

Genetic Effects on Postprandial Variations of Inflammatory Markers in Healthy Individuals

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Circulating levels of inflammatory markers predict the risk of cardiovascular disease (CVD), mediated perhaps in part by dietary fat intake, through mechanisms only partially understood. To evaluate post-fat load changes in inflammatory markers and genetic influences on these changes, we administered a standardized high-fat meal to 838 related Amish subjects as part of the Heredity and Phenotype Intervention (HAPI) Heart Study and measured a panel of inflammatory markers, including C-reactive protein (CRP), interleukin-1 β (IL-1 β), matrix metalloproteinase-1 and -9 (MMP-1 and MMP-9), and white blood cell (WBC) count, before and 4 h after fat challenge (CRP prechallenge only). Heritabilities ($h^2 \pm$ s.d.) of basal inflammatory levels ranged from $16 \pm 8\%$ for MMP-9 ($P = 0.02$) to $90 \pm 7\%$ for MMP-1 ($P < 0.0001$). Post-fat load, circulating levels of WBC, MMP-1, and MMP-9 increased by 16, 32, and 43% (all $P < 0.0001$), with no significant changes in IL-1 β . Postprandial changes over the 4-h period were modestly heritable for WBC (age- and sex-adjusted $h^2 = 14 \pm 9\%$, $P = 0.04$), but the larger MMP-1 and MMP-9 changes appeared to be independent of additive genetic effects. These results reveal that a high-fat meal induces a considerable inflammatory response. Genetic factors appear to play a significant role influencing basal inflammatory levels but to have minimal influence on post-fat intake inflammatory changes.

Obesity (2010) **18**, 1417–1422. doi:10.1038/oby.2009.416

INTRODUCTION

Levels of inflammatory markers in circulation predict cardiovascular disease (CVD) events (1,2), consistent with the emerging views of a strong inflammatory component to the pathogenesis of atherosclerosis and the transition to its clinical manifestations (3). Immune cells and their effector molecules are implicated in a number of pathogenic mechanisms in atherosclerosis, including endothelial dysfunction, leukocyte migration, extracellular matrix degradation, and platelet activation (3–6). For example, recent evidence suggests that proinflammatory cytokines, such as interleukin (IL)-6 and -1 β , can mediate the migration of leukocytes to vascular endothelium and induce the expression of acute-phase proteins (7). The expression and production of matrix metalloproteinase-1 and -9 (MMP-1 and MMP-9) by macrophages in the plaques have been suggested to play a critical role in plaque rupture via their ability to degrade extracellular matrix (4).

Circulating levels of several inflammatory cytokines are known to increase following dietary fat intake (8–15), although the potential contribution of the postprandial inflammatory response to CVD risk is not well established. The mechanisms

underlying the postprandial inflammatory response are unclear, although some evidence suggests that postprandial hypertriglyceridemia from a high-fat meal activates endothelial cells and initiates inflammatory cytokine production (16,17). Dietary fat intake induced expression of several inflammatory genes in mice (18,19). The degree to which genetic factors contribute to postprandial inflammation is unknown, although a genetic contribution to nonpostprandial circulating levels of inflammatory markers is well documented, with estimated heritabilities of 20–40% for white blood cell (WBC) and C-reactive protein (CRP) concentrations (20–22).

Given emerging evidence that the postprandial response in circulating lipids and/or inflammation factors may be associated with CVD risk (23,24), we have measured postprandial changes of inflammatory markers in response to a standardized high-fat challenge in subjects from large Amish families for the purpose of: (i) identifying factors associated with baseline levels and postprandial changes in inflammatory markers; and (ii) assessing the genetic contributions to the interindividual variation in baseline levels and postprandial changes in inflammatory markers. Inflammatory markers measured

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Received 4 March 2009; accepted 16 October 2009; published online 12 November 2009. doi:10.1038/oby.2009.416

in this study include WBC, CRP, IL-1 β , MMP-1, and MMP-9, all of which have been linked to various pathogenic stages of atherosclerosis or CVD risk in animal and clinical studies (1,2,4,7). Understanding the relative genetic and environmental contributions to inflammation may lead to the identification of modifiable factors that could shape individual risk and provide a future direction for CVD prevention.

