

## Dietary Modulation of Serum Platelet-derived Growth Factor-AB Levels<sup>1</sup>

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### Abstract

Epidemiological studies have demonstrated that diets high in vegetables and fruit are associated with a decreased risk of cancer and, possibly, cardiovascular disease. Certain constituents of vegetables and fruit inhibit the *in vitro* activity of platelet-derived growth factor (PDGF), a potent mitogen implicated in both cancer and cardiovascular disease. Few studies have measured PDGF in relationship to diet *in vivo*. Specifically, there are no data regarding changes in PDGF levels or mitogenic activity after a dietary intervention. In this study, 19 young, healthy individuals consumed four (9-day) experimental diets in random order: (a) control diet alone; (b) control diet plus carotenoid-rich vegetables; (c) control diet plus cruciferous vegetables; and (d) control diet plus soy foods. Compared to the control diet, there was a significant elevation in PDGF-AB serum levels when individuals were consuming the soy diet ( $P = 0.016$ ). Increased PDGF-AB levels were also noted for the carotenoid diet. There was no change from baseline levels when individuals were consuming the cruciferous diet. Overall, mitogenic activity did not change on any of the experimental diets. This study suggests that high soy and carotenoid diets increase serum levels of PDGF-AB. This may represent an additional mechanism by which diet influences individual risk of cancer; further investigation into the role of diet and growth factors is warranted.

### Introduction

Epidemiological studies have provided evidence for a protective effect of vegetable and soy consumption on the development of cancer and, to a lesser extent, cardiovascular disease. In a recent review of over 100 studies examining the relationships between vegetable and fruit consumption and human cancer,

decreased risks were associated most consistently with a high intake of raw and fresh vegetables (including carrots, leafy green vegetables, and cruciferous vegetables; Ref. 1). The majority of the animal studies that have examined soy or soybean isoflavones and experimental carcinogenesis reported decreased tumor development with soy consumption (2). In 21 human studies that analyzed 26 cancer sites, 10 showed statistically significant decreased risks associated with soy consumption, 15 showed no association or nonsignificant trends, and 1 study reported an increased risk (2).

Recent studies examining relationships between diet and cardiovascular disease suggest that vegetable consumption may be important (3-5). James *et al.* (6) have speculated that the markedly decreased incidence rates for cardiovascular disease in the Mediterranean as compared to Northern European countries may be due in part to differences in vegetable and fruit consumption. Numerous mechanisms have been suggested to explain these protective associations of diet with cancer and cardiovascular disease, including antioxidants and anticarcinogens present in certain vegetables (3, 7), and antiestrogenic compounds contained in soy (8). An alternative hypothesis that we have proposed recently is that dietary constituents may alter the activity of growth factors *in vivo* (9) and, thus, potentially affect cellular proliferation through positive or negative feedback mechanisms.

Growth factors have been linked etiologically to both cancer and cardiovascular disease, not only because of their ability to stimulate cellular proliferation, but because of their relationships with known oncogenes (10). One particular growth factor, PDGF,<sup>4</sup> has been under extensive study in many chronic diseases (11). PDGF is a potent growth factor that brings many cells from the G<sub>0</sub> to G<sub>1</sub> state and can play a key role in chemotaxis (12). PDGF is dimeric with two chains, A and B, that are linked by disulfide bonds; there are three different configurations, PDGF-AA, PDGF-AB, and PDGF-BB (12). Data suggest that PDGF is important in the initiation and progression of atherosclerotic lesions through activated macrophage and endothelial cell expression of the growth factor (11). There are also several studies that suggest PDGF is important in neoplastic cell proliferation through both autocrine and paracrine loops (11).

In a model for breast cancer, Cullen *et al.* (13) proposed recently that paracrine mechanisms may exist for PDGF, whereby other growth factors, such as peritumor stromal IGF-II, can stimulate the expression of PDGF mRNA in epithelial cell lines; this, in turn, stimulates the stromal cell to produce more IGF-II, leading to a positive feedback cycle. It is noteworthy that in at least one study, elevated plasma levels of PDGF have been found in breast cancer patients and were

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<sup>4</sup> The abbreviations used are: PDGF, platelet-derived growth factor; IGF, insulin-like growth factor.

associated significantly with poorer survival (14). In an extension of Cullen's model, we suggested that circulating endogenous factors (such as diet) may affect normal growth factor secretion and, thus, influence both autocrine and paracrine growth factor loops (9).

