Comparison of low-intensity blood flow-restricted training-induced muscular hypertrophy in eumenorrheic women in the follicular phase and luteal phase and age-matched men

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blood occlusion; gender; menstrual cycle; sex hormone; skeletal muscle mass

Summary
The purpose of this study was to compare the muscle hypertrophic response in women during both the follicular (FP) and the luteal phase (LP) of their menstrual cycles following short-term, low-intensity resistance training combined with blood flow restriction (BFR). Eight eumenorrheic women and five men, all previously untrained, performed unilateral low-intensity (30% of 1 repetition maximum) dumbbell curl training with BFR once a day for 6 days. The opposite arm served as an untrained control. This 6-day training programme was conducted during both menstrual cycle phases: the early FP and the mid LP. MRI-measured biceps muscle volume (MV) and isometric elbow flexion strength were measured in both arms before and 2 days after the final training bout. Significantly (P<0.05) greater muscle hypertrophy was observed in the LP (5–7%) than in the FP (3–7%). The absolute and relative changes in serum hormone concentrations between the two phases did not correlate (P>0.05) with the percentage change in MV between the LP and FP. There was no change in MV in the control arm for both cycle phases. Following training, isometric strength increased (P<0.01) in the LP, but not in the FP (P = 0.17). Relative strength (strength per unit MV) was similar pre- and post-training in both phases. The percentage changes in MV and strength were similar between the women (average of LP and FP) and men. Our results indicate that muscle hypertrophy and strength gain are higher in the LP than in the FP following 6 days of BFR training, although the sex difference in the training response is non-existent.

Introduction
In general, the relative increases in muscle cross-sectional area (CSA) and mass in response to high-intensity resistance training are similar for men and women, although absolute change in muscle size tends to be greater in men (Cureton et al., 1988; O’Hagan et al., 1995; Abe et al., 2000; Wals et al., 2008). In both sexes, the muscle hypertrophy can be attributed to increased accumulation of contractile protein, which occurs when the balance between protein synthesis and degradation shifts towards synthesis. In men, however, the resting blood concentration of testosterone is up to 10-fold higher than it is in women. Testosterone has a strong anabolic action and stimulates muscle protein synthesis (Bhasin et al., 1996; Ferrando et al., 1998; Ahtiainen et al., 2003). Thus, it would appear that women’s testosterone might have a similar anabolic effect as men’s testosterone and produce muscle hypertrophy in response to resistance training.

Blood concentrations of female sex hormones, such as oestrogen and progesterone, are known to change during the menstrual cycle and are high during the luteal phase (LP) and low during the early follicular phase (FP). Based on previous studies, estradiol is known for its anabolic effect on humans (Sipila et al., 2001; Sorensen et al., 2001; Brown, 2008) and animals (Kahler et al., 1997; Sitnick et al., 2006; Hatae et al., 2009), although some studies have reported no effect on muscle tissue accumulation and muscle protein metabolism (Toth et al., 2001; Maddalozzo et al., 2004; Aubertin-Leheudre et al., 2005). On the other hand, a limited number of studies have reported the catabolic effect of progesterone on muscle tissue (Landau & Lugibihl, 1961). During high-intensity resistance training, the appearance of significant muscle hypertrophy occurs at about 2 months into the training period. It is difficult to evaluate the effect of female sex hormones on muscle hypertrophy because there are conflicting anabolic (oestrogen) and catabolic (progesterone) hormonal alterations in response to training periods.
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over the length of the menstrual cycle. Therefore, to evaluate the effects of the female sex hormones, muscle hypertrophy would have to occur during either the 2-week LP or the 2-week FP. Muscular blood flow restriction (BFR) during resistance training has been shown to elicit muscle hypertrophy similar to that elicited by traditional high-intensity resistance training, but at much lower training intensities (Abe et al., 2005; Karabulut et al., 2010). Recently, we reported that rapid muscle hypertrophy occurred following only 1–2 weeks of low-intensity resistance training when subjects performed exercise bouts at a higher frequency (twice daily, at least 4 h between bouts) instead of a standard resistance training programme (Fujita et al., 2008; Yasuda et al., 2010). After an acute bout of low-intensity resistance exercise combined with BFR, mammalian target of rapamycin (mTOR) signalling pathway and muscle protein synthesis are increased (Fujita et al., 2007; Fry et al., 2010). Therefore, it is possible to compare the muscle hypertrophic response during the 2-week LP with that of the 2-week FP through the use of this training protocol. In this study, to compare the muscle hypertrophic potential during the LP with that of the FP, we evaluated the muscular hypertrophy occurring during the FP and the LP following 6 days of low-intensity BFR training in eumenorrheic women.

