levels returning to preexercise levels 30 min postexercise. STAT5 phosphorylation showed a trend to increase immediately after exercise (approximately 3.5-fold, P = 0.08) and was significantly increased 30 min postexercise (3.2-fold, P = 0.002) compared with preexercise levels. Phosphorylation levels of STAT5 returned to preexercise levels 60 min postexercise.

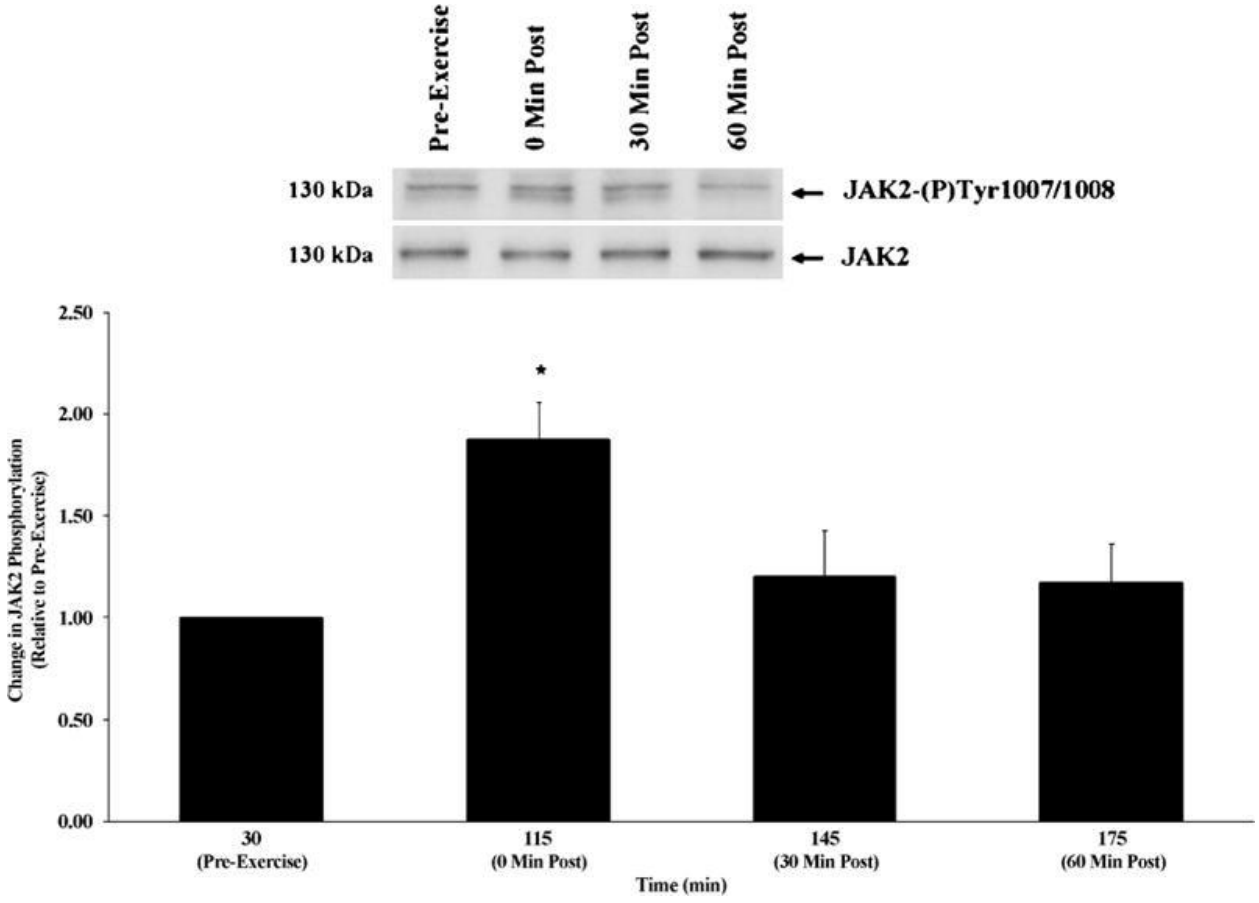

FIGURE 2-JAK2 phosphorylation at rest and after exercise. Top: immunoblot from one subject. Bottom: fold change in JAK2 phosphorylation relative to preexercise levels. Values are mean ± SE for 11 subjects. *Different from preexercise, P = 0.004.

