

Inability of HDL from abdominally obese subjects to counteract the inhibitory effect of oxidized LDL on vasorelaxation

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Abstract Abdominal obesity is associated with a decreased plasma concentration of HDL cholesterol and with qualitative modifications of HDL, such as triglyceride enrichment. Our aim was to determine, in isolated aorta rings, whether HDL from obese subjects can counteract the inhibitory effect of oxidized low density lipoprotein (OxLDL) on endothelium-dependent vasodilation as efficiently as HDL from normolipidemic, lean subjects. Plasma triglycerides were 74% higher ($P < 0.005$) in obese subjects compared with controls, and apolipoprotein A-I (apoA-I) and HDL cholesterol concentrations were 12% and 17% lower ($P < 0.05$), respectively. HDL from control subjects significantly reduced the inhibitory effect of OxLDL on vasodilation [maximal relaxation (E_{max}) = $82.1 \pm 8.6\%$ vs. $54.1 \pm 8.1\%$; $P < 0.0001$], but HDL from obese subjects had no effect (E_{max} = $47.2 \pm 12.5\%$ vs. $54.1 \pm 8.1\%$; NS). In HDL from abdominally obese subjects compared with HDL from controls, the apoA-I content was 12% lower ($P < 0.05$) and the triglyceride-to-cholesterol ester ratio was 36% higher ($P = 0.08$). E_{max} (OxLDL + HDL) was correlated with HDL apoA-I content and triglyceride-to-cholesterol ester ratio ($r = 0.36$ and $r = -0.38$, respectively; $P < 0.05$). We conclude that in abdominally obese subjects, the ability of HDL to counteract the inhibitory effect of OxLDL on vascular relaxation is impaired. This could contribute to the increased cardiovascular risk observed in these subjects.—Perségol, L., B. Vergès, P. Gambert, and L. Duvillard. Inability of HDL from abdominally obese subjects to counteract the inhibitory effect of oxidized LDL on vasorelaxation. *J. Lipid Res.* 2007. 48: 1396–1401.

Supplementary key words obesity • high density lipoprotein • oxidized low density lipoprotein • triglycerides • apolipoprotein A-I

Abdominal obesity and insulin resistance have been shown to be associated with an increased cardiovascular risk (1–3). Several atherogenic abnormalities are commonly observed in abdominally obese subjects, such as

hypertriglyceridemia, decreased HDL cholesterol concentration, hypertension, and increased oxidative stress or oxidized low density lipoprotein (OxLDL) concentration (4–6).

HDL particles have multiple antiatherosclerosis properties. Among them is the beneficial effect they exert on endothelium-dependent vasorelaxation. Indeed, HDL particles can counteract the inhibitory effect of OxLDL on endothelium-dependent vasodilation in isolated rabbit aorta rings (7). Moreover, the intravenous administration of reconstituted HDL either to hypercholesterolemic men or to ATP binding cassette A1-deficient heterozygotes, both with an initially impaired endothelium-dependent vasorelaxation, restored a normal vasorelaxation in these subjects (8, 9).

Endothelium dysfunction, a predictor for cardiovascular events, has been observed in overweight patients with visceral obesity and was demonstrated to be associated with body fat distribution (10–12). Because of qualitative modifications, HDL from these subjects may be less protective against impairments of endothelium-dependent vasorelaxation than HDL from lean normolipidemic subjects. Therefore, the purpose of our study was to determine whether or not HDL from abdominally obese nondiabetic patients could protect the vessel from the inhibitory effect of OxLDL on vasorelaxation.

METHODS

Subjects

Nineteen nondiabetic abdominally obese patients and 16 lean normolipidemic and normoglycemic control subjects were included in this study. This study was approved by the Ethics

Abbreviations: apoA-I, apolipoprotein A-I; E_{max} , maximal relaxation; eNOS, endothelial nitric oxide synthase; HOMA, homeostasis model assessment; NO, nitric oxide; OxLDL, oxidized low density lipoprotein; PAF-AH, platelet-activated factor-acetyl hydrolase; PON, paraoxonase.

