Palmitate-Induced Interleukin-6 Expression in Human Coronary Artery Endothelial Cells

Harald Staiger,1 Katrin Staiger,1 Norbert Stefan,1 Hans Günther Wahl,2 Fausto Machicao,1 Monika Kellerer,1,3 and Hans-Ulrich Häring1

Obesity-linked insulin resistance is associated with chronic inflammation and cardiovascular complications. Free fatty acids (FFAs) are prominent candidates for the molecular link between these disorders. In this study, we determined whether FFAs contribute to vascular inflammation via induction of interleukin (IL)-6 in coronary artery endothelial cells (CAECs) and coronary artery smooth muscle cells (CASMCs) and whether this is reflected in vivo. In contrast to our findings regarding IL-6 and gp130 (the glycoprotein of 130 kDa) expression, IL-6 receptor mRNA expression was very low in these cells. Palmitate, but not linoleate, induced a significant increase in IL-6 mRNA expression in CAECs (P < 0.001) and, to a less relevant extent, in CASMCs (P < 0.01). gp130 remained unaffected. As to potency, palmitate was comparable with the IL-6–inducer IL-1β.

To substantiate our in vitro data, we examined the plasma FFA pattern in 54 healthy human subjects and studied the relation of individual FFAs with plasma IL-6. IL-6 levels correlated with palmitate, but not with other abundant FFAs, even after adjusting for body fat (r = 0.33, P < 0.05) and total FFAs (r = 0.29, P < 0.05). We show here that the common plasma FFA palmitate induces high levels of IL-6 in CAECs. Furthermore, palmitate correlates with IL-6 in vivo. This points to a potential contribution of palmitate to vascular inflammation. Diabetes 53:3209–3216, 2004

The metabolic syndrome, comprised of disorders such as obesity, insulin resistance, and type 2 diabetes, as well as hypertension, dyslipidemia, endothelial dysfunction, and atherosclerosis, is the prevailing metabolic disorder in Western industrialized countries. This syndrome is thought to be caused by environmental factors, such as high-caloric food intake and sedentary lifestyle, combined with a genetic predisposition. High levels of plasma free fatty acids (FFAs) are often seen in patients suffering from the metabolic syndrome and are mainly the result of high fat intake and/or increased adipose tissue lipolysis. It is well known that abnormally high plasma FFA levels have multiple detrimental effects on peripheral tissues (i.e., muscle, liver, pancreas). FFAs, especially saturated FFAs, impair glucose metabolism in skeletal muscle, attenuate insulin clearance and enhance VLDL production in the liver, and provoke β-cell dysfunction (rev. in 1). Therefore, FFAs are important players in the pathogenesis of the metabolic syndrome.

Many, if not all, features of the metabolic syndrome are closely associated with chronic inflammation (rev. in 2). One of the most interesting inflammatory cytokines known to be elevated in obesity, insulin resistance, and atherosclerosis is interleukin (IL)-6, which is released in substantial amounts not only by monocytes and hepatic Kupffer cells, but also by endothelial cells (3,4), skeletal muscle cells (5), and adipocytes (6). IL-6 is reported to be a pleiotropic cytokine involved in the regulation of acute-phase reactions, immune responses, and hematopoiesis. IL-6–mediated induction of acute-phase reactants in the liver increases blood viscosity and promotes thrombus formation (7,8). In addition, IL-6 impairs hepatocyte insulin signaling (9) and adipocyte production of the insulin-sensitizing hormone adiponectin (10). Furthermore, a functional polymorphism within the IL-6 gene promoter (C-174G) was recently shown to affect plasma lipids and insulin sensitivity (11-13). Therefore, IL-6 seems to be a crucial player in the metabolic syndrome as well.