Methods

Subjects

Eight women and five men, aged 23–37 years, were recruited from the University of Tokyo for the study (Table 1). The subjects in this study were physically active, but none had participated in regular resistance training for a minimum of 1 year prior to the start of the study. All subjects were normotensive (blood pressure <140/90 mm Hg) and were free of overt chronic disease as assessed by medical history, physical examination and complete blood chemistry and haematologic evaluation. Candidates who had smoked in the previous 5 years or who were taking medications or female hormone supplements were excluded. All of the women were having regular menstrual cycles (25–35 days in length). The subjects were informed of the methods, procedures and risks and signed an informed consent document before participation. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of the University of Tokyo, Japan.

Basal body temperature

During the orientation period, the women were informed on how to measure their own basal body temperature. Digital thermometers (CS52; Terumo Co Ltd., Tokyo, Japan) with scale steps of 0·01°C were used, and basal body temperature was measured orally for 90 s before rising in the morning. The subjects continued to measure and record their basal body temperature from 2 months before the start of the study and throughout the study period. The pattern of the basal body temperature was used to estimate the duration of the different phases of the cycles and determine the training schedule. During the training period, the cycle phases were subsequently validated by the measurement of serum ovarian hormones.

Training protocol and 1 repetition maximum testing

Subjects participated in 6 days of supervised unilateral dumbbell curl exercise training combined with BFR. The opposite arm served as the control. The contraction intensity and sets performed in the exercise protocols were chosen based on the previous findings of enhanced muscle adaptations following low-intensity resistance training with BFR (Abe et al., 2005; Fujita et al., 2008). Training involved four sets of low-intensity [predetermined 30% of 1 repetition maximum, (1-RM)] exercise training (30 reps followed by three sets of 15 reps, for a total of 75 contractions, with 30 s of rest between sets) once per day for 6 consecutive days. All subjects (both men and women) were right handed. During the first 6 days of training, half of the female subjects (n = 4) trained their right arm, and the other four female subjects trained their left arm. As for the men, three male subjects trained their right arm, and two male subjects trained their left arm. For both men and women, the opposite arm served as the control. During the second 6-day training period, the female subjects switched arms. The rest

Table 1 Changes in body weight and upper arm girth following 6 days of low-intensity blood flow restricted resistance training

<table>
<thead>
<tr>
<th></th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>Men</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29·1 (6·1)</td>
<td>29·1 (6·1)</td>
<td>24·0 (1·7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1·57 (0·02)</td>
<td>1·57 (0·02)</td>
<td>1·74 (0·05)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52·3 (4·5)</td>
<td>52·5 (4·5)</td>
<td>63·3 (5·8)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>21·4 (1·5)</td>
<td>21·3 (1·6)</td>
<td>20·8 (1·2)</td>
</tr>
<tr>
<td>Upper arm girth (cm)</td>
<td>Trained</td>
<td>25·3 (1·6)</td>
<td>25·6 (1·8)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25·5 (1·6)</td>
<td>25·9 (1·8)</td>
</tr>
</tbody>
</table>

BMI, body mass index.
period between the first and second 6-day training periods was one menstrual cycle, or about 30 days. For the women, data were collected in the early FP (the first training session was performed 3 days after the start of menstruation, when oestrogen and progesterone were low) and in the mid LP (the first training session was performed 19–25 days after the start of menstruation, when oestrogen and progesterone were elevated) of the menstrual cycle. The women performed 6 days of training during both the FP and LP, and the order of training was randomized. The 1-RM testing was conducted 3 days before the first training session, as described previously (Abe et al., 2000). Briefly, each subject performed a warm-up of three to five biceps curls using a moderate load. The load was then set as high as 80% of the predicted 1-RM. Following each successful lift, the load was increased by as much as 5% until the subject failed to lift the load through the entire range of motion. Borg’s Rating of Perceived Exertion (RPE), which is a scale (6–20) to measure subjective feelings of exertion and fatigue during exercise, was used immediately after the training sessions to assess exercise intensity (Borg, 1982).