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Committee of Dijon University Hospital, and written informed consent was obtained from each subject before the study.

Obese and control subjects were not taking any antioxidant or any drug that could affect lipid metabolism. Controls were included in our study if they had a waist circumference of <88 cm for women and <102 cm for men, fasting glycemia < 110 mg/dl, serum triglycerides < 150 mg/dl, and HDL cholesterol of >40 mg/dl for men and >50 mg/dl for women. Obese subjects were included if they had fasting glycemia < 110 mg/dl and waist circumference of >88 cm for women and >102 cm for men.

Chemical compounds

Acetylcholine, arterenol hydrochloride, sodium nitroprusside, butylated hydroxytoluene, and EDTA were purchased from Sigma Chemical Co. (Saint Quentin Fallavier, France).

Preparation of lipoproteins

The different lipoprotein subclasses were isolated from serum or plasma by sequential flotation ultracentrifugation according to their density. The LDLs (1.019 < d < 1.063 g/ml) used to form OxLDL were prepared in a 70.Ti rotor on an XL-90 ultracentrifuge (Beckman, Palo Alto, CA) by sequential flotation ultracentrifugation from 170 ml of fresh citrated plasma drawn from normolipidemic donors. Densities of plasma were adjusted by the addition of KBr. After a 15 h run at 50,000 rpm, the top fraction of the sample containing lipoproteins with d < 1.019 g/ml was removed. Finally, LDLs were prepared from the infranatant adjusted to a 1.063 g/ml density by a 24 h run at 45,000 rpm. HDLs (1.063 < d < 1.21 g/ml) of control and obese subjects were prepared from 20 ml of EDTA plasma in a 70.Ti rotor on an XL-90 ultracentrifuge (Beckman). After a 20 h run at 50,000 rpm, the top fraction of the sample containing lipoproteins with d < 1.063 g/ml was removed. Finally, HDLs were prepared from the infranatant adjusted to a 1.21 g/ml density by a 24 h run at 50,000 rpm. The different lipoprotein fractions were dialyzed overnight against TBS (10 mmol/l Tris and 150 mmol/l NaCl, pH 7.4). To avoid oxidation, native lipoproteins were supplemented with EDTA (final concentration, 200 μ mol/l) and butylated hydroxytoluene (final concentration, 20 μ mol/l) and kept at 4°C.

Oxidative modification of LDL

The oxidation of LDL was performed by incubating freshly prepared LDL, adjusted to a 1.2 g/l final concentration of proteins in TBS, with a copper sulfate solution (final concentration, 5 μ mol/l) for 24 h at 37°C. At the end, oxidation was stopped by the addition of EDTA (final concentration, 200 μ mol/l) and butylated hydroxytoluene (final concentration, 20 μ mol/l), and OxLDLs were kept at 4°C (13).

Serum and HDL biochemical measurements

Glycemia was measured on a Vitros analyzer (Ortho Clinical Diagnostics, Rochester, NY). Total cholesterol and HDL cholesterol, triglycerides, proteins, apolipoprotein A-I (apoA-I), and apoB were quantitated on a Dimension analyzer with dedicated reagents (Dade Behring, Newark, DE). LDL cholesterol was calculated using the Friedewald formula. Free cholesterol and phospholipids were measured on the same analyzer with Wako reagents (Neuss, Germany). Insulin was quantitated by radioimmunoassay (CisBio International, Gif sur Yvette, France).

Insulin resistance evaluation

The insulin resistance level was estimated using the homeostasis model assessment (HOMA) method (14). HOMA was cal-

culated with the following formula: $HOMA = \text{glucose} \times \text{insulin} / 22.5$, where glucose is the fasting glucose millimolar concentration and insulin is the fasting insulin concentration expressed in milliunits per liter.

