Eccentric exercise increases circulating levels of Fibroblast Activation Protein but not bioactive Fibroblast Growth Factor 21 in healthy individuals

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Abstract

The primary aim of the current investigation was to determine for the first time whether eccentric exercise would augment the release of the novel myokine, Fibroblast Growth Factor 21 (FGF21) and/or its regulatory enzyme Fibroblast Activation Protein (FAP) from skeletal muscle tissue into the systemic circulation of healthy human volunteers.

Eight physically active young healthy male volunteers [age 25.0 ± 3.8 years; body mass index (BMI) 23.1 ± 2.8 kg/m^2] completed 3 sets of 25 repetitions (with 5 min rest in between) of single-leg maximal eccentric contractions using their non-dominant leg, whilst the dominant leg served as a control. Arterialised blood samples from a hand vein and deep venous blood samples from the common femoral vein of the exercised leg, along with blood flow of the superficial femoral artery using Doppler ultrasound, were obtained before and after each exercise bout and every 20 minutes during the 3h recovery period. Muscle biopsy samples were taken at baseline, immediately and 3h and 48h post-exercise.

The main findings from this study showed there was no significant increase in total or bioactive FGF21 secreted from skeletal muscle into the systemic circulation in response to exercise. Furthermore, skeletal muscle FGF21 protein content was unchanged in response to exercise. However, there was a significant increase in arterialised and venous FAP concentrations with no apparent contribution to its release from the exercised leg. These findings raise the possibility that the elevated levels of FAP may play a role in the inactivation of FGF21 during exercise.

Key words. Bioactive FGF21; Fibroblast Activation Protein; Skeletal muscle; Eccentric exercise.

New & Noteworthy (75 words max on summarising what is new about the research).

The role of Fibroblast Growth Factor 21 (FGF21) as an exercise-induced myokine remains controversial and little is known about its activity in the circulation. This study shows eccentric exercise does not stimulate the release of total or bioactive FGF21 from human skeletal muscle. However, exercise augments the release of its regulatory enzyme Fibroblast Activation Protein (FAP) from tissue(s) other than skeletal muscle, which may play a role in the inactivation of FGF21.

Introduction

The endocrine ability of skeletal muscle to secrete particular proteins (referred to as myokines) into the systemic circulation to facilitate cross-talk with other tissues is well established (15). In particular, myokines secreted in response to exercise have a variety of positive effects on chronic diseases and metabolic disorders (13)

Fibroblast Growth Factor 21 (FGF21) has been identified as a novel myokine (6) and is a key regulator of energy metabolism and plasma glucose homeostasis (9). The major site of FGF21 production occur through the liver but also by adipose tissue, pancreas and skeletal muscle in response to specific stimuli, and functions in a paracrine and/or endocrine fashion in these tissues (16). Recently, FGF21 has been suggested to act as an exercise-induced myokine (Kim et al. 2013b), and muscular contractions have been identified as a key stimulus in inducing hepatic release of FGF21 in humans. Indeed, studies have shown FGF21 serum levels to be elevated after endurance exercise between 50-80% of VO_{2max} (1, 17). However, under those conditions most of the release of FGF21 into the systemic circulation occurred through the liver rather than skeletal muscle (5). Collectively, the results from the literature suggest that FGF21 may be a stress-induced hormone rather than an exercise-induced myokine.

Interestingly, most studies have used protocols consisting of exercise involving concentric contractions. Despite the growing interest in the metabolic benefits of eccentric training on young and elderly populations (12, 14, 19), there is a paucity of studies investigating the effects of eccentric exercise on myokine release in humans. Moreover, it should be noted that all studies to date involving FGF21 have only measured the total amount of FGF21 in circulation. However, the activity and stability of FGF21 in circulation and the mechanisms regulating these processes remain largely unknown. Recently, the Fibroblast Activation Protein (FAP) was described as a serine protease that cleaves and inactivates FGF21 (20) but the effects of exercise in this process have not been investigated.

The aim of this study was to evaluate for the first time the effects of exercise on both the bioactive and total forms of circulating FGF21 along with the levels of FAP, and test the hypothesis that performing maximal eccentric muscle contractions will augment the release of FGF21, along with other well established myokines such as IL6, from muscle tissue into the systemic circulation due to greater muscle structural disruption and increased inflammatory responses normally associated with eccentric exercise.

Method and Materials

Subjects

Eight young healthy males (age 25.0 ± 3.8 years, height 177.5 ± 7.2 cm, body mass 73.3 ± 11.8 kg, BMI 23.1 ± 2.8 kg/m^2) participated in the study. All subjects were physically active individuals but unaccustomed to maximal eccentric exercise. The study was approved by the University of Nottingham Medical School Research Ethics Committee in observance of the present regulations imposed by the Code of Ethics of World Medical Association (Declaration of Helsinki).

Prior to any participation, all subjects were fully briefed on all aspects of the study and thereafter signed an informed consent. All subjects took part in an individual medical screening consisting of completing health and physical activity questionnaires, having a 12-lead electrocardiogram and blood pressure measured, and a blood sample taken for routine screening. All eligible subjects were then asked to complete a 3-day food diary (2 weekdays and 1 weekend) in order to assess their habitual dietary intake that allowed the design of the 2-day isoenergetic standardised diet (50% energy from carbohydrate, 30% from fat and 20% from protein) that was required to be adhered to over the course of the experiment.

