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ORIGINAL ARTICLE

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# Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing work in humans

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**Abstract** This double-blind, placebo-controlled, crossover study investigated the effect of blackcurrant anthocyanin (BCA) intake on peripheral circulation during rest and during typing work by using near-infrared spectroscopy (NIRS), and it also assessed improvement in shoulder stiffness caused by poor local circulation. In a resting circulation study, nine healthy male subjects took capsules of BCA at a dosage of 17 mg $kg^{-1}$ or placebo (isoenergetic sugar). NIRS was used to measure left forearm blood flow (FBF) following venous occlusion and muscle oxygen consumption following arterial occlusion prior to and hourly for 4 h after ingestion of BCA. Plasma anthocyanin concentration was measured prior to ingestion and 1, 2, and 4 h later. FBF increased significantly 2 h after BCA ingestion [BCA 1.22 (0.13)-fold increase relative to pre-values vs placebo 0.83 (0.06) of pre-values; $P < 0.05$] and then tended to increase for a further 3 h after ingestion [BCA 1.26 (0.15)-fold increase relative to pre-values vs placebo 0.82 (0.07) of pre-values; $P = 0.078$]. There was, however, no significant difference in muscle oxygen consumption between BCA and placebo intake at any time point. In a typing work study, 11 healthy subjects took capsules of BCA (7.7 mg $kg^{-1}$) or placebo (isoenergetic sugar) daily for 2 weeks. The subjects then performed intermittent typing workload for 30 min in order to induce acute shoulder stiffness. During the workload, total hemoglobin and oxygenated hemoglobin (oxy-Hb) were determined using NIRS and myoelectric signals measured in the right trapezius muscle using electromyography (EMG). The viscoelasticity of the trapezius muscle was also evaluated using a muscle stiffness meter before and after the typing workload. BCA intake prevented the decrease in oxy-Hb significantly ($P < 0.05$), and also tended to alleviate the increase in root mean square (RMS) of the EMG during the typing workload, and also muscle stiffness after the workload. There was no improvement in typing performance with BCA intake. The results of this study suggest that intake of BCA may improve shoulder stiffness caused by typing work by increasing peripheral blood flow and reducing muscle fatigue.

**Keywords** Blackcurrant · Anthocyanin · Blood circulation · Near-infrared spectroscopy · Electromyography

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## Introduction

The peripheral circulation plays a pivotal role in maintaining blood flow necessary for both organ function and exchange of substances between blood and tissue, thereby maintaining the integrity of the inner environment. Insufficiency in the peripheral circulation is thought to be an important factor in the pathogenesis of various diseases. For example, localized circulatory insufficiency is known to contribute to the cervico-omobrachial syndrome, a disorder that is increasing in prevalence as a result of changes in labor conditions following the widespread introduction of computers (Matsumoto 1994).

Dietary anthocyanins have attracted considerable attention as a consequence of their beneficial health effects that include a reduction in the risk of coronary heart disease and prevention of several chronic diseases (Renaud and de Logeril 1992; Morazzoni and Bombardelli 1996). Recently, we reported that following oral intake of blackcurrant anthocyanins (BCA), four

anthocyanin components in the compound were absorbed into the blood stream (Matsumoto et al. 2001a). We also demonstrated that BCA prepared from blackcurrant fruits improved dark adaptation and visual display terminal (VDT) work-induced transient refractive alternations in humans (Nakaishi et al. 2000). In addition, this earlier study showed oral intake of BCA reduced subjective symptoms of fatigue of the head, neck, arm, eye, shoulder, and lower back during 2 h of mild VDT work. Similar findings were reported by Edwards et al. (2000) who used a general health questionnaire to show that intake of anthocyanosides resulted in a significant treatment effect on the severity of fatigue and sleep disturbance in the management of primary fibromyalgia. These findings suggest that some of the problems associated with fibromyalgia may derive from poor peripheral circulation.

Although there are no studies in humans, investigations in animals have shown that BCA improves blood circulation as a result of the combined effects of decreased platelet coagulability, and improved blood filterability and capillary penetration (Millet et al. 1984). We have also confirmed that BCA has a relaxing effect on the rat thoracic aorta (Nakamura et al. 2002). Taken together, these results indicate that BCA improves the peripheral circulation by increasing blood fluidity resulting from enhanced blood filterability and/or vasodilatation-induced increases in blood flow.

Tissue oxygenation dynamics are evaluated noninvasively by near-infrared spectroscopy (NIRS), which uses near infrared light to penetrate deep into biological tissue and measure the different absorption spectra between oxygenated and deoxygenated hemoglobin. Since the first report by Jobsis et al. (1977), the application of NIRS in human studies has developed rapidly and is now used extensively in research on skeletal muscles (Chance et al. 1990; Ferrari et al. 1997). Muscle oxygen consumption and muscle blood flow can be evaluated by NIRS using short periods of arterial and venous occlusion, respectively. The evaluation of muscle blood flow by the venous occlusion method correlates strongly with blood flow measured by plethysmography (De Blasi et al. 1994), and appears to provide an adequate technique for measuring in vivo flow (Homma et al. 1996). Recently, the relationship between stiff shoulder and circulation dynamics determined by NIRS in muscle tissue has been reported (Okubo et al. 2000). However, the effect of BCA on the peripheral circulation assessed by NIRS has not been investigated.

