THE ASSOCIATION OF CELECOXIB, ROFECOXIB, AND NON-SELECTIVE NONSTEROIDAL ANTI-INFLAMMATORY MEDICATIONS WITH INDICES OF THROMBOSIS AND ENDOTHELIAL FUNCTION IN THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS.

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INTRODUCTION

The Association of Celecoxib, Rofecoxib, and Non-selective Nonsteroidal Anti-Inflammatory Medications with Indices of Thrombosis and Endothelial Function in the Multi-Ethnic Study of Atherosclerosis

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Nonsteroidal anti-inflammatory medications (NSAIDs) are one of the most commonly prescribed and self-administered drugs in the United States (Singh 1999). However, up to 50% of patients taking these medications experience some variety of gastrointestinal side effect often resulting from erosive gastritis or GI bleeding (Wolfe 1999). The Cyclooxygenase-2 (COX-2) inhibitors rofecoxib and celecoxib were introduced to the market in 1999 and hailed as an effective, safer alternative to traditional NSAIDs in that the risk of GI side effects was far less with this new class of medications. COX-2 inhibitors became very widely prescribed, and despite controversy in recent years over their actual safety profile, pharmaceutical companies continue to pursue development of new COX-2 inhibitors.

The beneficial effects of aspirin, the prototypical NSAID, date back to 400 BC when Hippocrates recommended chewing leaves of the willow tree to relieve pain and fever. Not until the 19th century was the compound salicin extracted from willow bark, purified, and manufactured as the compound acetylated salicylic acid. In 1982 Sir John Vane won the Nobel Prize in Medicine for discovering aspirin's active mechanism as an inhibitor of prostaglandin synthetase (Levasque 2000). Over the ensuing years further understanding of aspirin's mechanism led the FDA in 1988 to propose aspirin use for the reduction of primary and recurrent myocardial infarction as well as ischemic strokes.

The majority of nonsteroidal anti-inflammatory medications act as non-selective inhibitors of the cyclooxygenase enzymes. These enzymes lead to the transformation of arachodonic acid (a derivative of the cellular phospholipid bilayer via actions of Phospholipase A_2) to prostaglandins, prostacyclin (PGI_2), thromboxanes (TXA_2), leukotrienes, and hydroxyeicosatetraenoic acids (HETEs). Initially formation of PGH_2

endoperoxide synthase oxygenates and cyclizes the arachidonic acid to form the cyclic peroxide prostaglandin G_2. Peroxidase activity then converts prostaglandin G_2 to prostaglandin H_2, a common precursor for all prostanoids. Prostaglandin H_2 is then metabolized through the actions of prostacyclin synthase, thromboxane synthase, and isomerase to yield cyclic prostanoids that are important to cardiovascular hemostasis: prostacyclin (prostaglandin I_2), thromboxane A_2, and prostaglandin E_2 (Khanapure 2007).

In the late 1980s several research labs across the country described experiments demonstrating that two separate forms of the cyclooxygenase enzyme may exist (Funk 2007). COX-1, generally thought to be the constitutive isoform, exists primarily within platelets and takes on certain "housekeeping" roles such as gastric cytoprotection, regulation of renal blood flow and platelet aggregation. COX-1 is the major enzyme responsible for producing thromboxane (TXA_2), a potent platelet-aggregating substance formed within the platelet that binds to a G-protein coupled receptor on the platelet plasma membrane. Thromboxane's role also includes vasoconstriction and proliferation of smooth muscle cells within blood vessels. The irreversible inhibition of COX-1 and the resulting decreased levels of thromboxane confer the cardioprotective benefits of aspirin (Funk 2007).

Conversely, the actions of COX-2 lead to the majority of prostacyclin (PGI_2) production, a potent inhibitor of platelet aggregation. Like thromboxane, prostacyclin is synthesized from arachidonic acid; however, the process takes place in arterial and venous tissues. Prostacyclin also promotes vasodilatation and inhibition of vascular smooth muscle cell proliferation. COX-2 exists within the endothelial cells and is largely inducible (the exception being constitutively in the kidney and brain). It carries the

responsibility of mediating the inflammatory response via the production of prostaglandins (Muhammad 2006). Non-selective NSAIDs cause varying degrees of COX-1 and COX-2 inhibition and lead to reversible platelet inhibition inadequate for cardioprotection (Hermann 2006).

The pharmaceutical industry quickly envisioned the potential of a medication that could selectively inhibit the enzyme responsible for the inflammatory and pain response without the consequences of inhibiting the prostaglandins responsible for gastric cytoprotection. Throughout the 1990's pharmaceutical companies focused efforts on the development of a product that would selectively inhibit COX-2. On December 31, 1998 the first COX-2 inhibitor, celecoxib, was approved by the FDA for the relief of the symptoms associated with osteoarthritis and rheumatoid arthritis. Its competitor, rofecoxib, was approved as safe and effective shortly thereafter on May 20, 1999. By 2001 approximately 58% of all NSAID prescriptions from specialists (31% from primary care providers) were for selective COX-2 inhibitors (De Smet 2006).

COX-2 inhibitors and increased cardiovascular risk

Concerns regarding the cardiovascular risk profile of this new class of medication were first publicly aired after a secondary *post hoc* analysis of the vioxx gastrointestinal outcomes research (VIGOR) study demonstrated that rofecoxib purported a significantly higher incidence of cardiovascular events compared with the non-selective anti-inflammatory naproxen (RR 2.38 [1.39-4.00]; p=0.002) (Bombardier 2000). Proponents of rofecoxib quelled an initial reaction by some that called for the withdrawal of

medication from the market, arguing that naproxen conferred a protective effect similar to aspirin, and therefore was an unfair comparison. Over the next four years these medications (including the newly approved valdecoxib) continued to hold a sizable portion of the market share of NSAIDs, although the concern surrounding the potential cardiovascular side effects lingered. These concerns would finally be validated with the publication of the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial that confirmed a significant increase in cardiovascular events compared with placebo (Bresalier 2005). Rofecoxib was withdrawn from the market on September 30, 2004 representing the largest prescription drug withdrawal in history (Topol 2004). The withdrawal resulted in a national uproar which included accusations of dishonesty on the part of the pharmaceutical industry and harsh criticisms and questions regarding the competence and integrity of the Food and Drug Administration for not taking action earlier in response to the VIGOR trial. The events created a backlash that can arguably be felt today through the apparent hypersensitivity the FDA appears to be demonstrating surrounding issues such as the safety of ECHO contrast.

Several prior studies also provided data to support the increase in MI in subjects using rofecoxib compared to naproxen (Bombardier 2000) and the increased risk of MI and sudden cardiac death in subjects using rofecoxib compared to celecoxib (Juni 2004). Most studies examining celecoxib, either compared to rofecoxib or a nonselective NSAID, have failed to show a similar increase in cardiovascular events (Celik 2006). One case-control study comparing 1718 case-patients to 6800 controls actually showed that the use of celecoxib was associated with a reduced risk of nonfatal MI when compared to rofecoxib or other NSAIDs (Kimmel 2005). However, the APC trial did

demonstrate a dose–dependent increase in CV risk in those patients on celecoxib compared to placebo (Solomon 2005). More studies are needed to better understand the impact of these medications upon cardiovascular events.

COX-2 inhibitors and proposed mechanisms of increased cardiovascular risk

The precise mechanisms for the apparent increase in risk imposed by COX-2 inhibitors are not yet completely understood; although several influences coxibs have over the cardiovascular system have been demonstrated. In the 1990's Dr. Garrett Fitzgerald and his colleagues proposed that the primary mechanism involves increased platelet activation and aggregation through selective blocking of prostacyclin formation with little inhibition of prothombotic platelet-derived thromboxane A_2 (TXA_2) (Fitzgerald 2001). The unopposed, platelet derived thromboxane A_2 is thought to lead to increased thrombosis and resulting ischemic events (Marwali 2006). Studies have shown celecoxib and rofecoxib at clinically used doses cause a 65-85% decrease in prostacyclin synthesis based on urinary metabolite measures (Funk 2007). However, no effect of COX-2 inhibitors on platelet thromboxane synthesis has been demonstrated. This theory later became known as the 'Fitzgerald Hypothesis' and is still thought to be the primary mechanism by which this class of medications confers cardiovascular risk.

Shinmura et al. demonstrated that COX-2 inhibition may inhibit the cardioprotective effects of the late phase of ischemic preconditioning using the rabbit model (Shinmura 2000). Ischemic preconditioning of the coronary circulation leads to an increase in myocardial COX-2 mRA levels, COX-2 protein expression, levels of

prostaglandin (PGE_2), and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin). Treatment with celecoxib eliminated the increase in prostaglandin and 6-keto-$PGF_{1\alpha,}$ thereby blocking the cardioprotective effects of ischemic preconditioning and demonstrating a protective role COX-2 may play during coronary ischemia.

More recently it has been suggested that COX-2 inhibitor's effect on cardiovascular risk may occur through alterations of the endothelium (Verma 2001). Several small trials have indicated that the effect on the endothelium is dependent on the specific coxib used. Specifically, Chenevard et al. found that celecoxib taken for two weeks caused improvement in endothelial function as measured by Flow Mediated Dilation (FMD) in a group of fourteen male patients with severe coronary artery disease when compared with placebo (Chenevard 2003). Widlansky et al. found a similar enhancement of endothelial function in hypertensive patients taking celecoxib for one week (Widlansky 2003). Contrarily, other authors have found no effect of rofecoxib on endothelial function in healthy adults (Verma 2001) as well as subjects with coronary artery disease, acute coronary syndrome, and rheumatoid arthritis (Title 2003, Lekakis 2007, Wong 2007). Although these studies were small in nature they provide initial evidence that different coxibs may have different effects on endothelial function. This difference in function could help to explain the apparently unequal distribution of cardiovascular risk between users of rofecoxib and celecoxib.

There are several potential and conflicting mechanisms which may account for COX-2 inhibitors altering endothelial function. COX-2 (along with COX-1) can be found in the vascular endothelium and may have effects on endothelial function though a variety of mechanisms. COX-2 inhibitors have been shown to decrease C-reactive

protein (CRP) (Lekakis 2007). CRP has posttranscriptional effects on endothelial NO synthase mRNA stability, leading to diminished NO bioavailability. Decreased NO bioavailability is directly associated with decreased endothelial function. Therefore, a decrease in CRP may improve endothelial function. COX-2 inhibitors have also been shown to decrease oxidized LDL; an effect that potentially leads to decreased oxidative stress on the endothelium resulting in improved endothelial function (Chenevard 2003).

Flow Mediated Dilation, endothelial function, and cardiovascular risk

Endothelial function is recognized as a 'barometer' of vascular health and predictor of cardiovascular events (Vita 2002). Dysfunction of endothelial cells is thought to occur very early in the process of atherosclerosis; therefore the assessment of endothelial function has been investigated as a useful prognostic tool for cardiovascular events (Verma 2003). Endothelial dysfunction is defined as a decrease in the functional levels of nitric oxide (NO) either through decreased production or decreased bioavailability of the substance. Vascular dilatation can occur through an endothelial dependent or independent process. Endothelium independent vasodilation is achieved with exogenous NO which has a direct effect on the smooth muscle of the vessel wall (Moens 2005). It is used to determine the maximum vasodilator response of the vasculature and has been shown to be affected by cardiovascular risk factors (Adams 1998). Endothelium dependent vasodilation is dependent on the regulatory efforts of the endothelial cells themselves.

The measurement of endothelium dependent function through brachial artery Flow Mediated Dilation (FMD) was first described in 1989 (Anderson 1989). Blood flow through a vessel causes shear stress that is sensed by the endothelium and results in the release of vasodilatory substances (Moens 2005). Specifically, in response to shear stress, calcium activated potassium channels open, hyperpolarizing the cell causing an influx of calcium. Increased levels of calcium leads to the production of NO through activation of NO synthase (eNOS) (Joannides 1995).

The measurement of FMD has evolved to become a widely used clinical research tool employed to assess endothelial function and further evaluate cardiovascular risk profiles. Brachial artery FMD has been shown to be highly correlated with the capacity for dilation in coronary circulation (Anderson 1995) reflecting on its potential, non-invasive predictability of coronary artery endothelial function. The ability for the coronary arteries to dilate is decreased in patients with atherosclerosis and those with cardiovascular risk factors (Moens 2005). The dilatory capacity of coronary arteries has also been shown to improve with risk reduction therapy (Vita 2000). Yeboah et al demonstrated that FMD is a predictor of cardiovascular events in adults over 65 (Yeboah 2007), while Shrimbo et al. demonstrated similar findings in a younger multi-ethnic cohort free of baseline cardiovascular disease (Shrimbo 2007). In summary, the measurement of brachial FMD is a widely accepted technique for accurately and reliably assessing endothelial function, thereby indicating level of cardiovascular risk.

