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Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers

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Fruits and vegetables have been thought to be beneficial in cardiovascular disease. The beneficial effects of fruits and vegetables may be explained by the antioxidants and other components contained therein. These nutrients may function individually or in concert to protect lipoproteins and vascular cells from oxidation, or by other mechanisms such as reducing plasma lipid levels (LDL cholesterol, triglycerides), and platelet aggregation response. Kiwi fruit which contains high amounts of vitamin C, vitamin E and polyphenols may be beneficial in cardiovascular disease; however very little is known about its cardioprotective effects. Platelets are involved in atherosclerotic disease development and the reduction of platelet activity by medications reduces the incidence and severity of disease. To this end, we evaluated whether consuming kiwi fruit modulated platelet activity and plasma lipids in human volunteers in a randomized cross-over study. We report that consuming two or three kiwi fruit per day for 28 days reduced platelet aggregation response to collagen and ADP by 18% compared with the controls ($P < 0.05$). In addition, consumption of kiwi fruit lowered blood triglycerides levels by 15% compared with control ($P < 0.05$), whereas no such effects were observed in the case of cholesterol levels. All these data indicate that consuming kiwi fruit may be beneficial in cardiovascular disease.

Introduction

Many risk factors for cardiovascular disease (CVD) including platelet hyperactivity, high blood cholesterol, triglycerides, obesity and diabetes are substantially influenced by dietary factors. Since these risk factors are modifiable therefore primary preventive efforts through dietary means hold much promise.¹ High intake of fruit and vegetables is such an important preventive measure by which the risk of cardiovascular disease can be reduced. The American Heart Association and other national agencies recommend a diet that includes ≥ 5 servings of fruit and vegetables daily.^{2,3} These recommendations are based primarily on the studies indicate that fruit and vegetable intake may reduce CVD risk through the beneficial combinations of micronutrients, antioxidants, phytochemicals, and fiber in fruits and vegetables.⁴⁻⁷ A decreased oxidative modification of

LDL may be one of the mechanisms by which fruits and vegetables reduce the risk of CVD and atherosclerotic progression. However, hyperactivity of platelets, and their adhesion and aggregation at the site of injury in atherosclerotic vessel walls, is also critically important in the pathogenesis of CVD.⁸⁻¹⁰ There is also an increasing evidence that acute clinical manifestations of coronary atherosclerotic disease are caused by plaque disruption and subsequent platelet–thrombus formation.¹¹⁻¹³ Coronary atherosclerotic lesions may appear in early life and, towards the end of the second decade of life, asymptomatic atherosclerotic lesions are present in most people living in industrialised societies.^{12,14} Therefore, platelet activity may play a major role in the development as well as in the stability of atherosclerotic plaques. In support of the pathophysiological role of platelets, platelet inhibitory drugs such as aspirin reduce the incidence of myocardial infarction, stroke and death from CVD in secondary prevention trials.¹⁵⁻¹⁸

Flavonoids are polyphenolic compounds, widely distributed in fruit and vegetables, many of which have antioxidant as well as anti-thrombotic properties.¹⁹⁻²² Certain polyphenols have been shown to be inhibitory to platelet aggregation than others, and

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consequently dietary studies involving flavonoids on anti-platelet effects are not conclusive.^{23–25} The difference in platelet inhibitory effects among polyphenols could be due to several reasons. Variations in digestion, absorption, and hepatic processing may limit the bioavailability of these compounds. It is also possible that compounds other than the polyphenols may be responsible for the platelet inhibitory activity. We recently examined the anti-platelet activity of several fruits and this activity was not related to the antioxidants potential of fruits.^{9,26} Despite the fact that kiwi fruit contain very significant amounts of antioxidants such as polyphenols and vitamins C and E,^{27,28} it was not included in the study. Recently it has been shown that kiwi fruit provide a dual protection against oxidative DNA damage, enhancing antioxidant levels and stimulating DNA repair and may be beneficial in cancer;²⁹ however, no information is available on the effects of kiwi fruit on platelet activity and plasma lipids, these two important risk factors of CVD, in human volunteers.

In this paper we report that consuming of two or three kiwi fruit per day for 28 days significantly reduced platelet aggregation in human volunteers in a randomized cross-over design. Moreover, plasma triglycerides levels were also reduced in these volunteers. Our study indicates that consuming kiwi fruit (two or three per day) may be beneficial in CVD.

