Expression of adrenomedullin in human epicardial adipose tissue: role of coronary status

Alina Silaghi,1,2,3 Vincent Achard,1,2 Odile Paulmyer-Lacroix,1,2 Taixan Scridon,4 Virginie Tassistro,1,2 Ilenea Duncea,3 Karine Clément,5 Anne Dutour,1,2 and Michel Grino1,2

1INSERM U626, 2Faculté de Médecine, Aix-Marseille Université, Marseille, France; 3Department of Endocrinology, Cluj Hospital, Cluj; 4Department of Cardiac Surgery, Cluj Heart Institute, Cluj, Romania; 5INSERM U872, Paris, France

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IT IS RECOGNIZED that body fatness distribution is related to atherogenesis. Indeed, peripheral adiposity exhibits an independent antiatherogenic effect (44), whereas accumulation of abdominal visceral white adipose tissue (vWAT) is associated with increased prevalence of coronary artery disease (CAD) (23). Fat depots around other visceral organs, such as the heart, can also play a role in the pathogenesis of cardiovascular disease (32). Epicardial WAT (eWAT) mass correlated positively with diastolic blood pressure (16) and, in obese patients, with indexes of insulin resistance and glucose intolerance (14). eWAT deposition, measured by magnetic resonance imaging, around the myocardium of moderately obese subjects was related to free fatty acid exposure, generalized ectopic fat excess, and peripheral vascular resistance (21). Accumulation of eWAT in obese patients could be involved in the pathogenesis of cardiovascular diseases, through an increased ventricular stiffness or through secretion of various locally acting substances (32). Because eWAT lacks fascia and shares the same vascularization with the myocardium (30), factors synthesized by eWAT could directly alter coronary homeostasis. Enhanced synthesis and secretion of deleterious adipokines, such as interleukin (IL)-1β, IL-6, or tumor necrosis factor-α (TNFα) (31), or decreased synthesis and secretion of beneficial adipokines, like adiponectin (15), have been found in eWAT of patients with CAD.

During and following acute myocardial infarction, adrenomedullin (AM), as well as other endogenous mediators, is elevated. AM is a 52-amino acid peptide initially isolated from a human pheochromocytoma. AM displays vascular and cardiac protective roles by exerting various inhibitory actions against vascular damage and remodeling (reduction of myocyte hypertrophy, fibroblast proliferation, and collagen synthesis). In addition, AM shows potent vasodilating [by stimulating nitric oxide (NO) synthesis and antagonizing angiotensin II and endothelin 1 actions], antioxidative [by inhibiting oxidative stress-induced Bax, P38 MAPK phosphorylation, and activation of the Akt-Bad-Bcl2 signaling pathway], angiogenic (synergistic with vascular endothelial growth factor), and anti-inflammatory (by stimulating regulatory-cytokine secretion, modulating vascular permeability, and inducing adhesion molecule expression) properties (10, 19, 22). Recent experimental studies have demonstrated that AM expression is a critical factor regulating myocardial tolerance to ischemia-reperfusion injury (11). AM acts through seven-transmembrane domain G protein-coupled receptors (AM1 and AM2), which associate the calcitonin receptor-like receptor (CRLR) and specific receptor activity-modifying proteins (RAMP2 or RAMP3, respectively) (19). AM is produced by different tissues and cell types in humans, such as adrenal gland, heart, vascular endothelial cells, and vascular smooth muscle cells (reviewed in Refs. 10, 19, 22). In addition, we and others have recently demonstrated that AM is synthesized by human (12, 24, 29, 41) and rodent (9, 35) WAT. To the best of our knowledge, there is no report investigating AM and its associated receptor expression and regulation in eWAT. We studied in paired surgical biopsies of subcutaneous WAT (sWAT) and eWAT obtained from patients

Address for reprint requests and other correspondence: M. Grino, INSERM U626, Faculté de Médecine Timone, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France (e-mail: michel.grino@gmail.com).

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with or without CAD (NCAD) their morphological characteristics, and the expression of AM, CRLR, RAMP2, and RAMP3 mRNA and protein by use of quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunocytochemistry, respectively. Because it is established that glucocorticoids stimulate AM mRNA in human coronary vascular smooth muscle cells (8), we investigated in sWAT and eWAT the status of the local glucocorticoid metabolism, i.e., the expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), the enzyme that catalyzes the conversion of inactive to active glucocorticoids (6, 40).

