Alpha Defensins 1, 2, and 3
Potential Roles in Dyslipidemia and Vascular Dysfunction in Humans

Abel López-Bermejo, Berta Chico-Julià, Antoni Castro, Mònica Recasens, Eduardo Esteve, Josefina Biarnés, Roser Casamitjana, Wifredo Ricart, José-Manuel Fernández-Real

Objectives—α-Defensins are natural antibiotics made by neutrophils that have been reported to modulate cholesterol metabolism and vascular function; however, their role in vivo remains largely unknown. We hypothesized that α-defensins 1 to 3 (DEFA1–3) are associated with serum lipids and vascular reactivity in humans.

Methods and Results—One hundred thirteen apparently-healthy White men, participants in a prospective study of cardiovascular risk factors, were assessed for a lipid profile, insulin sensitivity (Si, frequently-sampled intravenous glucose tolerance test), and non-stressed circulating DEFA1–3 (ELISA). In a subset of 52 subjects, vascular reactivity (high-resolution ultrasound of the brachial artery) was also assessed. Subjects in the highest quartile for plasma DEFA1–3 were found to be leaner and more insulin sensitive, and to have significantly reduced total and LDL-cholesterol, compared with subjects in the lowest quartile for circulating DEFA1–3 (P<0.0001 to P=0.002 for linear trend ANOVA). The associations with serum lipids persisted after adjustment for age, body mass index, insulin sensitivity, and smoking (which was associated with reduced plasma DEFA1–3 concentrations). Finally, endothelium-independent vasodilation increased with increasing circulating DEFA1–3 (P=0.003) and this association was not explained by age, body mass index, serum cholesterol, insulin sensitivity, or smoking.

Conclusions—Circulating DEFA1–3 are associated with serum cholesterol and vascular reactivity in humans. α-Defensins may have clinical implications in patients with either hypercholesterolemia or vascular dysfunction. (Arterioscler Thromb Vasc Biol. 2007;27:1166-1171.)

Key Words: human neutrophil peptide ■ defensin ■ cholesterol ■ insulin resistance ■ vascular function

The innate immune system, composed by cellular and humoral responses, is one of the first lines of defense against invading microorganisms. In recent years, it has become evident that alterations in the function of the innate immune system are intrinsically linked to metabolic pathways in humans.1–6

We have previously shown that bactericidal/permeability-increasing protein, a major constituent of neutrophils with antimicrobial function, possesses also antiinflammatory properties, and its serum concentration is associated with metabolic parameters, including plasma triglycerides, and HDL-cholesterol.7

Defensins (also known as human neutrophil peptides) are small cationic peptides (3 to 4 kDa) with broad antibacterial, antiviral, and antifungal properties.8 In humans, α- and β-defensins exist, which differ in their disulfide bond pattern. α-Defensins are predominantly found in neutrophils (mainly DEFA1–3) and in small intestinal Paneth cells, whereas β-defensins have been isolated from both leukocytes and epithelial cells (skin and mucus membranes). Although the main target for DEFA1–3 is the phagolysosomes in granulocytes and monocytes, some DEFA1–3 are also secreted and are present in the circulation.9,10

Besides their role in innate immunity, a number of biological functions for α-defensins have also been described. The peptides were shown both to bind to LDL particles and to favor its uptake by endothelial, smooth muscle cells, (SMC) and fibroblasts, as well as to upregulate its degradation in the latter.11 These events can implicate both the LDL-receptor and the LDL-receptor–related protein/α2-macroglobulin receptor,11,12 as α-defensins were shown to be ligands for these proteins.

α-Defensins bind also to endothelial cells in vitro,13 and it has been proposed that they may play a role in atherosclerosis, based on the fact that they accumulate in the intima of atherosclerotic vessels.14,15

Although these findings suggest possible roles of α-defensins in cholesterol metabolism and vascular function, to our knowledge, these experimental reports have not been followed by in vivo or clinical studies. We hypothesized that...
DEFA1–3 are related to serum lipids and vascular reactivity in humans and addressed this hypothesis by examining the clinical associations between non-stressed circulating DEFA1–3 and both serum lipids and vascular reactivity in apparently-healthy men.

