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Oral intake of encapsulated dried ginger root powder hardly affects human thermoregulatory function, but appears to facilitate fat utilization

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Abstract

The present study investigated the impact of a single oral ingestion of ginger on thermoregulatory function and fat oxidation in humans. Morning and afternoon oral intake of 1.0 g dried ginger root powder did not alter rectal temperature, skin blood flow, O_2 consumption, CO_2 production, and thermal sensation and comfort, or induce sweating at an ambient temperature of 28°C. Ginger ingestion had no effect on threshold temperatures for skin blood flow or thermal sweating. Serum levels of free fatty acids were significantly elevated at 120 min after ginger ingestion in both the morning and afternoon. Morning ginger intake significantly reduced respiratory exchange ratios and elevated fat oxidation by 13.5% at 120 min after ingestion. This was not the case in the afternoon. These results suggest that the effect of a single oral ginger administration on the peripheral and central thermoregulatory function is miniscule, but does facilitate fat utilization although the timing of the administration may be relevant.

Keywords: gingerol, shogaol, thermoregulation, heat balance, free fatty acid, fat oxidation

Introduction

The components of root of Ginger (*Zingiber officnale* Roscoe, Zingiberaceae) have been widely used for various medicinal purposes in Asia for thousands of years. A variety of active components of ginger have been identified (Shao et al. 2010; Tao et al. 2009; Yu et al. 2011). Among them, 6-, 8-, and 10-gingerols and 6-shogaol have been intensively investigated as remedies for medical use (Pertz et al. 2011; Someya et al. 2003; Suekawa et al. 1984; Suekawa et al. 1986a; Suekawa et al. 1986b). One of well-known effects of ginger consumption is a release of gastrointestinal symptoms (Ernst and Pittler 2000; Quinla and Hill 2003), e.g. administration at a dose of at least 1.0 g of dry extract of ginger root is effective in preventing nausea and vomiting associated with postoperative recovery or pregnancy (Chaiyakunapruk et al. 2006; Chittumma et al. 2007). As the ginger products used in these studies were in capsule form, it is unlikely that the positive effects of ginger are simply a response to the characteristic flavor and taste of ginger. Indeed, administration of 6-, 8-, or 10-gingerols or 6-shogaol (Yu et al. 2011; Zick et al. 2008) showed various pathophysiological effects, e.g. anti-inflammatory, anti-angiogenic, anti-tumorigenic and anti-pyretic properties (Bode et al. 2001; Brown et al. 2009; Grzanna et al. 2005; Kim et al. 2005; Suekawa et al. 1984), in *in vivo* studies where perception of ginger flavor and taste was not involved.

In Japan, one of the generally acknowledged beneficial effects of ginger consumption is an induction of a “warm sensation”, although the meaning and usage of the phrase is vague. Thermophysiologically, the “warm sensation” is mainly caused by a rise in skin temperature which is a function of skin blood flow under a constant ambient temperature (T_a) (Cabanac and Brinnel 1987). At least two

mechanisms are involved in increased skin blood flow. One is associated with a rise in core body temperature (T_{cor}). When T_{cor} rises, skin blood flow increase is driven by thermoregulatory centers to facilitate nonevaporative heat loss to restore T_{cor} to its initial level. The other mechanism involves the direct stimulation of warm-sensitive channels, such as transient receptor potentials (TRP) V1, V3 or V4 in the cutaneous tissues. Excitation of these channels elevates skin blood flow in a thermoregulatory reflex. In such a case, T_{cor} gradually lowers due to increased heat dissipation. Thus, the direction of a shift in T_{cor} can be the opposite. However, it has not been scientifically and clearly investigated how ginger consumption modifies thermoregulatory functions and affects T_{cor} in humans. Ueki et al. (2008) reported that in rats, an intraperitoneal injection of 6-gingerol lowered T_{cor} by depressing metabolic heat production, which suggested that active ginger components modify heat production systems in animals. Thus, the primary purpose of the present study was to investigate the impact of the intake of a practical dose of ginger root extract on heat balance (i.e. metabolic heat production and evaporative and nonevaporative heat loss), T_{cor} and blood energy substrate levels comprehensively in humans.

Recently, oral intake of ginger components for several weeks has been shown to modify fat metabolism in rodents with alcohol-induced fatty liver. The administration of ginger essential oil for 4 weeks decreased triglycerides (TG) content in the liver in mice (Liu et al. 2013) and serum levels of TG and total cholesterol (TC) in mice (Liu et al. 2013) and rats (Nwozo et al. 2014). Further, in healthy humans, oral intake of ginger capsules (3.0 g/day) for 45 days reduced serum TG levels (Alizadeh-Navaei et al. 2008). Thus, long-term administration of ginger components appears to enhance fat utilization in animals and humans. However, there is no data on how a single dose

of ginger affects fat oxidation in humans. Free fatty acids (FFA) derived from TG are known to be essential energy substrates, especially in nonshivering thermogenesis. Therefore, we also investigated the effects of ingestion of ginger powder on serum FFA levels and fat oxidation.

Methods

This study was approved by the Ethical Committee for Human Experimentation of the School of Medicine, Shimane University, Japan. The study consists of 4 series of experiments including a Preliminary study.

Dried ginger root powder

The two batches of ginger capsules and placebo capsules used in this study were manufactured by encapsulation and provided by Sunsho Pharmaceutical Co., Ltd, Shizuoka, Japan. Each ginger capsule contained 250 mg of dried ginger root powder. The placebo capsules contained 250 mg of starch. In all experiments, each subject ingested 4 ginger capsules or 4 placebo capsules, i.e. 1.0 g of dried ginger root powder or starch. The capsules were ingested with 200 ml of temperature-controlled warm water (37°C) to avoid thermal stimulation to the subjects. The dose of ginger powder was chosen according to foregoing studies (Gonlachanvit et al. 2003; Lien et al. 2003).

The active components of the ginger powder were measured as described by Yu et al. (2011) and Tao et al. (2009) with a slight modification. Briefly, high-performance liquid chromatography (HPLC) was combined with electrospray ionization/tandem mass spectrometry (LC–ESI–MS/MS) in a TSQ Quantum mass spectrometer (Thermo Fisher Scientific K.K., Kanagawa, Japan). HPLC was conducted in a Luna 3u C18(2)

100Å LC column (100 × 2.0 mm, Phenomenex, CA, USA) at 30°C. Samples were eluted with a mobile phase composed of acetonitrile–methanol (4:1, v/v) and water–acetic acid (100:0.1, v/v) in a 20:80 ratio for 5 min, ramped up to a 100:0 ratio after 10 min, and held for 5 min at a flow rate of 0.2 ml/min. MS/MS analyses were conducted in positive ion mode, and 6-, 8-, 10-, and 12-gingerols and 6-, 8-, and 10-shogaols were detected and quantified by selected reaction monitoring (SRM). Peaks were selected and their areas were calculated by Xcalibur 2.1 software (Thermo Fisher Scientific K.K., Kanagawa, Japan).

Table 1 summarizes the main active ginger components in the capsules used in this study. The second batch of ginger capsules was ordered and used to ensure the potency of the active ingredients of the capsules. The major active component was 6-gingerol in both ginger root powders. With the exception of 10-shogaol, there were only slight differences in the profiles of the active components of the two batches of powder.

Subjects

A total of 23 healthy male subjects volunteered for the experiment after giving informed consent. Prior to participation, they were well familiarized with test procedures and with instruments to be affixed to their bodies. One subject participated in two tests, once in the Preliminary Study and once in Experiment 1. The latter was done approximately one year after the former.

For at least one week prior to the start of each test, the subjects' clock times for waking up and going to bed were controlled to exact times by each subject. During this period, all subjects were forbidden from participating in any strenuous exercise in order to prevent unwarranted changes in body temperature. In addition, the subjects were not

allowed to have any food or beverage containing large amounts of ginger components, caffeine, alcohol or capsaicin for at least 3 days before measurements. For meals, the subjects consumed identical meals for dinner the day before, and for breakfast on the day of, the measurements. These restrictions were set to optimize a subject's condition for the detection of fine differences in physiological functions (Ohta et al. 2008; Ohta et al. 2008).

Preliminary study: serum levels of active ginger components

This study was performed to confirm the existence of active ginger components in the blood after ingestion of 1.0 g of ginger powder. Six subjects (mean age, height and body mass were 26 y (20-36), 174 cm (168-180) and 74 kg (58-87), respectively) were used. On the day of experiment, the subjects were instructed to arrive at the laboratory by 09:00 h after having breakfast (200 kcal of commercially-available balanced food). The subjects then remained in a room with a T_a of approximately 25.0°C and a relative humidity of approx. 50%, sitting on a chair in an upright position. After 30 min at rest, the subjects ingested ginger capsules. At 15, 30, and 60 min after ginger intake, a blood sample (2 ml) was taken from the vein at the right cubital region. During this period, subjects were allowed to watch TV and videos and read, but were encouraged to stay as quiet as possible. Serum was separated from the blood immediately and kept at -80°C until analyses.

To analyze free, or glucuronide- or sulfate-conjugated forms of 6-, 8-, 10-, and 12-gingerol and 6-, 8-, and 10-shogaol, main active ginger components, the serum samples were treated with β-glucuronidase or sulfatase (Yu et al. 2011) and analyzed by LC–ESI–MS/MS as described above in "*Dried ginger root powder*".