All subjects attended a preliminary laboratory session to familiarise themselves with the exercise testing procedure, which consisted of a series of sub-maximal and maximal eccentric, concentric and isometric knee-extensor contractions with their non-dominant leg using an isokinetic dynamometer (HUMAC NORM, CSMi solutions, MA, US). Subject also undertook three maximal isometric concentric contractions at 60 degrees for 3 seconds with 5 min rest in between for the assessment of maximum voluntary contraction (MVC).

Experimental Protocol

Following the familiarisation visit, all subjects attended the laboratory on 2 further occasions (main experimental visits) separated by 48h. On the first occasion, subjects arrived at the laboratory at ~08.00am following an overnight fast (10-12h), having abstained from heavy exercise and alcohol and having consumed the standardised mixed diet (described above) for the previous 24h. Subjects were then asked to rest on a bed in a semi-supine position and an intravenous cannula was inserted retrograde into the superficial hand vein of one arm for arterialised-venous blood sampling for the determination of bioactive and total FGF21, FAP and IL6 concentrations. The hand thereafter remained in a hand-warming unit (50-55^{o}C) with

the hand and air temperature continuously monitoring the arterialised-venous blood drainage (3). One further cannula was inserted anti-grade into the common femoral vein of the exercised leg (using ultrasound guidance and the Seldinger technique) for deep venous blood sampling. The cannulas were kept patent via a saline drip. Blood samples from each sampling line were obtained for baseline measurements, after each exercise bout and every 20 min during a 3h recovery period. It should be noted that due to anatomical differences in the location of the common femoral veins between individuals, 6/8 subjects had a femoral cannula successfully inserted into their exercising legs. Blood flow of the superficial femoral artery using Doppler ultrasound (Toshiba, Aplio 300) was also measured at the same time intervals. The product of blood flow and the differences between arterialised venous (from the hand) and deep venous (from the femoral vein) myokine concentrations at each time point were used to calculate the net flux of myokines across the exercising upper leg to provide an index of the contribution of local muscle release to their levels in the general circulation.

Baseline muscle biopsy samples were obtained from the vastus lateralis of both legs (control and exercised) using the needle biopsy technique for the determination of intramuscular protein levels of FGF21 using Western blotting. Muscle biopsy samples were also taken immediately post-exercise and after the 3h recovery period from the exercise leg only during which subjects were rested on a bed in a semi-supine position.

Following completion of all baseline measurements, subjects undertook a series of muscle function tests 10 min before the allocated exercise protocol to assess their (a) maximal isometric strength at a fixed (60 degrees from full extension) knee angle (best of 2 contractions), and (b) total work and peak torque attained during 10 maximal isokinetic concentric contractions of the knee extensors performed at 60 degrees per second. All subjects then performed 3 bouts of single-leg maximal eccentric muscle contractions using their non-dominant leg, whilst their dominant leg served as a control. Each exercise bout consisted of 25 contractions performed at a speed of 30 degrees per second with a 5-min rest interval between each exercise bout. Subjects then rested on a bed in a semi-supine position for 3h. Thereafter, they were provided with a standardised carbohydrate-rich lunch (consisting of pasta, tomato sauce and cheddar cheese) and asked to consume a standardised isoenergetic mixed diet providing 50% energy from carbohydrate, 30% from fat and 20% from protein for the next 48h. All subjects were then subsequently allowed to leave the laboratory.

Forty-eight hours after the end of the last bout of maximal eccentric muscle contractions, subjects returned to the laboratory after an overnight fast having abstained from heavy exercise and alcohol in the intervening period. Upon arrival to the laboratory subjects were asked to rest on a bed in a semi-supine position and a single arterialised blood sample was obtained from a superficial hand vein. Following this, a muscle biopsy sample was obtained from the vastus lateralis of both legs (control and exercised). Subjects subsequently performed the same muscle function tests conducted during the previous visit, using the previously exercised leg to assess the extent to which muscle function had been restored, following the performance of the eccentric protocol 48h beforehand. Following the completion of that visit, subjects were provided with lunch and then allowed to leave the laboratory.

Blood Analysis

Blood samples were centrifuged immediately after collection at 10,000g for 10 min at 4^{o}C to obtain plasma. Bioactive FGF21 (Eagle Biosciences, USA), total FGF21 (Biovendor, Research and Diagnostics products, Czech Republic), FAP and IL6 levels were determined using commercially available Enzyme-Linked ImmunoSorbant Assay (ELISA) kits according to manufactures instructions.

Muscle analysis

Muscle protein extraction was conducted by adding 50mg of frozen tissue to 300µl of HEPES (SIGMA H4034) homogenisation buffer and protease inhibitor cocktail (SIGMA P-8340). Samples were then homogenised on a polytron at a medium speed for 30s and left on ice for 20min. Samples were then transferred into an eppendorf tube and centrifuged at 10,000g for 20min at 4^{o}C. Supernatant was transferred into clean eppendorfs and SDS was then added. Protein was quantified using the Pierce Bovine Serum albumin (BSA) protein assay and stored at -80^{o}C.

