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The Association of Non-steroidal Anti-inflammatory Medications and Carotid Artery Intima-media Thickness in Male Veteran Twins

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Bachelor of Pharmacy

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Abstract

The Association of Non-steroidal Anti-inflammatory Medications and Carotid Artery Intima-media Thickness in Male Veteran Twins

By Chao Song

Objective: NSAID use is common and may increase cardiovascular risk through vascular mechanisms. We studied the relationship between NSAID use and carotid IMT in a sample of middle-aged veteran twins.

Methods: We studied 318 middle-aged male twins (159 pairs) aged 47-60 years were recruited from the Vietnam Era Twin Registry for the study. Common carotid artery intima-media thickness (cIMT) was measured using high resolution B-mode ultrasonography. Generalized estimating equation (GEE) models to adjust for possible confounding.

Results: The twins who took NSAIDs, on average, had a 17.8 μm greater carotid IMT than his twin who did not use NSAID (95% CI -4.5-40.0; monozygotic: 9.8, 95% CI -9.8-29.4; dizygotic 95.9, 95% CI: 59.8-132.0). Compared with twins who used aspirin, twins who non-aspirin NSAIDs had more significantly greater carotid IMT. The carotid IMT of twins who used non-aspirin NSAIDs was, on average, 31.7 μm higher than carotid IMT for his twin brother who did not (95% CI 1.7-62.7; monozygotic: 37.3, 95% CI 7.4-67.2; dizygotic 102.3, 95% CI: 56.3-148.3), while the carotid IMT of twins who used aspirin was, on average, 10.3 μm higher than carotid IMT for his twin brother who did not (95% CI -15.4-35.9; monozygotic: -7.0, 95% CI -32.4-18.4; dizygotic 93.2, 95% CI: 48.9-137.6).

Conclusion: Among middle-aged Vietnam era veterans, NSAID use was associated with carotid intima-media thickness. This suggests that NSAIDS may increase CVD risk through vascular mechanisms. These findings may help future efforts to evaluate and treat NSAID-related cardiovascular effects.

Keywords NSAIDs, Aspirin, Carotid IMT, Twin Study

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Contents

Background and Literature Review

Non-steroidal anti-inflammatory drugs (NSAIDs) have two subgroups: 1) Selective NSAIDs and 2) unselective NSAIDs (including aspirin). They are popular medications which could be prescribed and purchased over the counter to treat fever, chronic or acute pain and inflammation. The debate whether people could benefit and harm from using NSAIDs or aspirin have lasted over a century. The increased risk of morbidity and mortality related to gastric and duodenal ulcer disease have been realized by the public. Several of selective cyclooxygenase (COX)-2 inhibitors have been marked "black box" by FDA for their increased risks for cardiovascular events. In general, NSAIDs may increase the chance of a heart attack or stroke, especially in those with a history of heart disease.(Bhala et al., 2013; Fosbol et al., 2010; Trelle et al., 2011) Despite increasing evidence behind this risk, NSAIDs remain one of the most commonly used over the counter medications. As such, several questions in clinical practice remain, such as the possible need to perform surveillance studies of CVD risk in those taking chronic NSAIDs. NSAIDs may specifically have vascular effects, which may have implications on management of such patients. In contrast, aspirin is a specific type of NSAID may have different effects, as it has been found to be protective against CVD events.(Sutcliffe et al., 2013)

Despite increasing evidence behind this risk, NSAIDs remain one of the most commonly used over the counter medications. In a 2010 U.S. study, NSAID use increased 41% from 2005 to 2010.(Zhou, Boudreau, & Freedman, 2014)Around 12% of subjects reported regular NSAID use, and 38% using both prescription and OTC. Ibuprofen based drugs were most popular used OTC drugs. Also, 19.0% used aspirin at least three times per week. (Zhou et al., 2014) While NSAIDs help people by reducing inflammation, the side effects may affect people's kidneys, heart and digestive system. In a national survey, 56% exclusive OTC users believed OTC were safe, while

60 % of exclusive users of prescription NSAID believed they were safe.(Wilcox, Cryer, & Triadafilopoulos, 2005) Additionally, around half exclusive OTC users were not aware of nor believed they were at risk for side effects from NSAID and 26% percent of respondents used more than the recommended dose on the label.(Wilcox et al., 2005)

