The effects of a transcontinental flight on markers of coagulation and fibrinolysis in healthy men after vigorous physical activity

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The effects of a transcontinental flight on markers of coagulation and fibrinolysis in healthy men after vigorous physical activity


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ABSTRACT

Purpose: Athletes and military service members are known to undergo strenuous exercise and sometimes have to take long haul flights soon afterwards; however, its combined effect on many physiological functions is relatively unknown. Therefore, we examined the combined effects of a full-body muscle-damaging workout and transcontinental flight on coagulation and fibrinolysis in healthy, resistance trained men. We also determined the efficacy of a full-body compression garment in limiting their coagulation responses. Materials and Methods: Nineteen healthy, resistance trained men flew from Connecticut (CT) to California (CA), performed a full-body muscle-damaging workout and then flew back to CT. Ten participants wore full-body compression garments (FCG) for the duration of both flights and during all other portions of the study except workouts and blood draws, when they wore loose clothing. Nine controls wore loose clothing (CON) throughout the study. Blood samples were collected at 16 h and 3 h before the initial flight from CT, immediately after landing in CA, immediately before and immediately after the full-body workout in CA, immediately after landing in CT, and at 29 h after landing in CT. Plasma markers of coagulation included activated partial thromboplastin time (aPTT), prothrombin fragment 1+2 (PTF 1+2) and thrombin ant-thrombin (TAT). Markers of the fibrinolytic system included the tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) and D-Dimer. Results: Both FCG and CON groups exhibited a faster aPTT after the full-body workout compared to all other time points. Thrombin generation markers, TAT and PTF 1+2, increased significantly after the full-body workout and immediately after landing in CT. Additionally, tPA increased after the full-body workout, while PAI-1 increased before the flight to CA, after the full-body workout, and just after landing in CT. The D-Dimer significantly increased after the full-body workout and at 29 h post-flight in both groups. Between groups, aPTT was significantly faster and TAT elevated with the CON group at 29 h post-flight. Also, PAI-1 demonstrated higher concentrations immediately after landing in CT for the CON group. Conclusion: A full-body muscle-damaging workout in conjunction with a transcontinental flight activated the coagulation and fibrinolytic systems. Additionally, wearing a full-body compression garment may limit coagulation following a workout through the recovery period.

Introduction

More than 2 billion passengers fly annually worldwide, with estimated 300 million flights considered long-haul flights (> 5 h) (Anning, 2005). The combination of long-haul flights and limited space available for passengers traveling in economy class forces many travelers into periods of prolonged sitting, which in turn reduces venous return from the lower extremities due to compression of popliteal and femoral veins (Cruickshank et al., 1988). Additionally, the long periods inside an airplane may lead to dehydration and hemorrhage concentration (Moyes, 1988). Long-haul travel thus increases the risk of deep vein thrombosis (DVT) between 2- and 4-fold (Schwarz et al., 2003). Nonetheless, previous research regarding the effects of long haul flights on coagulation and fibrinolytic processes has yielded only conflicting results on blood parameters, including an increase...
in thrombin generation or fibrinolytic parameters (Schreijer et al., 2006), a decrease (Boccalon et al., 2005), or no changes (Schobersberger et al., 2002).

Regular endurance exercise, recommended for the avoidance of thrombotic events (Sacco et al., 2006), may also protect against the formation of deep vein thrombosis and pulmonary embolism (Gough et al., 1992). Both acute endurance exercise (Menzel and Hilberg, 2011) (marathon) and resistance exercise (Kupchak et al., 2013a) (6 sets of 10 repetitions of squats) can activate the coagulation cascade, through stripping away at the endothelium baselayer through increased blood viscosity, exhibited by a decrease in activated partial thromboplastin time (aPTT) (a measure of the intrinsic and common pathways) and increases in markers of thrombin formation (prothrombin fragments 1 and 2, PTF1+2 and TAT). Such exercises also activate the fibrinolytic systems, as seen by increases in the tissue plasminogen activator (tPA) (enzyme that catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown), plasminogen activator inhibitor (PAI-1) (main inhibitor of tPA) and D-Dimer (a marker of fibrin degradation). Even with the activation of both systems, however, most healthy individuals remain in a state of hemostatic balance.

Previous studies have shown an increased risk of venous thromboembolism (VTE), an absolute risk of 1 in 4600, for flights over a 4-h duration (Kuipers et al., 2007). Additionally, other studies have shown association between air travel time and VTE (Collins et al., 1979; Homans, 1954). Many factors contribute to VTE including both cabin-related and traveler-related risk factors.

The three main cabin-related risk factors known to increase the risk of developing VTE include extended sitting (long periods of immobility), hypoxia and low humidity. Sitting for an extended time (even < 3 h) is associated with a reduced velocity of venous blood flow in lower extremities (Wright & Osborne, 1952) and an increase in blood viscosity (Landgraf et al., 1994), a known risk factor for VTE (Dormandy & Edelman, 1973). The decreased air pressure and hypoxia in the airline cabin can limit fibrinolytic activity and lead to venous stasis (Maher et al., 1976). At the air pressure maintained for transcontinental US flights, arterial oxygen saturation is 93% in healthy individuals (Toff et al., 2006). In addition, the atmosphere inside commercial airliners is typically very dry, usually around 1% relative humidity, which can cause increased perspiration rates. The environmental factors inside the aircraft coupled with reduced access to ad lib. fluid intake can lead to dehydration and hemococoncentration. Yet, although a synergistic effect of these three risk factors may contribute to the formation of VTE, taken independently they likely do not explain clot formation during air travel (Schreijer et al., 2008).