METHODS AND PROCEDURES

Study subjects

The Amish Heredity and Phenotype Intervention (HAPI) Heart Study began in 2002 to identify novel loci that interact with specific environmental exposures to modify risk factors for CVD. Subjects recruited for this study were ≥ 20 years old and were excluded if they had severe hypertension (blood pressure $> 180/105$ mm Hg), malignancy, and kidney, liver, or untreated thyroid disease (25). This report includes the 838 subjects (456 men and 382 women) who completed the high-fat feeding intervention arm of the HAPI Heart Study. Subjects were also excluded from this arm if they had malabsorption disorders, lactose intolerance, gall bladder disease, or history of pancreatitis. Virtually all subjects can be connected into a single 14 generation pedigree (26). Our sample included 326 parent-offspring pairs, 633 sib pairs, 11 grandparent-grandchild pairs, 443 avuncular pairs, and 191 first cousin pairs that were informative for estimating heritabilities.

All subjects discontinued vitamins, supplements, and prescription medications beginning 7 days before participation. Subjects underwent a physical examination, including height and weight, using standard methods by a registered nurse during their first clinic visit at the Amish Research Clinic (Strasburg, PA). Blood pressure was measured in triplicate in the sitting position after the subject had been sitting quietly for 5 min by use of a standard sphygmomanometer, and the average of the measurements was calculated. Smoking status and medical history (including diabetes, heart attack, stroke, and cancer status) were assessed by questionnaires administered by trained study nurses. Fasting lipid profile (including total cholesterol, high-density lipoprotein cholesterol, and triglyceride) was assayed by the Quest Diagnostics (Horsham, PA). The protocol was approved by the Institutional Review Board at University of Maryland. Informed consent, including permission to contact relatives, was obtained before participation.

High-fat challenge

A high-fat meal was administered in the morning following an overnight 10-h fast at the Amish Research Clinic. This meal was a milkshake comprising heavy whipping cream, skim milk powder, and corn syrup, standardized to include 782 calories/m² of the subject's body surface area. The total energy content per 100 g of milkshake was 26.6 g total fat (77.6% of total calories), 97.8 mg cholesterol, 2.4 g protein (3.1% of total calories), and 14.8 g carbohydrates (19.2% of total calories). An indwelling angiocatheter was placed in the right antecubital vein for serial blood draws before milkshake consumption (time 0) and at 1, 2, 3, 4, and 6 h after ingestion. Blood was processed within 1–2 h and serum frozen at -80 °C until the lab assay.

Assessment of inflammatory biomarkers

Blood samples collected at 0 (T_0) and 4 h (T_4) after the high-fat challenge were assayed for inflammatory biomarkers. The number of samples measured at baseline ranged from 779 for IL-1B to 837 for WBC. Due to budgetary constraints, only a subset of samples was measured at the 4-h time point, ranging from 61% (476/779) for IL-1B to 98% (822/837) for WBC. Serum concentrations of IL-1 β were measured in triplicate and MMP-1 and MMP-9 concentrations in duplicate using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) by the University of Maryland Cytokine Biochemistry Core Laboratory (Baltimore, MD). The means of the replicate values were

used for data analyses. The assay detection ranges were 0.781–50 pg/ml for IL-1 β , 0.156–10 ng/ml for MMP-1, and 31.2–2,000 ng/ml for MMP-9. Values above and below the range were assigned the maximum and minimum values of detection, respectively. The intra-assay coefficients of variation were 5.5, 7.5, and 5.8%, for IL-1 β , MMP-1, and MMP-9, respectively. WBC counts were determined by complete blood count using Coulter LH750 Hematology Analyzer (Beckman Coulter, Fullerton, CA), and high-sensitive CRP (interassay coefficients of variation = 4.8%) were determined by endpoint nephelometry, as assayed by Quest Diagnostics.

