Journal of the American College of Cardiology
© 2010 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 55, No. 2, 2010
ISSN 0735-1097/10/$36.00
doi:10.1016/j.jacc.2009.08.039

Treatment of Aspirin-Resistant Patients With Omega-3 Fatty Acids Versus Aspirin Dose Escalation

Eli I. Lev, MD,*† Alejandro Solodky, MD,*† Naama Harel,* Aviv Mager, MD,*† David Brosh, MD,*† Abid Assali, MD,*† Milton Roller, MD,*† Alexander Battler, MD,*† Neal S. Kleiman, MD,‡ Ran Kornowski, MD*†

Petah Tikva and Tel-Aviv, Israel; and Houston, Texas

Objectives The aim of this study was to evaluate whether addition of omega-3 fatty acids or increase in aspirin dose improves response to low-dose aspirin among patients who are aspirin resistant.

Background Low response to aspirin has been associated with adverse cardiovascular events. However, there is no established therapeutic approach to overcome aspirin resistance. Omega-3 fatty acids decrease the availability of platelet arachidonic acid (AA) and indirectly thromboxane A2 formation.

Methods Patients (n = 485) with stable coronary artery disease taking low-dose aspirin (75 to 162 mg) for at least 1 week were screened for aspirin response with the VerifyNow Aspirin assay (Accumetrics, San Diego, California). Further testing was performed by platelet aggregation. Aspirin resistance was defined by ≥2 of 3 criteria: VerifyNow score ≥550, 0.5-mg/ml AA-induced aggregation ≥20%, and 10-μmol/l adenosine diphosphate (ADP)-induced aggregation ≥70%. Thirty patients (6.2%) were found to be aspirin resistant and randomized to receive either low-dose aspirin + omega-3 fatty acids (4 capsules daily) or aspirin 325 mg daily. After 30 days of treatment patients were re-tested.

Results Both groups (n = 15 each) had similar clinical characteristics. After treatment significant reductions in AA- and ADP-induced aggregation and the VerifyNow score were observed in both groups. Plasma levels of thromboxane B2 were also reduced in both groups (56.8% reduction in the omega-3 fatty acids group, and 39.6% decrease in the aspirin group). Twelve patients (80%) who received omega-3 fatty acids and 11 patients (73%) who received aspirin 325 mg were no longer aspirin resistant after treatment.

Conclusions Treatment of aspirin-resistant patients by adding omega-3 fatty acids or increasing the aspirin dose seems to improve response to aspirin and effectively reduces platelet reactivity. (J Am Coll Cardiol 2010;55:114–21)

In recent years it has become apparent that the biologic response to aspirin therapy is not uniform; rather, there is wide interindividual variability in the response to aspirin (1,2). The prevalence of low response or "resistance" to the antiplatelet effects of aspirin is estimated to be <1% to 45%, depending on the assay used, cutoff value chosen, and population tested (3–8). Low response to aspirin has been associated with adverse clinical outcomes in patients with cardiovascular disease or cardiovascular risk factors (2,4,7,9) and with an increase in markers of myonecrosis after percutaneous coronary intervention (PCI) (5,6). Furthermore, 2 recent meta-analyses encompassing 15 to 20 studies and totaling almost 3,000 patients showed that aspirin resistance was associated with an odds ratio of almost 4 for development of cardiovascular events (10,11). Despite the emerging clinical evidence, there is no established treatment paradigm for the management of patients who are resistant to the effects of aspirin.

One potential approach to manage aspirin resistance is to increase the drug dose. Most patients receiving long-term aspirin therapy after an acute myocardial infarction (MI) or PCI are treated with low-dose aspirin (75 to 162 mg/day). Therefore, resistant patients could potentially be treated by increasing the dose to 325 mg/day. However, the Antithrombotic Trialists' Collaboration meta-analysis has shown that there is no difference in clinical efficacy for prevention of vascular events when comparing aspirin doses of 75 to 325 mg and even higher doses (12). Conversely, Lee et al. (13) have shown that, among patients with stable coronary artery disease (CAD), low aspirin dose (80 to 100 mg) was associated with the highest prevalence of resistance

From the *Cardiology Department, Rabin Medical Center, Petah Tikva, Israel; †Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; and the ‡Methodist DeBakey Heart Center, The Methodist Hospital, Houston, Texas.
Manuscript received June 12, 2009, accepted August 10, 2009.

compared with higher doses. Furthermore, Gurbel et al. (14) reported dose-dependent effects of aspirin on platelet function when using assays not based on arachidonic acid (AA). It is not known, however, whether increasing the dose in resistant patients taking low-dose aspirin would improve the response to the drug.

