Additive beneficial effects of lactotripeptides and aerobic exercise on arterial compliance in postmenopausal women

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ARTERIAL COMPLIANCE PLAYS an important role in the functional abilities of the vasculature. Central arterial compliance plays an important role in the functional abilities of the vasculature. Two active tripeptides, valine-proline-proline and isoleucine-proline-proline, were isolated from sour milk and were referred to as lactotripeptides (LTP). Because LTP appears to act as an angiotensin-converting enzyme inhibitor, it is plausible to hypothesize that LTP improves arterial compliance. We determined the effects of LTP ingestion alone or in combination with regular aerobic exercise on arterial compliance. A total of 55 postmenopausal women (50–65 yr old) were randomly divided into four groups: placebo, LTP, exercise and placebo (Ex + placebo), or exercise and LTP (Ex + LTP). LTP or placebo was administered orally for 8 wk. The exercise groups completed an 8-wk moderate aerobic exercise intervention. There were no differences in baseline arterial compliance and most other key dependent variables among the groups. Carotid arterial compliance increased significantly in the LTP (0.93 ± 0.07 vs. 0.99 ± 0.08 mm²/mmHg·10⁻¹), Ex + placebo (0.92 ± 0.04 vs. 1.00 ± 0.05 mm²/mmHg·10⁻¹), and Ex + LTP groups (0.86 ± 0.06 vs. 1.00 ± 0.06 mm²/mmHg·10⁻¹), whereas no such changes were observed in the placebo control group (0.86 ± 0.06 vs. 0.85 ± 0.07 mm²/mmHg·10⁻¹). The magnitude of increases in carotid arterial compliance was significantly greater in the Ex + LTP group (19 ± 4%) than in other groups. The improvements in arterial compliance with LTP were associated with the corresponding reductions in arterial blood pressure and plasma angiotensin II concentrations. We concluded that LTP ingestion improves carotid arterial compliance and that the combination of LTP ingestion and regular exercise is additive and synergistic in improving arterial compliance in postmenopausal women.

In typical clinical settings, dietary modifications are not prescribed in isolation but rather with other lifestyle interventions (e.g., regular exercise). We and others have previously reported that regular aerobic exercise is a clinically efficacious therapy for preventing and treating arterial compliance (1, 24, 25). It is not known whether LTP intake exerts additive or synergistic effects with other lifestyle modifications, including regular exercise. Accordingly, the second aim of the present study was to test the hypothesis that combined LTP and regular aerobic exercise is more efficacious in increasing carotid arterial compliance than either treatment alone. To test these hypotheses, we used a placebo-controlled study involving apparently healthy postmenopausal women.

METHODS

Subjects

A total of 55 apparently healthy, sedentary postmenopausal women volunteered to participate. Subjects were randomly assigned to one of the following interventions: placebo group (n = 13), LTP group (n = 15), exercise and placebo group (Ex + placebo; n = 12), or exercise and LTP group (Ex + LTP; n = 15). Subjects were nonsmokers, nonobese, and free of CVD as assessed by medical history. None of the subjects was taking cardiovascular-acting medications, including hormone replacement therapy. All subjects gave their written informed consent to participate. All procedures were reviewed and approved by the Ethical Committee of the University of Tsukuba.
**Lactotripeptide, Exercise, and Arterial Compliance**

**LTP Ingestion**

Subjects in the LTP and Ex + LTP groups ingested 2.8 g of casein hydrolysate powder containing 2.4 mg of VPP and 4.3 mg of IPP per day, and this daily dose was divided into eight tablets. An equivalent dose of sodium caseinate, which is the starting material for casein hydrolysate, was used as a placebo. LTP or placebo was administered orally for 8 wk. The dosage of the LTP was chosen based on the previous reports that the ingestion of 100 ml of sour milk containing 1.5 mg of VPP and 1.1 mg of IPP per day for 8 wk decreased blood pressure (5) and that IPP and VPP may have a dose-dependent effect on blood pressure (15).