As such, several questions in clinical practice remain, such as the possible need to perform surveillance studies of CVD risk in those taking chronic NSAIDs. NSAIDs may specifically have vascular effects, which may have implications on management of such patients. Furthermore, cyclooxygenase (COX)-2 inhibitors may associate with the highest risk based on outcomes studies. Carotid artery intima-media thickness (cIMT) is a marker of subclinical atherosclerosis and is predictive of future CVD events and may help to understand vascular consequences of NSAID use.(Bots, Hoes, Koudstaal, Hofman, & Grobbee, 1997; Chambless et al., 2000; Helfand et al., 2009)

We propose to study the relationship between NSAID using and cIMT in a cohort of middle-aged male veteran twins. Twin cohorts are advantageous for these studies in general, as twin pairs discordant on exposure provide a useful analog to the counterfactual design for causal inference.(McGue, Osler, & Christensen, 2010) Twins are matched for age, genetics (50% for dizygotic, and 100% for monozygotic), and share familial environments while growing up (e.g., diet, socioeconomic and parental factors), which allows us to carefully adjust for confounders, such health behaviors, genetic factors, and depression that may indirectly measure variations that influence NSAID use or non-use. (Juo et al., 2004)

Study Objective and Research Questions

The main objective of this study is to investigate whether NSAID use is associated with greater carotid IMT measured by high resolution B-mode ultrasonography using a twins-paired cohort study in which we compared twins who were NSAID users with twin brothers who were not.

Furthermore, we evaluated whether the relationship differed by type of NSAID, comparing aspirin with non-aspirin NSAIDs.

The study will test the following questions:

Primary:

1) Is NSAID use associated with greater carotid IMT?

   H_1: There is statistically significant association of NSAID use and greater carotid IMT in the middle age veteran twins.

   H_A: There is not a statistically significant association of NSAID use and greater carotid IMT in the middle age veteran twins.

Secondary:

1) Is aspirin use associated with greater carotid IMT?

   H_1: There is statistically significant association of aspirin use and greater carotid IMT in the middle age veteran twins.

   H_A: There is not a statistically significant association of aspirin use and greater carotid IMT in the middle age veteran twins.

2) Are traditional unselective NSAID use and selective NSAID use associated with greater carotid IMT?

   H_{1a}: There is a statistically significant association of traditional unselective NSAID use and greater carotid IMT in the middle age veteran twins.

   H_{Aa}: There is not a statistically significant association of traditional unselective NSAID use and greater carotid IMT in the middle age veteran twins.

H_{1b}: There is statistically significant association of selective NSAID use and greater carotid IMT in the middle age veteran twins.

H_{Ab}: There is not a statistically significant association of selective NSAID use and greater carotid IMT in the middle age veteran twins.

Methods

Study population

The Twins Heart Study is an investigation of the role of psychological, behavioral, and biologic factors in the development of subclinical CVD (Rooks, Veledar, Goldberg, Bremner, & Vaccarino, 2012; Vaccarino et al., 2011) All Participants were middle-aged male twin pairs, monozygotic and dizygotic, born between 1946 and 1956, recruited from the Vietnam Era Twin Registry.(Goldberg, Curran, Vitek, Henderson, & Boyko, 2002) The Vietnam Era Twin registry is a national sample of male monozygotic and dizygotic twins from all military branches who served on active duty during the Vietnam era. Twins with previous coronary heart disease history were excluded in this study.

Twin pairs were examined on the same date at a clinical research facility in the Emory University Hospital. Activity was limited to leisurely ambulation within the Emory facilities, and all assessments began and ended at the same time. Medical history and data collection occurred during a 24-hour admission under controlled conditions. Anthropometric measurements, blood samples and behavioral questionnaires for measurement of CVD risk factors were obtained. Zygosity was determined by DNA typing as previously described.(Forsberg, Goldberg, Sporleder, & Smith, 2010) The Emory Institutional Review Board approved the study protocol and informed consent was obtained from all study participants.

Measurement

Common cIMT was measured using high resolution B-mode ultrasonography with standard techniques.(Simon, Gariepy, Chironi, Megnien, & Levenson, 2002; van der Meer et al., 2004; Wang et al., 2003) The cIMT was measured on both the near and far walls of the left and right common carotid arteries at the distal 1.0 cm proximal to the bifurcation. Multiple angles were used to identify

the longitudinal image of cIMT showing the maximum cIMT. Measurements were made offline using semi-automated computerized analytical software (Carotid Tools, MIA Inc., Iowa City, Iowa). The average values of maximum cIMT for each of four segments (left and right carotid near and far walls) were used as the total mean of maximum cIMT for each twin. Three sonographers measured cIMT over the study period, and all were unaware of other twin data and blinded to medication use.