We hypothesized that BCA intake may enhance peripheral blood circulation with or without workload, and that BCA intake may be effective for preventing mild local hypoxia and disturbances in oxidative metabolism in muscles induced by exercise. In this study, we used NIRS to investigate the effect of BCA intake on peripheral circulation, a technique that measures peripheral circulation dynamics in a simple and noninvasive manner. As there is evidence that stiff shoulder induced experimentally is caused by local circulation insufficiency (Takenami et al. 2003), we also investigated the effect of BCA intake on local circulation insufficiency linked to stiff shoulder.

## Methods

### BCA concentrate

BCA concentrate was prepared from a commercially available blackcurrant juice using the method developed in a previous study (Matsumoto et al. 2001b). The concentrate contained 10.83% anthocyanins, consisting of 5.09% delphinidin 3-rutinoside (D3R), 1.48% delphinidin 3-glucoside (D3G), 3.76% cyanidin 3-rutinoside (C3R), and 0.50% cyanidin 3-glucoside (C3G). In this study, capsules of BCA were prepared and provided in a cross-over and double-blinded manner, with placebo capsules containing isoenergetic sucrose.

### Subjects

Nine healthy, male subjects [age 29.9 (1.1) years] and 11 healthy subjects [eight males and three females, age 39.0 (11.6) years] participated in the resting circulation study (study 1) and the typing work study (study 2), respectively. The subjects had been engaged in office work for at least 3 years, and although having worked on VDTs for approximately 3–6 h each day, this work was not similar to that of a computer programmer. None of the subjects had a serious concomitant disorder. The two studies were performed according to the Helsinki Declaration and were approved by the local ethical committee. The possible risks of the experiments were explained to all subjects with signed, informed consent being obtained prior to entry in the study. The two studies were double-blind, placebo-controlled, cross-over design, with a 2-week wash-out period.

The study consisted of two experiments: The measurement of peripheral circulation dynamics either at rest (study 1) or during typing work (study 2).

### Experimental design for study 1

On the day before the experiment, the subjects were instructed not to consume caffeine, nicotine or any foods rich in anthocyanin, such as vegetables, fruit, or juices, and during the 12-h prior to the experiment, not to ingest any food or beverages, with the exception of water. The experiment was started in the morning in a quiet room with the temperature controlled between 22 and 24°C. The subjects rested in the supine position for at least 10 min, and were then provided with capsules of either BCA concentrate [17 mg (kg body weight)$^{-1}$] or isoenergetic sugar [1.22 mg (kg body weight)$^{-1}$] to be taken orally with water (150 ml). Forearm blood flow (FBF) in the left arm was measured by NIRS before

ingestion of BCA, and then at hourly intervals for 4 h. Blood samples were collected from the right arm prior to ingestion and at 1, 2, and 4 h after ingestion.

## Experimental design for study 2

The subjects consumed BCA concentrate capsules [7.7 mg (kg body weight)$^{-1}$] or isoenergetic sugar for 2 weeks. This dose was the same as that used in our earlier study (Nakaishi et al. 2000), which demonstrated an improvement in fatigue after 2 h of a mild visual display task measured by a visual analog scale. During this 2-week period, the subjects maintained their usual diet, but were asked to avoid foods rich in anthocyanins, such as vegetables, fruits, and juices. The subjects were also requested not to consume caffeine or nicotine 12 h before the start of the measurements, and on the day of the experiment ate nothing except for a controlled meal consisting of energy 327.6 kcal, carbohydrate 70.8 g, protein 6.2 g, and fat 1.2 g. After the subjects had finished the meal, they ingested the BCA capsules, with measurements being carried out 2 h later. The subjects rested in a sitting upright position for at least 30 min in a temperature-controlled room at 22–24°C, followed by two maximum voluntary contractions (MVCs) performed by pulling the scapula in an upwards direction. This maneuver was achieved by raising both shoulders to maximal strength whilst grasping grips located on the side of the chair. There was a 5-min recovery period between each MVC. The subjects were then exposed to the typing workload in order to induce acute stiffness in the shoulder and neck. The subjects typed using only their fingers, with the wrists and elbows positioned above the keyboard with a shoulder flexion angle of 90°. The speed of typing was similar to that used routinely with typing software (Type-S, S.YAM.net, Tokyo, Japan). The typing workload consisted of six sets each involving 5 min typing followed by a 1-min rest, resulting in a total of 30 min workload. Three minutes after the last set, the subjects carried out three MVCs. The number of words typed each minute and the percentage of correct typing were calculated in order to allow comparison of typing performance between BCA and placebo.