In this study, we determined whether FFAs could play a role in inflammation via regulation of IL-6 production. In particular, we tested whether the FFAs palmitate (C16:0) and linoleate (C18:2 ω6) stimulate the expression of the IL-6 system (IL-6, IL-6 receptor [IL-6R], and the glycoprotein of 130 kDa [gp130]) in endothelial and smooth muscle cells from human coronary arteries. Moreover, we analyzed the plasma FFA patterns of 54 healthy subjects and examined the relation between individual and grouped (saturated and unsaturated) FFAs and plasma IL-6 concentrations.

RESEARCH DESIGN AND METHODS

Endotoxin detection. The endotoxin content of sterile-filtered solutions of BSA and BSA-bound fatty acids (concentrations described below) was measured with the HyCult biotechnology limulus amebocyte lysate chromogenic end point assay (Cell Sciences, Canton, MA) according to the manufacturer's instructions. Measurements were performed in duplicate.

Cell culture. Human coronary artery endothelial cells (CAECs) and coronary artery smooth muscle cells (CASMCs) as well as the corresponding cell
TABLE 1

<table>
<thead>
<tr>
<th>Condition for RT-PCR quantification of mRNAs</th>
<th>Forward primer (5′→3′)</th>
<th>Reverse primer (5′→3′)</th>
<th>T&lt;sub&gt;annealing&lt;/sub&gt; (°C)</th>
<th>No. of cycles</th>
<th>MgCl&lt;sub&gt;2&lt;/sub&gt; (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>CCAGCTTGAACCTCCTTCTC</td>
<td>GCTTGTTCCTCCTCATCCTC</td>
<td>63</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>IL-6R</td>
<td>GACACTGGCCTGTCATTG</td>
<td>GCTAACTGCGGAGGAACTTT</td>
<td>65</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td>gp130</td>
<td>ACAGAACAGCTCCAGTGGT</td>
<td>AATCTGGCTCCAGTGGAGG</td>
<td>70</td>
<td>45</td>
<td>3</td>
</tr>
</tbody>
</table>

Culture media were obtained from Clonetics/BioWhittaker (Verviers, Belgium). The cells, derived from healthy Caucasian donors, were cultured according to the supplier’s instructions. After reaching 80–90% confluence, the cultures were incubated for 20 h with 1.25% (wt/vol) fatty acid–free BSA (Sigma-Aldrich, Taufkirchen, Germany) for controls or the indicated concentrations of fatty acids (Sigma-Aldrich) bound to BSA, as recently described (14). In brief, fatty acids (200 mmol/l in ethanol) were diluted 1:25 into Krebs Ringer HEPES buffer containing 20% (wt/vol) BSA. This mixture was gently agitated at 37°C under nitrogen overnight. The control medium containing ethanol and BSA was prepared in a similar manner. These stock solutions were stored in aliquots under nitrogen at −20°C. At the highest fatty acid concentration used (500 μmol/l), BSA reached a concentration of 1.25% (wt/vol) in the medium. Human recombiant IL-1β and tumor necrosis factor-α (TNF-α; TEBU, Offenbach, Germany) were water soluble and therefore no carrier controls were performed. Trypsin C, fumonisin B<sub>1</sub>, C-2 ceramide, Wy14643, troglitazone, GW501516, and etomoxir were dissolved in DMSO; DMSO was then used as the carrier control. Cycloheximide were water soluble.

Flow cytometric determination of cell death and viability. Cells were treated as indicated. Detached cells were harvested from the supernatant by centrifugation and added to the nondenatured cells harvested by trypsinization. Cells were washed with PBS and double-stained with propidium iodide (1 μg/ml) and Annexin-V-Fluos (Roche Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. Stained cells were subjected to flow cytometry (FACSCalibur cytometer; Becton Dickinson, Heidelberg, Germany). The percentage of necrotic, apoptotic, and vital cells was calculated by CellQuest software (Becton Dickinson).