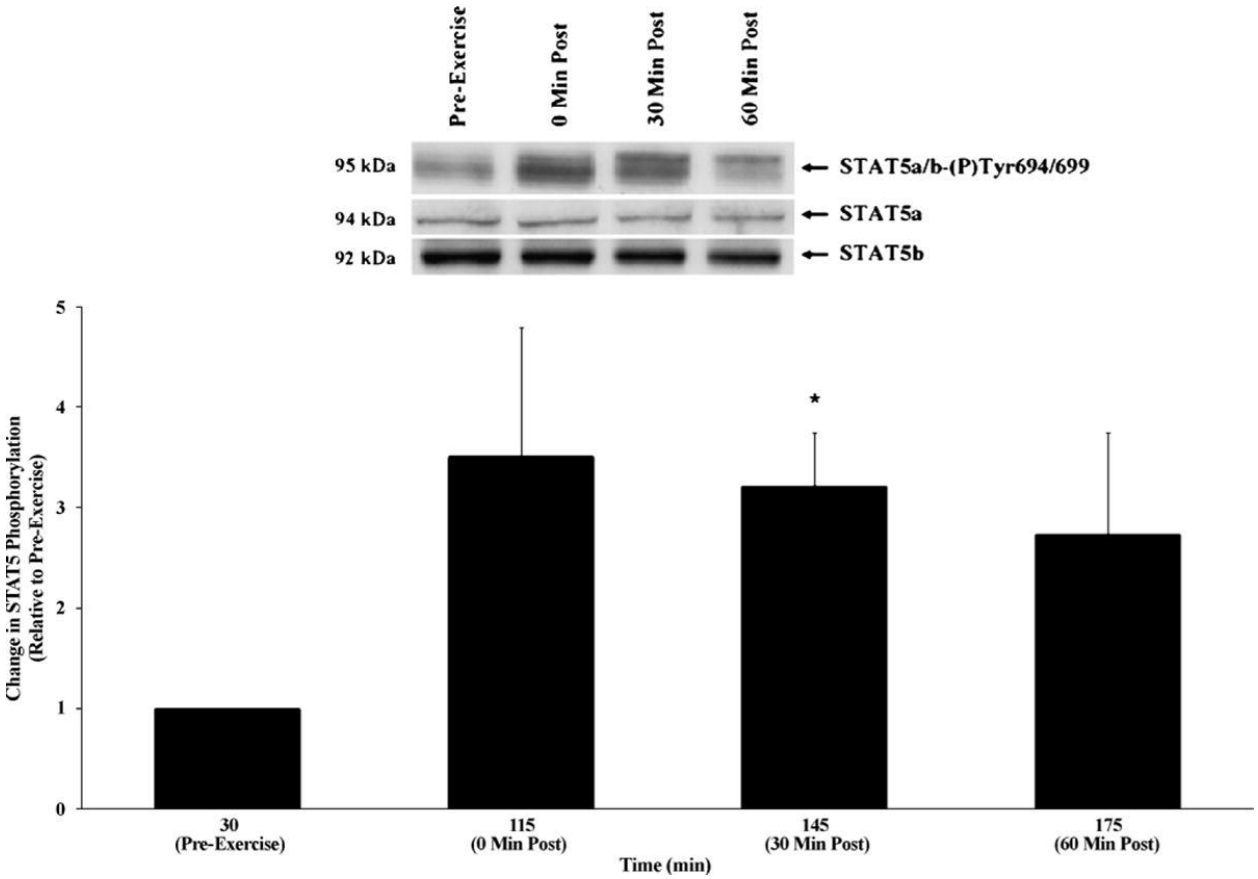

FIGURE 3-STAT5 phosphorylation at rest and after exercise. Top: immunoblot from one subject. Bottom: fold change in STAT5 phosphorylation relative to preexercise levels. Values are mean ± SE for 11 subjects. *Different from preexercise, P = 0.002.

Relationship between hormone variables and JAK2-STAT5 pathway.

No significant relationships were noted between GH measured by the general human GH assay and STAT5 phosphorylation. However, the absolute change in IF GH (30 to 115 min) and peak IF GH was significantly related to the fold change in STAT5 phosphorylation from preexercise to immediate postexercise ($r = 0.72$, $P = 0.019$; $r = 0.66$, $P = 0.037$, respectively).