Experiment 1: effects of ginger intake on thermal balance, T_{cor} and blood energy substrates levels

The experiment consisted of two series of measurements, i.e. in the morning and in the afternoon.

In the morning test, 5 subjects (mean age, height and body mass were 22 y (20-24), 170 cm (164-174) and 66 kg (58-78), respectively) were used. On the day of experiment, the subjects were instructed to arrive at the laboratory by 08:30 h after having breakfast (200 kcal of commercially-available balanced food). The subjects, wearing only shorts, entered a temperature-controlled room with a T_a of 28.0 ± 0.5°C and relative humidity of 50 ± 5%. The air velocity and lighting intensity were maintained constant. Subjects rested on a chair in an upright position and all devices for measurements were fitted within 30 min. At 09:00 h, the first blood sample (approx. 15 ml) was taken from the vein at the right cubital region. After another 30-min rest (09:30 h), the subjects ingested ginger capsules or a placebo. The second blood sample was taken 120 min after capsule intake (11:30 h). During the measurement period, subjects were allowed to watch one of two different animated movies. For 2 of the subjects, measurements with ginger intake were first performed and then control experiments using a placebo were made at least one week after completing the first study; for the other 3 subjects, the control tests were made at least one week prior to the test with ginger products.

In the afternoon test, 4 subjects (mean age, height and body mass 21 y (20-23), 170 cm (158-181) and 70 kg (54-90), respectively) were used. On the day of experiments, the subjects were instructed to arrive at the laboratory by 13:00 h after having a prescribed breakfast and lunch (200 kcal of commercially-available balanced food). The

subjects, wearing only shorts, entered the same room with the same environment as in the morning test. They sat on a chair at rest in an upright position and all devices for measurements were fitted within 30 min. At 13:30 h, the first blood sample (approx. 15 ml) was taken from the vein at the right cubital region. After another 30-min rest (14:00 h), the subjects ingested ginger capsules or a placebo. The second blood sample was taken 120 min after capsule intake (16:00 h). During the measurements, the subjects were allowed to watch one of two different animated movies. For 2 of the subjects, measurements with ginger intake were first performed and then control experiments using a placebo were made at least one week after completing the first study; for the other 2 subjects, the control tests were made at least one week prior to the test with ginger products.

Skin temperatures were recorded at 7 body sites (forehead, trunk, forearm, finger, thigh, calf, and foot) by skin thermistors (SZL-64, Techno Seven, Yokohama, Japan) held in place with surgical tape. The accuracy of the thermistors was estimated to be within ± 0.05°C. For finger temperature, the thermistor was attached at the ventral side of the left 4th finger tip. The skin temperature data were sampled every 3 sec with a data logger (K730, Techno Seven, Yokohama, Japan) through a scanning unit (X115, Technol Seven, Yokohama, Japan). Rectal temperature (T_{re}), as an indicator of T_{cor}, in each subject was measured every min with a thermistor probe introduced 15 cm into the rectum. Heart rate (HR) was estimated by the count of R-waves in one min on an ECG. T_{re} and HR data were sampled with a portable memory (VM4-064, VINE Co., Tokyo, Japan). Oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were recorded with a respiratory analysis system (AT-1100, ANIMA, Tokyo, Japan). Arterial blood pressure (BP) was measured every 30 min at the right upper arm with an automatic

sphygmomanometer (HDI/PulseWave, CR-2000, USA). In addition, the subjects voted scales of whole body thermal sensation and thermal comfort every 30 min according to a perceptual table (Table 2). The table was arranged based on previous studies (Grivel F and Candas V 1991; Shido et al. 2001).

Finger skin blood flow (skBF) at the tip of the left middle finger and left forearm skBF were continuously measured with Laser-Doppler flowmeters (TBF-LC1, Unique Medical Co., Tokyo, Japan). Sweating rates ($\dot{m}_{sw}$) at the thenar eminence (thenar area) of the left palm and the left forearm were measured by the ventilation method (Ogawa et al. 1967; Ogawa and Sugenoya 1993) with 1.0 cm^2 capsules (SKD-2000, Sukinos, Nagoya, Japan). The capsules were fixed to the skin of the left forearm with adhesive tape, and ventilated with air at a constant flow of 200 ml/min. The relative humidities of the inlet and outlet air were measured by capacitance hygrometers, and water contents and then $\dot{m}_{sw}$ were calculated with exclusive software (SKS-2000, Sukinos, Nagoya, Japan).

Blood was collected into three different types of tubes, one containing NaF for glucose assay, one containing EDTA-2K for plasma analyses, and the other for serum analyses. After hematocrit (Ht) was measured, the tubes were centrifuged at 4°C, 2500 rpm. All the assays except Ht were performed by Japan Clinical Laboratories, Inc, Kyoto. Briefly, plasma osmolality (Osm) was determined by freezing point depression. Plasma levels of Na, K, and Cl were measured by an ion selective electrode method. Total protein (TP) and albumin concentrations were measured by the Biuret and bromcresol green methods, respectively, and glucose, TG, and FFA were measured by the enzyme method. Plasma concentrations of ACTH were determined by RIA and those of cortisol were measured by ECLIA.

Experiment 2: effects of ginger intake on threshold T_{cor} for skBF and $\dot{m}_{sw}$

As an additional study, we determined threshold T_{cor}s for increases in skin blood flow and sweating to examine the influence of ginger administration on the functioning of human thermoregulatory centers. Since T_{cor} of humans rises rapidly in the morning (Ohta et al. 2008; Ohta et al. 2008), this experiment was performed in the afternoon when T_{cor} is relatively stable.

Four subjects (mean age, height and body mass were 22 y (20-24), 172 cm (163-176) and 65 kg (53-79), respectively) were used. On the day of the experiment, the subjects were instructed to arrive at the laboratory by 13:30 h after having a prescribed breakfast and lunch (200 kcal of commercially-available balanced food). The subjects, wearing only shorts, then entered the same with the same environment as in Experiment 1. The subjects sat on a chair in an upright resting position and all devices for measurements were fitted within 30 min. At 14:00 h, the subjects ingested ginger capsules or a placebo. At 30 min after ginger or placebo intake, the subjects immersed both legs in a water-bath (depth 28 cm; LTP-112, Tabai Espec, Osaka, Japan) in which the water temperature was controlled at 42.0 ± 0.1°C to induce thermal cutaneous vasodilation and sweating. The warm water immersion continued for 30 min. Skin temperatures and T_{re} were recorded as in Experiment 1. skBF and $\dot{m}_{sw}$ at the left forearm and chest were continuously measured, also as in Experiment 1.

Experiment 3: effects of ginger intake on serum FFA profile

The experiment was done in the morning following the results of fat utilization in Experiment 1 (see results and discussion).

Five subjects (mean age, height and body mass were 22 y (20-26), 174 cm (170-181) and 69 kg (58-78), respectively) were used. On the day of the experiment, the subjects were instructed to arrive at the laboratory by 09:00 h after eating breakfast (200 kcal of commercially-available balanced food). The subjects then entered a room with a T_a of approx. 25.0°C and a relative humidity of approx. 50%. They sat on a chair in an upright resting position. After 30 min, the subjects ingested either ginger capsules or a placebo. Just prior to ginger or placebo capsule intake (0 min), and at 30, 60, and 120 min after ginger or placebo intake, a blood sample (2 ml) was taken from the vein at the right cubital region. During the entire time, they were allowed to watch TV and videos and read, but were encouraged to stay as quiet as possible. Serum was separated from the blood immediately and kept at -80°C until analyses.

FFA profiles of the serum total lipids were determined by gas chromatography, as described previously (Hashimoto et al. 1999). FFAs were classified according to carbon number. Palmitic, stearic, oleic, linoleic, and α-linoleinic acids were grouped as long chain fatty acids (LCFA, carbon chain 16-18), whereas arachidonic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acid were grouped as very long chain fatty acids (VLCFA, carbon chain >20). The classification was made since VLCFAs are known to be mainly oxidized in peroxisomes, while LCFAs are mainly oxidized in mitochondria.

Data analyses and statistics

Mean skin (T_{sk}) and mean body (T_b) temperatures were calculated as follows: $T_{sk} = 0.07T_1 + 0.35T_2 + 0.14T_3 + 0.05T_4 + 0.19T_5 + 0.13T_6 + 0.07T_7$, $T_b = 0.7T_{re} + 0.3\ T_{sk}$,

where T_{1-7} were temperatures of the forehead, trunk, forearm, finger, thigh, calf and foot, respectively (Hirata et al. 1986).

For Experiments 1 and 3, the influences of ginger components on changes in all parameters measured (except blood data in Experiment 1) were assessed by repeated measures of two-way analysis of variance (ANOVA). The effects of ginger intake on all parameters (including blood parameters in Experiment 1) for each experiment were evaluated using Student's t-test. The results are presented as means + or ± SEMs. $P < 0.05$ were considered significant.

Results

Preliminary study: serum levels of active ginger components

Major active ginger components, such as 6- and 8-gingerols and 6-shogaol, were detected in the serum within 15 min after ginger capsule ingestion, and consistently existed in the blood for at least the following 45 min (Fig. 1 A, B, E). The majority of the chemicals were glucuronide- or sulfate-conjugated forms, indicating that orally ingested ginger components were rapidly absorbed and conjugated in the liver. In three subjects, serum concentrations of free 6-gingerol increased at 60 min after ginger intake (Fig. 1 A), and those of free 6-shogaol rose at 30 min after ginger ingestion (Fig. 1 E). In around a half of the subjects, free 6-gingerol and free 6-shogaol seem to appear in the serum at between 30 and 60 min and at between 15 and 30 min, respectively, after ginger capsule intake.

