

Effects of Low-Dose Aspirin on Serum C-Reactive Protein and Thromboxane B₂ Concentrations: A Placebo-Controlled Study Using a Highly Sensitive C-Reactive Protein Assay

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OBJECTIVES	We performed a placebo-controlled study to evaluate the effect of low-dose aspirin on serum C-reactive protein (CRP) levels.
BACKGROUND	Elevated circulating concentrations of CRP, an inflammatory marker, increase the risk of thrombotic cardiovascular diseases such as myocardial infarction (MI). Moreover, low-dose aspirin therapy has been reported to be more effective in preventing MI in men with higher CRP levels than it is in those with lower levels, raising the possibility that aspirin prevents thrombosis by reducing vascular inflammation. The effect of low-dose aspirin therapy on serum CRP levels in men has been addressed recently, but the results of the two studies conflict.
METHODS	Effects of aspirin (81 mg every day or 325, 81 or 40 mg every-third-day given for 31 days) on serum CRP, using a highly-sensitive assay, and on serum platelet-cyclo-oxygenase (COX)-1-derived thromboxane (Tx) B ₂ concentrations were studied simultaneously in 57 healthy volunteers (30 men and 27 women).
RESULTS	Trough platelet COX-1-derived serum Tx B ₂ concentrations decreased by 100% with daily aspirin and by 90%, 84% and 78% with 325, 81 and 40 mg aspirin every-third-day ($p < 0.001$). However, there were no significant changes in serum CRP levels from baseline with daily low-dose aspirin therapy, with any of the every-third-day aspirin regimens or with placebo treatment.
CONCLUSIONS	Low doses of aspirin that markedly inhibit platelet COX-1 activity, as manifested by a profound decline in platelet-derived serum Tx B ₂ concentrations, have no detectable effect on serum CRP levels in healthy men and women. (J Am Coll Cardiol 2001;37: 2036-41) © 2001 by the American College of Cardiology

Recently it has been proposed that inflammation plays an important role in the pathogenesis of atherosclerotic cardiovascular diseases (1). Furthermore, elevated serum concentrations of C-reactive protein (CRP), a marker of inflammation, portend an increased subsequent risk of myocardial infarction (MI), stroke and symptomatic peripheral vascular disease in healthy men and women (2,3). In men, low-dose aspirin (ASA) therapy appears to have its greatest protective effect against MI when serum CRP levels are relatively high (2). For example, in men with CRP levels in the top quartile, the relative risk reduction with 325 mg ASA every other day was 56% ($p = 0.02$) while in men with CRP levels in the lowest quartile, the relative risk reduction with the same dose of ASA was only 14% ($p = 0.77$). This intriguing observation raises the possibility that low-dose ASA therapy may actually protect against MI by reducing inflammation.

Two recent prospective studies have examined whether low-dose ASA treatment reduces serum CRP levels (4,5). Ikonomidis et al. (4) administered 300 mg ASA per day for three weeks or placebo to 40 men with coronary artery disease. Serum CRP levels were significantly lower after ASA therapy than they were after placebo (4). More recently, Feng et al. (5) treated 32 healthy men with 325 or 81 mg ASA per day for seven days. They found no significant effect of either dose of ASA on mean serum CRP levels.

In an attempt to further clarify the effect of low-dose ASA on serum CRP concentrations, we performed a prospective, randomized, placebo-controlled, double-blind study in 57 healthy men and women. We gave volunteers one of four low-dose ASA regimens or placebo for 31 days. Serum CRP levels were measured twice before therapy (day 0 and day 1), after four weeks of therapy (day 28), at the end of therapy (day 31) and two weeks after cessation of therapy (day 45). In addition, we measured serum platelet-derived thromboxane (Tx) concentrations at each time point so that we could compare ASA's anticipated serum Tx-lowering effect (6) with any possible "anti-inflammatory" effect of low-dose ASA, as manifested by a CRP-lowering effect.