## Analysis of anthocyanins in plasma samples

In study 1, venous blood samples were collected before and 1, 2, and 4 h after ingestion, followed by preparation of plasma samples from the whole blood using a method described in a previous report (Matsumoto et al. 2001a). This involved plasma being harvested immediately by centrifugation at 1,600 *g* for 15 min at 4°C. The plasma was then acidified with a one-fortieth volume of 6 M HCl, and a 4 ml portion of this mixture was loaded onto a Sep-Pak $C_{18}$ cartridge (Waters, Milford, Mass.). Prior to use, this cartridge was washed with 10 ml of methanol containing 5% trifluoroacetic acid (TFA) followed by equilibration with 10 ml of 5% TFA. After washing with 10 ml of 5% TFA, the anthocyanins were eluted with 5 ml methanol containing 5% TFA, and the eluate was carefully evaporated to dryness in vacuo below 35°C. The dried residue was then re-dissolved in 200 µl of 3% phosphoric acid, and 100 µl of this solution was analyzed for anthocyanin composition and quantification by HPLC using an HP 1100 Series HPLC system equipped with a Zorbax SB C-18 column (4.6 mm×250 mm, particle size 5 µm) and a photodiode array detector at 520 nm, using the conditions described in a previous report (Matsumoto et al. 2001a). Under these conditions, four anthocyanins were detected, with the peaks on the chromatogram being identified by measuring photodiode-array UV spectra from 200 to 600 nm.

## NIR spectrometer

The NIR spectrometer used in this study (NIRS, OMRON, HEO-200, Tokyo, Japan) measures oxygenated hemoglobin (oxy-Hb), deoxygenated-Hb and total Hb (t-Hb) every 0.5 s. However, this technique provides relative values only, and therefore physiological calibration was required in order to compare measurements between subjects. In the present study, the changes in FBF, or muscle oxygen consumption, were compared in a series of measurements using venous and arterial occlusion methods. In study 1, the separate type probes, comprising a light source and an optical detector, were attached 10 cm below the olecranon along the palmaris longus muscle of the left forearm, with the distance between the light source and the detector fixed at an optically stable position of 3 cm (Hampson et al. 1988). Changes in FBF were measured by venous occlusion (De Blasi et al. 1994), and changes in skeletal muscle oxygen consumption ($\dot{V}O_2$) were measured by brief arterial occlusion (Hamaoka et al. 1996), with both methods being based on experience and findings from previous studies. Venous occlusion was achieved by placing a pneumatic cuff around the upper arm and inflating to 60 mmHg. After placement of the NIRS on the forearm, the subjects rested for 15 min. At the beginning of the study, a pediatric pneumatic cuff was placed around the wrist and inflated to 250 mmHg pressure in order to exclude hand blood flow from the measurements. Thirty seconds after wrist occlusion, the arm cuff was inflated and venous occlusion was maintained for 50 s and then released. This maneuver was repeated five times consecutively with a 30-s interval between each measurement. FBF was calculated from NIRS data by evaluating the rate of increase in t-Hb after venous occlusion. Arterial occlusion was used to interrupt arterial blood flow by placing a pneumatic tourniquet on the upper arm at a pressure of 250 mmHg. After the subjects had rested, arterial occlusion was maintained for 1 min, followed by a 2-min recovery of oxy-Hb until stabilization. The rate of

decline in oxy-Hb with a stable t-Hb is a reflection of $\dot{V}O_2$ during arterial occlusion, with values being expressed relative to that of the resting value.

In study 2, the probes were attached 5 cm from the acromial end of the trapezius muscle of the right shoulder, with the distance between the light source and the detector being 3 cm. As calibration of the arterial occlusion method was impossible due to the measurement site, changes in t-Hb and oxy-Hb in the right shoulder trapezius muscle were evaluated by a method reported previously (Takenami et al. 2003). This involved changes in t-Hb and oxy-Hb being measured as a standard at the time whilst the subjects, in a sitting position, were requested to grasp grips attached to a chair and then perform MVC of the trapezius muscle by raising their shoulders to maximal strength. For oxy-Hb and t-Hb standardization, the baseline at rest was set at 0%, while the maximum point obtained during reactive hyperemia after MVC prior to typing was defined as 100%. The relative values were then calculated.

### Electromyography

In study 2, myoelectric signals were measured using EMG according to the method described in a previous report (Yoshitake et al. 2001). EMG was recorded bipolarly using a pair of surface vital electrodes of 1 cm diameter, with an inter-electrode distance of 3 cm. The electrodes were placed along the direction of the fibers of the right upper trapezius muscle, and myoelectric signals were recorded continuously at a sampling rate of 2,000 $s^{-1}$ by a PowerLab system (ADI Co. Ltd., Tokyo, Japan). The signal was then converted by an AD converter (UPS-800 Unique Medical Co. Ltd., Tokyo, Japan) and filtered at a band-pass of 100–3,000 Hz. The root mean square (RMS) value represented the square root of the average power of the myoelectric signal for a given interval. This RMS value was affected by recruitment of the motor unit, and the firing rate and electric properties of the muscle fibers, similar to that of the average rectified amplitude value of the EMG. We observed a relatively large variability in RMS values between individuals and, therefore, normalization of the RMS value was performed in order to minimize the disturbances during the measurements and also to allow comparison between individuals. This was achieved by dividing the RMS values by the RMS values measured during MVCs undertaken prior to the typing workload.

### Viscoelasticity of the shoulder muscle

The viscoelasticity of the trapezius muscle was evaluated using a muscle stiffness meter (muscle meter PEK-1, Imoto Machinery Co. Ltd, Tokyo, Japan) before and after the typing workload. This measurement was carried out five times, with the data expressed as the mean of arbitrary units of viscoelasticity recorded at the top of the right trapezius muscle (Yano et al. 1998).