Statistical analysis

All baseline inflammatory marker values were natural logarithm transformed to remove skewness. The differences between the (untransformed) T_4 and T_0 values (i.e., $T_4 - T_0$) were approximately normally distributed and were tested for deviation from 0 by a paired t -test. Partial correlations (r) between inflammation markers and covariates were computed by first estimating the proportionate reduction in the variance of the trait (e.g., inflammatory marker) in the model associated with inclusion of the covariate of interest and then taking the square root of this quantity, with the direction of correlation assigned based on the sign of the covariate beta value. In order to adjust for potential nonlinear effect of age and allow the effect of age to vary by sex, correlation estimates were adjusted for age, age², sex, and the corresponding age-by-sex interactions. Trait heritability (h^2) was defined as the proportion of the total trait variance attributable to additive genetic effects and was estimated by modeling the phenotypic covariance (conditional upon covariate effects) between any two individuals in the pedigree as a function of their degree of biological relatedness. Statistical analyses were conducted using variance component analyses as implemented in Sequential Oligogenic Linkage Analysis Routines to account for familial relatedness of the data (version 4.0.7; Southwest Foundation for Biomedical Research, San Antonio, TX) (27).

Our sample size provided at least 42, 76, 95, and 99% power to detect heritabilities of 10, 20, 30, and 40%, respectively, for basal inflammatory markers (at $\alpha = 0.05$). For postprandial inflammatory changes (including WBC, MMP-1, and MMP-9), our sample provided at least 36, 63, 87, and 97% power to detect heritabilities of 10, 20, 30, and 40%, respectively.

RESULTS

Baseline anthropometric and clinical characteristics

Baseline characteristics are presented in **Table 1**. The average age of study subjects was 44 (± 14) years, and 54.4% of the participants were male. Very few among this study were previously diagnosed with myocardial infarction, stroke, or diabetes. Whereas none of the female participants were current smokers, 20% of the men reported current use of a pipe, cigar, and/or cigarettes. Overall, the study population represented a group of relatively healthy Amish adults.

Effect of high-fat meal on inflammatory markers

The distributions of basal WBC, CRP, IL-1 β , MMP-1, and MMP-9 values were positively skewed. Postprandial changes in these markers were approximately normally distributed (see **Supplementary Figure S1** online). **Table 2** shows median levels (and interquartile ranges) of these markers at baseline (T_0) and 4-h postchallenge (T_4), as well as the mean changes ($T_4 - T_0$) in inflammatory response to the high-fat meal intake. Levels of WBC, MMP-1, and MMP-9 were significantly elevated at T_4 compared to T_0 , with increases of 16% for WBC count ($P < 0.0001$), 32.1% for MMP-1 ($P < 0.0001$), and 42.9%

($P = 0.0009$) for MMP-9. In contrast, median levels of IL-1 β were essentially unchanged after the consumption of the high-fat meal. Triglyceride levels also increased significantly in response to the high-fat meal, from 68.1 mg/dl at T_0 to 184.5 mg/dl at T_4 ($P < 0.0001$). The increase in triglyceride levels was positively correlated with changes in WBC count (age- and sex-adjusted $r = 0.12$, $P = 0.0007$) and MMP-1 (age- and sex-adjusted $r = 0.06$, $P = 0.06$).

Correlations among basal and postprandial inflammatory measures

The ln-transformed basal levels of WBC count, CRP, MMP-1, and MMP-9 levels were all significantly correlated with each other ($r = 0.13$ – 0.36 , $P < 0.0001$ for all). The strongest age- and sex-adjusted residual correlations occurred between WBC count and MMP-9 ($r = 0.36$, $P < 0.0001$) and between WBC and CRP ($r = 0.29$, $P < 0.0001$). In contrast, basal IL-1 β was significantly correlated only with basal levels of MMP-9 ($r = 0.10$, $P = 0.007$) and not with any of the other markers.