**Methods**

**Subjects**

One hundred thirteen apparently-healthy subjects, consecutively enrolled in a prospective study of cardiovascular risk factors in men, were studied. Subjects were identified from a census in our health area and were invited to participate in the study. Response rate was over 70%. None of these participants had evidence of metabolic disease other than nonmorbid obesity. Indeed, type 2 diabetes was ruled out by an oral glucose tolerance test according to criteria from the American Diabetes Association. Exclusion criteria for this group were: (1) body mass index (BMI) ≥40 kg/m²; (2) history or current clinical evidence of cardiovascular, hepatic, renal, neurological, or endocrine disease; (3) history of drug or alcohol abuse, defined as consumption of more than 80 g of alcohol per day, or serum γ-glutamyl transferase activity over twice the upper normal limit; (4) acute infectious or inflammatory disease; (5) medication use.

All subjects were of White origin and reported that their body weight had been stable for at least 3 months before the study.

Smoking was defined as consumption of one or more cigarettes per day in the last 6 months.

Informed written consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The experimental protocol was approved by the Ethics Committee of the Hospital of Girona.

**Measurements**

Subjects were studied in the postabsorptive state. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Subjects’ waists were measured with a soft tape midway between the lowest rib and the iliac crest; hip circumference was measured at the widest part of the gluteal region; and waist-to-hip ratio (WHR) was accordingly calculated. Blood pressure was measured in the supine position on the right arm after a 10-minute rest; a standard sphygmomanometer of appropriate cuff size was used and the first and fifth phases were recorded. Values used in the analysis are the average of 3 readings taken at 5-minute intervals.

**Insulin Sensitivity Studies**

Insulin sensitivity (S), insulin secretion (AIRg), and glucose effectiveness (SG) were measured by frequently-sampled intravenous glucose tolerance tests (FSIGT). In brief, the experimental protocol for FSIGT studies started between 8:00 and 8:30AM after an overnight fast in subjects without any clinical associations between non-stressed circulating DEFA1–3 and both serum lipids and vascular reactivity in apparently-healthy men.

Serum insulin concentrations were measured in duplicate by a single observer who was blind to the subjects’ clinical and biochemical characteristics.

**Analytical Methods**

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. HDL-cholesterol was quantified after precipitation with polyethylene glycol (PEG) at room temperature. LDL-cholesterol was estimated by the Friedewald formula. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II at room temperature. LDL-cholesterol was estimated by the Friedewald formula. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments). The coefficient of variation was 1.9%. Serum insulin concentrations were measured in duplicate by a monoclonal immuno-radiometric assay (IRMA, Medgenix Diagnostics). Intraassay and interassay coefficients of variation (CV) were less than 7%.

Serum C-reactive protein was determined by an immunoturbidimetric assay (Beckman) with intra- and interassay CV lower than 4% and sensitivity of 1.0 mg/L.

Serum soluble tumor necrosis factor (TNF) receptor 1 (sTNFR1) and receptor 2 (sTNFR2) levels were analyzed using commercially available solid-phase Enzyme Amplified Sensitivity Immunoassays: sTNFR1 and sTNFR2 EASIA ( Biosource Technologies Inc Europe S.A.). The intra- and interassay CV were <7% and <9%, respectively. sTNFR1 EASIA has no cross-reactivity with sTNFR2, and TNF-α does not interfere with the assay.

Serum DEFA1–3 concentrations were measured by an ELISA kit (HyCult Biotechnology) in EDTA plasma samples under basal conditions, ie, after an overnight fast in subjects without any symptoms or signs of clinical infection or disease. Samples were centrifuged at 4°C and plasma was rapidly separated to avoid leukocyte degradation or apoptosis. Sensitivity of the method is 50 pg/mL. The intra- and interassay CV were less than 10%.

**Brachial Artery Vascular Reactivity**

In a subset of 52 subjects who agreed to further participate in the study and whose clinical and biochemical characteristics did not differ significantly from the whole population (see below), vascular reactivity was also assessed.

A high resolution external ultrasound (128XP/10) mainframe with a 7.5-MHz linear array transducer (Toshiba SSH-140A) was used to measure changes in brachial artery diameter in response to reactive hyperemia and in response to 400 μg of sublingual glyceryl trinitrate (GTN), as described by Celermajer et al. The lumen diameter of the artery was defined as the distance between the leading edge of the echo of the near wall-lumen interface to the leading edge of the far wall-lumen interface echo. All scans were taken ECG-triggered coincident with the R wave, which corresponds to end diastole at the brachial artery. All images were recorded with a super-VHS videotape (Panasonic MD-830AG). Endothelial-dependent vasodilatation was secondary to hyperemia induced by inflation of a pneumatic tourniquet placed around the forearm, distal to the scanned part of the artery, at a pressure of 300 mm Hg for 5 minutes, followed by sudden deflation; it is expressed as the percentage of change in the arterial diameter one minute after hyperemia. Endothelial-independent vasodilatation is induced after sublingual administration of a 400-μg metered dose of GTN (Solitinrina, Almirall Prodesfarma, Barcelona, Spain) and expressed as the percentage of change in the arterial diameter 3 minutes later. Reactive hyperemia is calculated as the percentage change between the maximum flow recorded in the first 15 seconds after cuff deflation and the flow during the resting scan.