RT-PCR. RNA was isolated with peqGOLD TriFast according to the manufacturer’s instructions (Peqlab, Erlangen, Germany). Total RNA treated with RNase-free DNase I was transcribed into cDNA using Avian myoblastosis virus reverse transcriptase and the first strand cDNA kit from Roche Diagnostics. Quantitative PCR was performed with SYBR Green I dye on a high-speed thermal cycler with an integrated microvolume fluorometer, according to the manufacturer’s instructions (Roche Diagnostics). The primers were obtained from Invitrogen (Karlsruhe, Germany); PCR conditions are given in Table 1. Measurements were performed in triplicate. mRNA contents are given in arbitrary units (AU), with 1 AU corresponding to 1 ng mRNA/100 ng total RNA.

Cytokine protein quantification. Intracellular and secreted proteins (IL-6, TNF-α), as well as serum IL-6 proteins were quantified with Quantikine enzyme-linked immunosorbent assays (ELISAs) from R&D Systems (Wiesbaden, Germany). To measure cytokines in cell lysates, cell monolayers were washed with PBS and scraped off in PBS supplemented with 1 mmol/l EDTA, 1 mmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 0.5 μg/ml leupeptin, and 0.7 μg/ml pepstatin. After cells were lysed by sonication, the lysates were cleared by centrifugation. To determine TNF-α and IL-1β content, lysates were measured undiluted; to determine IL-6 content, lysates were diluted 1:10 with calibrator diluent. Measurements were performed in duplicate. To achieve standardization, the cellular protein of the lysates was determined with the Bradford method. Intracellular IL-6 protein content is given in picograms per milligram of cellular protein. Cytokines in cell culture supernatants were measured after being centrifuged in 1:100 diluted (IL-6) and undiluted (TNF-α and IL-1β) samples. Results are given in picograms (IL-6) or picograms (TNF-α and IL-1β) per milliliter. To determine IL-6 content in serum, the samples were cleared by centrifugation and measured without dilution. Serum IL-6 content is expressed in picograms per milliliter.

Determination of blood parameters. We recruited 54 healthy nondiabetic subjects (determined according to World Health Organization criteria) (15) from the Tübingen Family Study for type 2 diabetes, a cohort of ~1,200 anthropometrically (sex, age, BMI, waist-to-hip ratio, and percentage of body fat [determined by bioimpedance]) and metabolically (oral glucose tolerance test, hyperinsulinemic-euglycemic clamp) characterized Caucasians. The subjects were not taking any medication known to affect glucose tolerance, insulin sensitivity, or insulin secretion. All subjects gave informed written consent before the drawing of blood samples. Subject characteristics are presented in Table 2. The study was approved by the local ethics committee.

Fasting blood parameters were determined after a 10-h overnight fast. Serum IL-6 content was measured as described above. C-reactive protein was photometrically determined with the wide range C-reactive protein assay for the high-throughput chemistry system ADVIA 1650 (Bayer Diagnostics, Tarrytown, NY). Plasma FFA concentrations were determined by an enzymatic method (NEFAC kit; Waco Chemicals, Neuß, Germany). The fatty acid composition of the plasma FFA fraction was determined as recently described in detail (16,17). In brief, total plasma lipids were extracted and separated into five fractions (phospholipids, triglycerides, diglycerides, cholesterol esters, and FFA's) by thin-layer chromatography. Individual and grouped (saturated and unsaturated) FFAs are given as the percentage of the total FFA fraction.

Statistical analysis. In vitro data were analyzed by unpaired Student’s t test. Data from the human cohort were log transformed (ln) to approximate a normal distribution. These data were analyzed by linear regression analysis and ANOVA. Adjusted values were derived from a multivariate linear regression model. These transformations and tests were performed with the statistical software package JMP 4.0 (SAS Institute, Cary, NC). P < 0.05 was considered statistically significant. Data are given as means ± SE.