NSAID intake was collected as part of a clinical interview by a trained healthcare professional. NSAID use, dosage and times were recorded if a participant used NSAID for any reason. Dosages were categories into low dose level, mid dose level, and high dose level, according to dosage information for specific generic NSAIDs from drug.com. The detail cut-off points showed in Table 2.

Information on demographics (age), behavioral factors (smoking and physical activity), cardiovascular risk factors (body mass index, mean systolic blood pressure (SBP), lipid profile, diabetes) and depression was collected. Physical activity was measured using the Baecke Questionnaire of Habitual Physical Activity used in the Atherosclerosis Risk in Communities (ARIC) Study.(Richardson, Ainsworth, Wu, Jacobs, & Leon, 1995) A trained research nurse obtained information about medication usage. Diabetes was defined as a blood glucose ≥ 126 mg/dL or treatment with insulin or oral antihyperglycemic medications. Direct high-density lipoprotein was measured by homogeneous assays (Equal Diagnostics, Exton, PA). Body mass index was calculated as (weight in kg)/ (height in m^2). History of PTSD and depression were identified using The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders(DSM IV)["Structured Clinical Interview For DSM Disorders," 2014].

Statistics

All statistical analyses were done using Statistical Analysis Software (SAS, version 9.4). NSAID use was evaluated as the primary exposure of interest. Aspirin, selective COX-2 inhibitor and non-selective NSAID use were assessed in secondary analyses. Descriptive statistics were used to summarize baseline participant characteristics. Generalized estimating equations (GEE) were used to assess differences in baseline and account for pair-level clustering. The sociodemographics factors (age, education, race, employment), behavior factors (Baecke physical fitness, coffee, drug abuse, current tobacco use, drug/alcohol abuse, and total alcoholic drinks per day), cardiovascular risk factors (mean systolic blood pressure, low-density lipoprotein level, body mass index, diabetes), psychiatric comorbidities (Beck depression inventory, posttraumatic stress disorder), and important co-medications (antidepressant medication use, narcotic and other analgesic drug use, statin use, and anti-hypertension drug use) were considered as potential confounders.

Both unadjusted and adjusted models were evaluated using GEE models. Model 1 adjusted for sociodemographics factors, behavioral factor, cardiovascular risk factors, psychiatric comorbidities and co-medications. Model 2 additionally adjusted for genetic and familiar factor via comparing cIMT in twin pairs discordant for NSAID use. The interaction between NSAID use and zygosity was evaluated to investigate for genetic confounding.

Results

There were 292 twins (146 pairs) included in the study, including 86 pairs of monozygotic twins and 60 pairs of dizygotic twins. Among the participants, 113 (38.6%) used NSAIDs. Of them, 72 (63.7%) used aspirin or equivalent, 27 (23.9%) used non-selective NSAID and 14 (12.4 %) used selective COX-2 inhibitors. The descriptive characteristics of the twins by NSAID use were shown in the table 3. The twins who used NSAIDs, on average, had 0.7 years more education, but otherwise did not significantly differ in other demographic and socioeconomic factors. Twins who used NSAIDs were more likely to be diabetic (19% vs 4.5%), statin users (38.9% vs 15.9%), and users of angiotensin-converting enzyme inhibitors (ACEI) (25.0% vs 6.5%). Otherwise, no significant baseline differences were found.

The overall mean carotid IMT was 740.0 μm. There was a statistically significant greater carotid IMT among twins who took NSAIDs in unadjusted models, such that NSAID users had a 30.3 μm (95% CI: 7.2- 53.4), as shown in Table 4. The association was significant after additional adjustment for familial and genetic factors. In dizygotic twin pairs discordant for NSAID use, the brother who took NSAIDs, on average, had a 95.9 μm higher carotid IMT than his twin brother who did not use NSAID (95% CI: 59.8-132.0).

