



Consumption of ginger (*Zingiber Officinale Roscoe*) does not affect *ex vivo* platelet thromboxane production in humans

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Objectives: Ginger (*Zingiber Officinale Roscoe*) has been claimed to exert an anti-thrombotic effect in humans as ginger extracts inhibit cyclo-oxygenase activity of platelets *in vitro*. Effects of ginger consumption on *ex vivo* platelet function, however, are contradictory. We therefore investigated whether daily consumption of raw or cooked ginger decreases platelet cyclo-oxygenase activity as assessed by *ex vivo* maximally stimulated platelet thromboxane B₂ production.

Design: We carried out a randomized placebo-controlled cross-over study of 3×2 weeks.

Subjects: Eighteen healthy volunteers aged 22 ± 3 y (mean ± s.d.) participated in the study; there were no drop-outs.

Interventions: Subjects consumed 15 g of raw ginger root, 40 g of cooked stem ginger, or placebo daily for two weeks. We took fasted venous blood samples and measured thromboxane B₂ production in maximally stimulated platelet-rich plasma at days 12 and 14 of each treatment period.

Results: Mean decrease in thromboxane production relative to placebo was 1 ± 9% for ginger root, and -1 ± 8% for stem ginger, with no effect of treatment order ($P = 0.984$).

Conclusions: We cannot confirm the putative anti-thrombotic activity of ginger in humans.

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Descriptors: cyclo-oxygenase; ginger; humans; nutrition; thromboxane; platelets.

Introduction

We are interested in natural aspirin-like factors in foods as such factors may partly explain the relatively low incidence of cardiovascular diseases in oriental countries (Thom and Epstein, 1994; WHO MONICA Project, 1994; The World Health Organization MONICA Project, 1994).

It has been suggested that ginger exerts an anti-thrombotic activity (Srivastava and Mustafa, 1989), because ginger extracts inhibit platelet aggregation and thromboxane B₂ production *in vitro* (Srivastava, 1984; Srivastava, 1986). However data on effects of ginger consumption on blood platelet function, and specifically on cyclo-oxygenase activity, are scarce and contradictory (Dorso *et al*, 1980; Srivastava, 1989; Verma *et al*, 1993; Lumb, 1994).

We therefore performed a study investigating the effect of daily consumption of raw ginger root or cooked stem ginger on platelet cyclo-oxygenase activity as assessed by maximally stimulated *ex vivo* platelet thromboxane production in healthy volunteers.

Methods

Subjects

We recruited 24 subjects through announcements in the University newspaper and posters in student dormitories. We excluded one subject because of elevated values for serum creatinine and alanine amino transferase, and one

because of a marginally low value for mean red cell volume. We selected 9 men and 9 women at random from the remaining 22 subjects; all 18 volunteers completed the study.

Participants were healthy based on a medical questionnaire, none suffered from urinary protein or glucose, or high blood pressure. All had normal values for haematocrit, haemoglobin, mean red cell volume, erythrocyte sedimentation rate, plasma alanine amino transferase, gamma-glutamyl transferase, and creatinine, platelet count, thrombin time, prothrombin time, and activated partial thromboplastin time. The volunteers did not smoke and did not use any regular or homoeopathic medication. Mean age (± s.d.) was 22 ± 3 y and body mass index was 22 ± 2 kg/m².

We urged the participants not to consume any products containing ginger, or any fatty fish, and to maintain their normal eating and drinking habits and physical activity level during the study. We instructed subjects to avoid all regular and homoeopathic medicines from 1 month preceding the study until the end of the study. We supplied them with paracetamol which could be used as a pain medication. We urged subjects to record times of consumption of the supplements, health complaints, medications used, and any deviations from their normal physical activity and dietary habits in a diary.

The protocol was approved by the Medical Ethics Committee of the Department of Human Nutrition and was fully explained to the participants, who gave their written informed consent.

Design and treatments

We investigated the effects of daily consumption of ginger root and stem ginger relative to placebo on *ex vivo* maximally stimulated platelet thromboxane production. We carried out a randomized multiple cross-over study of 3 consecutive two-week periods during which each participant consumed all supplements in a different order. All subjects participated simultaneously.

Supplements consisted of 125 g of vanilla custard (Coberco, Arnhem, The Netherlands), containing either 15 g of Brazilian ginger root (Toko Rinus, Nijmegen, The Netherlands), 40 g of stem ginger (Ambition, Polak Import, Rotterdam, The Netherlands), or no ginger as a placebo. The custard served to mask the pungent taste of ginger. Stem ginger and ginger root originated from two separate batches. We bought vanilla custard at a local supermarket every few days and preserved all products in the dark at 4°C. We prepared all supplements in a similar way during the whole study.