**Exercise Intervention**

Subjects in the Ex + placebo and Ex + LTP groups underwent aerobic exercise training 3–5 days/wk (2 supervised and additional home-based trainings) for 8 wk. Initially, subjects performed walking or cycling 25 to 30 min/day, 3 to 4 days/wk at a relatively low intensity of exercise (~60% of their individually determined maximal heart rate). As their exercise tolerance improved, the intensity and duration of aerobic exercise were increased to 40–45 min/day, 4 to 5 days/wk, at an intensity of 70–75% of maximal heart rate.

**Measurements**

Carotid arterial compliance, resting arterial blood pressure, blood biochemistry, and maximal oxygen consumption (\(V_{O2max}\)) were measured before and after 8 wk of each intervention. Age and height were measured only before interventions. Subjects were instructed not to alter their dietary habit (other than placebo or LTP ingestion) throughout the intervention period. Before they were tested, subjects abstained from caffeine and fasted for at least 12 h. Subjects were studied 24 h after their last exercise training session and/or the last LTP ingestion to avoid the immediate (acute) effects.

**Arterial blood pressure.** Blood pressure at rest was measured over brachial artery using a semiautomated device (Form PWV/ABI; Colin Medical Technology, Komaki, Japan) with subjects in the supine position.

**Artery compliance.** Subjects were studied under quiet resting conditions while they were in the supine position. The common carotid artery was imaged using B-mode ultrasound (En Visor; Koninklijke Philips Electronics, Eindhoven, The Netherlands) equipped with a high-resolution linear-array transducer (7.5 MHz). Diameters were measured from the intima of the far wall to the media-adventitia of the near wall. Pulsatile changes in the common carotid artery diameter were analyzed 1 to 2 cm proximal to the bifurcation. Carotid arterial pressure waveforms were obtained with arterial planation tonometry incorporating an array of 15 micropiezoresistive transducers (Form PWV/ABI; Colin Medical Technology) (2) and calibrated by equating the carotid mean arterial and diastolic blood pressure to the brachial mean arterial and diastolic blood pressure (obtained via brachial sphygmomanometry). This calibration procedure is based on the assumption that mean arterial pressure does not change in large conduit arteries and that the diastolic pressure (as opposed to the systolic pressure) was not substantially different among brachial, carotid arteries. To characterize the mechanical properties of the arterial wall as comprehensively as possible, four different measures, cross-sectional arterial compliance, \(\beta\)-stiffness index, arterial distensibility, and incremental elastic modulus, were calculated as previously described in detail (10, 19, 25). The \(\beta\)-stiffness index and incremental elastic modulus provide indexes of arterial compliance adjusted for distending pressure.

**Blood biochemistry.** A blood sample was collected from the antecubital vein after an overnight fast. Serum concentrations of cholesterol and triglycerides were determined by using the standard enzymatic techniques. Plasma angiotensin II concentrations were measured by enzyme-linked immunosorbent assay.

**Maximal oxygen consumption.** All subjects underwent an incremental cycle exercise test (after 2 min at 40 W, with 20 W increases every 2 min) until volitional exhaustion. Oxygen consumption was measured with the metabolic cart throughout the exercise test.

**Statistical Analyses**

To determine the effect of each intervention on all outcome measures, repeated-measures ANOVA was used. When indicated by a significant main effect or interaction, specific mean comparisons were performed to identify significant differences within each intervention. In the case of a significant F value, a post hoc test using the Newman-Keuls method identified significant differences among mean values. Differences in subject characteristics and percent changes in carotid arterial compliance across the four groups were determined by ANOVA. All data are reported as means ± SE. Statistical significance was set a priori at \(P < 0.05\) for all comparisons.

**RESULTS**

In both exercise groups (Ex + placebo and Ex + LTP), the average frequency and duration of the exercise training were similar and were 4.4 ± 0.2 days/wk and 45 ± 2 min/day, respectively. The compliance/adherence to the placebo/LTP ingestion was 100% as verified by the pill counts. There were no side effects (e.g., dry cough, drowsiness) of LTP reported by the subjects throughout the study period.

