Altered lipoprotein subclass distribution and PAF-AH activity in subjects with generalized aggressive periodontitis

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Abstract In this study, we examined whether the documented increase of plasma triglycerides in patients with generalized aggressive periodontitis (GAgP) is associated with changes in lipoprotein subclass distribution and/or LDL-associated platelet-activating factor acetylhydrolase (PAF-AH) activity. Lipoprotein subclasses were analyzed in whole plasma samples using nuclear magnetic resonance methods. Compared with subjects without periodontitis (NP subjects; n = 12), GAgP subjects (n = 12) had higher plasma levels of large, medium, and small VLDL (35.0 ± 6.7 vs. 63.1 ± 9.6 nmol/l; $P = 0.025$), higher levels of intermediate density lipoprotein (24.8 ± 11.6 vs. 87.2 ± 16.6 nmol/l; $P = 0.006$), lower levels of large LDL (448.3 ± 48.5 vs. 315.8 ± 59.4 nmol/l; $P = 0.098$), and higher levels of small LDL (488.2 ± 104.2 vs. 946.7 ± 151.6 nmol/l; $P = 0.021$). The average size of LDL from NP and GAgP subjects was 21.4 ± 0.2 and 20.6 ± 0.3 nm, respectively ($P = 0.031$). Compared with NP subjects, GAgP subjects had a greater number of circulating LDL particles (961.3 ± 105.3 vs. 1,349.0 ± 133.2 nmol/l; $P = 0.032$). Differences in the plasma levels of large, medium, and small HDL were not statistically significant. NP and GAgP subjects had similar plasma levels of total LDL-associated PAF-AH activity; however, LDL of GAgP subjects contained less PAF-AH activity per microgram of LDL protein (1,458.0 ± 171.0 and 865.2 ± 134 pmol/min/μg; $P = 0.014$). These results indicate that, in general, GAgP subjects have a more atherogenic lipoprotein profile and lower LDL-associated PAF-AH activity than NP subjects. These differences may help explain the increased risk of GAgP subjects for cardiovascular disease.—Rufail, M. L., H. A. Schenkein, S. E. Barbour, J. G. Tew, and R. van Antwerpen. **Altered lipoprotein subclass distribution and PAF-AH activity in subjects with generalized aggressive periodontitis.** *J. Lipid Res.* 2005. **46:** 2752–2760.

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Periodontitis is characterized by chronic inflammation of the supporting structures of the teeth. The etiologic agents of this disease are mainly Gram-negative bacteria existing in a complex biofilm in the subgingival region (1). Lipopolysaccharides and other virulence factors of these bacteria have been shown to promote a host-mediated, tissue-destructive immune response that leads to gingival inflammation, destruction of periodontal tissue, loss of alveolar bone, and eventual exfoliation of teeth (2).

Many epidemiological studies have indicated an association between severe periodontitis and myocardial infarction or stroke (3–16), and additional studies have linked periodontal disease to subclinical indicators of atherosclerosis. For example, subjects with severe periodontitis have decreased flow-mediated dilation of the brachial artery (17), increased carotid artery intima-media thickness (18, 19), and a nearly 4-fold increased risk for the presence of carotid artery plaque (19). Periodontal inflammation has been shown to decrease the antiatherogenic potency of HDL (20) and to enhance lipopolysaccharide-mediated macrophage activation (21). Furthermore, infection with oral bacteria, such as *Porphyromonas gingivalis*, increases markers of systemic inflammation (17, 22–24); high serum antibody levels to *P. gingivalis* predict myocardial infarction in humans (25); and oral infection with *P. gingivalis* accelerates early atherosclerosis in apolipoprotein E-null mice (26). Together, these studies strongly indicate an association between chronic periodontal inflammation and increased cardiovascular risk.

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A clear molecular mechanism linking periodontitis to atherosclerosis remains to be defined. However, recent studies suggest that such a mechanism may involve the plasma lipoproteins. Several studies have indicated that severe periodontitis is associated with a modest decrease in HDL cholesterol, a modest increase in LDL cholesterol, and a more robust increase in plasma triglycerides (27–31).

Increased levels of plasma triglycerides, carried mostly by VLDL, are often associated with a predominance of small, dense low density lipoprotein particles (small LDLs) (32, 33). Compared with larger LDLs, small LDLs have a lower affinity for the apolipoprotein B/E receptor (34–37), a higher affinity for proteoglycans of the vascular wall (38), and a higher susceptibility to oxidation (39–44). These properties are generally thought to increase the atherogenicity of small LDLs, and subjects with a predominance of this LDL subclass in their plasma have a 3- to 6-fold increased risk of cardiovascular disease (45–47). Although subjects with severe periodontitis are known to have increased plasma triglycerides, the distribution of lipoprotein subclasses in these subjects has not been analyzed in depth.

A second factor that may influence the cardiovascular risk of subjects with severe periodontitis is the plasma concentration of LDL-associated platelet-activating factor acetylhydrolase (PAF-AH). PAF-AH is a calcium-independent phospholipase A_2 that hydrolyzes oxidized phospholipids in the LDL surface (48, 49). As oxidized phospholipids are generally proinflammatory and proatherogenic, PAF-AH may have antiatherogenic properties. Such properties are consistent with population studies showing that subjects with mutated, inactive PAF-AH are at an increased risk of stroke (50) and myocardial infarction (51). Furthermore, recent experiments have shown that local expression of PAF-AH exerts anti-inflammatory, antithrombotic, and antiproliferative effects while reducing the accumulation of oxidized LDL in the arteries of nonhyperlipidemic rabbits (52).

Plasma PAF-AH is mostly synthesized and secreted by monocyte-derived macrophages. Recent observations indicate that the monocytes of subjects with localized aggressive periodontitis (LAgP) have a propensity to differentiate into dendritic cells, which synthesize and secrete much less PAF-AH than macrophages (53). This raises the possibility that, compared with controls, LDLs from subjects with periodontitis may contain less PAF-AH activity and, as a result, lack the protective, antiatherogenic influence that this enzyme may have.

In this study, we investigated the lipoprotein subclass distribution and LDL-associated PAF-AH activity of subjects with generalized aggressive periodontitis (GAgP). Our results show that, compared with controls, GAgP subjects have a smaller average LDL particle size, a higher plasma concentration of small LDL particles, a higher total number of circulating LDL particles, and lower LDL-associated PAF-AH activity. These differences may in part be responsible for the increased cardiovascular risk of patients with periodontitis.

MATERIALS AND METHODS

Human subjects

This study was conducted with 12 healthy control subjects without periodontitis (NP subjects) and 12 subjects with GAgP (GAgP subjects). The gender, race, and age distributions of NP and GAgP subjects were matched as shown in **Table 1**. All subjects were nonhypertensive and nondiabetic. Subjects used no oral contraceptives, statins, or other medications. The study was approved by the Institutional Review Board of Virginia Commonwealth University, and all subjects participated with informed consent.