Another possible approach to the management of aspirin resistance is to add an additional substance that would inhibit the cyclooxygenase (COX)-1 pathway. Omega-3 fatty acids, which are components of dietary fish oil, might fulfill this role. Ingestion of fish oils leads to replacement of n-6 fatty acids (such as AA) by n-3 fatty acids in the cell membranes of various cells (15,16). In platelets this process leads to a reduction in the availability of platelet membrane AA, along with an increase in eicosapentaenoic acid (EPA) (15). The latter substance can compete with AA as a substrate of the COX-1 pathway. The net effect is an increase in the ratio of EPA to AA and indirectly a reduction in thromboxane A2 formation from AA (15,16). Studies of antiplatelet effects of fish oil, however, have been inconsistent (16,17). In patients naïve to aspirin, a daily dose of 3 to 4 g omega-3 fatty acids given over several weeks seems to have mild antiplatelet effects, such as weak inhibition of collagen-induced platelet aggregation and mild prolongation of bleeding time (16–18).

Recently Larson et al. (19) reported that omega-3 fatty acids alone had no significant effect on platelet aggregation but when combined with aspirin had a synergistic inhibitory effect. It should be noted that addition of omega-3 fatty acids (1 to 4 g/day) to aspirin in patients with cardiovascular disease has not been associated with an increased risk of bleeding complications (20,21). Given the mechanism of action of omega-3 fatty acids and the report by Larson et al. (19), we hypothesized that omega-3 fatty acids might act in synergy with aspirin among patients with low response to aspirin. Therefore, our aim was to evaluate whether adding omega-3 fatty acids or increasing the aspirin dose would improve the response to low-dose aspirin among patients who are aspirin resistant.

Methods

Patients. Stable patients with CAD, ages 18 to 80 years, who have been treated with aspirin at a dose of 75 to 162 mg for at least 1 week were included. Exclusion criteria were PCI or an acute coronary syndrome within 1 month of enrollment; treatment with glycoprotein IIb/IIIa inhibitors or thrombolytic agents within 1 month of enrollment; concomitant treatment with omega-3 fatty acid-containing products; concomitant treatment with ibuprofen, naproxene, or warfarin; thrombocytopenia ($<100 \times 103$ cells/mm^3); anemia (hemoglobin <10 g/dl); or renal insufficiency (creatinine >2.5 mg/dl). The study was approved by the Investigational Review Board (Helsinki committee) of the Rabin Medical Center, Israel, and all subjects provided written informed consent.

Abbreviations and Acronyms

AA = arachidonic acid
ADP = adenosine diphosphate
ARU = aspirin reaction units
CAD = coronary artery disease
COX = cyclooxygenase
EPA = eicosapentaenoic acid
MI = myocardial infarction
PCI = percutaneous coronary intervention
TXB2 = thromboxane B2

Screening for aspirin resistance. The VerifyNow Aspirin assay (Accumetrics, San Diego, California) was used for the purpose of screening for aspirin resistance. This assay is a point-of care, turbidometric-based system that uses citrate-anticoagulated blood and cartridges containing fibrinogen-coated beads and AA as the platelet agonist. Results are expressed as aspirin reaction units (ARU). According to the manufacturer's recommendation, a cutoff of ≥550 ARU indicates resistance to the antiplatelet effects of aspirin. However, from our previous cohort of 150 patients (5), a cutoff level of 550 ARU had a sensitivity of 70% and specificity of 90% for identification of aspirin resistance when compared with AA-induced platelet aggregation. Therefore we used a cutoff level of 500 ARU for screening, which was associated with sensitivity of 90% and specificity of 75% in our previous cohort. Patients with an ARU >500 underwent further testing by turbidimetric platelet aggregation in response to AA and adenosine diphosphate (ADP) (Fig. 1). Our aim was to identify 30 patients with aspirin resistance (Fig. 1).

Definition of aspirin resistance. Aspirin resistance was defined by the presence of at least 2 of the following 3 criteria: 0.5-mg/ml AA-induced platelet aggregation ≥20%, 10-μmol/l ADP-induced aggregation ≥70%, and VerifyNow ARU ≥550 (in contrast to the screening process in which an ARU >500 was used as the cutoff) (5). In case of concomitant treatment with clopidogrel (approximately 5% of the patients screened) aspirin resistance was defined on the basis of 2 of 2 criteria (AA-induced aggregation and VerifyNow Aspirin).

Randomization of aspirin-resistant patients. Patients found to be aspirin resistant were randomized 1:1 into 2 groups (n = 15 each): 1) treatment with the original dose of aspirin and, in addition, omega-3 fatty acids—4 capsules/day taken 2 in the morning and 2 in the evening, each capsule containing 360 mg EPA and 240 mg DHA (Omega Max 3, SupHerb, Israel), for a period of 30 days; and 2) treatment with aspirin 325 mg daily for a period of 30 days.

In addition, a control group of 15 patients was randomly selected from the patients with an ARU >500 who did not fulfill the criteria for aspirin resistance (Fig. 1). These patients continued to receive the same treatment (including the original aspirin dose), without change. The 2 study groups and control group underwent testing for response to aspirin after 30 days of treatment with the corresponding regimen.

