Effects of Lactotripeptide Supplementation on Tele-Monitored Home Blood Pressure and on Vascular and Renal Function in Prehypertension
— Randomized, Double-Blind, Placebo-Controlled, Cross-Over Study —

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Background: This randomized, double-blind, placebo-controlled, cross-over study was conducted to examine the effects of lactotripeptide supplementation on 7-day mean tele-monitored home blood pressure (BP), and also on the markers of vascular function and renal damage in Japanese subjects with prehypertension.

Methods and Results: A total of 26 subjects with prehypertension were randomly allocated to receive the active product (lactotripeptide tablet) or a placebo tablet for 8 weeks each in a cross-over manner. Urinary liver-type fatty acid-binding protein-to-creatine ratio (UFABPCR) and vascular function were measured at the end of each intervention. Home systolic and diastolic BP at the end of the lactotripeptide supplementation period was significantly lower than that at the end of the placebo period (P<0.05). On mixed linear model analysis there was a significant difference in the change in home diastolic BP after intervention between the 2 interventions (P=0.04). UFABPCR was significantly lower at the end of the lactotripeptide intervention period than at the end of the placebo period (P<0.05).

Conclusions: The beneficial effect of lactotripeptide supplementation on 7-day mean tele-monitored home BP was confirmed in Japanese subjects with prehypertension. In addition, this intervention also seemed to have a protective effect against the progression of renal function decline.

Key Words: Home blood pressure; Lactotripeptide; Prehypertension; Tele-monitoring
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Methods

Subjects
A total of 30 apparently healthy subjects aged 36–57 years (17 men and 13 women) with prehypertension (office systolic blood pressure [SBP]≤140 mmHg, diastolic blood pressure [DBP]≤90 mmHg) who were not under antihypertensive treatment were enrolled in this study. Office BP was measured on 2 occasions, ≤1 month apart, twice on each occasion, and the mean of the 2 measurements on each occasion was defined as the office BP. Prehypertension was defined based on the office BP measured on 2 occasions before the start of the run-in period. Patients with the following were excluded from the study: history of CVD, malignant disease, other chronic diseases, including CVD risk factors other than high BP (e.g., dyslipidemia [serum low-density lipoprotein cholesterol, LDL-C ≥160 mg/dL] or diabetes mellitus [glycohemoglobin A1c, HbA1c ≥6.5%]), body mass index ≥30 kg/m², current smoking, habitual use of supplements for BP reduction, and/or allergy to milk, and premenopause status. Subject data used to assess inclusion eligibility are listed in Table 1. Informed consent was obtained from all of the participants prior to the measurements. This study was conducted with the approval of the Ethics Guidelines Committee of Tokyo Medical University granted on 2 April 2018 (No. 4,071). In addition, in compliance with the Clinical Trials Act enforced in 2017, this study was additionally approved by a specific clinical research review committee of the University of Ryukyu (CRB7180002). Then, the study protocol was uploaded to the Japan Registry of Clinical Trials (https://jRCTs071180030).

Experiment Protocol
The study was conducted as a double-blind randomized placebo-controlled cross-over study, from September 2018 to March 2019. The randomization was conducted based on subject gender (men/women) and age (age≥/<50 years). During the study period, the subjects were asked to maintain the same lifestyle as before they entered this study. The active product was a tablet (lactotripeptide tablets) made from casein hydrolysate containing valine-proline-proline (Val-Pro-Pro; VPP) and isoleucine-proline-proline (Ile-Pro-Pro; IPP), which are casein-derived peptides; the active product contained 1.4 mg of VPP and 2.0 mg of IPP. The placebo tablets were prepared using sodium caseinate instead of casein hydrolysate. After enrolment, the subjects were randomly allocated to 2 groups that initially received lactotripeptide supplementation or placebo (a group that received lactotripeptide supplementation as the initial intervention in the first period followed by placebo in the second period and a group that received placebo as the initial intervention in the first period followed by lactotripeptide supplementation in the second period). Both the interventions were carried out for a period of 8 weeks each. A 2-week run-in period and a 4-week wash-out period between the 2 interventions were set up. Home BP was measured on tele-monitoring from the run-in period to the end of the study period. Vascular function parameters and other cardiovascular risk markers were measured in the run-in period and at the end of each intervention (Figure 1).