NON-SELECTIVE NSAIDS

There are no randomized, placebo-controlled studies evaluating non-selective nonsteroidal anti-inflammatory medications and cardiovascular risk. The available evidence includes approximately 16 observational studies that yield conflicting results: six demonstrating increased CV risk, five demonstrating decreased CV risk, and five showing no association (Cheng 2006). Despite the lack of definitive evidence, in February 2005 the FDA recommended a black box warning on over the counter NSAIDs highlighting the potential for increased risk of cardiovascular events: "Nonselective non-ASA NSAIDs may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. The risk may increase with duration of use. Patients with cardiovascular disease or risk factors for cardiovascular disease may be at greater risk" (Antman 2007).

Prior to this statement the cardiovascular safety profile of NSAIDs was largely taken for granted. It has been known that NSAIDs can increase salt and water retention potentially contributing to hypertension and subsequent cardiovascular events. However the inhibition of both COX-1 and COX-2 theoretically should not upset the balance of vasoactive eicosanoids and therefore not promote thrombosis (Cheng 2006). Some data suggests that certain non-ASA, nonselective NSAIDs may actually convey a cardioprotective benefit similar to aspirin as a result of their ratio of COX-1:COX-2 inhibition (Cheng 2006). Other data suggest that non-selective NSAIDs with higher degrees of COX-2 specificity may cause an imbalance in vasoactive eicosanoids. Further evidence that the association between non-selective NSAIDs and cardiovascular risk is

not purely a 'class effect' can be found in a meta-analysis of randomized trials published in 2006. The analysis demonstrated that when ibuprofen and diclofenac – but not naproxen - are used at higher doses, the risk of vascular events is moderately elevated to a level similar to that imposed by selective COX-2 inhibitors (Kearney 2006).

The issue is complex with poorly defined guidelines based on conflicting data in various patient populations. The approach to the use of these medications has direct and meaningful implications for the treatment of patients, especially those at high risk for cardiovascular events.

Significance

Prior to rofecoxib being withdrawn from the market in 2004, an estimated 80 million patients had already taken this medication worldwide. Pharmaceutical companies continue to invest in COX-2 inhibitors, making it paramount that we understand the mechanism by which particular drugs in this class confer cardiovascular risk. This study will help to clarify the effects celecoxib, rofecoxib and non-ASA nonsteroidal anti-inflammatory medications have on endothelial function and indices of thrombosis. Assessments of flow mediated dilation will be used to assess the vascular health of the subjects taking these medications. Any differences of effect may help to further elucidate why and how cardiovascular risk is differentially imposed within this class of controversial medications.

It is the intent of this discussion to explore mechanisms by which both COX-2 inhibitors and nonselective anti-inflammatory medications may influence cardiovascular risk. Specifically, I will present data from the Multi-Ethnic Study of Atherosclerosis in

order to help identify an association of nonsteroidal anti-inflammatory medications with endothelial function and indices of thrombosis. It is my hope that this discussion will lead to a more complete understanding of the cardiovascular risk associated with this class of medications that has had a dramatic effect on public health.

References

Adams MR, Robinson J, McCredie R, et al. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol*. 1998;32:123-27.

Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235-41.

Antman E, Bennet J, Daugherty A et al. Use of Nonsteroidal Antiinflammatory Drugs : An Update for Clinicians: A Scientific Statement from the American Heart Association. *Circulation*. 2007;115:1634-42.

Bennett J, Daugherty A, Herrington D. The Use of Nonsteroidal AntiInflammatory Drugs (NSAIDs) : A Science Advisory From the American Heart Association. *Circulation*. 2005;111:1713-16.

Bombardier C, Laine L, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis: VIGOR Study Group. *N Engl J Med*. 2000;343:1520-28.

Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med*. 2005;352:1092-102.

Celik T, Iyisoy A et al. Cyclooxygenase-2 inhibitors dilemma in cardiovascular medicine: Are all family members bad players? *International Journal of Cardiology*. 2007;116:259-60.

Chenevard R, Hurlemann D et al.Selective COX-2 Inhibition Improves Endothelial Function in Coronary Artery Disease. *Circulation*. 2003;107:405-9.

Cheng, J. Use of Non-Aspirin Nonsteroidal Antiinflammatory Drugs and the Risk of Cardiovascular Events. *The Annals of Pharmacotherapy*. 2006;40:1785-96.

Corretti MC, Anderson TJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39: 257–65.

De Smet BD, Fendrick AM, Stevenson JG, Bernstein SJ. Over and under-utilization of cyclooxygenase-2 selective inhibitors by primary care physicians and specialists: the tortoise and the hare revisited. *J Gen Intern Med*. 2006;21(7):694-7.

Fitzgerald GA, Cheng , Austin S. COX-2 inhibitors and the cardiovascular system. *Clin Exp Rheumatol*. 2001;25:S31-6.

Funk C, Fitzgerald, G. COX-2 Inhibitors and Cardiovascular Risk. *J Cardiovasc Pharmacol*. 2007;50:470-78.

Graham DJ, Campen D, et al. Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: nested case-control study. *Lancet*. 2005;365:475-481.

Hermann M. Ruschitzka F. Coxibs, non-steroidal anti-infammatory drugs and cardiovascular risk. *Intern Med J*. 2006;36:308-19.

Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314-19.

Joshi G, Gertler R, Fricker R. Cardiovascular Thromboembolic Adverse Effects Associated with Cyclooxygenase-2 Selective Inhibitors and Nonselective Antiinflammatory Drugs. *Anesthesia & Analgesia*. 2007;105:1793-1804.

Juni P, Nartey L, et al. Risk of cardiovascular events and rofexcoxib: cumulative meta-analysis. *Lancet*. 2004;364:2021-29.

Kearney P Baigent C, Godwin J, et al. Do selective cyclo-oxygenase-2 inhibitors and traditional non-seroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomized trials. *BMJ*. 2006:332:1302-08.

Khanapure S, Garvey D, Janero D. Eicosanoids in Inflammation: Biosynthesis, Pharmacology, and Therapeutic Frontiers. *Current Topics in Medicinal Chemistry*. 2007;7:311-40.

Kimmel SE, Berlin JA, et al. Patients exposed to rofecoxib and celecoxib have different odds of nonfatal myocardial infarction. *Annals of Internal Medicine*. 2005;142:157-164.

Lekakis JP, Vamvakou G, Andreadou I, et al. Divergent effects of rofecoxib on endothelial function and inflammation in acute coronary syndromes. *Int J Cardiol*. 2007;116:259-60.

Lévesque H, Lafont O. Aspirin throughout the ages: a historical review. *Rev Med Interne*. 2000; Mar;21 Suppl 1:8s-17s.

Marwali M, Mehta J. COX-2 inhibitors and cardiovascular risk. *Thrombosis Haemostasis*. 2006;96:401-406.

Moens A, Goovaertrs I, Claeys M, Vrints C. Flow Mediated Vasodilation: A diagnostic Instrument, or an experimental Tool? *Chest*. 2005;127:2254-63.

Shimbo D, Grahame-Clarke C, Miyake Y, Rodriguez C, Sciacca R, Di Tullio M, Boden-Albala B, Sacco R, Homma S. The association between endotheial dysfunction and

cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis*. 2007; 192:197-203.

Shinmura K, Tang XL, Wang Y, et al. Cyclooxygenase-e mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc Natl Acad Sci USA*. 2000;97:10197-202.

Silverstein FE, Faich G, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis. The CLASS study: A randomized controlled trial. *JAMA*. 2000;284:1247-1255.

Singh G, Triadafilopoulos G. Epidemiology of NSAID induced gastrointestinal complications. *J Rheumatol*. 1999;26:Suppl 56:18-24.

Solomon SD McMurray JJ, et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med*. 2005;352:1071-1080.

Title L, Giddens K, McInerney M. Effect of cyclooxygenase-2 inhibition with rofecoxib on endothelial dysfunction and inflammatory markers inpatients with coronary artery disease. *Journal of the American College of Cardiology*. 2003;42:1747-53.

Topol EJ. Failing the public health—rofecoxib, Merck, and the FDA. *N Engl J Med*. 2004; 351:1707–09.

Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. *Circulation*. 2003;108:2054-59.

Verma S, Raj SR, et al. Cyclooxygenase-2 blockade does not impair endothelial vasodilator function in healthy volunteers : randomized evaluation of rofecoxib versus naproxen on endothelium-dependent vasodilation. *Circulation*. 2001;104:2879-2882.

Verma S, Szmitko P. Coxibs and the endothelium. *Journal of the American College of Cardiology*. 2003;42:1754-56.

Vita JA, Keaney JF. Endothelial function: a barometer for cardiovascular risk? *Circulation*. 2002;106:640-42.

Warner T, Mitchell J. COX-2 selectivity alone does not define the cardiovascular risks associated with non-steroidal anti-inflammatory drugs. *Lancet*. 2008;371:270-273.

Widlansky, M, Price DT, Gokce N, et al. Short- and long-term COX-2 inhibition reverses endothelial dysfunction in patients with hypertension. *Hypertension*. 2003;42:310-15.
Wolfe, M, Lichtenstein D, Singh G. Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs. *N Engl J Med*. 1999;340:1888-99.

Wong M, Joang B McNeill K, et al. Effects of selective and non-selective cyclo-oxygenase inhibition on endothelial function in patients with rheumatoid arthritis. *Scand J Rheumatol*. 2007;36:265-69.

Yeboah J, Crouse J, Hsu F, Burke G, Herrington D. Brachial Flow-Mediated Dilation Predicts Incident Cardiovascular Events in Older Adults. *Circulation*. 2007;115:2390-97.

Chapter I

The Association of Celecoxib, Rofecoxib, and Non-selective Nonsteroidal Anti-Inflammatory Medications with Indices of Thrombosis in the Multi-Ethnic Study of Atherosclerosis

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Abstract

Background:
Cyclooxygeanse-2 (COX-2) inhibitors and non-selective nonsteroidal anti-inflammatory medications (NSAIDs) have been associated with increased cardiovascular risk. COX-2 inhibition may lead to an imbalance in prostanoids producing a prothrombotic state, thereby increasing cardiovascular events. Limited data exists on indices of thrombosis in patients taking these medications.

Methods:
Through a cross-sectional analysis of subjects within the Multi-Ethnic Study of Atherosclerosis (MESA), we investigated the association between use of celecoxib (n=235), rofecoxib (n=163), and non-selective NSAIDs (n=1121) use with d-dimer, fibrinogen, von Willebrand Factor (vWF), Factor VIII, ICAM-1, PAI-1 compared with controls (n=5180).

Results:
There was a statistically significant association of elevated d-dimer levels with use of celecoxib ($p<0.0001$), rofecoxib ($p=0.0014$) and non-selective NSAIDs ($p=0.0003$). Also, subjects taking celecoxib at high doses (>250mg daily) had significantly higher d-dimer levels than those taking lower doses (<150mg daily). These associations continued to be present after logarithmic transformation of d-dimer. There was an effect modification of aspirin use upon the relationship of COX-2 inhibitor and non-selective NSAID use and d-dimer that was not present after logarithmic transformation of d-dimer.

Conclusions:
The current analysis supports the hypothesis that the cardiovascular risk associated with COX-2 inhibitors and non-selective NSAID results from increased thrombotic proclivity.

Introduction

Non-steroidal anti-inflammatory medications (NSAIDS) are one of the most commonly self-administered and prescribed medications in the world (Singh 1999). The use of selective NSAIDs (COX-2 inhibitors) has been associated with increased cardiovascular risk. The APPROVe trial in 2005 demonstrated a clear increase in cardiovascular events in patients taking rofecoxib compared with placebo (Bresalier 2005). The subsequent withdrawal of rofecoxib from the market has led to an increased focus on this class of medications. Analysis of other trials and a series of observational studies have confirmed the increase in cardiovascular risk, and have provided data suggesting celecoxib also may be linked to cardiovascular events (Nartey 2004 & Solomon 2008).