The characteristics of the subject groups are presented in Table 1. Before the intervention, there were no group differences in age, height, body mass, body mass index, total cholesterol, HDL cholesterol, triglycerides, plasma angiotensin II level, or \(V_{O2max}\). Body mass decreased significantly but mildly with the exercise intervention (both Ex + placebo and Ex + LTP groups). \(V_{O2max}\) in the exercise groups (Ex + placebo and Ex + LTP groups) increased after exercise interventions (\(P < 0.05\)). There were no significant changes in total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides with any of the interventions. Plasma angiotensin II levels decreased 23–35% with LTP ingestion, but the differences did not reach statistical significance.

There were no differences in baseline carotid arterial compliance among the groups (Fig. 1A). After 8 wk interventions, carotid arterial compliance increased in the LTP group (\(P < 0.05\), Ex + placebo group (\(P < 0.05\)), and Ex + LTP group (\(P < 0.01\)). There was no significant change in carotid arterial compliance in the placebo control group. The percent change of carotid arterial compliance was significantly greater in the Ex + LTP group than in other groups (Fig. 1B).

There were no differences in baseline \(\beta\)-stiffness index or arterial distensibility among the groups. After 8 wk interventions, the \(\beta\)-stiffness index decreased in the Ex + LTP group (7.2 ± 0.5 vs. 6.3 ± 0.3 units; \(P < 0.01\)). There was no significant change in the \(\beta\)-stiffness index in the other groups. Arterial distensibility increased in the Ex + placebo group (3.65 ± 0.13 vs. 3.91 ± 0.14 mm/mmHg·10⁻²; \(P < 0.05\)) and the Ex + LTP group (3.42 ± 0.20 vs. 4.03 ± 0.18 mm/mmHg·10⁻²; \(P < 0.01\)) after 8 wk interventions. There was no significant change in arterial distensibility in the placebo control or LTP groups. Incremental elastic modulus significantly decreased in the Ex + LTP group (34.6 ± 2.8 vs. 28.0 ± 1.5 mmHg⁻¹·10⁻⁴; \(P < 0.01\)) after 8 wk interventions. There was no significant change in incremental elastic modulus in the placebo control, LTP, or Ex + placebo groups.
Before the intervention, there were no differences in baseline blood pressure at rest among the groups (Fig. 2). Blood pressure did not change in the placebo control group. Systolic blood pressures decreased significantly in the LTP and Ex + LTP groups (LTP, \( P < 0.05 \); and Ex + LTP, \( P < 0.01 \)). Diastolic blood pressure decreased only in the Ex + LTP group (\( P < 0.01 \)).

DISCUSSION

The main findings of the present investigation were as follows. A regular ingestion of LTP significantly increased carotid arterial compliance in postmenopausal women. This was associated with the corresponding reductions in arterial blood pressure. When the ingestion of LTP was superimposed on the exercise training intervention, the effects on arterial compliance were additive and synergistic as the magnitude of improvement was significantly greater in the combined inter-

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**Table 1. Selected subject characteristics**

<table>
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<tr>
<th></th>
<th>Placebo</th>
<th>LTP</th>
<th>Ex + Placebo</th>
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<td>( n )</td>
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<td>15</td>
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<td>HDL cholesterol, mmol/l</td>
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<td>Carotid arterial SBP, mmHg</td>
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<td>103 ± 4</td>
<td>112 ± 6</td>
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<td>Carotid arterial DBP, mmHg</td>
<td>72 ± 3</td>
<td>70 ± 3</td>
<td>76 ± 3</td>
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<td>( \Delta ) V(\text{O}_2)max, ml·kg(^{-1})·min(^{-1})</td>
<td>28.6 ± 1.3</td>
<td>26.9 ± 1.3</td>
<td>28.5 ± 1.0</td>
<td>28.3 ± 1.3</td>
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</table>

Values are means ± SE; \( n \), number of subjects. LTP, lactotripeptide; Ex, exercise training; SBP, systolic blood pressure; DBP, diastolic blood pressure; \( \text{V}\(\text{O}_2\)max\), maximal oxygen consumption. *\( P < 0.05 \) and †\( P < 0.01 \) vs. before intervention. ‡\( P < 0.05 \) vs. placebo.