Few studies have explored the effect of dietary constituents on PDGF activity. Genistein, a flavonoid found in soybeans, has been shown to inhibit the tyrosine kinase activity of PDGF and epidermal growth factor *in vitro* (15, 16). Furthermore, retinoids, natural and synthetic analogues of vitamin A that are under study as inhibitors of epithelial tumorigenesis (17), have been found to inhibit PDGF stimulation of DNA synthesis and cell division and, thus, hold the cells in an arrested G<sub>0</sub> or early G<sub>1</sub> state (18).

To date, there have been few *in vivo* investigations that have examined the effect of the human diet on growth factor activity. In a human feeding study that examined the *in vivo* effect of omega-3 fatty acid supplementation on gene regulation in mononuclear cells, nearly 70% of PDGF mRNA (both PDGF-AA and PDGF-BB) was down-regulated with fish oil supplementation (19). Other growth factors were not affected, including IGF-I and transforming growth factor  $\beta$ 1 (20). The inhibition of PDGF mRNA expression with fish oil consumption could represent a novel mechanism for the protective effect of omega-3 fatty acids in cardiovascular disease (19).

In our hypothesis, we speculated that dietary effects on PDGF would most likely occur at the cellular level (9); thus, in this study, we predicted that there would be a decrease in mitogenic activity due to the inhibitory effects present in soy- and carotenoid-rich foods. We also expected that if either of these two diets affected circulating growth factor levels, they would result in a decrease in measurable levels (particularly since elevated levels have been detected in cancer patients). In this study, both serum levels of PDGF-AB and serum-stimulated mitogenic activity were examined in humans taking part in a feeding trial that included high intakes of cruciferous vegetables, carotenoid-rich vegetables, or soy foods *versus* a control diet essentially devoid of phytochemicals. The data demonstrate that diets high in soy and carotenoids increase serum levels of PDGF-AB.

## Materials and Methods

**Subjects.** Twenty-three healthy male and female subjects consuming a typical American diet (low in fruit and vegetables) were recruited from the University of Minnesota community to participate in a vegetable feeding study conducted October 1992 through February 1993. Potential participants were screened for the following exclusion criteria: (a) medical history of gastrointestinal disorders; (b) food allergies; (c) weight loss or gain greater than 4.5 kg within the past year; (d) major changes in eating habits within the last year or significant short-term dietary changes (such as seen with high-intensity exercise regimens); (e) antibiotic use within the past 3 months; (f) body weight >130% of the ideal weight; (g) current treatment for a diagnosed disease; (h) alcohol intake >2 drinks/day; (i) use of oral contraceptives by female participants within the past month; (j) smoking; and (k) unwillingness to consume only and all foods provided in the study. In addition, participants were instructed to maintain their usual exercise levels, to take no medications (except for infrequent over-the-counter pain relievers), and to take no nutritional supplements during the feeding period. This study was approved by the Institutional Review Board Human Subjects Committee at the University of

Minnesota. Subjects provided informed written consent before admission into the study.

**Diets.** Subjects were randomized in the controlled cross-over feeding study and consumed four diets for 9 days each, with at least a 10-day washout in between. The four diets were: control, carotenoid, cruciferous, and soy. The control diet consisted of commonly consumed foods and no fruits and vegetables and was essentially phytochemical free. Foods consumed on the control diet were: crisped rice cereal, corn flake cereal, bagel, white bread, instant chicken noodle soup, rice, canned chicken, saltine and club crackers, 1% milk, margarine, processed American cheese, shortbread and chocolate sandwich cookies, and vanilla pudding. The remaining diets (referred to here as the intervention diets) consisted of the control diet plus specific plant foods. The carotenoid diet consisted of the control diet plus daily servings of 165 g of carrot coins (J. R. Simplot Co., Caldwell, ID), 125 g of carrot puree (Stahlbush Island Farms, Inc., Corvallis, OR), and 250 g of chopped spinach (Simplot, Caldwell, ID). The cruciferous diet consisted of the control diet plus daily servings of 390 g broccoli (Simplot, Boise, ID) and 300 g of cauliflower (Simplot, Boise, ID). The soy diet consisted of the control diet plus daily servings of firm tofu (Morinaga Nutritional Foods, Inc., Los Angeles, CA) and 45 g FriChik, a textured vegetable protein product (Worthington Foods, Inc., Worthington, OH). Compliance with the feeding regimen was assessed by a daily checklist, which required subjects to check off the study foods when eaten and list any additional nonstudy foods consumed.