A first scan was recorded after 10 minutes of resting in a quiet room in the supine position. Then the tourniquet was inflated for 5 minutes. A second scan was recorded during 90 seconds beginning 10 seconds before cuff deflation. After at least 10 more minutes of rest, a new control scan was recorded. A last scan was recorded after 2 minutes of GTN administration for 70 seconds. The arterial diameter was assessed in 4 different cardiac cycles for each condition, and the measurements were averaged.

Reproducibility of this technique at our center has been previously reported. Because agreement in measuring both EDV and EIV in our trained personnel was high, recorded scans in the present study were analyzed by a single observer who was blind to the subjects’ clinical and biochemical characteristics.

**DEFA1–3 are related to serum lipids and vascular reactivity in humans and addressed this hypothesis by examining the clinical associations between non-stressed circulating DEFA1–3 and both serum lipids and vascular reactivity in apparently-healthy men.**
TABLE 1. Clinical and Laboratory Variables According to Quartiles of Defa1–3 in the Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;225</th>
<th>225 to 290</th>
<th>291 to 430</th>
<th>&gt;430</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>12 (41)</td>
<td>10 (36)</td>
<td>7 (25)</td>
<td>6 (21)</td>
<td>0.07</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 [0.08]</td>
<td>0.95 [0.05]</td>
<td>0.94 [0.08]</td>
<td>0.91 [0.07]</td>
<td>0.019</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.7 [0.7]</td>
<td>5.4 [0.8]</td>
<td>5.4 [0.9]</td>
<td>5.0 [0.8]††</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>3.7 [0.7]</td>
<td>3.5 [0.7]</td>
<td>3.5 [0.8]</td>
<td>3.1 [0.8]† †</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.3 [0.4]</td>
<td>1.3 [0.3]</td>
<td>1.4 [0.3]</td>
<td>1.5 [0.4]</td>
<td>0.008</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1 (0.8–2.0)</td>
<td>1.0 (0.8–1.3)</td>
<td>0.9 (0.6–1.5)</td>
<td>0.9 (0.6–1.2)</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.4 [0.5]</td>
<td>5.3 [0.5]</td>
<td>5.3 [0.7]</td>
<td>5.3 [0.6]</td>
<td>ns</td>
</tr>
<tr>
<td>GGT, 2h glucose, mmol/L</td>
<td>7.3 [2.0]</td>
<td>7.1 [1.4]</td>
<td>6.9 [1.8]</td>
<td>6.4 [1.7]</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>64 (46–100)</td>
<td>66 (43–84)</td>
<td>64 (42–105)</td>
<td>55 (37–72)</td>
<td>ns</td>
</tr>
<tr>
<td>S₁, min⁻¹·ml/l·10⁻⁴</td>
<td>2.0 (1.0–2.8)</td>
<td>2.3 (1.8–2.6)</td>
<td>2.2 (1.0–4.1)</td>
<td>3.2 (2.3–4.8)†</td>
<td>0.002</td>
</tr>
<tr>
<td>A1Rg, min · mL/U</td>
<td>326 (167–448)</td>
<td>443 (199–538)</td>
<td>359 (214–578)</td>
<td>290 (106–438)</td>
<td>ns</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.0 (1.0–4.3)</td>
<td>3.0 (1.0–6.0)</td>
<td>2.0 (1.0–4.0)</td>
<td>1.0 (1.0–4.0)</td>
<td>ns</td>
</tr>
<tr>
<td>sTNFR1, µg/L</td>
<td>1.7 (1.4–2.2)</td>
<td>1.7 (1.2–2.1)</td>
<td>1.5 (1.2–1.7)</td>
<td>1.8 (1.4–2.1)</td>
<td>ns</td>
</tr>
<tr>
<td>sTNFR2, µg/L</td>
<td>4.3 (3.1–5.7)</td>
<td>5.2 (3.6–8.9)</td>
<td>6.9 (4.6–11.6)</td>
<td>5.3 (3.7–8.5)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Defa1–3 indicates α-defensins 1–3; BMI, body mass index; WHR, waist-to-hip ratio; SBP & DBP, systolic and diastolic blood pressure; GGT, oral glucose tolerance test; S₁, Sₙ, and A1Rg, insulin sensitivity, glucose effectiveness and acute insulin response to glucose (from frequently-sampled intravenous glucose tolerance tests); CRP, C-reactive protein; sTNFR1 & 2, soluble tumor necrosis factor receptor 1 & 2. Data are mean [SD] for Gaussian variables and median and interquartile range for non-Gaussian variables. P values shown on the right are for linear trend (ANOVA), except for smoking comparisons (Linear-by-Linear Association). Post-hoc comparisons (Bonferroni): *P<0.05 and †P<0.01, compared to first quartile. General linear models: †P<0.05 and §P<0.01, compared to first quartile, after adjustment for age (BMI), and age, smoking, BMI, and S (total and LDL-cholesterol).