Observational studies have indicated that non-selective NSAIDs may be associated with increased cardiovascular risk. The available evidence is limited to approximately 16 observational studies that yield conflicting results: six demonstrating increased CV risk, five demonstrating decreased CV risk, and five showing no association (Cheng 2006). NSAIDs are known to cause salt and water retention and are associated with increases in blood pressure; however, other mechanisms increasing cardiovascular risk may exist. Existing data indicating risk, combined with reasonably established mechanisms potentially conveying risk have raised enough concern that in February 2005 the FDA recommended a black box warning on over the counter NSAIDs highlighting the potential for increased risk of cardiovascular events.

A great deal of controversy exists surrounding the potential mechanistic effects that selective and non-selective NSAIDs have on cardiovascular risk. Thrombosis has

been suggested as the central mechanism for the increased cardiovascular risk associated with selective and perhaps non-selective NSAIDs (Fitzgerald 2001). Specifically COX-2 inhibitors and non-selective NSAIDs with high degrees of COX-2 selectivity are thought to increase platelet activation and aggregation through selective blocking of prostacyclin (PGI_2), an inhibitor of platelet aggretation, with little inhibition of prothombotic, platelet-derived thromboxane A_2 (TXA_2) (Ftizgerald 2001, Grosser2006). Widlansky et al demonstrated decreased urinary metabolites of PGI_2 after 1 week of celecoxib use (Widlansky 2003). The unopposed, platelet derived TXA_2 is theorized to increase thrombosis resulting in ischemic events (Marwali 2006). Although this mechanism has been generally accepted, there is a dearth of data on indices of thrombosis in patients taking these medications.

Recent meta-analysis have established the association of D-dimer [OR 1.7; 95% CI: 1.3-2.2)], fibrinogen [OR1.8; 95% CI: 1.6-2.0], and Von Willebrand factor (vWF) [OR 1.23; 95% CI: 1.13-1.33] with cardiovascular disease in long term prospective studies (Lowe 2005). Juhan-Vague et al showed an increase in myocardial infarction and sudden death in subjects with higher PAI-1 at baseline (Juhan-Vague 1996). Others have found elevated PAI-1 levels associated with unstable angina and acute myocardial infarction (Al-Nozha 1994 & Soeki 2000). Although ICAM-1 and Factor VIII are known to play roles in the formation of thrombosis and subsequent cardiovascular events, there has not been sufficient prospective data on their associations with cardiovascular disease (Lowe 2005).

D-dimer is a well described fibrin degradation product that is present during fibrinolysis that can be indicative of active thrombosis and predictive of future events.

D-dimer concentrations have been shown to be higher in patients with acute cardiovascular events (Bayes-Genis 2000, Kamikura 1997, Kruskal 1987, Van der Putten 2006); furthermore increased concentrations months after myocardial infarction have been associated with recurrent coronary events at a 2 year follow up (Moss 1999).

Fibrinogen forms the substrate for thrombin, which cross-links fibrin into an insoluble matrix. It is a determinant of blood viscosity and promotes platelet adherence and aggregation (Frennette 1996), a crucial step early in the process of myocardial infarction. The Fibrinogen Studies Collaboration conducted a meta-analysis on 154,211 subjects that demonstrated associations between fibrinogen levels and risk of cardiovascular disease in healthy, middle-aged adults (Danesh 2005).

Von Willebrand Factor interacts with the glycoprotein 1b/IX-V-receptor complex on the platelet's surface and is required for primary hemostasis in states of high shear stress (Van der Putten 2006). vWF is a predictor of cardiac events in patients with known vascular disease and is thought to play a direct role in the pathogenesis of myocardial infarction (Spiel 2008). The molecule has been shown to be as consistent of a measure as blood pressure or total serum cholesterol in decade to decade analysis making it a potentially suitable for long-term risk stratification for cardiovascular events (Danesh 2004).

In order to further define the mechanism by which NSAIDs confer cardiovascular risk, the association between indices of thrombosis and the use of COX-2 inhibitors and non-selective NSAIDs was examined in a large, multi-ethnic population based cohort.

Methods

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based study of 6814 Caucasian, African-American, Hispanic, and Chinese men and women aged 45-84. The patients were recruited from 6 centers in the United States: Baltimore, MD; Chicago, IL; Forsyth County, NC; New York, NY; Los Angeles, CA; and St. Paul, MN. The primary objective of MESA is to determine the characteristics related to the progression of subclinical cardiovascular disease. All subjects were free of diagnosed cardiovascular disease upon enrollment into the study. Subjects within the cohort had four examination visits over the course of approximately five years. All data from the current cross-sectional analysis were attained at the first visit. The study was approved by Institutional Review Boards at each center and all subjects gave informed consent. The details regarding recruitment, objectives, and design have been previously published (Bild 2002). All subjects within MESA that had the laboratory values of interest were used in the current analysis.

Clinical Evaluation

Participants within the cohort provided a complete medical history and had anthropometric and laboratory measurements collected upon entry into the study during visit one (July 2000-Augest 2002). The data for this cross-sectional study was obtained at visit one.

Laboratory Measurments

All assays were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT).

D-dimer: Fibrin fragment D-dimer is measured using an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ) on the Sta-R analyzer (Diagnostica Stago, Parsippany, NJ). The assay utilizes microlatex particles to which specific antibodies have been attached. In the presence of the antigen (D-dimer), the antibody-coated latex particles agglutinate to form aggregates that absorb more light. This increase in light absorption is a function of the antigen level present in the test sample. The normal reference range is 0.22 - 4.0 ug/mL, with expected normal values <0.4 ug/ml. The analytical CV for this assay is 8%.

Fibrinogen: Fibrinogen antigen is measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). The amount of fibrinogen present in the sample is quantitatively determined by immunochemical reaction. Complexes formed between antigen and antibody molecules scatter light passing through the sample. The intensity of the scattered light is proportional to the concentration of the antigen (fibrinogen) in the sample. Expected values for fibrinogen in normal, healthy individuals are 180 – 350 mg/dl. Intra-assay and inter-assay analytical CVs are 2.7% and 2.6%, respectively.

Von Willebrand factor (vWf): vWf is measured by an immunoturbidimetric assay on the Sta-R analyzer (liatest vWF; Diagnostica Stago, Parsippany, NJ). The assay utilizes latex particles to which specific antibodies have been attached. In the presence of antigien (vWF) the particles agglutinate to form aggregates, which absorb more light.

This increase in absorbance is proportional to the vWF present in the test sample. The results are presented as percent vWF, with an expected normal range of 50-160%. The intra-assay and inter-assay analytical CVs are 3.7% and 4.5%, respectively.

Factor VIII: Factor VIII levels are determined by measuring the clot time of a sample in factor VIII deficient plasma in the presence of activators utilizing the Sta-R analyzer (STA-Deficient VIII; Diagnostica Stago, Parsippany, NJ). The results are given as percent factor VIII, with reported normal plasma range of factor VIII in the adult population between 60 and 150%. The analytical CV for the factor VIII assay is 10%.

Soluble Intercellular Adhesion Molecule-1 (sICAM-1): sICAM-1 is measured by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). sICAM-1 is sandwiched by an immobilized monoclonal antibody and the enzyme-linked monoclonal antibody. The amount of ICAM-1 present is determined by colorimetric reaction. The laboratory analytical CV is 5.0%, with a healthy reference mean of 326 +/- 89 ng/mL. The assay range is 2.73 – 49.55 ng/ml.

Plasminogen Activator Inhibitor-1 (PAI-1): The PAI-1 assay was originally developed by Dr. Désiré Collen and colleagues (DeClerck, et al, 1988), and is sensitive to free PAI-1 (both latent and active) but not PAI-1 in complex with tissue plasminogen activator. The Laboratory for Clinical Biochemistry Research has extensive experience with this assay (Macy, et al, 1993), having used it in over 6,000 epidemiological participants to date. The analytical CV for this assay is 3.5%. The significant diurnal change in PAI-1 levels and the potential for contamination by platelets makes attention to the details of blood drawing particularly important (Macy, et al, 1993; Tracy & Bovill 1995). The expected normal range is 5 -66 ng/mL.

Statistical Analysis

The data are presented as mean +/-SD for continuous variables and the frequencies of subjects in each category for categorical variables. Student T-tests were used to compare continuous covariates and Chi-Square tests were used to compare frequencies. Normality of the outcome variables of interest was assessed. The distribution of D-dimer was skewed, therefore all analysis were also performed after logarithmic transformation of D-dimer was applied.

As data was collected from 6 separate centers in the US, intraclass (ie intrasite) correlations were assessed and they were not statistically different from 0. Multiple linear regression analysis was used to investigate the association between indices of thrombosis (D-dimer, fibrinogen, Von Willebrand Factor, Factor VIII, ICAM-1 and PAI-1) and selective (COX-2 inhibitors) and non-selective NSAID use. The aggregate of subjects using either celecoxib or rofecoxib were initially compared to controls not using a COX-2 inhibitor. The subjects using celecoxib and rofecoxib were than individually compared to non-users of COX-2 inhibitors. Users of non-selective NSAIDs were excluded from the control groups. Multiple linear regression analysis was also performed to investigate the association between the indices of thrombosis and non-selective NSAIDs. Similarly, subjects using COX-2 inhibitors were excluded from the control group. ANCOVA was used to compare the variables among the low, medium, and high doses of celecoxib. Analysis is presented with adjustment for conventional covariates: age, gender, race, hypertension, diabetes, tobacco use, total cholesterol, and BMI. In addition, CRP was added as a covariate to attempt to control for the inherent indication bias of NSAID use; NSAIDs are often prescribed for chronic inflammatory states. In

order to assess for the potential effect modification of aspirin use on the relationship of COX-2 inhibitor and non-selective NSAID use with indices of thrombosis, a separate model evaluating the interaction between aspirin use and NSAID use (COX-2 and non-selective) was employed. A correction for multiple comparisons was not made as all analyses were prespecified. If patients were coded as using both non-selective NSAIDs and COX-2 inhibitors they were excluded from the analysis. All analysis was done using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Subjects

In the MESA cohort there were 1121 users of non-selective NSAIDs, 235 users of celecoxib, and 163 users of rofecoxib. There were 56 subjects using low dose celecoxib (<150mg daily), 123 using moderate dose (151-250mg daily), and 46 using high dose (>250mg daily). The doses are reported as aggregates of actual total intake indicated by each subject and not necessarily the prescribed dosage and frequency. There were no subjects taking a cumulative dose of celecoxib >400mg daily. Users of COX-2 inhibitors were more likely to be older, male, Caucasian, hypertensive, diabetic and have higher BMIs. Non-selective NSAID users also tended to be Caucasian, male, and have higher BMIs; however, they were younger and less likely to be diabetic compared with controls. Users of COX-2 inhibitors and non-selective NSAIDs both tended to self report arthritis and have higher levels of inflammatory markers compared with controls (Table 1).

Association of COX-2 inhibitors and non-selective NSAID use with d-dimer

Subjects taking COX-2 inhibitors (celecoxib and rofecoxib users combined) had significantly higher d-dimer levels when compared with controls (0.5923 vs 0.3475, $p<0.0001$) after adjustment for age, gender, race, tobacco use, hypertension, diabetes mellitus, total cholesterol, body mass index, C-reactive protein, aspirin use and the interaction of aspirin with celecoxib. Similarly only subjects taking celecoxib had a significantly higher d-dimer compared to controls not taking any other selective or non-selective NSAID (0.6485 vs 0.3460, $p<0.0001$) after controlling for covariates (Table 2). Those taking rofecoxib also had a significantly higher d-dimer compared to controls not taking any other selective or non-selective NSAID (0.5228 vs 0.3444, $p=0.0014$) after controlling for covariates. The associations with celecoxib and rofecoxib held up to logarithmic transformation of d-dimer at α levels of 0.0413 and 0.0200 respectively. When the presence or absence of arthritis was used as a covariate in the model in place of CRP, similar associations were found.

Subjects taking non-selective NSAIDs had significantly higher d-dimer levels than controls (0.4040 vs 0.3429, $p=0.0003$) after adjusting for the same covariates. The association of logarithmically transformed d-dimer with non-selective NSAIDs yielded an α level of 0.0588.

Effect Modification of Aspirin on the relationship between COX-2 inhibitor and non-selective NSAID use and D-dimer (Table 3)

Through investigating the association of COX-2 inhibitor use (celecoxib and rofecoxib users combined) and d-dimer levels, an interaction between COX-2 inhibitor use and concurrent aspirin use was found (p=0.0004). Subjects taking COX-2 inhibitors (celecoxib and rofecoxib combined) without concurrent aspirin had significantly higher d-dimer levels than subjects not taking COX-2 inhibitors or aspirin (0.6419 vs 0.3246, p<0.0001) after controlling for covariates. However, in subjects taking both COX-2 inhibitors and aspirin there was no statistical difference in d-dimer levels when compared to controls taking aspirin alone (0.4047 vs 0.4341, p=0.7935).