Measurement of paraoxonase activity

Paraoxonase (PON) activity was measured as described in fresh HDL samples within 2 days of isolation (15). It was assayed in tubes containing Tris-HCl buffer (150 mmol/l NaCl and 1 mmol/l CaCl₂, pH 7.4) and 100 μ g of proteins of HDL (final volume, 800 μ l). The reaction was initiated by adding paraoxon (1 mmol/l), and the absorbance was monitored at 412 nm for 1 h. The amount of 4-*p*-nitrophenol formed was calculated from the molar extinction coefficient (12,800 mol⁻¹ cm⁻¹). PON activity was expressed as nmol of 4-*p*-nitrophenol formed per minute per milligram of apoA-I under the experimental conditions.

Vasoreactivity on rabbit aorta rings

Vasoreactivity experiments were performed on rabbit (Charles River, l'Arbresle, France) aorta rings as described previously (13). Our protocol was approved by the local ethics committee for animals. Aortic rings were first contracted by 0.3 μ mol/l arterenol hydrochloride, a concentration giving 75% of the maximal contraction. Then, the rings were relaxed by cumulative additions of acetylcholine in the 1 nmol/l to 0.01 mmol/l concentration range. After washout and a 30 min recovery period, aortic rings were incubated for 2 h with either OxLDL or OxLDL + HDL. The concentration of proteins in the organ bath (native or oxidized lipoproteins) was constantly 1 g/l for each lipoprotein subclass, as used in many previous studies conducted with rabbit aortic rings (7, 13, 16). At the end of the incubation period, aortic segments were again contracted with arterenol hydrochloride and progressively relaxed with acetylcholine or sodium nitroprusside. Sodium nitroprussate, a nitric oxide (NO) donor, was used to check that the defects in vasodilation we observed were endothelium-dependent.

The maximal relaxation (E_{max}) induced by acetylcholine and expressed as a percentage of the contraction to arterenol hydrochloride (0.3 μ mol/l) was determined from experimental data. To limit variations in the results attributable to intrinsic properties of the vessels, experiments were performed in quadruplicate on aorta rings from four different rabbits and the results were averaged.

Statistical procedures

Statistical analysis was performed using the Statview statistical software. Data are expressed as means \pm SD. The comparison of quantitative data was performed with the Mann-Whitney *U*-test. Correlation coefficients for the whole population were determined by linear regression analysis. We also performed a multiple regression analysis in which the dependent variable was the maximal vasorelaxation and the independent variables were the HDL triglyceride-to-cholesterol ester ratio and the HDL apoA-I percentage. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical and biochemical characteristics

The clinical and biochemical characteristics of obese and control subjects are shown in **Table 1**. No statistically

TABLE 1. Clinical and biochemical characteristics of lean control and obese subjects

Characteristic	Controls (n = 15)	Obese Subjects (n = 19)
Age (years)	43.5 ± 16.6	42.8 ± 14.6
Sex (male/female)	5/10	3/16
Body mass index (kg/m ²)	23.05 ± 1.60	35.66 ± 4.64 ^a
Homeostasis model assessment	1.55 ± 0.23	2.31 ± 1.72
Waist circumference (cm)	83.4 ± 9.1	116.7 ± 11.6 ^a
Fasting glycemia (mg/dl)	97 ± 8	90 ± 10
Total cholesterol (mg/dl)	208 ± 21	185 ± 32
HDL cholesterol (mg/dl)	58 ± 14	48 ± 11 ^b
LDL cholesterol (mg/dl)	139 ± 20	113 ± 29
Triglycerides (mg/dl)	76 ± 38	129 ± 68 ^a
ApoA-I (mg/dl)	153 ± 24	134 ± 26 ^b
ApoB (mg/dl)	92 ± 18	84 ± 20

ApoA-I, apolipoprotein A-I. Values are expressed as means ± SD.

^a*P* < 0.005.

^b*P* < 0.05.

significant differences between obese individuals and controls were observed for total cholesterol and LDL cholesterol. The plasma triglyceride level was 41% higher (*P* < 0.005) in the obese group compared with the control group, and plasma apoA-I and HDL cholesterol were 13% and 17% lower, respectively (*P* < 0.05).

