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Effects of low-intensity resistance exercise with blood flow restriction on coagulation system in healthy subjects

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Summary

Recent studies have demonstrated that even a low-intensity resistance exercise can effectively induce muscle hypertrophy and strength increase when combined with moderate blood flow restriction (BFR) into the exercising muscle. Although serious side effects of low-intensity resistance exercise with BFR have not been reported, a concern of thrombosis has been suggested, because this type of exercise is performed with restricted venous blood flow and pooling of blood in extremities. Thus, the purpose of this study was to investigate the effects of low-intensity resistance exercise with BFR on coagulation system in healthy subjects. Ten healthy men (25·1 ± 2·8 year) performed four sets of leg press exercises with and without BFR (150–160 mmHg) at an intensity of 30% of one-repetition maximum (1RM). In each exercise session, one set with 30 repetitions was followed by three sets with 15 repetitions. Blood samples were taken before, and 10 min, 1, 4 and 24 h after the exercise. Prothrombin fragment 1 + 2 (PTF) and thrombin–antithrombin III complex (TAT) were measured as markers of thrombin generation, whereas D-dimer and fibrin degradation product (FDP) were measured as markers of intravascular clot formation. Changes in plasma volume (PV) were calculated from haemoglobin and hematocrit values. PV reduction was significantly greater after the exercise with BFR than without ($P<0·05$). However, neither markers of thrombin generation nor intravascular clot formation increased after the exercises. These results suggest that low-intensity resistance exercise with BFR does not activate coagulation system in healthy subjects.

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Introduction

Recent studies have demonstrated that even a low-intensity resistance exercise can effectively increase muscular size and strength when combined with moderate blood flow restriction (BFR) into the exercising muscle [reviewed in (Wernbom *et al.*, 2008; Manini & Clark, 2009)]. Although serious side effects of low-intensity resistance exercise with BFR have not been reported (Nakajima *et al.*, 2006), a concern of thrombosis has been suggested (Manini & Clark, 2009), because this type of exercise is performed with restricted venous blood flow and pooling of blood in extremities. To tackle this concern, we have previously performed a study and reported that low-intensity resistance exercise with BFR increases neither plasma fibrin degradation product (FDP) nor D-dimer, markers of intravascular clot formation, indicating that this type of exercise would not activate coagulation system and cause intravascular clot formation (Nakajima *et al.*, 2007). However, Zaar *et al.* (2009) reported in their recent study that plasma thrombin–antithrombin III complex (TAT), a marker of thrombin generation, increased during a 10 min of lower body negative pressure (LBNP) at 30 mmHg, though plasma D-dimer did not increase. They suggested that blood pooling in lower extremities and reduction in central blood volume (BV) induced by LBNP activated the thrombin-generating part of the coagulation system. It has been demonstrated that BFR causes LBNP-like effects on hemodynamic responses (Takano *et al.*, 2005; Iida *et al.*, 2007), e.g. reductions in stroke volume and cardiac output.

It should be noted that the study on LBNP (Zaar *et al.*, 2009) did not combine exercise to LBNP, so that the effect cannot be directly compared to that of resistance exercise with BFR.

However, low-intensity resistance exercise with BFR has been shown to reduce stroke volume during the exercise (Takano *et al.*, 2005) and plasma volume (PV) after the exercise (Nakajima *et al.*, 2007). In addition, this type of exercise is normally performed 3–5 sets with continuous BFR including 30–60 s rest intervals between sets (Wernbom *et al.*, 2008).

Exercise-induced thrombin generation seems to be a complex function of exercise type (Hilberg *et al.*, 2005), intensity (Weiss *et al.*, 1998) and duration (Mockel *et al.*, 2001; Hilberg *et al.*, 2003b). It has been considered that metabolic and/or adrenergic factors play some roles in exercise-induced thrombin generation, though the precise mechanism remains unclear. For example, Herren *et al.* (1992) reported a positive correlation between postexercise blood lactate and TAT concentrations, whereas Wallen *et al.* (1999) reported that an adrenaline infusion as well as a fatiguing exercise increased plasma TAT. Although resistance exercise with BFR is normally performed with a relatively low intensity for a short duration (Wernbom *et al.*, 2008), this type of exercise has been shown to induce strong metabolic stress (Suga *et al.*, 2009) with increases in concentrations of blood lactate and catecholamines (Takarada *et al.*, 2000; Nakajima *et al.*, 2007).