### Visual analog scale

A questionnaire was used for the assessment of subjective pain in the shoulder, neck, forearm, and upper arm, with the subjects being instructed to respond by placing a mark on a visual analog scale (VAS) showing the magnitude of the pain (Kirshner and Guyatt 1985). The VAS was recorded at five time intervals, i.e. before and after the typing workload, immediately after the last measurement, and 1 and 2 h after completion of the experiment.

### Blood pressure and heart rate

In study 1, systolic blood pressure and heart rate were measured in the right arm during the experiment using a plethysmograph (EC-5R Hokanson Inc., USA). In study 2, mean blood pressure and heart rate were measured in the left arm using a non-invasive automated sphygmomanometer (TM-2421, A&D Co. Ltd., Tokyo, Japan).

### Statistics

Statistical analyses were carried out using SAS version 8.0, with the data expressed as means (SE). The changes following BCA or placebo intake were analyzed by two-way ANOVA with Dunnet's post-hoc test, while changes in viscoelasticity of the shoulder muscle between pre- and post-intake were compared using Student's paired $t$-test. Statistical significance was set at $P < 0.05$.

## Results

### Study 1

#### *Anthocyanin content in plasma*

The plasma concentration of the four BCA components D3R, C3R, D3G and C3G after ingestion were determined by HPLC, and the results are summarized in Fig. 1. None of the anthocyanins were detected in the plasma before ingestion. The level of total anthocyanins in the plasma reached a maximum 1 h after intake of the BCA concentrate, and then decreased gradually to about half of the maximum level by 4 h. This pattern of changes was similar to those observed in our earlier study (Matsumoto et al. 2001a).

#### *NIRS indicators*

The changes in FBF were expressed as a ratio of the pre-ingestion values (Fig. 2A). When the differences in FBF were compared at each time point, BCA intake was found to increase FBF significantly 2 h after ingestion [BCA 1.22 (0.13)-fold pre-values vs placebo 0.83

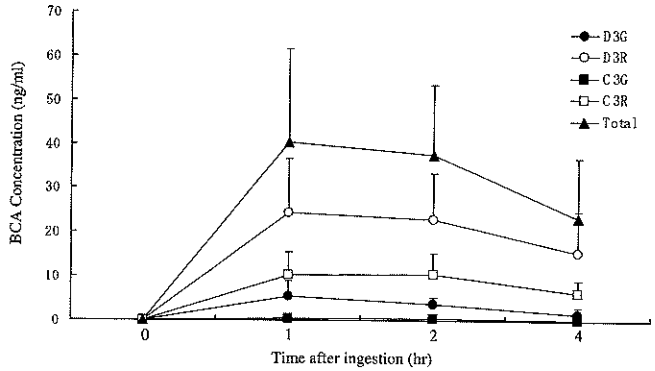


**Fig. 1** Time course of changes in the concentration of blackcurrant anthocyanin (BCA) components

(0.06)-fold pre-values; $P < 0.05$] with this enhanced flow tending to increase further by 3 h [BCA 1.26 (0.15)-fold pre-values vs placebo 0.82 (0.07)-fold pre-values; $P = 0.086$]. There was no significant difference in skeletal muscle oxygen consumption between BCA and placebo intake (Fig. 2B).

## Study 2

### *NIRS indicators*

The changes in maximum t-Hb during reactive hyperemia induced by MVC after a typing workload are shown in Fig. 3. After BCA intake, the maximum t-Hb

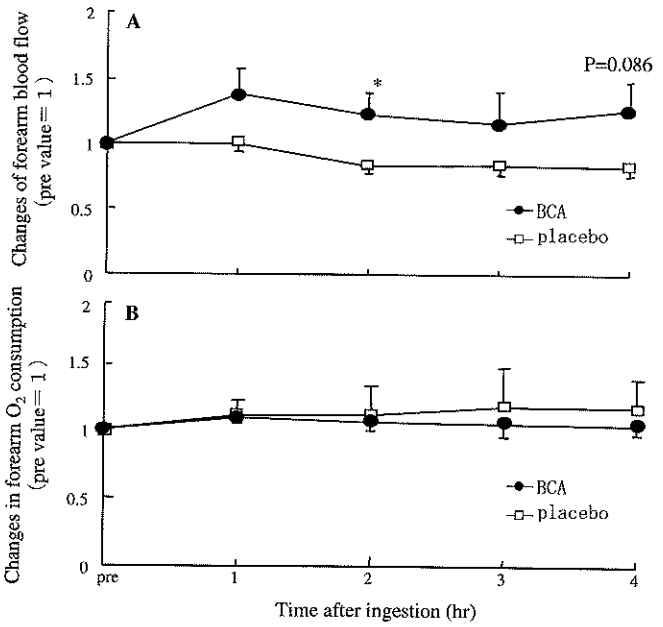


**Fig. 2** A comparison of changes in forearm blood flow (**A**) and oxygen consumption (**B**) measured by near infra-red spectroscopy between the BCA (•) and placebo (□) intake groups. The values shown are relative to the pre-intake measurements. The *P* values represent the comparison between BCA and placebo, $*P < 0.05$