Follow-up phase. All 30 patients included in the 2 treatment groups were invited to participate in a follow-up phase performed approximately 1 year (13 ± 4 months) after the

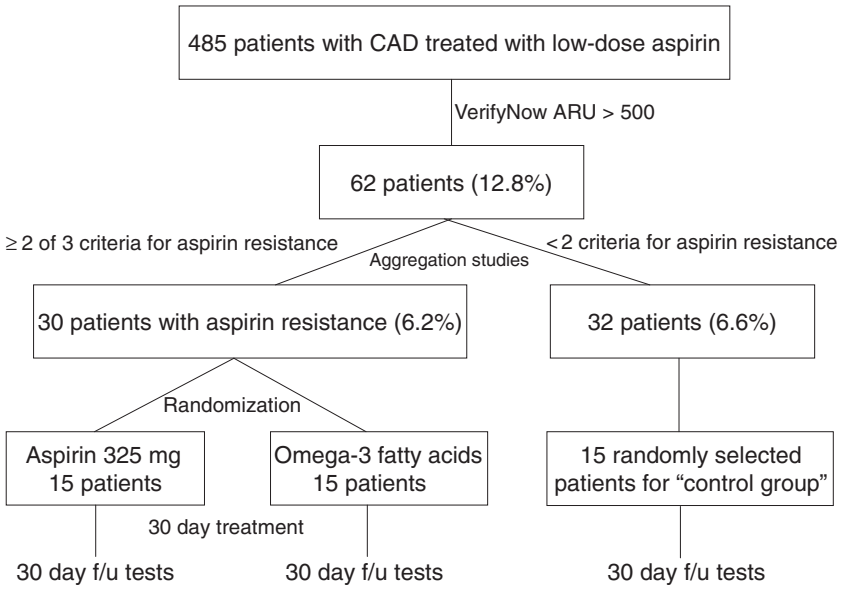

Figure 1 **Algorithm of the Study**

We aimed to identify 30 patients with aspirin resistance. Patients with stable coronary artery disease (CAD) (n = 485) were screened for resistance by the VerifyNow Aspirin assay (Accumetrics). Sixty-two patients (12.8%) had an aspirin reaction unit (ARU) score >500 and underwent further testing by platelet aggregation. Thirty patients (6.2%) were found to be resistant and were randomized to 1 of the 2 treatment groups. They were re-tested after 30 days of treatment. f/u = follow-up.

30-day treatment period. Patients were tested at conditions identical to the first baseline sample, after discontinuing the corresponding treatment (omega-3 fatty acids supplementation or high-dose aspirin) and reverting back to the original low-dose aspirin. The purpose of this phase was to examine the stability of aspirin response at the baseline conditions. Some of the patients were treated with low-dose aspirin when contacted; others were asked to take low-dose aspirin (without omega-3 fatty acids) for a period of at least 1 week before testing. A total of 24 patients (12 in each group) participated in the follow-up phase. The other 6 patients were not available or interested in participating or had significant changes in their medical management.

Blood sampling. All blood samples were obtained from an antecubital vein, with a 21-gauge needle, and collected in tubes containing 3.2% citrate. The tubes were filled to capacity and gently mixed. The blood samples were processed within 2 h of blood collection.

Evaluation of response to aspirin. In addition to the VerifyNow Aspirin assay, the following tests were performed at all time points.

LIGHT TRANSMITTANCE (TURBIDIMETRIC) PLATELET AGGREGATION. Light transmittance (turbidimetric) platelet aggregation was performed in platelet-rich plasma with a platelet count adjusted to approximately 250 × 103/mm^3. Platelets were stimulated with 0.5 mg/ml (1.6 mmol/l) AA and 5- and 10-μmol/l ADP. Aggregation was performed with a BioData PAP-4 platelet aggregometer (BioData, Horsham, Pennsylvania). The extent of aggregation was defined as the maximal light transmission ≤6 min after addition of the agonist, with platelet-poor plasma used as reference.

PLASMA LEVELS OF THROMBOXANE B2 (TXB2). Plasma levels of TXB2 were determined from frozen plasma samples (kept at −70°C until testing), with an enzyme immunoassay, according to the manufacturer's instructions (Cayman Chemical Co., Ann Arbor, Michigan).

Compliance at the initial interview. Compliance to aspirin (taken ≥1 week) was verified separately by the patient's attending physician, study nurse, and study physician. In cases of uncertainty, patients were not enrolled. Two patients with an ARU >500 had an exceptionally high level of AA-induced aggregation (>40%) and were asked to be re-tested after 1 week of aspirin treatment (1 patient became responsive and was withdrawn from the study; the other remained resistant but with lower levels of AA-induced aggregation than originally measured). In the 2 study groups, compliance to the specific regimen was verified by phone call after 2 weeks of treatment.