Therefore, it is hypothesized that low-intensity resistance exercise with BFR would activate thrombin-generating part of coagulation system, even when markers of intravascular clot formation did not increase. In the present study, we measured both markers of thrombin generation and intravascular clot formation to comprehensively investigate the effects of low-intensity resistance exercise with BFR on coagulation system.

Methods

Subjects

Ten healthy men volunteered to participate in the present study. Their mean age, height and body mass were 25·1 ± 2·8 year, 172·2 ± 5·2 cm and 68·5 ± 7·0 kg, respectively. All subjects were fully informed about the experimental procedures and the purpose of the study and gave their written informed consent prior to participation. The institutional review board of human research of the Japan Aerospace Exploration Agency and the ethics committee of the University of Tokyo approved this study.

Experimental procedure

Subjects participated in three experimental sessions. The first session was a preliminary session to determine one-repetition maximum (1RM) of seated leg press exercise. In the second and third sessions, which were separated by 24 h, the subjects performed four sets of leg press exercise at an intensity of 30% 1RM either with or without BFR, the order of which was counterbalanced across subjects. In each exercise session, one set with 30 repetitions was followed by three sets with 15 repetitions. The subjects were instructed to maintain a cadence of a 1-s concentric phase and a 1-s eccentric phase. A rest period of 1 min was allowed between sets.

When the subjects performed the exercise with BFR, the proximal portions of their thighs were compressed at the pressure of 150–160 mmHg by electronically controlled air-pressure belts as previously described (Nakajima *et al.*, 2008). The compression was kept throughout the exercise session including the rest periods between sets and was released immediately after the session.

Blood sampling and analysis

The subjects came to the laboratory in the morning and rested for 30 min before the pre-exercise blood collection. While the subjects rested in a seated position, venous blood samples (10 ml for each sampling) were taken from the antecubital vein and collected into test tubes with EDTA-2Na as well as with 3·2% sodium citrate. Postexercise blood samples were obtained at 10 min, 1, 4 and 24 h after the exercise. Test tubes with 3·2% sodium citrate were centrifuged (4°C, 1006 *g*) for 10 min to isolate plasma, and removed plasma was stored at −20°C until analysis.

Blood samples were measured for haemoglobin (Hb), hematocrit (Hct, %) and plasma concentrations of prothrombin fragment 1 + 2 (PTF), TAT, D-dimer and FDP. Hb (g 100 ml^{-1}) was measured by the cyanomethemoglobin method (Coulter haemoglobinometer; Beckman Coulter Inc., Brea, CA, USA), whereas Hct was measured by micro-hematocrit using ultra centrifugation. Plasma concentrations of PTF, TAT, D-dimer and FDP were measured at commercially available laboratories (SRL Inc., Tokyo, Japan) by following methods: PTF, enzyme-linked immunosorbent assay (ELISA); TAT, enzyme immunoassay (EIA); D-dimer and FDP, latex immunoassay (LIA).

Percentage changes in BV and PV were derived from the following equation (Dill & Costill, 1974; Nakajima *et al.*, 2008);

$$BV_B/BV_A = Hb_A/Hb_B$$

$$\%\Delta PV = 100 * (Hb_B/Hb_A) * [(1 - Hct_A * 10^{-2})/ (1 - Hct_B * 10^{-2})] - 100$$

where A is the value at rest (Pre), and B is the value after the exercise.