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**Fig. 1.** A: arterial compliance before and after intervention. B: percent changes in arterial compliance before and after intervention. *\( P < 0.05 \) and **\( P < 0.01 \) vs. before intervention. †\( P < 0.01 \) vs. placebo; ‡\( P < 0.05 \) vs. lactotripeptides (LTP); ‡\( P < 0.05 \) vs. exercise training (Ex) + placebo.

**Fig. 2.** Changes in arterial blood pressure at rest. Before intervention, values (in mmHg) are shown above each bar. *\( P < 0.05 \) and **\( P < 0.01 \) vs. before intervention. SBP, systolic blood pressure; DBP, diastolic blood pressure.
vention than either treatment alone. These results suggest that the regular LTP intake has isolated as well as additive effects on improving the elastic property of the central artery.

The origin of the fermented milk consumption in Japan can be traced back to about 100 years ago when it was observed that Mongolians who consumed fermented milk products enjoyed good health. Since then, fermented milk products have been found to exert a number of health benefits including antihypertensive effects (5). The sequences of the individual milk proteins displaying active properties have been isolated and collectively termed as LTP (15). These tripeptides escape from intestinal degradation and reach the circulation undegraded, thus being bioavailable to the vascular system (3). LTP is an easily available nutritional supplement, and a drink containing LTP has been a favorite drink for Japanese children. In the present study, we examined the effects of LTP on arterial compliance in postmenopausal women. Central artery compliance increased significantly after 8 wk of the LTP ingestion. To the best of our knowledge, the present study is the first to demonstrate the beneficial effects of LTP on arterial compliance in humans.

The exact mechanisms responsible for the LTP ingestion-induced improvement in carotid arterial compliance are not known but likely to be multiple. Animal studies have reported that LTP ingestion decreases angiotensin II production via the inhibition of ACE and that these effects are more pronounced in the aortic tissue (15). In the present study, plasma angiotensin II concentration decreased 23–35% with LTP ingestions, and this decrease in plasma angiotensin II was associated with increases in arterial compliance. Thus LTP may have improved arterial compliance through the inhibition of ACE and the resultant decreases in angiotensin II. It should, however, be emphasized that the plasma concentration of angiotensin II may not represent the tissue concentration of angiotensin II in the arterial wall and might be misleading. For example, a chronic treatment of ACE inhibitor resulted in decreases in blood pressure and arterial stiffness without changes in the plasma angiotensin II level (21), and the effects of LTP on ACE activity are more pronounced in the aortic tissue in animal studies (14, 15). Additionally, the inhibition of ACE is also known to decrease the degradation of the vasodilator bradykinin and the subsequent elevations in prostaglandins and nitric oxide (NO) (9). NO is an important factor for modulating arterial compliance as arterial distensibility decreases significantly during the intra-arterial infusion of NO synthase inhibitor (28). Taken together, LTP ingestion may improve arterial compliance via the inhibition of ACE activity and the subsequent elevations in prostaglandins and nitric oxide (NO) (9). NO is an important factor for modulating arterial compliance via the inhibition of ACE activity and the subsequent elevations in prostaglandins and nitric oxide (NO) (9). NO is an important factor for modulating arterial compliance in humans.