Traveler-related risk factors can also play a role in the development of VTE. Many of these risk factors, including being overweight or obese (Body Mass Index (BMI) ≥ 25 kg·m⁻²), having a history of VTE, recent major surgery, or exercise that causes muscle damage can increase the odds of clot formation during a flight (Landgraf et al., 2002). The more risk factors that apply to an individual, the greater his/her chance of developing a thrombosis through flight.

Despite the increased risk of DVT after long-haul flights (Kuipers et al., 2006), anticoagulant therapy carries its own risks (bleeding). Use of prophylactic anticoagulants (Vitamin K antagonists (Coumadin) lower levels of factors II, VII, IX and X). The heparins and fondaparinux work indirectly through antithrombin. Rivaroxaban and apixaban directly inhibit Factor Xa and several anticoagulants directly inhibit thrombin), which is therefore not typically justified. Yet, the balance of risk may be different for athletes and military warfighters who undergo strenuous exercise soon before they travel for long distances. Since vigorous exercise can also affect hemostasis, a long-haul flight coupled with a full body muscle-damaging workout can disrupt hemostatic balance in favor of coagulation.

Use of a full-body compression garment allows the traveler to avoid many of the risks of pharmacologic prophylaxis and requires little instruction or training. Previous studies have demonstrated that compression gear such as compression stockings have a positive effect on limiting the formation of DVT (Sachdeva et al., 2014). Compression stockings have also been used by runners to enhance recovery from an athletic performance (Beliard et al., 2015), and there is anecdotal evidence that compression gear diminishes their DVT risk (Hadfield, 2013).
Another factor in our study is diurnal variation of both coagulation and fibrinolytic markers: our study takes place over several days, with blood sampling performed during different times of the day. Although aPTT, PTF 1+2 and TAT are generally shown to have low biological variability (<10%), the fibrinolytic parameters (tPA) and their inhibitor (PAI-1) have a higher degree of variability. tPA is characterized by an early morning drop and a peak in the afternoon, while PAI-1 typically exhibits the opposite time-course (Siahkouhian et al., 2013). Furthermore, studies of diurnal variation in D-Dimer concentrations have yielded conflicting results (Iverson et al., 2002; Trifiletti et al., 2000).

The effects of long-haul travel on coagulation and fibrinolytic parameters have been ambiguous (Boccalon et al. 2005; Schobersberger et al. 2002; Schreijer et al., 2006). Still less is known about the hemostatic effects following a trans-continental flight preceded by a full-body, muscle-damaging workout such as those typical of athletes and military service members. The purpose of this study is therefore to determine the coagulant and fibrinolytic responses from a full-body, muscle-damaging workout followed by a transcontinental flight in physically active young adults. A secondary aim of the study is to investigate the utility of wearing a full-body compression garment in reducing coagulation activation. We hypothesized that exercise-induced activation of the coagulation and fibrinolytic systems would be augmented after a transcontinental flight and that wearing a full-body compression garment during travel would limit hemostatic responses.

Materials and methods

Experimental approach

This study was part of a larger investigation of jet-lag and trans-continental flight stress and recovery (Kraemer et al., 2016). In this study, we focused on the effects of long-wear compression garments on the coagulation and fibrinolytic systems and their role in recovery. We overlaid our analyses for this study on the basic format of the primary investigation. We therefore provide here an overview of results from the larger investigation as context for our findings from this particular study, including experimental understanding of our systems interpretation.

Subjects

Healthy males between the ages of 18 and 35 years completed a medical history and physical activity questionnaire and were screened by a physician to ensure eligibility. All subjects were involved with progressive heavy resistance training with complementary endurance training, each performed three to four times a week. Resistance training was characterized by periodized, multiple sets, whole body, large muscle group exercises with targets for muscle strength and size. Subjects were mostly former high school and college athletes, who participated in a variety of sports including football, basketball, track and field, wrestling, and baseball and some were former military service members who were accustomed with intense weekly physical training routines. Subjects were non-tobacco users and reported no history of cardiovascular disease. Exclusion criteria included the following to limit confounding effects on the variables being measured or ability to complete exercise protocol: 1) diagnosis of liver, kidney, or gastrointestinal disease, or severe metabolic or endocrine disorder; 2) history of blood clotting disorders or venous thromboembolisms; 3) reported use of cholesterol-lowering, blood pressure, or nonsteroidal anti-inflammatory medications; 4) reported use of anticoagulant medications (e.g. Coumadin); 5) reported use of hormonal substances including testosterone, anabolic steroids, or growth hormones; 6) current musculoskeletal or orthopedic injuries; 7) fear of flying and 8) other conditions or medications considered by the medical monitor to have potential impact on participant safety or the results of the investigation. The Institutional Review Board for use of human subjects in research at the University of Connecticut approved this study. All subjects provided written informed consent after having the study risks and benefits carefully explained to them and their questions answered.