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Abbreviations and Acronyms

- ASA = aspirin (acetylsalicylic acid)
- BMI = body mass index
- COX = cyclo-oxygenase
- CRP = C-reactive protein
- IL-6 = interleukin-6
- MI = myocardial infarction
- Tx = thromboxane

METHODS

Fifty-seven men and women participated in this placebo-controlled, double-blind, randomized study, which was approved by a human study subcommittee of the Dallas VA Medical Center's Research Committee. Informed, written consent was obtained from each participant. All participants considered themselves to be healthy.

Participants had not taken any ASA or nonsteroidal anti-inflammatory drug in the 14 days before beginning the study. None had a history of ASA allergy, nor had been diagnosed with peptic ulcer disease or any other disease of the stomach or duodenum, cancer, HIV infection, liver disease, renal disease, diabetes, coronary artery disease or chronic pulmonary disease. Individuals with obesity, hypertension, hyperlipidemia or a family history of early-onset atherosclerotic cardiovascular disease were not excluded.

Aspirin powder and placebo (methycellulose) were packaged by a pharmacist in identical-appearing opaque capsules. Volunteers were randomly assigned to a 31-day treatment with a regimen as listed in Table 1. Groups 3, 4 and 5, who took ASA every third day, received a placebo capsule on the days when ASA was not administered. Pilot studies had indicated that platelet function was markedly inhibited even three days after low-dose ASA.

Two baseline serum samples were obtained: one the day before randomization (day 0) and the other after randomization and immediately before the first dose of ASA or placebo was taken (day 1). Two additional serum samples were collected on treatment days 28 and 31 (just before that day's ASA or placebo dose), and a final sample was obtained on day 45 (14 days after the end of treatment). All 57 subjects completed the 45-day study, and all 285 serum samples were available for the assays. Serum samples were coded before assay so that assays could be performed in blinded fashion.

Table 1. Demographic Variables in Five Treatment Groups*

Group	1. Placebo (n = 11)	2. ASA 81 qd (n = 13)	3. ASA 325 q3d (n = 12)	4. ASA 81 q3d (n = 11)	5. ASA 40 q3d (n = 10)	Entire Group (n = 57)
Men/Women	7/4	6/7	7/5	4/7	6/4	30/27
Age (yr)†	42 ± 3	42 ± 4	40 ± 3	39 ± 3	39 ± 5	40 ± 2
African American/Caucasian/Hispanic	6/3/2	9/4/0	7/5/0	7/4/0	5/4/1	34/20/3
BMI (kg/m ²)‡	27.0 ± 2.2	28.6 ± 1.4	28.5 ± 1.8	25.7 ± 1.1	25.7 ± 1.6	27.2 ± 0.7
Nonsmoker/smoker/ex-smoker	6/4/1	6/3/3‡	6/5/1	5/5/1	4/4/2	27/21/8

*None of the demographic variables differed significantly among treatment groups by analysis of variance (p = 0.44 to 0.91). Each treatment was administered for 31 days; †mean (± SEM); ‡smoking status unknown in one subject.

ASA = aspirin; BMI = body mass index; qd = every day; q3d = every third day.

The CRP assay used a monoclonal antibody to CRP coated on polystyrene beads. The intensity of light scattering after the addition of serum was measured by nephelometry (Dade Behring Laboratories, Newark, Delaware). This assay has been referenced to the World Health Organization standard and is sensitive in the range of 0.175 to 60 mg/l. Interassay and intra-assay coefficients of variation were each <5%. Serum Tx B₂ concentrations were measured by radioimmunoassay as previously described (7). This assay was sensitive in the range of 25 to 1,000 µg/l. Interassay and intra-assay coefficients of variation were 10.9% and 9.2%, respectively.