HDL composition

Table 2 illustrates the composition of HDL in the two groups of subjects. Compared with HDL from lean control subjects, HDL from obese patients displayed a 42% higher triglyceride content (*P* < 0.05) and a 12% decrease in apoA-I content (*P* < 0.001). The HDL triglyceride-to-cholesteryl ester ratio was 36% higher in obese patients compared with controls, with borderline significance (*P* = 0.08). No significant difference was observed between the two groups for PON activity.

OxLDL influence on endothelium-dependent vasorelaxation

As described previously, preincubation of isolated arterial segments with OxLDL inhibited the endothelium-

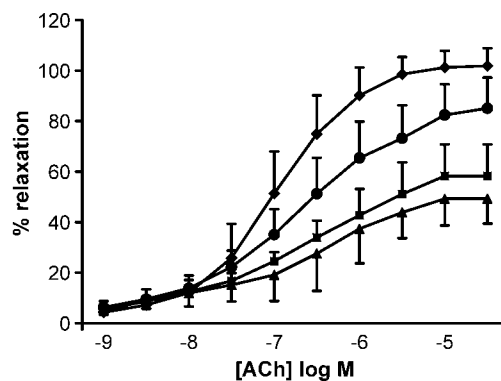


Fig. 1. Influence of HDL from obese and lean control subjects on the inhibitory effect of oxidized low density lipoprotein (OxLDL) on endothelium-dependent vasorelaxation in rabbit aorta rings. The graph shows mean concentration-response curves for acetylcholine (ACh) in aortas contracted with 0.3 μmol/l arterenol hydrochloride and preincubated for 2 h with OxLDL alone (squares), with OxLDL and HDL from control lean subjects (circles), with OxLDL and HDL from obese patients (triangles), or in Krebs buffer (diamonds). The concentration of proteins was 1 g/l for each lipoprotein subclass. Values represent means ± SD.

dependent relaxation induced by acetylcholine: the E_{max} to acetylcholine decreased from $101.2 \pm 6.9\%$ in segments incubated with Krebs solution to $54.1 \pm 8.1\%$ in segments treated with OxLDL (*P* < 0.0001) (Fig. 1).

Ability of HDL to counteract the inhibitory effect of OxLDL on endothelium-dependent vasodilation

In control subjects, the E_{max} of rabbit aortic rings was significantly greater after preincubation with OxLDL + HDL than after preincubation with OxLDL alone ($E_{max} = 82.1 \pm 8.6\%$ vs. $54.1 \pm 8.1\%$; *P* < 0.0001). On the contrary, the addition of HDL from obese patients to OxLDL did not modify the E_{max} compared with incubation with OxLDL ($E_{max} = 47.3 \pm 12.5\%$ vs. $54.1 \pm 8.1\%$; NS) (Fig. 1).

With HDL alone, E_{max} was $94.8 \pm 4.7\%$ in control subjects and $92.0 \pm 7.2\%$ in obese patients (difference not significant). In all experiments, sodium nitroprussiate-mediated endothelium-independent relaxation was not modified (data not shown).

Correlations

The E_{max} obtained after coincubation of the vessels with OxLDL and HDL [$E_{max}(\text{OxLDL} + \text{HDL})$] was correlated positively with the percentage of apoA-I in HDL ($r = 0.36$, *P* < 0.05) and negatively with the HDL triglyceride-to-cholesteryl ester ratio ($r = -0.38$, *P* < 0.05) (Fig. 2). The correlation between $E_{max}(\text{OxLDL} + \text{HDL})$ and HDL triglycerides was borderline significant ($r = 0.30$, *P* = 0.09). $E_{max}(\text{OxLDL} + \text{HDL})$ was not correlated with the percentage of HDL free cholesterol, phospholipids, total proteins, or cholesteryl esters. In multiple regression analysis including HDL apoA-I percentage and HDL triglyceride-to-cholesteryl ester ratio in the model, both