Protein separation was carried out using SDS-PAGE, using a 14% gel which was then transferred overnight onto a hydrophobic polyvinylidene difluoride (PVDF) membrane (GE Healthcare). The PVDF membranes were then incubated in BSA blocking buffer on a rocker for 1h at room temperature. FGF21 (Eli Lilly, IN) primary antibody was diluted in 3% BSA and TBS-T at ratio of 1:4000 and was used to incubate the membranes overnight at 4^{o}C. Following this, the membranes were soaked in TBS-T 3 times for 10min to wash off non-specific binding and then incubated with anti-mouse horseradish peroxidase (HRP) secondary antibody (Dako, Denmark) at 1:2000 dilution containing 1% blocking reagent for 1h at room

temperature. The PVDF membrane was then soaked in TBS-T 3 times for 10min. All immunoreactive proteins were visualized using ECL (Amersham Biosciences, UK) and quantified by densitometry using the Quantity One 1-D Analysis Software version 4.5 (Bio-Rad Laboratories, Inc., USA). Actin (Cell Signaling, USA) was used as a housekeeping protein.

Statistical Analysis

Data analysis was carried out using GraphPad Prism 7 Software (GraphPad Software Inc., San Diego, CA). Isometric and isokinetic measurements including fatigue index were analysed using a paired sample t-test with Bonferroni corrections. Blood hormones and enzymes, blood flow, western blot data and eccentric peak torque measurements were analysed using one-way repeated measures analysis of variance (ANOVA) using Bonferroni corrections. Data are reported as means ± SEM, and statistical significance was set at $P<0.05$.

Results

Exercise Tests

There was no difference in maximal isometric peak torque between baseline (206 ± 17 Nm) and 48-hours after exercise (201 ± 27 Nm). Maximal isokinetic concentric peak torque also showed no significant difference between baseline (139 ± 17 Nm) and 48h post-exercise (122 ± 20 Nm). Furthermore, there was no difference in isokinetic concentric total work output between baseline (1489 ± 149 Nm) and 48h post-exercise (1326 ± 195 Nm). There was no significant difference in the amount of fatigue experienced when conducting maximal isokinetic concentric contractions during baseline and 48h post-exercise.

Maximal eccentric peak torque showed no significant difference between set 1 (87 ± 12 Nm), set 2 (75 ± 10 Nm) and set 3 (75 ± 11 Nm). However, maximal eccentric contraction total work per repetition showed a significant decrease over time ($P<0.01$) between set 1 (20 ± 3 Nm), set 2 (16 ± 2 Nm) and set 3 (14 ± 2 Nm).

Total Plasma FGF21

Total FGF21 in arterialised blood showed no significant differences between baseline and all subsequent time points **(Figure 1A).** Similarly, there was no significant change in total FGF21 in the deep venous blood of the exercised leg in response to exercise and subsequent 3h recovery **(Figure 1B).** As a consequence, there was no difference over time in the arterio-venous (a-v) differences of total FGF21 **(Figure 1C)** or its flux across the exercised upper leg **(Figure 1D)**.

Bioactive Plasma FGF21

There were no changes in the concentrations of the bioactive form of FGF21 in arterialised blood **(Figure 2A)** and deep venous blood of the exercised leg **(Figure 2B)** from baseline to all exercise and post-exercise time points. Thus, there were no significant changes over time in a-v differences of bioactive FGF21 **(Figure 2C)** or its flux across the exercised upper leg **(Figure 2D)**. The ratio of bioactive to total FGF21 was 0.65 ± 0.21 at baseline and was unaffected by exercise (maintained at 0.83 ± 0.22 over the 3h recovery period).

Plasma Fibroblast Activation Protein (FAP)

There was an increase ($P<0.05$) in arterialised FAP levels during the exercise period **(Figure 3A)**. Similarly, deep venous FAP in the exercised leg also showed a significant increase during the exercise period ($P<0.05$) **(Figure 3B)**. Both concentrations returned to basal levels within

the first 20 min of the recovery period. However, the a-v differences of FAP were similar between baseline and all exercise and recovery time points **(Figure 3C).** As a result, there was no net flux of FAP across the exercised leg at any time point studied **(Figure 3D).**

Plasma IL6 levels

Arterialised plasma IL6 concentrations showed a significant increase ($P<0.0001$) in response to the 3h recovery from exercise (**Fig. 4A**). On the other hand, deep venous IL6 concentrations in the exercised leg showed an earlier increase in response to the second and third bout of exercise ($P<0.05$) and the subsequent 3h recovery period ($P<0.0001$; **Fig.4B**). However, there was a greater increase in deep venous IL6 concentrations compared with the arterialised levels resulting in negative ($P<0.001$) a-v differences between 40 and 160 min of recovery period (**Fig. 4C**), indicating a net release ($P<0.01$) of IL6 from the working muscle over that period (**Fig. 4D**).