The secondary analysis results were presented in the Table 5, Table 6 and Table 7. There are 73 participants who reported aspirin use. Of them, 42 participants used less than 100 mg aspirin per day, and only 4 reported use aspirin more than 500mg per day. For aspirin use, no statistically significant difference was found in the adjusted individual model. However, within discordant dizygotic twin pairs, the carotid IMT of twins who used aspirin was, on average 93.2μm higher than carotid IMT for his dizygotic twin brother who not (95% CI: 48.9- 137.6), while a non- significant lower carotid IMT was observed within monozygotic twins (-7.0, 95%CI; -32.4-18.4). For non-aspirin

NSAID user as individual, there was a statistically significant association of non-aspirin NSAID use with greater carotid IMT, which was 31.7μm on average (95% CI 1.7- 62.7). In addition, statistical significance was found with non-aspirins use in monozygotic and dizygotic twins after additionally adjusting for familial and genetic factors. In monozygotic twins, twins who use non-aspirin NSAID had, on average, a 37.3 μm higher carotid IMT than did his brother who not (95% CI: 7.4- 67.2). In the dizygotic twins, the carotid IMT of the twin who used non-aspirin NSAID was, on average, 102.3 μm higher than the IMT of his twin brother who not (95% CI: 56.3-148.3).

There were no significant differences in carotid IMT among unselective NSAID users and selective NSAID users in crude and adjusted individual models. After within-pair analysis, a significant effect modifier of zygotic type was observed in unselective NSAID use, but not in selective NSAID use. In the dizygotic twins, the carotid IMT of the twin who used unselective NSAID was, on average, 110.7 μm higher than the IMT of his twin brother who not (95% CI: 47.3-174.2). For selective NSAID users, the carotid IMT was not significantly increased, compared wto their dizygotic twins (60.7, 95% CI: -5.4-126.9). A statistically insignificantly increased trend also appeared in the twin who use selective NSAID compared with his dizygotic twin brother who not (60.7, 95%CI: -5.4, 126.9).

Among non-aspirin use, 26% used high doses, and 36% used low doses. The results based on dose level was presented in Table 7. In unadjusted models, there were no significant differences of carotid IMT in non-aspirin NSAID use with low and mid-level doses, and the carotid IMT of the twin who used high dose non-aspirin NSAIDs was, on average, 194.1 μm higher than the IMT of his dizygotic twin brother who did not (95%CI: 183.1- 205.2). The interaction term of high dose non-aspirin NSAID use and zygosity was significant after adjustment. After adjusting confounders, the carotid IMT of high dose non-aspirin NSAID users was, on average, 137.9 μm higher than the

IMT of his dizygotic twin brother who not (95%CI: 52.3- 223.6); the estimate was 2.1 μm for monozygotic twins (95%CI: -28.6- 32.8).

Discussion

In this study of middle-aged, male Vietnam era veteran twins, NSAID use was significantly associated with increased carotid IMT after adjusting for multiple covariates. Through twin modeling, by comparing within MZ twin pairs, we were able to demonstrate significant genetic confounding. Adjustment for genetic factors explained the association with aspirin such that the result was no-longer significant within discordant MZ pairs. However, for non-aspirin NSAID use, the association was significant despite adjustment for genetic factors. Most impressively, non-aspirin NSAID use associated with a 100 micron increased carotid IMT within DZ twin pairs. Given that carotid IMT, on average, increases by 10 micron each year, this implies a 10-year increased vascular age in the twin who was taking NSAIDS compared to his brother who was not taking them.

Our findings are supported by other studies that demonstrate a hypertensive effect due to NSAID use, as well as increased cardiovascular events likely related to that. A 2-3mg average blood pressure increase could have a measurable influence on cardiovascular risk.(Borer & Simon, 2005) All non-selective NSAIDs, including aspirin as the secondary prevention of cardiovascular events, could increase mean arterial pressure by 5.0 mmHg(Pope, Anderson, & Felson, 1993) via increasing sodium and water retention.[20] These are also consistent with previous epidemiologic studies. (Adams et al., 2011; Huang, Hsiao, Wen, & Tsai, 2006; Kurth, Hennekens, Buring, & Gaziano, 2004) Many NSAIDs, not only COX-2 inhibitors(Mamdani et al., 2004; Wong et al., 2007), have "black box" warnings about cardiovascular risk; naproxen(Huang et al., 2006) and aspirin, which may be considered safer NSAIDs, might be potential risk factors of incident CVD according to population-based studies. (Bhala et al., 2013; Trelle et al., 2011)