**Blood flow reduction by external limb compression**

A method for providing external compression around a limb has been previously reported (Yasuda et al., 2010). A specially designed elastic cuff belt (30 mm wide) for the arm (KAATSU Master; Sato Sports Plaza, Tokyo, Japan) was placed around the most proximal portion of the training arm during bilateral dumbbell curl exercise sessions. On the first day of training, the cuff belt air pressure was 80 mm Hg. As subjects adapted to the occlusive stimulus during the early phase of training, the belt air pressure was increased up to 100 mm Hg after the third or fourth training day. Blood flow to the arm muscles was restricted for a total of about 5 min (1 min of preparation time and 4 min of training time) during each training session, with the belt air pressure released immediately upon completion of the session.

**Muscle cross-sectional area and muscle volume**

Multislice MRI images of the upper arms (both the training arm and the control arm) were obtained using a General Electric Yokogawa Signa 0.2-T scanner (Milwaukee, WI, USA). A T1-weighted, spin-echo, axial plane sequence was performed with a 520-ms repetition time and a 20-ms echo time. Subjects rested quietly in the magnet bore in a supine position with their arms extended. The lateral epicondyle of the humerus was used as the origin point, and continuous transverse images with 1.0-cm slice thickness (0.2-cm interslice gap) were obtained from the lateral epicondyle of the humerus to the acromial process of the scapula for each subject. All MRI data were transferred to a personal computer for analysis using specially designed image analysis software (TomoVision Inc., Montreal, Canada). For each slice, elbow flexor muscle CSA was digitized, and the muscle volume (cm³) per slice was calculated by multiplying muscle CSA (cm²) by slice thickness (cm). We have previously determined that the coefficient of variation (CV) of this measurement was <1.0%. This measurement was completed at baseline and 2 days after the final training session (post-testing). Muscle volume was determined using brachium flexor data.

**Maximum isometric strength**

Maximum voluntary isometric strength of the elbow flexor was measured (Biodex System 3 dynamometer; Sakai Medical Instrument, Tokyo, Japan) in both the training and the control arms. Each subject was comfortably seated on a chair, and the training arm was placed on a firm and stable table at chest level, with the elbow joint positioned at an angle of 90° (0° at full extension). The upper arm was maintained in the horizontal plane while the hand grasped the Biodex lever in the pronated position. After being briefed on the testing procedures and warming up with submaximal contractions, each subject was instructed to perform two bouts of maximal isometric contractions, and the maximal value was adopted. This test was performed at baseline and was repeated 2 days after the final training session (post-testing).

**Blood hormonal concentrations**

Venous blood samples were collected from an antecubital vein between 9:00 and 9:30 am after a 30-min rest period following an overnight fast. The blood was drawn 2 days before the start of training and again on the fifth day of training. The average of the two values was used as data. Serum estradiol (E₂), progesterone and testosterone concentrations were measured at commercially available laboratories (SRL Inc., Tokyo, Japan) using an electrochemiluminescent immunoassay. Haematocrit was measured using a microcentrifugation technique, and haemoglobin was determined using a cyanomethemoglobin method (Coulter haemoglobinometer).

**Upper arm girth**

Exercise-induced acute arm girth change, which is an index used to temporarily induce transcapillary fluid shifts and subsequent muscle swelling, was determined using a flexible tape measure at 60% distal to the upper arm. Measurements were taken before and immediately after the training sessions. The CV for this measurement in our laboratory was 1.3%.

**Statistical analyses**

StatView 4.5 (Abacus Concepts, Piscataway, NJ, USA) was used to compute the data, and the results were expressed as means and standard deviations (SDs) for all variables. To compare the women with the men, we used the mean values of the LP and FP for the women. Statistical analyses were performed using a two-way analysis of variance (ANOVA) with repeated measures [Group (FP and LP or Women and Men) × Time (pre- and post-training)] to evaluate the training effects for all dependent variables. When
appropriate, post hoc paired t tests were used to assess within-group changes. All baseline characteristics and percentage changes in anthropometric variables, skeletal muscle volume and muscular strength were compared between groups using a one-way ANOVA. Statistical significance was set at P<0.05.

Results

Blood hormone concentrations in women and men

Serum estradiol and progesterone concentrations were higher (P<0.05) in the LP than in the FP, but the serum testosterone concentration was similar in both phases. In contrast, the men had higher (P<0.05) serum testosterone concentrations than both the LP and FP women (Fig. 1). There were no differences (P>0.05) between the FP and LP in haematocrit (40.1±3.8% and 40.5±2.9%, respectively) and haemoglobin (13.0±1.5 and 13.0±1.1 g dl⁻¹, respectively) concentrations.