TABLE 2. HDL composition in lean control and obese subjects

Constituent	Controls (n = 15)	Obese Subjects (n = 19)
Proteins (%)	45.4 ± 4.7	47.8 ± 7.3
Phospholipids (%)	24.6 ± 4.3	22.7 ± 5.2
Free cholesterol (%)	3.3 ± 0.8	3.1 ± 0.6
Cholesteryl esters (%)	23.4 ± 3.4	24.6 ± 4.9
Triglycerides (%)	2.6 ± 1.1	3.7 ± 2.0 ^a
ApoA-I (%)	38.4 ± 5.5	33.9 ± 6.5 ^b
Triglycerides/cholesteryl esters	0.11 ± 0.04	0.15 ± 0.08
Paraoxonase activity (nmol/min/mg apoA-I)	1.36 ± 1.05	1.50 ± 0.96

Values are expressed as means ± SD. HDL composition is expressed as the percentage of total HDL mass.

^a*P* < 0.05.

^b*P* < 0.01.

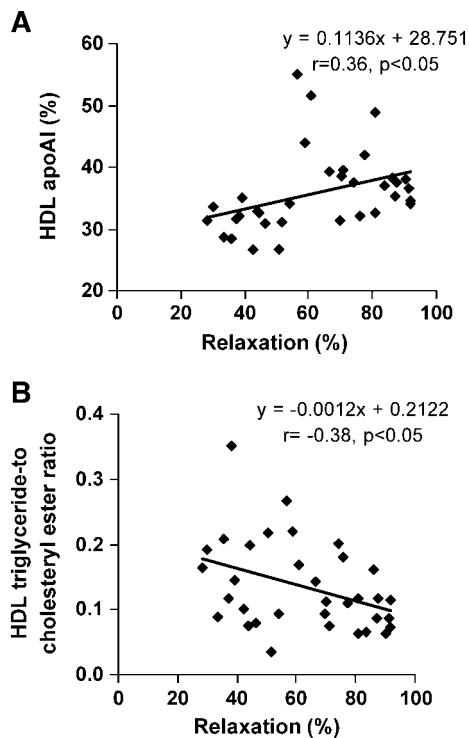


Fig. 2. Correlation between HDL apolipoprotein A-I content (A) and triglyceride-to-cholesteryl ester ratio (B) and maximal endothelium-dependent relaxation in rabbit aortic rings after preincubation with HDL and OxLDL.

variables were associated with $E_{max}(\text{OxLDL} + \text{HDL})$ ($r = 0.41, P < 0.05$ and $r = -0.42, P < 0.01$, respectively).

DISCUSSION

In this study, we have demonstrated that, contrary to HDL from lean control subjects, HDL from abdominally obese patients is unable to counteract the inhibitory effect of OxLDL on endothelium-dependent vasodilation. Moreover, we have shown that both the triglyceride enrichment of HDL particles and the reduction in HDL apoA-I observed in abdominally obese patients are associated with the decrease in the vasorelaxant effect of HDL.

The deleterious effect of OxLDL on vascular reactivity is related mainly to a decreased bioavailability of NO, a powerful vasorelaxant molecule. Indeed, lysophosphatidylcholine, a product of the oxidation of phosphatidylcholine, activates the production of superoxide anion that reacts with NO to produce peroxynitrites, decreasing NO bioavailability (17, 18). Moreover, lysophosphatidylcholine can be incorporated into cytoplasmic membranes, inducing the uncoupling of receptors for vasodilatory ligands and G proteins, which decreases NO production (19). Furthermore, OxLDL can deplete caveolae of free cholesterol, leading to the displacement of endothelial nitric oxide synthase (eNOS) to an intracellular compart-

ment where it cannot be activated (20). Moreover, OxLDL particles decrease the stability of eNOS mRNA (21).