Statistical Methods

Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and SD for Gaussian variables, and median and interquartile range for non-Gaussian variables. Parameters that did not fulfill normal distribution were mathematically transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Pearson test) and multiple regression in a stepwise manner. One-way ANOVA followed by analysis of covariance using general linear models (to correct for effect modifiers, such as age, BMI, serum cholesterol, insulin sensitivity, or smoking), with Bonferroni correction as the post-hoc test, were used to seek differences in plasma Defa1–3 concentrations among groups. Levels of statistical significance were set at P<0.05.

For a given value of P=0.05, the study had an 80% power to detect significant correlations between serum Defa1–3 and metabolic and vascular parameters (Pearson coefficient of at least 0.26 and 0.38, respectively) in bilateral tests. The study was also powered to detect significant differences of at least 0.4 SD in either total or LDL-cholesterol between subjects in the highest and lowest quartiles for Defa1–3, and differences of at least 0.6 SD in either endothelium-dependent or independent vasodilation between subjects with Defa1–3 above and below the corresponding median values.

Results

Please see supplemental materials, available online at http://atvb.ahajournals.org, for clinical and biochemical variables of the study subjects.

Circulating Defa1–3, Body Mass Index, and Serum Cholesterol in Apparently-Healthy Men

To study whether Defa1–3 are associated with metabolic parameters in humans, the study subjects were stratified according to Defa1–3 quartiles. Significant associations were evident between circulating Defa1–3 and BMI, WHR, serum lipids, and S₁ (P<0.02 to P=0.001 for linear-trend ANOVA for comparisons across Defa1–3 quartiles; Table 1). Figures 1 and 2 show the associations between circulating Defa1–3 and BMI, S₁, and both total and LDL-cholesterol (subjects in the second and third quartiles of circulating Defa1–3 had similar anthropometric and biochemical variables and are therefore shown together).

We further tested whether the associations between circulating Defa1–3 and metabolic parameters were independent of known effect modifiers. After adjustment for age (in the case of BMI) and age, smoking, BMI, and S₁ (in the case of serum lipids), subjects in the highest quartile for plasma Defa1–3 were shown to be significantly leaner (P<0.01) and to exhibit lower total and LDL-cholesterol (P<0.05), compared with subjects in the lowest quartile (Table 1).

Despite these associations with metabolic parameters, circulating Defa1–3 were unrelated to inflammatory markers (C-reactive protein and both sTNFR1 and 2; Table 1).
Circulating DEFA1–3 and Vascular Function in Apparently-Healthy Men

In a subpopulation of 52 apparently-healthy men (please see supplemental materials), vascular reactivity of the brachial artery was also assessed. We documented significantly increased endothelium-independent vasodilation (EIV) in those subjects with DEFA1–3 values above versus below the median for the study population [mean (95% CI): 18% (15% to 22%) versus 12% (9% to 15%), respectively; P<0.005 (non-adjusted) and P=0.017 (adjusted for age, smoking, BMI, total or LDL-cholesterol, S_I and basal artery diameter)]. This difference was remarkable in overweight (BMI ≥25 kg/m²) subjects (n=40; adjusted P<0.005; Figure 3). No significant differences were found in endothelium-dependent vasodilation in either the whole group or in overweight (adjusted P=0.39; Figure 3) subjects.