Table 1 Baseline characteristics of 838 Amish subjects

Characteristics	Mean \pm s.d. ^a
Age, years	44 \pm 14
BMI, kg/m ²	26.6 \pm 4.4
Systolic blood pressure, mm Hg	121 \pm 15
Diastolic blood pressure, mm Hg	77 \pm 9
Fasting triglyceride, mg/dl	68.1 \pm 41.4
Fasting total cholesterol, mg/dl	208 \pm 46
Fasting HDLc, mg/dl	56 \pm 14
Fasting LDLc, mg/dl	138 \pm 43
HDLc/LDLc ratio	0.44 \pm 0.22
Previous MI, n (%) ^b	8 (0.96)
Previous stroke, n (%) ^b	4 (0.48)
Diabetes, n (%) ^b	5 (0.61)
Current smoking status, men only, n (%) ^b	
Cigarette smokers	11 (2.4)
Pipe, cigar, and cigarette smokers	91 (20)

HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; MI, myocardial infarction.

^aUnless otherwise indicated. ^bSelf-reported.

Additional analyses were carried out to determine relations between basal levels of WBC, MMP-1, and MMP-9 and the respective postprandial changes. Higher basal levels were significantly correlated with smaller postprandial changes in WBC ($r = -0.14$, $P < 0.0001$) and MMP-9 ($r = -0.35$, $P < 0.0001$) whereas there was no correlation between basal and postprandial MMP-1 change ($r = -0.02$, $P = 0.5$).

Predictors of basal levels of inflammatory levels

Age was positively correlated with higher basal levels of CRP ($r = 0.34$, $P < 0.0001$) and MMP-1 ($r = 0.14$, $P < 0.0001$). Basal MMP-9 levels were higher in men (age-adjusted geometric mean = 477.0 ng/ml and 422.2 ng/ml for men and women, respectively, $P = 0.007$) whereas basal CRP levels were higher in women (age-adjusted geometric mean = 0.8 mg/l and 1.0 mg/l for men and women, respectively, $P = 0.003$). Neither age nor sex was associated with WBC or IL-1 β . The inflammatory markers were correlated with only a few covariates at levels greater than $r = 0.2$ (Table 3). In particular, higher BMI was significantly associated with higher basal CRP ($r = 0.45$, $P < 0.0001$) level, and higher fasting triglyceride was associated with higher basal WBC level ($r = 0.21$, $P < 0.0001$). In men, smokers had higher WBC count, CRP, and MMP-9 basal levels compared to nonsmokers. The age-adjusted geometric mean levels for smokers vs. nonsmokers were WBC = 5.7 vs. 5.0 thous/ μ l, CRP = 1.0 vs. 0.7 mg/l, and MMP-9 = 551.8 vs. 454.8 ng/ml ($P < 0.01$ for all). Smoking was not significantly associated with IL-1 β or MMP-1 basal levels.

Predictors of postprandial changes in inflammatory levels

Correlations of the baseline characteristics were generally much weaker with postprandial inflammatory changes than with basal inflammatory levels (Table 3). For example, postprandial changes in WBC count were correlated with only BMI, diastolic blood pressure, and fasting triglycerides, whereas postprandial changes in MMP-9 were correlated with only total cholesterol. None of these variables was significantly correlated with postprandial changes in MMP-1. In men, nonsmokers had a greater increase in WBC levels in response to fat intake than smokers in the age-adjusted model (age-adjusted WBC change = 0.76 thous/ μ l and 0.57 thous/ μ l, respectively; $P = 0.04$). In contrast,

Table 2 Serum concentrations of inflammatory markers in response to a high-fat meal

Inflammatory markers	Serum level of inflammatory markers				Postprandial changes, $T_4 - T_0$	
	T_0		T_4		Mean (s.e.)	P^a
	N	Median (IQR)	N	Median (IQR)		
WBC (thous/ μ l)	837	5.2 (4.5–5.9)	822	6.0 (5.2–6.9)	0.8 (0.03)	<0.0001
CRP (mg/l) ^b	818	0.9 (0.4–1.9)	—	—	—	—
IL-1 β (pg/ml)	779	0.8 (0.8–1.3)	476	0.8 (0.8–0.8)	–0.07 (0.07)	0.50
MMP-1 (ng/ml)	780	3.1 (1.8–5.0)	682	3.7 (2.4–6.0)	0.66 (0.04)	<0.0001
MMP-9 (ng/ml)	780	454.0 (294.3–707.6)	477	459.4 (326.8–714.3)	83 (12.0)	0.0009

CRP, C-reactive protein; IL, interleukin; IQR, interquartile range; MMP, matrix metalloproteinase; WBC, white blood cell count.