Home BP Measurement
Home BP was measured using a tele-monitoring system that stored and transmitted data to a secure website (HEM-7251G, Omron Healthcare, Kyoto, Japan), to avoid

| Table 1. Subject Data for Inclusion Eligibility |
|-----------------------------------------------|
| Variables | Mean±SD or n (%) |
| Age (years) | 49±6 |
| Female | 9 (3) |
| BMI (kg/m²) | 23±3 |
| Office SBP (mmHg) | 126±6 |
| Office DBP (mmHg) | 84±4 |
| Serum LDL-C (mg/dL) | 115±26 |
| HbA1c (%) | 5.5±0.3 |

BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycohemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Figure 1. Study design.
reporting bias. In the morning, home BP was measured twice at an interval of 90 s under the same conditions (i.e., ≤1 h after the subject had woken up and passed urine, before he/she had taken breakfast and/or the active/placebo product) with the patient in the seated position. The measuring device recorded the BP and pulse rate wirelessly and transmitted the data to a central Web server (located at Tokyo Medical University) via the Internet. We were thus able to check the subject data in real time. Subjects with a ≥7-day mean home BP >150/95 mmHg were requested to withdraw from this study and to take anti-hypertensive medication. Mean home BP was calculated as the mean of the BP measured on 7 days just before the start and at the end of each intervention period. Office BP was also measured in the right upper arm using the oscillometric method, with the patients in the seated position (HEM-907; Omron Healthcare).

Vascular Function Tests
Vascular function parameters were measured after the participants had rested for ≥15 min in the supine position in an air-conditioned room (24–26°C). Flow-mediated vasodilatation (FMD) and the reactive hyperemia index (RHI) were measured first, followed by measurement of the brachial-ankle pulse wave velocity (brachial-ankle PWV) and the radial augmentation index (rAI).

Radial AI
Blood pressure and rAI were measured after the subjects had rested for ≥5 min in the seated position. BP was measured in the right upper arm using the oscillometric method (SBP(pos)/DBP(pos); HEM-907; Omron Healthcare). Immediately after this measurement, the left radial arterial waveform was recorded using an arterial applanation tonometry probe equipped with an array of 40 micropiezoresistive transducers (HEM-9010AI; Omron Healthcare). Subsequently, the first and second peaks of the radial pressure waveform (SBP1 and SBP2) were automatically detected. rAI was calculated as follows: (SBP2−DBP(pos))/ (SBP1−DBP(pos))×100 (%).  

Brachial-Ankle PWV
Brachial-ankle PWV was measured once using a volume-plethysmograph (Form/ABI, Omron Healthcare), as previously described.  

FMD
The method of measurement of FMD is described elsewhere in detail. The subjects were instructed to fast overnight and to abstain from alcohol, smoking, caffeine and anti-oxidant vitamins for ≥12 h prior to the measurements. Just prior to the measurements, they were asked to rest in the sitting position in a quiet, dark, air-conditioned room (22–25°C) for 5 min, and then again in the supine position for ≥15 min. BP was measured using the oscillometric method (UA 767; A&D, Saitama, Japan).

Then, using a 10-MHz linear array transducer for high-resolution ultrasound, longitudinal images of the right brachial artery were recorded at baseline and then continuously from 30 s prior to ≥2 min after cuff deflation following supra-systolic compression (to 50 mmHg over SBP) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring brachial artery diameter (Unex, Nagoya, Japan). The changes in diastolic diameter were continuously recorded. Then, FMD was estimated as the percent change of the diameter of the brachial artery at maximum dilatation during reactive hyperemia as compared with baseline. The reproducibility of the measurement of FMD at the present institute is described elsewhere.

RHI
The methodology for measurement of RHI is described elsewhere. During the measurement of FMD, before the forearm cuff occlusion, a peripheral arterial tonometry device (Endo-PAT2000; Itamar Medical, Caesarea, Israel) was placed on the index finger of each hand. Then, the pulse amplitude detected by this device was electronically recorded throughout the period of measurement of FMD. The extent of reactive hyperemia was calculated as the ratio of the average pulse amplitude of the device signal over a 1-min time interval starting 1.5 min after cuff deflation to the average pulse amplitude of the device signal over a 2.5-min time period before cuff inflation. RHI was calculated as the ratio of the reactive hyperemia between the 2 hands.

Laboratory Measurements
As markers of glucose metabolism, fasting blood glucose (mg/dL), serum insulin (μU/mL), and homeostatic model assessment of insulin resistance (HOMA-IR) were measured. In addition, serum LDL-C, Hba1c, CRP, creatinine, and sodium were also measured. In urine samples, liver-type fatty acid-binding protein (L-FABP) was measured on enzyme-linked immunosorbent assay (ELISA) using the L-FABP ELISA KIT. Then, the urinary L-FABP-to-creatinine ratio (UFABPCR) was calculated.