Inclusion criteria for GAgP

Both NP subjects and GAgP subjects were identified at the Clinical Research Center for Periodontal Diseases at Virginia Commonwealth University. To qualify as a GAgP subject, patients had at least eight teeth affected by 5 mm attachment loss or more, at least three of which were not first molars or incisors. Initial diagnosis of GAgP was established after puberty and before the subject's 31st birthday.

Lipoprotein subclass analysis

Lipoprotein particle concentrations and size were measured in total plasma samples by proton NMR spectroscopy (LipoScience, Inc., Raleigh, NC) (54, 55). The following lipoprotein subclasses were distinguished: large VLDLs (>60 nm), medium-sized VLDLs (35–60 nm), small VLDLs (27–35 nm), intermediate density lipoproteins (IDLs; 23–27 nm), large LDLs (21.2–23 nm), small LDLs (18–21.2 nm), large HDLs (8.8–13 nm), medium-sized HDLs (8.2–8.8 nm), and small HDLs (7.3–8.2 nm). Plasma concentrations of triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were derived from NMR analyses by assuming that the particles contain normal amounts of cholesterol and triglycerides. Previous studies have shown that these derived plasma concentrations correlate well with standard laboratory tests (54).

Isolation of LDLs

Blood samples (50 ml) were collected after an overnight fast in purple-top vacutainers containing EDTA. Samples were immediately centrifuged for 20 min at 3,000 g and 4°C, after which the supernatant plasma was supplemented with gentamicin (5 μg/ml), aprotinin (0.2 U/ml), leupeptin (50 μg/ml), NaN_3 (0.02%), and EDTA (1 mM). LDL (d = 1.019–1.063) was isolated from plasma samples by sequential flotation ultracentrifugation using a Beckman Ti70 rotor, essentially as described by Schumaker and Puppione (56). Isolated LDL was dialyzed overnight in PBS (10 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA, and 0.02% NaN_3, pH 7.4) and stored under argon at 4°C until use. The protein concentration of each LDL sample was determined by a modified Lowry method, using BSA as a standard (57).

TABLE 1. Gender, race, and age distribution of NP and GAgP subjects

Variable	NP Subjects	GAgP Subjects
Gender (female/male)	6/6	6/6
Race (African American/Caucasian)	11/1	11/1
Age (years; mean ± SD)[a]	34 ± 9	41 ± 6

GAgP, generalized aggressive periodontitis; NP subjects, subjects without periodontitis.

[a] Multiple regression analysis established that age did not correlate with any of the parameters measured in this study.
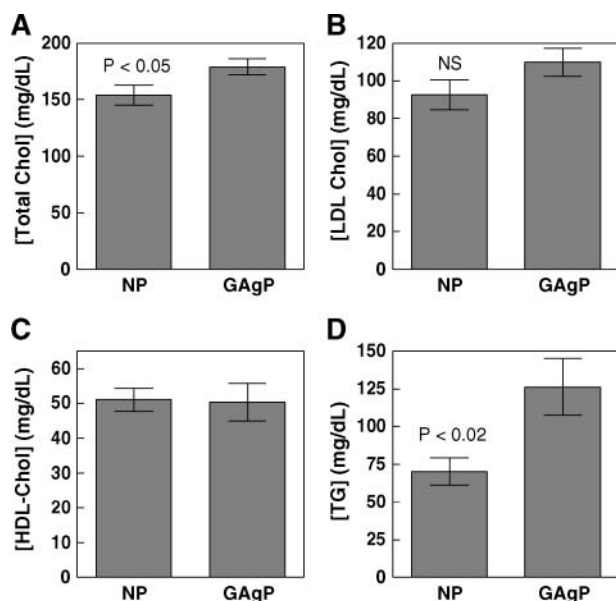
Fig. 1. Plasma lipid levels in subjects without periodontitis (NP subjects) and generalized aggressive periodontitis (GAgP) subjects. A: Total plasma cholesterol. B: LDL-associated cholesterol. C: HDL-associated cholesterol. D: Total plasma triglycerides (TG). Error bars indicate SEM.

PAF-AH assay

LDL-associated PAF-AH activity was measured using the method of Miwa et al. (58), with minor modifications, as described by Narahara, Miyakawa, and Johnston (59). Briefly, 0.05 µCi of

[³H]PAF (Perkin-Elmer, Boston, MA) and 13 µg of nonlabeled PAF (Avanti Polar Lipids, Alabaster, AL) were dried under N_2 and resuspended in 125 µl of a buffer containing 120 mM Tris, pH 7.5, and 8 mg/ml fatty acid-free BSA (Sigma Chemical Co., St. Louis, MO). This substrate solution was mixed with 10 µg of LDL, after which deionized water was added to a total assay volume of 500 µl. Subsequently, the preparation was incubated for 15 min at 37°C in a shaking-water bath. The reaction was stopped by the addition of 500 µl of 14% trichloroacetic acid and incubated on ice for an additional 10 min. Next, the sample was centrifuged for 10 min at 4°C, after which 100 µl of the supernatant, containing [³H]acetate released by the enzyme activity, was assayed for radioactivity in a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Inc., Fullerton, CA).

Statistical analyses

Lipoprotein subclasses from NP and GAgP subjects, as well as LDL-associated PAF-AH activities of these subjects, were compared using the unpaired t-test. Correlations of LDL-associated PAF-AH activity with plasma levels of small LDLs and LDL size were analyzed using linear regression. All statistical analyses were performed using GraphPad Prism software (San Diego, CA).

