

Differences in prevalence and severity of coronary artery calcification between two non-Hispanic white populations with diverse lifestyles

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Abstract

Background: Comparison of atherosclerosis and its risk factors among diverse populations may provide insights into the pathogenesis of the disease. We investigated differences in traditional coronary artery disease (CAD) risk factors and presence and quantity of coronary artery calcification (CAC), a marker of subclinical coronary atherosclerosis, between two diverse non-Hispanic white populations living in the US.

Methods and results: Members of the Old Order Amish (OOA), a unique culture with a physically active rural lifestyle who rarely use prescription medications, were compared to another non-Hispanic white population with a more typical US lifestyle, Epidemiology of Coronary Artery Calcification (ECAC), Study participants from Rochester, Minnesota. Although age- and sex-adjusted presence and quantity of CAC in those with detectable CAC were similar between study groups, there were significant differences in the distribution of many traditional CAD risk factors. OOA had significantly less abdominal adiposity and history of cigarette smoking but a less advantageous lipid profile than ECAC participants. Importantly, after adjusting for CAD risk factors, presence of CAC and quantity of CAC among those with detectable CAC were significantly higher among OOA than ECAC participants.

Conclusions: Identification of factors contributing to differences in subclinical disease across groups could increase our understanding of mechanisms for coronary atherosclerosis.

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Atherosclerosis, the major cause of coronary artery disease (CAD), is a multi-factorial disease. The prevalence and extent of atherosclerosis and its risk factors vary substantially among populations. These differences may be due to differ-

ences in genetic susceptibility and lifestyle factors and their interactions [1]. Hence, the comparison of the prevalence and extent of atherosclerosis and its risk factors among diverse populations is an important approach to provide insights into the pathogenesis of the disease [1].

The Old Order Amish (OOA) of Lancaster County, Pennsylvania is a genetically homogeneous closed non-Hispanic white founder population with uniform socioeconomic status and rural lifestyle. The OOA have a unique culture that

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distinguishes them from most other subpopulations living in the United States (US) [2]. Farming is the predominant Amish occupation, and the Amish lifestyle is characterized by a high level of physical activity because of both their rural orientation and their cultural prohibition against driving automobiles and using modern farm equipment or electricity in their homes. The OOA tend to have very low levels of smoking and alcohol use and typically use prescription medications sparingly [3]. The OOA attend their own schools and their formal education typically ends at the 8th grade.

The impact of the Amish lifestyle on atherosclerotic CAD risk is unknown. To address this issue, we sought to compare the prevalence and quantity of coronary artery calcification (CAC), a subclinical measure of coronary atherosclerosis, between members of the OOA and another non-Hispanic white population with a more typical US lifestyle. The non-Amish comparison group is comprised of participants from the community-based Epidemiology of Coronary Artery Calcification (ECAC) Study based in Rochester, Minnesota. While several previous studies have compared CAC between different ethnic/racial groups within the US [4–7] and among whites living on different continents [8], ours is the first study to compare different non-Hispanic white populations within the US, and especially populations with vastly different lifestyles.

CAC can be quantified non-invasively and accurately with electron beam computed tomography (EBCT) [9]. A direct relationship exists between CAC and both histologic and *in vivo* intravascular ultrasound measures of atherosclerotic plaque [10]. CAC quantity is an independent predictor of angiographically defined CAD, after controlling for established CAD risk factors [11], and CAC predicts future CAD events in asymptomatic and symptomatic adults [12,13].

Toward a greater understanding of CAD risk factors and pathogenesis, the purpose of the current study was to investigate: (1) differences in traditional CAD risk factors and the presence and quantity of CAC between these two diverse non-Hispanic white populations; (2) differences in the presence and quantity of CAC between these populations after adjusting for differences in traditional CAD risk factors.

1. Methods

1.1. Study population

The Amish Family Calcification Study (AFCS) and ECAC Study are ongoing community-based studies whose goals are to examine environmental and genetic risk factors for CAC. The studies are approved by their respective Institutional Review Boards and all participants have given informed consent.