Experimental design

The study involved a battery of physiological tests, a muscle-damaging resistance exercise protocol,
and two trans-continental flights over five days (Figure 1). Twenty subjects were assigned by Ponderel Index (Babar, 2016); pairs were then randomly split into two groups at study enrollment: the intervention group (FCG) wore a full-body compression garment throughout the study except during workouts and blood draws, when they wore loose-fitting clothes. Controls (CON) wore loose-fitting clothes throughout the entire study. Since one enrolled subject could not make the flight, nineteen recreationally-trained men (i.e. with weight training and endurance training for over 6 months) participated in the study: a ten-subject compression group (FCG) and a nine-subject control group (Table 1).

For the duration of the study, subjects were asked not to ingest any alcohol; to refrain from taking any oral pain medications, including NSAIDs; and to abstain from heavy lifting for a period beginning 3 days prior to till the end of the study. Subjects were also asked to limit time (<5 min) and utilize lower water temperatures (temperature was not monitored) when bathing or showering after completing the exercise protocol; to abstain from any strenuous or moderate physical activity (i.e. lifting, running) other than those activities required for tasks of daily living; and to use no pain-relieving modalities including heat, ice, or massage. In addition, a registered dietitian screened subjects for any unusual (i.e. ketogenic, low fat diets) diets or supplements which might have compromised variables measured in the study and monitored food intake during their participation by utilizing dietary logs. Meals were not uniform for the subjects during the study, but each subject ate approximately the same time. No attempt was made to limit caffeine ingestion, as only water was allowed 4 h prior to testing, and only normal caffeine ingestion was observed.

**Familiarization**

Subjects were screened, consented to the study, and had their anthropometrics recorded at the University of Connecticut (UConn) Human Performance Laboratory (HPL). The body height was recorded to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany) while the subjects were not wearing shoes. Body mass was measured to the nearest 0.1 kg on a calibrated digital scale (OHAUS Corp., Florham Park, NJ) with subjects wearing only a t-shirt and shorts.

**Table 1. Subject characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>FCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.2 ± 2.3</td>
<td>23.1 ± 2.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177.5 ± 6.3</td>
<td>174.8 ± 5.3</td>
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<tr>
<td>Body Mass, kg</td>
<td>84.3 ± 9.0</td>
<td>84.9 ± 10.1</td>
</tr>
<tr>
<td>BMI, kg·m⁻²</td>
<td>26.8 ± 2.8</td>
<td>27.8 ± 3.2</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>15.1 ± 6.4</td>
<td>15.3 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means and standard deviation; CON, Control group; FCG, Compression Garment group; CO, n = 9; CG, n = 10.
BMI was calculated as body mass (kg)/height (m²). The body composition (% body fat) was measured by a trained technician via the 3-site Jackson–Pollock skinfold technique (Jackson & Pollock, 1978). All subjects were measured by the same technician using a caliper (Bodycare Harpenden Caliper, England) at the chest, abdomen and thigh. Subjects were then familiarized with the study’s muscle-damaging protocol.

**Clothing**

Subjects were assigned by the Ponderel Index (Mass/Height³) (measure of leanness, and shown to have a lower false positive rate in athletes) (Babar, 2016) and then randomized into either the compression garment (FCG) group or the control (CON) group. The compression garment used was the UnderArmour Recharge™ garment (75% nylon and 25% spandex) [UnderArmour, Baltimore, MD, USA], which consisted of a long-sleeved shirt and leggings. These garments have been shown to effectively improve recovery after high-intensity resistance training workouts (Kraemer et al., 2010). Compression garments were worn at all times during the study (including sleep) except during the muscle-damaging workout, during brief showers, and blood draws.

**Timeline**

Day 1: Subjects arrived at UConn HPL at 1300 EDT and provided a urine sample to determine hydration status. Hydration status was measured using a refractometer. A urine specific gravity of <1.020 was used to define adequate hydration. Subjects were then seated for 10 min and provided a blood sample (baseline/BL).

Day 2: Subjects arrived at the UConn HPL 0600 EDT and provided a urine and blood sample (before the flight to CA/1CT). Subjects then put on a compression garment or loose-fitting clothing. Following testing, at 0600 EDT, participants were transported by bus for 30 min to Bradley International Airport (BDL) in Hartford, CT for a departing flight at 0900 EDT. The flight was direct to Los Angeles International Airport (LAX) flying on a Boeing 737–800 airliner with participants traveling in coach class, randomly ticketed throughout the plane with a travel time of approximately 6 h and 20 min and arrived at 1220 PDT. Participants were then transported to the California State University-Fullerton (CSUF) HPL by coach bus with a travel time of 1 h. At the CSUF HPL, the FCG group removed their compression garments and all subjects gave a blood and urine sample (after the flight to CA/2CA) at 1500 PDT. FCG subjects put their compression garments back on immediately after the blood draw. All subjects were then transported to a hotel, where they were instructed to relax and stay within walking distance of the hotel. Participants were also instructed as to turn in by 2200 PDT to get ready for the next day’s events. FCG subjects wore their compression garments to sleep.