Our study demonstrated that genetic factors, together with early environments factors, influence the association of NSAID use and carotid IMT. After fully adjusting for genetic effects in monozygotic twins who share 100% genetic information, the change of results are more than 10%. Second, the statistical interaction terms of zygotic type and difference of carotid IMT within pair concealed the effect modification function of genetic effects. As mentioned in several previous studies, the potential genetic effects on the association of NSAID use and CVD worked on different and complex pathways. First, genetic effects might be the confounder in this association. The use pattern of NSAID use could change due to cytochrome P450 polymorphism. (Beinema et al., 2007; Visser et al., 2005) At same time, genetic variant on chromosome 10q11 is associated with carotid intima-media thickness as reported.(Kiechl et al., 2010) Second, genetic variation could play as an effect modifier. Recent study reported the genetic variation of COMT modified the association of aspirin and incident CVD.[(Hall et al., 2014)] Additionally, NSAIDs altered expression of an array of genes associated with cardiovascular events (Palayoor et al., 2012). Potential gene effects should be considered in a future study involving NSAIDs and risk of cardiovascular events.

Our findings highlight possible concerns with aspirin use in genetically susceptible individuals. Nearly 60 million people in U. S. take aspirin regularly to get potential 21% reduction(Berger, Brown, & Becker, 2008) of risks for future cardiovascular events. However, almost 10-year increased vascular age was observed in dizygotic twins who used aspirin compared to their brothers who not. Genetically susceptible individuals could highly increase risk of cardiovascular events rather gain benefit because of dramatic increased carotid IMT. For example, in the population who have susceptible COMT genetic variant, hazard ratios of aspirin on IMT increased 3 times and preventive effect of aspirin use had been reversed to harm. (Hall et al., 2014) Additionally, previous study reported one third of aspirin users could not reach effective blood concentration because of drug resistant. (Grosser et al., 2013) During prescribing aspirin, reduced

benefits of aspirin and potential risks of CVD for susceptible population should be considered. The high prevalence and long term of NSAID use appeal more attention on increasing cardiovascular risk of chronic NSAIDs use. In U. S, the population of regular NSAID use kept increasing from 2005.(Zhou et al., 2014) The convenient over-the-counter availability of nonselective NSAIDs in many countries throughout the world facilitated NSAIDs consumption. The prevalence of NSAID use could still be underestimated due to multiple NSAID use pattern, such as the combination of COX-2 inhibitor and aspirin. It is uncertain about the accumulative effect of NSAID use on carotid IMT. Considered the high prevalence and increasing trend of long term NSAID use, the potential heavier health burden on cardiovascular disease appeal for further researches and related pharmaceutical administration of NSAID use.

In conclusion, we found that among middle-aged Vietnam era veterans, NSAID use, especially non-aspirin NSAID use, was associated with carotid intima-media thickness. We also discovered evidence of genetic confounding in the association between aspirin use and cIMT; the reasons for this are not clear. These findings should be verified in future clinical trials of commonly used over-the-counter NSAIDs, in consideration of restricting use in individuals above a certain risk threshold, especially those with hypertension or known vascular disease.

Strengths and Weaknesses

The twin-pair study design brought a lot of benefits when addressing NSAID use and carotid IMT's association. First, twins were reprehensive samples of population. The twins differ little from non-twin siblings and twins looks like quasi-random sample beyond population in which they have been derived.(Keller, Medland, & Duncan, 2010) Second, the twins study design allows the further analysis on varying family environments and widely different genetic makeup.(Schousboe et al., 2003) Monozygotic twins shared nearly 100% genetic information, and dizygotic twins share only about 50%. The results stratified on zygotic types offer more information on the roles of genetic effects, shared environment and unique environment.

In our study, other potential confounders, including sociodemographics factors, behavior factors, cardiovascular risk factors, psychiatric comorbidities and important co-medication, were considered in adjustment. Emory Twins Study was conducted for an investigation of the role of psychological, behavioral, and biologic factors in the development of subclinical CVD. The details of psychological, behavioral, and biologic factors offered the opportunity to full adjust confounders.

There are several limitations in this study. Dose information, was limited particularly for those who ingested NSAIDs on an as-needed basis, and duration of use was not available. As such, we were unable to assess for a reliable dose-response relationship. The effect on cardiovascular events of NSAIDs is time dependent. The duration information could be critical for the further analysis. Second, the lack of follow-up medication information might cause potential misclassification, and reduced the significance of association. Third, all study participants were middle-aged male veterans, and therefore the results may not apply to women and different age groups. Finally, it is still possible that there was residual confounding due to unmeasured factors although this was minimized through the twin design.