We prepared supplements with raw ginger each working day: we thinly peeled ginger roots, cut them into small pieces (Magimix, Micave BV, Utrecht, The Netherlands), and weighed out portions of 15 g. We prepared the placebo and stem ginger supplements three times a week. We rinsed the stem ginger, cut it into pieces, and divided it into portions of 40 g. We weighed out portions of 125 g of vanilla custard.

Participants came to the Department on working days to consume their supplements of raw ginger, and took their supplements home for the weekends on Fridays. We handed out the placebo and stem ginger supplements three times a week. We supplied all supplements in closed boxes containing cooling elements, and instructed all subjects to keep their supplements in a refrigerator and to mix the custard with the ginger immediately prior to consumption.

We weekly determined body weights of the participants using a digital scale (Berkel ED 60-T, Rotterdam, The Netherlands), and checked the diary and measured food intake using a 24 h recall (Cameron and van Staveren, 1988; Stichting Nederlands Voedingsstoffenbestand, 1986).

Blood sampling and analysis

Venous blood was sampled using a butterfly needle system (Becton Dickenson, Meylan, France) after an overnight fast on days 12 and 14 of each treatment period while the participant was lying down. We discarded the first 3 mL of blood to prevent activation, and drew the next 18 mL slowly into 3.8% sodium-citrate tubes 1:10 v/v (Sarstedt, Etten-Leur, The Netherlands). We prepared platelet-rich plasma by centrifugation (Sigma, Osterode, Germany) at room temperature for 10 min at 200 g, removed the platelet-rich plasma, prepared platelet-poor plasma by centrifuging the residual blood for 15 min at 2000 g, and normalized the platelet-rich plasma to 250×10^9 platelets per litre by adding autologous platelet-poor plasma. We counted platelets before and after dilution (Coulter, Coulter Corporation, Miami, FL) and stimulated 450 μL of normalized platelet-rich plasma with arachidonic acid (final concentration 1.5 mM) (Bio Data Corporation, Horsham, USA) in an aggregometer (37°C, 900 rpm; Payton Aggregation Module, Salm en Kipp, Breukelen, The Netherlands). We then took out 50 μL of the aggregate exactly 10 min after the addition of arachidonic acid, added it to 950 μL buffer containing 9 g/L NaCl, 0.01 mol/L EDTA, 3 g/L bovine gamma-globulin, 0.005% Triton-X-100, and

0.05% sodium-azide in 50 mM phosphate buffer, pH 6.8 (NEN Research Products, Du Pont, Boston, MA), immediately submerged the samples in liquid nitrogen, and stored them at -80°C until analysis. We measured thromboxane B_2 production in duplicate using a thromboxane B_2 [^{125}I] RIA kit (NEN Research Products, Du Pont, Boston, MA). We analyzed all samples from a particular subject within one run. Within person variation over a 2-day period was 12% after placebo, 10% after raw and 8% after cooked ginger treatment.

Plasma of two healthy volunteers who had not used any medication for at least one month served as quality control for thromboxane measurements. We once sampled venous blood from these volunteers, and stimulated *ex vivo* platelet thromboxane B_2 production in platelet rich plasma as described above. We stored aliquots of aggregates, and measured thromboxane B_2 production at every radio immuno assay. Within assay variation for thromboxane measurements was 9%; between run variation was 9%.

Statistics

We checked the data for normality using residual analysis (Snedecor and Cochran, 1989). We averaged the two values of thromboxane production obtained for each subject on days 12 and 14 of each treatment period, and analyzed differences in thromboxane B_2 production, body weight, and intake of energy and macronutrients between treatments using the General Linear Models of the Statistical Analyses System (SAS Institute Inc., 1989) with subject and treatment as class variables; we used $P < 0.05$ to indicate significant difference. We introduced a period term into the model to check for time effects, and a treatment-by-period interaction term to check for carry-over effects. Values in the text are means \pm s.d.

Results

Daily treatment with 15 g of ginger root or 40 g of stem ginger for 14 days did not affect maximum *ex vivo* platelet thromboxane B_2 production ($P = 0.616$). Mean thromboxane production was 2994 ± 566 nmol/ 10^{11} platelets after treatment with raw ginger, 3044 ± 546 after cooked ginger, and 3045 ± 609 after placebo. The average effects on platelet thromboxane B_2 production relative to placebo were $-1 \pm 9\%$ (\pm s.d.) on raw ginger, and $1 \pm 8\%$ on cooked ginger. There was neither a treatment sequence ($P = 0.984$), nor a time effect ($P = 0.932$).