AFCS participants were recruited from the OOA of Lancaster County, Pennsylvania between March 2002 and June 2005. Subjects were initially recruited into the AFCS on the basis of their prior participation in an earlier family study of

bone mineral density, although recruitment guidelines were later modified to allow other interested individuals in the community to participate. All first and second degree relatives of these new participants were also invited to participate in the AFCS. Recruitment efforts were made without regard to cardiovascular disease status and extensive analyses have revealed no correlations in AFCS participants between bone mineral density and CAC after accounting for age (data not shown). Women who were pregnant or lactating were not eligible to participate.

Participants were evaluated for traditional CAD risk factors at the Amish Research Clinic in Strasburg, Pennsylvania and an EBCT examination of the heart was conducted in Timonium, Maryland. A total of 596 participants at least 40 years of age were examined. Sixty participants with a history of myocardial infarction (MI), stroke, or a coronary angiogram with a detectable blockage were excluded. An additional 17 participants were excluded from analysis because of missing data ($n=8$) or extreme values for measured risk factors (± 4 standard deviations from the mean for AFCS participants; $n=9$). The final AFCS sample consisted of 519 (217 men) individuals. These AFCS participants were compared to participants in the ECAC Study.

The ECAC Study participants were recruited through the Rochester Family Heart Study (RFHS), a community-based study of 3974 individuals, 5–90 years of age, which was conducted between 1984 and 1991. RFHS participants with a history of coronary or non-coronary heart surgery or women who were pregnant or lactating were excluded from participation in the ECAC Study. Levels of traditional CAD risk factors in ECAC participants are similar, in many ways, to other non-Hispanic whites living in the US [14]. ECAC Study participants had baseline CAD risk factor assessment and EBCT examinations of the heart between 1991 and 1998 and follow-up assessments and EBCT examinations between December 2000 and February 2005. For temporal comparability with the AFCS, only data from the follow-up visits were used in the current analysis. A total of 732 individuals at least 40 years of age were examined. Thirty-four with a history of MI, stroke, or a coronary angiogram with a detectable blockage were excluded. An additional 68 participants were excluded from analysis because of missing data ($n=54$) or extreme values for measured risk factors (± 4 standard deviations from the mean for ECAC Study participants; $n=14$). The final ECAC Study sample thus consisted of 630 (290 men) individuals.

1.2. Risk factor assessment

A panel of standard CAD risk factors was assessed in both studies using identical methodologies except for blood pressure. Height and weight were measured and body mass index (BMI; kg/m^2) was calculated. Waist circumference was measured at the umbilicus, hips at the level of maximal circumference, and waist-to-hip ratio was calculated. Standard enzymatic methods were used to measure total cholesterol, high-density lipoprotein (HDL)-cholesterol, and

triglycerides after an overnight fast. Low-density lipoprotein (LDL)-cholesterol was calculated with the Friedewald equation [15]. Systolic blood pressure (SBP; mmHg) and diastolic blood pressure (DBP; mmHg) were measured in the right arm using a standard sphygmomanometer in the AFCS and a random-zero sphygmomanometer (Hawksley and Sons) in the ECAC Study. Self-reported history of physician-diagnosed diabetes and hypertension, as well as smoking history was also documented. Use of blood pressure-lowering medications and lipid-lowering medications was recorded.

1.3. Coronary artery calcification

CAC was measured in both studies by EBCT scanning to detect and quantify CAC using Imatron C-150 scanners (Imatron Incorporated, South San Francisco, California). The common, standard protocol used in both studies included thirty to forty 3 mm contiguous transverse tomograms between the aortic root and the apex of the heart, gated to 80% of the RR interval. One cardiologist (JAR) and one diagnostic radiologist (PFS) were involved in both studies. CAC was defined as a hyperattenuating focus in a coronary artery $\geq 1.00 \text{ mm}^2$ and having CT number >130 Hounsfield Units. Quantity of CAC was defined as the CAC score in the four epicardial arteries using the method of Agatston and colleagues [16].

1.4. Statistical analyses

Prior to analysis, the distributions of the continuously measured risk factors were tested (and confirmed) for normality using the Anderson–Darling test [17]. Triglyceride values were natural log-transformed to reduce skewness. Means and standard deviations were computed for continuous variables, and frequencies and percentages were computed for discrete variables. CAD risk factors were compared between study groups after adjusting for age and sex.

The distribution of CAC quantity had a mass of values at zero and a highly skewed set of non-zero values. Therefore, a two-part model was used to assess the association between CAD risk factors and (1) the presence of CAC and (2) the quantity of CAC among those with detectable CAC. CAC score was natural log-transformed (i.e., $\ln(\text{CAC score})$) to reduce skewness. This approach has the virtue of allowing risk factors for initiation of detectable CAC to be different from those affecting quantity of detectable CAC.