RESULTS

Endotoxin content of FFA preparations. The solution of 1.25% (wt/vol) fatty acid–free BSA (control condition)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>n (F/M)</td>
<td>54 (31/23)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.8 ± 1.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ± 0.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25.5 ± 1.3</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>Myristate (C14:0)</td>
<td>2.86 ± 0.11</td>
</tr>
<tr>
<td>Pentadecanoate (C15:0)</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Palmitate (C16:0)</td>
<td>27.8 ± 0.4</td>
</tr>
<tr>
<td>Stearate (C18:0)</td>
<td>12.1 ± 0.6</td>
</tr>
<tr>
<td>Arachidate (C20:0)</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Behenate (C22:0)</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Σ Saturated fatty acids</td>
<td>47.3 ± 0.8</td>
</tr>
<tr>
<td>Palmitoleate (C16:1 ω7)</td>
<td>4.31 ± 0.19</td>
</tr>
<tr>
<td>Oleate (C18:1 ω9)</td>
<td>31.6 ± 0.8</td>
</tr>
<tr>
<td>Viscenate (C18:1 ω7)</td>
<td>3.33 ± 0.22</td>
</tr>
<tr>
<td>Linoleate (C18:2 ω6)</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>Ω-Linolenate (C18:3 ω3)</td>
<td>2.25 ± 0.19</td>
</tr>
<tr>
<td>γ-Linolenate (C18:3 ω6)</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Dihomo-γ-linolenate (C20:3 ω6)</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Arachidonate (C20:4 ω6)</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Eicosapentaenoate (C20:5 ω3)</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Docosatetraenoate (C22:4 ω6)</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Docosapentaenoate (C22:5 ω3)</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Docosahexaenoate (C22:6 ω3)</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

Σ Unsaturated fatty acids | 56.3 ± 0.8 |

Data are means ± SE. Individual and grouped fatty acids are given as the percentage of the total plasma FFA fraction.
contained 2.0 ng/ml endotoxin. The 500-μmol/l palmitate solution (in 1.25% BSA) revealed an endotoxin content of 2.8 ng/ml. These very low endotoxin concentrations are, according to earlier studies in human umbilical vein endothelial cells (18) and human CAECs (19), not expected to induce significant amounts of IL-6. The 500-μmol/l linoleate solution (in 1.25% BSA) exhibited an endotoxin content of 19.7 ng/ml. For this still low concentration, however, we could not exclude minor endotoxin effects on IL-6 production.

**Effect of FFAs on the mRNA expression of the IL-6/IL-6R system in coronary artery cells.** The expression of IL-6, IL-6R, and the co-receptor protein gp130 was studied in human CAECs and CASMCs after they were treated with BSA (control), 500 μmol/l linoleate, or 500 μmol/l palmitate for 20 h. Microscopically, no significant cytotoxic FFA effects could be detected under these conditions. As assessed by flow cytometry, the viable cell fractions (≥80%), the apoptotic and necrotic cell fractions (~4% each), as well as the fractions containing undistinguishable late apoptotic/necrotic cells (~10%) of BSA- and palmitate-treated cultures were not significantly different. As presented in Fig. 1, neither cell type expressed remarkable amounts of IL-6 mRNA (~6 fg/μg total RNA). However, gp130 mRNA was expressed in both cell types and its level was at least twice as high in CASMCs as in CAECs. IL-6 mRNA was also readily detectable in both cell types. Although the IL-6R components, IL-6R and gp130, were not affected by any FFA in both cell types, the saturated FFA palmitate induced an 18-fold increase of IL-6 mRNA expression in CAECs (~2,800-fold more potent than IL-1β (n = 3, P = 0.0003) and TNF-α (100 ng/ml), two well-known stimuli of IL-6 expression. Under these conditions, palmitate turned out to be 3- and 10-fold more potent than IL-1β (n = 3, P = 0.0021) and TNF-α (n = 3, P = 0.0002), respectively, in CAECs (Fig. 3A). In CASMCs, palmitate was two- and threefold more effective than IL-1β (n = 4, NS) and TNF-α (n = 4, P = 0.0171), respectively (Fig. 3B). To further assess the fatty acid specificity of IL-6 gene induction, we treated CAECs and CASMCs with other saturated and unsaturated long-chain FFAs, such as stearate (C18:0), palmitoleate (C16:1