Bivariate and Multiple Regression Analyses

On bivariate correlations, DEFA1–3 were significantly associated with BMI (r=−0.29, P=0.002), WHR (r=−0.23, P=0.014), total and LDL cholesterol (r=−0.29, P=0.002 and r=−0.27, P=0.003, respectively), serum triglycerides (r=−0.26, P=0.006), and S_I (r=0.27, P=0.005). Significant associations were also evident between circulating DEFA1–3 and EIV (r=0.29, P=0.040 and r=0.40 P=0.011, for all subjects and for overweight subjects, respectively).

A significant effect of smoking on circulating DEFA1–3 was evident, with smokers exhibiting reduced DEFA1–3 versus nonsmokers [mean (95% CI): 324 pg/mL (206 to 441 pg/mL) versus 432 pg/mL (362 to 502 pg/mL), respectively; P<0.05]. This effect was independent of age, BMI, total or LDL-cholesterol, and S_I (P<0.05, adjusted for these covariates).

On multiple regression analysis, BMI (β=−0.26, P=0.005), total or LDL-cholesterol (β=−0.22, P=0.015), and smoking (β=−0.18, P=0.044), but not age or S_I, were independent predictors of circulating DEFA1–3, explaining 8%, 4%, and 3% of its variance, respectively (the proportion of DEFA1–3 variance explained by either total or LDL-cholesterol increased to 11% in overweight subjects; n=89).

On multiple regression analysis, BMI (β=−0.26, P=0.005), total or LDL-cholesterol (β=−0.22, P=0.015), and smoking (β=−0.18, P=0.044), but not age or S_I, were independent predictors of circulating DEFA1–3, explaining 8%, 4%, and 3% of its variance, respectively (the proportion of DEFA1–3 variance explained by either total or LDL-cholesterol increased to 11% in overweight subjects; n=89).

On multiple regression analysis, BMI (β=−0.26, P=0.005), total or LDL-cholesterol (β=−0.22, P=0.015), and smoking (β=−0.18, P=0.044), but not age or S_I, were independent predictors of circulating DEFA1–3, explaining 8%, 4%, and 3% of its variance, respectively (the proportion of DEFA1–3 variance explained by either total or LDL-cholesterol increased to 11% in overweight subjects; n=89).


**Discussion**

α-Defensins are related to at least 2 modifiable cardiovascular risk factors: obesity and smoking. Inverse associations are readily apparent in healthy subjects. In addition, α-defensins are inversely related to serum total and LDL-cholesterol and directly related to vasodilation in response to an NO donor.

α-Defensins are abundant proteins, comprising half of the azurophilic granule protein and 5% of the total protein content of human neutrophils. Although their normal plasma concentrations are low, they can reach concentrations in the micromolar range in patients with sepsis or bacterial meningitis. Other than this increase during bacterial infections (which is likely to respond to mobilization of stored peptides in phagocytes in response to bacterial products), the regulation of α-defensins in vivo is largely unknown. In fact, it has been proposed that the expression of α-defensins is constitutive under normal conditions. This is in contrast to β-defensins, whose expression is known to be upregulated by both bacterial products and proinflammatory cytokines.

Our data suggest for the first time possible roles of obesity and smoking decreasing circulating DEFA1–3. In this sense, we have recently reported that obesity is associated with decreased bactericidal/permeability-increasing protein (a natural antibiotic also found in azurophilic granules of neutrophils).7,25

Besides their role in the defense against invading microorganisms, DEFA1–3 are suggested to be novel modulators of lipid metabolism and vascular function. To our knowledge, ours is also the first description of a clinical association between DEFA1–3 and both serum lipids and vascular reactivity. A relative deficiency in these α-defensins was found to be associated with a more atherogenic lipid profile; decreased α-defensins was also associated with poorer vaso-motor responses to an endothelium-independent vasodilator, a known independent risk factor for atherosclerosis.24 Although the strength of the association of DEFA1–3 with each of the variables examined in our study was relatively low, it is well known that risk factors for atherosclerosis tend to cluster, and therefore small individual effects may be clinically relevant if added together.

Our data concur with those by Higazi et al who showed that the peptides were able to bind to LDL particles, to interact with both the LDL-receptor and the LDL-receptor-related protein/α2-macroglobulin receptor, and to favor the uptake and degradation of LDL particles in vascular cells. They also concur with data from the same group showing that α-defensins were able to bind to the LDL-receptor–related protein/α2-macroglobulin receptor on smooth muscle cells and inhibit the phenylephrine-induced smooth muscle contraction of rat aortic rings.