**Blood Collection.** Fasting venous blood was drawn into 15-ml vacutainer tubes (Fisher, Inc.) on the morning of the 10th day of the feeding period. Blood was allowed to clot for 1 h at room temperature and then placed immediately on ice. Serum samples were obtained by centrifugation at 1500  $\times$  g for 10 min. Aliquots were removed and stored in the dark at  $-70^{\circ}\text{C}$  until analysis. Platelet count was measured in a routine hematology laboratory on the day of sampling.

**PDGF ELISA.** Serum levels of PDGF-AB were measured in duplicate with the use of a commercially available immunoassay kit (R&D Systems, Inc., Minneapolis, MN).

**Cell Culture Condition.** Cells (normal human foreskin fibroblasts; ATCC 1634) were cultured routinely in DMEM with 4500 mg/liter D-glucose and 10% fetal bovine serum (GIBCO-BRL, Grand Island, NY). Cells were grown at 37°C and 5% CO<sub>2</sub> and were screened periodically for *Mycoplasma* contamination. Mitogenic activity was measured with the use of the method of Raines and Ross (21), modified as described below.

**Mitogenic Assay.** Quiescent test cultures of normal human fibroblasts (ATCC 1634) were obtained by allowing cells to grow to confluence (4–5 days); then cells were test plated at  $1 \times 10^4$ /well in 1% calf serum. Four days after plating, the growth medium was changed to 1% plasma-derived human serum. Seventy-two h later, test samples were added to the wells (20 and 40  $\mu\text{l}$ /well) in triplicate and incubated for 20 h at 37°C. Sixteen to 19 h after sample addition, wells were examined for toxicity. At 20 h, proliferation was measured by [<sup>3</sup>H]thymidine incorporation; 0.5 ml of fresh medium (serum free) containing 1  $\mu\text{Ci/ml}$  [<sup>3</sup>H]thymidine was added to each well for a 4-h labeling period. Cells were harvested by aspirating the media, washing wells twice with 1 ml of ice-cold 5% trichloroacetic acid, solubilizing trichloroacetic acid-insoluble material in 0.25 NaOH (0.8 ml), and counting 0.6 ml of this solution in a scintillation counter. Serum stimulation of proliferation was compared with control wells (100  $\mu\text{l}$  of PBS alone). In a separate experiment, neutralizing antibodies (range of