A similar interaction was found when examining subjects taking celecoxib (p=0.0010) or rofecoxib (p=0.0022) individually. In patients not taking aspirin, subjects using celecoxib had significantly higher d-dimer levels than those not taking the medication (0.7000 vs 0.3233, p<0.0001) after adjustment of covariates. In aspirin users there was not a significant difference in d-dimer between users of celecoxib and non-users (0.4488 vs 0.4326, p=0.9129). Likewise in subjects not taking aspirin, higher levels of d-dimer were found in users of rofecoxib than in non-users (0.5720 vs 0.3213, p<0.0001). This association between users and non-users of rofecoxib and d-dimer did not exist in patients taking aspirin (0.3483 vs 0.4316, p=0.6201).

The relationship between the use of non-selective NSAIDs and d-dimer levels is also modified by aspirin use (p=0.0003). In subjects not taking aspirin, users of NSAIDs had significantly higher d-dimer levels than non-users of selective or non-selective NSAIDs (0.4158 vs 0.3221, p<0.0001) after adjustment for covariates. In those taking

aspirin, there was no statistical difference in d-dimer levels between users and non-users of non-selective NSAIDs (0.3582 vs 0.4278, p=0.4088).

After logarithmic transformation of d-dimer there was no significant interaction between aspirin use and celecoxib (p=0.6013), rofecoxib (p=0.2136), and non-selective NSAID (p=0.1647).

Association of COX-2 inhibitors and non-selective NSAID use and fibrinogen.

Users of COX-2 inhibitors (celecoxib and rofecoxib combined) had significantly lower fibrinogen levels compared with non-users of selective or non-selective NSAIDs (339.3416 vs 347.4964, p=0.0133) after adjustment for covariates (Table 2). Subjects taking celecoxib had significantly lower fibrinogen levels than those not taking selective or non-selective NSAIDs (337.4621 vs 347.0358, p=0.0239). Contrarily, there was no significant difference in fibrinogen levels between users and non-users of rofecoxib when compared to controls not taking selective or non-selective NSAIDs (339.3539 vs 346.5336, p=0.1969).

Association of COX-2 inhibitors and non-selective NSAIDs with other indices of thrombosis

Users of celecoxib, rofecoxib, and non-selective NSAIDs had higher levels of Factor VIII and ICAM-1 than controls not using NSAIDs after adjustment for covariates; however, these differences were not statistically significant at an α level of 0.05 (Table 2). There was no statistical association or consistent trend between the use of selective or non-selective NSAIDs and levels of PAI-1 and vWF.

Dose effect of COX-2 inhibitors on indices of thrombosis (Table 4)

Subjects taking <150mg (low dose), 151-250mg (moderate dose), and >250 (high dose) a day had respective d-dimer levels of 0.2764, 0.7095, and 1.3838 after adjustment for covariates. There was a statistically significant difference in d-dimer levels between subjects taking low and high dose celecoxib (p=0.0161) and a trend towards significance between subjects taking moderate and high doses (p=0.0962) in the multivariate model. After logarithmic transformation of d-dimer there continued to be significantly higher d-dimer levels in subjects taking high dose versus those taking low dose celecoxib (p=0.0169). There was also significantly higher logarithmically transformed d-dimer levels in subjects taking moderate doses versus those taking low doses (p=0.0440).

Subjects taking low, moderate, and high doses of celecoxib had respective Von Willebrand Factor levels of 119.9140, 143.0674, and 197.1260. The difference in vWF levels between subjects taking low and moderate doses of celecoxib was statistically significance (p=0.0471).

There was no statistically significant difference of ICAM-1, factor VIII, PAI-1, and fibrinogen in subjects taking low, moderate, and high doses of celecoxib after adjustment for covariates.

There was no significant dose effect of rofecoxib on any of the indices of thrombosis after multivariate analysis.

Discussion

The cross-sectional analysis of this population based cohort demonstrates an 87% increase in d-dimer levels in celecoxib users and a 52% increase in rofecoxib users

compared with controls not using selective or non-selective NSAIDs. A large body of data supports the hypothesis that COX-2 inhibition leads to a reduction in PGI_2 formation and enhances the response to thrombotic stimuli (Grosser 2006). Concordantly, data from several clinical trials and a multitude of observational studies have demonstrated an increase in cardiovascular events with rofecoxib (Bresalier 2005, Bombardier 2000, Juni 2004) and suggest that celecoxib may also impart risk depending on the patient population and dosages used. Soloman et al. investigated the cardiovascular risk of celecoxib through a pooled analysis of 6 placebo controlled trials and 16,070 patient-years of follow up, demonstrating a 60% increase in hazard for cardiovascular events, which was largely dependent on dosage and baseline cardiovascular risk (Soloman 2008). It is plausible that the prothrombotic mechanism attributed to COX-2 inhibitor use manifests as elevated d-dimer levels in these patients.

The present analysis shows a 12 fold increase in d-dimer levels between subjects taking low dose vs high dose celecoxib. This dramatic dose related association in subjects using celecoxib may have clinical implications. Previous data from Soloman et al. demonstrated a dose regimen effect ($p=0.0005$) of celecoxib on cardiovascular events through analysis of The Adenoma Prevention with Celecoxib (APC) trial and the Prevention of Spontaneous Adenomatous Polyps (PreSAP) trial (Soloman 2006). The former trial studied celecoxib 200mg twice daily and 400mg twice daily, whereas the latter study used 400mg once daily (Soloman 2005 & Arber 2006). Those patients taking the highest doses of celecoxib had significantly more events than those taking the lower doses. Although the cumulative doses taken by subjects in the MESA cohort were lower than those in APC and PreSAP, the clinical data demonstrating a dose related increase in

events in these trials is concordant with dose related increases in d-dimer in the MESA cohort assuming the mechanism of thrombotic proclivity holds true in celecoxib users.

Grosser et al. have postulated that administration of low dose aspirin with concomitant COX-2 inhibitor use may diminish the hazard associated with COX-2 inhibitors (Grosser 2006). When taken with a COX-2 inhibitor, aspirin causes the irreversible inhibition of COX-1 in platelets, creating an anti-thrombotic effect and countering the unbalanced COX-2 inhibition. COX-1 knockdown mice genetically mimic the effects of low dose ASA (Yu 2005). The elimination of COX-1 in these mice reduces the prothrombotic effect of COX-2 inhibition (Y Cheng as cited in Grosser 2006). The only human trial that prespecifies the potential mitigating effect of aspirin is TARGET (Grosser 2006), which compared the COX-2 inhibitor, lumiracoxib, with ibuprofen and naproxen (Schnitzer 2004). Although the trial did not have sufficient power to address cardiovascular outcomes, the relative risk of myocardial infarction in subjects taking lumiracoxib was reduced from 2.37 to 1.36 compared with naproxen, if the subjects were taking concomitant aspirin (Grosser 2006). In the current analysis there was a significant interaction between aspirin use and COX-2 inhibitor use (celecoxib and rofecoxib combined and individually). There was a significant association present between elevated d-dimer levels and COX-2 inhibitor use in non-aspirin users that was not present in subjects using aspirin. This data may provide serologic evidence to support the biologically plausible mechanism of aspirins attenuating effect on the thrombotic risk associated with COX-2 inhibition. However, the interaction of COX-2 inhibitors with aspirin use did not remain significant when logarithmically transformed d-dimer is used as the outcome variable, casting doubt on the validity of this interaction.

Data have suggested the potentially hazard limiting effects of aspirin therapy when added to COX-2 inhibitors is not limited to COX-2 inhibitors. A recent case control analysis with over 4900 cases of acute myocardial infarction (MI) indicated that long term treatment with non-selective NSAIDs is associated with an increased risk of non-fatal MI and that this risk was concentrated in subjects not taking concomitant aspirin (Garcia 2005). The current analysis demonstrates an interaction between nonselective NSAIDs and aspirin use. The association between non-selective NSAID use and elevated d-dimer levels was abolished if the subjects were also taking aspirin; providing potential serologic evidence of aspirin imparting a decrease in thrombosis related hazard in subjects taking non-selective NSAIDs. Again, the interaction between non-selective NSAIDs and aspirin use did not remain significant when the log of d-dimer was used, limiting the validity of this interaction.

Among NSAIDs there exists a continuum of COX-2 selectivity. If the degree of COX-2 selectivity does determine cardiovascular risk, those non-selective NSAIDs with higher degrees of COX-2 selectivity may impart increased cardiovascular risk. Medications such as diclofenac, the most commonly used NSAID worldwide, and meloxicam have levels of COX-2 selectivity approaching that of celecoxib where as ibuprofen and naproxen tend to be less COX-2 selective (Grosser 2006). The current analysis is limited by the fact that the individual non-selective NSAIDs used are not known. Nevertheless, an association between elevated d-dimer levels and non-selective NSAID use exits; there was an 18% increase in d-dimer levels in non-selective NSAID users compared with controls. This association provides serologic evidence for the

possible increased thrombotic potential (or increased ongoing thrombosis) in users of non-selective NSAIDs.

Fibrinogen levels are lower in patients taking celecoxib and non-selective NSAIDs but not significantly different in users taking rofecoxib compared to controls. Levels of this prothrombotic substrate are not lower in rofecoxib users indicating a potential role fibrinogen may play in the biological mechanism that leads to the increase in cardiovascular risk associated with rofecoxib compared with celecoxib and non-selective NSAIDs.

Although there are biologically plausible mechanisms and trial data that are concordant with elevation of d-dimer levels in users of NSAIDs, no conclusions regarding causality can be made in this cross-sectional, observational study. This real world cohort provides benefits regarding the external validity of the findings. However, the indications for taking both selective and non-selective NSAIDs are often conditions that inherently have higher states of inflammation which could themselves affect d-dimer levels. Although the milieu of confounders involved in the inflammatory (and thrombotic) cascades likely precludes any precise statistical correction, attempts to control for this indication bias were made through adjustment for c-reactive protein, a sensitive marker of inflammatory states.

These indices of thrombosis are also well known markers of systemic inflammation. Elevations in these indices are not necessarily in the causal pathway of cardiovascular events. Instead, they may be systemic markers of low-grade arterial inflammation (Lowe 2005). The role of COX-2 in arterial inflammation is not completely understood; however, COX-2 expression in the arterial wall is known to be

limited to atherosclerotic lesions (Cipollone 2001). It remains unclear whether the presence of COX-2 in the arterial wall confers a protective or plaque destabilizing effect (Lowe 2005). In the current analysis the presence of COX-2 inhibition could potentially impede any protective effect of the COX-2 enzyme in the artery, thereby increasing atherosclerotic plaque inflammation and predisposing the plaque to rupture. The increase in local arterial inflammation may manifest as an increase in d-dimer levels.

The nature of the analysis necessitates comparisons of several medications with multiple indices of thrombosis, which can raise questions of statistical integrity. However, there is a consistent relationship between the use of both non-selective and selective (celecoxib and rofecoxib) NSAIDs and d-dimer. Furthermore, concordant associations exist with d-dimer across increasing doses of celecoxib. The consistency of associations demonstrated with this marker of thrombosis support the validity of these finding and suggest they are not the result of a statistical phenomena.

This population based, multi-ethnic cohort likely represents many of the patients that are taking these medications across the United States and throughout much of the world. However, the conclusions drawn cannot be applied to populations with established cardiovascular disease; although data suggest those populations may be the most affected by the prothrombotic nature of COX-2 inhibition (Solomon 2008).

In summary the association demonstrated by the current analysis supports the hypothesis that the cardiovascular risk associated with COX-2 inhibitors and non-selective NSAIDs results from increased thrombosis. It also suggests that aspirin may have a moderating effect on the relationship between these medications and thrombotic risk.