The habitual diet of the subjects as measured by 24 h recall supplied on average 11 ± 5 MJ/d (2629 ± 1195 kcal/d, mean \pm s.d.), of which $32 \pm 9\%$ was fat, $13 \pm 3\%$ protein, $53 \pm 8\%$ carbohydrate, and $2 \pm 3\%$ alcohol. Mean body weight increased by 0.3 ± 1.1 kg during the study. There were no changes in intake of macronutrients ($P = 0.12$ for fat; 0.10 for carbohydrate; 0.73 for protein; 0.84 for alcohol) and energy ($P = 0.23$) between treatment periods. Subjects did not consume any fatty fish. All participants consumed the raw ginger supplements under our supervision on working days and reported that they had consumed all other supplements at home. There was no evidence from the diaries of changes in physical activity patterns or any deviation that might have affected the results.

No adverse reactions to the supplements were reported. One subject took 7 tablets of Paracetamol spread over 3 consecutive days because of toothache, and another took 2

tablets per day for 2 consecutive days because of a headache. One subject took 2 × 20 mg of Temazepam during treatment with stem ginger, and 4 × 20 mg during treatment with ginger root. Temazepam is a sedative and was taken because of a sleeping disorder unrelated to the study. All participants denied having used acetylsalicylic acid from 1 month preceding the study until the end of the study.

Discussion

We found that daily consumption of large amounts of ginger did not affect *ex vivo* platelet thromboxane production in healthy volunteers. Data from the diaries as well as data on food consumption did not reveal any confounding effects of medication, physical activity levels, or dietary patterns. We bought all ginger at once before the study to exclude differences between batches, stored the products in the dark at 4°C to diminish changes in composition of the ginger, and prepared the supplements in a standardized way (see Methods section) to prevent differences in composition of the supplements during the study. The compounds known to affect *in vitro* platelet activity (gingerols, shogaols and zingerone) generally exist in the fleshy centre of the rhizome (Fulder, 1993); we used these parts of the ginger in the raw ginger supplements. We earlier showed in similar volunteers that even as little as 3 mg/d of acetylsalicylic acid decreased maximally stimulated *ex vivo* thromboxane B₂ production by 39 ± 8% (Janssen *et al.*, 1995); we could thus calculate that the power of this study was 90% to pick up an effect of 6% or more.

Our data disagree with results of Dorso (1980) and Verma *et al.* (1993). Dorso (1980) described a total inhibition of stimulated *ex vivo* platelet aggregation after consumption of large amounts of ginger-grapefruit marmalade; this study was done in only one subject, and the result may be due to chance. Verma *et al.* (1993) showed that consumption of butter increased platelet aggregation induced by adenosine diphosphate (ADP) and epinephrine, and that this increase was neutralized by simultaneously administering 5 g of powdered ginger for 7 d. Our results are in accordance with *ex vivo* results of Lumb (1994) and Srivastava (1989). Lumb (1994) found no effects of consumption of 2 g of dried ginger on bleeding time and whole blood platelet aggregation. Platelet aggregation and bleeding time measurements, however, are much less specific for determination of platelet cyclo-oxygenase activity than measurement of platelet thromboxane production; results of Verma *et al.* (1993) may indicate that ginger consumption affects secretion in platelets (George and Shattil, 1991). Lumb (1994) could have missed possible long-term effects of ginger consumption, as he studied effects of only a single dose. It is highly unlikely that we missed possible long-term effects as blood platelets have a mean life-time of 7–10 d. This indicates that our daily treatment with high doses of ginger during 14 d must have been long enough to trace effects on platelet thromboxane production. Srivastava (1989) found no significant effect of consumption of 5 g/d of raw ginger for 7 days on serum thromboxane production in 7 healthy volunteers, but the power of

Srivastava's study was too low to pick up relevant effects due to the large variation in the outcome variable.

We fed the participants 15 g of raw ginger root or 40 g of stem ginger. These are very high doses. Consumption of higher doses is impractical because of the pungent taste. If any inhibitors of cyclo-oxygenase activity are present in ginger they are probably not absorbed or are rendered ineffective during first-pass metabolism in the body. We think it is unlikely that daily consumption of raw or cooked ginger affects platelet cyclo-oxygenase activity. However, it remains worthwhile to search for other possible aspirin-like agents in foods.

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