Experiment 1: effects of ginger intake on thermal balance, T_{cor} and blood energy substrates levels

In the morning tests, there were no significant differences between the ginger and placebo ingestion groups in changes of T_{re}, T_{sk}, finger and forearm skBFs, and palm and forearm $\dot{m}_{sw}s$ (Fig. 2 A, B, C, D, E, F). Furthermore, ginger intake did not affect the scores of whole body thermal sensation and thermal comfort (Fig. 2G, H). Figure 3 shows changes in $\dot{V}_{O_2}$, and respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$, R) after ingestion of ginger or placebo capsules. Oral intake of ginger powder had no significant effects on $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. In R, however, there were significant differences in the time course ($F_{4,16}$ = 4.332, P = 0.015) and interaction between ginger intake and time ($F_{4,16}$ = 3.432, P = 0.033) after ginger or placebo ingestion. In addition, R values at 60 (P = 0.043), 90 (P = 0.008) and 120 (P = 0.001) min after ginger ingestion were significantly lower than that just before ginger intake. Oral ginger intake did not modify BP and HR (data not shown).

Blood parameters except FFA measured in the morning test are summarized in Table 3. In both ginger- and placebo-treated groups, blood levels of K, TP, albumin, and TC slightly but significantly increased at 120 min after ginger or placebo ingestion. In addition, Ht tended to increase in this 120 min period. Although plasma Osm and other electrolyte levels did not change, our observations suggest a slight occurrence of hemoconcentration (blood water loss) simply due to a time elapse. Oral ginger intake seemed to have no specific effect on biochemical data of the blood (except FFA, see Fig. 6) measured in this study.

Figures 4 and 5 show changes in T_{re}, T_{sk}, finger and forearm skBFs, palm and forearm $\dot{m}_{sw}s$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and R after ingestion of ginger or placebo in the afternoon tests. There were no significant differences between the ginger and placebo ingestion

groups in changes of all parameters measured including R. Oral ginger intake did not modify BP and HR (data not shown). Blood parameters except FFA measured in the afternoon test are summarized in Table 4. After ginger ingestion, plasma Osm slightly but significantly decreased and blood TG levels significantly decreased. However, oral ginger intake did not appear to have any particular effect on blood levels of electrolytes, protein or stress hormones.

There were remarkable changes in serum FFA levels due to the ginger powder. In the morning tests, FFA levels after ginger ingestion were markedly and significantly greater than before ingestion (P = 0.005) (Fig. 6 A). Placebo ingestion also significantly elevated serum FFA levels (P = 0.043), although the P value was rather large. In the afternoon tests, ginger ingestion significantly elevated serum FFA levels (P = 0.040), while placebo intake had no significant affect on serum FFA levels (P = 0.125) (Fig. 6 B).

Experiment 2: effects of ginger intake on threshold T_{cor} or skBF and $\dot{m}_{sw}$

The onsets of skin vasodilation and thermal sweating were identified by the prompt increases of skBF and $\dot{m}_{sw}$, respectively, in each measurement as done in our previous studies (Hara et al. 2007; Shido et al. 1999). The time at the onset of cutaneous vasodilation after commencing the leg water immersion, and the T_b corresponding to the vasodilation onset, were defined as a skin vasodilation latency and threshold T_b for skin vasodilation, respectively. In the same manner, latency of thermal sweating and threshold T_b for sweating were determined.

The threshold T_bs for forearm and for chest skin vasodilation were not affected by ginger ingestion (Fig. 7 A, B). Latencies for skin vasodilation at the two sites after

ginger intake did not differ from those after placebo intake (Fig. 7 C, D). Similarly, ginger powder ingestion did not affect threshold T_bs or latencies for forearm and chest thermal sweating (Fig. 7 E, F, G, H).

Experiment 3: effects of ginger intake on serum FFA profile

Figure 9 shows changes of mol% of LCFAs (Fig. 8 A) and VLCFAs (Fig. 8 B) after ginger or placebo ingestion. There were significant effects of ginger intake ($F_{1,4}$ = 51.880, P = 0.002) and time ($F_{3,12}$ = 6.941, P = 0.006) on the mol% of LCFAs (for interaction, ($F_{3,12}$ = 14.974, P < 0.001)). In addition, the mol% of LCFAs after ginger ingestion was significantly lower than that after placebo ingestion at 30 (P = 0.006), 60 (P = 0.045) and 120 min (P = 0.006) after oral intakes. Similarly, ginger ingestion and time had significant influence on the mol% of VLCFAs. The mol% of VLCFAs was significantly greater in subjects treated with ginger than that in subjects administered with placebo at 30, 60 and 120 min after oral ingestion (statistical values are the same as in the mol% of LCFAs).

Discussion

The present results show that in human subjects, oral administration of 1.0 g of dry extract of ginger roots does not alter T_{re} level for 120 min after ginger intake. This observation was consistent for the two test periods of the day, i.e. the morning and afternoon (Figs. 2 A and 4 A). In an additional study (data not shown), it was established that T_{re} did not vary for 240 min after ginger intake. Thus, it appears that ingestion of encapsulated ginger powder has no acute effects on T_{cor} of humans in a resting condition. As for the heat loss system, T_{sk}, skBFs, and $\dot{m}_{sw}s$ were not affected by ginger intake in

either the morning or afternoon tests, suggesting that oral ginger administration had no effects on the evaporative or nonevaporative heat loss systems. Furthermore, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were not significantly altered in either of the two testing periods (Figs. 3 A, B and 5 A, B). The results suggest that, in contrast to results with rats (Ueki et al. 2008), oral ingestion of ginger components has no significant impact on metabolic heat production in humans. As the ginger powder was encapsulated, subjects participating in this study were unable to perceive the characteristic flavor and taste of ginger. Thus, it can be concluded that a single oral ginger ingestion with unperceptable ginger flavor or taste barely registers any change in heat balance and T_{cor} in humans for at least 120 min after administration at T_a 28°C.

Behavioral thermoregulation is usually a function of thermal sensation and comfort (Chatonnet and Cabanac 1965). For instance, when a subject does not feel hot and unpleasant, behavioral thermoregulation for dissipating heat and/or reducing heat production is not induced. Thus, we checked the influence of ginger ingestion on thermal sensation and comfort. However, ginger ingestion again had no significant effect on these thermal perceptions (Figs. 2 G, H and 4 G, H). Taken together, oral ingestion of 1.0 g of dry ginger powder seems to have no particular influence on either the autonomic or behavioral thermoregulatory effectors in humans.

In a simple thermoregulatory model, T_{cor} of endotherms is controlled within a temperature zone between thresholds for heat dissipation and heat production (interthreshold zone) by thermoregulatory centers at a constant T_a (Romanovsky 2004; Shido and Sugimoto 2011). When T_{cor} is changed for whatever reason and reaches either of these thresholds, the corresponding effector mechanism is activated and T_{cor} is

returned to a temperature between the two thresholds. Thermoeffector thresholds have been known to be shifted by various chemicals and pathophysiological conditions (Romanovsky 2004; Romanovsky et al. 2005; Shido and Sugimoto 2011). Thus, we determined threshold temperatures for nonevaporative (skin vasodilation) and evaporative (sweating) heat losses after ginger ingestion at a time when free active ginger components, 6-gingerol and 6-shogaol, were likely to exist in the blood (Preliminary study and Experiment 2). However, such thresholds did not differ from those after placebo ingestion (Fig. 7). These results may suggest that ginger intake has no significant effect on thermoregulatory centers in humans.

Findings in our Preliminary study (Fig. 1) and another study (Yu et al. 2011) showed that ginger components ingested orally are absorbed and rapidly conjugated with glucuronide or sulfate in the liver within 15 min after ingestion, which means that active free ginger components rarely exist in the blood in high concentrations. Indeed, free 6-gingerol and 6-shogaol were detected in only 3 of the 6 subjects in the Preliminary study. Thus, it would seem that practical doses of active ginger components would not affect tissues or organs directly through the blood stream. However, orally-ingested ginger components do directly modulate liver and gastrointestinal function via inhibition or activation of their receptors such as serotonin receptors (Pertz et al. 2011; Walstab et al. 2013) or TRPV1 (Iwasaki et al. 2006; Rabellato and Islam 2014). There are various liver-brain signal transduction systems in animals (Izumida et al. 2013; Tsukita et al. 2012). More specifically, high–fat feeding facilitates thermogenesis of brown adipose tissue, a major effector in thermoregulation, via afferent signals from the liver vagal nerve (Tsukita et al. 2012). In addition, it has been proposed that febrile signals from the intraabdominal tissues are transmitted to thermoregulatory

centers via vagal afferent nerves and induce fever in rodents (Romanovsky et al. 1998; Roth and De Souza 2001). Thus, we hypothesized that active ginger components would produce thermoregulatory signals in the liver and/or gastrointestinal system and would be sent to thermoregulatory centers via vagal or other afferent fibers, altering thermoregulatory functions. This apparently did not occur in the subjects of the present study.