Statistical analysis. Continuous variables are presented as mean ± SD. Comparisons between aspirin-resistant and responsive patients (Table 1) were performed by unpaired Student *t* tests and chi-square tests for continuous and categorical variables, respectively. Intragroup comparisons (between the 2 time points) were performed by paired Student *t* tests. Intergroup comparisons (between the 2 study groups) of the magnitude of change induced by the specific treatment were performed by 2-factor analysis of variance with repeated measures (for group- and time-

Table 1 Clinical Characteristics and Concurrent Medications

	Aspirin-Resistant (n = 30)	Aspirin-Responsive (n = 455)
Age (yrs)	66 ± 9	67 ± 10
Women	10 (33)*	73 (16)*
BMI (kg/m^2)	27 ± 6	28 ± 12
Diabetes	11 (37)	170 (37)
Hypertension	18 (60)	291 (64)
Hyperlipidemia	29 (97)	404 (89)
Current smoker	5 (17)	48 (11)
Prior MI	19 (63)	255 (56)
Prior CABG	11 (37)	149 (33)
Prior PCI	26 (87)	403 (89)
Medications		
Aspirin 100 mg	29 (97)	421 (93)
Aspirin 75–81 mg	1 (3.3)	23 (5.1)
Aspirin 150–162 mg	0	11 (2.4)
Clopidogrel	0	22 (4.8)
Statins	29 (97)	404 (89)
Beta-blockers	21 (70)	321 (71)
ACE inhibitors/ARBs	19 (63)	266 (59)
Calcium-blockers	3 (10)	67 (15)

Values are mean ± SD or n (%). *p = 0.02.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass graft; MI = myocardial infarction; PCI = percutaneous coronary intervention.

dependence). Analyses were performed with SPSS version 11.0 statistical software (SPSS, Inc., Chicago, Illinois), and statistical significance was set at $p < 0.05$.

Results

To achieve the pre-specified goal of 30 patients with aspirin resistance (as defined by ≥2 of 3 criteria), screening of 485 patients was required (aspirin resistance rate of 6.2%) (Fig. 1). Patients with aspirin resistance had similar clinical characteristics to those of aspirin-responsive patients, except for a higher proportion of women among resistant patients (33.3% vs. 16%, p = 0.02) (Table 1). Patients with aspirin resistance were also treated with similar medications to aspirin-responsive patients (Table 1). It should be noted that concomitant clopidogrel treatment was not contraindicated. Among the 22 patients treated with clopidogrel, 2 had an ARU >500, but neither satisfied any criteria for aspirin resistance and therefore did not enter the study groups (they were also not included in the control group).

Platelet reactivity and response to aspirin in the study groups. The 30 patients with aspirin resistance were randomized to receive either omega-3 fatty acids (omega group, n = 15) or aspirin 325 mg (aspirin group, n = 15) for a period of 30 days. Clinical characteristics and concomitant medical treatment were similar among the 2 groups and did not significantly differ from the characteristics of the total aspirin-resistant cohort (presented in Table 1). In both the omega and aspirin groups, significant reductions in platelet aggregation and the VerifyNow Aspirin score were observed after 30 days of treatment (Table 2). Plasma levels of TXB2 were also reduced in both groups after 30 days of treatment (57% relative reduction in TXB2 levels in the omega group, and a 40% decrease in the aspirin group) (Fig. 2). Comparison of the magnitude of reductions in platelet reactivity and TXB2 levels between the 2 groups revealed that there were no significant intergroup differences, except for a trend for difference in 10-μmol/l ADP-induced platelet aggregation, with greater reduction in the omega group (relative reduction of 14.9% in the omega group vs. 6.9% in the aspirin group, p = 0.06) (Fig. 3).

Rates of aspirin resistance after treatment. After 30 days of treatment with omega-3 fatty acids, 3 patients (20%) remained resistant to aspirin (≥2 of 3 criteria), and 4 other patients fulfilled only 1 criterion for aspirin resistance (3 of them had ADP-induced aggregation ≥70%, and 1 had AA-induced platelet aggregation ≥20%). In the aspirin group after 30 days of treatment with aspirin 325 mg, 4 patients (26.7%) were still resistant to the effects of aspirin, and 5 other patients fulfilled 1 criterion for aspirin resistance (all had ADP-induced aggregation ≥70%). Thus, 80% of the patients in the omega group and 73% of the patients in the aspirin group were no longer resistant to aspirin, according to the definition employed in our study.

Safety. During the 30-day treatment period no bleeding episodes were reported in either group. One patient treated with omega-3 fatty acids reported belching for a few days that subsided gradually and did not require discontinuation of the drug.

Follow-up phase. Twenty-four patients (12 in each group) participated in this phase—performed approximately 1 year after the treatment period. As shown in Figure 4, in both groups platelet aggregation in response to AA and ADP increased significantly after discontinuation of the corre-

Table 2 Platelet Reactivity and Response to Aspirin in the Omega-3 Fatty Acid Versus Aspirin Groups at Baseline and After 30 Days of Treatment

	Omega Group			Aspirin Group		
	Baseline	Post	p Value	Baseline	Post	p Value
VerifyNow Aspirin (ARU)	566 ± 35	461 ± 47	<0.0001	554 ± 19	476 ± 57	0.0003
Aggregation 0.5-mg/ml AA (%)	19.1 ± 7	13.4 ± 5	0.003	18.9 ± 7	13 ± 6	0.01
Aggregation 5-μmol/l ADP (%)	68 ± 10	54.7 ± 11	0.0005	66.3 ± 6	56.7 ± 6	0.0002
Aggregation 10-μmol/l ADP (%)	77.1 ± 7	65.6 ± 11	0.002	74.7 ± 6	69.5 ± 6	0.007

VerifyNow Aspirin assay (Accumetrics).