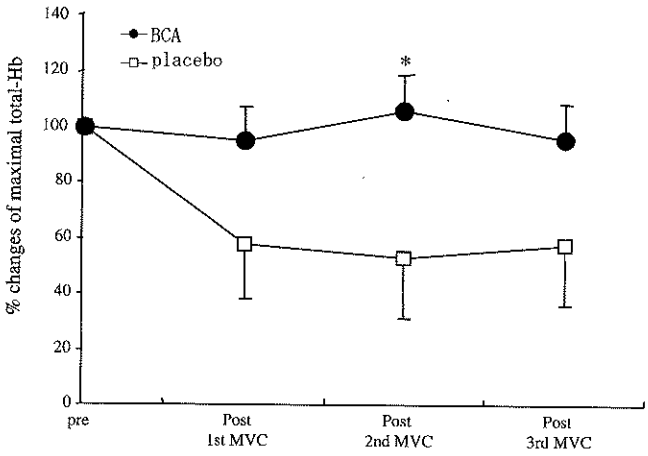


**Fig. 3** A comparison of changes in maximal total hemoglobin (*total-Hb*) after maximum voluntary contractions (*MVC*) after typing workload between the BCA (•) and placebo intake groups (□) measured by NIRS. The *P* values represent the comparison between BCA and placebo, $*P < 0.05$

after typing remained unchanged [95.0 (12.1)% of the pre-value at the first MVC after the workload, 106.0 (12.8)% at the second MVC, and 95.6 (13.2)% at the third MVC]. In contrast, following placebo intake, maximum t-Hb after typing decreased to 57.9 (12.0)%, 53.2 (21.6)% ($P < 0.05$), and 57.6 (21.7)% of the pre-value, at the first, second, and third sets, respectively. The changes in oxy-Hb during the typing workload are summarized in Fig. 4, which shows that oxy-Hb levels were significantly higher in the BCA intake group compared to the placebo intake group, at the first, second, third, and fourth workload sets ($P < 0.05$).

### *Electromyography*

The percentage changes in the RMS of the EMG signal during the typing workload are shown in Fig. 5. The RMS values tended to increase gradually during typing workload with both BCA and placebo intake. During

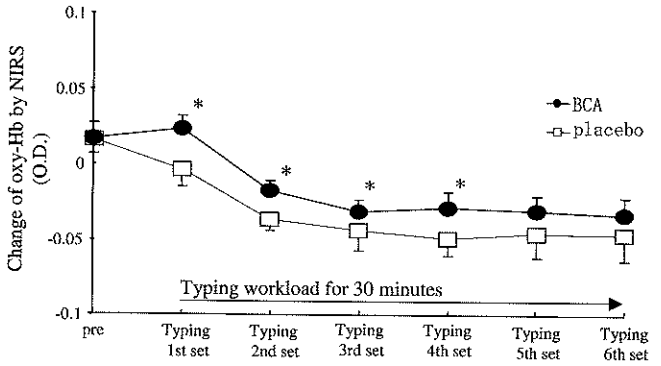


**Fig. 4** A comparison of changes in oxygenated hemoglobin (*oxy-Hb*) during typing workload between the BCA (•) and placebo intake groups (□) measured by NIRS. The *P* values represent the comparison between BCA and placebo, $P < 0.05$

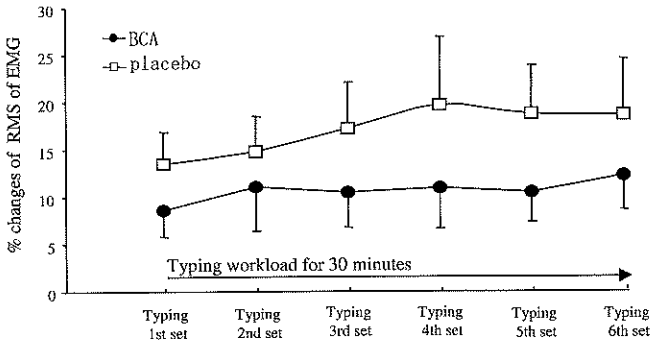


**Fig. 5** A comparison of changes in root mean square (*RMS*) measured by EMG during typing workload between the BCA (●) and placebo intake groups (□). The *P* values represent the comparison between BCA and placebo. $*P < 0.05$

typing, the RMS values recorded following intake of placebo were always higher than with BCA, although this difference was not statistically significant.

*The viscoelasticity of the trapezius muscle*

The changes in viscoelasticity of the trapezius muscle are shown in Fig. 6. The viscoelasticity of the trapezius muscle increased significantly after the typing workload in the placebo group only [placebo group 56.1 (1.3) to 57.9 (1.3), $P < 0.05$; BCA group 56.3 (1.3) to 57.5 (0.9)]. We did not observe any significant difference in the viscoelasticity of the trapezius muscle between the BCA and placebo intake.