A plausible interpretation of the experimental data so far is that α-defensins can induce LDL-cholesterol catabolism and improve vascular tone in vivo, and that decreased production of these peptides in neutrophils of obese subjects and smokers can result in a functional deficiency of these peptides. However, arguments can also be found for opposing roles of DEFA1–3 in cholesterol metabolism and vascular reactivity, and we cannot exclude the possibility that the decrease of DEFA1–3 observed in our obese subjects and smokers could respond to increased deposition of these peptides in the vascular wall.

---

**TABLE 2. Stepwise Multiple Regression Analyses of LDL Cholesterol and Endothelium-Independent Vasodilation as Dependent Variables in Apparently-Healthy Subjects**

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>LDL Cholesterol</th>
<th>EV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.05</td>
<td>0.62</td>
</tr>
<tr>
<td>Smoking</td>
<td>−0.06</td>
<td>0.51</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.03</td>
<td>0.82</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DEFA1–3, pg/mL</td>
<td>−0.23</td>
<td>0.018*</td>
</tr>
<tr>
<td>S, min⁻¹·ml/L·10⁻⁴</td>
<td>−0.20</td>
<td>0.036*</td>
</tr>
<tr>
<td>Basal artery diameter</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; S, insulin sensitivity (from frequently-sampled intravenous glucose tolerance tests); DEFA1–3: α-defensins 1–3.

*Predictive variables.

Adjusted R² for LDL cholesterol: DEFA1–3: 0.08; S: 0.03.

Adjusted R² for EV: BMI 0.22; DEFA1–3: 0.09.
Along these lines, early studies reported that α-defensins immunostaining was increased in atherosclerotic versus normal human arteries. More recently, it was shown that α-defensins inhibit the fibrinolytic effect of tissue plasminogen activator and the vascular dilatation in response to bradykinin. Based on these observations, it has been proposed that α-defensins may exert detrimental actions in the vasculature.

It should be noted that the experimental conditions of these in vitro studies (supraphysiological versus physiological doses of peptides, specific cellular or tissue characteristics, and reagents and techniques used) may be responsible for some of the apparent paradoxical effect of α-defensins described so far, and that some of the apparently opposing roles of these peptides in the vasculature are reminiscent of those reported for tissue-plasminogen activator, which causes vasodilation at low doses and vasoconstriction at high doses.

As to the mechanisms linking α-defensins with both metabolic parameters and vascular reactivity, a contribution of protein kinase C isozymes is possible, as the enzyme, a possible target of α-defensins, is known to be involved in insulin signaling, cholesterol metabolism and vascular function. In summary, circulating nonstressed α-defensins are associated with serum cholesterol and vascular reactivity in humans. α-Defensins may have clinical implications in patients with either hypercholesterolemia or vascular dysfunction. Further studies in experimental models of dyslipidemia and atherosclerosis and clinical studies in patients with these diseases are merited.

**Sources of Funding**

This work was supported, in part, by grants PI041407 (to A.L.B.) and PI041383 and CB06/03 (Ciber Fisiopatologia de la Obesidad, to J.M.F.-R.) from the Fondo de Investigación Sanitaria, Health Institute Carlos III, Spain), and grant BFU2004–03654/BFI (to J.M.F.-R.) from the Fondo de Investigación Sanitaria, Health Institute Carlos III, Spain), and grant BFU2004 – 03654/BFI (to J.M.F.-R.) from the Fondo de Investigación Sanitaria, Health Institute Carlos III, Spain, Spain. A.L.B. is a Research Investigator of the Fund for Scientific Research “Ramón y Cajal” (Ministry of Education and Science, Madrid, Spain).

**Disclosures**

None.

**References**

Alpha Defensins 1, 2, and 3: Potential Roles in Dyslipidemia and Vascular Dysfunction in Humans
Abel López-Bermejo, Berta Chico-Julía, Antoni Castro, Mònica Recasens, Eduardo Esteve, Josefina Biarnés, Roser Casamitjana, Wifredo Ricart and José-Manuel Fernández-Real

Arterioscler Thromb Vasc Biol. 2007;27:1166-1171; originally published online February 15, 2007;
doi: 10.1161/ATVBAHA.106.138594

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/5/1166

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2007/02/15/ATVBAHA.106.138594.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/