Day 3: Subjects awoke between 0700 to 0800 PDT and were then instructed to rest until testing. At 1300 PDT, subjects were transported to the CSUF HPL. FCG subjects removed the compression garments and put on loose-fitting clothing for testing and the full-body, muscle-damaging workout. All subjects had their hydration status obtained and a blood sample was taken (before the full-body workout/3CA). Subjects then performed the muscle-damaging physical testing protocol (1400 PDT). The protocol was performed at an outdoor grass athletic field on the campus of California State University – Fullerton. Prior to administration of the exercise protocol, each participant performed a warm-up protocol that consisted of 5 min on a cycle ergometer with light resistance and a constant speed of 60 rpm, followed by a series of standard dynamic stretches that included forward lunges, lateral lunges, knee hugs, quad pulls and straight-leg march. The participants then completed the exercise protocol, which consisted of the following exercises selected to induce muscle damage (Kraemer et al., 2016): 5 sets of 10 repeated countermovement jumps, 5 sets of 10 plyometric push-ups, 5 sets of 10 Nordic hamstring curls and 5 sets of 10 standard pushups. All exercises were performed with 60 s rest between sets. These exercises were followed by a repeated sprint protocol: subjects performed 15–20 m sprints with a maximum of 10 m deceleration distance (thereby producing eccentric damage). Sprints were performed every minute, with a rest period between sprints for the time remaining in each minute. The exercise protocol was performed by the entire group, led by an exercise leader, while other members of the research team cheered...
and encouraged individual performance. Upon completion of the exercise protocol, the participants immediately returned to the laboratory at 1500 PDT for post-exercise blood and urine sampling. At the start of the exercise, the temperature was 71°F and the humidity was 63%.

Following the exercise protocol, participants provided a blood and urine sample (immediately after the full-body workout, 4CA), showered, and put back on the prescribed clothing. Later that evening, all participants and research team arrived at the CSFU HPL at 2000 PDT, where they were then transported by coach bus for 1 h to LAX for the departing flight at 2330 PDT. The flight was direct to BDL with a travel time of approximately 5 h and 20 min and arrived at 0750 EDT. Subjects were transported by coach bus for 30 min to the UConn HPL for post-travel testing.

Day 4: Following arrival at BDL at 0750 EDT, subjects were transported by chartered coach to the UConn HPL (30 min travel time), where a blood and urine sample were obtained at 0900 EDT (after the flight to CT/5CT). Subjects were then released and instructed to not perform any strenuous or moderate physical activity.

Day 5: Subjects were instructed to awake between 0700 to 0800 EDT, and then consume only water to remain hydrated before testing at 1300 EDT. Subjects arrived at UConn HPL at 1300 EDT, and provided a urine sample and blood sample (after 29 h post-flight/6CT). After testing, all subjects were released and monitored over the next few days to ensure satisfactory recovery.

**Blood and urine collection**

Blood and urine samples were collected at baseline [BL] (Day 1 – 1300 EDT), before the flight to CA [1CT] (Day 2 – 0600 EDT), after the flight to CA [2CA] (Day 2 – 1500 PDT), immediately before the full-body workout [3CA] (Day 3 – 1300 PDT), immediately after the full-body workout [4CA] (Day 3 – 1500 PDT), after the flight to CT [5CT] (Day 4 – 0900 EDT) and after 29 h post-flight [6CT] (Day 5 – 1300 EDT). In each case, whole blood was collected from an antecubital vein without stasis into serum, EDTA, and sodium citrate vacutainers, while the subject was in a seated position. Serum and EDTA vacutainers were centrifuged at 1,500 x g for 15 min. While, sodium citrate vials were centrifuged at 2,000 x g for 30 min to extract platelet poor plasma. The serum, sodium citrate plasma, and EDTA-plasma was separated into several individual cryovials and subsequently flash frozen with liquid nitrogen. The frozen serum and plasma in the cryovials were immediately stored and then shipped on dry ice to the UConn HPL. On arrival, specimens were stored in a –80°C ultra-low freezer until biochemical analysis was conducted. Participants provided a urine sample and hydration state was confirmed by measuring urine specific gravity (USG) with a handheld urine refractometer (Model Reichert TS400, Reichert, New York). A USG < 1.020 indicated euhydration. Subjects were given frequent verbal reminders to stay hydrated, and especially to drink 0.5 liters of water at night and in the morning. All subjects met this requirement. If a participant’s USG was > 1.020 prior to any testing session, he was instructed to drink water until his USG was < 1.020.