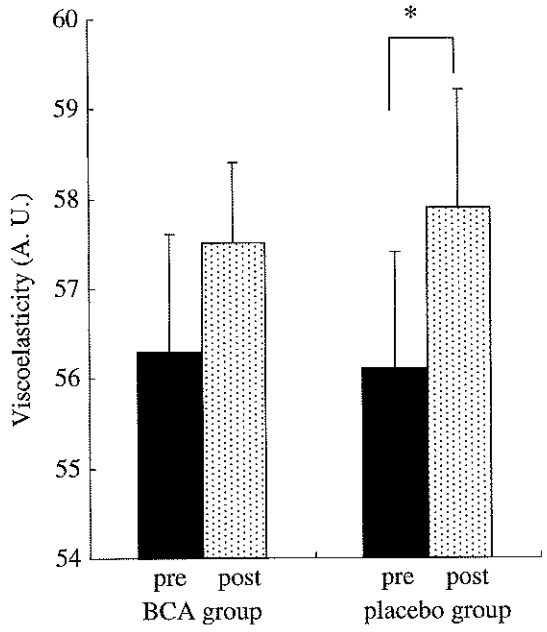


**Fig. 6** A comparison of changes in viscoelasticity of the trapezius muscle after typing workload between the BCA and placebo intake groups.The *P* values represent the comparison between pre and post, $*P < 0.05$

*Typing performance*

Typing performance was similar with BCA and placebo [number of words typed per minute: placebo 107.4 (12) vs BCA 108.4 (14.1); percentage of correct typing: placebo 95.4 (8.6% vs BCA 96.1 (9.4)%].

*Visual analog scale*

The changes in the VAS for subjective pain are summarized in Fig. 7, with no significant difference being observed between BCA and placebo intake throughout the experiment.

*Blood pressure and heart rate*

There was no significant difference in blood pressure or heart rate between the BCA and placebo groups, with no significant change in these measurements being detected in either treatment group during the two experiments. (Fig. 8)

## Discussion

To our knowledge, this is the first study investigating the physiological effects of oral anthocyanin intake on blood circulation dynamics. In this study, BCA intake was shown to have several beneficial effects on the circulation that included an increase in resting FBF, prevention of the decrease in muscle oxygenation that occurs during typing, and normalization of the decrease in the hyperemic reaction induced by typing. We consider all these effects may be attributable to BCA preventing the decrease in the peripheral circulation induced by work. However, this improvement in circulation did not affect typing performance.

Effects of the BCA concentrate on peripheral blood flow at rest

Following BCA intake, FBF tended to increase compared to that measured with placebo. We also observed that this increase in FBF occurred 1–2 h after intake, and coincided with the rise in plasma concentrations of the major anthocyanin components of BCA. This suggests that the absorbed BCA entered the plasma within 1 h after intake and initiated a vasodilatory action. This acute effect may be caused by rapid absorption of anthocyanin in the stomach (Passamonti et al. 2003). The increase in FBF appeared to be sustained for longer than 2 h, at which stage the plasma concentration of the anthocyanins was decreasing as shown in Fig. 2.

We have previously investigated whether plasma concentrations of anthocyanins correlated with antioxidant activity after oral intake of BCA in humans (Matsumoto et al. 2002). The antioxidant activity