Materials and methods

Materials

Collagen, ADP and arachidonic acid were obtained from Chrono-Log (Havertown, USA). Uric acid, ascorbic acid, EDTA, acetic acid, sodium hydroxide, metaphosphoric acid and phosphate-buffered saline (PBS) tablets were obtained from Sigma (Poole, UK). All other reagents used were of analytical grade quality.

Subjects

Thirty (12 males and 18 females) healthy volunteers took part in this study. Volunteers were aged 20–51 years and their mean BMI was 22.43 ± 0.52 kg/m². The volunteers were recruited by means of advertisements at the Institute for Nutrition Research, University of Oslo, Oslo, Norway. Volunteers were requested to maintain normal diet during the trial. Subjects were allocated randomly to two groups ($n = 15$), each of which was given different order of kiwi fruit doses. Group A took two kiwi fruit per day in the first period and three kiwi fruit per day in the second period, whereas group B took two and three kiwi fruit per day. Each volunteer consumed two and three kiwi fruit per day for successive 28-day periods separated by at least 2-week wash-out periods. The study protocol was approved by the Ethical Committee of Ullevål University Hospital, Oslo.

Healthy subjects were recruited after assessment of their medical and dietary history. Exclusion criteria were the presence of overt vascular, haematological or respiratory disease, hypertension, infection, frequent consumption of drugs which affect platelet function (e.g., aspirin, paracetamol, ibuprofen, steroid, habitual consumption of omega-3 fatty acid supplements).

Preparation of kiwi fruit extract

In order to investigate the effects of kiwi fruit extract on human platelet aggregation *in vitro*. Extracts consisting of 100% fruit juice were freshly prepared on the day of the assay from kiwi fruit. To prepare 100% fruit juice, the fruits were peeled and the pulp after being weighed was homogenised. The resulting homogenate was centrifuged at $3000 \times g$ for 10 min. The supernatant was collected and the pH was adjusted to 7.4 with sodium hydroxide. The pH-adjusted kiwi fruit extract (KFE) was then used for *in vitro* platelet aggregation experiments.

Platelet aggregation study

Overnight fasted blood was collected through siliconized needles into plastic syringes: coagulation was prevented by mixing 9 vol of blood with 1 vol sodium citrate (final concentration, 13 mM). Platelet-rich plasma (PRP) was obtained by centrifugation of citriated blood for 15 min at $180 \times g$. The supernatant platelet-rich plasma ($2\text{--}3 \times 10^8$ cells/ml) was collected and used for platelet aggregation.²⁴

Platelet aggregation was monitored on a platelet aggregometer (Chrono-Log) at a constant stirring speed of 1000 rpm at 37°C, as described before.²⁴ The aggregating agents used were ADP (4 and 8 μM), and collagen (4 and 8 μg/ml). Platelet aggregation was followed over 10 min. Platelet aggregation was performed within 2 h after the blood was taken. Platelet aggregation is expressed as the area under the curve. To determine the effect of KFE on platelet aggregation *in vitro*, PRP (450 μl) was incubated with 50 μl of KFE for 15 min at 37°C prior to the addition of an aggregating agent. Since maximal amplitude of aggregation of PRP was obtained with either 4 μg/ml collagen, 8 μM ADP, and 500 μg/ml arachidonic acid, these concentrations of agonists were used and aggregation was followed as described above. Controls were run in parallel replacing fruit extract with 50 μl of PBS. Inhibition of platelet aggregation is expressed as the decrease in the area under the curve compared with the control.

In order to determine the effects of consumption of kiwi fruit on platelet aggregation response in human volunteers, blood samples were taken at study entry (day 0), and after day 28 of fruit consumption, between 08:00 and 10:00 h after an overnight fast. PRP was prepared for platelet aggregation experiments as described before.

Determination of plasma vitamin C

Blood was collected in heparin on ice and was centrifuged at $2000 \times g$ for 10 min at 4°C . Then $600 \mu\text{l}$ of plasma were mixed with $600 \mu\text{l}$ of 10% metaphosphoric acid in a tube and was snap frozen. The samples were stored at -80°C until required for determination of total vitamin C concentration (vitamin C and dehydroascorbic acid) by HPLC, as published before.³⁰

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1997).³¹ The FRAP assay was used to measure the concentration of total antioxidants in plasma using Technicon RA 1000 system (Technicon Instruments Corporation, New York, USA). An intense blue colour with absorption maximum at 600 nm was measured.