^a P value for testing whether $T_4 - T_0$ is significant different from 0, adjusted for age and sex. ^bIndividuals with CRP levels >10 mg/l ($n = 17$) were removed from analysis.

Table 3 Correlations (*r*) between baseline characteristics and inflammatory markers at baseline and postprandial changes

Baseline characteristics	ln-Transformed baseline level (ln T_0)					Postprandial changes ($T_4 - T_0$)		
	WBC	CRP	IL-1 β	MMP-1	MMP-9	WBC	MMP-1	MMP-9
BMI	0.19***	0.45***	0.01	0.04	0.12**	0.07*	-0.06	-0.005
SBP	0.08*	0.12***	0.04	0.07*	-0.01	0.04	-0.02	0.04
DBP	-0.001	0.12**	0.01	0	-0.01	0.08*	-0.06	-0.01
ln-TG	0.21***	0.17***	0.01	0.13**	0.01	0.09**	-0.003	0.04
Total cholesterol	-0.08*	0	0.01	0	-0.13***	0	-0.04	-0.09*
HDLc/LDLc	-0.11**	-0.16**	0.01	-0.05*	0.06	0.01	0.06	0.05

Model was adjusted for age, age², sex, age-by-sex and age²-by-sex interactions.

CRP, C-reactive protein; DBP, diastolic blood pressure; HDLc, high-density lipoprotein cholesterol; IL-1 β , interleukin-1 β ; LDLc, low-density lipoprotein cholesterol; MMP, matrix metalloproteinase; SBP, systolic blood pressure; TG, fasting triglyceride; WBC, white blood cell.

*** $P \leq 0.001$; ** $0.001 < P \leq 0.01$; * $0.01 < P \leq 0.05$.

Table 4 Heritability estimates ($h^2 \pm$ s.e.) of inflammatory markers

	WBC	CRP	IL-1 β	MMP-1	MMP-9
ln-Transformed baseline level (ln T_0)					
Age- and sex-adjusted ^a	0.40 \pm 0.10	0.45 \pm 0.10	0.41 \pm 0.09	0.90 \pm 0.07	0.16 \pm 0.08
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.02$
Multivariable-adjusted ^b	0.35 \pm 0.10	0.42 \pm 0.10	NA	0.89 \pm 0.07	0.15 \pm 0.08
	$P < 0.0001$	$P < 0.0001$		$P < 0.0001$	$P = 0.02$
Postprandial change ($T_4 - T_0$)					
Age-, sex-adjusted ^a	0.14 \pm 0.09	—	—	0.09 \pm 0.08	0
	$P = 0.04$			$P = 0.10$	
Multivariable-adjusted ^b	0.12 \pm 0.09	—	—	NA	0.004 \pm 0.13
	$P = 0.07$				$P = 0.5$

CRP, C-reactive protein; IL-1 β , interleukin-1 β ; MMP, matrix metalloproteinase; NA, not applicable because none of the covariates in **Table 3** were significantly associated with the trait; —, not estimated because data is not available (CRP) or no significant postprandial change was observed (IL-1 β).

^aModel was adjusted for age, age², sex, age-by-sex and age²-by-sex interactions. ^bModel was adjusted for age, age², sex, age-by-sex and age²-by-sex interactions, smoking status (if significant), and all significant covariates listed in **Table 3**.

smoking status was not significantly associated with postprandial changes in either MMP-1 or MMP-9.