AA = arachidonic acid; ADP = adenosine diphosphate; ARU = aspirin reaction units.

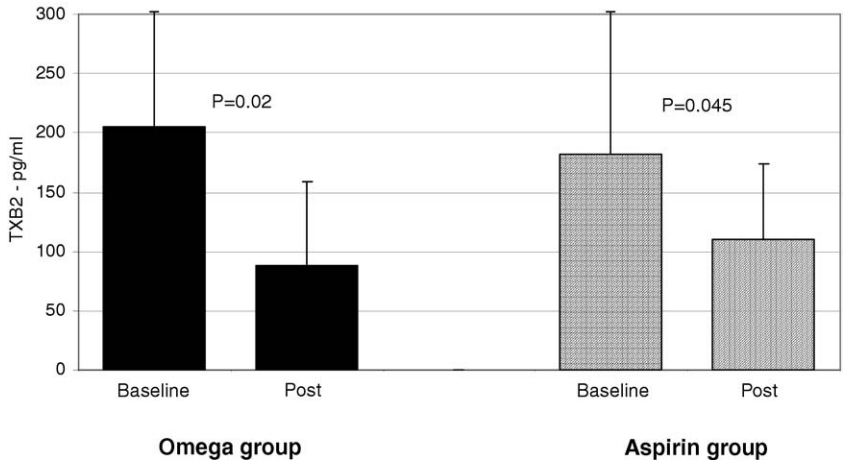

Figure 2 **Plasma TXB2 Levels at Baseline and After 30 Days of Treatment**

Plasma thromboxane B2 (TXB2) levels at baseline and after 30 days of treatment with omega-3 fatty acids (omega group) or 325 mg of aspirin (aspirin group). In both groups a significant reduction was noted from baseline after 30 days of treatment (57% relative reduction with p = 0.02 in the omega group, and 40% relative reduction with a p = 0.045 in the aspirin group). There were no significant differences between the groups at baseline or after treatment.

sponding treatment regimen and returned to levels similar to the baseline levels (in both groups there were no significant differences between the baseline and follow-up levels in any of the aggregation assays). Similar findings were noted with the VerifyNow Aspirin assay (data not shown).

Control group. The control group consisted of 15 patients who had a VerifyNow ARU >500 but did not satisfy criteria for aspirin resistance. The purpose of the control group was to examine whether there is significant variability in response to aspirin over a 30-day period, without any change in medical treatment. In contrast to the study groups, there were no significant differences in platelet aggregation or VerifyNow score between the baseline and 30-day measurements in the control group (Table 3).

Discussion

To our knowledge, this is the first study that examined potential therapeutic approaches to manage resistance to the antiplatelet effects of low-dose aspirin. Two approaches were evaluated: addition of omega-3 fatty acids or aspirin dose escalation (to 325 mg daily). We identified 30 patients with stable CAD who were aspirin resistant, on the basis of AA and ADP-induced platelet aggregation and the VerifyNow Aspirin assay criteria. These patients were randomized to receive 1 of the 2 treatment protocols for 30 days. Both therapeutic approaches were associated with a reduction in platelet aggregation, VerifyNow Aspirin score, and plasma TXB2 levels and therefore with improved response to aspirin. In addition, the majority of patients treated by both treatment

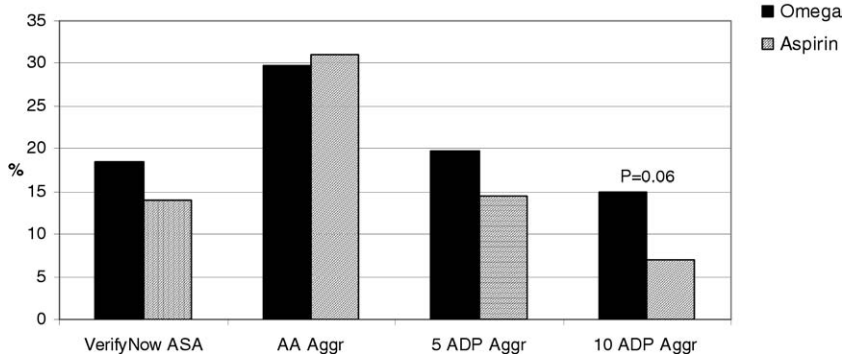

Figure 3 **Comparison of the Relative Reductions From Baseline to Post-Treatment in the 2 Groups**

Comparison of the magnitude of relative reductions from baseline to post-treatment in platelet aggregation (Aggr) and the VerifyNow Aspirin assay (Accumetrics) score between the omega group and the aspirin group. There were no significant differences between the 2 groups, except for a tendency for greater reduction in 10-μmol/l adenosine diphosphate (ADP)-induced platelet aggregation in the omega group (p = 0.06). AA = arachidonic acid.