Current prevention/treatment guidelines for postmenopausal women emphasize lifestyle modifications such as regular exercise and dietary modifications. Regular aerobic exercise prevents and reverses the decrease in central arterial compliance that occurs with aging. We (12, 18, 24, 25) and others (1, 22) have reported that arterial compliance is enhanced by regular aerobic exercise although some conflicting evidence exists (26). In the present study, the 8 wk moderate exercise training in postmenopausal women increased arterial compliance. Perhaps the most novel finding of the present study is that a combination of LTP ingestion with regular aerobic exercise was more efficacious in improving arterial compliance than either treatment alone. These results indicate that the effects of LTP intake and regular exercise are additive and synergistic in nature in improving the elastic property of the artery. Importantly, this can be accomplished with a combination of lifestyle modifications that can be performed easily by most, if not all, postmenopausal women.

Additive beneficial effects on arterial compliance suggest that physiological mechanisms underlying the increase in arterial compliance may be distinct between regular exercise and LTP intake. Aerobic exercise training decreases plasma concentration of endothelin-1 (13), and endogenous endothelin-1 participates in the regular exercise-induced increase in central arterial compliance (1). Moreover, endurance training-induced improvements in arterial compliance may be mediated through reductions in α-adrenergic vasoconstrictor tone (23). To the best of our knowledge, there have been no reports in humans indicating that the activity of ACE decreases with regular exercise. Therefore, regular exercise and LTP ingestion do not appear to share the same physiological mechanisms to improve the elastic property of the artery, and this may be the reason as to why the effects of regular exercise and LTP ingestion appear to be additive and synergistic.

Most measures of arterial stiffness/compliance are somewhat dependent on arterial pressure. It is possible that the improvement of the arterial compliance with LTP ingestion may be mediated by the corresponding changes in arterial blood pressure. To address this, we have calculated the β-stiffness index and incremental elastic modulus, indexes of arterial compliance adjusted for distending pressure. The results indicate that the improvement in the arterial compliance after the combination of exercise and LTP ingestion remained statistically significant even when the data were expressed as the β-stiffness index and incremental elastic modulus. However, the change in β-stiffness index and incremental elastic modulus observed after the LTP or Ex + placebo groups did not reach statistical significance. Thus some of the treatment effects on arterial compliance we observed after a single treatment with LTP or exercise may have been due to the epiphenomenon of blood pressure changes.

There are several other limitations of this study that should be emphasized. First, the present study has a relatively small sample size due primarily to the highly rigorous inclusion/exclusion criteria employed to screen healthy postmenopausal women. However, the power calculation indicated that we had a sufficient number of subjects in the present study. Indeed, we were able to demonstrate the significant effects of interventions. Second, the present study would be a relatively short intervention period. However, the elastic property of the artery changes very rapidly to lifestyle-intervention stimuli as early as 2–4 wk, and the effects would reach nadir before the 8-wk period (4, 8). Indeed, the decrease in blood pressure induced at 8 wk after LTP treatment (5). Furthermore, arterial distensibility increased after aerobic exercise training of 8 wk (7).

Our findings have a number of potentially important clinical implications, especially for high-risk postmenopausal women. The loss of endogenous estrogen production is thought to induce a decrease in central arterial compliance by advancing deterioration of endothelial function, fibroblast and vascular smooth muscle cell proliferation, and collagen accumulation in the aortic wall, all of which could lead to the higher incidence of coronary heart disease in women after menopause (11). Lifestyle modification is the recommended first-line therapeu-
tic approach for postmenopausal women. Because LTP is a nutritional supplement in tablet form, it is ideally suited as a preventive measure for such elevated risk populations as postmenopausal women. Additionally, it can be combined with other lifestyle modifications (i.e., regular exercise) to produce greater benefits for arterial health. The results of the present study may create a new strategy for improving central arterial compliance in postmenopausal women at high risk of developing CVD.

In conclusion, the present study demonstrated that LTP ingestion alone increases arterial compliance in postmenopausal women and that combining LTP ingestion with regular aerobic exercise produces additive and synergistic effects that are greater than either treatment alone. As such, habitual aerobic exercise and LTP ingestion may be effective lifestyle modifications for minimizing and reversing the loss in carotid arterial compliance with advancing age in women.

GRANTS

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REFERENCES