Muscle FGF21 Protein Content

There were no significant differences in FGF21 muscle protein content in the exercised leg between baseline and all subsequent post-exercise time points **(Figure 5).** There were also no significant differences in FGF21 muscle protein content in the control leg between baseline and 48h post-exercise **(Figure 5).**

Discussion

The present study demonstrated maximal eccentric contractions do not cause the release of total or bioactive FGF21 from human skeletal muscle tissue into the systemic circulation. This suggests, under the conditions of the present study, FGF21 may not be considered an exercise-induced myokine. This was further supported by the observation that there was no increase in skeletal muscle FGF21 protein content in the exercised leg in response to eccentric contractions for up to 48h into recovery period. Interestingly, exercise significantly increased the levels of circulating FAP, although it did not appear to be released from skeletal muscle. Despite the absence of an effect on FGF21, maximal eccentric exercise was of sufficient stimulus to cause the release of IL6 from skeletal muscle.

The finding from this study that FGF21 is not released from skeletal muscle in response to maximal eccentric exercise supports the data acquired by Hansen *et al.*, (5) demonstrating no release of FGF21 from the exercising leg in response to sub-maximal concentric exercise in humans. Similarly, data presented by Kim *et al.*, (11) conducted on mice showed that exercise increased hepatic but not skeletal muscle FGF21 mRNA. Collectively, the data from the present and the aforementioned studies indicate that in response to both sub-maximal and maximal contractions, FGF21 is not released from skeletal muscle. Moreoever, the studies by (5, 11) indicate that the liver is the main source of FGF21 secretion into systemic circulation under resting and exercised conditions in humans. It should be noted, in the current study FGF21 protein content in skeletal muscle was measured for up to 48h post exercise unlike the Kim *et al.,* (11) and Hansen *et al.,* (5) studies that measured mRNA only. Although exercise did not cause the induction of FGF21 protein in skeletal muscle, the possibility of FGF21 as a non-exercise myokine exerting localised autocrine or paracrine effects cannot be totally discarded. Indeed, mitochondrial dysfunction induced by autophagy deficiency in skeletal muscle tissue results in the secretion of FGF21 (10). Subsequently, mitochondrial dysfunction induced in transgenic mice resulted in significant increases in plasma FGF21 and its mRNA levels in skeletal muscle (8). Collectively, these findings imply under particular non-exercise conditions such as mitochondrial dysfunction FGF21 may act as a myokine.

Previous studies have shown FGF21 to be significantly increased in the systemic circulation in response to acute and long-term aerobic exercise (1, 17). However, the present study showed no changes in arterialised FGF21 in response to maximal eccentric exercise. A possible explanation for these conflicting reports may be differences in exercise modality and/or

intensity employed by those studies. Indeed, the current study used contractions involving one-leg which is less demanding on systemic stress than contractions using multiple limbs with larger muscle mass involved. The studies by Cuevas-Ramos *et al.,* (1) and Tanimura *et al.,* (17) used submaximal whole-body treadmill exercise at 75-85% of $VO_{2max.}$ This may suggest that in order to augment the release of FGF21 from the liver into circulation, exercise involving substantial muscle mass may be required.

A novel aspect of the present study was the measurement of the bioactive form of FGF21 in response to exercise, whereas all studies to date involving exercise and FGF21 have only measured total FGF21. This is of clear importance, as it was recently shown that a large percentage of total FGF21 is not active (18). Indeed, in the present study ~65% of the total FGF21 was found to be in the bioactive form at baseline. Furthermore, maximal eccentric exercise did not increase the circulating bioactive form of FGF21, and there was no release from human skeletal muscle tissue into the systemic circulation. Interestingly, exercise significantly increased arterialised and venous FAP concentrations. The actions of FGF21 are attenuated by its proteolytic cleavage in plasma by FAP (4). However, FAP has many other targets, and while its activity has been shown to be increased in liver and plasma from patients with liver disease (7), the impact of exercise on FAP activity, and hence FGF21 biology, has remained largely unknown. In the present study, the effect of exercise on FAP was transient as its concentration returned to basal levels within the first 20 min of the recovery period and coincided with a significant transient increase in arterialised blood glucose levels (from 4.09 +/- 0.15 to 4.45 +/- 0.21 mmol/l), likely as a result of enhanced hepatic glucose output. Future studies should determine to what extent altered exercise-induced hepatic energy metabolism may account for the release of FAP observed in the present study. While no previous studies have investigated the response of FAP during exercise in humans, Zhen *et al.*, (20) and Dunshee *et al.*, (2) have suggested that FAP plays a key role in preventing the release of bioactive FGF21 into systemic circulation. However, the ratio of bioactive to total FGF21 remained unchanged in the present study, and there was no release of FAP by skeletal muscle. Future research into the role of FAP released, and the tissues involved, during exercise in humans may provide useful information into identifying and targeting FAP as a mechanism to increase the bioactive levels of circulating FGF21 in order to optimise the use of exercise as a treatment in preventing metabolic disorders.