![A](brooklynbridgespan.jpg) ![B](brooklynbridgespan.jpg)

**FIG. 1.** Effect of FFAs on the expression of the IL-6/IL-6R system in CAECs (A) and CASMCs (B). The cells were treated with 1.25% BSA (control), 500 μmol/l linoleate, or 500 μmol/l palmitate for 20 h. IL-6, IL-6R, and gp130 mRNA were quantified by RT-PCR. Data are means ± SE of three independent experiments. *Significantly different from control (CAECs: P = 0.0003; CASMCs: P = 0.0089; t test).

![Graph](brooklynbridgespan.jpg)

**FIG. 2.** Concentration dependence of palmitate-induced IL-6 mRNA expression in CAECs and CASMCs. The cells were treated with the indicated palmitate concentrations; 20 h later, IL-6 mRNA was quantified by RT-PCR. Data are means ± SE of four independent experiments. *Significantly different from basal (250 μmol/l palmitate: P = 0.0357 for CAECs, P = 0.0014 for CASMCs; 500 μmol/l palmitate: P = 0.0499 for CAECs, P = 0.0019 for CASMCs; t test).
ω7), oleate (C18:1 ω9), and the poorly metabolizable palmitate analog 2-bromo-palmitate, at 500 μmol/l each. At this concentration, stearate turned out to be toxic to both cell types and, therefore, was not evaluated. In CAECs, palmitoleate (n = 4, P = 0.0163) and oleate (n = 4, P = 0.0339) significantly increased IL-6 mRNA expression five- and eightfold, respectively, but were far less potent than palmitate (Fig. 4A). 2-Bromo-palmitate showed no inducive effect at all (Fig. 4A). All FFAs except linoleate significantly raised the IL-6 mRNA content of CASMCs (n = 4, P < 0.01 each) (Fig. 4B). It is worth noting, however, that the induction factors seen in CASMCs (two- to threefold) were markedly lower than those observed in CAECs, as was already shown for palmitate (Fig. 1).

**FFA- and cytokine-stimulated IL-6 protein expression in coronary artery cells.** As expected from the mRNA data, palmitate (500 μmol/l) also promoted IL-6 protein expression (Fig. 5). Under control conditions, no IL-6 protein was detectable. The protein concentrations reached after palmitate treatment in CAECs were about twofold higher than those seen in CASMCs (Fig. 5A and B). Furthermore, the palmitate-induced IL-6 levels were comparable with those stimulated by IL-1β in both cell types (Fig. 5). Thus, the significant difference in IL-6 mRNA contents between palmitate- and IL-1β-treated CAECs (Fig. 3A) was not observed at the protein level. This result probably reflected differences in the kinetics of IL-6 induction, possibly caused by differences in the underlying molecular mechanisms triggered by these two stimuli. Consistent with the mRNA data, TNF-α was a less potent inducer of IL-6 protein expression in both cell types (Fig. 5). The unsaturated FFA linoleate (500 μmol/l) did not induce IL-6 protein expression in CAECs (Fig. 5A). It was surprising that linoleate provoked IL-6 protein expression to some extent in CASMCs (Fig. 5B). This induction probably reflected the minor nonsignificant increase in IL-6 mRNA induced by linoleate in these cells (Fig. 1B).