RESULTS

Cholesterol and triglyceride levels

Total plasma cholesterol, LDL cholesterol, HDL cholesterol, and total plasma triglyceride levels of 12 NP subjects and 12 GAgP subjects were compared to verify the documented increase in plasma lipids (27–31) of subjects with severe periodontitis. **Figure 1A**, **B** show a modest but sta-
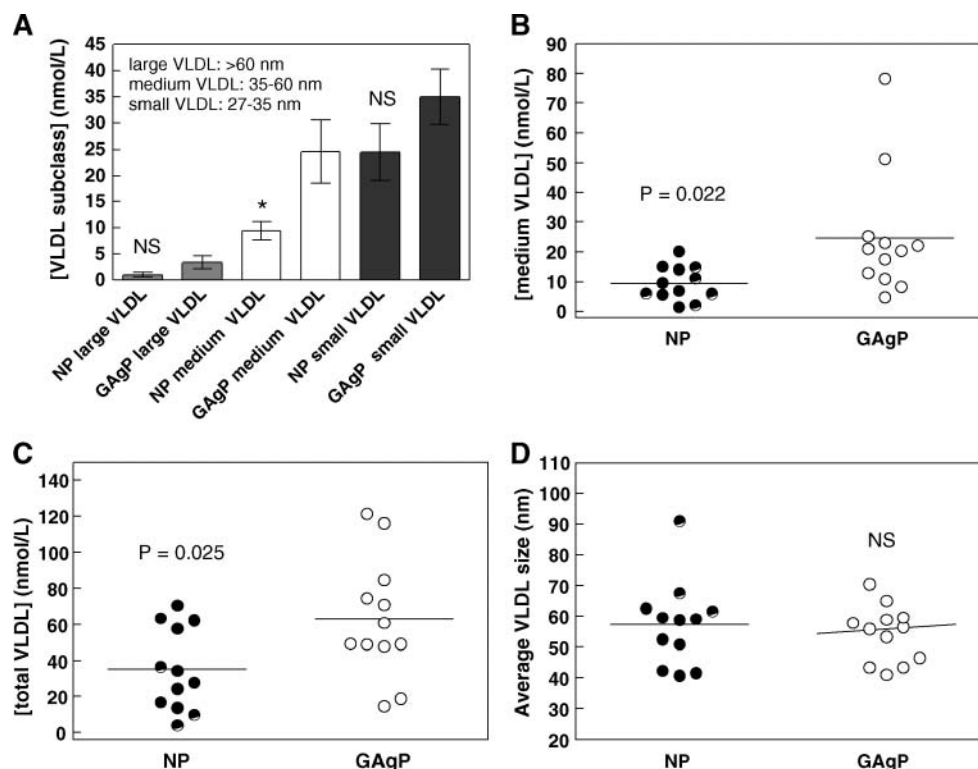

Fig. 2. A: VLDL subclass distribution in NP and GAgP subjects. Error bars indicate SEM (* $P < 0.05$). B: Plasma concentrations of medium-sized VLDL in NP and GAgP subjects. C: Plasma concentrations of total VLDL in NP and GAgP subjects. D: Average size of VLDL in NP and GAgP subjects.
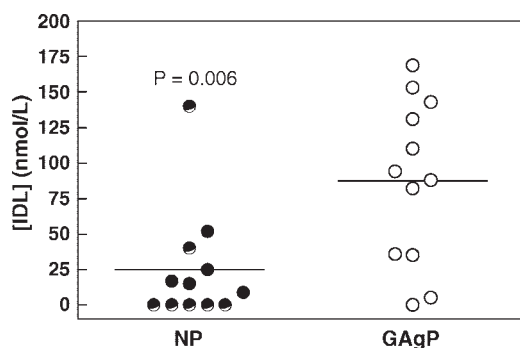
Fig. 3. Plasma concentration of intermediate density lipoprotein (IDL) in NP and GAgP subjects. (Note that the NP subject with the highest IDL concentration is not the sole Caucasian in the group.)

tistically significant increase of total plasma cholesterol (154.1 ± 8.7 vs. 178.9 ± 7.1 mg/dl; $P = 0.038$) and a minor, nonsignificant increase in LDL-associated cholesterol (92.6 ± 8.0 vs. 109.8 ± 7.5 mg/dl; $P = 0.130$) in GAgP subjects. No statistically significant difference in HDL-associated cholesterol was observed (Fig. 1C). In contrast, plasma triglyceride levels were increased significantly in GAgP subjects (70.4 ± 9.1 vs. 126.3 ± 18.9 mg/dl; $P = 0.014$) (Fig. 1D). These data are in general agreement with published results (27–31).

VLDL and IDL subclass distribution

Increased levels of plasma triglycerides (Fig. 1D) are reflected in the VLDL and IDL subclass distributions of NP and GAgP subjects (**Figs. 2**, **3**). On average, each of the three VLDL subclasses is increased in GAgP subjects, but only for medium-sized VLDL is the increase statistically significant ($P = 0.022$) (Fig. 2A, B). Compared with controls, total VLDL plasma concentration is significantly higher in GAgP subjects ($P = 0.025$) (Fig. 2C). The average size of VLDL in NP and GAgP subjects is similar (57.4 ± 4.0 vs. 54.4 ± 2.6 nm, respectively; $P = 0.54$) (Fig. 2D). The most significant difference in triglyceride-rich lipoproteins between NP and GAgP subjects is found in the IDL density class (Fig. 3). Although a few GAgP subjects have low plasma IDL levels (Fig. 3), the average IDL plasma concentration of the 12 GAgP subjects is almost 4-fold higher than the average IDL concentration of NP subjects (87.2 ± 16.6 vs. 24.8 ± 11.6 nmol/l; $P = 0.006$). Modest increases in each of the VLDL subclasses (Fig. 2A) and the major increase in the plasma concentration of IDL (Fig. 3) together account for the significant increase in the plasma triglycerides of GAgP subjects (Fig. 1D).

LDL subclass distribution

Because increased plasma triglyceride levels are often associated with a predominance of small LDL particles, we compared the LDL subclass distributions of NP and GAgP subjects. **Figure 4A** shows that, compared with NP subjects, GAgP subjects on average have a lower plasma level of large LDLs (448.3 ± 48.5 vs. 315.8 ± 59.4 nmol/l; $P = 0.098$) and a higher plasma level of small LDLs (488.2 ± 104.2 vs. 946.7 ± 151.6 nmol/l; $P = 0.021$). The variation in small LDL levels among GAgP subjects is considerable
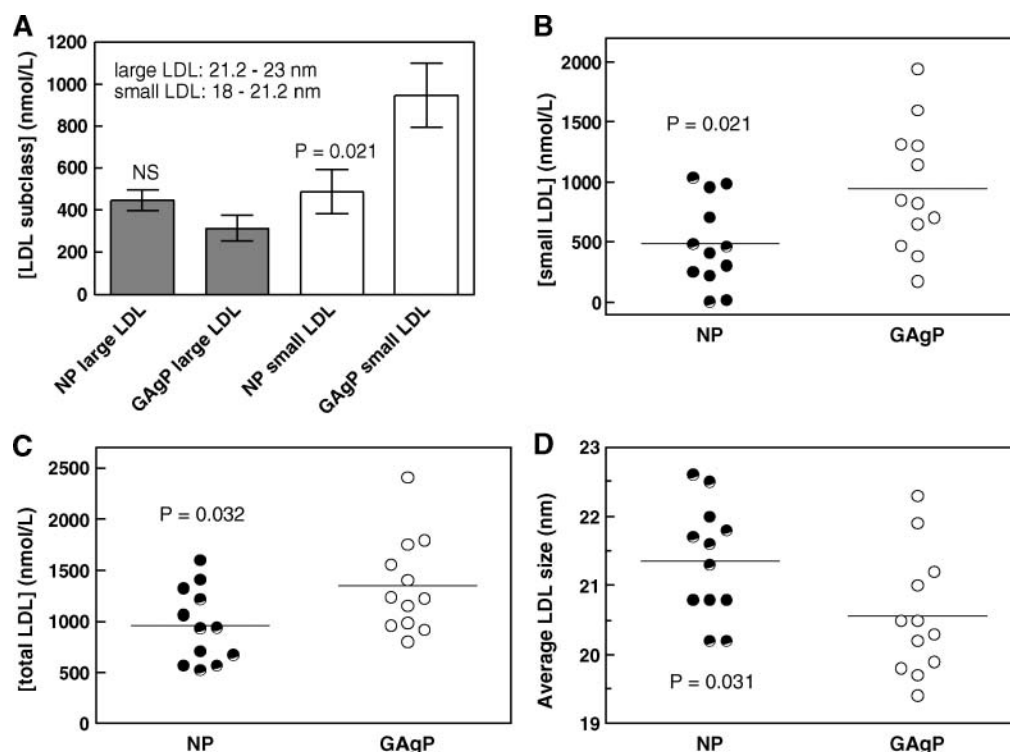