**Biochemical assays**

aPTT was measured from sodium citrate platelet-poor plasma using an automated clinical analyzer (Quest Diagnostics, Willimantic, CT). The serum total creatine kinase was measured in duplicate from samples utilizing liquid creatine kinase reagents (Sekisui Diagnostics, Canton, MI) and assayed according to manufacturer’s instructions. The intra-assay coefficient of variation (CV) was 3.9%, respectively. PTF 1+2 and TAT were measured in duplicate by ELISA from sodium citrate platelet-poor plasma (Enzygnost®; Dade Behring Marberg, Newark, DE). The intra-assay CVs were 5.3% and 6.9% and the inter-assay CVs were 9.8% and 8.9%, respectively. D-Dimer, tPA antigen (Ag), and PAI-1 Ag were determined from sodium citrate platelet poor plasma (Diagnostica Stago, Inc., Mount Olive, NJ) by ELISA. The intra-assay CVs were 7.0, 4.7 and 6.3% and inter-assay CVs were 9.2, 5.8 and 10.3%, respectively. Myoglobin was measured from EDTA-plasma in duplicate by ELISA (CALBiotech, Spring Valley, CA). The intra-assay CV was 5.6%, and the inter-assay CV was 7.9%. All ELISAs were performed on a Versamax tunable microplate reader (Molecular
Devices, Sunnyvale, CA) at the appropriate wavelength according to the manufacturer’s directions.

**Statistical analyses**

Data (means and standard deviation) were analyzed using SPSS version 20.0. Statistical power was determined to range from 0.83 to 0.96 for the n sizes used in this study (nQuery Advisor; Statistical Solutions, Saugus, MA, USA). Reliability range for the dependent variables intra-class correlation coefficients were $R \geq 0.85$. Tests for normality of distribution (Kolmogorov–Smirnov Chi-square test) and homogeneity of variance (Levine’s test) were for all data sets that were analyzed where statistical assumptions for linear statistics were met. Any variables that did not meet the assumptions were logarithmically (log10) corrected and tested again. To compare demographic characteristics between the experimental groups, an independent t-test was utilized, where no significant differences were observed with the matching process.

A 2-way analysis of variance with repeated measures for treatment and time was used to statistically evaluate the experimental data. When appropriate, Fisher’s LSD post hoc tests were used to determine pairwise differences. An alpha level of $p \leq 0.05$ was defined as being statistically significant.

**Results**

In our study, a trans-continental flight alone did not activate the coagulation and fibrinolytic systems. However, a full-body exercise protocol activated the coagulation system, and the fibrinolytic system. The trans-continental flight following the exercise protocol further maintained a hypercoagulable state into recovery. But, the wearing of a full-body compression garment did have a positive effect on limiting coagulation (decreased clot formation).

**Markers of coagulation**

aPTT, a marker for activation of the intrinsic and common pathways of coagulation, exhibited a faster clotting time for both groups immediately post-workout compared to BL. Additionally, the CON group showed a significantly faster aPTT compared with the FCG group at 29 h post-flight ($p = 0.024$) (Table 2). TAT significantly rose for both groups immediately post-workout, after landing in CT, and at 29 h post-flight. The FCG group showed a significantly lower concentration of TAT at 29 h post-flight ($p = 0.049$) compared with the CON group, respectively (Table 2). PTFI+2, another marker of thrombin formation, increased for CON and FCG groups immediately post-workout, and after landing in CT, respectively (Table 2). However, no significant difference in PTFI+2 levels between the CON and FCG groups was exhibited at any time points.

**Markers of fibrinolysis**

Both groups exhibited a significant rise in tPA concentrations immediately post-workout, which returned to baseline levels after landing in CT (Table 3). However, there was no significant difference in tPA concentrations between groups at any time points. Both groups showed significant increases in PAI-1 concentrations before the flight to CA, immediately post-workout, and after landing in CT. Between groups, the FCG group had a significantly lower PAI-1 concentration compared with the CON group after landing in CT ($p = 0.017$) (Table 3). D-Dimer concentrations rose significantly in the CON group immediately post-workout, after landing in CT, and at 29 h post-flight, while the FCG group concentrations rose significantly immediately post-workout and at 29 h post-flight only. D-Dimer levels showed no significant difference between the FCG and CON groups at any time point (Table 3).

**Markers of muscle damage**

Both groups did not show an increase in serum CK activity from baseline levels (CON = 110 U·L$^{-1}$, FCG = 123 U·L$^{-1}$) at 1CT (CON = 145 U·L$^{-1}$, FCG = 157 U·L$^{-1}$), 2CA (CON = 136 U·L$^{-1}$, FCG = 138 U·L$^{-1}$) and 3 CA (CON = 153 U·L$^{-1}$, FCG = 158U·L$^{-1}$). However, following the muscle damaging protocol, both groups showed a significant increase ($p < 0.05$) from baseline at 4CA (CON = 282 U·L$^{-1}$, FCG = 245 U·L$^{-1}$), 5 CT (CON = 686 U·L$^{-1}$, FCG =
Table 2. Changes in coagulation markers, aPTT, TAT and PTF 1+2, following a trans-continental flights and a muscle damaging workout.