Statistical Analysis
Home SBP/DBP was defined as the primary outcome. Data were insufficient to calculate the sample size in Japanese subjects. Therefore, the sample size was determined based on the results of previous studies that examined the effect of lactotripeptide supplementation on FMD (mean no. subjects, 10–15 in each arm) and studies that examined its effect on BP assessed on 24-h BP monitoring on a single day (mean no. subjects, 30). Based on this, the number of subjects needed for this study was determined to be 30.

The baseline characteristics and the pre- and post-intervention data are expressed as mean ± SD. Mean home SBP/DBP and home heart rate (home HR) were calculated as the mean of the measurements conducted on 7 days just before the start and at the end of each intervention period. Then, the differences in the variables between the placebo and active product interventions were assessed on paired t-test. In addition, the treatment effects on home SBP/DBP/HR were calculated as the change in the parameters from the beginning to the end of each intervention period. Random subject-effect models of restricted maximum likelihood analyses for linear mixed models were applied to evaluate the treatment effects. The intervention and the initial
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Result

Figure 2 shows a flow-chart of subject enrolment. A total of 52 subjects were invited to enroll in the study, and 30 subjects were initially entered into the study. Of the 30 subjects, one had a 7-day mean home SBP/DBP >150/95 mmHg, and was requested to withdraw from the study and start anti-hypertensive medication. Then, an attempt was made to enroll one of 2 substitutes into the study, but the BP data measured during the run-in period could not be obtained for this patient, and therefore, this subject was not included in the analyses. In addition, another subject had an episode of urinary colic and was receiving medication for urolithiasis, one subject had influenza, and one subject was taking a phenylalanine supplement, which potentially affects BP. Then, after the aforementioned subjects were excluded from the analyses, the data of a total of 26 subjects were included in the present analyses (Figure 2). Of the 26 subjects, 13 initially received the lactotripeptide intervention, while the remaining 13 initially received the placebo intervention.

Table 2 lists the BP variables at each intervention (lactotripeptide supplementation/placebo). While the home SBP/
The results were inconsistent.

Studies examined the BP-lowering effect of interventions on 24-h ambulatory BP monitoring on a single day, but most studies used measurement of office BP, and therefore, did not account for the white-coat phenomenon. Although some studies examined the BP-lowering effect of interventions on 24-h ambulatory BP monitoring on a single day, the results were inconsistent. Presumably because of day-to-day BP variability, previous studies that used 24-h ambulatory BP monitoring and 1-day home BP monitoring failed to confirm a significant BP-lowering effect of lactotripeptides, and, although the white coat phenomenon may have been excluded in these studies, the day-to-day BP variability was not. In addition, most of these studies were conducted in subjects with hypertension, and few data on the effects of lactotripeptide intervention in individuals with prehypertension are available. The strengths of the present study, conducted in subjects with prehypertension, are as follows: (1) the study was a randomized, double-blind, placebo-controlled, cross-over study; and (2) the BP-lowering effect of lactotripeptide supplementation was assessed on 7-day mean tele-monitored home BP. Home BP monitoring by this method, involving BP measurement on multiple days, would be expected to be less influenced by the white coat phenomenon and day-to-day BP variability; furthermore, tele-monitoring may provide more reliable BP data than self-monitoring. Unlike 24-h ambulatory BP monitoring, diurnal variations in BP cannot be assessed on home BP monitoring. Nonetheless, the present study, with the aforementioned strengths, has confirmed the BP-lowering effect of lactotripeptide supplementation in subjects with prehypertension.

Vascular dysfunction, such as increased arterial stiffness and endothelial dysfunction, and renal dysfunction are well-known to be associated with an elevated risk of hypertension. Although the precise mechanisms underlying the BP-lowering effect of lactotripeptide supplementation have not yet been clarified in detail, a plausible mechanism is thought to be the effect of lactotripeptides in inhibiting the activity of angiotensin-converting enzyme (ACE), which results in inhibition of conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and also an increase in the release of vasodilatory peptides such as bradykinins. These actions provide beneficial effects on the vasculature and kidney function, and some previous studies have demonstrated that lactotripeptide supplementation improves the arterial stiffness and endothelial function, In subjects with prehypertension, however, the extent of deterioration of the arterial stiffness and endothelial