Statistical analysis

The data are reported as means ± SD, unless otherwise stated. Statistical analysis was performed using StatView 5.0 for Windows (SAS Institute Inc., Cary, NC, USA). Box and whisker plots were used to display the changes in markers of blood coagulation. The central line of the box represents the median value. The box represents the 25th and 75th percentiles, whereas the whiskers represent the 10th and 90th percentiles. Markers beyond the whiskers are outliers. The data were analysed with a two-factor (condition × time) repeated-measures ANOVA. $P<0·05$ was considered significant.

Results

PV reduction was significantly greater ($P<0{\cdot}05$) after the exercise with BFR ($-4{\cdot}6 \pm 3{\cdot}5\%$) than without ($0{\cdot}9 \pm 3{\cdot}7\%$). However, for PTF and TAT, neither condition × time interactions (PTF, $P = 0{\cdot}28$; TAT, $P = 0{\cdot}19$) nor main effects for time (PTF, $P = 0{\cdot}84$; TAT, $P = 0{\cdot}77$) were observed (Fig. 1). Similarly, neither condition × time interactions (D-dimer, $P = 0{\cdot}76$; FDP, $P = 0{\cdot}17$) nor main effects for time (D-dimer, $P = 0{\cdot}09$; FDP, $P = 0{\cdot}61$) were observed for D-dimer and FDP (Fig. 2). Note that, except a few outliers, most of the data for FDP are around 2 μg ml^{-1}, so that the boxes are compressed (Fig. 2b).

Discussion

The present study showed that neither markers of thrombin generation (PTF and TAT) nor markers of intravascular clot formation (D-dimer and FDP) increased after four sets of low-intensity leg press exercise with BFR. These results suggest that moderate BFR does not activate coagulation system when combined with exercise. In addition, it is suggested that three sets of 30 s rest period (90 s in total) are not sufficient to activate coagulation system even under the condition of BFR.

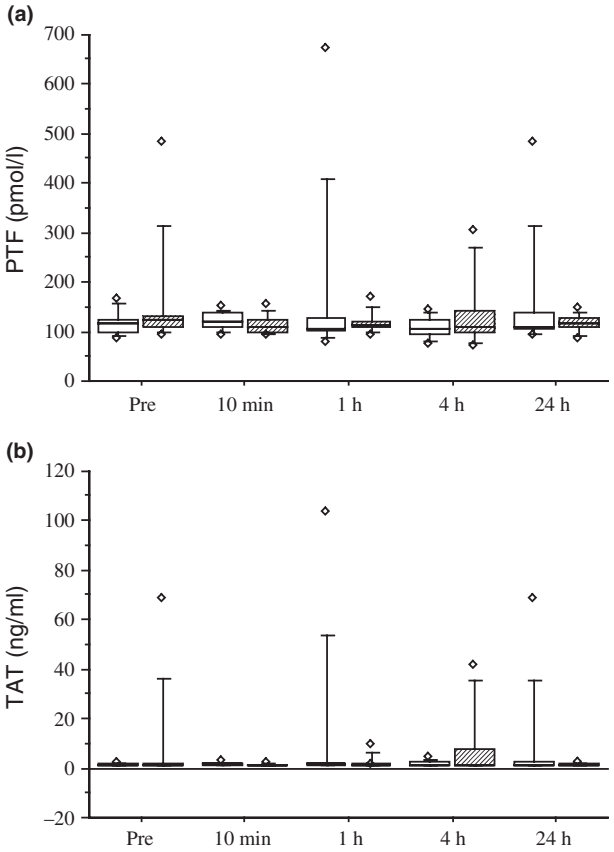

Figure 1 Plasma concentrations of prothrombin fragment 1 + 2 (a) and thrombin–antithrombin III complex (b) measured before and after the exercises with (open box) and without (hatched box) blood flow restriction. The central line of the box represents the median value. The box represents the 25th and 75th percentiles, whereas the whiskers represent the 10th and 90th percentiles. Markers beyond the whiskers are outliers.