Statistical analyses. All statistical analyses were performed using Systat version 8.0.1 for Windows (SPSS Inc., 1998, Chicago, Illinois). Serum CRP and Tx measurements were not normally distributed. Therefore, CRP and Tx results for each treatment group were expressed as median and interquartile ranges (rather than mean and SEM). Moreover, nonparametric analysis of variance (Kruskal-Wallis test) was used to compare baseline demographic and serologic values among the five treatment groups. Comparisons of changes in serum CRP and Tx values from baseline as a function of treatment group were analyzed by analysis of variance using the nonparametric Mann-Whitney U statistic. A general multivariate linear model was used to determine whether gender, age, race, body mass index (BMI), smoking habit or treatment had independent effects on serum CRP concentrations. In all statistical tests, two-tailed probability (p) values <0.05 were considered statistically significant.

RESULTS

Baseline serum CRP levels. Serum CRP varied considerably among subjects, from undetectable levels (<0.175 mg/l) to >20 mg/l. Median CRP values were close to 1.5 mg/l, and mean values were close to 3 mg/l. Univariate analysis of variance indicated that baseline CRP levels were significantly affected by BMI (high > low), gender (female > male) and age (older > younger) but not by race, smoking habit or treatment group to which subjects had been randomly assigned. In the general multivariate linear model, however, only BMI independently affected baseline CRP (p < 0.001), although gender had a borderline effect (p = 0.067). Table 2 presents median and mean (95% confidence interval) baseline CRP levels (average of day 0

Table 2. Median and Mean (95% CI) Baseline Serum CRP Concentration in Men and Women as a Function of BMI*

BMI Tertile†	Men (n = 30)			Women (n = 27)		
	Median BMI (kg/m ²)	Median CRP (mg/l)	Mean (95% CI) CRP (mg/l)	Median BMI (kg/m ²)	Median CRP (mg/l)	Mean (95% CI) CRP (mg/l)
1st	23.25	0.51	0.90 (0.19, 1.61)	21.50	1.25	1.31 (0.74, 1.88)
2nd	25.80	0.86	2.83 (0, 6.35)	25.90	3.23	4.66 (0.89, 8.42)
3rd	33.05	3.20	3.45 (1.75, 5.16)	32.40	5.64	5.71 (2.50, 8.93)

*Baseline serum CRP was the average of the CRP value on day 0 and day 1; †each BMI tertile in men consisted of 10 participants, while each BMI tertile in women consisted of nine participants.

BMI = body mass index; CI = confidence interval; CRP = C-reactive protein.

Table 3. Median (Interquartile Range) Serum CRP Levels (mg/l) in 57 Subjects Before, During and 14 Days After 31 Days of Treatment With ASA or Placebo

Treatment Group	Before Treatment		During Treatment		After Treatment
	Day 0	Day 1	Day 28	Day 31	Day 45
1. Placebo (n = 11)	1.80 (0.92-5.53)	1.72 (0.86-5.33)	2.31 (0.75-6.2)	1.52 (0.98-7.79)	2.61 (0.9-6.89)
2. ASA 81 qd (n = 13)	2.64 (1.7-4.66)	2.84 (1.65-5.47)	3.01 (1.69-8.25)	3.19 (2.38-7.02)	2.78 (1.46-3.5)
3. ASA 325 q3d (n = 12)	1.52 (0.71-3.42)	1.37 (0.93-3.36)	1.68 (1.05-4.52)	2.23 (1.57-3.45)	3.03 (0.88-3.78)
4. ASA 81 q3d (n = 11)	1.03 (0.53-1.85)	1.14 (0.52-1.72)	0.61 (0.41-1.64)	0.80 (0.3-1.68)	0.70 (0.31-2.67)
5. ASA 40 q3d (n = 10)	0.99 (0.56-2.46)	0.95 (0.51-2.18)	2.11 (0.49-5.49)	1.71 (0.94-4.12)	2.05 (0.72-4.18)

None of the changes in serum CRP after treatment differed significantly from those before treatment.

ASA = aspirin; CRP = C-reactive protein; qd = every day; q3d = every third day.