Furthermore, we tried to determine secreted IL-6 protein in the conditioned media of cells treated with BSA, FFAs (palmitate and linoleate), and cytokines (TNF-α and IL-1β). However, for still unresolved technical reasons, we were not able to quantify IL-6 protein in the media containing BSA or BSA-bound FFAs. TNF-α (100 ng/ml) and IL-1β (2 ng/ml), however, provoked high levels of secreted IL-6 protein in culture supernatants of CAECs (1.07 ± 0.16 and 12.27 ± 3.37 ng · ml⁻¹ · 20 h⁻¹, respectively).
and CASMCs (6.77 ± 4.41 and 9.46 ± 4.21 ng·ml⁻¹·20 h⁻¹, respectively). In addition, we examined whether palmitate and linoleate (500 μmol/l each) stimulate TNF-α and IL-1β protein expression or release. In both cell types, neither intracellular nor secreted TNF-α protein was detectable after BSA or FFA treatment. Moreover, CAECs did not produce measurable amounts of intracellular or secreted IL-1β protein. By contrast, CASMCs expressed relevant levels of intracellular IL-1β protein (not shown) that was also secreted into the culture medium (73.5 ± 10.8 [control] vs. 130.2 ± 14.9 [palmitate] vs. 85.8 ± 11.0 [linoleate] pg·ml⁻¹·20 h⁻¹). Palmitate, but not linoleate, induced a significant twofold rise in secreted IL-1β protein (n = 4, P = 0.0216).

**Potential mechanisms of palmitate-induced IL-6 expression.** Regarding the molecular mechanism underlying palmitate-induced IL-6 mRNA expression, we examined the involvement of mitochondrial β-oxidation, ceramide formation, and activation of peroxisome proliferator-activated receptors (PPARs), a class of FFA-activated nuclear receptors and transcription factors. We did so by treating the cells with the carnitine palmitoyl transferase 1 inhibitor etomoxir, inhibitors of the ceramide synthesis pathway (triacsin C, C-2 ceramide, and finally isoform-specific PPAR agonists (Wy14643, troglitazone, and GW501516). None of these treatments, however, revealed an important role for any of these pathways (data not shown).

Because we demonstrated that palmitate induces IL-1β production in CASMCs, we also tested whether IL-6 expression depends on IL-1β production. To examine this, we incubated the cells with the protein synthesis inhibitor cycloheximide (20 μg/ml) before and during palmitate (500 μmol/l) treatment. Cycloheximide significantly reduced the amount of IL-1β protein secreted into the medium (n = 4, P = 0.0273) (Fig. 6A) but did not reduce IL-6 mRNA expression (Fig. 6B), thereby excluding the possibility that IL-1β production mediates IL-6 gene expression. By contrast, cycloheximide significantly increased the amount of IL-6 mRNA content of palmitate-treated CASMCs (n = 4, P = 0.0014) as well as BSA- (n = 4, P < 0.0001) treated CASMCs (Fig. 6B). Similar effects of cycloheximide were also found in CAECs (data not shown).

**Relation of IL-6 and FFAs in human blood.** To investigate the relation of individual FFAs and IL-6 in vivo in humans, fatty acid patterns of the fasting plasma FFA fraction, fasting plasma FFA concentrations, and fasting serum IL-6 concentrations were determined in 54 healthy participants of the Tübingen Family Study for type 2 diabetes. In these subjects, no indications for acute

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**FIG. 5. Induction of IL-6 protein expression by palmitate, linoleate, IL-1β, and TNF-α in CAECs (A) and CASMCs (B). The cells were treated with 1.25% BSA (control), 500 μmol/l palmitate, 500 μmol/l linoleate, 2 ng/ml IL-1β, or 100 ng/ml TNF-α for 20 h. After cell lysis, intracellular IL-6 protein was quantified by ELISA. IL-6 protein contents are given as picograms per milligram of cellular protein. Data are means ± SE of three (CASMCs) or five (CAECs) independent experiments.**