In conclusion, the present study provides evidence that single-leg maximal eccentric contractions do not cause the release of either total or bioactive FGF21 from skeletal muscle

into systemic circulation in humans. In accordance, skeletal muscle FGF21 protein content did not increase in response to exercise supporting the notion that FGF21 is neither induced nor released from skeletal muscle in response to maximal eccentric exercise. Finally, the data from this study showed a significant increase in circulating FAP levels during maximal eccentric contractions with no apparent contribution to its release from the exercised leg. These findings raise the interesting possibility that the elevated levels of FAP may play a role in the inactivation of FGF21 during exercise.

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Figure legends

Figure 1. Effect of exercise on plasma total FGF21 concentrations at baseline, during exercise (sets 1,2,3; S1-S3) and subsequent recovery (0-180min and 48h). Total FGF21 (pg/ml) in arterialised blood, n=8 (A), deep venous blood of the exercised leg, n=6 (B), arterio-venous (a-v) differences of the exercised leg, n=6 (C), and net flux (ng/ml) across the exercised leg, n=6 (D). Values represent means ± SEM. The shaded grey area represents the exercise period (sets 1,2,3).

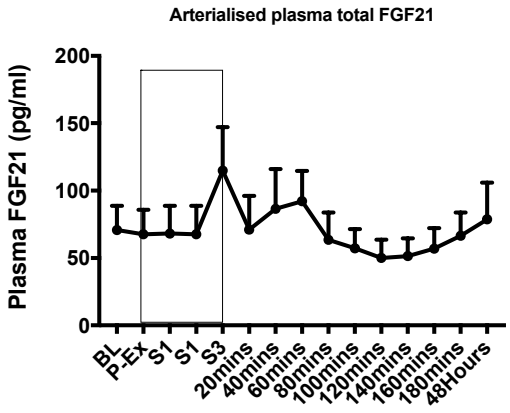
Figure 2. Effect of exercise on plasma bioactive FGF21 concentrations at baseline, during exercise (sets 1,2,3; S1-S3) and subsequent recovery (0-180min and 48h). Bioactive FGF21 (pg/ml) in arterialised blood, n=8 (A), deep venous blood of the exercised leg, n=6 (B), a-v differences of the exercised leg, n=6 (C), and net flux (ng/ml) across the exercised leg, n=6 (D). Values represent means ± SEM. The shaded grey area represents the exercise period (sets 1,2,3).

Figure 3. Effect of exercise on plasma FAP concentrations at baseline, during exercise (sets 1,2,3; S1-S3) and subsequent recovery (0-180min and 48h). Plasma FAP (ng/ml) in arterialised blood, n=8 (A), deep venous blood of the exercised leg, n=6 (B), a-v differences of the exercised leg, n=6 (C), and net flux (μg/min) across the exercised leg (n=6) (D). Values represent means ± SEM. * denotes $P < 0.05$ from baseline. The shaded grey area represents the exercise period (sets 1,2,3).

Figure 4. Effect of exercise on plasma IL6 concentrations at baseline, during exercise (sets 1,2,3; S1-S3) and subsequent recovery (0-180min and 48h). Plasma IL6 (pg/ml) in arterialised blood, n=8 (A), deep venous blood of the exercised leg, n=6 (B), a-v differences of the exercised leg, n=6 (C), and release from the exercised leg, n=6 (D). Values represent means ± SEM. ** denotes $P < 0.01$, *** denotes $P < 0.001$, and $^{\#}$ denotes $P < 0.0001$ from baseline. The shaded grey area represents the exercise period (sets 1,2,3).

Figure 5. Effect of exercise on skeletal muscle FGF21 protein content in the exercised leg before (Ex Pre), immediately post-exercise (Ex Post), 3h post-exercise (Ex 3h Post), 48h post-exercise (Ex 48h Post), and in the control leg before (Con Pre) and 48h post-exercise (Con 48h Post). Values represent means ± SEM; n=8.