Genetic contributions to baseline and postprandial variation in inflammatory variables

Heritability of basal inflammatory factors (**Table 4**) ranged from 0.16 \pm 0.08 for MMP-9 ($P = 0.02$) to 0.90 \pm 0.07 for MMP-1 ($P < 0.0001$) in the age- and sex-adjusted model. Adjusting for covariates that were significantly correlated with the basal levels of each corresponding inflammatory markers did not change the results substantially. Heritabilities for postprandial inflammatory changes were much lower than those for basal levels. Only WBC change appeared to be modestly heritable (age- and sex-adjusted $h^2 = 0.14 \pm 0.09$, $P = 0.04$, and multivariable-adjusted $h^2 = 0.12 \pm 0.09$, $P = 0.07$).

DISCUSSION

Consistent with previous studies suggesting that dietary fat may have important effects on inflammatory markers (8–15,24,28–32), we have shown significant increases in

three: WBC count (16%), MMP-1 (32%), and MMP-9 (43%), following a standardized fat load. The postprandial increase in WBC count we observed is concordant with previous observations that blood leukocytes, particularly neutrophil counts, can increase within 3 h after fat (50 g/m²) intake in healthy normolipidemic young men (11,24). Our observation of postprandial increases in MMP-1 and MMP-9 are novel whereas the absence of significant postprandial increases in IL-1 β in our data are consistent with previous report (28).

The determinants of basal levels and postprandial changes in inflammatory markers are largely unknown. Although some traditional cardiovascular risk factors (e.g., BMI, lipids, and blood pressure) are correlated with basal levels of some of these markers, the correlations with postprandial changes are substantially smaller. Consistent with earlier studies, we observed strong evidence for genetic contributions to basal inflammatory levels, with residual heritability estimates ranging from 15 to 89% after adjustment for the effects of other measured environmental covariates. Whereas strong genetic effects on serum CRP, WBC count, and IL-1 β levels have

been previously reported, few, if any, studies have provided heritability estimates for MMP-1 and MMP-9, two collagenases which affect atherosclerotic plaque stability via their ability to degrade extracellular matrix (33). In contrast to the strong genetic contributions to baseline levels, we detected only very weak evidence for genetic influences on postprandial inflammatory changes after fat intake; in fact, only for WBC count postprandial change was there evidence for even modest heritability ($14 \pm 9\%$; $P = 0.04$). It is possible that we were unable to detect stronger genetic effects on the postprandial variations in inflammation because the standardized fat challenge in this study was so strong that it overwhelmed the genetic control of the inflammatory response. Alternatively, despite the large number of family members studied, we may have had insufficient power to detect lower heritabilities (e.g., $h^2 < 20\%$). To our knowledge, heritabilities of postprandial changes in inflammatory markers have not been reported previously.

The increase in inflammatory activities in response to fat intake may result from elevated triglyceride-rich particles in the postprandial state. Hydrolysis of triglyceride in chylomicrons can change these particles into smaller remnant forms, which are hypothesized to be atherogenic because they can penetrate arterial tissue and accumulate within the subendothelial space (34). Chylomicron remnants as well as fatty acids released during lipoprotein lipase-mediated triglyceride hydrolysis are able to induce endothelial activation and dysfunction, expression of cellular adhesion molecules, and activation of monocytes and neutrophils, which can lead to the increase in the recruitment and activation of inflammatory cells to vascular endothelium and ultimately, the formation of foam cells (7,11,16,24,32,35). Postprandial triglyceride levels, particularly those measured 2–4 h after meal consumption, have been found to be more highly associated than fasting triglyceride levels with incident cardiovascular events in women, independent of levels of other lipids or traditional cardiovascular risk factors (23). Elevated postprandial triglycerides were also associated with increased common carotid intima-media thickness, suggesting that postprandial triglyceride may be crucial in the early stage of atherosclerosis (36,37). The significant positive correlations between postprandial triglyceride change and postprandial changes in WBC count and MMP-1 observed in this study support the hypothesis that inflammatory changes are related to postprandial triglycerides and may further imply a potential role of inflammation in early atherosclerosis.