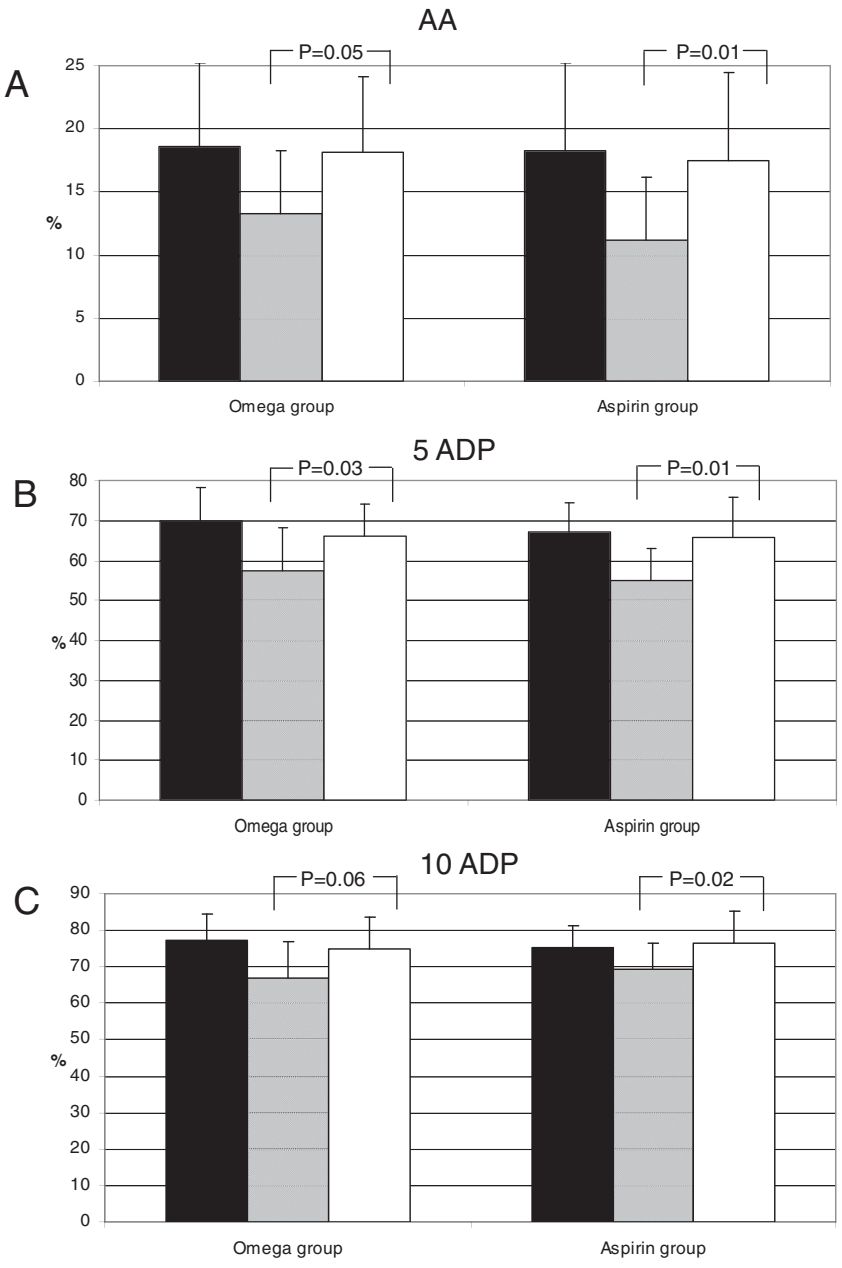

Figure 4 **Platelet Aggregation at Baseline, After Treatment, and During Follow-Up**

Platelet aggregation in response to arachidonic acid (AA) and adenosine diphosphate (ADP) in the omega and aspirin groups (12 patients in each group) at 3 time points: **(A)** baseline—low-dose aspirin **(solid bars)**; **(B)** after 30 days of treatment with the corresponding regimen—omega-3 fatty acids or high-dose aspirin **(gray bars)**; and **(C)** low-dose aspirin—after discontinuing the corresponding treatment regimens for at least 1 week (**open bars**—follow-up phase). In both groups, aggregation decreased after treatment and increased back to levels similar to the baseline levels after discontinuing the treatment regimens (p values for **B** vs. **C** time points).

protocols were no longer resistant to aspirin, according to the criteria employed in our study.

Aspirin-resistance rate. The prevalence of aspirin resistance found in our study (6.2%) is similar to that reported by Gum et al. (4) in patients with stable cardiovascular disease (5.2%). Both studies employed similar criteria for definition of aspirin resistance, although we also incorporated the VerifyNow Aspirin assay. Previous studies that reported higher rates of aspirin resistance (12% to 30%) studied somewhat different patient populations, such as patients undergoing PCI (5,6), and/or used different criteria for aspirin resistance (e.g., only the VerifyNow Aspirin assay) (6,7). Lower rates of aspirin resistance (<1% to 6%) were reported when using only AA-induced platelet aggregation as the criteria for resistance (14) and/or testing the effects of 325 mg aspirin (8). Our finding of a higher proportion of