HDL particles counteract the inhibitory effect of OxLDL on vascular relaxation both by their antioxidant properties and by stimulating the production of NO by endothelial cells. HDLs are removed from LDL lipid peroxides and oxidized derivatives of cholesterol via cholesteryl ester transfer protein (22). PON, platelet-activated factor-acetyl hydrolase (PAF-AH), and LCAT, three HDL-associated enzymes, hydrolyze oxidized cholesterol or hydroperoxides (23). ApoA-I contributes to the removal of oxidized lipids from LDL and promotes the formation of phosphatidylcholine core aldehydes, which are hydrolyzed by PON or PAF-AH (24, 25). Furthermore, HDL allows eNOS to stay in caveolae by preventing the depletion of free cholesterol induced by OxLDL (26). As far as NO production is concerned, it can be stimulated by HDL. Indeed, HDL increases the expression of the eNOS gene and upregulates eNOS activity by inducing its phosphorylation through different signaling pathways involving Akt, mitogen-activated protein kinases, and calcium-calmodulin-dependent kinases (27–30).

The inability of HDL from abdominally obese subjects to counteract the inhibitory effect of OxLDL on endothelium-dependent vasodilation indicates that one or several of the HDL protective effects detailed above is deficient.

Previous works have reported impaired antioxidative activity of HDL in patients with decreased HDL cholesterol concentration and in patients with the metabolic syndrome (31, 32). In those studies, the activities of PON, PAF-AH, and LCAT were not decreased, but HDLs were triglyceride-enriched. Our abdominally obese subjects are similar to the patients from those studies in the sense that they presented a low HDL cholesterol concentration and a triglyceride enrichment of HDL. It was recently demonstrated that the replacement of cholesteryl esters by triglycerides in HDL abrogates the HDL-mediated protection against LDL oxidation (32). Enrichment in triglycerides reduces the exposure of apoA-I to the aqueous phase as a result of its increased penetration into the lipid core of HDL and thus could decrease the capacity of apoA-I to transfer oxidized lipids from LDL (33). Thus, a decreased antioxidative capacity of HDL in our patients could contribute to the decreased capacity of HDL to counteract the inhibitory effect of OxLDL on vasorelaxation. In our work, the decreased antioxidative capacity of HDL in obese subjects may be reinforced by the fact that we measured a decreased percentage of apoA-I in HDL. Indeed, as detailed above, apoA-I has its own antioxidative capacity.

The modification of apoA-I conformation induced by HDL triglyceride enrichment could also have consequences on NO production by endothelial cells. Indeed, binding of HDL particles to cell surface receptors, such as scavenger receptor class B type I, implied in the signaling pathways activating eNOS could be impaired (28).

We recently demonstrated that HDL from type 2 diabetic patients was also unable to counteract the inhibitory effect of OxLDL on vascular relaxation and that the HDL triglyceride enrichment appeared to play an important

role in the decreased vasorelaxant effect of HDL (34). The results of our present study indicate that the loss of the vasorelaxant effect of HDL in type 2 diabetic patients appears at a very early stage, before the development of hyperglycemia, when plasma triglyceride concentration is very moderately increased, in a less important manner than in type 2 diabetes mellitus.

In conclusion, our study demonstrated the inability of HDL from abdominally obese subjects to counteract the inhibitory effect of OxLDL on vascular relaxation. The HDL triglyceride enrichment and the decreased apoA-I content could be major determinants of this abnormality. Thus, in abdominally obese subjects, HDLs appear less atheroprotective for at least two reasons: because their plasma concentration is decreased and because their compositional changes impair their intrinsic antiatherogenic properties, especially their vasorelaxant effect, as we demonstrate here. **LE**