Since individuals in the same family participated in each study group, analyses of risk factor associations were conducted accounting for the correlations among related individuals. These analyses utilized generalized estimating equations (GEE) with an exchangeable working correlation structure in which all pair-wise correlations between participants from the same family were equal [18]. In the ECAC Study, families consisted primarily of sibships. Although a more complex pedigree structure characterized the Amish

participants, AFCS families were defined for this analysis on the basis of sibships.

GEE models with the logic link function were fit to assess the association between age, sex, and each of several selected CAD risk factors (waist circumference, total cholesterol to HDL-cholesterol ratio, LDL-cholesterol, SBP, DBP, and smoking history) and presence of detectable CAC in each study group. All selected CAD risk factor models (except age and sex) were adjusted for age and sex. Parameter estimates from these models were interpreted as the estimated odds ratio associated with a 1-unit increase in the standard deviation of a quantitative predictor variable or a change in status of a categorical predictor variable.

GEE models with the identity link function were fit to examine the association between age, sex, and each of the same group of selected CAD risk factors and $\ln(\text{CAC score})$ among those with detectable CAC in each study. All selected CAD risk factor models (except age and sex) were adjusted for age and sex. Parameter estimates from these models were exponentiated and interpreted as the estimated relative increase in CAC score associated with a 1-unit increase in the standard deviation of a quantitative predictor variable or a change in status of a categorical predictor variable.

The association of study group (i.e., AFCS versus ECAC) with the presence and quantity of CAC was evaluated by pooling AFCS and ECAC Study participants and then adjusting for age, sex, waist circumference, total cholesterol to HDL-cholesterol ratio, SBP, use of lipid-lowering or blood-pressure lowering medications, and smoking status. Due to significant collinearity ($p < 0.0001$), several additional variables were not included in the multivariable models. Neither hypertension nor DBP were considered because of their strong associations with SBP. Because LDL-cholesterol was strongly associated with total cholesterol to HDL-cholesterol ratio and was also associated with use of lipid-lowering medication, this variable was not included in the multivariable models. The differences between study groups in mean lipid and blood pressure levels were re-analyzed and the final models were refit in the subset of participants who were not taking blood pressure- or lipid-lowering medications.

Interactions of age, male sex, and study group with the selected CAD risk factors included in the models were considered. A significance level of $p = 0.05$ was used for all analysis. All tests were 2-sided. We were not able to conduct sex-specific analyses due to sample size considerations.

2. Results

Levels of CAD risk factors are contrasted between AFCS and ECAC participants in Table 1. AFCS participants were significantly younger than ECAC participants ($p < 0.0001$). After adjusting for age and sex, there were significant differences in CAD risk factors between the two study groups. AFCS participants had significantly higher mean levels of total cholesterol and LDL-cholesterol but lower

Table 1

Characteristics and coronary artery calcification (CAC) measurements in Amish Family Calcification Study (AFCS) and Epidemiology of Coronary Artery Calcification (ECAC) Study participants