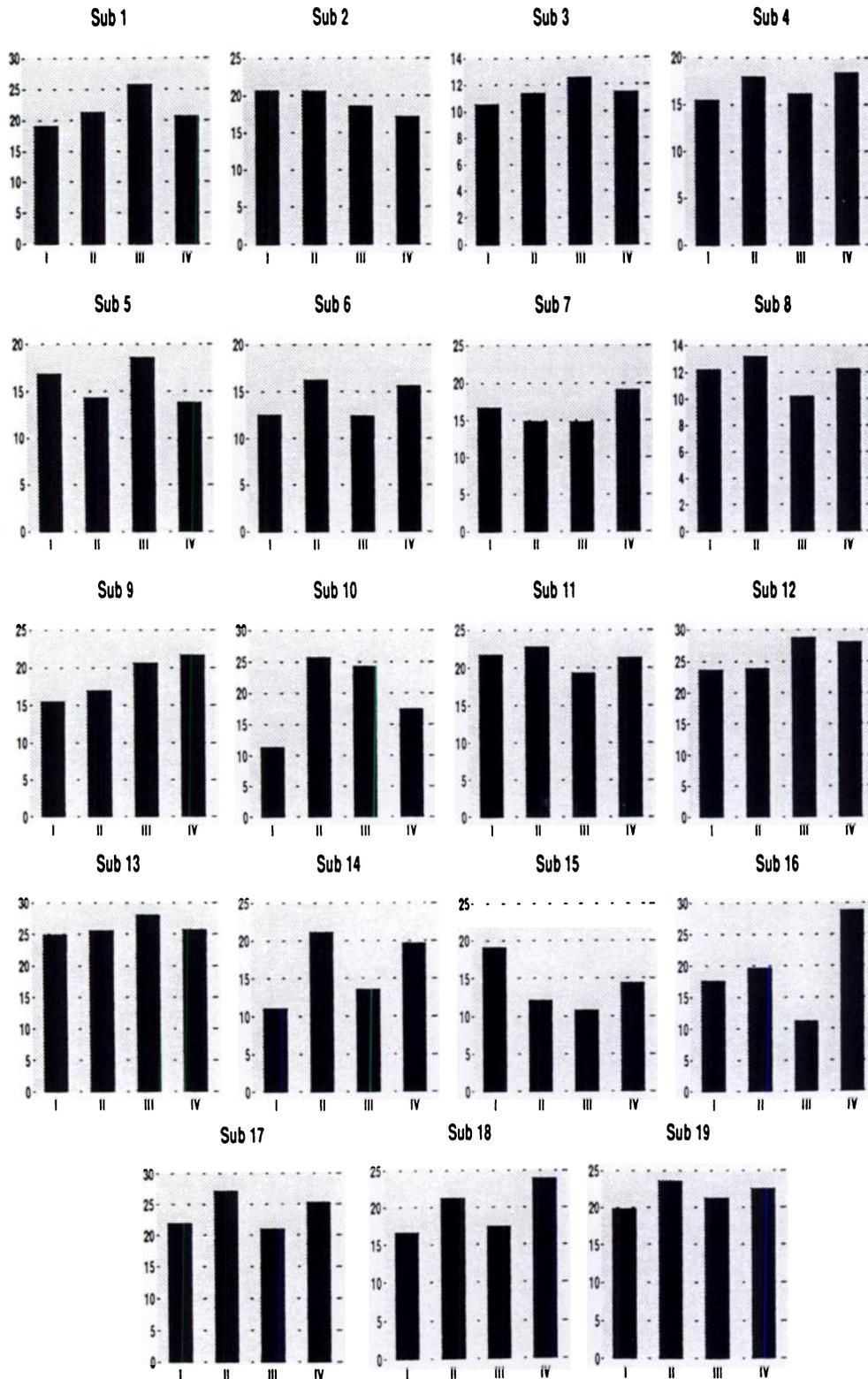


Fig. 1. Individual PDGF-AB serum levels (nanograms/ml) for subjects (Sub) 1-19 consuming diets I-IV. Diet I, control; diet II, carotenoid; diet III, cruciferous; and diet IV, soy.

concentration, 0.01 to 10 ng/ml) for PDGF-AA and PDGF-BB (R&D Systems, Inc.) were added to a standard mitogenic assay to determine the proportion of mitogenic activity attributable to PDGF.

**Statistical Analysis.** Since each individual served as his or her own control, data were analyzed with the use of paired *t* tests with the Epilog statistical computer program (Epicenter Software, Pasadena, CA). Log transformations were performed to normalize the dependent variable. The level of significance was set at  $P = 0.017$  to compensate for multiple comparisons with the use of the same control group (Bonferroni correction; Ref. 22).

## Results

Nineteen of the 23 initial recruits, 11 men and 8 women, completed all four experimental diet treatments. Four women dropped out of the study before completion of all four diets due to unexpected pregnancy ( $n = 1$ ), logistical problems ( $n = 2$ ), and noncompliance ( $n = 1$ ). The remaining 19 individuals demonstrated good compliance with the diets, as evidenced by their individual checklists.

Fig. 1 demonstrates the serum PDGF-AB levels (ng/ml) of each individual when consuming the control and intervention diets for 9 days. There was a nonsignificant elevation in PDGF-AB serum levels (adjusted for platelet count) when consuming the carotenoid diet (II) compared to the control diet (I; mean difference = 2.23 ng/ml; SD = 4.52;  $P = 0.065$ ). When PDGF-AB levels measured at completion of the cruciferous diet (III) were compared to the control diet (I), there was no change (mean difference = 0.94 ng/ml; SD = 4.72;  $P = 0.588$ ). Finally, in comparing the soy diet (IV) with the control diet (I), there was a statistically significant elevation in PDGF-AB serum levels (mean difference = 2.63 ng/ml; SD = 4.11;  $P = 0.016$ ).

We next assayed the serum of study individuals to determine if the increased serum levels of PDGF-AB were associated with a measurable increase in mitogenic activity. Table 1 shows mitogenic activity expressed as fold elevation of [ $^3$ H]thymidine incorporation over baseline (unstimulated levels) for each of the four diet treatments using 20  $\mu$ l of serum. No significant change in activity was observed when any of the intervention diets were compared with the control diet for each individual. Similarly, no changes were detected with the use of a larger volume of serum (data not shown).