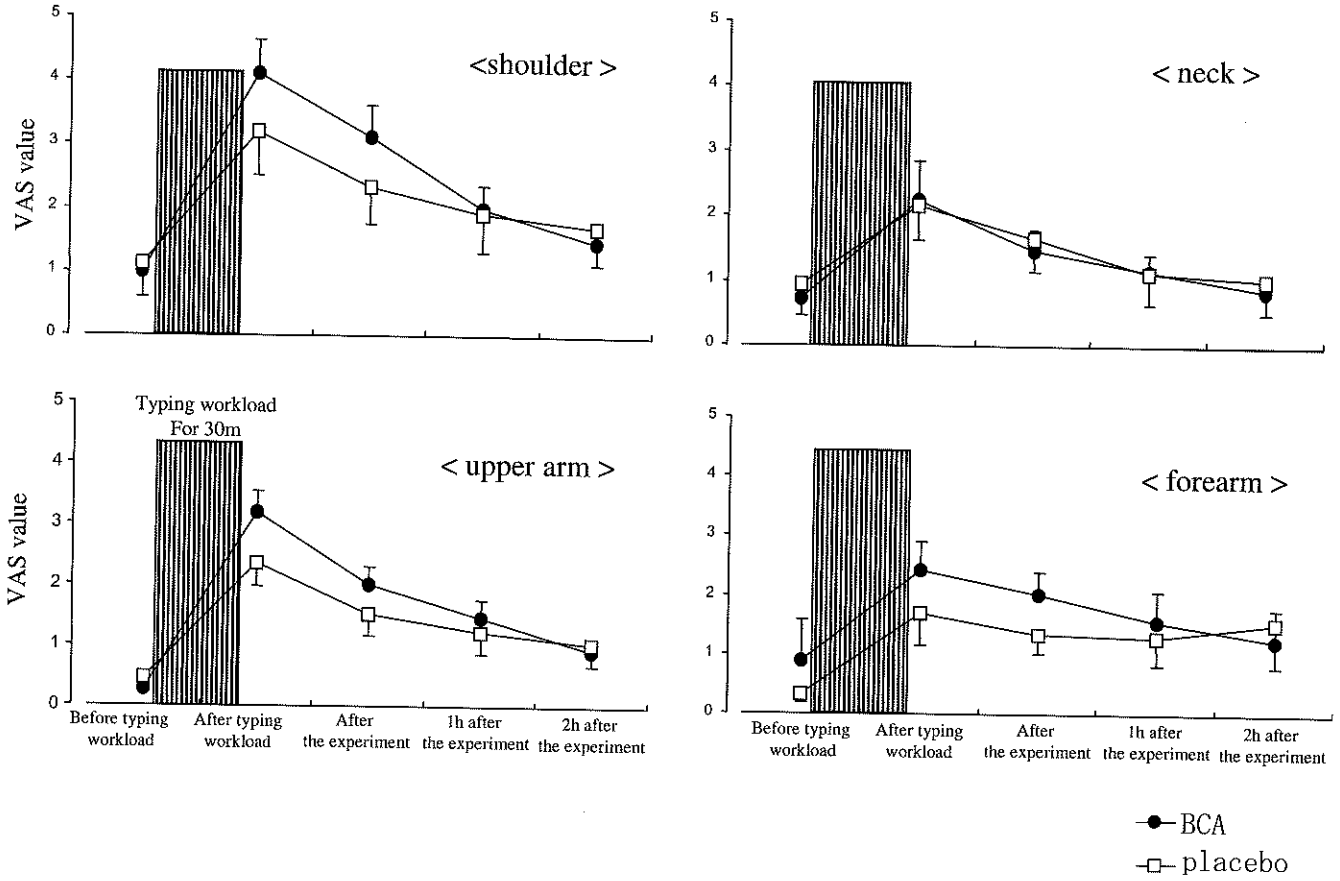


**Fig. 7** A comparison of changes of subjective pain in visual analog score (*VAS*) values after typing workload between the BCA (●) and placebo intake groups (□). They gray bar indicates typing workload for 30 min. There were no differences between the BCA and placebo intake groups, $P > 0.05$

increased rapidly until 2 h post-administration, with high levels being maintained until 8 h post-administration. The plasma anthocyanin levels also increased rapidly until 2 h post-administration, similar to the antioxidant activity. However, a rapid decrease in anthocyanin levels occurred between 2 and 8 h, and therefore the pattern in the latter half of the investigation was distinctly different from that of antioxidant activity. A possible explanation of this sustained effect may be that anthocyanins are converted into metabolites that also have antioxidant activity.

The similarity in the time-related changes in plasma antioxidant activity and FBF after BCA intake supports the concept that the changes in FBF we observed are related to anthocyanin metabolites. Duffy et al. (2001) reported that antioxidant therapy by vitamin E supplementation augmented resting FBF and reduced forearm vascular resistance. These data suggest that the increase in peripheral blood flow associated with BCA supplementation may be caused by the antioxidant action of BCA.

We have demonstrated in rat thoracic aorta that BCA-induced endothelium-dependent vasorelaxation is mediated by increased production of the endothelial-derived vasodilatation factor nitric oxide (NO) (Nakamura et al. 2002). This confirms evidence from other studies that have shown delphinidin, a non-glycoside anthocyanin found in red wine, relaxes blood vessels by increasing NO production as a result of elevated $Ca^{2+}$ concentrations in the endothelial cells (Martin et al. 2002). Polyphenols have also been shown to inhibit the production of peroxinitrate from NO (Nagai et al. 2002). Taken together, these results imply that BCA containing a large amount of glycosides of delphinidin and anthocyanins have the potential to increase peripheral blood flow by the combined action of increased production of NO by endothelial cells and reduced breakdown of NO by free radicals. The anticipated effect of either of these changes would be vasorelaxation.

## Effects of BCA concentrate on the peripheral circulation during a typing workload

In study 2, we aimed to investigate the effects of BCA on shoulder stiffness in subjects with daily subjective symptoms of mild shoulder stiffness. We demonstrated that during typing work, oxy-Hb measured by NIRS was significantly higher after intake of BCA than with placebo until the fourth set of the typing work (Fig. 4). When considered in conjunction with the results of study 1, this finding implies that the oxygen supply to