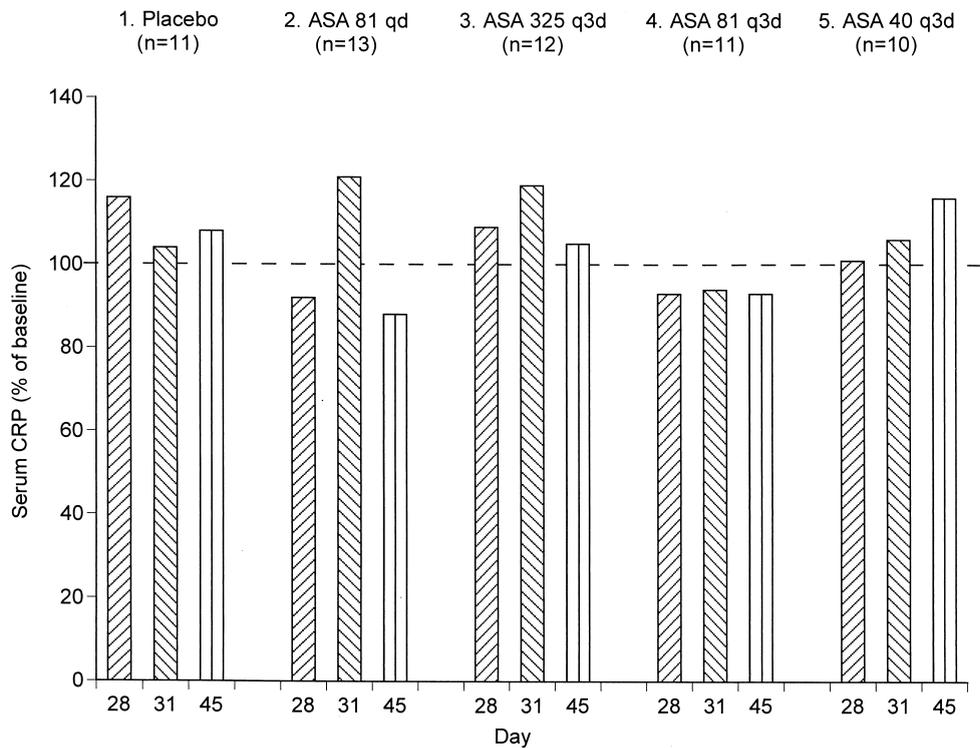


Figure 1. Serum C-reactive protein (CRP) (expressed as a percentage of average baseline CRP levels) in each treatment group. Median changes are shown during treatment (day 28 and 31) and 14 days after treatment (day 45). Changes were not significant in any treatment group. ASA = aspirin; qd = every day; q3d = every third day.

Table 4. Median (Interquartile Range) Serum Tx Levels in 57 Subjects Before, During and 14 Days After 31 Days of Treatment With ASA or Placebo

Treatment Group	Before Treatment		During Treatment		After Treatment
	Day 0	Day 1	Day 28	Day 31	Day 45
1. Placebo (n = 11)	354 (307, 530)	375 (293, 530)	411 (348, 441)	466 (317, 572)	344 (277, 595)
2. ASA 81 qd (n = 13)	441 (406, 681)	549 (396, 706)	0* (0, 30)	0* (0, 42)	412 (307, 535)
3. ASA 325 q3d (n = 12)	298 (176, 377)	311 (209, 384)	47* (29, 69)	40* (32, 43)	285 (238, 335)
4. ASA 81 q3d (n = 11)	247 (197, 382)	327 (261, 419)	64* (0, 214)	78* (30, 200)	258 (207, 397)
5. ASA 40 q3d (n = 10)	449 (334, 562)	446 (407, 597)	102* (48, 114)	87* (47, 146)	322 (232, 380)

Changes in serum Tx during ASA treatment (groups 2 to 5) differed significantly (*) from those before treatment ($p < 0.001$).
ASA = aspirin; Tx = thromboxane; qd = every day; q3d = every third day.

and day 1) by BMI tertile in men and women. C-reactive protein in men or women in the highest BMI tertiles were four- to sixfold higher than those in the lowest BMI tertiles. **Effects of ASA regimens or placebo on serum CRP measurements.** Table 3 presents the median and interquartile ranges for serum CRP levels for each treatment group at baseline (day 0 and day 1), during therapy (day 28 and day 31) and on day 45 (14 days after completing treatment). Serum CRP levels did not change significantly from baseline in any treatment group (Fig. 1). In the general multivariate linear model, treatment had no independent effect on CRP levels. The only variable that independently affected serum CRP at the end of the treatment period (day 31) was BMI ($p = 0.03$). Two weeks after completion of therapy, both BMI ($p = 0.001$) and gender ($p = 0.01$) independently affected serum CRP.