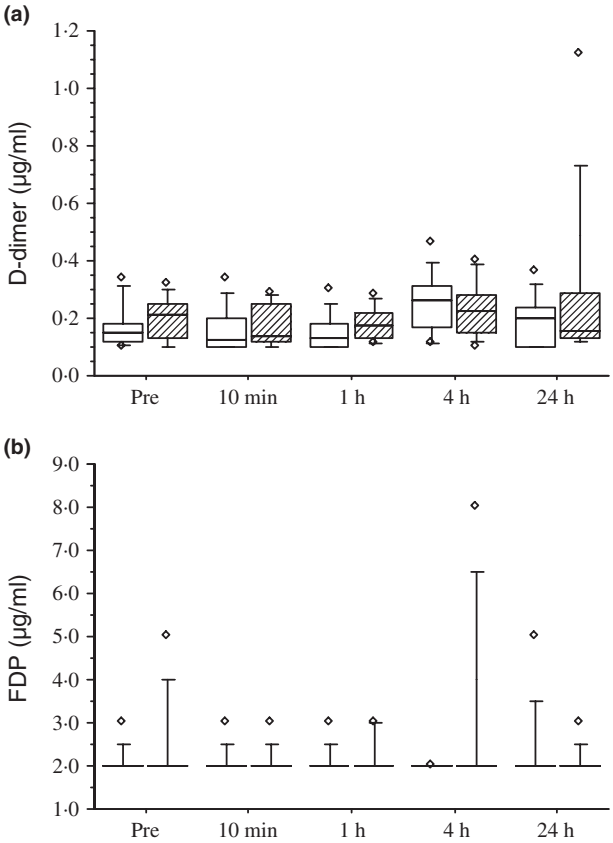

Figure 2 Plasma concentrations of D-dimer (a) and fibrin degradation product (b) measured before and after the exercises with (open box) and without (hatched box) blood flow restriction. The central line of the box represents the median value. The box represents the 25th and 75th percentiles, whereas the whiskers represent the 10th and 90th percentiles. Markers beyond the whiskers are outliers.

On the other hand, LBNP-induced reduction in central BV has been suggested to activate thrombin generation (Zaar *et al.*, 2009). Zaar *et al.* (2009) reported that LBNP reduced central BV along with stroke volume and cardiac output. It has been demonstrated that BFR causes LBNP-like effects on hemodynamic responses during exercise (Takano *et al.*, 2005) as well as at rest (Iida *et al.*, 2007). However, Takano *et al.* (2005) reported that although stroke volume decreased during the exercise with BFR, cardiac output increased in a similar manner in both conditions with and without BFR because of a larger increase in heart rate during the exercise with BFR. Therefore, it can be assumed that the larger increase in heart rate during the exercise with BFR would compensate for the reduction in central BV, so that thrombin generation would not be activated. In addition, it can also be assumed that, even under the condition of BFR, muscle contractions would, at least partially, facilitate venous return and prevent thrombin generation.

Although a precise mechanism of exercise-induced thrombin generation remains unclear, it has been considered that

metabolic and/or adrenergic factors play some roles in exercise-induced thrombin generation (Herren *et al.*, 1992; Wallen *et al.*, 1999). In the present study, however, low-intensity resistance exercise with BFR, which has been shown to cause marked increases in concentrations of blood lactate and catecholamines (Takarada *et al.*, 2000; Nakajima *et al.*, 2007), did not cause increases in markers of thrombin generation. Therefore, it is likely that an exercise-induced thrombin generation would be more dependent on exercise duration than on metabolic and/or adrenergic factors, and the exercise duration in the present study would not be sufficient to induce thrombin generation. This notion is in line with previous studies by Hilberg and colleagues demonstrating that TAT and D-dimer increased after 60–90 min exercise at anaerobic threshold (Hilberg *et al.*, 2003a), whereas these markers did not increase after 15–90 s of maximal exercise (Hilberg *et al.*, 2003b), in which blood lactate concentration increased significantly.

In conclusion, the present study showed that neither markers of thrombin generation (PTF and TAT) nor markers of intravascular clot formation (D-dimer and FDP) increased after four sets of low-intensity leg press exercise with BFR. These results suggest that low-intensity resistance exercise with BFR does not activate coagulation system in healthy subjects.

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