MATERIALS AND METHODS

Patients. The study was conducted in accordance with the guidelines proposed in The Declaration of Helsinki and was approved by the Cluj University and Hospital ethics committee. All patients gave their informed consent. We studied 22 subjects with no endocrine, hepatic, or systemic disease, who underwent cardiac surgery for CAD (7 men, 5 women, aged between 45 and 72 yr), aortic or mitral valve replacement, or myxoma resection (NCAD, 5 men, 5 women, aged between 36 and 62 yr) in the cardiovascular surgical unit of Cluj University, respectively. Because it is established that glucocorticoids stimulate AM mRNA in human coronary vascular smooth muscle cells (8), we investigated in sWAT and eWAT the status of the local glucocorticoid metabolism, i.e., the expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), the enzyme that catalyzes the conversion of inactive to active glucocorticoids (6, 40).

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Blood collection. Peripheral blood was drawn into dry tubes on the morning of the surgery after an overnight fast. After centrifugation (1,500 g, 10 min, 4°C), the resulting serum was stored at −70°C until assay. Glycemia, total and HDL cholesterol, and triglycerides were measured using automated enzymatic assays (Vitros, Ortho-Clinical Diagnostics, Rochester, NY); coefficients of variation (CVs) were 0.60, 0.77, and 0.88%, respectively. Circulating insulin and cortisol levels were measured using immunometric chemiluminescent assays (DPC, La Garenne-Colombes, France) with a CV of 5.3 and 4%, respectively.

Adipose tissue collection. Adipose biopsy samples were obtained before the initiation of cardiopulmonary bypass, within 15–20 min after the beginning of surgery, from areas that had not previously been injured mechanically or cauterized. sWAT samples were obtained from the site of chest incision, and eWAT biopsies were taken near the proximal tract of the right coronary artery. Biopsies were immediately frozen in liquid nitrogen or fixed in 4% paraformaldehyde in phosphate buffer and subsequently embedded in paraffin. A paraffin-embedded tissue block containing a portion of the anterior interventricular branch of left coronary artery and the adjacent eWAT and myocardium was obtained from a routine autopsy performed in the department of forensic medicine, Assistance Publique-Hôpitaux de Marseille, France. Five micrometer-thick sections were cut using a Leica microtome, apposed onto slides (Superfrost Plus; CML, Nemours, France), and counterstained with eosin and hematoxylin. Brightfield images were observed with a DM-RB microscope (Leica Microsystems, Wetzlar, Germany) at a ×10 magnification and digitized using a Coolsnap charge-coupled device camera (Coolsnap, Princeton Instruments, Evry, France). Adipocyte surface was measured using Image software, and the differentiating preadipocytes, defined as small-sized interadipocyte cells having a central round vacuole reminiscent of a lipid droplet, were manually counted in five randomly chosen fields (each measuring 0.43 mm²) for each patient and each WAT depot.

Immunohistochemistry. Polyclonal antibodies to AM, CRLR, and RAMP2 and -3 (generously provided by Drs. F. Boudouresque and L’H. Ouafik) were raised in female New Zealand rabbits after repeated immunizations with the peptides (Bachem, Voisins Le Bretonneux, France) corresponding to amino acids 1–52, 89–119, 59–81, and 34–55 of human AM, CRLR, RAMP2 and RAMP3, respectively, and emulsified with complete Freund’s adjuvant. The specificity of the antisera has been previously described (7, 39).