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>1CT</th>
<th>2CA</th>
<th>3CA</th>
<th>4CA</th>
<th>5CT</th>
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<tr>
<td><strong>aPTT (sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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</tr>
<tr>
<td>Mean</td>
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<td>29.3</td>
<td>32.0</td>
<td>30.0</td>
<td>27.3</td>
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<tr>
<td>SD</td>
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<td>SD</td>
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<td><strong>FCG</strong></td>
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<tr>
<td>Mean</td>
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<td>0.81</td>
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<td>222</td>
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<td>303</td>
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<tr>
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<td>58</td>
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<td>180</td>
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<td>210</td>
<td>343</td>
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<td>253</td>
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<td>43</td>
<td>81</td>
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Values are means ± standard deviation; CON, Control group = 9; FCG, Compression Group = 10; aPTT, activated Partial Thromboplastin Time; TAT, Thrombin–Antithrombin Complex; PTF 1+2, Prothrombin Fragment 1+2; BL, Baseline; 1CT, morning before flight to CA; 2CA, immediately after the flight to CA (Day 2 in CA); 3CA, the next day immediately before full-body workout (Day 3 in CA); 4CA, immediately post-workout (Day 3 in CA); 5CT, immediately post-flight to CT (Day 4 in CT); 6CT, 1 day post-flight (Day 5 in CT). Letters represent significantly different for time between each group – a significantly different from BL; b significantly different from 1CT; c significantly different from 2CA; d significantly different from 3CA; E significantly different from 4CA; F significantly different from 5CT; G significantly different from 6CT (p < 0.05). # – FCG significantly different from CON group within same time point (p < 0.05).

Table 3. Changes in fibrinolytic markers, tPA, PAI-1 and D-Dimer, following a trans-continental flights and a muscle damaging workout.

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<tr>
<th></th>
<th>BL</th>
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<th>2CA</th>
<th>3CA</th>
<th>4CA</th>
<th>5CT</th>
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<tr>
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<td>5.20</td>
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<td>4.78</td>
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<td><strong>PAI-1 (ng.mL⁻¹)</strong></td>
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<td>7.61</td>
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<td><strong>D-Dimer (ng.mL⁻¹)</strong></td>
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<tr>
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<td>Mean</td>
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<td>253.7</td>
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<td>198.8</td>
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<tr>
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<td>259.9</td>
<td>365.3</td>
<td>280.1</td>
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<td>148.4</td>
<td>204.1</td>
<td>149.1</td>
<td>142.9</td>
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</table>

Values are means ± standard deviation; CON, Control group = 9; FCG, Compression Group = 10; tPA, tissue Plasminogen Activator; PAI-1, Plasminogen Activator Inhibitor; BL, Baseline; 1CT, morning before flight to CA; 2CA, immediately after the flight to CA (Day 2 in CA); 3CA, the next day immediately before full-body workout (Day 3 in CA); 4CA, immediately post-workout (Day 3 in CA); 5CT, immediately post-flight to CT (Day 4 in CT); 6CT, 1 day post-flight (Day 5 in CT). Letters represent significantly different for time between each group – a significantly different from BL; b significantly different from 1CT; c significantly different from 2CA; d significantly different from 3CA; E significantly different from 4CA; F significantly different from 5CT; G significantly different from 6CT, (p < 0.05). # – FCG significantly different from CON group within same time point (p < 0.05).
536 U·L$^{-1}$) and 6CT (CON = 508 U·L$^{-1}$, FCG = 350 U·L$^{-1}$). Between groups, the CON group exhibited a significantly greater CK activity ($p < 0.05$) than FCG group at 4CA, 5CT and 6CT. Plasma myoglobin was also analyzed and showed no increase from baseline (CON = 1.7 nmol·L$^{-1}$, FCG = 2.0 nmol·L$^{-1}$) at 1CT (CON = 2.3 nmol·L$^{-1}$, FCG = 2.3 nmol·L$^{-1}$), 2CA (CON = 1.9 nmol·L$^{-1}$, FCG = 2.2 nmol·L$^{-1}$) and 3CA (CON = 2.4 nmol·L$^{-1}$, FCG = 2.1 nmol·L$^{-1}$) time points. Plasma myoglobin did significantly increase in both groups after the muscle damaging protocol at 4CA (CON = 10.2 nmol·L$^{-1}$, FCG = 8.0 nmol·L$^{-1}$) and 5CA (CON = 4.3 nmol·L$^{-1}$, FCG = 3.6 nmol·L$^{-1}$), but returned to baseline concentrations and 6CA (CON = 2.3 nmol·L$^{-1}$, FCG = 1.9 nmol·L$^{-1}$). Additionally, the CON group exhibited greater concentrations at 4CA compared to the FCG group ($p < 0.05$) (Kraemer et al., 2016).

**Discussion**

Athletes and military service members are known to undergo strenuous exercise and sometimes have to take long haul flights relatively soon afterwards; however, its combined effect on many physiological functions are relatively unknown. Thus, this investigation was designed to examine the effects of a trans-continental flight preceded by a full-body muscle-damaging workout on the coagulation and fibrinolytic systems. Additionally, we examined whether the use of a full-body compression garment can limit the activation of coagulation. The exercise protocol was demanding even for our resistance-trained subjects, as evidenced by an increase in plasma myoglobin concentrations (Kraemer et al., 2016). Marked increases in the coagulation and fibrinolytic systems were observed post-exercise, and activation of coagulation continued following the trans-continental flight. However, wearing a full-body compression garment limited the degree of coagulation post-exercise and post-flight.