Future Directions

The doubt, how much people could benefit from long term NSAID use is still waiting for more clear and strong evidences. With people seek for higher quality of life, the duration and dose of NSAIDs increases to relief pain or prevent cardiovascular effects. The effects of NSAIDs have a lot of room to dig.in

There are three directions worthy for further research. First, how genetic effects works on the association of NSAID and carotid IMT, especially for the effect of aspirin use and cardiovascular events. Based on our study, we observed that aspirin use could cause harm for genetic susceptible population rather than the preventive effects as people expected. More future work could focus on how to specific gene information and define the genetic susceptible population. Second, the potential dose response and time dependent relationship of NSAID use and carotid IMT would be interesting. It would offer more useful information for the NSAID use duration and pharmaceutical administration. Third, it also worthy to investigate that the association of carotid IMT and specific type of NSAID. The difference of effects of different generic NSIADs on anti-inflammatory and relieving pain has been reported in the previous studies, and the slightly differences of greater carotid IMT in difference generic NSAIDs were observed in our study. The sample size limited to get statistical significant results but it could be tested in the larger size study.

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Tables

Table 1. Measurements of variables

Constructs		Measures
Outcome	Carotid IMT	Continuous
Exposure	NSAID Use Aspirin Use Traditional NSAID Use Selective NSAID Use	Dichotomous • No • Yes
	Non Aspirin NSAID Dosage	Categorical
Socio-demographics factors		
	Age	Continuous
	Education level	
	Employed	Dichotomous • No • Yes
	Marriage Status	Categorical • Married • Widowed • Divorced • Separated • Never Married
Cardiovascular Factors		
	Mean SBP LDL Level, mg/dl BMI, kg/m^2	Continuous
	Diabetes	Dichotomous
Behavior factors		
	Baecke physical fitness Coffee, cups per day Total drinks, cups per day	Continuous
	Drug abuse Current smoker Current alcohol user	Dichotomous • No • Yes
Psychiatric comorbidities		
	Beck depression inventory	Continuous
	PTSD	Dichotomous
Comedication	Antidepressant user Narcotics combination user Other analgesics Statin User CCB user ACEI user ARB user	Dichotomous

Abbreviations: SBP, systolic blood pressure; PTSD, posttraumatic stress disorder; CCB, calcium channel blockers; ACEI, angiotensin-converting-enzyme inhibitor; ARB, Angiotensin II receptor antagonist

Table 2. The Dosage Level of NSAIDs

NSAID	Low Dose Level	Mid Dose Level	High Dose Level
Unselective			
Ibuproffen	0-1800	1800-2400	>2400
Indomethacin	0-75	50-75	>75
Naproxen	0-750	750-1000	>1000
Nabumetone	0-1000	1000-2000	>2000
Oxaprozin	0-600	600-1200	>1200
Diclofenac	0-50	50-150	`>150
Etodolac	0-600	600-800	>800
Selective			
Meloxicam	0-7.5	7.5-15	>15
Celecoxib	0-200	200-400	>400
Valdecoxib	0-10	10-40	>40
Piroxicam	0-10	10-40	>40

Table 3. Selected Characteristics by NSAIDs use, Emory Twins Study (n=318)

Characteristics *	Take NSAIDs (n=72) Mean (SD)	Do not take NSAIDs (n=246) Mean (SD)	p-value
Socio-demographics factors			
Age, years	55.5 (2.7)	54.1 (2.9)	0.92
Education level, years	14.8 (2.3)	14.1 (2.2)	0.02
Employed, %	75.0	82.5	0.17
Marriage Status, %			0.11
Married	83.3	77.2	
Widowed	4.2	1.6	
Divorced	6.9	15.5	
Separated	4.2	2.0	
Never Married	1.4	3.7	
Cardiovascular Factors			
Mean SBP, mmHg	130.5 (14.9)	129.3 (16.0)	0.33
LDL Level, mg/dl	122.6 (37.0)	126.8 (32.0)	0.35
BMI, kg/m^2	29.9 (4.7)	28.8 (4.5)	0.05
Diabetes, %	19.4	4.5	<.001
Behavior factors			
Baecke physical fitness	7.2 (1.9)	7.4 (1.8)	0.30
Coffee, cups per day	2.5 (4.6)	2.5 (3.2)	0.89
Drug abuse, %	9.7	16.3	0.19
Current smoker, %	17.1	17.7	0.11
Total drinks, cups per day	5.7 (11.6)	4.7 (8.6)	0.49
Current alcohol user	3 (4.2)	10 (4.1)	0.99
Psychiatric comorbidities			
Beck depression inventory	4.1 (5.8)	5.0 (6.8)	0.30
PTSD, %	6.9	6.1	0.78
Co-medications			
Antidepressant user, %	16.7	13.8	0.57
Narcotics combination user, %	5.6	3.6	0.53
Other analgesics, %	1.4	4.1	0.69
Statin User, %	38.9	15.0	<.001
CCB user, %	5.6	2.9	0.28
ACEI user, %	25.0	6.5	<.001
ARB user, %	4.2	4.5	0.91

Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drug; SD, standard deviation; SBP, systolic blood pressure; PTSD, posttraumatic stress disorder; CCB, calcium channel blockers; ACEI, angiotensin-converting-enzyme inhibitor; ARB, Angiotensin II receptor antagonist.

* All variables presented as means(SD) unless otherwise indicated.

Table 4. The Association of Mean carotid IMT differences and NSAID use (n=292 individuals, 86 discordant MZ pairs, and 60 discordant DZ pairs)

Model		Carotid IMT(μm) (95%CI)	p-value
Unadjusted			
Individual Model		30.3 (7.2, 53.4)	0.01
Within Pair Model	MZ	4.3 (-20.8, 29.4)	0.74
	DZ	52.6 (20.0, 85.3)	0.002
Adjusted*			
Individual Model		17.8 (-4.5, 40.0)	0.12
Within-Pair Model		23.6 (-1.9, 49.1)	0.07
Stratification**	MZ	9.8 (-9.8,29.4)	0.32
	DZ	95.9 (59.8,132.0)	<.0001

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; carotid IMT, carotid artery intima-media thickness; CI, confidence interval; MZ, monozygotic; DZ, dizygotic.

* Covariates include age, education level, relationship status, mean systolic blood pressure, IL-6, LDL level, body mass index, Baecke physical fitness, coffee, drug abuse, current smoker, drug abuse, total drink per day, current alcohol use, diabetes, Beck depression inventory, PTSD, antidepressant medication use, narcotics combination medication use, other analgesics, statin use, anti-hypertension use. The estimates express the difference in cIMT (μm) associated with NSAIDs use.

**P-value for interaction term (NSAID use x zygotic) in adjusted model is 0.01.

Table 5. The Association of Mean carotid IMT differences and aspirin and non-aspirin NSAID (n=292 individuals, 86 discordant MZ pairs, and 60 discordant DZ pairs)

Model		Aspirin use (N=72) Carotid IMT(μm) (95% CI)	p-value	Non-Aspirin NSAID use (N=41) Carotid IMT(μm) (95% CI)	p-value
Unadjusted					
Individual Model		30.1 (4.2, 56.1)	0.02	28.7 (-3.8, 61.3)	0.08
Within-Pair Model	MZ	2.0 (-24.7, 28.8)	0.88	6.0 (-29.7, 41.7)	0.74
	DZ	52.3 (14.8, 89.9)	<.001	73.8 (14.4, 133.2)	0.01
Adjusted*					
Individual Model		10.3(-15.4, 35.9)	0.43	31.7 (1.7, 62.7)	0.03
Within-Pair Model		21.3 (-7.7, 50.3)	0.15	48.3 (-28.5, 125.1)	0.22
Stratification **	MZ	-7.0 (-32.4, 18.4)	0.59	37.3 (7.4, 67.2)	0.01
	DZ	93.2 (48.9, 137.6)	<.001	102.3(56.3, 148.3)	<.001

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; carotid IMT, carotid artery intima-media thickness; CI, confidence interval; MZ, monozygotic; DZ, dizygotic.

* Covariates include age, education level, relationship status, mean systolic blood pressure, IL-6, LDL level, body mass index, Baecke physical fitness, coffee, drug abuse, current smoker, drug abuse, total drink per day, current alcohol use, diabetes, Beck depression inventory, PTSD, antidepressant medication use, narcotics combination medication use, other analgesics, statin use, anti-hypertension use. The estimates express the difference in cIMT (μm) associated with aspirin/non-aspirin NSAID use.

** P-values of interaction term of difference of medication use within pairs and zygotic type were 0.001 for aspirin use and 0.04 for non-aspirin use.