Immunohistochemistry was performed as previously described (1). After dewaxing and rehydration, slides were heated in a microwave oven for 5 min in a solution containing 10 mmol/l sodium citrate and 1 mmol/l EDTA, pH 6.0 to maximize antigen retrieval, incubated in 1% H₂O₂ for 20 min to inactivate endogenous peroxidases, and blocked with 0.5% blocking reagent (PerkinElmer, Courtaboeuf, France) in PBS for 2 h. Slides were incubated overnight in PBS containing 0.5% blocking reagent and the AM, CRLR, RAMP2, or RAMP3 antisera, diluted 1:2,000, 1:3,000, 1:1,500, and 1:2,000, respectively, washed three times, and subsequently incubated for 2 h with biotinylated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200 and for 2 h with the avidin-biotin complex. Control sections included incubation without the primary antisera. The signal was revealed using diaminobenzidine chromogen substrate in the presence of 0.1% H₂O₂, and sections were counterstained with Mayer’s hematoxylin. Brightfield images were observed with a DM-RB microscope and digitized. For double labeling, background autofluorescence was quenched before the assay by use of an in-house-built photobleaching box. eWAT sections were blocked as described above and subsequently incubated overnight in PBS containing 0.5% blocking reagent, the anti-AM monoclonal, and a monoclonal antibody against CD68 (clone 514H12; Serotec, Cergy Saint Christophe, France) diluted 1:20. Slides were subsequently rinsed three times in PBS and incubated for 2 h with fluorescein-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG (both diluted 1:200; Jackson ImmunoResearch, West Grove, PA). Preparations were then observed using a True Confocal Scanner microscope (×63 magnification) coupled to 4D software. Controls included omission of the rabbit immune serum and the monoclonal antibody.

qRT-PCR. Total RNA was extracted using an RNasy minikit (Qiagen, Courtaboeuf, France). The RNA concentration was determined spectrophotometrically. RT was performed using 0.5 μg of RNA and MMLV transcriptase (Promega, Charbonnières, France). RT product (10 ng, diluted 1:1,000 for 18S) was amplified for 40 cycles on an Mx3005P thermocycler (Stratagene, Strasbourg, France) using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and specific primers (Table 1). PCR aliquots were visual-
ized on 2% agarose gel by ethidium bromide staining to confirm the presence of only one product. The amount of mRNA was determined from standard curves generated using cDNAs corresponding to bases 157–1372 of AM mRNA, 629–951 of CRLR mRNA, 141–458 of RAMP2 mRNA, 24–335 of RAMP3 mRNA, and 584–683 of 11β-HSD-1 mRNA and normalized against the 18S rRNA. Results are expressed as the percentage of sWAT NCAD.

Statistical analysis. All data were analyzed using the Statview analysis program. Baseline measurements were analyzed using the unpaired t-test. We used the Mann-Whitney U-test to compare mRNA expression and preadipocytes density (average of values measured in the 5 fields) between CAD and NCAD patients, and the paired Wilcoxon test to compare mRNA expression and preadipocytes density between sWAT and eWAT, as previously described (31). The Kolmogorov-Smirnov test was used to compare adipocyte size between CAD and NCAD patients in sWAT or eWAT. The association between 11β-HSD-1 and AM mRNAs was assessed by the Spearman rank correlation test.

RESULTS

Patients’ characteristics. Anthropometric and clinical characteristics and medications of the patients are summarized in Tables 2 and 3. Note that circulating cortisol was not different between groups.

Morphological analysis of eWAT. Figure 1 shows a bright-field view of a section through the anterior interventricular branch of the left coronary artery, counterstained with hematoxylin and eosin, demonstrating the close relationship between epicardial white adipose tissue (eWAT) and the coronary artery and the myocardium. The lack of fascia between eWAT and the coronary artery and the adjacent myocardium. The mean adipocyte surface (mean ± SE of averaged values measured for each patient on 5 randomly chosen fields) was larger in sWAT and eWAT obtained from CAD compared with the corresponding depots of NCAD patients (27.63 ± 2.44 vs. 17.79 ± 1.45 μm² × 10³ in sWAT; 23.31 ± 1.26 vs. 19.68 ± 1.54 μm² × 10³ in eWAT, respectively, P < 0.0001, Kolmogorov-Smirnov test). Differentiating preadipocyte density (mean ± SE of averaged values measured for each patient on 5 randomly chosen fields) was comparable between sWAT and eWAT obtained from NCAD subjects (7.5 ± 1.0 vs. 11.3 ± 2.8 cells/mm², P = 0.48) and in sWAT from CAD patients (6.9 ± 1.6 cells/mm², P = 0.156 vs. sWAT from NCAD). It increased in eWAT obtained from CAD patients (20.8 ± 3.0 cells/mm²) compared with sWAT from CAD (P = 0.0004) or eWAT from NCAD (P = 0.0207).