JACC Vol. 55, No. 2, 2010
January 12, 2010:114–21

Table 3 Platelet Reactivity at Baseline and After 30 Days in the Control Group

	Baseline	30 Days
VerifyNow Aspirin (ARU)	519 ± 16	513 ± 19
Aggregation 0.5-mg/ml AA (%)	9.1 ± 3	9.5 ± 2.5
Aggregation 10-μmol/l ADP (%)	64.5 ± 10	61.9 ± 9
Aggregation 5-μmol/l ADP (%)	51.6 ± 12	52.8 ± 10

VerifyNow Aspirin assay (Accumetrics).
Abbreviations as in Table 2.

women among aspirin-resistant patients, compared with aspirin-responsive patients, is consistent with previous studies (4,6,13). Higher rates of aspirin resistance among women might contribute to the recently reported failure of aspirin to reduce the risk of a first MI in women, in contrast to its beneficial primary prevention effects in men (22).

Aspirin dose effect. Considerable research has been dedicated to study the dose-effect relationship of aspirin. In the Antithrombotic Trialists' Collaboration, a meta-analysis that included more than 200,000 patients, low-dose aspirin was associated with proportional risk reduction of vascular events similar to that of high-dose aspirin but with fewer gastrointestinal side effects and less bleeding (12). Furthermore, previous studies have shown that aspirin at low daily doses of 50 to 100 mg is an effective antithrombotic agent that generally causes efficient inhibition of COX-1 as demonstrated by ≥95% inhibition of serum TXB2 (23,24). However, Hart et al. (25) reported an incremental reduction in serum TXB2 levels with increasing doses of aspirin (81, 325, or 1,300 mg). In the ASPECT (Aspirin Induced Platelet Effect) study, Gurbel et al. (14) reported that dose-dependent effects of aspirin (81, 162, and 325 mg) were assay-dependent and were observed when using assays such as collagen- and ADP-induced platelet aggregation, PFA-100, and urinary 11-dehydroTXB2 but not AA-based assays. Therefore, although these studies indicate aspirin dose-response with respect to TXB2 synthesis (14,25), there does not seem to be a clear and consistent dose-effect relationship for aspirin, as assessed by platelet inhibition or clinical outcomes. This is in apparent disagreement with our study, which showed improved response to aspirin when increasing the dose from a low dose (75 to 162 mg) to 325 mg. However, in contrast to previous studies, we examined the effect of dose escalation only in patients with aspirin resistance, which might be a unique state. Lee et al. (13) have shown that low aspirin doses (≤100 mg) were associated with an increased prevalence of aspirin resistance compared with higher doses. Although the mechanisms for aspirin resistance are probably multiple and have not been well-established (24,26), in some cases there might be a contribution of reduced bioavailability of aspirin (e.g., reduced absorption) (26) or of increased platelet turnover and platelet hyper-reactivity (24,26,27). In both circumstances—reduced drug bioavailability, and increased platelet turnover—an increase in the dose of aspirin might be associated with better inhibition of its target—platelet COX-1.

Effect of omega-3 fatty acids on response to aspirin. Treatment of aspirin-resistant patients with omega-3 fatty acid supplementation was associated in our study with improved response to aspirin and reduced platelet reactivity, at least as effective as that achieved by increasing the aspirin dose. The mechanism of action probably involves a reduction in the availability of platelet membrane AA, along with an increase in EPA, which can compete with AA as a substrate of the COX-1 pathway (15,16). The increased ratio of EPA to AA likely leads to a reduction in thromboxane A2 formation from AA (15,16). Indeed, we have observed a 56.8% relative reduction in plasma TXB2 levels when omega-3 fatty acids were added to low-dose aspirin for 30 days. These results are supported by the study of Engström et al. (28), who showed that in 4 healthy volunteers addition of fish oil to low-dose aspirin augmented the inhibition of serum TXB2 by aspirin. Larson et al. (19) have also reported that omega-3 fatty acids enhance the antiplatelet effects of aspirin (measured by platelet aggregation) in healthy volunteers. We extended these findings to patients with aspirin resistance. It should be emphasized that the addition of omega-3 fatty acids (1 to 4 g/day) to aspirin in cardiovascular patients has not been associated with an increased risk of bleeding (20,21). Furthermore, omega-3 fatty-acid supplementation might have favorable effects in the secondary prevention of CAD and its complications (21,29,30). Taken together, the clinical and platelet function studies suggest that omega-3 fatty-acid supplementation might be a promising approach to optimize response to aspirin.

Study limitations. First, although we attempted to verify compliance to aspirin and the treatment regimens before and during the study, by several measures, we cannot absolutely rule out inadequate compliance. Second, unlike the 2 study groups, the control group did not consist of patients with aspirin resistance but rather patients with a VerifyNow Aspirin score >500 ARU who did not satisfy aspirin resistance criteria. Therefore, it is not a "classic" matched control group. However, it does indicate that in patients with stable CAD treated with low-dose aspirin there were no significant changes in the measured platelet indexes over a 30-day period. Therefore, the reductions in platelet reactivity and TXB2 levels we observed in the study groups are likely not the result of "spontaneous" fluctuations over time but are related to the effects of the therapeutic regimens given. Finally, the study groups are of relatively limited size for inter-group comparisons.