A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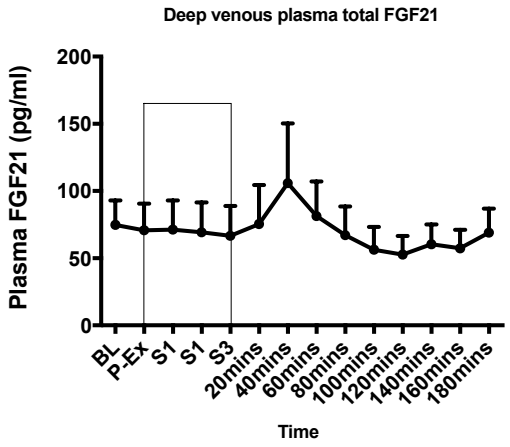
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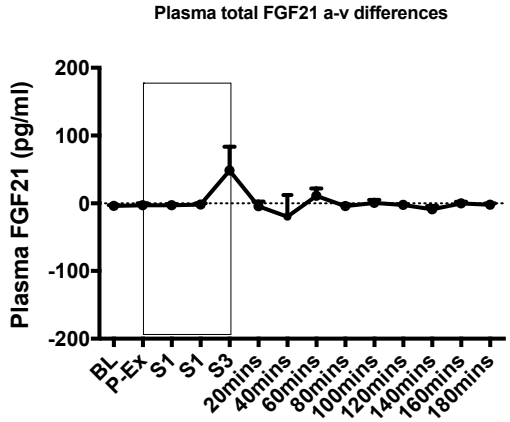
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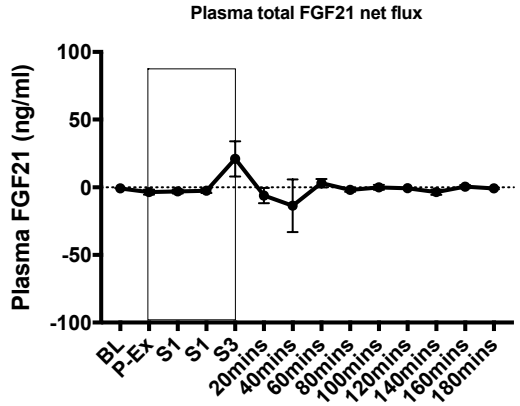
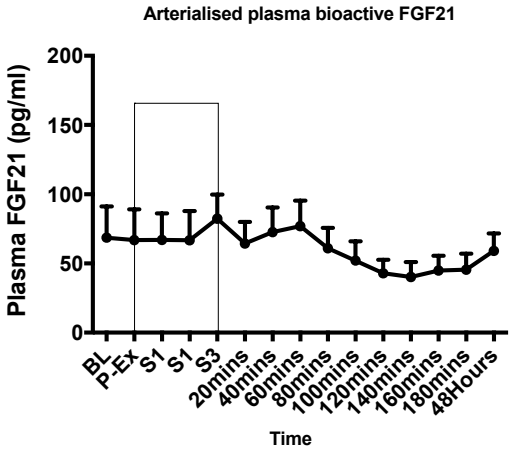
200
100
0
-100
-200
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins

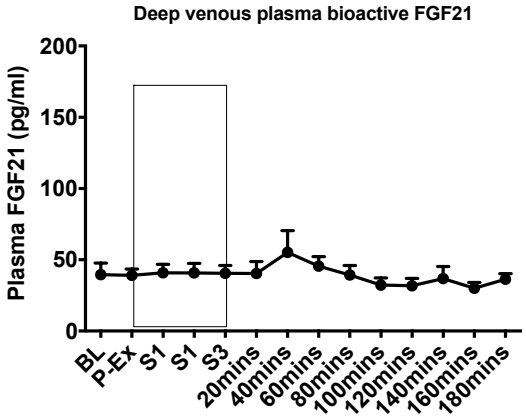
D


Plasma total FGF21 net flux
Plasma FGF21 (ng/ml)
100
50
0
-50
-100
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins

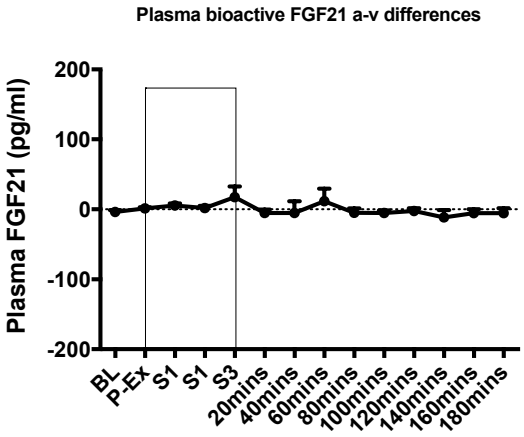
A


Arterialised plasma bioactive FGF21
Plasma FGF21 (pg/ml)
200
150
100
50
0
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins
48Hours
Time