A single oral ingestion of ginger powder elevated serum FFA levels within 120 min after ingestion in healthy subjects in both morning and afternoon test periods (Fig. 6). In the morning tests, placebo ingestion also significantly elevated serum FFA levels. The calculated ratios of serum FFA levels pre- and post-ginger or placebo ingestion showed that ginger ingestion increased serum FFA levels by 3.01 ± 0.42 fold, whereas placebo intake increased them by 2.06 ± 0.35 fold. There were significant differences between the two ratios ($P = 0.020$). Since in the morning tests, the magnitude of rise in FFA concentrations was greater after ginger ingestion than after placebo intake, it may be concluded that a single oral ingestion of 1.0 g of ginger powder plays a significant role in an increase in serum FFA levels in humans. FFA is derived from TG by lipase activity. In rats, dietary ginger was shown to enhance lipase activity in the liver and white adipose tissue (Prakash and Srinivasan 2012). Similar changes in lipase activity may also be induced in humans by oral ginger intake.

The present study also showed that oral ginger intake significantly reduced R, although only in the morning tests (Fig. 3 C). This suggests the possibility of ginger components facilitating fat oxidation. The rates of fat oxidation were calculated using stoichiometric equations of thermal equivalents of oxygen for non-protein R (Frayn 1983) (Fig. 9). Fat oxidation was significantly elevated by 13.5% at 120 min after

ginger ingestion, while it decreased by 2.2% after placebo intake. Thus, one oral dose increased fat oxidation, i.e. β-oxidation of FFA, in human subjects, though timing may be of relevance. β-oxidation of FFA occurs in both mitochondria and peroxisome of hepatocytes (Nguyen et al. 2008), though the FFA types differ. VLCFAs are oxidized in peroxisomes, whereas LCFAs are oxidized in mitochondria. As shown in Figure 9, the mol% of LCFAs decreased and VLCFAs increased after ginger intake, suggesting that β-oxidation in mitochondrial pathways may have been involved in ginger-induced fat oxidation in the present study subjects.

Several enzymes are involved in β-oxidation of FFA. Ginger phytochemical-supplemented diets were supplied to animals for 6 weeks after which proteomic analysis revealed significant increases in acetyl-CoA acyltransferase 1 and enoyl CoA hydratase, which participate in the β-oxidation of FFA (Beattie et al. 2011). Moreover, 6-gingerol significantly increased mRNA and protein levels of hepatic peroxisome proliferator-activated receptor α (PPARα), known to be an upstream nuclear receptor to activate β-oxidation (Beattie et al. 2011). However, the impact of a single dose of ginger on such fat oxidation pathways remains unclear. Further experiments are warranted to clarify the mechanisms promoting β-oxidation of FFA through oral ginger ingestion.

In sum, in human subjects, a single oral intake of 1.0 g of dried ginger root powder failed to induce autonomic and behavioral thermoregulation for at least 120 min after ingestion in morning and afternoon tests. Serum FFA levels were significantly elevated at 120 min after ginger ingestion for those two time periods. Interestingly, ginger intake significantly reduced R and facilitated fat oxidation only in the morning tests. A single oral use of ginger does not appear to induce thermoregulatory events in humans

in the daytime. However, fat utilization is enhanced by ginger administration, though timing may have some relevance.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legends

Fig. 1 Changes in serum concentrations of 6-, 8-, 10- and 12-gingerol, 6-, 8- and 10-shogaol, and their glucuronide and sulfate conjugates after oral administration of 1.0 g of ginger powder. Values are means and vertical bars are + SEMs. A, 6-gingerol; B, 8-gingerol; C, 10-gingerol; D, 12-gingerol; E, 6-shogaol; F, 8-shogaol; G, 10-shogaol

Fig. 2 Changes in rectal temperature (T_{re}) (A), mean skin temperature (T_{sk}) (B), finger (C) and forearm (D) skin blood flows (skBF), and palm (E) and forearm (F) sweating rates ($\dot{m}_{sw}$), thermal sensation (G) and thermal comfort (H) after ginger (●) or placebo (○) ingestion in the morning test. Values are means in 30 min and vertical bars are + SEMs.

Fig. 3 Changes in oxygen consumption ($\dot{V}_{O_2}$) (A), CO_2 production ($\dot{V}_{CO_2}$) (B) and respiratory exchange ratio (R) (C) after (●) or placebo (○) ingestion in the morning test. Values are means in 30 min and vertical bars are + SEMs. For R, there were significant differences in the time course ($F_{4,16} = 4.332$, $P = 0.015$) and interaction between ginger intake and time ($F_{4,16} = 3.432$, $P = 0.033$) after ginger and placebo ingestions. * R value in the ginger intake group was significantly different from that at 0 min (just before ginger ingestion) ($P < 0.05$).

Fig. 4 Changes in rectal temperature (T_{re}) (A), mean skin temperature (T_{sk}) (B), finger

(C) and forearm (D) skin blood flows (skBF), and palm (E) and forearm (F) sweating rates ($\dot{m}_{sw}$), thermal sensation (G) and thermal comfort (H) after ginger (●) or placebo (○) in the afternoon test. Values are means in 30 min and vertical bars are + SEMs.

Fig. 5 Changes in oxygen consumption ($\dot{V}_{O_2}$) (A), CO_2 production ($\dot{V}_{CO_2}$) (B) and respiratory exchange ratio (R) (C) after ginger (●) or placebo (○) ingestion in the afternoon test. Values are means in 30 min and vertical bars are + SEMs.

Fig. 6 Serum free fatty acid (FFA) levels before (closed columns) and after (open columns) ginger or placebo ingestion in the morning (A) and afternoon (B) tests. Values are means and vertical bars are + SEMs. * significantly different from the FFA level before ginger or placebo ingestion.

Fig. 7 Threshold mean body temperatures (T_b) and latencies for forearm (left panels) and chest (right panels) vasodilation (A, B, C, D) and thermal sweating (E, F, G, H) in subjects ingested with ginger powder or placebo. Values are means and vertical bars are + SEMs.

Fig. 8 Changes of the mole percentage (mol%) of long-chain fatty acids (LCFA) (A) and very long-chain fatty acids (VLCFA) (B) in the serum after ginger (●) or placebo (○) ingestion. Values are means and vertical bars are + SEMs. There were significant effects of ginger intake ($F_{1,4}$ = 51.880, P = 0.002) and time ($F_{3,12}$ =

6.941, $P = 0.006$) on both mol% of LCFA and VLCFA (for interaction, ($F_{3,12} = 14.974$, $P < 0.001$)). * the value in the ginger intake group was significantly different from that of placebo intake group ($P < 0.05$).

Fig. 9 Changes in fat oxidation after ginger (●) or placebo (○) ingestion. Values are means in 30 min and vertical bars are + SEMs. There were significant effects of ginger intake ($F_{1,4} = 9.165$, $P = 0.039$) and time ($F_{4,16} = 3.523$, $P = 0.030$) on percentages of fat oxidation (for interaction, ($F_{4,16} = 3.947$, $P < 0.020$)). * the value in the ginger intake group was significantly different from that of placebo intake group ($P = 0.012$).