Table 6. The Association of Mean carotid IMT differences and Unselective/ Selective NSAIDs (n=292 individuals, 86 discordant MZ pairs, and 60 discordant DZ pairs)

Model		Unselective NSAIDs (N=27) Carotid IMT(μm) (95% CI)	p-value	Selective NSAIDs (N=12) Carotid IMT(μm) (95% CI)	p-value
Unadjusted					
Individual Model		34.4 (-4.8, 73.5)	0.08	13.2 (-40.2, 66.6)	0.63
Within-Pair Model	MZ	22.8 (-31.4, 77.0)	0.41	-18.8 (-77.3,39.7)	0.53
	DZ	110.4 (21.9, 198.8)	0.01	29.7 (-66.8, 126.2)	0.55
Adjusted*					
Individual Model		25.9 (-9.4, 61.2)	0.15	30.2 (-19.1, 79.6)	0.23
Within-Pair Model		51.4 (14.9, 87.9)	0.006	-5.6 (-77.0, 65.7)	0.87
Stratification**	MZ	34.6 (5.6, 63.5)	0.19	31.5 (-57.5,120.5)	0.49
	DZ	110.7 (47.3, 174.2)	<.001	60.7 (-5.4, 126.9)	0.07

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; carotid IMT, carotid artery intima-media thickness; CI, confidence interval; MZ, monozygotic; DZ, dizygotic

* Covariates include age, education level, relationship status, mean systolic blood pressure, IL-6, LDL level, body mass index, Baecke physical fitness, coffee, drug abuse, current smoker, drug abuse, total drink per day, current alcohol use, diabetes, Beck depression inventory, PTSD, antidepressant medication use, narcotics combination medication use, other analgesics, statin use, anti-hypertension use. The estimates express the difference in cIMT (μm) associated with unselective/selective NSAID use.

** P-values of interaction term of difference of NSAID use within pairs and zygotic type were 0.02 for unselective NSAID use and 0.84 for selective NSAID use

Table 7. The Association of Mean carotid IMT Differences and Non-aspirin NSAIDs by dose (n=292 individuals, 86 discordant MZ pairs, and 60 discordant DZ pairs)

Model		Low Dose NSAIDs (N=15) Carotid IMT(μm)	p-value	Middle Dose NSAIDs (N=8) Carotid IMT(μm)	p-value	High Dose NSAIDs (N=11) Carotid IMT(μm)	p-value
Unadjusted							
Individual Model		47.5 (-4.0, 99.0)	0.10	13.8 (-55.9, 93.5)	0.08	18.4 (-41.3, 78.1)	0.08
Within-Pair Model	MZ	14.3(-61.1, 89.7)	0.71	-52.3(-12.2, 17.5)	0.14	-6.9 (50.0, 36.2)	0.75
	DZ	82.1 (-23.1, 187.3)	0.13	38.5 (-35.6, 112.6)	0.30	194.1(183.1, 205.2)	<.001
Adjusted*							
Individual Model		45.7 (-1.0, 92.5)	0.05	31.8 (-35.1, 98.8)	0.35	22.1 (-33.0, 77.3)	0.43
Within-Pair Model		27.7 (-41.5, 96.8)	0.43	42.6 (-16.7, 101.9)	0.16	26.3 (-26.5 79.1)	0.33
Stratification**	MZ	42.2 (3.1, 81.4)	0.03	72.2 (-3.2, 147.6)	0.06	2.1 (-28.6,32.8)	0.89
	DZ	77.0 (-3.7, 157.7)	0.06	166.0 (92.4, 239.6)	<.001	137.9 (52.3, 223.6)	<.001

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; carotid IMT, carotid artery intima-media thickness; CI, confidence interval; MZ, monozygotic; DZ, dizygotic

* Covariates include age, education level, relationship status, mean systolic blood pressure, IL-6, LDL level, body mass index, Baecke physical fitness, coffee, drug abuse, current smoker, drug abuse, total drink per day, current alcohol use, diabetes, Beck depression inventory, PTSD, antidepressant medication use, narcotics combination medication use, other analgesics, statin use, anti-hypertension use. The estimates express the difference in cIMT (μm) associated with non-aspirin NSAID use by dose.

** P-values of interaction term of difference of non-NSAIDs within pairs and zygotic type were 0.60 for low dose, 0.32 for middle dose and 0.0004 for high dose.