Conclusions and Clinical Implications

There is accruing evidence that resistance to the antiplatelet effects of aspirin is associated with an increased risk of adverse cardiovascular events (2,4–7,9–11). However, currently there is no established therapeutic approach to manage and overcome aspirin resistance in patients treated with low dose of the drug. We have demonstrated by using various platelet assays that either adding omega-3 fatty acids or

increasing the aspirin dose can improve response to aspirin and reduce residual platelet reactivity in stable patients with CAD. Treatment with both regimens was associated with a reduction in aspirin-resistance rates. We did not examine the clinical impact of such therapeutic interventions or the impact of routine monitoring of aspirin response. Further larger studies are required to assess whether our findings can be translated to a clinical benefit in patients with cardiovascular disease found to be resistant to aspirin and treated with the therapeutic regimens tested in our study.

Reprint requests and correspondence: Dr. Eli I. Lev, Rabin Medical Center, The Sackler Faculty of Medicine, 39 Jabotinsky Street, 49100 Petah Tikva, Israel. E-mail: elil@clalit.org.il or elev@tmhs.org.

REFERENCES

1. Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. Can J Cardiol 1995;11:221–7.
2. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002;105:1650–5.
3. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001;88:230–5.
4. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003;41:961–5.
5. Lev EI, Patel R, Maresh K, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. J Am Col Cardiol 2006;47:27–33.
6. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. J Am Coll Cardiol 2004;43:1122–6.
7. Chen WH, Chang X, Lee PY, et al. Aspirin resistance and adverse clinical events in patients with coronary artery disease. Am J Med 2007;120:631–5.
8. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. J Am Coll Cardiol 2005;46:1705–9.
9. Eikelboom JW, Hankey GJ, Thom J, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. Circulation 2008;118:1705–12.
10. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ 2008;336:195–8.
11. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Huisman MV. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. Arch Intern Med 2007;167:1593–9.
12. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324:71–86.
13. Lee PY, Chen WH, Ng W, et al. Low-dose aspirin increases aspirin resistance in patients with coronary artery disease. Am J Med 2005;118:723–7.
14. Gurbel PA, Bliden KP, DiChiara J, et al. Evaluation of dose-related effects of aspirin on platelet function results from the Aspirin-Induced Platelet Effect (ASPECT) study. Circulation 2007;115:3156–64.
15. Smith WL. Cyclooxygenases, peroxide tone and the allure of fish oil. Curr Opin Cell Biol 2005;17:174–82.
16. Engler MM, Engler MB. Omega-3 fatty acids: role in cardiovascular health and disease. J Cardiovasc Nurs 2006;21:17–24.
17. Knapp HR. Dietary fatty acids in human thrombosis and hemostasis. Am J Clin Nutr 1997;65:1687S–98S.
18. Mori TA, Beilin LJ, Burke V, Morris J, Ritchie J. Interactions between dietary fat, fish and fish oils and their effects on platelet function in men at risk of cardiovascular disease. Arterioscler Thromb Vasc Biol 1997;17:279–86.
19. Larson MK, Ashmore JH, Harris KA, et al. Effects of omega-3 acid ethyl esters and aspirin, alone and in combination, on platelet function in healthy subjects. Thromb Haemost 2008;100:634–41.
20. Harris WS. Expert opinion: omega-3 fatty acids and bleeding—cause for concern? Am J Cardiol 2007;99:44C–6C.
21. Eritsland J, Arnesen H, Gronseth K, Fjeld NB, Abdelnoor M. Effect of dietary supplementation with n-3 fatty acids on coronary artery bypass graft patency. Am J Cardiol 1996;77:31–6.
22. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352:1293–304.
23. Patrono C, Coller B, FitzGerald GA, Hirsh J, Roth G. Platelet-active drugs: the relationships among dose, effectiveness, and side effects: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126 Suppl:234S–64S.
24. Maree AO, Fitzgerald DJ. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation 2007;115:2196–207.
25. Hart RG, Leonard AD, Talbert RL, et al. Aspirin dosage and thromboxane synthesis in patients with vascular disease. Pharmacotherapy 2003;23:579–84.
26. Gasparyan AY, Watson T, Lip GY. The role of aspirin in cardiovascular prevention: implications of aspirin resistance. J Am Coll Cardiol 2008;51:1829–43.
27. Guthikonda S, Lev EI, Patel R, et al. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. J Thromb Haemost 2007;5:490–6.
28. Engström K, Wallin R, Saldeen T. Effect of low-dose aspirin in combination with stable fish oil on whole blood production of eicosanoids. Prostaglandins Leukot Essent Fatty Acids 2001;64:291–7.
29. Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 1999;354:447–55.
30. von Schacky C, Angerer P, Kothny W, et al. The effect of dietary omega-3 fatty acids on coronary atherosclerosis: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 1999;130:554–62.

Key Words: aspirin ■ omega-3 fatty acids ■ platelets.