Although traveler-related risk factors can present problems for long-haul flights, our subjects were deemed low risk, as only a few of them had a BMI above 25 and their body fat % was within the normal range. Additionally, hemo-concentration and dehydration caused by the 1% humidity inside the aircraft was not a problem as subjects were well-hydrated as hydration status were checked immediately before and immediately after each flight. The largest risk to subjects was thus the exercise protocol.

The exercise protocol used in the present study is known to induce muscle damage (Kraemer et al., 2016). Subjects’ risk for VTE was therefore increased due to three underlying factors: hypercoagulability, stasis, and vein wall damage (Anning, 2005). In our study, a hypercoagulable state existed after the full-body muscle damaging workout; stasis was introduced by two trans-continental flights of approximately 5 h in duration (35 h apart), with little movement initiated by the subjects, and vein wall damage was likely introduced from the exercise protocol, as these types of exercise protocols increase blood viscosity (hematocrit) stripping away at the endothelium (Kupchak et al., 2013a).

An acute bout of high-intensity exercise activates the coagulation cascade and produces a hypercoagulable state (Herren et al., 1992). To examine this process, we measured aPTT, a measure of the intrinsic and common pathways of coagulation. Our study reaffirmed that intense exercise shortens the length of coagulation (Kupchak et al., 2013a; Weiss et al., 1998). Shortening of aPTT is strongly related to von Willebrand factor (vWF) an adhesive protein that is released from endothelium cells during exercise (Lekakis et al., 2008), and an increase in Factor VIIIa has been proposed as a cause. Moreover, the diminished liver blood flow along with the muscle damage impairing kidney function during exercise could prolong the clearance of blood clotting factors (Cadroy et al., 2002), along .

We also utilized aPTT to examine the effects of coagulation on a trans-continental flight. Schreijer et al. (2010) hypothesized that the intrinsic pathway may be responsible for air travel-related activation of coagulation. In the present study, we demonstrated that aPTT was not decreased following a transcontinental flight to CA (2CA). This result was similar to those of previous study (Boccalon et al., 2005). Nor was aPTT affected on the return flight to CT (5CT).

No changes were observed due to diurnal variation (difference between BL and 1CT) in PTF 1+2
and TAT, whose biological variability is considered generally low. Both markers were elevated after a bout of intense exercise, demonstrating the formation of thrombin. The increase in PTF 1+2 and TAT in response to a bout of intense exercise is consistent with previous studies (Kupchak et al., 2013b; Weiss et al., 1998). This rise in formation of thrombin is typically transient and returns to baseline concentrations fairly quickly depending on the type and intensity of the exercise. The increase in thrombin markers are thought to occur mechanistically via a number of factors, including aerobic metabolism coupled with lactate formation, platelet activation, lysis of red blood cells and tissue damage (Bärtsch et al., 1995; Dufaux et al., 1991; Herren et al., 1992; Prisco et al., 1998). Tissue damage is the most likely cause here, since this exercise protocol has been shown to cause increases in both myoglobin and CK levels (Kraemer et al., 2016).

Unlike previous studies, we also examined the effects of a trans-continental flight post-exercise. We observed a significant increase in both PTF 1+2 and TAT immediately following the second trans-continental flight to CT (5CT) and in the follow-up measures the next day (6CT). Previous studies have documented inconsistent results in thrombin activity after a flight, showing increased (Schreijer et al., 2006), decreased (Boccalon et al., 2005), and no changes in formation of thrombin markers (Schobersberger et al., 2002). However, in the present study, the muscle-damaging workout preceding the trans-continental flight back to CT (5CT) might be a mitigating factor in the increased concentrations of PTF 1+2 and TAT. Thus, the increased levels of thrombin generation markers immediately after the full-body workout, after landing from CA, and at 29 h post-flight may be attributed to the tissue damage (increased myoglobin and CK) incurred from the exercise protocol, which has been linked to tissue factor mediated coagulation activation (Möckel et al., 2001; Prisco et al., 1993).

Additionally, the increases in coagulation levels in the body are commonly balanced by a subsequent increase in the fibrinolytic system. During this process, a fibrin clot is broken down to its soluble form by the enzyme plasmin. Plasmin is activated by tissue plasminogen activator (tPA), which is produced and released from the endothelium (Kooistra et al., 1994). In our study, tPA increased only after the full-body workout (4CA), signifying the effect was attributable to the exercise and not to the travel. This result is consistent with our previous studies, which showed that exercise increases concentration of tPA in the blood (Kupchak et al., 2013b). tPA is stimulated through multiple mechanisms, including increased shear stress causing endothelium damage (Levin et al., 1984), generation of thrombin (Levin et al., 1993), and catecholamine release (Brommer et al., 1982).

Additionally, other investigators have shown that air travel not preceded by exercise had either no effect or a potential decrease on tPA concentrations (Boccalon et al. 2005; Schobersberger et al., 2002). Since no change was seen between samples taken in the afternoon (BL) and early morning (1CT), the change we saw in tPA concentrations was not due to diurnal variation. Even though tPA is known to have a high variability between day and night values. Our results are similar to those of Parker et al. (2011), who demonstrated that tPA concentrations return to baseline levels 24 h after completing a marathon and then flying for more than 4 h.