Figure 1

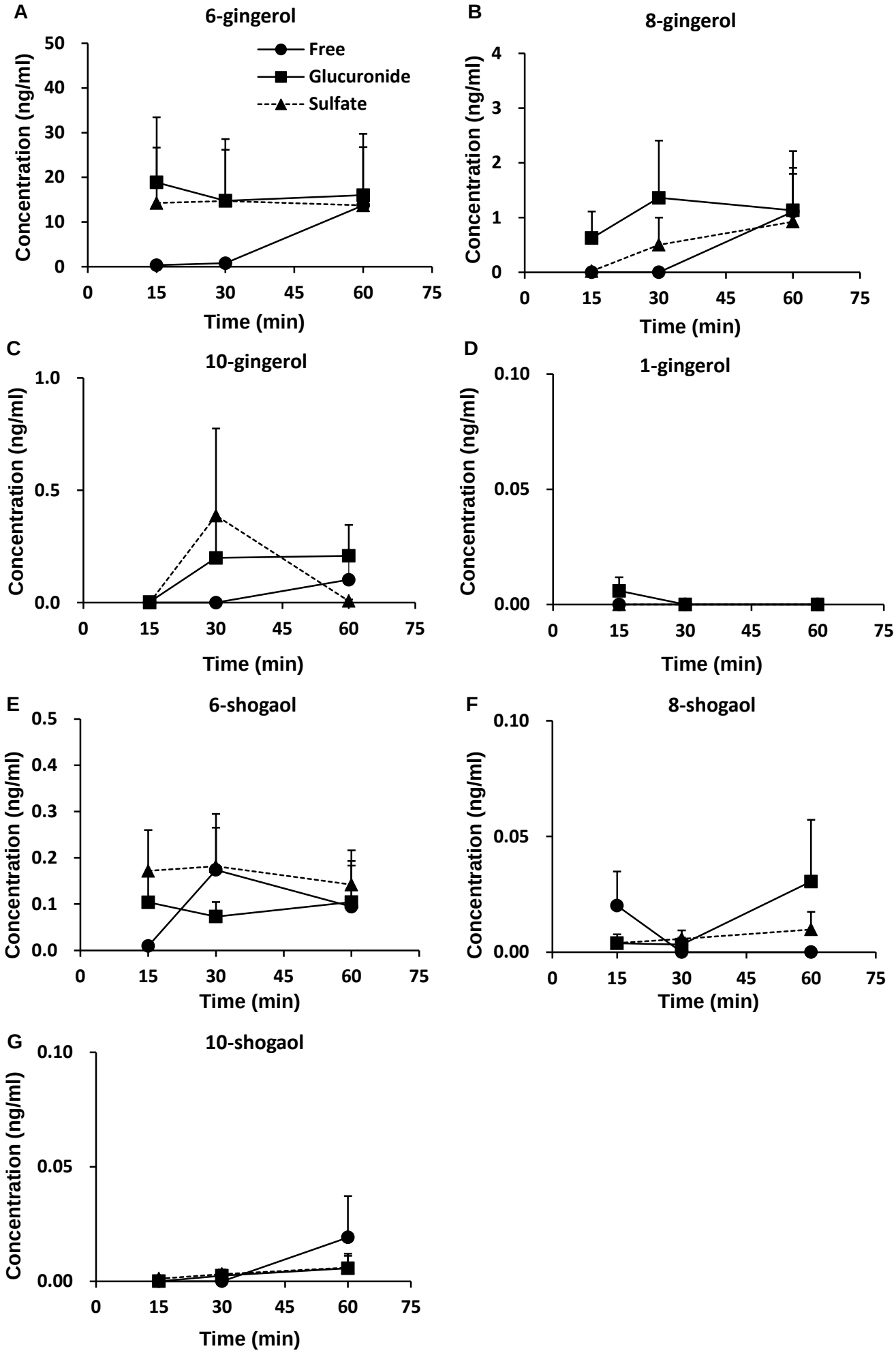

A
6-gingerol
Free
Glucuronide
Sulfate
Concentration (ng/ml)
Time (min)
B
8-gingerol
C
10-gingerol
D
1-gingerol
E
6-shogaol
F
8-shogaol
G
10-shogaol

Figure 2

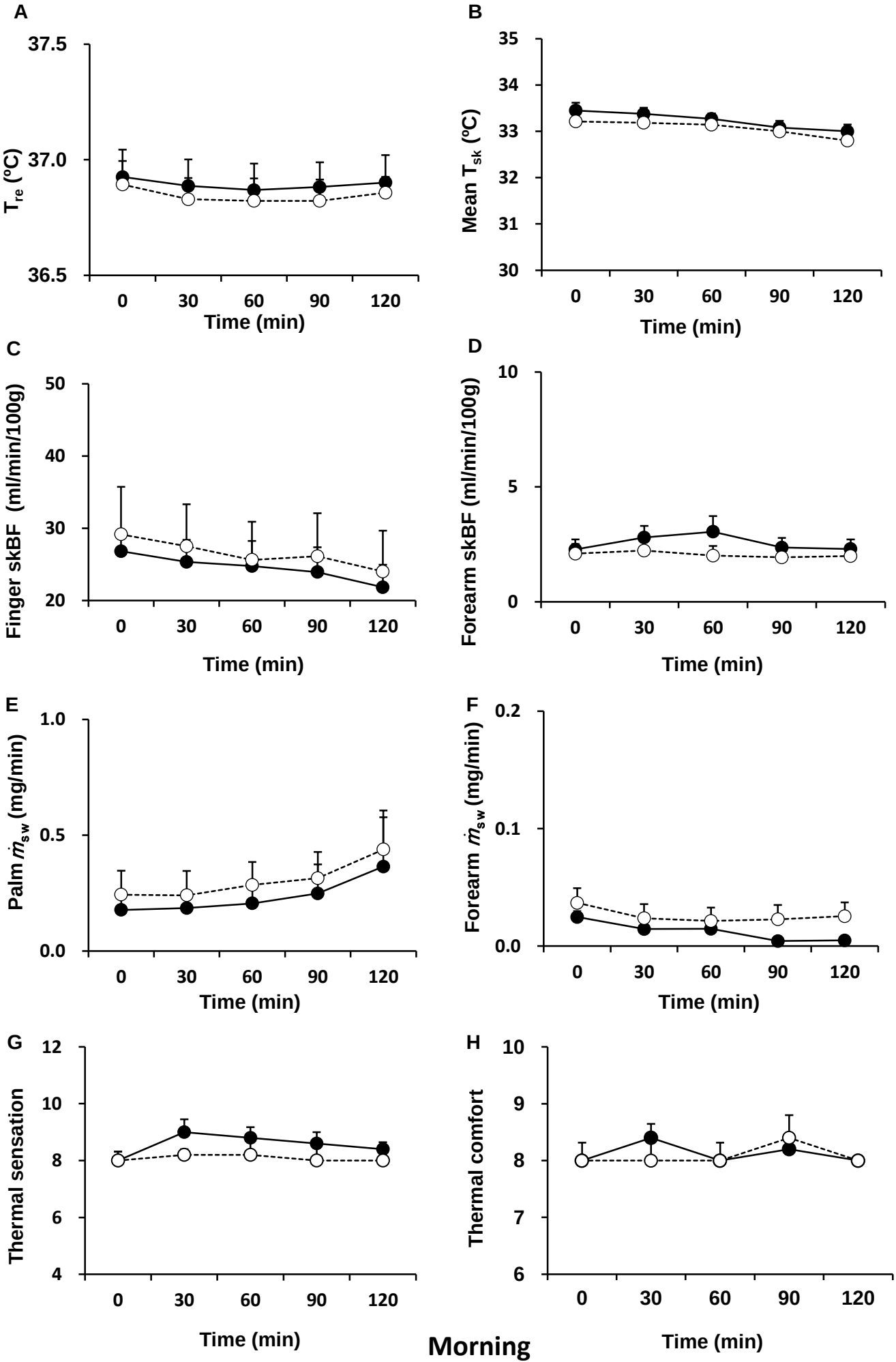

A
37.5
37.0
36.5
T_{re} (ºC)
0
30
60
90
120
Time (min)
B
35
34
33
32
31
30
Mean T_{sk} (ºC)
0
30
60
90
120
Time (min)
C
50
40
30
20
Finger skBF (ml/min/100g)
0
30
60
90
120
Time (min)
D
10
5
0
Forearm skBF (ml/min/100g)
0
30
60
90
120
Time (min)
E
1.0
0.5
0.0
Palm $\dot{m}_{sw}$ (mg/min)
0
30
60
90
120
Time (min)
F
0.2
0.1
0.0
Forearm $\dot{m}_{sw}$ (mg/min)
0
30
60
90
120
Time (min)
G
12
10
8
6
4
Thermal sensation
0
30
60
90
120
Time (min)
H
10
9
8
7
6
Thermal comfort
0
30
60
90
120
Time (min)
Morning

Figure 3

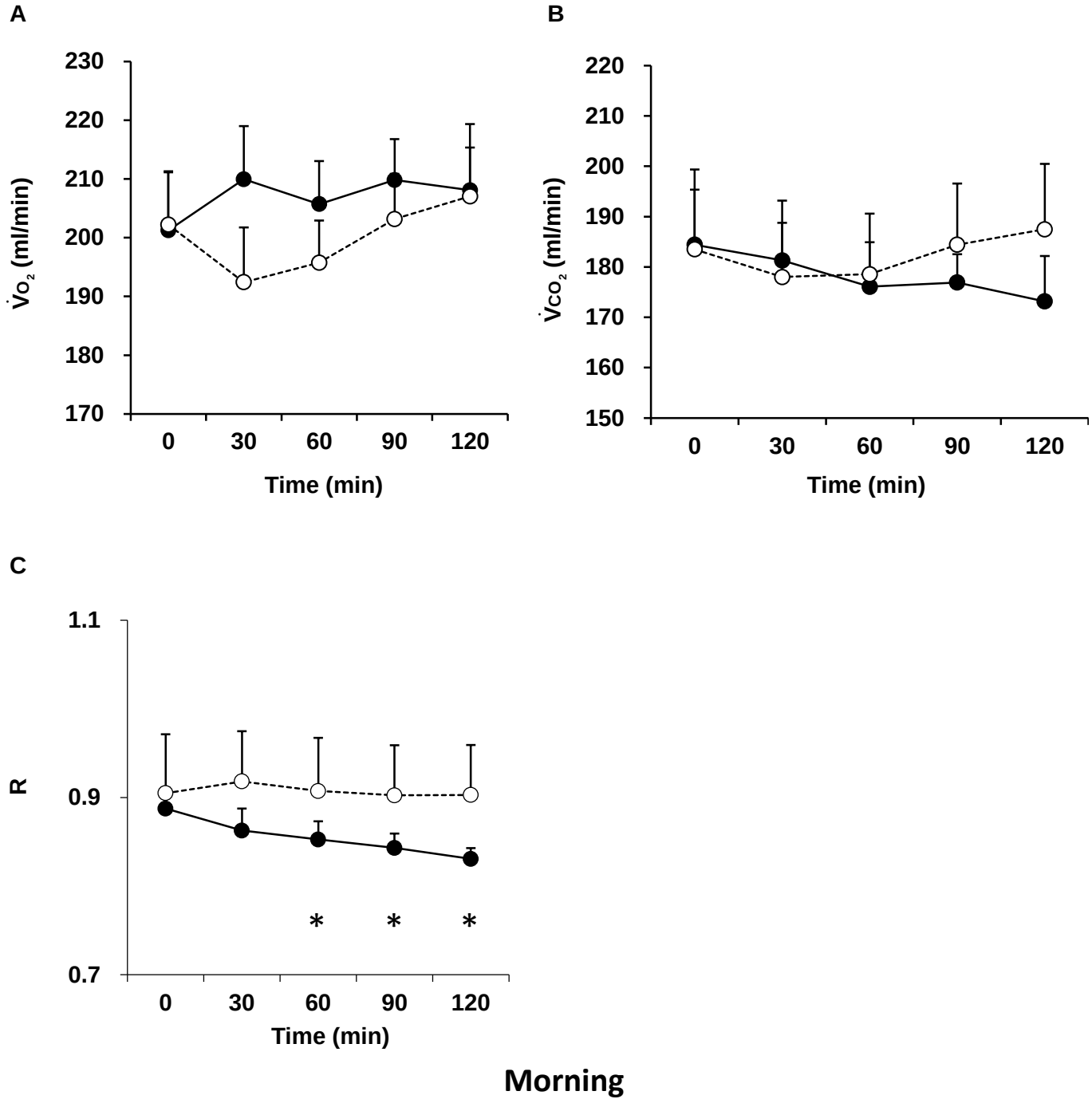

A
230
220
210
200
190
180
170
$\dot{V}O_2$ (ml/min)
0
30
60
90
120
Time (min)
B
220
210
200
190
180
170
160
150
$\dot{V}CO_2$ (ml/min)
0
30
60
90
120
Time (min)
C
1.1
0.9
0.7
R
*
*
*
0
30
60
90
120
Time (min)
Morning