DISCUSSION

The aim of the present investigation was to examine the response of the JAK2-STAT5 pathway to acute aerobic exercise, a potent stimulator of GH release in humans. The main findings from this study show that 1) JAK2 and STAT5a and STAT5b isoforms are expressed in human skeletal muscle; 2) an increase in the phosphorylation of JAK2 and STAT5 occurs in response to acute aerobic exercise in young males; and 3) a positive relationship exists between the exercise-induced increase in IF GH in circulation and the phosphorylation of the STAT5 pathway in skeletal muscle. To our knowledge, this is the first study to investigate the response of the JAK2-STAT5 pathway to exercise.

As hypothesized, both JAK2 and STAT5 were phosphorylated in response to exercise and returned to baseline levels within 30 and 60 min of exercise cessation, respectively. Similar to JAK2, STAT5 demonstrated peak tyrosine phosphorylation immediately after exercise, but unlike the upstream kinase, STAT5 demonstrated considerable variability in its response, and consequently, statistical significance was achieved only at 30 min postexercise. To date, there have been no other published studies examining the response of this pathway to exercise; however, our timeline of phosphorylation is consistent with other STAT5 research. Recently, Jorgensen et al. (12) reported increased skeletal muscle STAT5b phosphorylation 30-60 min after infusion of an exogenous GH bolus in humans. In addition, Tannenbaum et al. (24) reported that in rats, hepatic STAT5 DNA-binding was highest during the first 15-60 min of a natural GH pulse.

Exogenous GH is a proven regulator of the JAK2-STAT5 pathway in cellular and animal models (6,24). Exercise is a powerful stimulator of endogenous GH release in humans and, thus, was considered a unique model to investigate the STAT5 pathway. As expected, circulating human GH and IF GH increased in response to acute exercise (approximately 26-fold and 15-fold, respectively), substantiating our claim that exercise provides an attractive model to investigate the GH-mediated STAT5 pathway in vivo.

In addition to the novelty of using an endogenous GH pulse, the results from the present study were of interest because the magnitude of circulating GH and coinciding activation of the JAK-STAT pathway could be investigated. Previously, Jorgensen et al. (12) demonstrated increased STAT5b phosphorylation after administration of an exogenous GH bolus that produced peak GH values that were much greater than those observed in the present study (94 ± 5 vs 17 ± 2 $\mu g \cdot L^{-1}$) and were in excess of that typically observed under normal physiological conditions. Therefore, the present study provides unique insight into the activation of this pathway under normal physiological conditions.

A temporal relationship ($r = 0.94$) between spontaneous GH pulses in circulation and STAT5 activity in the liver has been previously reported in rats (24). In the present study, a significant positive relationship was observed between the increase in circulating IF GH and the increase in STAT5 phosphorylation ($r = 0.72$) in skeletal muscle. Although purely speculative at this time, possibly, this significant relationship between IF GH (and not human GH) and STAT5 phosphorylation highlights the sensitivity of this assay for measuring GH capable of binding to the GHR and initiating postreceptor signaling.

Circulating GHBP and IGF-I were also measured based on their potential influence on circulating GH concentrations. In agreement with others (19,21), both hormones increased in response to exercise. Unfortunately, the mechanism for the exercise-induced increase in circulating IGF-I still remains unknown. Similar to other studies (21), we speculate that the increase in IGF-I was independent of circulating GH because the timeframe for GH-induced release of IGF-I from the liver likely exceeds the timeframe studied. It was also speculated that local production of IGF-I from skeletal muscle could not account for an increase in circulating IGF-I of this magnitude. GHBP is produced during proteolytic cleavage of the GHR by tumor necrosis factor-[alpha]-converting enzyme (TACE) (22). To date, no research has examined the

effects of exercise on TACE activity, but in vitro research has suggested a number of potential regulators (i.e., MAPK, PKC, ROS), some of which are known to be activated by exercise (4,28,33). Exercise-induced activation of these intracellular regulators may explain the transient increase in GHBP observed in the present study.