	AFCS (<i>N</i> = 519) mean ± S.D. or <i>N</i> (%)	ECAC (<i>N</i> = 630) mean ± S.D. or <i>N</i> (%)	<i>P</i> ^a
Age (years)	56.3 ± 11.1	60.3 ± 9.2	<0.0001
Male sex	217 (41.8)	290 (46.0)	0.15
Waist (cm)	93.3 ± 11.4	96.3 ± 13.7	0.0012
Hip (cm)	106.7 ± 10.5	106.3 ± 10.6	0.65
Waist/hip ratio	0.88 ± 0.07	0.91 ± 0.10	<0.0001
Height (cm)	163.5 ± 8.9	168.8 ± 9.6	<0.0001
Weight (kg)	76.0 ± 14.3	81.4 ± 16.4	<0.0001
Body mass index	28.4 ± 5.0	28.5 ± 5.0	0.53
Cholesterol (mmol/L)	5.5 ± 1.01	5.2 ± 0.87	<0.0001
HDL-cholesterol (mmol/L)	1.48 ± 0.40	1.45 ± 0.41	0.21
Cholesterol/HDL-cholesterol ratio	4.0 ± 1.1	3.8 ± 1.1	0.0060
Triglycerides (mmol/L)	1.03 ± 0.58	1.48 ± 0.73	–
ln(triglycerides)	−0.11 ± 0.50	0.28 ± 0.47	<0.0001
LDL-cholesterol (mmol/L)	3.59 ± 0.91	3.04 ± 0.76	<0.0001
Systolic BP (mmHg)	119.7 ± 15.6	121.4 ± 15.7	0.59
Diastolic BP (mmHg)	72.0 ± 8.8	70.5 ± 9.7	0.0101
Physician-diagnosed hypertension	47 (9.1)	159 (25.2)	<0.0001
Physician-diagnosed diabetes	12 (2.3)	30 (4.8)	0.0436
Ever smoked cigarettes	84 (16.2)	299 (47.5)	<0.0001
Current smoker	44 (8.5)	51 (8.1)	0.74
Former smoker	40 (7.7)	248 (39.4)	<0.0001
BP-lowering medications	32 (6.2)	142 (22.5)	<0.0001
Lipid-lowering medications	19 (3.7)	144 (22.9)	<0.0001
Coronary artery calcification (CAC)			
Detectable CAC	274 (52.8)	375 (59.5)	0.22
CAC score	302.9 ± 615.4	305.7 ± 562.0	
Median	99.9	97.2	–
ln(CAC score)	4.2 ± 2.0	4.3 ± 2.0	0.26

S.D.: standard deviation; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BP: blood pressure.

^a All risk factors (except age and sex) were adjusted for age and sex.

mean levels of ln-transformed triglycerides ($p < 0.0001$ for each). AFCS participants were significantly less likely to report a prior history of hypertension, or to report that they ever smoked cigarettes than ECAC participants ($p < 0.0001$ for each). As expected, substantially fewer AFCS than ECAC participants were taking blood pressure-lowering medications (6.2% versus 22.5%) or lipid-lowering medications (3.7% versus 22.9%) ($p < 0.0001$ for each). Although BMI was not significantly different ($p = 0.53$) between study groups, waist circumference and waist-to-hip ratio were significantly less among AFCS than ECAC participants ($p = 0.0012$ and < 0.0001 , respectively). The prevalence of CAC and mean ln(CAC score) among those with detectable CAC were not statistically different between AFCS and ECAC participants after adjusting for age and sex.

2.1. Association of selected CAD risk factors with CAC

Age and male sex were significantly associated with presence of CAC in each study group ($p < 0.0001$ for each). Cholesterol to HDL-cholesterol ratio, SBP, and history of ever having smoked cigarettes were significantly associated with presence of CAC in each study group after adjusting for age and sex ($p < 0.01$ for all) (Fig. 1). LDL-cholesterol

was significantly associated with presence of CAC in AFCS participants ($p < 0.0001$) while waist circumference was significantly associated with the presence of CAC in ECAC participants ($p = 0.0002$) (Fig. 1).

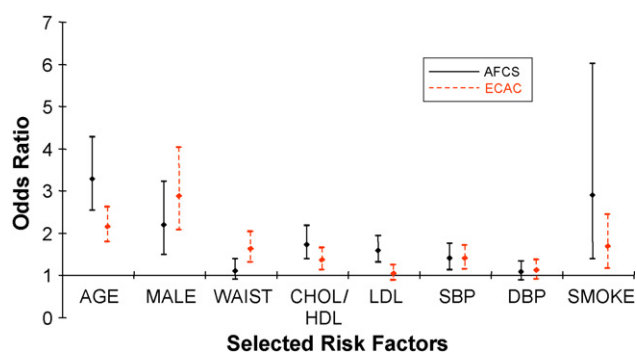


Fig. 1. Associations of selected risk factors with presence of detectable coronary artery calcification among Amish Family Calcification Study (AFCS) and Epidemiology of Coronary Artery Calcification (ECAC) Study participants. All selected CAD risk factors (except age and sex) were adjusted for age and sex. Odds ratio (95% confidence interval) for a 1-unit change in standard deviation of continuous risk factor or change in status of dichotomous risk factor. WAIST: waist circumference; CHOL: cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; SMOKE: ever having smoked cigarettes.