Figure 4

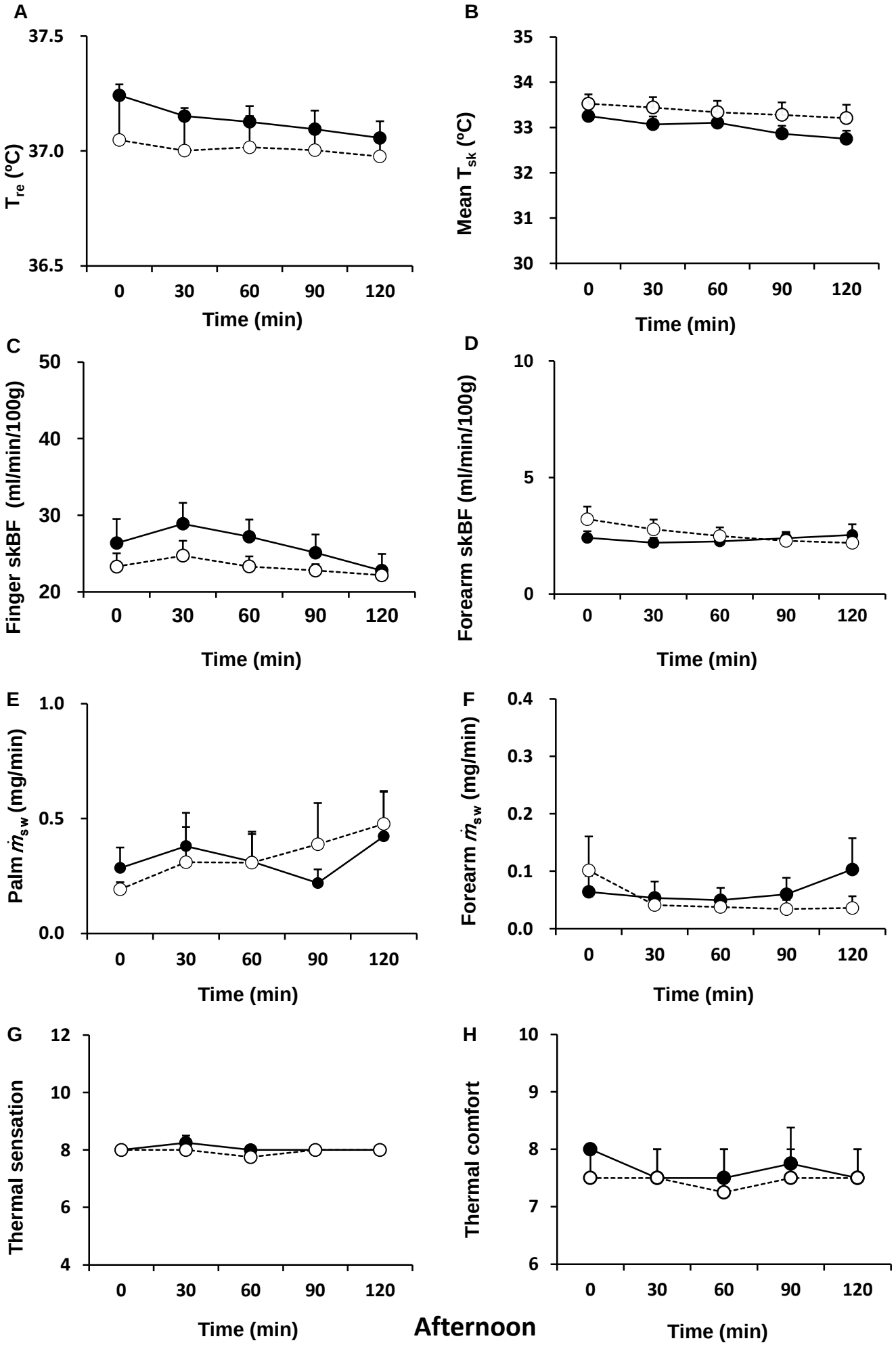

A
37.5
37.0
36.5
T_{re} (ºC)
0
30
60
90
120
Time (min)
B
35
34
33
32
31
30
Mean T_{sk} (ºC)
0
30
60
90
120
Time (min)
C
50
40
30
20
Finger skBF (ml/min/100g)
0
30
60
90
120
Time (min)
D
10
5
0
Forearm skBF (ml/min/100g)
0
30
60
90
120
Time (min)
E
1.0
0.5
0.0
Palm $\dot{m}_{sw}$ (mg/min)
0
30
60
90
120
Time (min)
F
0.4
0.3
0.2
0.1
0.0
Forearm $\dot{m}_{sw}$ (mg/min)
0
30
60
90
120
Time (min)
G
12
10
8
6
4
Thermal sensation
0
30
60
90
120
Time (min)
H
10
9
8
7
6
Thermal comfort
0
30
60
90
120
Time (min)
Afternoon

Figure 5

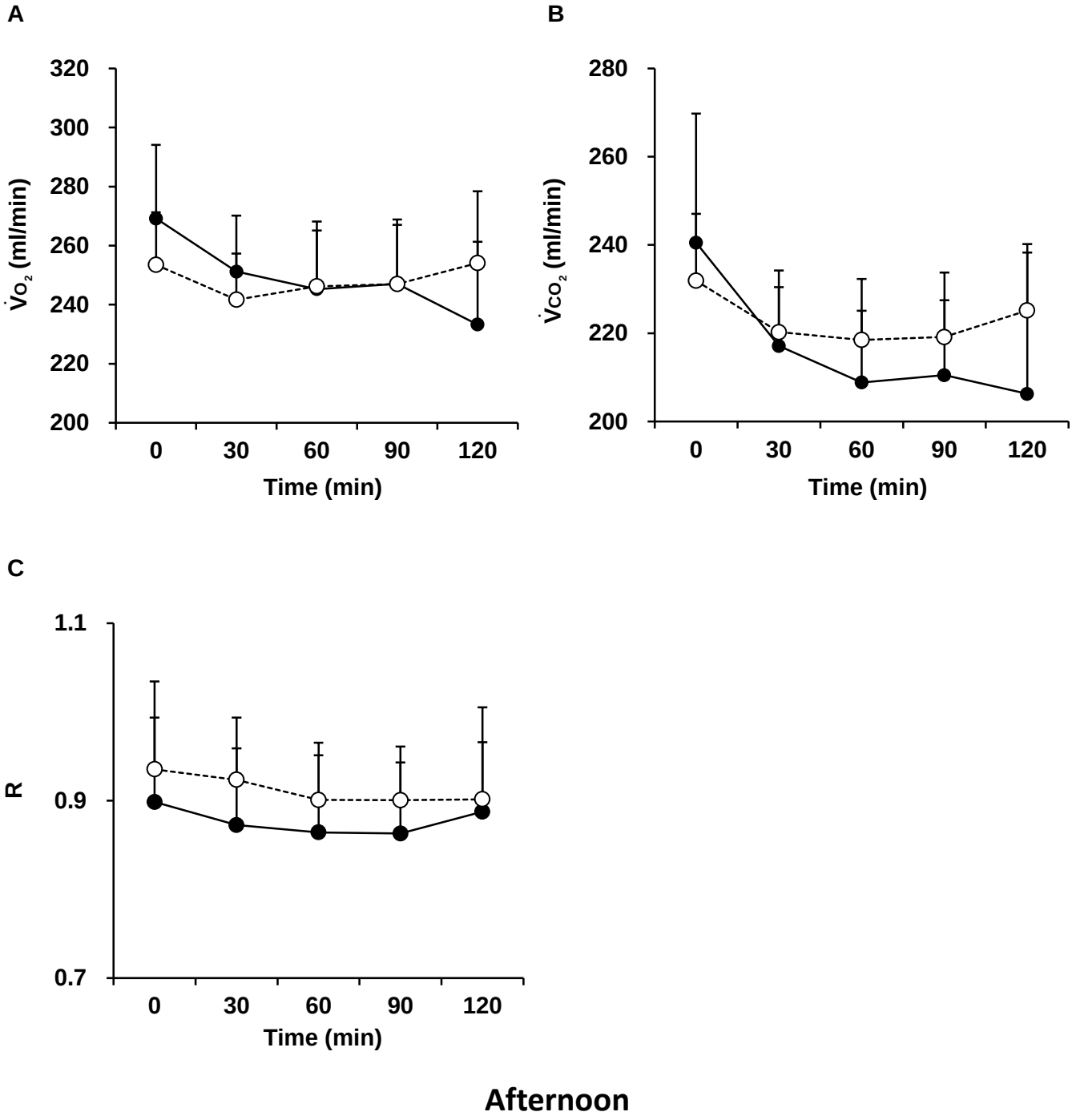

A
320
300
280
260
240
220
200
V̇O2 (ml/min)
0
30
60
90
120
Time (min)
B
280
260
240
220
200
V̇CO2 (ml/min)
0
30
60
90
120
Time (min)
C
1.1
0.9
0.7
R
0
30
60
90
120
Time (min)
Afternoon