Determination of plasma lipids

Plasma triglycerides, HDL and total cholesterol concentrations were determined using reagents from Genzyme diagnostics (UK) following the protocol. LDL-cholesterol was calculated using the Friedwald formula.³²

Statistical analysis

Results are presented as the mean \pm SEM. Results were analysed by the Student's *t*-test. Values for the area under the curve for platelet aggregation in the absence and in the presence of different concentrations of

fruit extract were evaluated by a computer-assisted programme. Other statistical analyses were performed using ANOVA where appropriate, values were considered to be significantly different when $P < 0.05$.

Results

Effects of kiwi fruit extract on human platelet aggregation *in vitro*

One hundred percent kiwi fruit extract (KFE) (w/v) was used in this study. Incubation of KFE (expressed as weight of pulp used to prepare KFE) inhibited ADP-induced platelet aggregation in a dose-dependent manner (Figure 1). ADP-induced aggregation was inhibited by 11% with 5 mg KFE, 54% with 10 mg KFE, and 96% with 20 mg KFE, compared with controls. KFE also inhibited collagen-induced platelet aggregation, however, the level of inhibition was lower with the 5- and 10-mg KFE incubations compared with those observed with ADP-induced platelet aggregation (Table 1). Inhibition of arachidonic acid-induced platelet aggregation exhibited a very different profile with only 32% inhibition at the highest KFE level (20 mg) tested, and nothing at all at the lower concentrations of KFE. The IC_{50} (minimum concentration required for 50% inhibition of platelet aggregation induced by ADP in $500 \mu\text{l}$ PRP) of KFE extract was around 10 mg KFE. The KFE extract inhibited both collagen- and ADP-induced platelet aggregation to a greater extent, whereas it had very little inhibitory effects on arachidonic acid-induced aggregation.

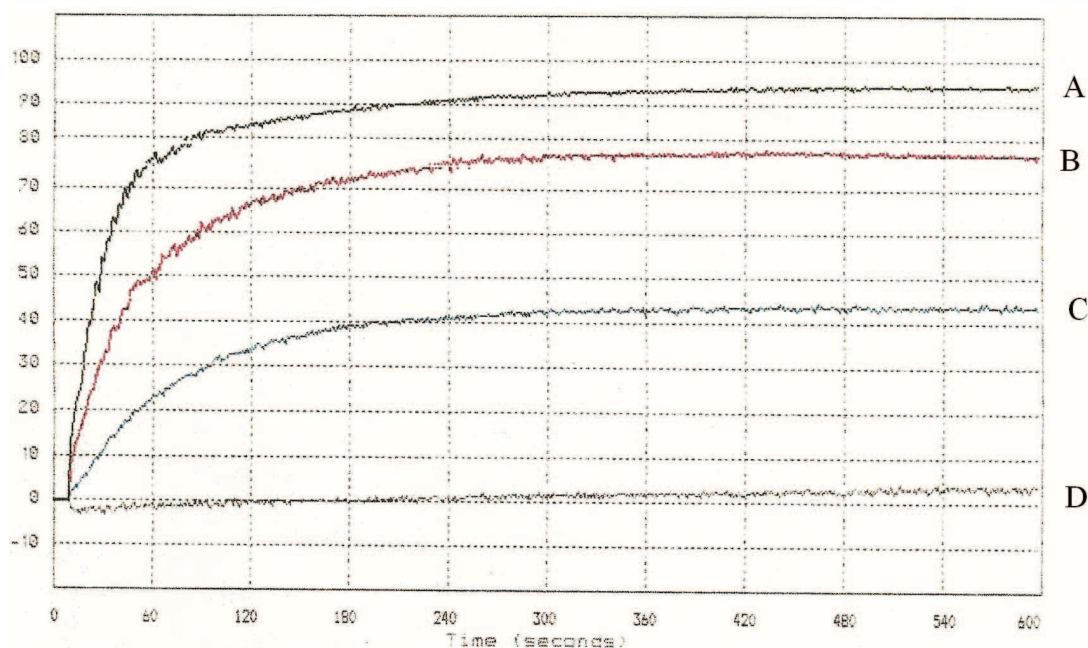
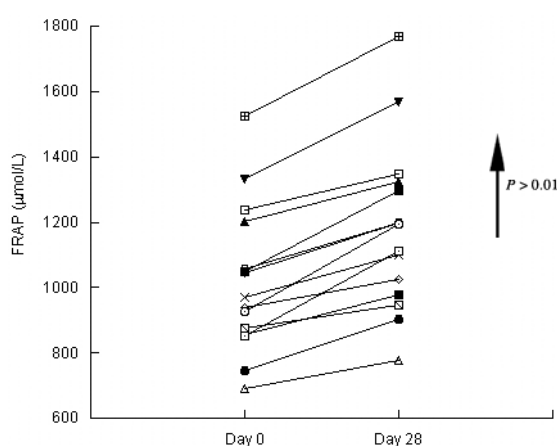


Figure 1. Effect of different amounts of kiwi fruit extracts on platelet aggregation by ADP *in vitro*. PRP ($450 \mu\text{l}$) was incubated with different amounts (0, 5, 10, and 20 mg) of kiwi fruit extract for 15 min at 37°C prior to the addition of ADP ($8 \mu\text{M}$). For details, please see Materials and methods. (A) Control; (B) 5 mg KFE; (C) 10 mg KFE; and (D) 20 mg KFE.