Effects of ASA regimens or placebo on serum Tx measurements. Median and interquartile ranges for serum Tx levels at baseline, during therapy and 14 days after the end of therapy are shown in Table 4. Baseline serum Tx levels among the five treatment groups did not differ significantly. Serum Tx levels did not change significantly during placebo therapy (group 1), whereas they decreased significantly ($p < 0.001$) from baseline with each ASA regimen (Fig. 2). Compared with placebo, serum Tx on day 28 or 31 was inhibited by 100% with ASA 81 mg/day (group 2), 90% with ASA 325 mg every-third-day (group 3), 84% with ASA 81 mg every-third-day (group 4) and 78% with ASA 40 mg every-third-day (group 5). On day 45, 14 days after ASA therapy was discontinued, serum Tx levels had returned to near baseline levels and to levels that were similar to those of the placebo group.

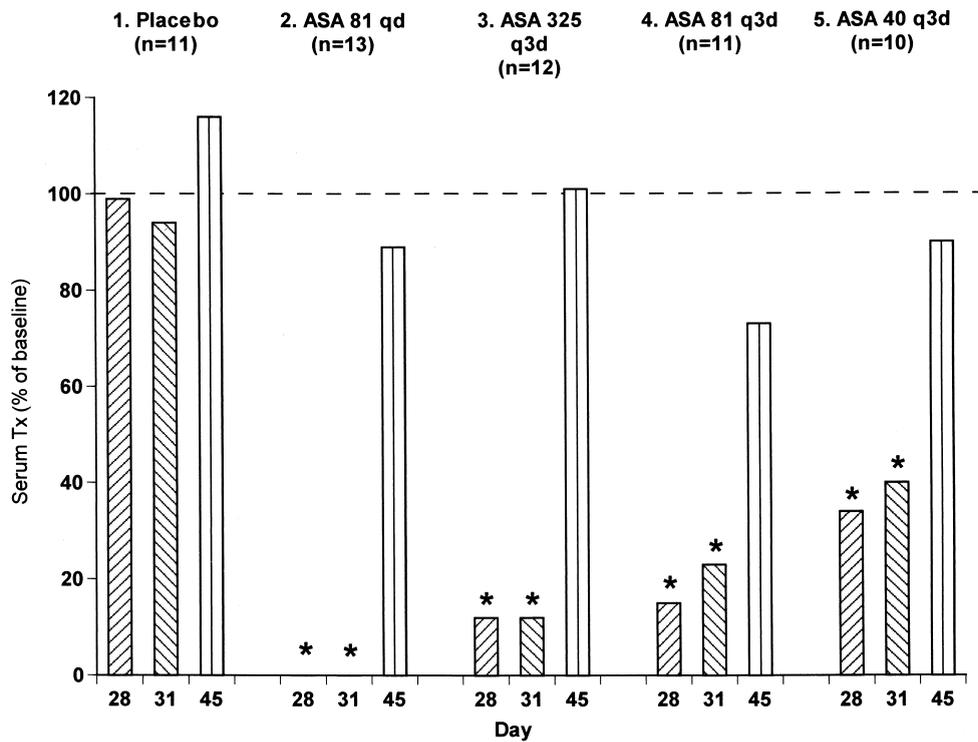


Figure 2. Serum thromboxane (Tx) (expressed as a percentage of average baseline Tx levels) in each treatment group. Median changes are shown during treatment (day 28 and 31) and 14 days after treatment (day 45). Changes were statistically significant ($*p < 0.001$) in groups 2, 3, 4 and 5, but not in group 1. ASA = aspirin; qd = every day; q3d = every third day.