Figure 6

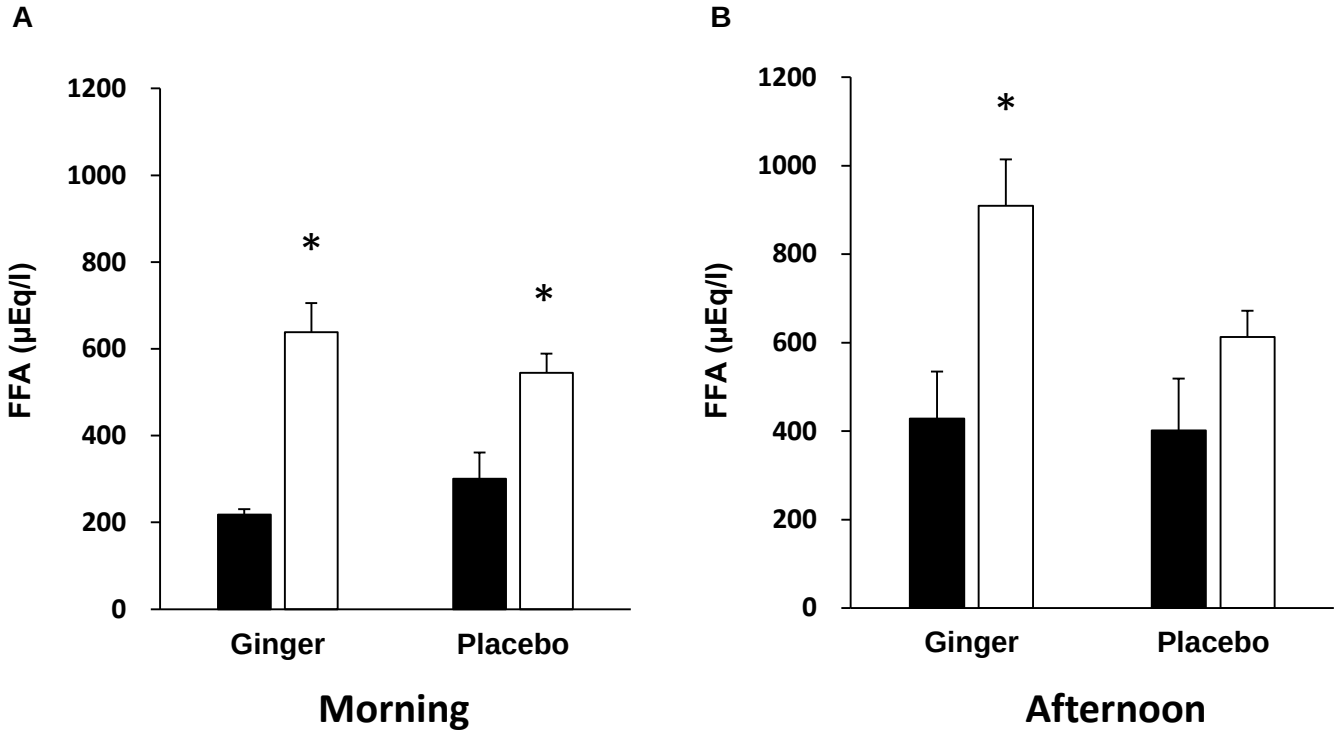

A
1200
1000
800
600
400
200
0
FFA (µEq/l)
*
*
Ginger
Placebo
Morning
B
1200
1000
800
600
400
200
0
FFA (µEq/l)
*
Ginger
Placebo
Afternoon

Figure 7

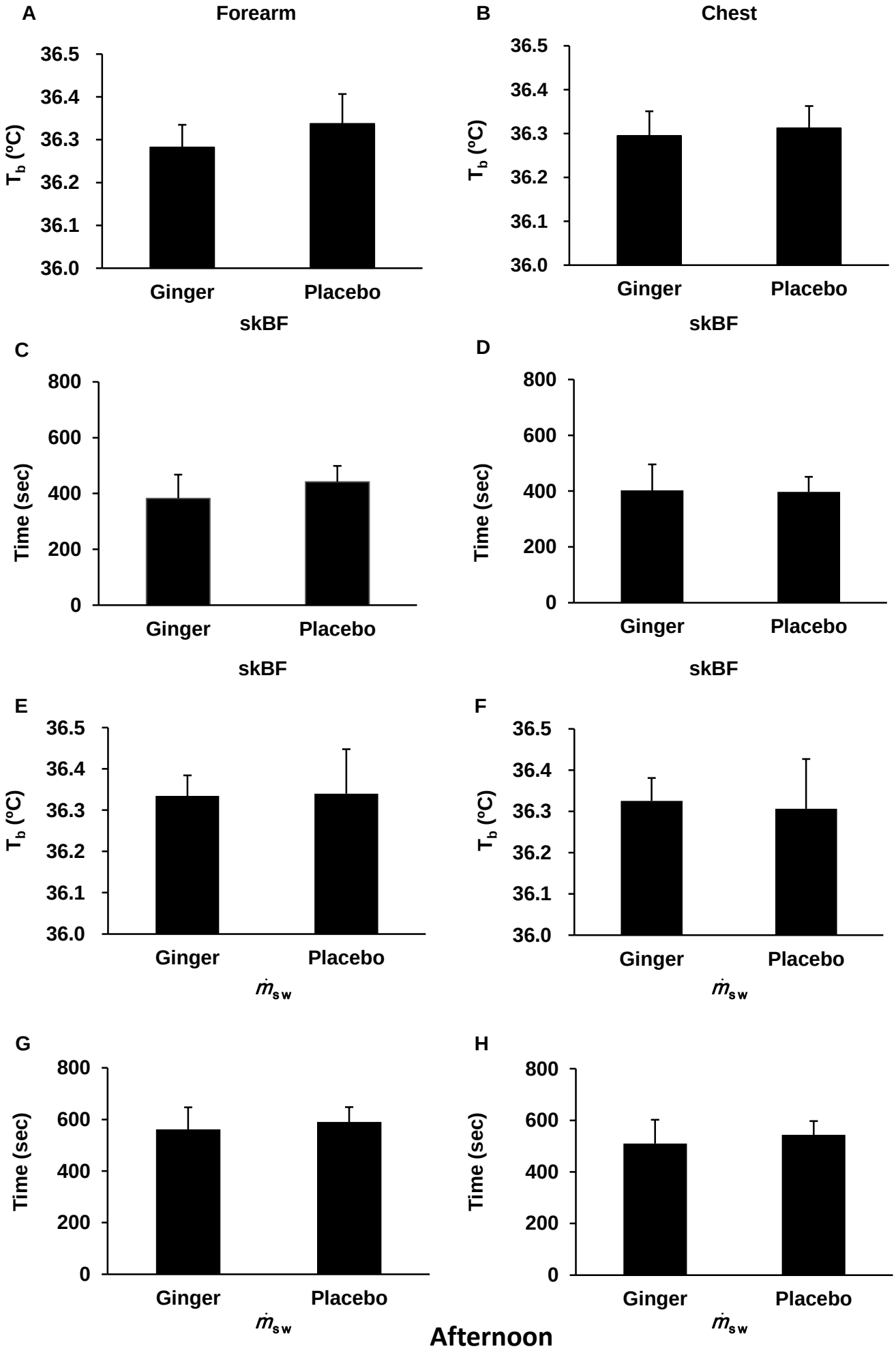
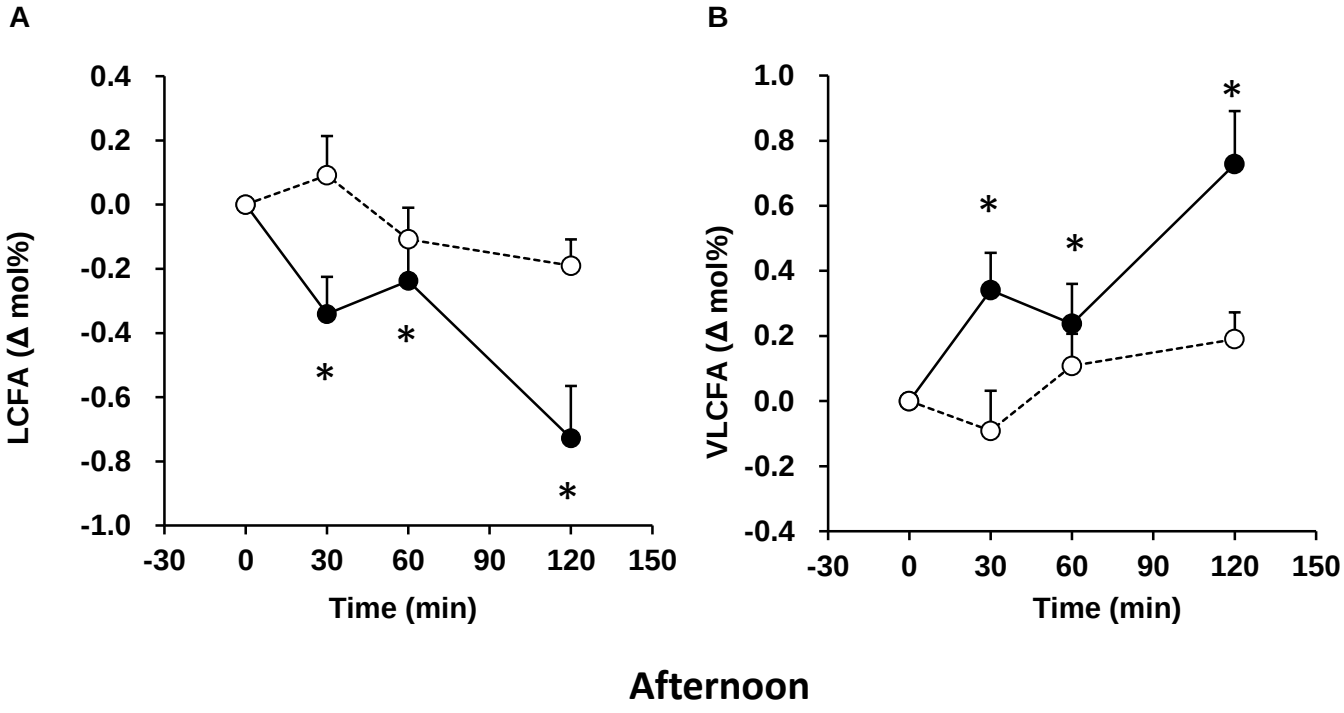
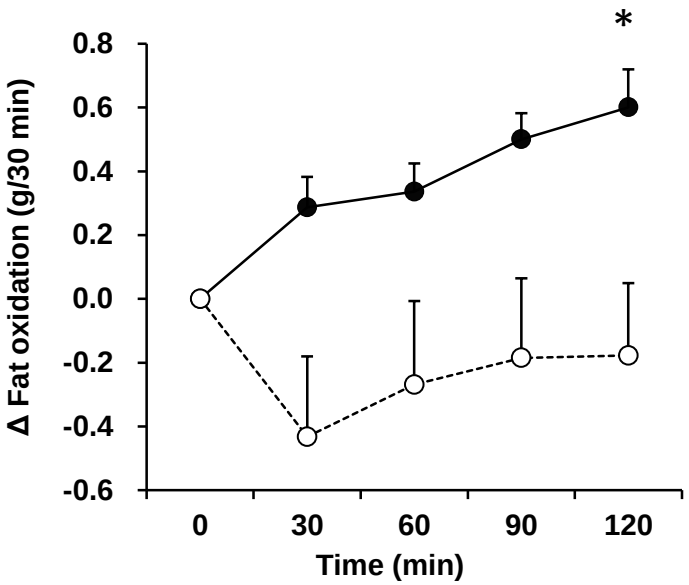