Fig. 4. A: LDL subclass distribution in NP and GAgP subjects. Error bars indicate SEM. B: Plasma concentrations of small, dense low density lipoprotein particles (small LDLs) in NP and GAgP subjects. C: Plasma concentrations of total LDL in NP and GAgP subjects. D: Average size of LDL in NP and GAgP subjects.
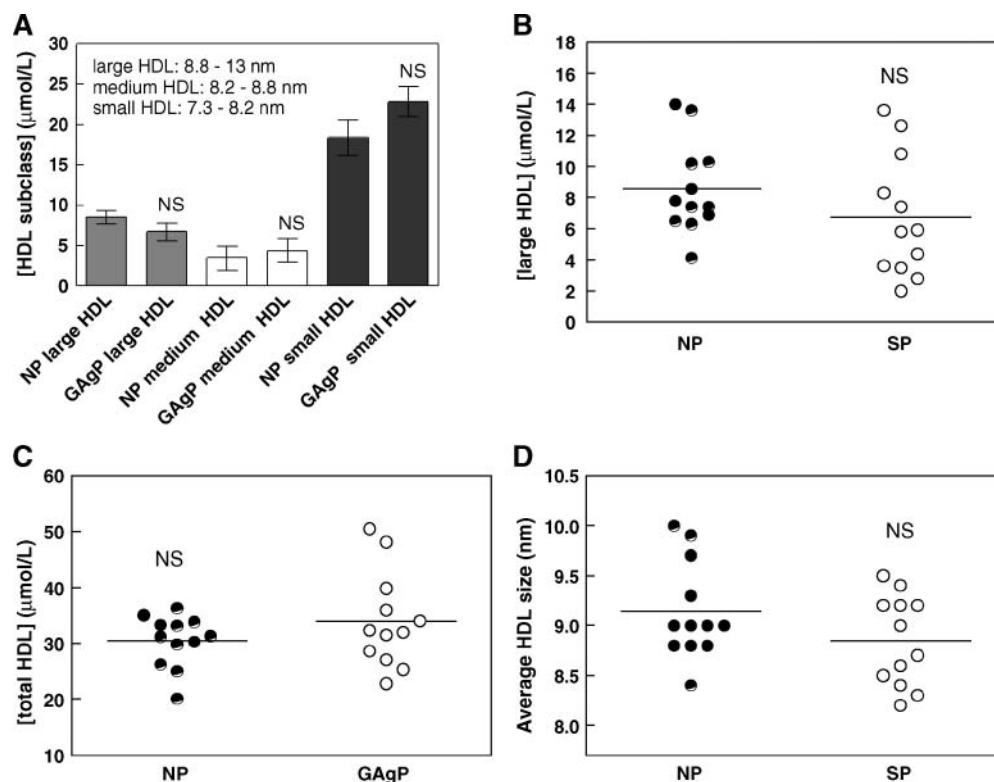
Fig. 5. A: HDL subclass distribution in NP and GAgP subjects. Error bars indicate SEM. B: Plasma concentrations of large HDLs in NP and GAgP subjects. C: Plasma concentrations of total HDL in NP and GAgP subjects. D: Average size of HDL in NP and GAgP subjects.

(Fig. 4B); however, on average, small LDL levels are ~2-fold higher in GAgP subjects. Importantly, the total number of circulating LDL particles is significantly higher in GAgP subjects (961.3 ± 105.3 vs. 1,349 ± 133.2 nmol/l; $P = 0.032$) (Fig. 4C). The average size of LDLs from NP and GAgP subjects is 21.4 ± 0.2 and 20.6 ± 0.3 nm, respectively ($P = 0.031$) (Fig. 4D). Although the average size of LDL particles is smaller in GAgP subjects, the greater number of circulating LDL particles in these subjects leads to somewhat increased levels of LDL-associated cholesterol (Fig. 1B).

HDL subclass distribution

Although differences in HDL subclass distribution between NP and GAgP subjects were not significant, certain trends were observable. On average, compared with NP subjects, GAgP subjects had somewhat lower plasma levels of large HDLs and somewhat higher levels of small HDLs (**Fig. 5A**, **B**). Thus, the average size of HDLs in GAgP subjects (8.9 ± 0.1 nm) was smaller than the average size of HDLs in NP subjects (9.1 ± 0.1 nm), but this difference was not statistically significant ($P = 0.14$) (Fig. 5D). Average plasma levels of total HDL were similar in NP and GAgP subjects (30.5 ± 1.4 vs. 34.0 ± 2.5 μmol/l; $P = 0.22$) (Fig. 5C). The lack of significant differences between the HDL subclass distributions of NP and GAgP subjects (Fig. 5) accounts for the lack of difference in HDL-associated cholesterol levels (Fig. 1C).

LDL-associated PAF-AH activity

Figure 6 compares the LDL-associated PAF-AH activity of NP and GAgP subjects. The data show no difference between the total LDL-associated PAF-AH activity of the two experimental groups (Fig. 6A). However, on average, GAgP subjects have a higher number of circulating LDL particles than NP subjects (Fig. 4C). Therefore, on a per particle basis (i.e., per microgram of LDL protein), GAgP subjects have a lesser amount of LDL-associated PAF-AH activity than NP subjects (1,014.0 ± 192.8 vs. 1,458.0 ± 171.0 pmol/min/μg; $P = 0.099$) (Fig. 6B).

Figure 6 shows that the LDL-associated PAF-AH activity of one of the GAgP subjects is quite different from the other values of the GAgP group. For this particular GAgP subject (who is not the sole Caucasian subject of the group), both total LDL-associated PAF-AH activity (Fig. 6A) and LDL-associated PAF-AH activity per microgram of LDL protein (Fig. 6B) are higher than the means of the group plus two standard deviations. Using this criterion to exclude the outlying measurement, the LDL-associated PAF-AH activities of NP and GAgP subjects are 1,458.0 ± 171.0 and 865.2 ± 134 pmol/min/μg, respectively ($P = 0.014$).