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REFERENCES

1. Yusuf, S., S. Hawken, S. Ounpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen, A. Budaj, P. Pais, J. Varigos, et al. and INTERHEART Study Investigators. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. **364**: 937–952.
2. Saeely, C. H., S. Aczel, T. Marte, P. Langer, G. Hoefle, and H. Drexel. 2005. The metabolic syndrome, insulin resistance, and cardiovascular risk in diabetic and nondiabetic patients. *J. Clin. Endocrinol. Metab.* **90**: 5698–5703.
3. Rutter, M. K., J. B. Meigs, L. M. Sullivan, R. B. D'Agostino, and P. W. Wilson. 2005. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes*. **54**: 3252–3257.
4. Taskinen, M. R. 1995. Insulin resistance and lipoprotein metabolism. *Curr. Opin. Lipidol.* **6**: 153–160.
5. Couillard, C., G. Ruel, W. R. Archer, S. Pomerleau, J. Bergeron, P. Couture, B. Lamarche, and N. Bergeron. 2005. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. *J. Clin. Endocrinol. Metab.* **90**: 6454–6459.
6. Weinbrenner, T., H. Schroder, V. Eскурriol, M. Fito, R. Elosua, J. Marrugat, and M. I. Covas. 2006. Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. *Am. J. Clin. Nutr.* **83**: 1–2.
7. Matsuda, Y., K. Hirata, N. Inoue, M. Suematsu, S. Kawashima, H. Akita, and M. Yokoyama. 1993. High density lipoprotein reverses inhibitory effect of oxidized low density lipoprotein on endothelium-dependent arterial relaxation. *Circ. Res.* **72**: 103–109.
8. Bisoendial, R. J., G. K. Hovingh, J. H. Levels, P. G. Lerch, I. Andresen, M. R. Hayden, J. J. Kastelein, and E. S. Stroes. 2003. Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. *Circulation*. **107**: 2944–2948.
9. Spieker, L. E., I. Sudano, D. Hurlimann, P. G. Lerch, M. G. Lang, C. Binggeli, R. Corti, F. Ruschitzka, T. F. Luscher, and G. Noll. 2002. High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation*. **105**: 1399–1402.
10. Steinberg, H. O., H. Chaker, R. Leaming, A. Johnson, G. Brechtel,

- and A. D. Baron. 1996. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J. Clin. Invest.* **97**: 2601–2610.
11. Brook, R. D., R. L. Bard, M. Rubenfire, P. M. Ridker, and J. Rajakopal. 2001. Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults. *Am. J. Cardiol.* **88**: 1264–1269.
12. Arcaro, G., M. Zamboni, L. Rossi, E. Turcato, G. Covi, F. Armellini, O. Bosello, and A. Lechi. 1999. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. *Int. J. Obes.* **23**: 936–944.
13. Deckert, V., L. Persegol, L. Viens, G. Lizard, A. Athias, C. Lallemand, P. Gambert, and L. Lagrost. 1997. Inhibitors of arterial relaxation among components of human oxidized low-density lipoproteins. Cholesterol derivatives oxidized in position 7 are potent inhibitors of endothelium-dependent relaxation. *Circulation*. **95**: 723–731.
14. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. **28**: 412–419.
15. Gowri, M. S., D. R. Van der Westhuysen, S. R. Bridges, and J. W. Anderson. 1999. Decreased protection by HDL from poorly controlled type 2 diabetic subjects against LDL oxidation may be due to the abnormal composition of HDL. *Arterioscler. Thromb. Vasc. Biol.* **19**: 2226–2233.
16. Jacobs, M., F. Plane, and R. K. Bruckdorfer. 1990. Native and oxidized low-density lipoproteins have different inhibitory effects on endothelium-derived relaxing factor in the rabbit aorta. *Br. J. Pharmacol.* **100**: 21–26.
17. Deckert, V., A. Brunet, F. Lantoine, G. Lizard, E. Millanvoe-van Brussel, S. Monier, L. Lagrost, M. David-Dufilho, P. Gambert, and M. A. Devynck. 1998. Inhibition by cholesterol oxides of NO release from human vascular endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **18**: 1054–1060.
18. Kugiyama, K., S. A. Kerns, J. D. Morrisett, R. Roberts, and P. D. Henry. 1990. Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. *Nature*. **344**: 160–162.
19. Jessup, W. 1996. Oxidized lipoproteins and nitric oxide. *Curr. Opin. Lipidol.* **7**: 274–280.
20. Wever, R., E. Stroes, and T. J. Rabelink. 1998. Nitric oxide and hypercholesterolemia: a matter of oxidation and reduction? *Atherosclerosis*. **137**: 51–60.
21. Blair, A., P. W. Shaul, I. S. Yuhanna, P. A. Conrad, and E. J. Smart. 1999. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. *J. Biol. Chem.* **274**: 32512–32519.
22. Christison, J. K., K. A. Rye, and R. Stocker. 1995. Exchange of oxidized cholesteryl linoleate between LDL and HDL by cholesteryl ester transfer protein. *J. Lipid Res.* **36**: 2017–2026.
23. Mackness, M. I., P. N. Durrington, and B. Mackness. 2000. How high-density lipoprotein protects against the effects of lipid peroxidation. *Curr. Opin. Lipidol.* **11**: 383–388.
24. Navab, M., S. Y. Hama, C. J. Cooke, G. M. Anantharamaiah, M. Chaddha, L. Jin, G. Subbanagounder, K. F. Faull, S. T. Reddy, N. E. Miller, et al. 2000. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J. Lipid Res.* **41**: 1481–1494.
25. Ahmed, Z., A. Ravandi, G. F. Maguire, A. Emili, D. Draganov, B. N. La Du, A. Kuksis, and P. W. Connelly. 2001. Apolipoprotein A-I promotes the formation of phosphatidylcholine core aldehydes that are hydrolyzed by paraoxonase (PON-1) during high density lipoprotein oxidation with a peroxy nitrite donor. *J. Biol. Chem.* **276**: 24473–24481.
26. Uittenbogaard, A., P. W. Shaul, I. S. Yuhanna, A. Blair, and E. J. Smart. 2000. High density lipoprotein prevents oxidized low density lipoprotein-induced inhibition of endothelial nitric-oxide synthase localization and activation in caveolae. *J. Biol. Chem.* **275**: 11278–11283.
27. Drew, B. G., N. H. Fidge, G. Gallon-Beaumier, B. E. Kemp, and B. A. Kingwell. 2004. High-density lipoprotein and apolipoprotein A-I increase endothelial NO synthase activity by protein association and multisite phosphorylation. *Proc. Natl. Acad. Sci. USA*. **101**: 6999–7004.
28. Mineo, C., I. S. Yuhanna, M. J. Quon, and P. W. Shaul. 2003. High density lipoprotein-induced endothelial nitric-oxide synthase