A
Forearm
36.5
36.4
36.3
36.2
36.1
36.0
T_b (°C)
Ginger
Placebo
skBF
B
Chest
36.5
36.4
36.3
36.2
36.1
36.0
T_b (°C)
Ginger
Placebo
skBF
C
800
600
400
200
0
Time (sec)
Ginger
Placebo
skBF
D
800
600
400
200
0
Time (sec)
Ginger
Placebo
skBF
E
36.5
36.4
36.3
36.2
36.1
36.0
T_b (°C)
Ginger
Placebo
$\dot{m}_{sw}$
F
36.5
36.4
36.3
36.2
36.1
36.0
T_b (°C)
Ginger
Placebo
$\dot{m}_{sw}$
G
800
600
400
200
0
Time (sec)
Ginger
Placebo
$\dot{m}_{sw}$
H
800
600
400
200
0
Time (sec)
Ginger
Placebo
$\dot{m}_{sw}$
Afternoon

Figure 8



Afternoon

Figure 9



Morning

Table 1 Main active components in the two batches of ginger powder used in this study

	Powder 1	Powder 2
6-gingerol (mg/g)	12.008	11.249
8-gingerol (mg/g)	3.491	2.813
10-gingerol (mg/g)	2.508	1.979
12-gingerol (mg/g)	0.003	0.019
6-shogaol (mg/g)	2.263	1.534
8-shogaol (mg/g)	0.154	0.288
10-shogaol (mg/g)	1.064	0.336

Table 2 Scales of thermal sensation and thermal comfort

Scale	Thermal sensation	Thermal comfort
2	Very cold	Very pleasant
4	Cold	Pleasant
6	Slightly cold	Slightly pleasant
8	Neither cold nor warm	Neither pleasant nor unpleasant
10	Slightly hot	Slightly unpleasant
12	Hot	Unpleasant
14	Very hot	Very unpleasant

Table 3 Biochemical data except FFA for blood samples of subjects before and after ginger or placebo ingestion in the morning tests.

	Ginger		Placebo	
	0 min	120 min	0 min	120 min
Na, mEq/l	141.0 ± 0.6	139.8 ± 0.4	140.2 ± 0.9	139.4 ± 0.8
K, mEq/l	4.0 ± 0.1	4.5 ± 0.1*	3.9 ± 0.1	4.5 ± 0.1*
Cl, mEq/l	103.3 ± 1.1	102.5 ± 0.6	103.0 ± 0.9	103.4 ± 1.6
Ca, mEq/l	9.3 ± 0.1	9.4 ± 0.1	9.3 ± 0.1	9.5 ± 0.2*
Osmolality, mOsm/l	280.2 ± 1.4	278.8 ± 1.7	280.2 ± 1.6	277.8 ± 1.2
Hematocrit, %	44.1 ± 1.2	45.7 ± 1.2*	45.4 ± 1.2	47.0 ± 0.9[#]
Glucose, mg/dl	88.8 ± 3.2	82.0 ± 2.0	90.2 ± 4.8	89.2 ± 2.8
Total protein, g/dl	7.1 ± 0.1	7.5 ± 0.2*	7.3 ± 0.2	7.7 ± 0.2*
Albumin, g/dl	4.5 ± 0.1	4.7 ± 0.1*	4.5 ± 0.1	4.7 ± 0.2*
Triglyceride, mg/dl	93.0 ± 22.2	93.0 ± 27.0	110.0 ± 44.9	78.0 ± 22.9
Total cholesterol, mg/dl	176.2 ± 20.5	185.8 ± 22.6*	182.0 ± 22.1	190.0 ± 23.2*
Cortisol, μg/dl	13.5 ± 2.4	11.1 ± 1.4	12.2 ± 2.0	10.2 ± 0.6
ACTH, pg/ml	19.6 ± 4.4	24.1 ± 3.4	16.2 ± 2.7	19.0 ± 2.5

Values are the means ± SEMs (n = 5). * Significantly different from values at 0 min ($P < 0.05$). 0 min, just before ginger or placebo ingestion; 120 min, 120 min after ginger or placebo ingestion. [#] n = 4.

Table 4 Biochemical data except FFA for blood samples of subjects before and after ginger or placebo ingestion in the afternoon tests.

	Ginger		Placebo	
	0 min	120 min	0 min	120 min
Na, mEq/l	140.8 ± 0.8	140.0 ± 0.6	139.3 ± 0.3	139.8 ± 0.9
K, mEq/l	3.9 ± 0.1	4.1 ± 0.2	3.9 ± 0.1	4.2 ± 0.1
Cl, mEq/l	103.3 ± 1.1	102.5 ± 0.6	103.0 ± 0.9	102.8 ± 1.4
Ca, mEq/l	9.4 ± 0.1	9.3 ± 0.1	9.3 ± 0.1	9.4 ± 0.2
Osmolality, mOsm/l	280.3 ± 1.0	277.5 ± 0.9*	280.3 ± 2.5	280.5 ± 2.1
Hematocrit, %	46.2 ± 0.7	46.9 ± 1.0	46.6 ± 1.1	47.1 ± 0.8[#]
Glucose, mg/dl	95.5 ± 4.8	84.5 ± 1.7	94.5 ± 7.4	85.0 ± 2.5
Total protein, g/dl	7.3 ± 0.2	7.6 ± 0.2	7.4 ± 0.1	7.6 ± 0.1*
Albumin, g/dl	4.6 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
Triglyceride, mg/dl	93.5 ± 12.3	81.8 ± 13.1*	153.5 ± 28.3	121.5 ± 23.8
Total cholesterol, mg/dl	163.3 ± 20.1	171.0 ± 21.0	152.0 ± 16.9	153.5 ± 18.8
Cortisol, μg/dl	9.2 ± 1.0	9.7 ± 1.8	9.0 ± 1.3	9.3 ± 1.1
ACTH, pg/ml	16.3 ± 2.3	21.4 ± 7.7	18.1 ± 5.6	17.7 ± 4.9

Values are the means ±SEMs (n = 4). * Significantly different from values at 0 min ($P < 0.05$). 0 min, just before ginger or placebo ingestion; 120 min, at 120 min after ginger or placebo ingestion. [#] n = 3.