The main regulatory enzyme of the fibrinolytic system is PAI-1, which functions as the primary inhibitor of tPA. PAI-1 exhibited an increase before the flight to CA (1CT) compared to BL. This result is likely due to the circadian rhythm of PAI-1, which peaks in the morning and is the lowest levels in the evening (Siahkouhian et al., 2013). We also demonstrated increased PAI-1 concentrations immediately following the full-body workout (4CA). Previous studies have found varied levels of PAI-1 post-exercise based on the type of exercise performed (Bärtsch et al., 1995; Hilberg et al., 2003; Sumann et al., 2007) and the training status of subjects (Kvernmo & Osterud, 1997). Interestingly, in our study PAI-1 remained elevated after landing in CT. This could be explained by either of two factors: 1) After landing in CT, blood was drawn at approximately 0900 EDT, when PAI-1 concentrations would be at their peak, or 2) hypoxia is known to trigger release of PAI-1 (Li et al., 2005) so could be a risk factor for the increased levels following the trans-continental flight.
D-Dimer was examined to measure in vivo activation of the fibrinolytic system, and is formed after fibrin is degraded. During long-distance flights, Jacobson et al. demonstrated that approximately 8.2% of all passengers had elevated D-Dimer levels. Their result was not limited to economy class passengers (Jacobson et al., 2003). In the present study, we did not see a rise in D-Dimer immediately after arriving in CA (2CA). However, we did find an increase in D-Dimer concentrations immediately after the full-body workout (4CA), which is consistent with other exercise studies, especially endurance exercise (Kupchak et al., 2013b; Röcker et al., 1990). We did find increased concentrations after landing in CT, and at 29 h post-flight. Even though we saw an increase after landing in CT, this increase was not due circadian variation because we found no change between BL (taken at 1300) and 1CT (performed at 0600 the next day). These results are not unprecedented, for Parker et al. (2012) demonstrated elevated levels of D-Dimers immediately after completing a marathon, and participants who had traveled >4 h to the race had higher immediate post exercise levels than controls who had traveled less than 2 h (Parker et al., 2012). However, our data suggest that performing a muscle-damaging workout followed by a trans-continental flight may place an individual at risk for venous thrombosis, due to the number of subjects who exhibited D-Dimer values greater than 500 ng·mL⁻¹ (clinical threshold for excluding VTE) immediately after the full-body workout (n = 2 for CON; n = 4 for FCG) and at 29 h post-flight (n = 1 for CON; n = 1 for FCG).

In the present study, compression gear did exhibit a positive effect on limiting coagulation, specifically at 29 h post-flight, when the CON group showed a significant decreased aPTT compared to the FCG group. Additionally, the thrombin generation marker, TAT, had a lower concentration in the FCG group at 29 h post-flight. This could be attributed to the compression garment limiting muscle damage (Kraemer et al., 2016) in turn limiting thrombin generation. A potential explanation for this observation is the compression garment may hold the limbs in what is known as “dynamic casting,” thereby reducing soft tissue injury (Kraemer et al., 2001a). This effect is important because it can limit excessive movement and vibration of the muscles providing an ideal environment for muscle recovery (Kraemer et al., 2001b). Kraemer et al. (2010) demonstrated significantly lower muscle swelling by ultrasound technology in a group using a compression garment compared to control. This could also explain the positive effect of compression garments on PAI-1 concentrations after landing in CT, even though a prior study has shown that compression garments do not improve markers of inflammation (Pruscino et al., 2013), which have been linked to PAI-1 levels (Devaraj et al., 2003). Furthermore, compression garments may reduce risk of VTE by compressing veins, thereby diminishing venous stasis and increasing venous return and arterial blood flow (Kraemer et al., 2000). This is important since venous stasis has been linked to a decline in intravascular oxygen tension and the formation of thrombus in the legs (Yan et al., 1999).

In summary, a trans-continental flight alone did not activate the coagulation and fibrinolytic
systems. However, an acute exercise protocol was able to elicit a response on the blood hemostatic system, exhibited by the activation of the coagulation system (aPTT was decreased, while TAT and PTF 1+2 were elevated). In order to balance coagulation, the fibrinolytic system (increases in tPA, PAI-1 and D-Dimer) was also activated. The transcontinental flight following the exercise protocol further maintained a hypercoagulable state as TAT, PAI-1 and D-Dimers remained elevated into recovery. The wearing of a full-body compression garment did have a positive effect on coagulation, as individuals showed lower levels of TAT and PAI-1 and a slower aPTT in recovery compared to controls. The results suggest an increased susceptibility of clot formation in individuals who travel cross-country via plane to participate in athletic events. Therefore individuals who travel long distances may be at a higher risk for a DVT or PE. However, the use of mechanical prophylaxis such as full body compression may be a viable option, as our research demonstrated that these individuals exhibited a slower aPTT and lower levels of TAT and PAI-1 into recovery.

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Declaration of interest

The authors report no conflicts of interest.

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