Both GH and STAT5 have characteristics that make them challenging to study in vivo. GH is released in a sporadic pulsatile manner with significant changes in GH concentrations occurring in short timeframes, which complicates tracking of cellular responses in vivo. In addition, cell culture research demonstrates a 4- to 5-h refractory period after maximal STAT5 activation, where subsequent phosphorylation cannot take place (6). Because spontaneous GH pulses before exercise may have an effect on intracellular signaling during exercise, GH samples were taken for 30 min before the baseline biopsy and for 85 min before the onset of exercise. As illustrated in Figure 1, resting serum GH values were within the normal resting range but appeared to increase slightly in anticipation of the initial muscle biopsy. This occurred in all subjects but was considered to have a minimal effect on intracellular signaling because GH levels returned to baseline levels before the onset of exercise, and the magnitude of the anticipatory increase in GH was much less than that observed in response to exercise. Despite the experimental session being completed in the morning after an overnight fast, when GH levels should be at their lowest, we cannot exclude the fact that a GH pulse occurred before sampling and may have played a role in affecting the STAT5 pathway before the exercise stimulus. Admittedly, based on the design of the present study, we cannot conclude that GH was solely responsible for the phosphorylation of STAT5. A number of cytokines are activated in response to exercise that may have influenced the STAT5 pathway in either a positive or inhibitory manner. Numerous cytokines have been reported to activate the STAT5 pathway; however, many of these cytokines (i.e., erythropoietin or thrombopoietin) have not been reported to increase in response to exercise under normal environmental conditions (2,8). Although the present study cannot directly link the phosphorylation of the STAT5 pathway to circulating GH, it provides preliminary evidence for future research investigating the possible direct (or indirect) role of exercise-induced GH on the STAT5 pathway in human skeletal muscle.

The "downstream" physiological effect(s) of enhanced STAT5 phosphorylation in skeletal muscle were not specifically assessed in the present study. However, the demonstration that this pathway is activated in response to exercise, along with the previously reported results in vitro, allow a number of attractive possibilities to be postulated, including roles for exercise-induced GH in skeletal muscle hypertrophy and lipid metabolism. Of particular interest is research linking GH-mediated STAT5 activation to the expression of IGF-I. Evidence exists that GH-induced STAT5 phosphorylation promotes IGF-I expression in C2C12 myoblasts (6). In addition, a recent study by Klover and Hennighausen (13) reported that mice deficient of skeletal muscle STAT5 had a 60% reduction in skeletal muscle IGF-I mRNA. Most of the research examining the response of skeletal muscle IGF-I to exercise has focused on resistance exercise but has reported increases in skeletal muscle IGF-I mRNA 3 h after a single bout of resistance exercise (3). Although no known research has examined the metabolic consequences of STAT5 in skeletal muscle, Asada et al. (1) reported that the GHR-JAK2-STAT5 pathway was a likely candidate for GH-induced lipolysis in 3T3-L1 adipocytes. In addition, it was recently reported that the STAT5a isoform was responsible for pyruvate dehydrogenase 4 (PDK4) gene expression (29) and decreases in the expression of fatty acid synthase mRNA and protein levels in

adipocytes (11). Findings in adipocytes advocate for further investigation of the metabolic role of STAT5 in skeletal muscle.

We identified the expression of both STAT5 isoforms in human skeletal muscle. To our knowledge, this is the first study demonstrating the existence of the STAT5a isoform in healthy human skeletal muscle. Webb et al. (27) compared the STAT5 phosphorylation in GH-deficient and "normal" healthy controls. These researchers reported that only half of their healthy subjects expressed STAT5b in skeletal muscle and none expressed STAT5a. The reasoning for this discrepancy is currently unknown but could be attributed to the examination of different muscle groups (biceps vs vastus lateralis) or methodological differences in the preparation of muscle lysates.

As illustrated in Figures 2 and 3, the JAK2 and STAT5 phosphospecific antibodies identified double bands of similar molecular weight on their respective blots. Muscle lysates treated with calf intestinal alkaline phosphatase demonstrated decreased band intensity in both bands on the STAT5 blot and the bottom band only on the JAK2 blot (data not shown). The decreased band intensity on these blots verified that these bands represented phosphorylated protein and, consequently, were selected for quantification.

In summary, the results from the present study demonstrate that JAK2 and STAT5 are phosphorylated in response to acute exercise and suggest that exercise-associated increases in circulating GH may be a candidate for regulating this pathway in humans. To date, most of the exercise research investigating intracellular signaling has focused on the IRS-PI3K-AKT and MAPK pathways as regulators of muscle hypertrophy and metabolism. The findings from the present study support future research to examine the physiological role of the JAK2-STAT5 pathway in skeletal muscle.

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