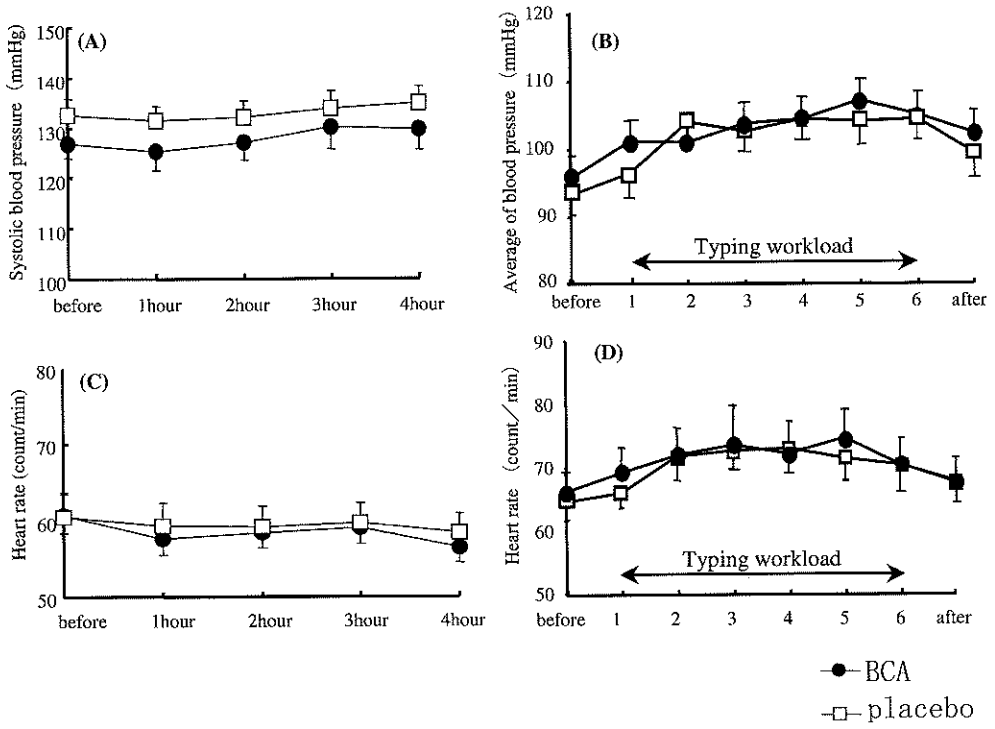


**Fig. 8A–D** Comparison of changes in blood pressure and heart rate during typing workload between the BCA (●) and placebo intake groups (□). **A** Systolic blood pressure in study 1. **B** Heart rate in study 1. **C** Mean blood pressure in study 2. **D** Heart rate in study 2

the right shoulder trapezius muscle during the workload may have been increased by BCA intake, and that any decrease in blood flow induced by the workload was attenuated. Furthermore, as there was no significant difference in Hb levels between the two treatments, we consider that the increased oxy-Hb associated with BCA intake was due not to an increase in venous blood congestion, but rather to increased arterial inflow into the muscle tissue (Fig. 3).

In addition, we observed that RMS values measured by EMG during typing were always higher following intake of placebo compared to BCA. Larsson et al. (1993) measured blood flow in the microcirculation in the upper portion of the trapezius muscle in healthy women using laser-Doppler flowmetry; they demonstrated that during accumulated local fatigue there was a negative correlation between flow and mean power frequency of EMG. In a later study, they also showed that impaired regulation of the microcirculation in the local muscle was of central importance in chronic trapezius myalgia, as patients with this condition had significant lower local blood flow in the painful side compared to the opposite side (Larsson et al. 1999). Muscle tension was also somewhat elevated in that study, as demonstrated by a small, but statistically significant increase in RMS-EMG.

It would be expected under placebo conditions that as muscle fatigue increased, recruitment of muscle fibers to compensate for the decrease in development of force would result in an increase in RMS values. On the other hand, with BCA intake, blood flow in the skeletal muscle and oxygen supply to the tissue may be increased thereby reducing the workload on the muscle.

We observed that the maximum Hb response was reduced after the typing workload with placebo, whereas it did not change significantly between pre- and post-workload with BCA (Fig. 3). This finding suggests that local hypoxia had occurred after the workload carried out under placebo conditions. Taylor and Bronks (1996) investigated the effects of environmental hypoxic changes on quadriceps EMG and metabolite accumulation; they showed that hypoxia resulted in significantly higher plasma lactate and ammonia concentrations, and a marked reduction in total exercise capacity. The EMG responses of the quadriceps muscles also tended to be greater during hypoxia, although this difference did not reach statistical significance. Gonzales et al. (1999) have also reported that reductions in leg muscle blood flow associated with exercise-induced dehydration reduced delivery of substrates and metabolites such as lactate, glucose, free fatty acids, and glycerol. It is therefore possible that when the circulation is disturbed by compression of the blood vessels resulting from continuous muscle contraction, removal of metabolites such as lactic acid and $CO_2$ becomes insufficient and leads to the development of muscle stiffness.

In this study, muscle stiffness increased significantly after the workload in the placebo experiments. In contrast, BCA intake suppressed the development of muscle stiffness, leading us to speculate that BCA intake reduced the circulatory disturbance induced by typing workload and facilitated the removal of unfavorable metabolites.

In study 2, there was no significant change in systemic circulatory response following BCA intake as demonstrated by the absence of changes in blood

pressure and heart rate. We therefore consider that the increased blood flow caused by BCA was most likely due to a direct effect on peripheral blood vessels. Because shoulder stiffness has no clear diagnostic criteria, subjective symptoms are important in the diagnosis. We used a VAS to evaluate these subjective symptoms, but found no clear difference in these assessments between BCA and placebo intake. This indicates that BCA intake has little effect on improving subjective symptoms of acute stiff shoulder caused by typing workload. The reason for this may be that in this study, shoulder stiffness was induced experimentally and, although we were able to measure a beneficial effect of BCA on acute local circulation disturbances, this change may not have been reflected by an improvement in chronic shoulder stiffness. Further studies are therefore required to elucidate the clinical effect of BCA intake on chronic shoulder stiffness.

## Conclusion

This study demonstrated that anthocyanins in BCA are transferred to the bloodstream leading to an increase in resting peripheral blood flow without any increase in oxidative metabolism. In addition, BCA intake inhibited the decrease in t-Hb and oxy-Hb that occurred in a model of experimentally induced disturbances to the local circulation. During workload, BCA intake tended to alleviate the increase in load in the trapezius muscle and also suppressed the development of muscle stiffness.

In conclusion, by enhancing peripheral circulation, BCA intake may be effective for preventing mild hypoxia and disturbance of oxidative metabolism in muscles.

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