Table 1. Characteristics of Subjects Using COX-2 Inhibitors, Non-selective NSAIDs, and Controls

Variable	Non-selective NSAID (N=1121)	Celecoxib (N=235)	Rofecoxib (N=163)	Controls (N=5180)
Age (years)	60.30 ± 9.82*	66.67 ± 8.97*	64.90 ± 9.50*	62.23 ± 10.31
Gender (%)				
Male	728 (64.48) *	155 (65.40) *	104 (63.80) *	2560 (49.12)
Female	401 (35.52)	82 (34.60)	59 (36.20)	2652 (50.88)
Race (%)				
Caucasian	572 (50.66) *	95 (40.08)	66 (40.49) *	1860 (35.70)
African-American	296 (26.22)	24 (10.13)	54 (33.13)	1451 (27.84)
Hispanic	229 (20.28)	72 (30.38)	33 (20.25)	1170 (22.45)
Chinese	32 (2.83)	46 (19.41)	10 (6.13)	731 (14.03)
BMI (kg/m2)	29.74 ± 5.88*	30.38 ± 6.00*	30.63 ± 6.08*	27.83 ± 5.23
Total cholesterol	195.58 ± 34.60	187.32 ± 32.91*	191.62 ± 35.58	194.32 ± 36.05
Hypertension (%)				
No	610 (54.03)	89 (37.55) *	62 (38.04) *	2960 (56.79)
Yes	519 (45.97)	148 (62.45)	101 (61.96)	2252 (43.21)
Diabetes (%)				
No	1042 (92.30) *	204 (86.08) *	143 (87.73)	4695 (90.08)
Yes	87 (7.71)	33 (13.92)	20 (12.27)	517 (9.92)
Smoking (%)				
Never	514 (45.65) *	126 (53.39)	74 (45.40)	2663 (51.26)
Former	449 (39.88)	88 (37.29)	65 (39.88)	1861 (35.82)
Current	163 (14.48)	22 (9.32)	24 (14.72)	671 (12.92)
CRP	4.84 ± 7.54*	5.82 ± 9.73*	4.45 ± 5.57*	3.42 ± 5.15
IL-6	1.63 ± 1.30*	1.84 ± 1.84*	1.71 ± 1.24*	1.52 ± 1.16
Arthritis (%)				
No	576 (51.02) *	36 (15.19) *	39(23.93) *	3637 (69.81)
Yes	541 (47.92)	198 (83.54)	121(74.23)	1518 (29.14)

Values are mean ± SD unless otherwise indicated. BMI indicates body mass index; Hypertension as defined by the JNC VI guidelines (≥ 140/90 nmHg); Diabetes, current pharmacologic treatment; CRP, C-reactive protein; IL-6, Interleukin-6; Arthritis, self-reported. *denotes significant difference from controls at alpha ≤ 0.05. Student *t* test for continuous variables and X^2 for categorical variables.

Table 2. The Association of COX-2 Inhibitors and Non-selective NSAIDs with Indices of Thrombosis

Variable	Non-selective NSAIDs		Celecoxib		Rofecoxib	
	No	Yes	No	Yes	No	Yes
D-dimer, ug/mL (N) *p* value	0.3429 (5177)	0.4040 (1121) 0.0003	0.3460 (5180)	0.6485 (235) <0.0001	0.3444 (5180)	0.5228 (163) 0.0014
Log D-dimer (N) *p* value	- 1.5300 (5177)	- 1.4767 (1121) 0.0588	- 1.5221 (5180)	- 1.4063 (235) 0.0413	- 1.527 (5180)	- 1. 3723 (163) 0.0200
Fibrinogen, mg/dl (N) *p* value	347.7819 (5176)	337.2472 (1121) < 0.0001	347.0358 (5179)	337.4621 (234) 0.0239	346.5336 (5179)	339.3539 (163) 0.1969
vWF, % (N) *p* value	137.3974 (756)	142.7299 (175) 0.2610	138.2897 (757)	129.0824 (36) 0.3266	137.6026 (757)	160.0176 (20) 0.0596
Factor VIII, % (N) *p* value	162.7825 (5173)	162.9433 (1121) 0.9410	163.0693 (5176)	170.6004 (235) 0.0847	162.8175 (5176)	167.7171 (163) 0.3454
ICAM-1, ng/mL (N) *p* value	271.8738 (2002)	278.0909 (457) 0.0666	270.7957 (2003)	278.1178 (81) 0.3460	270.6372 (2003)	283.7084 (57) 0.1961
PAI-1, ng/mL (N) *p* value	26.6337 (739)	27.6350 (171) 0.6665	25.8691 (740)	26.2380 (36) 0.9344	25.8253 (740)	17.7542 (18) 0.1837

P-values reflect comparison between medication users and non-users. All values are adjusted for age, gender, race, total cholesterol, hypertension, diabetes mellitus, BMI, cigarette smoking, CRP. D-dimer values are also adjusted for ASA and the interaction of ASA with each medication. vWF indicates von Willibrand Factor.

Table 3. The Effect Modification of Aspirin upon the Relationship of Non-selective NSAIDs and COX-2 Inhibitor Use with D-dimer Levels.

Variable	Non-selective NSAIDs		Celecoxib		Rofecoxib	
	No	Yes	No	Yes	No	Yes
Aspirin use (N) *p* value	0.4278 (1033)	0.3582 (159) 0.4088	0.4326 (1033)	0.4488 (52) 0.9129	0.4316 (1033)	0.3483 (39) 0.6201
No aspirin use (N) *p* value	0.3221 (3962)	0.4158 (920) <0.0001	0.3233 (3962)	0.7000 (177) <0.0001	0.3213 (3962)	0.5720 (118) <0.0001

P-values reflect comparison between users and non-users of non-selective NSAIDs, Celecoxib, and Rofecoxib.
All d-dimer values are adjusted for age, gender, race, total cholesterol, hypertension, diabetes mellitus, BMI, cigarette smoking, CRP.

Table 4. The Association of Indices of Thrombosis with Celecoxib by Dose

Variable	Celecoxib Dose			Dose Comparison (*p* value)		
	Low (< 150 mg)	Medium (151-250 mg)	High (> 250 mg)	Low-Med	Med-High	Low-High
D-dimer, ug/mL (N)	0.2764 (56)	0.7096 (123)	1.3838 (46)	0.2366	0.0962	0.0161
Log D-Dimer (N)	- 1.4038 (56)	- 1.0854 (123)	- 0.9305 (46)	0.0440	0.3735	0.0169
Fibrinogen, mg/dl (N)	369.9805 (56)	353.2222 (124)	363.3666 (44)	0.1630	0.4443	0.6594
vWF, % (N)	119.9140 (10)	143.0674 (23)	197.1260 (4)	0.2831	0.1384	0.0471
Factor VIII, % (N)	184.1622 (56)	179.2979 (125)	190.4181 (44)	0.6500	0.3493	0.6421
ICAM-1, ng/mL (N)	278.9053 (23)	291.9215 (47)	318.6576 (11)	0.6115	0.4636	0.2970
PAI-1, ng/mL (N)	38.6518 (10)	30.5307 (23)	15.7584 (4)	0.2971	0.2671	0.1038

All values are adjusted for age, gender, race, total cholesterol, hypertension, diabetes mellitus, BMI, cigarette smoking, CRP.
All doses represent total daily dose.

References

Al-Nozah M, Garder AM, Al-Momen AK, Noah MS, Jawaid M. Haemostatic variables inpatients with unstable angina. *Int J Cardiol*. 1994;43:269-77.

Arber N, Eagle C, Spicak J, et al for the Prevention of Sporadic Adenomatous Polyps (PreSAP) Investigators. Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med*. 2006;355:885–95.

Bayes-Genis A, Mateo J, Santalo M, et al. Procoagulant and proinflammatory marker of coronary ischemia in acute coronary syndromes. *Cardiovasc Res.* 1998;40:389-95.

Bild DE, Bluemke DA, Burke GL et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–81.

Bombardier, C., et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N. *Engl. J. Med*. 2000;343:1520-28.

Boneu B, Aptel I, Nguyen F et al. A new fast assay to determine D-Dimers, has performances comparable to classical ELISA for diagnosis of deep vein thrombosis. *Thromb Haemost*, supp. 159, abstract PD 651, June 1997.

Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med*. 2005;352:1092-102.

Catella-Lawson, F., et al. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J. *Pharmacol. Exp. Ther*. 1999;289:735-41.

Cipollone F, Prontera, B, Pini M, et al. Overexpression of functionally couples cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaqe instability. *Circulation*. 2001;104-921-27.

Danesh J, Lewington S, Thompson SG. et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: and individual participant meta-analysis. *JAMA*. 2005;294:2848.

Danesh J, Wheeler JG, Hirshfield GM. Et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350:1387-97.

DeClerck P, Alessi M, Verstreken M, Kruithof E, Juhan-Vague I, Collen D (1988) Measurement of plasminogen activator inhibitor 1 (PAI-1) in biological fluids with a

murine monoclonal antibody based enzyme-linked immunosorbent assay. *Blood*. 1988;71:220-225.

Fibrinogen Studies Collaboration. Collaborative meta-analysis of prospective observational studies of plasma fibrinogen and cardiovascular disease. *Eur J Cardiovasc Priv Rehabil*. 2004;11:9-17.

Fitzgerald GA, Cheng , Austin S. COX-2 inhibitors and the cardiovascular system. *Clin Exp Rheumatol*. 2001;25:S31-6.

Francis CW, Conneghan DG, Scott WL, et al. Increased plasma concentration of cross-linked fibrin polymers in acute myocardial infarction. *Circulation*. 1987;75:1170-77.

Frenette PS, Wagner DD. Adhesion molecules-Part II: blood vessels and blood cells. *N Engl J Med*. 1996;335:43-55.

Garcia Rodriguez, L.A., Gonzalez-Perez, A. Long-term use of traditional non-steroidal anti-inflammatory drugs and the risk of myocardial infarction in the general population. *BMC Med*. 2005;3:17.

Grosser T, Fries S, Fitzgerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest*. 2006;116:4-15.

Juhan-Vague I, Pyke SD, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. *Circulation*. 1996;94:2057-63.

Juni, N, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet*. 2004;364:1995-96.

Kamikura Y, Wada H, Yamada A, et al. Increased tissue factor pathway inhibitor in patients with acute myocardial infarction. *Am J Hematol*. 1997;55:183-87.

Kruskal JB, Commerford PF, Franks JJ et al. Fibrin and fibrinogen-related antigens in patients with stable and unstable coronary artery disease. *N Engl J Med.* 1987;317:1361-5.

Lowe GDO. Circulating inflammatory markers and risks of cardiovascular and non-cardiovascular disease. *Journal of Throm Haemost*. 2005;3:1618-27.

Lowe GDO, Danesh J, Lewington S, et al. Tissue plasminogen activator antigen and coronary heart disease: prospective study and meta-analysis. *Eur Heart J* 2004;25:252-9.

Macy E, Meilahn E, DeClerck P, Tracy R. Sample preparation for plasma measurement of plasminogen activator inhibitor-1 antigen in large population studies. *Arch Path Lab Med*. 1993;177:67-70.

Moss AJ, Goldstein RE, Marder VJ, et al. Thrombogenic factors and recurrent coronary events. *Circulation*. 1999;99:2517-22.

Nartey JP, Reichenbach S, Sterchi R et al. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet*. 2004;364:2021-29.

Schnitzer, T., et al.. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. *Lancet*. 2004;64:665-674.

Singh G, Triadafilopoulos G. Epidemiology of NSAID induced gastrointestinal complications. *J Rheumatol*. 1999;26:Suppl 56:18-24.

Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M, for the Adenoma Prevention with Celecoxib (APC) Study Investigators. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med*. 2005;352:1071–80.

Solomon, SD, Pfeffer MA, McMurray JJ et al. Effect of Celecoxib on Cardiovascular Events and Blood Pressure in Two Trials for the Prevention of Colorectal Adenomas. *Circulaiton*. 2006;114:1028-35.

Solomon SD, Wittes J, Finn PV, et al. Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials: the cross trial safety analysis. *Circulation*. 2008;117:2104-13.

Spiel AO, Gilbert JC, Jilma B von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. *Circulation*. 2008;117:1449-59.

Tracy R, Bovill E. (1995) Plasminogen activator inhibitor-1. In E Beutler, M Lichtman, B Coller, T Kipps (Eds.), Williams Hematology, pps. L110—L111. New York; McGraw Hill.

Yu, Y., et al. Differential impact of prostaglandin H synthase 1 knockdown on platelets and parturition. *J. Clin. Invest*. 2005; 115:986-95.

Van der Putten R, Glatz J, Hermens W. Plasma markers of activated hemostasis in the early diagnosis of acute coronary syndromes. *Clinica Chimica Acta*. 2006;371:37-54

Soeki T, Tamura Y, Fukuda N, Ito S. Plasma and platelet plasminogen activator inhibitor-1 in patients with acute myocardial infarction. *Jpn Cric J*. 2000;64:547-53.

Widlansky, M, Price DT, Gokce N, et al. Short- and long-term COX-2 inhibition reverses endothelial dysfunction in patients with hypertension. *Hypertension*. 2003;42:310-15.