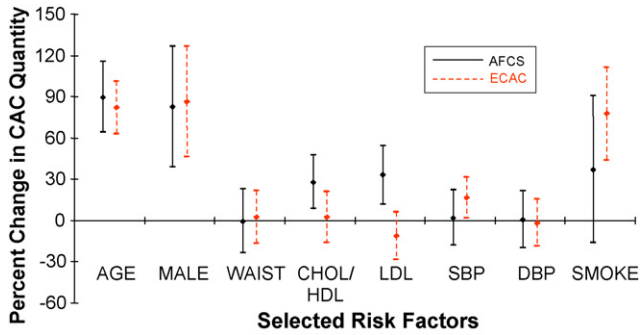


Fig. 2. Associations of selected risk factors with quantity of coronary artery calcification (CAC) among Amish Family Calcification Study (AFCS) and Epidemiology of Coronary Artery Calcification (ECAC) Study participants with detectable CAC. All selected CAD risk factors (except age and sex) were adjusted for age and sex. Percent change (95% confidence interval) in CAC score for a 1-unit change in standard deviation of continuous risk factor or change in status of dichotomous risk factor. WAIST: waist circumference; CHOL: cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; SMOKE: ever having smoked cigarettes.

Fig. 2 shows the association of age, sex, and the age- and sex-adjusted association of selected CAD risk factors with CAC quantity among those with detectable CAC. Only age and sex were significantly associated with CAC quantity in both studies. Cholesterol to HDL-cholesterol ratio and LDL-cholesterol were significantly associated with ln(CAC score) ($p=0.0048$ and 0.0023 , respectively) among AFCS participants. SBP was significantly, but weakly, associated with ln(CAC score) ($p=0.0268$) while having ever smoked cigarettes was significantly associated with ln(CAC score) ($p<0.0001$) among ECAC participants.

2.2. Multivariable GEE models

In a multivariable logistic regression model that included age, sex, and the selected CAD risk factors and study group as predictor variables, age, male sex, cholesterol to HDL-cholesterol ratio, SBP, taking lipid-lowering medications, having a history of smoking cigarettes, and being Amish were positively and significantly associated with the presence of detectable of CAC (data not shown; $p<0.01$ for all). After accounting for these risk factors, AFCS participants had a 1.6 time higher odds of having CAC than ECAC participants (95% C.I.: 1.1–2.2, $p=0.0053$; data not shown). There were no statistically significant interactions of age, sex, or study group with the selected CAD risk factors. The excess risk of having detectable CAC in AFCS participants relative to ECAC participants for the same CAD risk factor profile is illustrated in Fig. 3, which shows the predicted probability of having detectable CAC for participants with mean waist circumference of 95.0 cm, cholesterol to HDL-cholesterol ratio of 3.9, and SBP of 120.6 mmHg, who never smoked cigarettes and were not taking lipid-lowering or blood pressure-lowering medications. The predicted probability of presence of detectable CAC increased with older age.

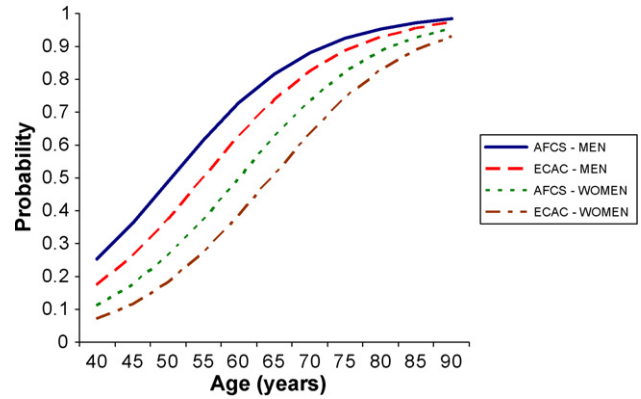


Fig. 3. Predicted probability of presence of detectable coronary artery calcification among Amish Family Calcification Study (AFCS) and Epidemiology of Coronary Artery Calcification (ECAC) Study participants.

AFCS men had the highest probability of having detectable CAC while ECAC woman had lowest probability of having detectable CAC across the age range.