In separate standard mitogenic assays, the addition of neutralizing antibodies to PDGF-BB and PDGF-AA at a concentration of 10 ng/ml reduced proliferation (3.38- to 2.04-fold and 4.00- to 2.49-fold, respectively). The addition of higher concentrations of antibody did not reduce proliferation further. Thus, it was determined that approximately one-third of the serum stimulation of fibroblast proliferation could be inhibited, indicating that the majority of the stimulation was likely due to growth factors other than PDGF.

## Discussion

In this study, we have demonstrated that diets high in soy and carotenoids increase serum levels of PDGF-AB compared to a carotenoid-free, low soy control diet. This was unexpected, particularly due to the observation that PDGF levels have been found to be elevated in certain cancer patients (14). We speculate that the mechanism of this effect is related to PDGF-inhibitory constituents of soy (*i.e.*, the effect of genistein on PDGF tyrosine kinase activity; Refs. 15 and 16) and carote-

**Table 1** Mitogenic activity expressed as fold elevation over background using 20- $\mu$ l aliquots of serum. Listed *P* values are the result of the mean difference in fold elevation for each intervention diet (II, III, and IV) compared with diet I. (diet II,  $P = 0.848$ ; diet III,  $P = 0.321$ ; diet IV,  $P = 0.683$ ).

Subject	Diets <sup>a</sup>			
	Diet I (control)	Diet II (carotenoid)	Diet III (cruciferous)	Diet IV (soy)
1	6.48	6.24	6.67	6.28
2	5.53	4.56	5.17	4.84
3	5.73	5.27	5.32	6.26
4	7.20	8.00	8.18	6.26
5	6.10	5.65	4.94	7.46
6	8.74	8.97	8.36	8.91
7	7.00	6.46	6.71	7.11
8	5.44	4.99	5.15	5.26
9	7.77	8.40	7.45	8.10
10	5.58	6.74	5.91	6.10
11	5.80	7.11	6.78	6.10
12	5.51	5.81	5.59	5.96
13	5.56	6.26	6.04	5.63
14	4.88	4.67	4.58	4.35
15	4.70	4.05	4.21	4.41
16	8.30	8.68	8.46	9.23
17	6.96	6.77	7.19	6.33
18	8.25	8.04	8.24	7.50
19	12.29	11.04	9.37	ND <sup>b</sup>

<sup>a</sup> Each value is the mean of three individual measurements.

<sup>b</sup> ND, not determined.

noids (*i.e.*, the decrease in PDGF stimulation of DNA synthesis; Ref. 18). It is possible that circulating levels of PDGF-AB are raised in response to high soy and carotenoid diets to maintain a constant mitogenic effect. If circulating genistein from a high soy diet inhibits the binding of PDGF to cell-surface receptors, then it is possible that more PDGF would need to be made available in the circulation to compensate for this inhibition.

We also showed that a diet high in cruciferous vegetables does not alter PDGF-AB serum levels. This finding is of interest because epidemiological studies have suggested that a diet high in cruciferous vegetables is associated with a decreased risk of cancer (1). Our data suggest that this effect is not mediated by alterations in serum PDGF-AB and, furthermore, that the increased levels of PDGF-AB associated with carotenoid- and soy-enriched diets are specific to those diets and are not a nonspecific effect of dietary modulation.

No differences were observed in mitogenic activity between the control and intervention diets. There are at least three possible explanations for these results: (a) only about one-third of the proliferation measured in the mitogenic assay was due to PDGF, and the assay may lack adequate sensitivity to identify small changes in mitogenic activity; (b) mitogenic activity may be truly unchanged, and although PDGF-AB levels are increased on these two diets, other growth factors that contribute to the overall mitogenic activity of serum are reduced due to reciprocal regulation. Possible growth factors that may be regulated in this way and may also be affected by dietary modulation include the two homodimers of PDGF (PDGF-AA and PDGF-BB), IGF-1, and transforming growth factor  $\beta$  (23–25); they will be examined together with PDGF-AB in a subsequent study; and (c) mitogenic inhibition may result from the presence of genistein and carotenoids in the two diets, but the increased levels of PDGF-AB on these diets compensate for any losses in stimulatory capacity.

These observations not only add to our understanding of the potential interactions between diet and cancer and cardiovascular disease but also raise many questions. The current study has shown that levels of PDGF-AB can be elevated by a short period (9 days) of controlled feeding with a high soy or carotenoid diet, but it has not demonstrated whether elevation is transient or sustained with longer periods of feeding. The biological significance of the change in PDGF-AB levels also remains unknown. Additional studies to address these important questions are currently in progress.

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