Chapter II

The Association of Celecoxib, Rofecoxib, and Non-selective Nonsteroidal Anti-inflammatory Medications with Endothelial Function in the Multi-Ethnic Study of Atherosclerosis

Stewart G Allen, MD

Abstract

Background:
Cyclooxygenase-2 (COX-2) inhibitors and non-selective nonsteroidal anti-inflammatory medications (NSAIDs) have been associated with an increase in cardiovascular events. Debate exists as to the potential mechanism(s) that are responsible for this association. Endothelial function may be influenced by the imbalance of prostanoids caused by blockade of COX-2 and hold a key role in conferring the cardiovascular risk of these medications.

Methods:
Using subjects within the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, we performed a cross-sectional analysis in order to investigate the association between use of celecoxib (n=113), rofecoxib (n=80), and non-selective NSAIDs (n=528) and brachial artery flow mediated dilatation (FMD) compared with controls (n=2768).

Results:
Users of COX-2 inhibitors tended to be older, female and have higher BMIs while users of non-selective NSAIDs tended to be younger and used tobacco more compared with controls. Both groups have higher markers of inflammation compared with controls. No significant association of FMD with celecoxib (p=0.8426), rofecoxib (p=0.0675) or non-selective NSAIDs (p=0.3659) was found with multivariate analysis.

Conclusions:
The current analysis casts further doubt on the hypothesis that endothelial dysfunction mediates the cardiovascular risk imposed by COX-2 inhibitors and non-selective NSAIDs. There was also no evidence that COX-2 inhibitors have a favorable impact on endothelial function.

Introduction

Cyclooxygenase-2 (COX-2) inhibitors have clearly been linked to an increase in cardiovascular events (Bresalier 2005, Natey 2004, Solomon 2008). However, there has been much debate surrounding the potential mechanistic effects that COX-2 inhibitors have on cardiovascular risk. The most widely accepted hypothesis is that COX-2 inhibition causes alterations in the downstream products of arachadonic acid metabolism leading to decreased levels of prostacyclin (PGI_2) in favor of prothombotic thromboxane A_2 (TXA_2) (Fitzgerald 2001). The imbalance in these two substrates may manifest in increased cardiovascular events through vascular thrombosis; however, other detrimental mechanisms may exist.

There is less certainty regarding the cardiovascular risk imposed by traditional, non-selective NSAIDs. Studies investigating associations with cardiovascular events have yielded inconsistent results (Cheng 2006). Non-selective NSAIDs have varying degrees of COX-2 specificity depending on the agent used. Those agents with higher degrees of COX-2 specificity may also cause an imbalance between PGI_2 and TXA_2, similar to that seen with COX-2 selective inhibitors (Grosser 2006).

COX-2 is a major source of endothelium-derived PGI_2. In addition to its effect as a potent inhibitor of platelet aggregation, PGI_2 also promotes vasodilation. Conversely, thromboxane (TXA_2) acts as an inducer of platelet aggregation and an endothelium-derived contracting factor (Verma 2003). The resulting imbalance between PGI_2 and COX-1 derived TXA_2 from selective blockade of COX-2, may promote endothelial dysfunction.

Endothelial cell dysfunction may be the earliest event in the process of atherosclerosis formation (Verma 2003). Endothelial function is recognized as a 'barometer' of vascular health and predictor of cardiovascular events (Vita 2002) as vascular endothelial cells regulate vasomotor tone, platelet aggregation, and smooth muscle cell proliferation (Moens 2005). Brachial artery flow mediated dilation (FMD) has been shown to be highly correlated with the capacity for dilation in the coronary circulation reflecting on its potential, non-invasive, predictability of coronary artery endothelial function (Anderson 1995). The ability for the coronary arteries to dilate is decreased in patients with atherosclerosis and those with cardiovascular risk factors (Moens 2005). The dilatory capacity has also been shown to improve with risk reduction therapy (Vita 2000). FMD itself has been identified as a predictor of cardiovascular events in younger and older populations alike (Shrimbo 2007, Yeobah 2007). The technique has evolved to become a widely used non-invasive, tool for accurately and reliably assessing endothelial function and thereby indicating level of cardiovascular risk.

Prior studies examining COX-2 inhibitors and endothelial function have been inconclusive. The limited data indicates COX-2 inhibition does not impair endothelial function (Verma 2001, Title 2003, Lekakis 2007, Wong 2007); however, some studies have suggested an actual improvement in FMD after COX-2 inhibitor use (Chevenard 2003, Widlansky 2003). Less data exists for non-selective NSAID; indicating no effect of naproxen or indomethacin on endothelial function (Verma 2001, Wong 2007). Prior studies have had small sample sizes, limited power, and yielded conflicting results depending on the agent used and populations investigated. We set forth to evaluate the

association of celecoxib, rofecoxib, and non-selective NSAIDs in a large, population based, multi-ethnic cohort free of coronary artery disease.

Methods

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based study of 6814 Caucasian, African-American, Hispanic, and Chinese men and women aged 45-84. The patients were recruited from 6 centers in the United States: Baltimore, MD; Chicago, IL; Forsyth County, NC; New York, NY; Los Angeles, CA; and St. Paul, MN. The primary objective of MESA is to determine the characteristics related to the progression of subclinical cardiovascular disease. All subjects were free of diagnosed cardiovascular disease upon enrollment into the study. Subjects within the cohort had four examination visits over the course of approximately five years. All data from the current cross-sectional analysis were attained at the first visit. The scans were equally distributed across all sites except the Baltimore, MD site which was excluded secondary to the lack of expected FMD response within that center. All other subjects with adequate FMD scans from MESA were used in the current analysis. The study was approved by Institutional Review Boards at each center and all subjects gave informed consent. The details regarding recruitment, objectives, and design have been previously published (Bild 2002).

Clinical Evaluation

Participants within the cohort provided a medical history, including a complete medication inventory, and had anthropometric and laboratory measurements obtained upon entry into the study during visit one (July 2000-Augest 2002).

Flow Mediated Brachial Artery Vasodilation

All FMD scans were performed at the first visit. Participants abstained from food (other than juice or water) and tobacco use for six hours prior to the brachial artery vasodilatation scan. If necessary, a small snack, mostly carbohydrates and no fat content were given 90 minutes prior to the endothelial function test.

The subject was supine during the examination and a blood pressure cuff was placed around the right and then the left upper arm and connected to an automated sphyngnomanometer. If there was greater than a 15mmHg difference in blood pressure between two arms, the subject was excluded from the brachial artery vasodilatation study. The sonographer placed the occlusion blood pressure cuff over the proximal right forearm, just below the antecubital fossa. All images were taken using GE Logiq 700 ultrasound machines using ML probes and were captured at 9 MHz. Baseline images of the brachial artery were taken throughout the initial blood pressure cuff inflation. The occlusive blood pressure cuff was inflated to 200 mmHg (if SBP >200mmHg, cuff was inflated to 50 mmHg above SBP) and deflated after 5 minutes. Ultrasound images were recorded from 15 seconds before to 2 minutes after deflation of the BP cuff as maximal vasodilation typically takes place at one minute following cuff deflation.

Acquisition of the ultrasound images was synchronized with the electrocardiogram so that the brachial artery diameters could be captured during diastole. Videotapes of the acquired images were analyzed at the Wake Forest University Cardiology Image Processing Laboratory with a previously validated semiautomated system (Herrington 2001). The readings of these digitized images generated the baseline and maximal diameters of the brachial artery from which the % brachial Flow Mediated Dilation was calculated with the formula:

$$\%FMD = \frac{\text{Maximum diameter} - \text{baseline diameter}}{\text{Baseline diameter}} \times 100\%$$

Statistical Analysis

The data are presented as mean +/-SD for continuous variables and frequencies for categorical variables. Student T-tests were used to compare continuous covariates between groups and chi-square was used to compare frequencies. Linear regression was used to evaluate the unadjusted association of brachial artery measurements (baseline diameter, maximal diameter, and % change in brachial artery diameter) with celecoxib, rofecoxib, and non-selective NSAID use. Multiple linear regression analysis was used to investigate this same relationship. Analysis is presented with adjustment for covariates known to be associated with FMD: age, gender, race, hypertension, diabetes, tobacco use, cholesterol, BMI, HMG CoA reductase inhibitor use, ACE inhibitor use, and B-blocker use. In addition, C-reactive protein (CRP) was added as a covariate to attempt to control for the inherent indication bias; users of NSAID tend to have chronic

inflammatory states. Lastly, as data was collected from 5 separate centers in the US, intraclass (ie intrasite) correlations were assessed to assure low levels of variability in the distribution of NSAID users. All analysis was done using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Subjects

In the MESA cohort there are 257 users of celecoxib, 180 users of rofecoxib, and 1129 users of non-selective NSAIDs. FMD scans were adequately performed on 113 celecoxib users, 80 rofecoxib users, and 528 non-selective NSAID users. FMD scans were also adequately performed on 2768 control subjects. Users of COX-2 inhibitors tended to be older, female, have higher BMIs, and higher CRP levels compared with controls. Non-selective NSAID users were younger, more likely to be Caucasian, smoked more, and also had higher levels of CRP.

Flow Mediated Dilation and COX-2 inhibitor use

Users of celecoxib and rofecoxib combined (n=193) had significantly lower FMD compared with controls (3.80 ± 2.38 vs 4.37 ± 2.81; $p=0.0065$). However, after adjustment for covariates there was no significant association of COX-2 inhibitor use (celecoxib and rofecoxib combined) with FMD (Table 2). Also, there was no association between COX-2 inhibitor use (celecoxib and rofecoxib combined) and baseline or maximum lumen diameters.

Celecoxib users had a lower, unadjusted point estimate for FMD compared with controls (3.92 $\pm$ 2.31 vs 4.37 $\pm$ 2.81; p=0.0918). After multivariate analysis there was no significant association of celecoxib with FMD (p=0.8426). There were no associations of celecoxib use with either baseline or maximum lumen diameters.

Rofecoxib users had significantly lower FMD than controls (3.55 $\pm$ 2.47 vs 4.37 $\pm$ 2.81; p=0.0097). However, this association was significantly weakened after controlling for covariates (p=0.0675). Correction for age alone was sufficient to yield an α level > 0.05. There were no associations of rofecoxib use with baseline or maximum lumen diameters.

Flow Mediated Dilation and non-selective NSAID use

There was no association of non-selective NSAIDs with FMD in the univariate or multivariate model. NSAID users did have significantly lower baseline and maximum diameters compared with controls (4.20 $\pm$ 0.85 and 4.38 $\pm$ 0.85 versus 4.35 $\pm$ 0.82 and 4.53 $\pm$ 0.81 respectively; p<0.0001); however this difference was not significant in the multivariate model (Table2).

Discussion

In this population based cohort of multi-ethnic subjects free of coronary artery disease, there was no association between brachial FMD and use of celecoxib, rofecoxib, or non-selective NSAIDs compared with controls. This cross-sectional analysis represents the largest study to date investigating the potential relationship of these widely prescribed medications with endothelial function.

Our data contributes to a limited body of research that has yielded conflicting results. Although a COX-2 imposed amplification of increased TXA_2 and decreased PGI_2 producing a substrate prone to endothelial dysfunction is theoretically sound; previous data suggests that COX-2 inhibition may actually have a beneficial effect on endothelial function in this patient population. Chenevard et al. found a significant increase in FMD after treatment with Celecoxib 200mg bid for 14 days compared to placebo (Chenevard 2003). The authors note that the aspirin therapy all subjects received throughout the study may block TXA_2 induced platelet aggregation and vasoconstriction, thereby eliminating any deleterious effect of COX-2 inhibition on endothelial function. Widlansky et al. found a similar increase in FMD in subjects given celecoxib 200mg bid for 7 days without concomitant aspirin use giving more support to a possible, intrinsic, beneficial effect of COX-2 inhibitors on the vasculature (Widlansky 2003). The improvement in FMD in those studies may be secondary to beneficial pleiotropic effects of COX-2 inhibitors.

Specifically, celecoxib has been shown to decrease high sensitivity CRP and oxidized LDL (Chenevard 2003). CRP is known to be a marker for inflammation, involved in the atherosclerotic process and a risk factor for cardiovascular events (Ridker 2001). CRP also has been shown to have post-transcriptional effects on endothelial NO synthase mRNA stability, leading to decreased NO bioavailability, thereby promoting endothelial dysfunction (Verma 2002). Therefore, a decrease in CRP imposed by COX-2 inhibition may lead to an improvement in endothelial function. Concordantly, the decrease in oxidized LDL previously described by Chenevard et al. may also lead to

improved endothelial function, as oxidized LDL modulates the production and release of NO (Diaz 1997).