Among those with detectable CAC, age, male sex, cholesterol to HDL-cholesterol ratio, taking lipid-lowering medications, taking blood pressure-lowering medications, having a history of smoking cigarettes, and being Amish were positively and significantly associated with ln(CAC score) (data not shown; $p<0.05$ for all). After adjusting for these risk factors, AFCS participants had an estimated 66% relative increase in CAC score compared to ECAC participants (data not shown; $p=0.0014$). There were no statistically significant interactions of age, sex, or study group with the selected CAD risk factors. Fig. 4 shows the predicted CAC score for participants with detectable CAC and mean waist circumference of 97.3 cm, cholesterol to HDL-cholesterol ratio of 4.1, and SBP of 124.3 mmHg who never smoked cigarettes and were not taking lipid-lowering or blood pressure-lowering medications. As expected, the predicted CAC score increased with older age. Among those with detectable CAC, AFCS men had the highest predicted CAC score while ECAC woman had the

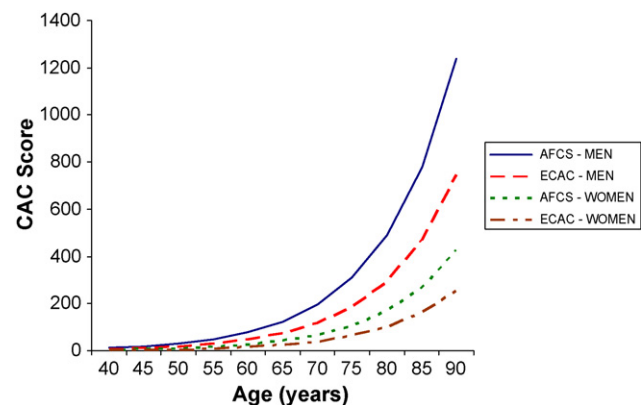


Fig. 4. Predicted quantity of coronary artery calcification (CAC score) among Amish Family Calcification Study (AFCS) and Epidemiology of Coronary Artery Calcification (ECAC) Study participants with detectable CAC.

lowest predicted CAC score, which parallels predictions for presence of detectable CAC.

Among the subset of participants not taking lipid-lowering or blood-pressure lowering medications (470 AFCS and 401 ECAC participants), after adjusting for age and sex, mean cholesterol (5.54 mmol/L versus 5.28 mmol/L; $p < 0.0001$), LDL-cholesterol levels (3.59 mmol/L versus 3.17 mmol/L; $p < 0.0001$), and DBP levels (71.5 mmHg versus 70.5 mmHg, $p = 0.02$) remained significantly higher in AFCS than ECAC participants. Mean SBP (118.5 mmHg versus 118.0 mmHg; $p = 0.06$) and cholesterol to HDL-cholesterol ratio (3.9 versus 3.8; $p = 0.06$) were marginally higher in AFCS than ECAC participants, respectively. Importantly, inferences from the multivariable models to assess associations between study group and CAC were similar to those from models in the entire study group.

3. Discussion

Substantial differences in the prevalence and extent of atherosclerosis across populations were documented by the International Atherosclerosis Project in 1968, in which autopsies from sets of aortas and coronary arteries were analyzed from >23,000 individuals in 14 countries [19]. Despite variation in atherosclerosis prevalence and severity, known CAD risk factors were associated with the prevalence and extent of atherosclerosis across all populations [20]. Moreover, within each population, those who died of CAD had more coronary atherosclerosis than those who died of unrelated causes [19].

Differences in prevalence and quantity of CAC across asymptomatic white populations living on continents with different cardiovascular disease rates have been examined [8]. After adjusting for CAD risk factors among 17,563 men and women from the US, Brazil, and Portugal, significant differences in prevalence and quantity of CAC were found. CAC differences paralleled the respective cardiovascular disease mortality rates in the three countries. Prevalence and quantity of CAC has also been compared among 6814 asymptomatic white, black, Hispanic, and Chinese men and women from the US participating in the Multi-Ethnic Study of Atherosclerosis (MESA) [5]. After adjusting for traditional CAD risk factors, the prevalence and quantity of CAC were found to be significantly higher in whites than the other three racial/ethnic groups.

The non-Hispanic white AFCS and ECAC populations provide an interesting contrast because they live within the US and yet have very different lifestyle and cultural practices. Participants in both studies were recruited from the community and were not self-referred or physician-referred. ECAC participants come from predominantly rural Olmsted County, MN in close proximity to the Mayo Clinic and CAD rates are thought to be lower than other non-Hispanic white populations in the US [21]. The OOA are also a predominantly rural population, and are characterized by

a strongly cohesive social order that includes eschewal of many modern technologies and relatively little use of modern medicine. The prevalence of CAD in the OOA is unknown. CAD event rates in other cohesive religious groups in the US (e.g., Seventh-day Adventists [22,23] and Mormons [24,25]) have been reported to be relatively low, but these studies are rather dated and may not reflect the OOA today.