Increases in muscle size and strength during the luteal phase versus the follicular phase

Before training, there were no significant differences in body weight and upper arm girth of the training and control limbs of the LP and FP women (Table 1). After training, the elbow flexor muscle volume was increased (P<0.05) in the training arm of both the FP and LP women (Table 2). The percentage change in muscle volume was higher in the LP than in the FP (P<0.05, Fig. 2). There were no significant correlations between the relative change in muscle volume and serum hormone concentrations in the LP and FP groups (Fig. 3). In addition, the absolute and relative changes in estradiol and progesterone concentrations between the two phases (LP minus FP) did not correlate (P>0.05) with the percentage change in muscle volume. Although the percentage change was similar, maximum voluntary isometric strength was increased in the LP (6.1%, P<0.01), but not in the FP (5.4%, P = 0.17) (Table 3). There were no changes (P>0.05) in muscle volume and isometric strength in the control arm for the LP and the FP. Maximum isometric strength per unit muscle volume was similar between pre- and post-training in the training (FP: pre 0±27, post 0±27 Nm cm⁻³; LP: pre 0±27, post 0.28 Nm cm⁻³) and control arms.

Comparison of increases in muscle size and strength between women and men

After training, muscle volume was increased (P<0.05) in the training arm, but not in the control arm, in both the women and the men (Table 2). The percentage change in muscle volume was similar in both sexes (Fig. 2). Maximum isometric strength was also increased in the women and the men (P<0.05), and the percentage change was similar in both groups (men: 9.0±7.8%; women: 6.0±7.5%).

Relationship between muscle hypertrophy and rating of perceived exertion or acute arm girth change

Immediately after exercise sessions, the mean RPE in the LP and FP were 15.5±1.8 and 15.4±1.0, respectively, in the women. A similar RPE was recorded in the men (15.8±0.7). The mean changes in the upper training arm girth before and immediately after exercise sessions were 25.8±1.8 and 26.4±1.8 cm, respectively, in the LP; 25.4±1.5 and 26.0±1.5 cm, respectively, in the FP; and 26.4±2.0 and 27.2±2.0 cm, respectively, in the men. The acute change in absolute upper arm girth was correlated with a training-induced increase in muscle volume in the LP (r = 0.71, P = 0.04), but not in the FP (r = −0.15, P = 0.72) and the men (r = 0.19, P = 0.76). There was no significant correlation between increase in muscle volume and RPE.

Discussion

The present study demonstrates some novel and important findings: (i) muscle hypertrophy was higher in the LP than in the FP following 6 days of low-intensity BFR training, (ii) the training-induced increase in muscle volume during the LP was associated with a temporary acute change in upper arm girth immediately after the training sessions and (iii) there was no significant difference in the percentage increase in muscle volume...
Table 2  Change in elbow flexor muscle volume (cm$^3$) following 6-days of low-intensity blood flow restricted resistance training.

<table>
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<th>Follicular phase</th>
<th>Luteal phase</th>
<th>Men</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Trained arm</td>
<td>115.7 (15.4)</td>
<td>119.7 (15.2)*</td>
<td>115.2 (12.0)</td>
</tr>
<tr>
<td>Control arm</td>
<td>116.4 (13.0)</td>
<td>116.2 (13.5)</td>
<td>117.9 (13.7)</td>
</tr>
</tbody>
</table>

*P<0.05 versus Pre.

Table 3  Change in maximum voluntary isometric strength (Nm) following 6-days of low-intensity blood flow restricted resistance training.

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<th>Follicular phase</th>
<th>Luteal phase</th>
<th>Men</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Trained arm</td>
<td>30.9 (4.8)</td>
<td>32.5 (5.0)</td>
<td>30.8 (4.5)</td>
</tr>
<tr>
<td>Control arm</td>
<td>33.0 (5.1)</td>
<td>33.0 (4.8)</td>
<td>30.9 (4.0)</td>
</tr>
</tbody>
</table>

*P<0.05 versus Pre.

Figure 2  Percentage change in muscle volume of trained (solid bars) and control arm (open bars) in the follicular (FP) and luteal (LP) phase (panel a) and in men and women (panel b). *P<0.05 versus FP.