As PAF-AH activity is more strongly associated with small LDLs (60), we analyzed the relationship between LDL-associated PAF-AH activity and the plasma concentration of small LDLs. **Figure 7A** shows that specific LDL-associated PAF-AH activity in NP and GAgP subjects (expressed as picomoles of product formed per minute per
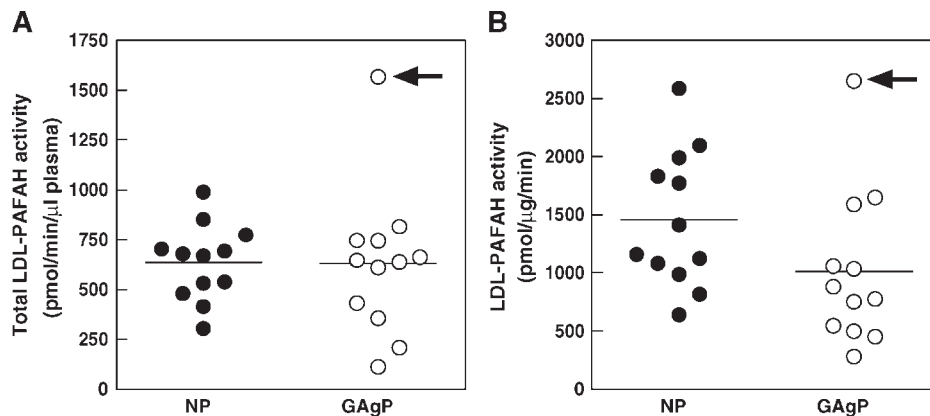
Fig. 6. LDL-associated platelet-activating factor acetylhydrolase (PAF-AH) activity in NP and GAgP subjects, expressed as total activity per microliter of plasma (A) and as specific activity per microgram of LDL protein (B). Arrows indicate values that are higher than the means of the group plus two standard deviations.

microgram of LDL protein) correlated negatively with the plasma level of small LDLs. Similarly, specific LDL-associated PAF-AH activity correlated positively with average LDL particle size (Fig. 7B).

DISCUSSION

The present study shows that the documented increase of plasma triglycerides in GAgP patients (27–31) (Fig. 1) is the result of modest increases in VLDL (Fig. 2) and a more robust increase in the plasma concentration of IDL (Fig. 3). The strong increase in IDL may be particularly important, as several studies have indicated that this lipoprotein class is especially atherogenic (61–63). IDL levels are strongly and independently correlated with carotid artery intima-media thickness (63) and are an independent risk factor for aortic atherosclerosis in hemodialysis patients (61). Indeed, the plasma concentration of IDL appears to be more predictive of atherosclerosis progression than LDL, as assessed by coronary artery angiography or carotid artery ultrasonography (62). Hence, increased plasma levels of IDL (Fig. 3) may contribute to the increased cardiovascular risk of GAgP subjects.

The relatively high triglyceride levels in the plasma of GAgP subjects are comparable to the levels found in subjects with a typical pattern-B lipoprotein profile (64). This profile is characterized by increased plasma triglycerides (VLDL and IDL), low plasma levels of HDL, and a predominance of the small LDLs (45–47). Recent studies have indicated that the concentration of small LDLs remains relatively low when plasma triglyceride levels are between 0.5 and 1.5 mmol/l (44–133 mg/dl) but increases sharply when triglyceride concentrations reach 133 mg/l or greater (64). The mean plasma TG concentration in GAgP subjects (126 mg/dl) (Fig. 1D) is close to the TG concentration that causes a shift to the predominance of small LDLs (~133 mg/dl or 1.5 mmol/l). Indeed, GAgP subjects with plasma triglyceride levels greater than the mean (126 mg/dl) have higher plasma levels of small LDLs than GAgP subjects with plasma triglyceride levels below the mean.

This study shows that the documented increase of plasma triglycerides in GAgP subjects is associated with an increase in the plasma concentration of small LDLs (Fig. 4A, B) and with a higher total number of circulating LDL particles (Fig. 4C). Both parameters indicate increased cardiovascular risk. Compared with larger LDLs, small LDLs have a lower affinity for the apolipoprotein B/E re-
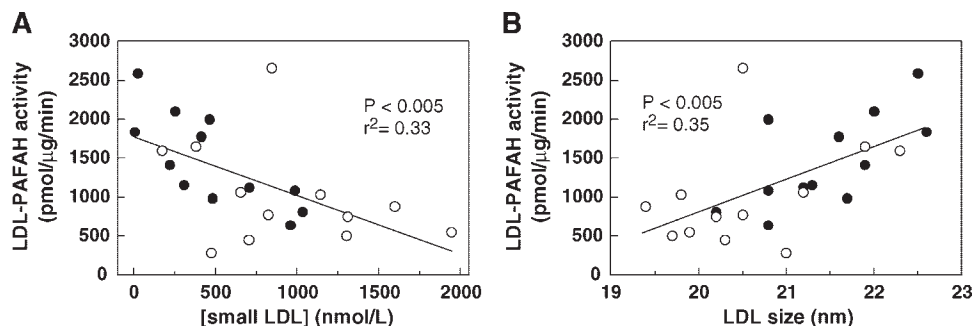

Fig. 7. LDL-associated PAF-AH activity correlates negatively with the number of small LDL particles in the plasma (A) and positively with the average LDL size (B). Compared with NP subjects (closed circles), GAgP subjects (open circles) have higher plasma concentrations of small LDLs, lower LDL-associated PAF-AH activity per microgram of LDL protein, and a smaller average LDL size.

ceptor (34–37), a higher affinity for proteoglycans of the vascular wall (38), and a lower content of antioxidants (39–44). Each of these properties is atherogenic, and subjects with a clear predominance of small LDLs have a 3- to 6-fold increased risk of cardiovascular disease (45–47). Additional studies have shown that LDL particle number may be a better marker for cardiovascular risk than LDL cholesterol (65). Hence, the combination of increased levels of small LDLs and increased total LDL particle numbers, as seen in GAgP subjects, may be particularly atherogenic.

Our results show that NP and GAgP subjects have similar plasma levels of HDL cholesterol (Fig. 1C). Subtle differences in the distribution of HDL subclasses in NP and GAgP subjects may exist, but these differences did not reach statistical significance in our group of subjects (Fig. 5). The data, therefore, suggest that neither the plasma level of total HDL nor the distribution of HDL subclasses is a major factor in the observed increase in cardiovascular risk of subjects with periodontitis. However, a recent study by Pussinen et al. (20) indicates that periodontal infections may alter the antiatherogenic potency of HDL, indicating that HDL may affect the cardiovascular risk of subjects with periodontitis in ways that are unrelated to the plasma concentration of these particles.