Table 1. Mean percentage inhibition of platelet aggregation *in vitro* by kiwi fruit extracts induced by three different aggregating agents

Kiwi fruit extract (mg)	Inhibition of platelet aggregation (%)		
	Arachidonic acid (500 µg/ml)	Collagen (2 µg/l)	ADP (8 µM)
5	0	0.53 ± 0.21	11 ± 4.3
10	0	18 ± 4.5	54 ± 12
20	32 ± 8	96 ± 11	96 ± 14

For details please see Materials and methods.

**Figure 2.** Effect of consuming kiwi fruit on plasma FRAP values in volunteers. FRAP was measured in the plasma of volunteers at day 0 and after consuming two kiwi fruit per day for 28 days. For details, please see Materials and methods.

Effects of consumption of kiwi fruit on plasma antioxidants in human volunteers

Of these 30 subjects, all except two volunteers completed the two phases of kiwi fruit consumption. During the supplementation period no significant change in their mean BMI was observed. The kiwi fruit were well tolerated without any adverse effect. Dietary antioxidants (antioxidant levels, and total vitamin C) were measured in plasma before and after the trial period. Figure 2 shows that the plasma levels of FRAP increased significantly after two or three kiwi fruit per day. FRAP levels were increased very significantly in both the groups after consuming fruit (two or three kiwi fruit per day) ($P < 0.01$), although no statistically significant differences between these two groups were observed. Similar to FRAP values, plasma vitamin C levels in these volunteers were also increased significantly, increasing from $65.92 \pm 9.0 \mu\text{M}$ (day 0) to $102 \pm 23.1 \mu\text{M}$ (day 28) ($P < 0.01$) and from $68 \pm 15.2 \mu\text{M}$ to $92.38 \pm 14.7 \mu\text{M}$ ($P < 0.01$) after consuming two and three kiwi fruit per day, respectively.

Platelet aggregation

Table 2 shows the platelet aggregation response to different concentrations of ADP and collagen at day

Table 2. Effect of kiwi fruit consumption on platelet aggregation

Agents	Two kiwi fruit per day		Three kiwi fruit per day	
	Day 0	Day 28	Day 0	Day 28
	% Aggregation			
ADP, 4 µM	70 ± 3.2	53 ± 3.2*	69 ± 1.8	55 ± 2.1*
ADP, 8 µM	69 ± 3.1	55 ± 4.1*	68 ± 2.3	52 ± 3.2*
Collagen, 4 µg/ml	68 ± 4.2	52 ± 2.3*	67 ± 3.1	53 ± 3.1*
Collagen, 8 µg/ml	70 ± 3.9	51 ± 4.3*	70 ± 2.2	50 ± 3.5*

For details please see Materials and methods, * $P < 0.05$.

0 and at 28 days after consuming two or three kiwi fruit per day. Platelet response to both low and high concentrations of ADP or collagen was inhibited by kiwi fruit consumption. Consuming two kiwi fruit inhibited ADP-induced platelet aggregation significantly (18% in case of 4 µM ADP and 15% in case of 8 µM ADP compared with those at day 0 ($P < 0.05$)). Similar reduction in platelet aggregation in response to collagen was observed when volunteers consumed two or three kiwi fruit per day for 28 days (Table 2). After the wash out period (minimum 2 weeks) platelet aggregation response returned to the base line level (day 0).

Plasma lipids

Table 3 shows the values of plasma concentrations of total cholesterol, HDL, LDL, and triglycerides at day 0 and day 28. Mean total cholesterol, LDL, and HDL cholesterol values in all groups were unchanged from days 0 to 28 in both groups, whereas triglyceride concentrations were significantly lowered on day 28 ($1.16 \pm 0.45 \text{ mmol/l}$ at day 0 vs. $0.87 \pm 0.29 \text{ mmol/l}$, $P < 0.05$, at day 28 and 1.19 ± 0.35 vs. $0.84 \pm 0.35 \text{ mmol/l}$, $P < 0.05$, in the case where volunteers consumed two and three kiwi fruit per day, respectively), compared with those on day 0 in both groups ($P < 0.05$). After the wash-out period (minimum 2 weeks), plasma triglyceride concentrations returned to the base line level.