Table 5. Comparison of Studies Evaluating Effects of Low-Dose ASA on Serum CRP

	Ikonomidis et al. (4)	Feng et al. (5)	Feldman et al. (Current Study)
Population	Angina pectoris	Healthy	Healthy
Number of men/women	40/0	32/0	30/27
Mean age (yr)	55	29	40
BMI (kg/m ²)	—	—	27.2 kg/m ²
ASA dose(s)	300 mg/day	81 mg/day and 325 mg/day*	81 mg/day and 325, 81, 40 mg q3d
Duration of therapy	21 days	7 days	31 days
Placebo group	Yes	No	Yes
CRP assay method	Nephelometry	ELISA	Nephelometry
Baseline CRP (median)	1.40 mg/l	—	1.47 and 1.57 mg/l
Baseline CRP (mean)	—	0.9 mg/l	3.02 and 3.19 mg/l
Effect of ASA on CRP	↓ of 0.4 mg/l (p < 0.05)	↓ of 0.1 mg/l (not significant)	↑ of 0.3 mg/l† (not significant)

*These two doses produced similar effects and were combined by authors; †median change for the 46 subjects who received any of the four ASA doses.

ASA = aspirin; BMI = body mass index; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbent assay; q3d = every third day.

DISCUSSION

CRP, atherosclerosis and body mass. C-reactive protein, a member of the pentraxin family, is the prototypic marker of inflammation. In addition to being a risk marker for atherosclerosis, it promotes tissue factor release from monocytes, phagocytosis and shedding of cell adhesion molecules. Furthermore, CRP co-localizes with complement in the atherosclerotic lesion (8–12). C-reactive protein is one of many proteins produced by the liver in response to cellular injury due to trauma, infarction or infection (13). Release of CRP from the liver into the circulation after cell injury is stimulated by the proinflammatory cytokine interleukin-6 (IL-6) (14). Obesity is also associated with elevated IL-6 and CRP levels (15,16). Thus, CRP is a possible link between obesity and atherosclerotic vascular disease. In our study, BMI was the most important demographic factor that affected serum CRP levels among individual participants. Female gender also had a modest effect on CRP, as in a previous study (17), while age, smoking and race had no detectable independent effects. While serum CRP levels were higher in our older participants than they were in their gender-matched younger counterparts, this was probably related to other factors, such as BMI, than it was to age per se.

Effect of ASA on CRP and serum Tx. The profound antiplatelet effect of low doses of ASA in this study, as demonstrated by a marked, dose-related decline in platelet-derived serum Tx B₂ concentrations, contrasted strikingly with an absence of any detectable effect of ASA on serum CRP levels. Our CRP findings are in close agreement with recent results of Feng et al. (5) but differ from those of Ikonmidis et al. (4). The three studies are compared in Table 5. To our knowledge, our study is the first to examine the effect of ASA therapy on highly-sensitive CRP levels in women and used the largest number of doses to date.

Our present CRP findings seem to argue against the hypothesis that low-dose ASA is anti-inflammatory. How-

ever, it is possible that a treatment period longer than 31 days, an observation period longer than 45 days or higher ASA doses than we tested might have lowered serum CRP. Furthermore, high oral doses of the cyclo-oxygenase (COX) inhibitor ibuprofen reduce signs and symptoms of endotoxin-mediated inflammation without affecting high serum CRP levels (18). Thus, low-dose aspirin might, at least theoretically, reduce vascular inflammation without reducing serum CRP levels. Until a specific marker of vascular inflammation is available, it will not be possible to prove or disprove this possibility. For the present, the beneficial effect of low-dose ASA in preventing arterial thrombosis and in acute coronary syndromes seems much more likely to be mediated by its effect on platelet COX-1 activity than by an anti-inflammatory action.

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