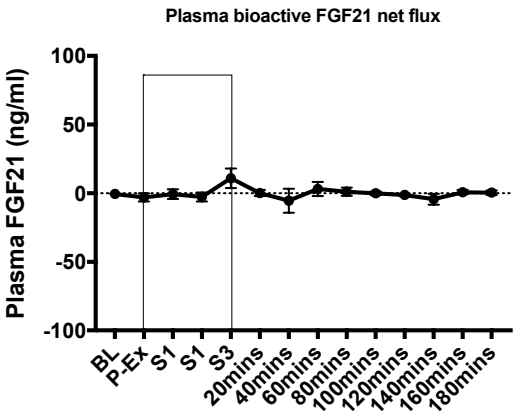
B

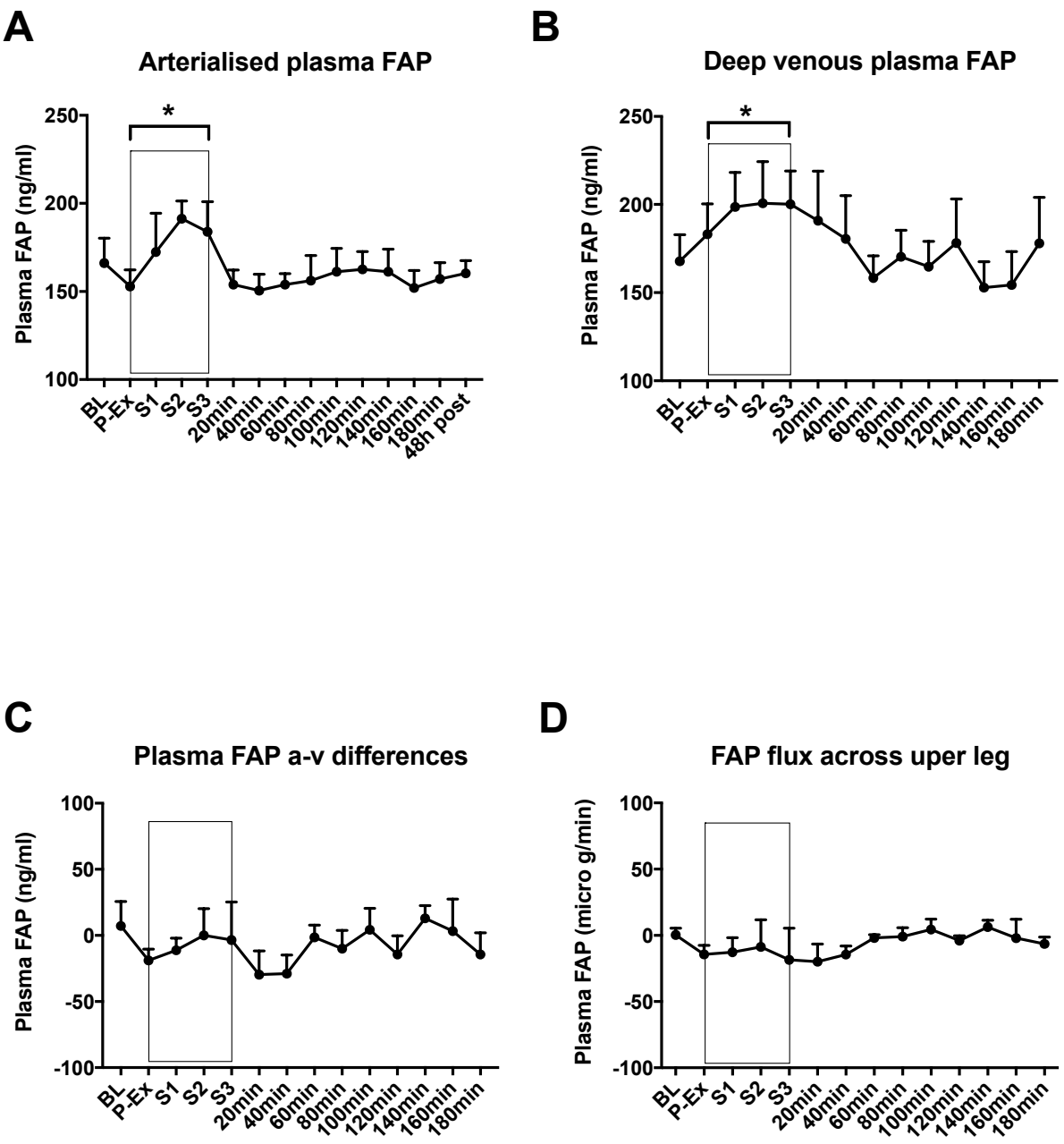

Deep venous plasma bioactive FGF21
Plasma FGF21 (pg/ml)
200
150
100
50
0
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins

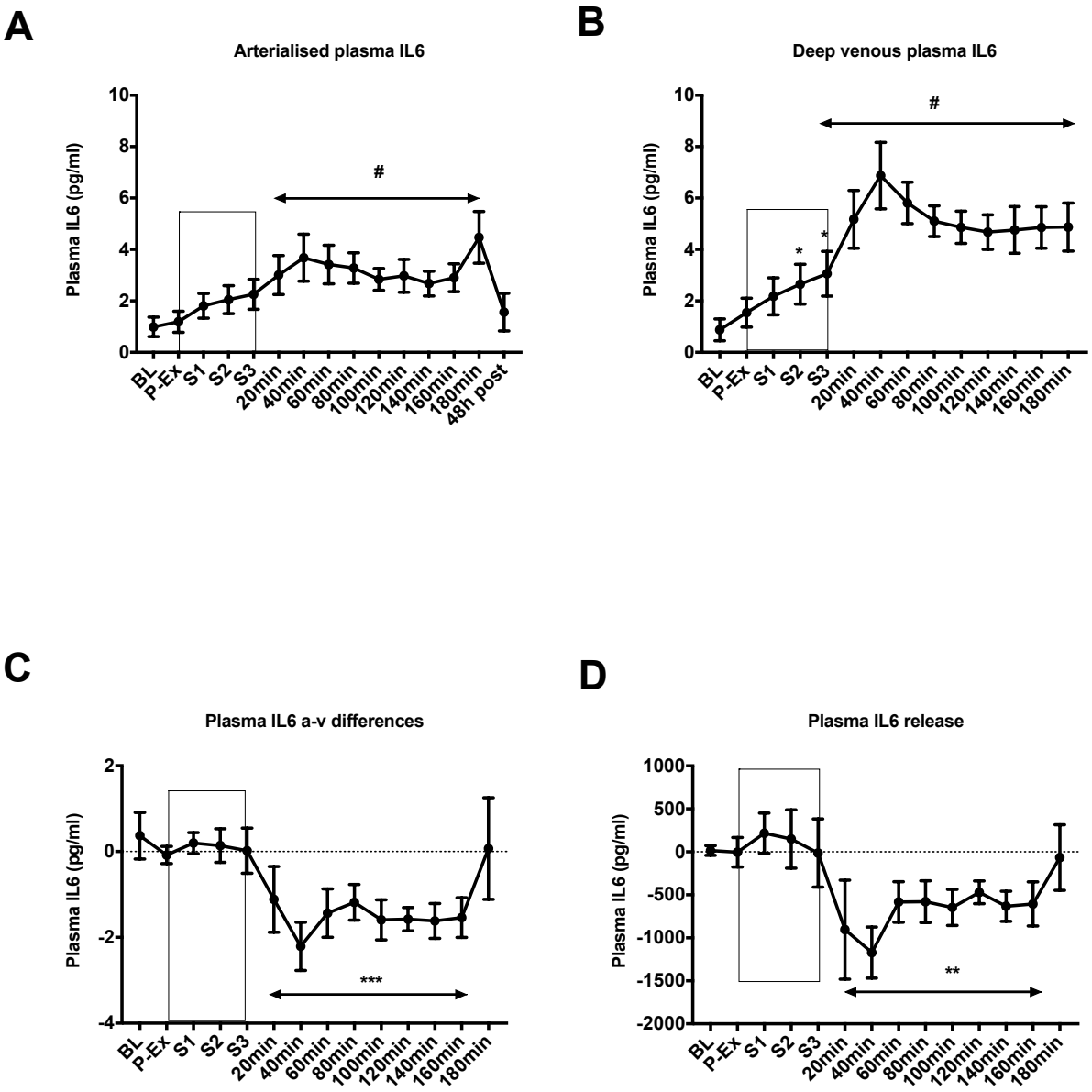
C

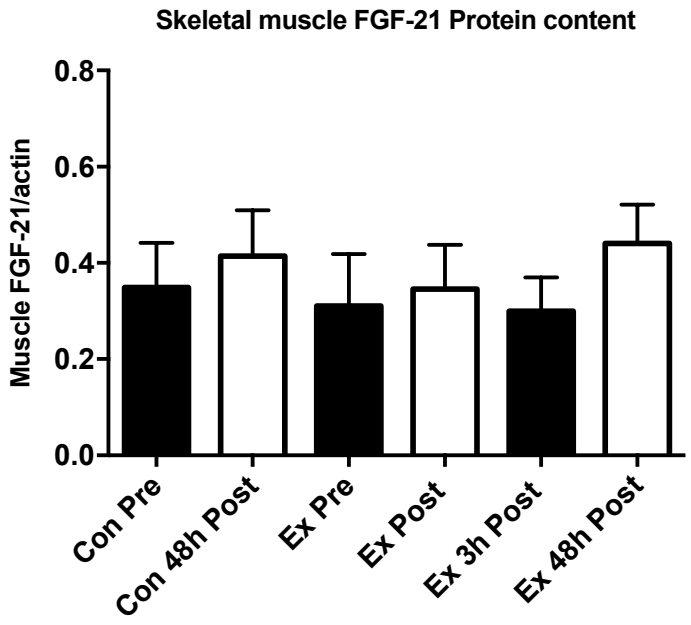

Plasma bioactive FGF21 a-v differences
Plasma FGF21 (pg/ml)
200
100
0
-100
-200
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins

D


Plasma bioactive FGF21 net flux
Plasma FGF21 (ng/ml)
100
50
0
-50
-100
BL
P-Ex
S1
S1
S3
20mins
40mins
60mins
80mins
100mins
120mins
140mins
160mins
180mins


A
Arterialised plasma FAP
Plasma FAP (ng/ml)
250
200
150
100
*
BL
P-Ex
S1
S2
S3
20min
40min
60min
80min
100min
120min
140min
160min
180min
48h post
B
Deep venous plasma FAP
Plasma FAP (ng/ml)
250
200
150
100
*
BL
P-Ex
S1
S2
S3
20min
40min
60min
80min
100min
120min
140min
160min
180min
C
Plasma FAP a-v differences
Plasma FAP (ng/ml)
100
50
0
-50
-100
BL
P-Ex
S1
S2
S3
20min
40min
60min
80min
100min
120min
140min
160min
180min
D
FAP flux across uper leg
Plasma FAP (micro g/min)
100
50
0
-50
-100
BL
P-Ex
S1
S2
S3
20min
40min
60min
80min
100min
120min
140min
160min
180min


A
Arterialised plasma IL6
Plasma IL6 (pg/ml)
10
8
6
4
2
0
#
BL
P-Ex
S1
S2
S3
20min
40min
60min
80min
100min
120min
140min
160min
180min
48h post
B
Deep venous plasma IL6
Plasma IL6 (pg/ml)
#
*
*
C
Plasma IL6 a-v differences
Plasma IL6 (pg/ml)
2
0
-2
-4
***
D
Plasma IL6 release
Plasma IL6 (pg/ml)
1000
500
0
-500
-1000
-1500
-2000
**


Skeletal muscle FGF-21 Protein content
Muscle FGF-21/actin
0.8
0.6
0.4
0.2
0.0
Con Pre
Con 48h Post
Ex Pre
Ex Post
Ex 3h Post
Ex 48h Post