Figure 3  Relationships between change in muscle volume and resting serum estradiol (panel a), progesterone (panel b) and testosterone (panel c) concentrations in both the follicular (●) and luteal (○) phase. © 2011 The Authors
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volume between the women (averaged value of the LP and FP) and men.

Previous studies (Fujita et al., 2007; Fry et al., 2010) demonstrated that a single bout of low-intensity (20% 1-RM) resistance exercise with BFR increased both vastus lateralis protein synthesis and Akt/mTOR signalling pathway in humans. During the menstrual cycle phase in eumenorrheic women, circulating estradiol and progesterone concentrations were higher during the LP than during the early FP. After oestrogen administration, activation of muscle protein synthesis is observed (Vasconsuelo et al., 2008; Hatae et al., 2009). Oestrogen receptor mRNA and transcriptional activity in exercising muscle increased after an acute bout of high-intensity exercise (Cartoni et al., 2005; Dieli-Conwright et al., 2009). Therefore, it is expected that an elevation of anabolic responses may be observed during the LP compared with the early FP, and the training-induced muscle hypertrophy and strength gain may be higher in the LP than in the FP. In the present study, muscle hypertrophic response was higher in the LP than in the FP following 6 days of low-intensity BFR training. However, our results showed that there was no significant correlation between the training-induced muscle hypertrophy and blood estradiol concentration in the LP and the FP. Our results did not support the findings of previous animal and human studies that elevated oestrogen levels can produce a greater hypertrophic response compared with that produced by low oestrogen levels. On the other hand, a previous study has reported the catabolic effects of progesterone on muscle tissue (Landro & Lugibihl, 1961). In the present study, however, there was no significant correlation between serum progesterone concentration and training-induced muscle hypertrophy. Therefore, our results may indicate that the function of progesterone in skeletal muscle is not yet clear.

Our results indicated that the exercise-induced acute change in arm girth, which is an index to temporarily induce fluid shifts, was associated with training-induced increase in muscle volume in the LP, but not in the FP. The reasons why a significant correlation does not appear in both the LP and the FP are unclear, but premenstrual fluid retention causes temporary tissue swelling and may generate an interactive effect on cell swelling during the LP. Previous studies reported that cell volume alteration induced by acute change in extracellular osmolality is an important regulator of protein metabolism. The acute cell swelling has been shown to stimulate muscle protein synthesis and suppress proteolysis (Berneis et al., 1999; Miller et al., 2006), thereby promoting net protein accretion. Therefore, the correlation seen in the LP can support the difference in advantage of the muscle hypertrophic response between the two phases.

There was no significant difference in the percentage increase in muscle volume between the women (averaged value of the LP and FP) and men in our study. In general, it is well known that relative increases in muscle size are similar between men and women after 3–4 months of high-intensity resistance training (Cureton et al., 1988; O’Hagan et al., 1995; Abe et al., 2000; Walts et al., 2008). Acute resistance exercise determines an increase in muscle protein synthesis, and this response is similar between young men and women tested during the FP (Dreyer et al., 2010). Therefore, resistance training–induced muscle hypertrophic responses are equivalent between men and women when training is performed two to three times per week on a regular basis. Interestingly, when the training sessions are held mainly during the FP of the menstrual cycle rather than being spread over the entire cycle, it was found that resistance-trained FP women achieve greater strength (Reis et al., 1995). These differences in strength gain are largely attributed to neural adaptation because the changes in muscle CSA are similar between the two training frequency groups. In our study, the results, when applied to a higher-frequency training model, are probably greater in the LP than in the FP and, as such, are in disagreement with the study of Reis et al. (1995). Further research is needed to clarify the reasons for the different results.

A number of limitations of this study should be mentioned. First, the assumptions of training only apply to the protocol used in this study, which was 6 days of low-intensity BFR training. Second, only one exercise was examined, arm curl, so we cannot imply similar results for other muscle groups. Third, our training model was low-intensity resistance exercise combined with BFR, so it is uncertain if the results pertain to subjects who perform high-intensity resistance training protocols. Lastly, because we did not measure muscle biochemical adaptations, it is unknown whether there are differences in gene and protein expression between the LP and FP following the training. Additional research is needed to address these issues.

In conclusion, our results suggest that in eumenorrheic women, hypertrophic potential and increased isometric strength are higher in the LP than in the FP following 6 days of low-intensity BFR resistance training, although the sex difference in the training response is non-existent.

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