An additional factor that may increase the atherogenicity of lipoproteins in GAgP subjects is the LDL-associated PAF-AH activity. PAF-AH hydrolyzes PAF and oxidized phospholipids that have proinflammatory, proatherogenic properties. A specific mutation that inactivates PAF-AH (Val279→Phe) has been shown to increase the risk of stroke (50) and myocardial infarction (51). In addition, recent experiments have shown that local expression of PAF-AH exerts anti-inflammatory, antithrombotic, and antiproliferative effects while reducing the accumulation of oxidized LDL in the arteries of nonhyperlipidemic rabbits (52). Together, these studies support the notion that PAF-AH plays an antiatherogenic role. Our present results show that, on average, PAF-AH activity per microgram of LDL protein is lower in GAgP subjects than in NP subjects (Fig. 6B). A plausible explanation for this difference is the increased number of circulating LDL particles in GAgP subjects (Fig. 4C). Because the total amount of LDL-associated PAF-AH activity in NP and GAgP subjects is the same (Fig. 6A), the observed increase in LDL particle number in GAgP subjects results in a decrease of specific LDL-associated PAF-AH activity (i.e., PAF-AH activity per microgram of LDL protein). Such a decline may decrease the antiatherogenic influence of PAF-AH, leading to increased cardiovascular risk. This mechanism may be important in a more general sense, not only in periodontitis. PAF-AH activity is more strongly associated with small LDLs (60), a subclass of particles that is more susceptible to oxidation than larger LDLs (39–44). Figure 7 shows that specific LDL-associated PAF-AH activity correlates negatively with the plasma concentration of small LDLs in both NP and GAgP subjects. In other words, the higher the plasma concentration of an LDL subclass that is particularly vulnerable to oxidation (small LDLs), the lower the specific activ-

ity of the enzyme that may protect this subclass against the atherogenic effects of oxidation (PAF-AH). For this reason, increases in the plasma level of small LDLs, such as those seen in our group of GAgP subjects, may be particularly atherogenic. This notion is consistent with increased plasma titers of antioxidized LDL antibodies found in subjects with periodontitis (66–69).

Although PAF-AH expression increases when monocytes differentiate into macrophages or dendritic cells (53, 70), dendritic cells express much lower levels of the enzyme than macrophages. We have shown previously that, in culture, monocyte-derived dendritic cells of subjects with LAgP (then called localized juvenile periodontitis) synthesize and secrete much less PAF-AH activity than monocyte-derived macrophages (53). As monocytes from LAgP subjects have a propensity to differentiate into dendritic cells (53), we hypothesized that plasma levels of PAF-AH may be lower in periodontitis patients. Our results show that this is not the case in the current group of GAgP subjects (Fig. 6A). LDL-associated PAF-AH activity per microgram of LDL is lower in GAgP subjects than in NP subjects (Fig. 6B), but this appears to be attributable to increased LDL particle numbers (see above), not to decreased production of PAF-AH.

In conclusion, the combined influence of an altered lipoprotein profile and decreased LDL-associated PAF-AH activity may contribute to the increased risk in GAgP subjects of cardiovascular disease and stroke. Therefore, it may be beneficial to test GAgP subjects for lipoprotein subclass distribution and specific PAF-AH activity so that referral for treatment can be recommended if necessary.

REFERENCES

1. Socransky, S. S., and A. D. Haffajee. 2005. Periodontal microbial ecology. *Periodontol. 2000.* **38:** 135–187.
2. Offenbacher, S. 1996. Periodontal diseases: pathogenesis. *Ann. Periodontol.* **1:** 821–878.
3. Mattila, K. J., M. S. Nieminen, V. V. Valtonen, V. P. Rasi, Y. A. Kesaniemi, S. L. Syrjala, P. S. Jungell, M. Isoluoma, K. Hietaniemi, and M. J. Jokinen. 1989. Association between dental health and acute myocardial infarction. *BMJ.* **298:** 779–781.
4. Syrjanen, J., J. Peltola, V. Valtonen, M. Iivanainen, M. Kaste, and J. K. Huttunen. 1989. Dental infections in association with cerebral infarction in young and middle-aged men. *J. Intern. Med.* **225:** 179–184.
5. DeStefano, F., R. F. Anda, H. S. Kahn, D. F. Williamson, and C. M. Russell. 1993. Dental disease and risk of coronary heart disease and mortality. *BMJ.* **306:** 688–691.

6. Kweider, M., G. D. Lowe, G. D. Murray, D. F. Kinane, and D. A. McGowan. 1993. Dental disease, fibrinogen and white cell count: links with myocardial infarction? *Scott. Med. J.* **38:** 73–74.

7. Mattila, K. J., M. S. Valle, M. S. Nieminen, V. V. Valtonen, and K. L. Hietaniemi. 1993. Dental infections and coronary atherosclerosis. *Atherosclerosis.* **103:** 205–211.

8. Mattila, K. J., V. V. Valtonen, M. Nieminen, and J. K. Huttunen. 1995. Dental infection and the risk of new coronary events: prospective study of patients with documented coronary artery disease. *Clin. Infect. Dis.* **20:** 588–592.

9. Beck, J., R. Garcia, G. Heiss, P. S. Vokonas, and S. Offenbacher. 1996. Periodontal disease and cardiovascular disease. *J. Periodontol.* **67:** 1123–1137.

10. Joshipura, K. J., E. B. Rimm, C. W. Douglass, D. Trichopoulos, A. Ascherio, and W. C. Willett. 1996. Poor oral health and coronary heart disease. *J. Dent. Res.* **75:** 1631–1636.

11. Grau, A. J., F. Buggle, C. Ziegler, W. Schwarz, J. Meuser, A. J. Tasman, A. Buhler, C. Benesch, H. Becher, and W. Hacke. 1997. Association between acute cerebrovascular ischemia and chronic and recurrent infection. *Stroke.* **28:** 1724–1729.

12. Loesche, W. J., A. Schork, M. S. Terpenning, Y. M. Chen, C. Kerr, and B. L. Dominguez. 1998. The relationship between dental disease and cerebral vascular accident in elderly United States veterans. *Ann. Periodontol.* **3:** 161–174.

13. Morrison, H. I., L. F. Ellison, and G. W. Taylor. 1999. Periodontal disease and risk of fatal coronary heart and cerebrovascular diseases. *J. Cardiovasc. Risk.* **6:** 7–11.

14. Joshipura, K. J., H. C. Hung, E. B. Rimm, W. C. Willett, and A. Ascherio. 2003. Periodontal disease, tooth loss, and incidence of ischemic stroke. *Stroke.* **34:** 47–52.