**FIG. 6. Effect of cycloheximide on IL-1β protein production (A) and IL-6 mRNA expression (B) in CASMCs. The cells were preincubated for 30 min with 20 μg/ml cycloheximide (CHX) and then treated for 20 h with CHX plus 1.25% BSA (control) or 500 μmol/l palmitate. Data are means ± SE of four independent experiments. A: IL-1β protein secreted into the cell culture supernatant was measured by ELISA. #Significantly different from palmitate (P = 0.0273; t test). B: IL-6 mRNA was quantified by RT-PCR. *Significantly different from control (P < 0.0001; t-test); #Significantly different from palmitate (P = 0.0014; t-test).**
Inflammation were seen (leukocytes: 5,737 ± 262 μl⁻¹; C-reactive protein: 0.20 ± 0.03 mg/dl). Of the total FFA concentration, 44% were saturated and 56% were unsaturated (39% monounsaturated, 17% polyunsaturated) (Table 2). Among the saturated FFAs, palmitate and stearate prevailed (64 and 28% of all saturated FFAs, respectively). Oleate and linoleate were the most frequent unsaturated FFAs (56 and 23% of all unsaturated FFAs, respectively). All other fatty acids were <5% of the total FFA fraction (Table 2). Although the circulating FFA concentration was not associated with serum IL-6 (Fig. 7A), there was a significant, positive correlation between serum IL-6 and the palmitate content of the FFA fraction (Fig. 7B). This correlation persisted when IL-6 levels were adjusted for the percentage of body fat, an important determinant of circulating IL-6 concentrations (Fig. 7C). IL-6 did not correlate with stearate (unadjusted: \( r = 0.10, P = 0.5 \); adjusted: \( r = 0.10, P = 0.5 \)), oleate (unadjusted: \( r = -0.09, P = 0.5 \); adjusted: \( r = -0.14, P = 0.3 \)), or linoleate (unadjusted: \( r = 0.03, P = 0.8 \); adjusted: \( r = 0.01, P = 1.0 \)). To assess whether the association between serum IL-6 levels and the palmitate content of the FFA fraction was independent of the total FFA concentration, we adjusted the IL-6 levels for circulating FFA levels. As depicted in Fig. 7D, this association remained significant. Again, IL-6 was not correlated with stearate (unadjusted: \( r = 0.10, P = 0.5 \)), oleate (unadjusted: \( r = -0.08, P = 0.6 \)), or linoleate (unadjusted: \( r = 0.05, P = 0.7 \)) after adjusting for total FFAs. Furthermore, none of the minor fatty acids (<5% of total FFAs) (Table 2) were associated with serum IL-6 concentrations. Finally, no correlation was seen between IL-6 and the whole saturated or unsaturated FFA subfraction, respectively.

**DISCUSSION**

Recent clinical data support a role for FFAs in vascular inflammation, endothelial dysfunction, and atherosclerosis (20–22). The molecular mechanisms underlying these associations are, however, far from clear. Whereas monounsaturated and polyunsaturated FFAs, such as oleate (C18:1 \( \omega 9 \)), linoleate (C18:2 \( \omega 6 \)), arachidonate (C20:4 \( \omega 6 \)), eicosapentaenoate (C20:5 \( \omega 3 \)), and docosahexaenoate (C22:6 \( \omega 3 \)), have been convincingly demonstrated to modulate endothelial functions (23–28), very little is known about the role of saturated FFAs in vascular complications.

Here we provide evidence that the saturated FFA palmitate (C16:0), one of the most prominent FFAs in human blood (~28% of total FFAs) (this study; 16), induces substantial amounts of the inflammatory cytokine IL-6 in CAECs and minor amounts in CASMCs. By contrast, linoleate (C18:2 \( \omega 6 \)), a polyunsaturated FFA with controversial effects on endothelial activation (25, 26), did not promote IL-6 expression in CAECs and had only a minor, possibly endotoxin-mediated, IL-6–inducing effect in CASMCs. As to the FFA specificity of this cellular response, palmitate was by far the most potent IL-6–inducing FFA in CAECs. In CASMCs, palmitate as well as the unsaturated FFAs oleate and palmitoleate induced similar levels of IL-6 mRNA expression. The absolute amounts of IL-6 induced by any of these FFAs in CASMCs was, however, markedly lower than those produced by palmitate in CAECs. Therefore, we consider the CAECs to be the main source of palmitate-provoked IL-6 production in the vasculature. Overall, our results allow us to add palmitate, as a novel potent inducer of IL-6 expression, to the list of known stimuli of IL-6 expression, such as IL-1\( \alpha \), IL-1\( \beta \), TNF-\( \alpha \), and lipopolysaccharides (4, 29). Whether palmitate is able to induce IL-6 in other known IL-6–producing cells, such as monocytes, Kupffer cells, and adipocytes, remains to be elucidated.