In the present study, CRP levels are higher in users of COX-2 inhibitors and non-selective NSAIDs, as the indication for these medications are often inflammatory disease states. Chronic inflammatory disease states are thought to affect the vascular endothelium and have a concordant increase in cardiovascular risk (Hermann 2006). The relationship between and CRP and any beneficial effect COX-2 inhibitors may have on the endothelium via decreasing this inflammatory marker is confounded by intrinsically higher CRP levels in this patient population. Also, the cross-sectional nature of the current analysis prevents comparison of CRP before initiation, during treatment, and after discontinuation of COX-2 inhibitors or non-selective NSAIDs. Therefore, we are unable to draw any distinct conclusions regarding the effect COX-2 inhibitors may have had on endothelial function via changes in CRP in the specific patients taking these medications.

The potential beneficial pleiotropic effects of COX-2 inhibitors may negate any endothelial dysfunction imparted by the imbalance of TXA_2 and PGI_2. Lekakis et al found no effect of rofecoxib on FMD in 43 patients with acute coronary syndromes, despite a decrease in CRP (Lekakis 2007). Other studies have also failed to show any change in FMD after treatment with rofecoxib, naproxen or indomethacin (Verma 2001, Title 2007, Wong 2007).

The results of the present analysis cannot be generalized to populations that have coronary artery disease (CAD). Subjects within the MESA cohort are free of CAD upon entry into the study and are likely to have normal levels of NO. Contrarily, patients that do have CAD may produce lower levels of NO at baseline. Also, the expression of COX-

2 is upregulated in atherosclerosis (Schonbeck 1999). The lower levels of NO in patients with CAD, when combined with an increase in COX-2 expression and the imbalance of PGI_2 and TXA_2 imposed by COX-2 inhibition, may result in an exaggerated effect on endothelial function; an effect that may be avoided in subjects free of CAD (Verma 2003).

Several other limitations of the current study exist. It remains possible that COX-2 inhibitors and non-selective NSAIDs influence endothelial function through other mechanisms at the cellular level that are not evaluated by FMD (Title 2003). Also, endothelium-independent vasodilatation with nitroglycerin was not ascertained; therefore no conclusions can be drawn regarding the isolated impact of these medications upon the vascular smooth muscle.

In this cohort significantly more subjects using COX-2 inhibitors were also using HMG CoA reductase inhibitors compared with controls. The accuracy of statistical corrections is limited by the number of subjects used in this cohort and the large number of covariates known to affect FMD. Therefore, the influence of concomitant treatment with HMG CoA reductase inhibitors may be unrecognized. Also, we do not know the treatment length of any of the medications included in the analysis.

Perhaps the largest limitation of the current analysis is the indication bias that exists. Often COX-2 inhibitors and non-selective NSAIDs are prescribed for chronic inflammatory states. Our data demonstrates significantly higher CRP levels in subjects taking non-selective NSAIDs, celecoxib or rofecoxib compared with controls. Attempts to control for the presence of chronic inflammation were made by including CRP in the multivariate model.

The large, multi-ethnic population within MESA is likely representative of patients that are prescribed COX-2 inhibitors and non-selective NSAIDs throughout the United States. Our data indicate that the cardiovascular risk associated COX-2 inhibitors and non-selective NSAIDs is not mediated by endothelial function. Furthermore, there does not appear to be a favorable affect of celecoxib, rofecoxib or non-selective NSAIDs on endothelial function.

Table 1. Characteristics of Subjects Using Non-selective NSAIDs, COX-2 Inhibitors, and Controls

Variable	Non-selective NSAIDs (N = 528)	Celecoxib (N = 113)	Rofecoxib (N = 80)	Controls (N = 2768)
Age (years)	59.18 ± 9.38*	68.01 ± 9.00*	66.14 ± 9.74*	61.41 ± 9.99
Gender (%)				
Male	211 (39.96)*	39 (34.51)*	31 (38.75)*	1456 (52.60)
Female	317 (60.04)	74 (65.49)	49 (61.25)	1312 (47.40)
Race (%)				
Caucasian	266 (50.38)*	38 (33.63)	30 (37.50)	830 (29.99)
African-American	112 (21.21)	21 (18.58)	17 (21.25)	634 (22.90)
Hispanic	127 (24.05)	31 (27.43)	21 (26.25)	724 (26.16)
Chinese	23 (4.36)	23 (20.35)	12 (15.00)	580 (20.95)
BMI (kg/m2)	29.56 ± 5.76*	30.18 ± 5.95*	29.29 ± 5.65*	27.42 ± 4.99
Total cholesterol	194.98 ± 34.60	188.82 ± 34.57	193.49 ± 35.53	194.50 ± 35.30
Hypertension (%)				
No	296 (56.06)	44 (38.94)*	31 (38.75)*	1637 (59.14)
Yes	232 (43.94)	69 (61.06)	49 (61.25)	1131 (40.86)
Diabetes (%)				
No	491 (92.99)	100 (88.50)	72 (90.00)	2515 (90.86)
Yes	37 (7.01)	13 (11.50)	8 (10.00)	253 (9.14)
Smoking (%)				
Never	241 (45.64)*	67 (59.29)	39 (48.75)	1504 (54.34)
Former	216 (40.91)	37 (32.74)	31 (38.75)	930 (33.60)
Current	71 (13.45)	9 (7.96)	10 (12.50)	334 (12.07)
CRP	4.27 ± 6.05*	4.98 ± 8.35*	4.24 ± 5.52	3.26 ± 5.17
ACE inhibitor use (%)				
No	465 (88.07)	93 (88.30)*	69 (86.25)	2466 (89.09)
Yes	63 (11.93)	20 (17.70)	11 (13.75)	302 (10.91)
β –blocker use (%)				
No	482 (91.29)	106 (93.81)	69 (86.25)	2517 (90.93)
Yes	46 (8.71)	7 (6.19)	11 (13.75)	251 (9.07)
HMG CoA reductase inhibitor use (%)				
No	465 (88.07)	84 (74.34)*	55 (68.75)*	2397 (86.60)
Yes	63 (11.93)	29 (25.66)	25 (31.25)	371 (13.40)

Values are mean ± SD unless otherwise indicated. BMI indicates body mass index; Hypertension as defined by the JNC VI guidelines (≥ 140/90 nmHg); Diabetes, current pharmacologic treatment; CRP, C-reactive protein. *denotes significant difference from controls at alpha ≤ 0.05. Student *t* test for continuous variables and X^2 for categorical variables

Table 2. The Association of Brachial Artery Reactivity Measurements between Users and Non-users of Non-selective NSAIDs and COX-2 Inhibitors.

Variable	Non-selective NSAIDs		Celecoxib		Rofecoxib	
	No (N=2768)	Yes (N=528)	No (N=2768)	Yes (N=113)	No (N=2768)	Yes (N=80)
Baseline diameter (mm)	4.34	4.29 $p = 0.1725$	4.35	4.36 $p = 0.8616$	4.35	4.34 $p = 0.8606$
Maximum diameter (mm)	4.52	4.47 $p = 0.1158$	4.53	4.54 $p = 0.8256$	4.53	4.50 $p = 0.6447$
FMD (%)	4.42	4.30 $p = 0.3659$	4.36	4.41 $p = 0.8426$	4.37	3.82 $p = 0.0675$

P-values reflect comparison between medication users and non-users. All values are adjusted for age, gender, race, total cholesterol, hypertension, diabetes mellitus, BMI, cigarette smoking, CRP, β-blocker, ACE inhibitor use and HMG CoA reductase inhibitor use.

References

Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235-41.

Bild DE, Bluemke DA, Burke GL et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–81.

Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med*. 2005;352:1092-102.

Cheng, J. Use of Non-Aspirin Nonsteroidal Antiinflammatory Drugs and the Risk of Cardiovascular Events. *The Annals of Pharmacotherapy*. 2006;40:1785-96.

Chenevard R, Hurlimann D, Bechir M. et al. Selective COX-2 inhibition improves endothelial function in coronary artery disease. *Circulation*. 2003;107:405-9.

Diaz MN, Frei B, Vita JA, et al. Antioxidants and atherosclerotic heart disease. *N Engl J Med*.. 1997;337:408-16.

Fitzgerald GA, Cheng , Austin S. COX-2 inhibitors and the cardiovascular system. *Clin Exp Rheumatol*. 2001;25:S31-6.

Grosser T, Fries S, Fitzgerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest*. 2006;116:4-15.

Hermann M, Rushitzka F. Coxibs, non-steroidal anti-inflammatory drugs and cardiovascular risk. *Internal Medicine Journal*. 2006;36:308-19.

Herrington DM, Liexiang Fan, Matella D, et al. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. *J Cardiovasc Risk*. 2001;8:319-28.

Lekakis JP, Vamvakou G, Andreadou I, et al. Divergent effects of rofecoxib on endothelial function and inflammation in acute coronary syndromes. *Int J Cardiol*. 2007;116:259-60.

Moens A, Goovaertrs I, Claeys M, Vrints C. Flow Mediated Vasodilation: A diagnostic Instrument, or an experimental Tool? *Chest*. 2005;127:2254-63.

Nartey JP, Reichenbach S, Sterchi R et al. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet*. 2004;364:2021-29.

Ridker PM. High-sensitivity C-ractive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*. 2001;103:1813-8.

Schonbeck U, Sukhova GK, Graber P, et al. Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions. *Am J Pathol*. 1999;155:1281-91.

Shimbo D, Grahame-Clarke C, Miyake Y, Rodriguez C, Sciacca R, Di Tullio M, Boden-Albala B, Sacco R, Homma S. The association between endotheial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis*. 2007; 192:197-203.

Solomon SD, Wittes J, Finn PV, et al. Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials: the cross trial safety analysis. *Circulation*. 2008;117:2104-13.

Title L, Giddens K, McInerney M et al. Effect of cyclooxygenase-2 inhibition with rofecoxib on endothelial dysfunction and inflammatory markers in patients with coronary artery disease. *J Am Coll Cardiol*. 2003;42:1747-53.

Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. *Circulation*. 2003;108:2054-59.

Verma S, Raj SR, Shewchuk L et al. Cyclooxygenase-2 blockade does not impair endothelial vasodilator function in healthy volunteers: randomized evaluation of rofecoxib versus naproxen on endothelium-dependent vasodilation. *Circulation*. 2001;104:2879-82.

Verma S, Szmitko P. Coxibs and the endothelium. *J Am Coll Cardiol*. 2003;42:1754-1756.

Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation*. 2002;102:994-99.

Widlansky, M, Price DT, Gokce N, et al. Short- and long-term COX-2 inhibition reverses endothelial dysfunction in patients with hypertension. *Hypertension*. 2003;42:310-15.

Wong M, Joang B McNeill K, et al. Effects of selective and non-selective cyclo-oxygenase inhibition on endothelial function in patients with rheumatoid arthritis. *Scand J Rheumatol*. 2007;36:265-69.

Yeboah J, Crouse J, Hsu F, Burke G, Herrington D. Brachial Flow-Mediated Dilation Predicts Incident Cardiovascular Events in Older Adults. *Circulation*. 2007;115:2390-97.

Chapter III

Propensity Score Analysis and the Association of COX-2 Inhibitors with D-dimer in the Multi-Ethnic Study of Atherosclerosis

Stewart G Allen, MD

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The evidence based pursuit and analysis of data compiled from observational studies is ever-expanding. Although the data derived from these studies are not able to provide the validity of those from randomized controlled trials, they are nonetheless important and warrant special statistical consideration.

The propensity score, named by Rosenbaum & Rubin in 1983, was developed to analyze data from observational studies in a manner that would reduce bias. The goal was to somehow create a method in which each subject in an observational study could be assigned a score that was based on the cumulative presence of potential confounders or covariates. This score could be used to create two groups equally matched by their potential confounders, thereby creating a 'quasi-randomized' experiment (D'Agostino 1995). Their work expanded on earlier exploration by Mettinen in 1976 who proposed that summarizing confounders into a single score may reveal relationships that would otherwise be hidden in traditional multivariate models (Glynn 2005). Specifically, Mettinen focused on two statistical functions: 1) the relationship between potential confounders and the outcome variable in the unexposed (disease risk score) and 2) the relationship between potential confounders and exposure in the non-diseased (exposure score). It is this later exposure score that was eventually developed into the propensity score by removing its focus on the non-diseased (Glynn 2006).