Discussion

The kiwi fruit extracts inhibited both ADP and collagen-induced platelet aggregation *in vitro*, but not arachidonic acid-induced aggregation, indicating that the thromboxane pathway does not appear to be involved, as we observed in the case of tomato extract.²⁶ This is quite different than that of aspirin's mode of action in platelets. Aspirin's anti-platelet action involves inhibition of the cyclooxygenase enzyme in platelets, leading to a decreased formation of prostaglandin G_2 , a precursor of TxA_2 .^{17,18} This blocks the formation of TxA_2 , a platelet aggregation agonist. Since kiwi fruit extract does not inhibit the

Table 3. Effects of kiwi fruit consumption on plasma lipids

Plasma lipids	Two kiwi fruit per day		Three kiwi fruit per day	
	Day 0	Day 28	Day 0	Day 28
	mM			
Total cholesterol	5.14 ± 0.87	5.20 ± 0.87	5.12 ± 0.77	5.22 ± 0.74
HDL cholesterol	1.55 ± 0.43	1.57 ± 0.34	1.59 ± 0.40	1.60 ± 0.36
LDL cholesterol	3.28 ± 0.76	3.32 ± 0.81	3.23 ± 0.81	3.24 ± 0.83
Triglycerides	1.16 ± 0.45	0.87 ± 0.29*	1.19 ± 0.35	0.84 ± 0.35*

For details please see Materials and methods, * $P < 0.05$.

thromboxane pathway, it is possible that the mode of action is upstream of platelet activation/aggregation processes. At present we do not know the nature of the anti-platelet factors in kiwi fruit.

This study was, therefore, designed to assess the effects of kiwi fruit consumption on the cardiovascular risk profiles in healthy volunteers. Kiwi fruit consumption increased plasma antioxidants and vitamin C levels in these volunteers as expected. Consuming two or three kiwi fruit reduced platelet aggregation to a similar extent. In addition, consumption of kiwi fruit reduced plasma triglyceride levels without affecting cholesterol levels in these volunteers. There were no correlations between individual changes in platelet aggregation response and plasma lipids and vitamin C values. In addition, the magnitude of these effects of kiwi fruit is not related to the number of kiwi fruit consumed. We do not have any explanation for this. The inhibitory effects on platelet aggregation response and lowering effects on plasma triglyceride levels of kiwi fruit disappeared during the wash-out period. This indicates that the effects of kiwi fruit on platelets and plasma lipids are reversible. We restricted our study with relatively small number of kiwi fruit (two, or three), as consuming larger number of fruits is known to have laxative effect. However, further work is necessary to determine the structure and mechanism of action of the active principles in kiwi fruit responsible for lowering platelet aggregation response as well as plasma triglycerides in humans.

Epidemiological studies as well as laboratory experiments have provided very strong evidence that fruits and vegetables and specific antioxidants are beneficial for human health.^{33,34} The protective effects of vegetables and fruit may in part be attributed to antioxidant vitamins and specific polyphenols that display powerful inhibition of oxidative stress. However, we have earlier reported that the anti-platelet potential of the fruits appeared to be unrelated to their antioxidant activity.^{9,24,26} Although further research on identification of the active compounds requires for definitive conclusions.

Given the role of blood lipids in the development of atherosclerosis and CVD and the positive roles of

fruits and vegetables on plasma lipids,^{35–37} we have also assessed the effects of kiwi fruit on plasma lipids. Lowering of plasma triglycerides by kiwi fruit was observed despite these volunteers maintaining their regular diet during the supplementation periods. None of the volunteers reported any loss of appetite or changes in their food intake during the supplementation. The mechanism of action is not known; it is possible that the presence of certain components in kiwi fruit may be responsible for the lipid-lowering effects. However, further work is required to understand the mechanism of action. It must be noted that the lipid-lowering effects of fruits and vegetables were also reported before.^{35–37}

In conclusion, consuming kiwi fruit is an effective way of inhibiting platelet aggregation induced by collagen and ADP in human volunteers. Our data thus provide evidence that consuming kiwi fruit has the potential to increase the effectiveness of thrombosis prophylaxis. Modulation of platelet reactivity towards collagen, ADP and plasma triglyceride levels by kiwi fruit could be of potentially prophylactic and therapeutic benefit in preventing and halting pathological processes that lead to CVD.

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