Concerning the molecular mechanism of palmitate-induced IL-6 production, we currently can exclude only the involvement of mitochondrial \( \beta \)-oxidation, ceramide formation, PPAR activation, and IL-1\( \beta \) production. One sur-
prising finding was that inhibition of protein biosynthesis markedly enhanced IL-6 mRNA expression not only in palmitate-treated cells, but also in control cells. This observation opens the possibility that IL-6 induction represents de-repression of the IL-6 gene by enhanced degradation of an actively synthesized repressor protein. Clearly, further studies are needed to clarify this issue.

Furthermore, we found that CAECs as well as CASMCs do not express appreciable amounts of IL-6R. This finding argues against a local auto-or paracrine role of this inflammatory cytokine on vasoreactivity and atherosclerosis. Therefore, in our opinion, two other probably synergistic functions of vasculature-derived IL-6 (rev. in 8) seem to be more conceivable: 1) endocrine activation of the hepatic acute-phase response provoking the release of strong risk factors for cardiovascular disease, such as C-reactive protein (30,31) and fibrinogen (32,33); and 2) local paracrine activation of monocytes and platelets favoring fibrinogen deposition and thrombus formation in the vessel wall. In the case of an endocrine function, it could be postulated that palmitate-induced vascular IL-6 secretion influences plasma IL-6 concentrations, but this remains to be shown. However, with regard to the immense surface of the endothelium (~350 m²) and the ubiquity of palmitate in the circulation, we assume that vascular IL-6 production in response to palmitate, as quantified herein on a cellular basis, is substantial and should indeed contribute to serum IL-6 levels.

In our analysis of the plasma FFA profile, we found, in accordance with earlier findings (16), that palmitate (C16:0), stearate (C18:0), oleate (C18:1 ω9), and linoleate (C18:2 ω6) are by far the most prominent FFA species in human plasma and, therefore, could be of systemic importance. Among these and other minor FFAs, only palmitate correlated with IL-6 levels in healthy human subjects. Moreover, this association was independent of the total FFA concentration and body fat, a well-known source of palmitate and IL-6. This observation 1) supports our in vitro finding that palmitate is an important adipose tissue-derived determinant of IL-6 production and 2) strengthens our idea that, in addition to adipose tissue, other tissues (with the vasculature being a prime candidate) also significantly contribute to plasma IL-6 concentrations. Therefore, it is tempting to speculate that in obesity-linked disorders, hypertrophic adipose tissue contributes essentially to systemic IL-6 concentrations in two ways: directly via IL-6 secretion by adipocytes and indirectly via release of palmitate, which, in turn, elevates vascular IL-6 production. Such a prominent role for adipose tissue in chronic vascular inflammation would be in keeping with many clinical findings (rev. in 2,34,35).

In summary, we have shown that the plasma FFA palmitate, in physiological concentrations, is able to induce considerable amounts of IL-6 mRNA and protein in endothelial cells of human coronary arteries. Furthermore, palmitate, but no other plasma FFAs, correlated in vivo with IL-6 concentrations independently of body fat mass. Therefore, we postulate that 1) palmitate is an important determinant of IL-6 production in vitro as well as in vivo and 2) elevated plasma concentrations of palmitate (e.g., as found in the obese state) contribute to vascular IL-6 secretion and chronic inflammation.

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