The use of propensity scoring has steadily been increasing in the literature. Between 1998 and 2000 fewer than 9 papers using this method can be found in the literature. By 2003 approximately 177 publications employed this novel method. The treatments studied include medications (34%), surgical interventions (28%),

interventional catheterization (7%), and other procedures and lifestyle interventions (Glynn 2006).

Propensity scoring allows the variables included in the score to potentially be even more balanced than if they were randomized (Rosenbaum 1999). The use of the propensity score negates the limited number of covariates that are allowed in traditional adjustment models by providing a scalar summary of the covariate information (DAgostino 1998). However, the persistent deficit is that unmeasured and perhaps unrecognized confounders can be balanced only through complete randomization.

The propensity score is defined as the conditional probability of being treated given only the individual's covariates: $e(X)=pr(Z=1 \mid X)$. There are three primary techniques that apply the previously generated propensity scores to arrive at a statistical conclusion: matching, stratification and regression adjustment (D'agostino 1998). The propensity score used in each of these techniques is identical and determined by logistic regression or discriminant analysis; however, the application of the score in each of these techniques differs (D'Agostino 1998).

Matching allows for the analysis of a cohort in which there are far fewer patients treated with a therapy than those not treated. Control subjects are selected whose covariate profile is most similar to that of the treated subjects. The selection process attempts to match specific individuals in the treatment group with individuals in the control group who have the same or the closest propensity score (Rosenbaum 1985).

Stratification and regression are less commonly employed than matching. Stratification groups individuals into strata based on the propensity scores, then compares control and treatment subjects who are in the same strata (D'Agostino 1998). Regression

uses a set of covariates to generate a propensity score, and then adjusts for this score in the general linear model (often times with a smaller subset of covariates). The advantage to this method is the allowance of more complicated models with interactions and higher order terms.

Propensity scoring provides specific advantages in pharmacoepidemilogy that are unmatched by traditional analytic techniques. One of the greatest challenges in studies examining medication usage and outcome variables is confounding by indication. This issue is very difficult if not impossible to totally overcome. The use of propensity scoring directs the focus of the analysis specifically on the indications for use and non-use (Glynn 2006). This focus ultimately allows for the recognition and description of the users of a drug that have no comparable subjects in the non-user group, thereby having important implications for interpretation of the data.

The advantages propensity scoring may have over more traditional techniques are somewhat theoretical. Prior comparisons by Shah et al. in 43 studies examining 78 exposure-outcome associations by propensity scores and regression analysis demonstrated a 10% rate of statistical discordance between the methods (Shah 2005). There was a tendency of propensity score analysis to favor the null hypothesis. Sturmer et al. compared 69 studies that reported propensity scores and regression analysis. The analysis found that 13% of the propensity score estimates differed by more than 20% from the regression model estimates (Sturmer 2006).

Care must also be taken to prevent the introduction of new bias through implementation of the propensity scores. A high correlation between the propensity score and the exposure can lead to a statistical overestimation of the exposure effect

(Glynn 2006). Also, when examining event outcomes propensity score analysis has been shown to be inferior to standard regression methods when there are large (>8) numbers of events per confounder. Conversly, when the outcomes are rarer (<8 events per confounder) Cepeda et al. found that propensity score based analysis was less biased, more robust and more precise than standard regression techniques (Cepeda 2003).

Propensity Score matching to evaluate the association between COX-2 inhibitors and d-dimer levels in the Multi-Ethnic Study on Atherosclerosis (MESA).

Logistic regression was first used using in order to generate a coefficient for each variable included in the analysis (age, gender, race, BMI, total cholesterol, hypertension, diabetes, smoking and CRP). The product of the variable and the generated coefficient produced risk units. These risk units are added together and their sum was added to the intercept of the model (Blackstone 2002). The resulting score is the propensity score (having the units of logit units) and represents the probability of treatment assignment of each subject based on the observed covariates (D' Agostino 1998). The logit units can be converted to probabilities with the equation: PROB=EXP(LOGIT)/(1+EXP(LOGIT). The control group is then formed by looking for propensity scores or probabilities that most closely match the scores of the treatment group.

Table 1 demonstrates the baseline characteristics of the population before and after propensity score matching. Significant differences exist between users of COX-2 inhibitors (celecoxib and rofecoxib combined) and controls initially with respect to age, gender, race, BMI, total cholesterol, hypertension, diabetes mellitus, and CRP. After

propensity score matching there are no significant differences in baseline characteristics between groups.

There is a significant association between COX-2 inhibitor use and d-dimer levels using the control group constructed by propensity score matching (p=0.0292) (Table 2). This association held up after logarithmic transformation of d-dimer (*p*=0.0261).

In order to further address the indication bias, a propensity model was formed matching patients based on age and the presence or absence of arthritis (addition of other covariates did not allow for successful matching). The association between COX-2 inhibitor use and d-dimer remained before (p=0.0325) and after logarithmic transformation of d-dimer (p=0.0.0439).

Table 1. Characteristics of Subjects Using COX-2 inhibitors, Non-selective NSAIDs, and Controls before and after propensity score matching.

Variable	BEFORE PROPENSITY MATCHING			AFTER PROPENSITY MATCHING		
	COX-2 Inhibitors (N = 410)	Controls (N = 5212)	*p* value	COX-2 Inhibitors (N = 406)	Controls (N = 406)	*p* value
Age (years)	66.00 ± 9.24	62.23 ± 10.31	<0.0001	66.05 ± 9.23	68.67± 10.00	0.3564
Gender (%)						
Male	145 (35.37)	2560 (49.12)	<0.0001	143 (35.22)	150 (36.95)	0.6090
Female	265 (64.63)	2652 (50.88)		263 (64.78)	256 (63.05)	
Race (%)						
Caucasian	163 (39.76)	1860 (35.70)	0.0084	163 (40.15)	154 (37.93)	0.9160
African-American	129 (31.46)	1451 (27.84)		125 (30.79)	131 (32.27)	
Hispanic	81 (19.76)	1170 (22.45)		81 (19.95)	81 (19.95)	
Chinese	37 (9.02)	731 (14.03)		37 (9.11)	40 (9.85)	
BMI (kg/m2)	30.39 ± 6.03	27.83 ± 5.23	<0.0001	30.31 ± 5.98	30.03 ± 6.46	0.5161
Total cholesterol	189.20 ± 34.20	194.32 ± 36.05	0.0056	189.33 ± 34.17	189.96 ± 33.16	0.7929
Hypertension (%)						
No	155 (37.80)	2960 (56.79)	<0.0001	155 (38.18)	141 (34.73)	0.3074
Yes	255 (62.20)	2252 (43.21)		251 (61.82)	265 (65.27)	
Diabetes (%)						
No	356 (86.83)	4695 (90.08)	0.0359	353 (86.95)	347 (85.47)	0.5415
Yes	54 (13.17)	517 (9.92)		53 (13.05)	59 (14.53)	
Smoking (%)						
Never	205 (50.12)	2663 (51.26)	0.5844	205 (50.49)	202 (49.75)	0.9775
Former	156 (38.14)	1861 (35.82)		154 (37.93)	156 (38.42)	
Current	48 (11.74)	671 (12.92)		47 (11.58)	48 (11.82)	
CRP	5.19 ± 8.23	3.42 ± 5.15	<0.0001	5.13 ± 8.18	4.96 ± 7.72	0.7661

Values are mean ± SD unless otherwise indicated. BMI indicates body mass index; Hypertension as defined by the JNC VI guidelines (≥ 140/90 nmHg); Diabetes, current pharmacologic treatment; CRP, C-reactive protein; IL-6, Interleukin-6; Arthritis, self-reported. *denotes significant difference from controls at alpha ≤ 0.05. Student *t* test for continuous variables and X^2 for categorical variables.

Table 2. The association of COX-2 Inhibitors with indices of thrombosis using propensity scoring

	D-dimer, ug/ML	Log D-dimer	Fibrinogen, mg/dl	vWF, %	Factor VIII, %	ICAM-1, ng/mL	PAI-1, ng/mL
COX-2 Inhibitor non-users	0.4155	-1.3348	363.3448	145.0556	175.7054	285.1526	31.1132
(N)	(405)	(405)	(406)	(54)	(405)	(151)	(53)
COX-2 Inhibitor users	0.6344	-1.1868	358.9059	151.0714	178.6272	293.3683	29.6852
(N)	(405)	(405)	(404)	(56)	(405)	(138)	(54)
p value	0.0292	0.0261	0.4373	0.6009	0.5524	0.4571	0.8231

P-values reflect comparison between medication users and non-users. All values are adjusted for age, gender, race, total cholesterol, hypertension, diabetes mellitus, BMI, cigarette smoking, CRP. vWF indicates von Willibrand Factor.

References

Blackstone E. Comparing apples and oranges. *J Thorac Cardiovasc Surg*. 2002;123:8-15.

Cepeda, MS, Boston JT, Farrar, et al. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. *Amer J Epidemiol*. 2003;158:280-87.

D'agostino R. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Statist. Med*. 17;1998:2265-81.

Glynn R, Schneeweiss S, Sturmer T. Indications for propensity scores and review of their use in pharmacoepidemilogy. *Basic & Clinical Pharmacology & Toxicolosgy*. 2006;98:253-59.

Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. *Amer Statist*. 1985;39:33-38.

Shah B, Laupacis J, Hux E. Propensity score methods gave similar results to traditional regression modeling in observational studies: a systematic review. *J Clin Epidemiol*. 2005;58:550-59.

Sturmer T, Joshi M, Glynn R, et al. A review of the application of propensity score methods yielded increased use, advantages in specific settings, but no substantially different estimates compared with conventional multivariable methods. *J Clin Epidemiol*. 2006;59:437-47.

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Education

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Training

Fellow in cardiovascular research and clinical Cardiology, Wake Forest University School of Medicine (7/1/06-present), Winston-Salem, NC
Chief resident in Internal Medicine, Thomas Jefferson University, Frankford-Torresdale hospital (7/1/2004-6/30/2005), Philadelphia, PA
Residency in Internal Medicine, Medical University of South Carolina (7/1/2002-6/30/2004) Charleston, SC
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Certifications and Licensure

Specialist in Clinical Hypertension, 2005
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Research Experience

Cardiovascular Research Fellow
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NIH Research Training Fellow: *"The effects of acute ischemic preconditioning on neurologic injury in a rat model of spinal cord ischemia."* Poster presented at Wake Forest University School of Medicine, 1998.

Professional Appointments and Activities

Academic Hospitalist
Frankford-Torresdale Hospital, Philadelphia, PA (7/05-6/06)
Manuscript Reviewer
Circulation, American Heart Journal, Journal of Thrombosis and Haemostasis, Gynecological Endocrinology

Professional Organizations and Positions held

American Heart Association (7/1/2006-present)
American College of Cardiology (11/19/2007-present)
Southern Medical Association, Resident Advisory Committee 2002-2004

Alpha Epsilon Delta (premedical honor society), president 1995-96
Beta Beta Beta (biology honor society)

Honors and Awards

Selected as Spotlight Speaker for American Heart Association, 2008
Chief Medical Resident, Thomas Jefferson University, Frankford-Torresdale hospital
Nominated for Golden Apple Teaching Award, 2003
Selected as member of Resident Advisory Committee of Southern Medical Association 2002 - 2004
Selected as speaker for Medical Student Alumni Association, 2002
Recipient of merit based scholarship, Wake Forest University School of Medicine, 80% tuition
Deans list, Wake Forest University, 8 semesters

Publications

Rudock M, Liu Y, Zieglerb J, Allen S, Lehtinen A, Freedman B, Carr J, Langefeld C and Bowden D. Association of polymorphisms in cyclooxygenase (COX)-2 with coronary and carotid calcium in the Diabetes Heart Study. *Atherosclerosis* 2009;203:459-65.

Seminars and Courses Attended

American Heart Association Spotlight Speaker training program, Dallas, Tx, 2008.
American Heart Association 32nd 10-day Seminar on the Epidemiology and Prevention of Cardiovascular Disease. Lake Tahoe, CA, 2006.
The American Society of Hypertension specialists program. New Orleans, LA, 2005.

Lectures Given

"Women and Cardiovascular Disease" lecture given to Wake Forest University School of Medicine class of 2009.
"The association of COX-2 inhibitors with endothelial function." presented at Cardiology research conference, Wake Forest University School of Medicine, 2008.
"A career in Internal Medicine" lecture given to Medical Student Alumni Association, 2002.

Professional Interests

clinical cardiology, NSAIDs, endothelial function, hypertension.