activation is mediated by Akt and MAP kinases. *J. Biol. Chem.* **278**: 9142–9149.

29. Nofer, M., Jr., M. van der Giet, I. Tolle, K. Wolinska, H. A. von Wnuck Lipinski, H. A. Baba, U. J. Tietge, A. Godecke, I. Ishii, B. Kleuser, et al. 2004. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor SIP3. *J. Clin. Invest.* **113**: 569–581.
30. Kuvin, J. T., M. E. Rămet, A. R. Patel, N. G. Pandian, M. E. Mendelson, and R. H. Karas. 2002. A novel mechanism for the beneficial vascular effects of high-density lipoprotein cholesterol: enhanced vasorelaxation and increased endothelial nitric oxide synthase expression. *Am. Heart J.* **144**: 165–172.
31. Hansel, B., P. Giral, E. Nobecourt, S. Chantepie, E. Bruckert, M. J. Chapman, and A. Kontush. 2004. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. *J. Clin. Endocrinol. Metab.* **89**: 4963–4971.
32. Kontush, A., E. C. de Faria, S. Chantepie, and M. J. Chapman. 2005. A normotriglyceridemic, low HDL-cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity. *Atherosclerosis*. **182**: 277–285.
33. Curtiss, L. K., D. J. Bonnet, and K. A. Rye. 2000. The conformation of apolipoprotein A-I in high-density lipoproteins is influenced by core lipid composition and particle size: a surface plasmon resonance study. *Biochemistry*. **39**: 5712–5721.
34. Persegol, L., B. Verges, M. Foissac, P. Gambert, and L. Duvillard. 2006. Inability of HDL from type 2 diabetic patients to counteract the inhibitory effect of oxidised LDL on endothelium-dependent vasorelaxation. *Diabetologia*. **49**: 1380–1386.