We found significant differences between AFCS and ECAC participants in the distribution of many traditional CAD risk factors. The pattern of risk factor differences between these two populations is complex, with the AFCS having less abdominal obesity and less cigarette smoking, but a potentially less advantageous lipid profile. The difference in prescription medication usage is striking and is undoubtedly related to differences in attitudes toward medical care and preventive health. This cultural difference is highlighted by the fact that over one-third of ECAC participants (229/630 = 36%) used prescribed lipid-lowering and/or blood pressure-lowering medications compared to fewer than 10% of AFCS participants (49/519 = 9%).

The AFCS participants may be exposed to additional CAD risk factors that are not reflected in the traditional CAD risk factors considered here. Moreover, the excess of CAC in the AFCS appears to exist despite their presumed higher levels of physical activity [26], although this study was not designed to examine the association between physical activity, diet, and CAC. Nutritional differences may partially explain the excess CAC in the OOA. Our review of 95 randomly selected food frequency questionnaires from subjects in our Amish Family Diabetes Study revealed an average daily energy intake of 1789 ± 702 kcal (women) and 2518 ± 1023 kcal (men) with 41%, 43% and 16% of calories consumed as carbohydrate, fat, and protein, respectively (Steinle and Shuldiner, unpublished). Of the fat consumed, 63% was estimated to be unsaturated fat, with daily cholesterol intake of 559 ± 437 mg. Food frequency data suggest that the OOA consume an atherogenic diet that is relatively high in energy, saturated fat, cholesterol, and sodium.

Socioeconomic factors are associated with CAD [27]. After adjusting for traditional CAD risk factors, lower educational attainment was associated with higher prevalence of CAC in non-Hispanic whites but with lower prevalence of CAC in Hispanics from MESA [28] and across all ethnic groups in the Coronary Artery Risk Development in Young Adults Study [29]. While the OOA end their formal education at the 8th grade, almost all ECAC participants had at least a 12th grade education.

There may be differences between populations in genetic susceptibility to coronary atherosclerosis and even within what are considered to be relatively homogeneous populations such as from Great Britain, there is evidence for population stratification [30]. Also, gene-environment interactions involving differences in the social, behav-

ioral, and physical environment of the two study groups could contribute to differences in subclinical coronary atherosclerosis.

3.1. Limitations

The excess of CAC in AFCS participants may be an artifact of differences in medical care utilization between the two populations. The OOA eschew many modern medical practices, do not participate in state or federal health insurance programs, and typically pay cash for medical treatment. In contrast, many residents of Rochester, MN receive their medical care at the Mayo Clinic and may utilize advanced medical therapies and technologies at a greater rate than the OOA. Coronary revascularization procedures were exclusion criteria for the current studies but these procedures may be underused in the OOA population. Therefore, residents of Rochester, MN with a history of revascularization (and therefore, at higher risk for having CAC) were excluded from participation in the ECAC study while OOA individuals who might have had indications for revascularization, but did not have an angiogram and thus did not receive revascularization, were allowed to participate in the AFCS. Because the analyses were based on cross-sectional data, we cannot provide further insight into pathophysiological mechanisms that underlie the associations found in this study.

3.1.1. Conclusions

The findings suggest that traditional CAD risk factors do not explain all the differences between populations in the prevalence and extent of subclinical coronary atherosclerosis. Differences could be due to additional lifestyle, environmental, and/or biochemical factors not reflected in the traditional CAD risk factors or the context-dependent effects of such factors across different populations. Conducting studies across populations is one method to investigate context-dependent effects and to identify new factors involved in the pathogenesis of atherosclerosis. An understanding of context-dependent effects will help identify high-risk individuals and allow clinicians to tailor specific therapeutic interventions for their patients.