The HAPI Heart Study has several unique features that are well suited for evaluating the determinants of the postprandial response of inflammatory factors to a high-fat challenge. These include a well-controlled intervention, the inclusion of large multiplex families and the uniformity of lifestyle and socioeconomic status, which serve to reduce nongenetic variability and boost the power to discern genetic determinants of traits. Nonetheless, several limitations need to be acknowledged. First, the high-meal challenge with 77.6% calories from fat represents a relatively extreme fat exposure such that the postprandial inflammatory changes observed in this study may not reflect the changes to a more moderate dietary fat intake. Second, the

study did not include an external control group to evaluate the potential diurnal variations in inflammatory markers (38). To minimize the potential inflammatory variation over time, the high-fat meal intervention was performed in the morning after an overnight fast for all study participants. However, it is still possible that diurnal variation, to some extent, could influence the postprandial changes observed in this study. Lastly, the Amish lifestyle is characterized by a relatively high degree of physical activity relative to other populations (39). The degree to which physical activity modifies the effects of fat intake on inflammatory levels is unknown, although the relatively consistent associations between postprandial changes in inflammatory markers observed in this study and those reported from other studies (11,24) suggests that our finding that these changes are heritable in the Amish may be applicable also to other populations. It is also possible that the environmental homogeneity of the Amish lifestyle may boost our power to detect the genetic effects (heritability) on these traits.

In conclusion, our results provide strong evidence that inflammatory markers are under the influence of both modifiable risk factors, including exposure to dietary fat, and genetic factors. These findings suggest possible mechanisms linking genetic and environmental factors to inflammation and may ultimately provide insight into future CVD prevention. Additional studies are needed to identify loci that may functionally influence basal levels and to determine the relevance of the inflammatory response to a single high-fat meal.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

ACKNOWLEDGMENTS

This work supported by NIH research grants U01 HL72515 and R01 088119, the University of Maryland General Clinical Research Center, grant M01 RR 16500, the Clinical Nutrition Research Unit of Maryland (P30 DK072488), and the Baltimore Veterans Administration Medical Center Geriatric Research and Education Clinical Center. We thank our Amish research volunteers for their long-standing partnership in research, and the research staff at the Amish Research Clinic for their hard work and dedication. Y.-C.C. was supported by the Merck Foundation fellowship.

DISCLOSURE

The authors declared no conflict of interest.

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REFERENCES

1. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA* 1998;279:1477–1482.
2. Danesh J, Whincup P, Walker M *et al*. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000;321:199–204.
3. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–126.
4. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868–874.
5. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes: part II: acute-phase reactants and biomarkers of endothelial cell activation. *Circulation* 2006;113:e152–e155.
6. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes: part IV: matrix metalloproteinases and biomarkers of platelet activation. *Circulation* 2006;113:e382–e385.

7. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004;109:112–110.
8. Esposito K, Nappo F, Giugliano F *et al*. Meal modulation of circulating interleukin 18 and adiponectin concentrations in healthy subjects and in patients with type 2 diabetes mellitus. *Am J Clin Nutr* 2003;78:1135–1140.
9. Nappo F, Esposito K, Cioffi M *et al*. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002;39:1145–1150.
10. Blackburn P, Després JP, Lamarche B *et al*. Postprandial variations of plasma inflammatory markers in abdominally obese men. *Obesity (Silver Spring)* 2006;14:1747–1754.
11. Van Oostrom AJ, Sijmonsma TP, Rabelink TJ, Van Asbeck BS, Cabezas MC. Postprandial leukocyte increase in healthy subjects. *Metab Clin Exp* 2003;52:199–202.
12. Lundman P, Boquist S, Samnegård A *et al*. A high-fat meal is accompanied by increased plasma interleukin-6 concentrations. *Nutr Metab Cardiovasc Dis* 2007;17:195–202.
13. MacEaney OJ, Harrison M, O’Gorman DJ *et al*. Effect of prior exercise on postprandial lipemia and markers of inflammation and endothelial activation in normal weight and overweight adolescent boys. *Eur J Appl Physiol* 2009;106:721–729.
14. Ceriello A, Assaloni R, Da Ros R *et al*. Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation* 2005;111:2518–2524.
15. Volek JS, Judelson DA, Silvestre R *et al*. Effects of carnitine supplementation on flow-mediated dilation and vascular inflammatory responses to a high-fat meal in healthy young adults. *Am J Cardiol* 2008;102:1413–1417.
16. Hennig B, Toborek M, McClain CJ. High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. *J Am Coll Nutr* 2001;20:97–105.
17. Norata GD, Grigore L, Raselli S *et al*. Post-prandial endothelial dysfunction in hypertriglyceridemic subjects: molecular mechanisms and gene expression studies. *Atherosclerosis* 2007;193:321–327.
18. Kim S, Sohn I, Ahn JI *et al*. Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model. *Gene* 2004;340:99–109.
19. Liao F, Andalibi A, deBeer FC, Fogelman AM, Luscis AJ. Genetic control of inflammatory gene induction and NF-kappa B-like transcription factor activation in response to an atherogenic diet in mice. *J Clin Invest* 1993;91:2572–2579.
20. de Maat MP, Bladbjerg EM, Hjelmberg JB *et al*. Genetic influence on inflammation variables in the elderly. *Arterioscler Thromb Vasc Biol* 2004;24:2168–2173.
21. Dupuis J, Larson MG, Vasan RS *et al*. Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q. *Atherosclerosis* 2005;182:307–314.
22. Tang W, Hong Y, Province MA *et al*. Familial clustering for features of the metabolic syndrome: the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study. *Diabetes Care* 2006;29:631–636.
23. Bansal S, Buring JE, Rifai N *et al*. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007;298:309–316.
24. van Oostrom AJ, Sijmonsma TP, Verseyden C *et al*. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res* 2003;44:576–583.
25. Mitchell BD, McArdle PF, Shen H *et al*. The genetic response to short-term interventions affecting cardiovascular function: rationale and design of the Heredity and Phenotype Intervention (HAPI) Heart Study. *Am Heart J* 2008;155:823–828.
26. Agarwala R, Biesecker LG, Hopkins KA, Francomano CA, Schaffer AA. Software for constructing and verifying pedigrees within large genealogies and an application to the Old Order Amish of Lancaster County. *Genome Res* 1998;8:211–221.
27. Blangero J, Williams JT, Almasy L. Variance component methods for detecting complex trait loci. *Adv Genet* 2001;42:151–181.
28. Meksawan K, Venkatraman JT, Awad AB, Pendergast DR. Effect of dietary fat intake and exercise on inflammatory mediators of the immune system in sedentary men and women. *J Am Coll Nutr* 2004;23:331–340.
29. Han SN, Leka LS, Lichtenstein AH *et al*. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *J Lipid Res* 2002;43:445–452.
30. Lopez-Garcia E, Schulze MB, Fung TT *et al*. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004;80:1029–1035.
31. Esposito K, Nappo F, Giugliano F *et al*. Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects. *Am J Clin Nutr* 2003;77:139–143.
32. van Oostrom AJ, Rabelink TJ, Verseyden C *et al*. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis* 2004;177:175–182.
33. Jones CB, Sane DC, Herrington DM. Matrix metalloproteinases: a review of their structure and role in acute coronary syndrome. *Cardiovasc Res* 2003;59:812–823.
34. Proctor SD, Mamo JC. Retention of fluorescent-labelled chylomicron remnants within the intima of the arterial wall – evidence that plaque cholesterol may be derived from post-prandial lipoproteins. *Eur J Clin Invest* 1998;28:497–503.
35. Alipour A, Elte JWF, van-ázaanen HCT, Rietveld AP, Cabezas MC. Postprandial inflammation and endothelial dysfunction. *Biochem Soc Trans* 2007;35:466–469.
36. Karpe F, de Faire U, Mercuri M *et al*. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis* 1998;141:307–314.
37. Boquist S, Ruotolo G, Tang R *et al*. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. *Circulation* 1999;100:723–728.
38. Scales WE, Vander AJ, Brown MB, Kluger MJ. Human circadian rhythms in temperature, trace metals, and blood variables. *J Appl Physiol* 1988;65:1840–1846.
39. Rampersaud E, Mitchell BD, Pollin TI *et al*. Physical activity and the association of common FTO gene variants with body mass index and obesity. *Arch Intern Med* 2008;168:1791–1797.