15. Dorfer, C. E., H. Becher, C. M. Ziegler, C. Kaiser, R. Lutz, D. Jorss, C. Lichy, F. Buggle, S. Bultmann, M. Preusch, et al. 2004. The association of gingivitis and periodontitis with ischemic stroke. *J. Clin. Periodontol.* **31:** 396–401.

16. Grau, A. J., H. Becher, C. M. Ziegler, C. Lichy, F. Buggle, C. Kaiser, R. Lutz, S. Bultmann, M. Preusch, and C. E. Dorfer. 2004. Periodontal disease as a risk factor for ischemic stroke. *Stroke.* **35:** 496–501.

17. Amar, S., N. Gokce, S. Morgan, M. Loukideli, T. E. Van Dyke, and J. A. Vita. 2003. Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. *Arterioscler. Thromb. Vasc. Biol.* **23:** 1245–1249.

18. Beck, J. D., J. R. Elter, G. Heiss, D. Couper, S. M. Mauriello, and S. Offenbacher. 2001. Relationship of periodontal disease to carotid artery intima-media wall thickness: the Atherosclerosis Risk In Communities (ARIC) Study. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1816–1822.

19. Engebretson, S. P., I. B. Lamster, M. S. Elkind, T. Rundek, N. J. Serman, R. T. Demmer, R. L. Sacco, P. N. Papapanou, and M. Desvarieux. 2005. Radiographic measures of chronic periodontitis and carotid artery plaque. *Stroke.* **36:** 561–566.

20. Pussinen, P. J., M. Jauhiainen, T. Vilkuna-Rautiainen, J. Sundvall, M. Vesanen, K. Mattila, T. Palosuo, G. Alfthan, and S. Asikainen. 2004. Periodontitis decreases the antiatherogenic potency of high density lipoprotein. *J. Lipid Res.* **45:** 139–147.

21. Pussinen, P. J., T. Vilkuna-Rautiainen, G. Alfthan, T. Palosuo, M. Jauhiainen, J. Sundvall, M. Vesanen, K. Mattila, and S. Asikainen. 2004. Severe periodontitis enhances macrophage activation via increased serum lipopolysaccharide. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2174–2180.

22. Loos, B. G., J. Craandijk, F. J. Hoek, P. M. Wertheim-van Dillen, and U. van der Velden. 2000. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J. Periodontol.* **71:** 1528–1534.

23. Noack, B., R. J. Genco, M. Trevisan, S. Grossi, J. J. Zambon, and E. De Nardin. 2001. Periodontal infections contribute to elevated systemic C-reactive protein level. *J. Periodontol.* **72:** 1221–1227.

24. D'Aiuto, F., D. Ready, and M. S. Tonetti. 2004. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J. Periodontal Res.* **39:** 236–241.

25. Pussinen, P. J., G. Alfthan, J. Tuomilehto, S. Asikainen, and P. Jousilahti. 2004. High serum antibody levels to *Porphyromonas gingivalis* predict myocardial infarction. *Eur. J. Cardiovasc. Prev. Rehabil.* **11:** 408–411.

26. Lalla, E., I. B. Lamster, M. A. Hofmann, L. Bucciarelli, A. P. Jerud, S. Tucker, Y. Lu, P. N. Papapanou, and A. M. Schmidt. 2003. Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. *Arterioscler. Thromb. Vasc. Biol.* **23:** 1405–1411.

27. Noack, B., I. Jachmann, S. Roscher, L. Sieber, S. Kopprasch, C. Luck, M. Hanefeld, and T. Hoffmann. 2000. Metabolic diseases and their possible link to risk indicators of periodontitis. *J. Periodontol.* **71:** 898–903.

28. Cutler, C. W., E. A. Shinedling, M. Nunn, R. Jotwani, B. O. Kim, S. Nares, and A. M. Iacopino. 1999. Association between periodontitis and hyperlipidemia: cause or effect? *J. Periodontol.* **70:** 1429–1434.

29. Cutler, C. W., R. L. Machen, R. Jotwani, and A. M. Iacopino. 1999. Heightened gingival inflammation and attachment loss in type 2 diabetics with hyperlipidemia. *J. Periodontol.* **70:** 1313–1321.

30. Iacopino, A. M., and C. W. Cutler. 2000. Pathophysiological relationships between periodontitis and systemic disease: recent concepts involving serum lipids. *J. Periodontol.* **71:** 1375–1384.

31. Cutler, C. W., and A. M. Iacopino. 2003. Periodontal disease: links with serum lipid/triglyceride levels? Review and new data. *J. Int. Acad. Periodontol.* **5:** 47–51.

32. Berneis, K. K., and R. M. Krauss. 2002. Metabolic origins and clinical significance of LDL heterogeneity. *J. Lipid Res.* **43:** 1363–1379.

33. Lada, A. T., and L. L. Rudel. 2004. Associations of low density lipoprotein particle composition with atherogenicity. *Curr. Opin. Lipidol.* **15:** 19–24.

34. Campos, H., K. S. Arnold, M. E. Balestra, T. L. Innerarity, and R. M. Krauss. 1996. Differences in receptor binding of LDL subfractions. *Arterioscler. Thromb. Vasc. Biol.* **16:** 794–801.

35. de Graaf, J., J. C. Hendriks, D. W. Swinkels, P. N. Demacker, and A. F. Stalenhoef. 1993. Differences in LDL receptor-mediated metabolism of three low density lipoprotein subfractions by human monocyte-derived macrophages: impact on the risk for atherosclerosis. *Artery.* **20:** 201–230.

36. Galeano, N. F., R. Milne, Y. L. Marcel, M. T. Walsh, E. Levy, T. D. Ngu'yen, A. Gleeson, Y. Arad, L. Witte, M. al-Haideri, et al. 1994. Apoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride-rich LDL of normal size. *J. Biol. Chem.* **269:** 511–519.

37. Nigon, F., P. Lesnik, M. Rouis, and M. J. Chapman. 1991. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J. Lipid Res.* **32:** 1741–1753.

38. Hurt-Camejo, E., G. Camejo, B. Rosengren, F. Lopez, O. Wiklund, and G. Bondjers. 1990. Differential uptake of proteoglycan-selected subfractions of low density lipoprotein by human macrophages. *J. Lipid Res.* **31:** 1387–1398.

39. Tribble, D. L., L. G. Holl, P. D. Wood, and R. M. Krauss. 1992. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis.* **93:** 189–199.

40. Chait, A., R. L. Brazg, D. L. Tribble, and R. M. Krauss. 1993. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.* **94:** 350–356.

41. Dejager, S., E. Bruckert, and M. J. Chapman. 1993. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. *J. Lipid Res.* **34:** 295–308.