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References

- [1] Kuller LH. Ethnic differences in atherosclerosis, cardiovascular disease and lipid metabolism. *Curr Opin Lipidol* 2004;15:109–13.
- [2] Weyer SM, Hustey VR, Rathbun L, et al. A look into the Amish culture: what should we learn? *J Transcult Nurs* 2003;14:139–45.
- [3] Streeten EA, McBride DJ, Lodge A, et al. Osteoporosis in the Old Order Amish: A population at decreased risk for fracture. *J Bone Miner Res* 2004;19:308–13.
- [4] Araneta MR, Barrett-Connor E. Subclinical coronary atherosclerosis in asymptomatic Filipino and white women. *Circulation* 2004;110:2817–23.
- [5] Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation* 2005;111:1313–20.
- [6] McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation* 2006;113:30–7.
- [7] Budoff MJ, Nasir K, Mao S, et al. Ethnic differences of the presence and severity of coronary atherosclerosis. *Atherosclerosis* 2006;187:343–50.
- [8] Santos RD, Nasir K, Rumberger JA, et al. Difference in atherosclerosis burden in different nations and continents assessed by coronary artery calcium. *Atherosclerosis* 2006;187:378–84.
- [9] Wexler L, Brundage B, Crouse J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. *Circulation* 1996;94:1175–92.
- [10] Rumberger JA, Sheedy 2nd PF, Breen JF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. *Circulation* 1995;92:2157–62.
- [11] Bielak LF, Rumberger JA, Sheedy II PF, Schwartz RS, Peyser PA. Probabilistic model for prediction of angiographically defined obstructive coronary artery disease using electron beam computed tomography calcium score strata. *Circulation* 2000;102:380–5.
- [12] Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD. Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol* 2000;36:1253–60.
- [13] Keelan PC, Bielak LF, Ashai K, et al. Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation* 2001;104:412–7.
- [14] Peyser PA, Bielak LF, Chu JS, et al. Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. *Circulation* 2002;106:304–8.
- [15] Friedewald WT, Levy RI, Fredrickson DS. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [16] Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;15:827–32.
- [17] Stephens MA. EDF statistics for goodness of fit and some comparisons. *J Am Stat Assoc* 1974;69:730–7.
- [18] Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13–22.
- [19] Strong JP, Solberg LA, Restrepo C. Atherosclerosis in persons with coronary artery disease. *Lab Invest* 1968;18:527–37.
- [20] Strong JP. Atherosclerotic lesions. Natural history, risk factors, and topography. *Arch Pathol Lab Med* 1992;116:1268–75.
- [21] American Heart Association. Heart Disease and Stroke Statistics, 2006 update. Dallas, Tex.: 2006.
- [22] Lemon FR, Walden RT. Death from respiratory system disease among Seventh-Day Adventist men. *JAMA* 1966;198:117–26.
- [23] Phillips RL, Kuzma JW, Beeson WL, Lotz T. Influence of selection versus lifestyle on risk of fatal cancer and cardiovascular disease among Seventh-day Adventists. *Am J Epidemiol* 1980;112:296–314.

- [24] Lyon JL, Wetzler HP, Gardner JW, Klauber MR, Williams RR. Cardiovascular mortality in Mormons and non-Mormons in Utah, 1969–1971. *Am J Epidemiol* 1978;108:357–66.
- [25] Williams RR, Hasstedt SJ, Wilson DE, et al. Evidence that men with familial hypercholesterolemia can avoid early coronary death. An analysis of 77 gene carriers in four Utah pedigrees. *JAMA* 1986;255:219–24.
- [26] Snitker S, Mitchell BD, Shuldiner AR. Physical activity and prevention of type 2 diabetes (letter). *Lancet* 2003;361:87–8.
- [27] Kaplan GA, Keil JE. Socioeconomic factors and cardiovascular disease: a review of the literature. *Circulation* 1993;88:1973–98.
- [28] Diez Roux AV, Detrano R, Jackson S, et al. Acculturation and socioeconomic position as predictors of coronary calcification in a multiethnic sample. *Circulation* 2005;112:1557–65.
- [29] Yan LL, Liu K, Daviglus ML, et al. 15-year risk factor progression, and coronary artery calcium in young adulthood and early middle age: the Coronary Artery Risk Development in Young Adults Study. *JAMA* 2006;295:1793–800.
- [30] Clayton DG, Walker NM, Smyth DJ, et al. Population structure, differential bias and genomic control in a large-scale, case-control association study. *Nat Genet* 2005;37:1243–6.