### Table 3. Vascular and Renal Function Variables at Intervention End

| Variables | Lactotripeptides | Placebo | P-value |
|-----------|-----------------|---------|---------|
| BAidia (mm) | 4.0±0.1 | 4.0±0.1 | 0.54 |
| FMD (%) | 5.0±0.5 | 4.8±0.5 | 0.62 |
| RHI | 1.75±0.41 | 1.75±0.39 | 0.97 |
| Brachial-ankle PWV (cm/s) | 1.27±1.49 | 1.25±1.36 | 0.36 |
| rAI (%) | 71±16 | 70±18 | 0.59 |
| Serum Cr (mg/dL) | 0.75±0.12 | 0.77±0.14 | 0.18 |
| UFABPCR (µg/g Cr) | 1.85±1.73 | 2.37±2.12 | 0.02 |
| UACR (mg/g Cr) | 6.8±7.9 | 6.9±6.5 | 0.97 |
| FENa (%) | 0.7±0.4 | 0.6±0.4 | 0.27 |
| FBG (mg/dL) | 98±7 | 100±6 | 0.09 |
| Serum insulin (µU/mL) | 5.1±3.2 | 5.3±5.2 | 0.75 |
| HOMA-IR | 1.23±0.77 | 1.31±1.30 | 0.66 |
| Serum CRP (mg/L) | 0.14±0.30 | 0.25±1.05 | 0.62 |

BAidia, diameter of the brachial artery before hyperemia; Cr, creatinine; CRP, C-reactive protein; FBG, fasting blood glucose; FENa, urinary fractional sodium excretion; FMD, flow-mediated dilatation of the brachial artery; HOMA-IR, homeostatic model assessment of insulin resistance; PWV, pulse wave velocity; rAI, radial augmentation index; RHI, reactive hyperemia index; UACR, urinary albumin-to-creatinine ratio; UFABPCR, urinary liver-type fatty acid-binding protein-to-creatinine ratio.
function might be mild, and in the present study, the lactotripeptide intervention had no effect on either of these parameters. In the present study, the extent of renal function decline was examined at the end of the interventions, whereas the change in the degree of renal function decline resulting from the interventions was not examined. Therefore, the effect of lactotripeptide supplementation on renal damage could not be clearly evaluated in the present study; UFABPCR, however, was lower after the lactotripeptide intervention than after the placebo intervention. To the best of our knowledge, no study until now has examined the effect of lactotripeptide supplementation on renal damage. UFABPCR is a marker not only of acute kidney injury, but also of chronic kidney disease. Therefore, the present study suggests that lactotripeptide supplementation serves to protect against the progression of renal function decline.

Study Limitations

There were several limitations of the present study, as follows. First, the dose relationship of the BP-lowering effect of lactotripeptides was not examined in this study. Second, the subjects consisted exclusively of Japanese subjects and, of the women, only postmenopausal women were included, and there were no subjects of other ethnicities or premenopausal women. Third, while abnormal BP variability is also a known risk factor for the development of CVD, the effect of lactotripeptide supplementation on abnormal BP variability was not examined in this study. Fourth, in the present study, significant reduction in BP by the lactotripeptide intervention was not confirmed. The present study was conducted from September 2018 to March 2019; in this context, seasonal variations in home BP (home BP higher in the winter than in other seasons) have also been demonstrated. This could be a plausible explanation for the absence of significant BP reduction after the lactotripeptide intervention in the present study. Fifth, unlike in the case of home BP, the change of which following the intervention was assessed, the vascular and renal functions were not compared before and after the intervention in this study. Sixth, while salt intake affects BP, the present study did not control daily salt intake. And seventh, the number of subjects was relatively small.

Clinical Perspectives

In subjects with prehypertension, lifestyle modifications and other BP-lowering modalities are recommended for preventing CVD, and prevention of the development of hypertension from prehypertension is the first step. Nutritional treatment may represent an alternative BP-lowering modality. In addition to the known effect of lactotripeptides in lowering BP via the inhibition of ACE, the present study also noted a possible protective effect of lactotripeptides against renal damage, which is a known risk factor for the development of hypertension. The next step is to clarify whether lactotripeptide supplementation would also be effective for preventing the development of hypertension in subjects with prehypertension.

Conclusions

The beneficial effect of lactotripeptide supplementation on 7-day mean tele-monitored home BP was confirmed in Japanese subjects with prehypertension. In addition, this intervention also seems to exert a protective effect against renal damage.

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Disclosures

The authors declare no conflicts of interest.

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