42. de Graaf, J., H. L. Hak-Lemmers, M. P. Hectors, P. N. Demacker, J. C. Hendriks, and A. F. Stalenhoef. 1991. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler. Thromb.* **11:** 298–306.

43. Tribble, D. L., M. Rizzo, A. Chait, D. M. Lewis, P. J. Blanche, and R. M. Krauss. 2001. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am. J. Med.* **110:** 103–110.

44. Tribble, D. L., J. J. van den Berg, P. A. Motchnik, B. N. Ames, D. M. Lewis, A. Chait, and R. M. Krauss. 1994. Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. *Proc. Natl. Acad. Sci. USA.* **91:** 1183–1187.

45. Austin, M. A., J. L. Breslow, C. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. 1988. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *J. Am. Med. Assoc.* **260:** 1917–1921.

46. Gardner, C. D., S. P. Fortmann, and R. M. Krauss. 1996. Association of small low-density lipoprotein particles with the incidence of

coronary artery disease in men and women. *J. Am. Med. Assoc.* **276:** 875–881.

47. Lamarche, B., I. Lemieux, and J. P. Despres. 1999. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, patho-physiology and therapeutic aspects. *Diabetes Metab.* **25:** 199–211.

48. Tselepis, A. D., and M. J. Chapman. 2002. Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor-acetylhydrolase. *Atheroscler. Suppl.* **3:** 57–68.

49. Karasawa, K., A. Harada, N. Satoh, K. Inoue, and M. Setaka. 2003. Plasma platelet activating factor-acetylhydrolase (PAF-AH). *Prog. Lipid Res.* **42:** 93–114.

50. Hiramoto, M., H. Yoshida, T. Imaizumi, N. Yoshimizu, and K. Satoh. 1997. A mutation in plasma platelet-activating factor acetylhydrolase (Val279→Phe) is a genetic risk factor for stroke. *Stroke.* **28:** 2417–2420.

51. Yamada, Y., S. Ichihara, T. Fujimura, and M. Yokota. 1998. Identification of the G994→T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. *Metabolism.* **47:** 177–181.

52. Arakawa, H., J. Y. Qian, D. Baatar, K. Karasawa, Y. Asada, Y. Sasaguri, E. R. Miller, J. L. Witztum, and H. Ueno. 2005. Local expression of platelet-activating factor-acetylhydrolase reduces accumulation of oxidized lipoproteins and inhibits inflammation, shear stress-induced thrombosis, and neointima formation in balloon-injured carotid arteries in nonhyperlipidemic rabbits. *Circulation.* **111:** 3302–3309.

53. Al-Darmaki, S., H. A. Schenkein, J. G. Tew, and S. E. Barbour. 2003. Differential expression of platelet-activating factor acetylhydrolase in macrophages and monocyte-derived dendritic cells. *J. Immunol.* **170:** 167–173.

54. Otvos, J. D. 1997. Handbook of Lipoprotein Testing. American Association for Clinical Chemistry Press, Washington, DC. 497–508.

55. Otvos, J. D. 2002. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin. Lab.* **48:** 171–180.

56. Schumaker, V. N., and D. L. Puppione. 1986. Sequential flotation ultracentrifugation. *Methods Enzymol.* **128:** 155–170.

57. Peterson, G. L. 1983. Determination of total protein. *Methods Enzymol.* **91:** 95–119.

58. Miwa, M., T. Miyake, T. Yamanaka, J. Sugatani, Y. Suzuki, S. Sakata, Y. Araki, and M. Matsumoto. 1988. Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J. Clin. Invest.* **82:** 1983–1991.

59. Narahara, H., I. Miyakawa, and J. M. Johnston. 1995. The inhibitory effect of 1,25-dihydroxyvitamin D3 on the secretion of platelet-activating factor acetylhydrolase by human decidual macrophages. *J. Clin. Endocrinol. Metab.* **80:** 3121–3126.

60. McCall, M. R., M. La Belle, T. M. Forte, R. M. Krauss, Y. Takanami, and D. L. Tribble. 1999. Dissociable and nondissociable forms of platelet-activating factor acetylhydrolase in human plasma LDL: implications for LDL oxidative susceptibility. *Biochim. Biophys. Acta.* **1437:** 23–36.

61. Shoji, T., Y. Nishizawa, T. Kawagishi, K. Kawasaki, H. Taniwaki, T. Tabata, T. Inoue, and H. Morii. 1998. Intermediate-density lipoprotein as an independent risk factor for aortic atherosclerosis in hemodialysis patients. *J. Am. Soc. Nephrol.* **9:** 1277–1284.

62. Krauss, R. M. 1998. Atherogenicity of triglyceride-rich lipoproteins. *Am. J. Cardiol.* **81:** 13B–17B.

63. Krauss, R. M. 1998. Triglycerides and atherogenic lipoproteins: rationale for lipid management. *Am. J. Med.* **105 (Suppl.):** 58–62.

64. Packard, C. J. 2003. Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochem. Soc. Trans.* **31:** 1066–1069.

65. Cromwell, W. C., and J. D. Otvos. 2004. Low-density lipoprotein particle number and risk for cardiovascular disease. *Curr. Atheroscler. Rep.* **6:** 381–387.

66. Schenkein, H. A., J. C. Gunsolley, A. M. Best, M. T. Harrison, C. L. Hahn, J. Wu, and J. G. Tew. 1999. Antiphosphorylcholine antibody levels are elevated in humans with periodontal diseases. *Infect. Immun.* **67:** 4814–4818.

67. Schenkein, H. A., C. R. Berry, D. Purkall, J. A. Burmeister, C. N. Brooks, and J. G. Tew. 2001. Phosphorylcholine-dependent cross-reactivity between dental plaque bacteria and oxidized low-density lipoproteins. *Infect. Immun.* **69:** 6612–6617.

68. Schenkein, H. A., C. R. Berry, J. A. Burmeister, C. N. Brooks, S. E. Barbour, A. M. Best, and J. G. Tew. 2003. Anti-cardiolipin antibodies in sera from patients with periodontitis. *J. Dent. Res.* **82:** 919–922.

69. Schenkein, H. A., C. R. Berry, J. A. Burmeister, C. N. Brooks, A. M. Best, and J. G. Tew. 2004. Locally produced anti-phosphorylcholine and anti-oxidized low-density lipoprotein antibodies in gingival crevicular fluid from aggressive periodontitis patients. *J. Periodontol.* **75:** 146–153.

70. Elstad, M. R., D. M. Stafforini, T. M. McIntyre, S. M. Prescott, and G. A. Zimmerman. 1989. Platelet-activating factor acetylhydrolase increases during macrophage differentiation. A novel mechanism that regulates accumulation of platelet-activating factor. *J. Biol. Chem.* **264:** 8467–8470.