Immunohistochemical analysis of eWAT. Figure 2, A–K, shows the results of the immunohistochemical analysis of AM, CRLR, and RAMP2 and -3 expression in eWAT from an NCAD patient. AM was expressed in blood vessel (Fig. 2A), in stromal areas (Fig. 2A, arrows), and in isolated stromal cells close to adipocytes (Fig. 2E, arrows), whereas mature adipocytes were not labeled. CRLR, RAMP2, and RAMP3 proteins were expressed in vessel walls (Fig. 2, B–D, respectively) and in isolated stromal cells close to adipocytes (Fig. 2, F–H, respectively, arrows). The localization of AM, CRLR, and RAMP2 and -3 proteins was comparable between sWAT and eWAT (not shown). The AM immunohistochemical labeling of blood vessels (Fig. 2I) and the

Table 2. Body composition and biological parameters in NCAD and CAD patients

<table>
<thead>
<tr>
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<th>NCAD (n = 10)</th>
<th>CAD (n = 10)</th>
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<tbody>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>78±4</td>
<td>98±7</td>
</tr>
<tr>
<td><strong>Men: waist circumference, cm</strong></td>
<td>107±7</td>
<td>127±9</td>
</tr>
<tr>
<td><strong>Waist/hip ratio</strong></td>
<td>0.85±0.01</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td><strong>Women: waist circumference, cm</strong></td>
<td>106±6</td>
<td>126±9</td>
</tr>
<tr>
<td><strong>Waist/hip ratio</strong></td>
<td>0.83±0.01</td>
<td>0.97±0.02</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, mmHg</strong></td>
<td>125±10</td>
<td>135±12</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, mmHg</strong></td>
<td>75±10</td>
<td>85±14</td>
</tr>
<tr>
<td><strong>Fasting glucose, mg/dl</strong></td>
<td>89±10</td>
<td>105±28</td>
</tr>
<tr>
<td><strong>Fasting insulin, μIU/l</strong></td>
<td>8.5±4</td>
<td>10.8±7.1</td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/l</strong></td>
<td>5.2±1.3</td>
<td>4.9±1.2</td>
</tr>
<tr>
<td><strong>HDL-cholesterol, mmol/l</strong></td>
<td>1.23±0.22</td>
<td>1.12±0.31</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/l</strong></td>
<td>1.79±0.56</td>
<td>1.55±1.07</td>
</tr>
<tr>
<td><strong>Coriolis, ng/ml</strong></td>
<td>214±40</td>
<td>225±84</td>
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Data are presented as means ± SD. CAD, coronary artery disease; NCAD, no CAD; BMI, body mass index.

Fig. 1. Brightfield view of a section through the anterior interventricular branch of the left coronary artery, counterstained with hematoxylin and eosin, demonstrating the close relationship between epicardial white adipose tissue (eWAT), coronary vessels, and myocardium. Bar, 250 μm.
density of AM-positive isolated stromal cells close to adipocytes (Fig. 2J, arrows) were increased in eWAT obtained from a CAD patient compared with NCAD subjects. In addition, sections of eWAT obtained from CAD patients showed an increased frequency of AM-positive cells having a central round vacuole (Fig. 2J, crossed arrow), reminiscent of a lipidic droplet, thus strongly suggesting a differentiating preadipocyte phenotype. Control experiments, that is, incubation without the primary antiserum, resulted in a lack of signal, demonstrating the specificity of the sera and of the immunohistochemical procedure (Fig. 2K). We next sought to determine whether AM was synthesized within macrophages. Therefore, we performed a double-labeling fluorescent immunohistochemistry experiment for AM and CD68. Confocal analysis of a double-labeled eWAT section obtained from an NCAD patient (Fig. 2, L–Q) shows that some of the AM-positive stromal cells colocalized CD68 immunoreactivity.

**qRT-PCR analysis.** Figure 3 depicts the results of the qRT-PCR analysis of AM, CRLR, and RAMP2 and -3 in sWAT and eWAT obtained from the 10 NCAD and the 12 CAD patients. AM mRNA levels showed an important increase in sWAT and eWAT obtained from CAD patients compared with NCAD subjects, whereas they did not differ in sWAT or eWAT obtained from NCAD patients. CRLR mRNA concentrations were comparable in sWAT obtained from NCAD or CAD subjects and decreased in eWAT irrespectively of the coronary status. However, although the above-mentioned variation reached significance only in CAD patients, statistical analysis of the pooled data indicated a significant decrease of CRLR mRNA expression in eWAT compared with sWAT (Wilcoxon paired test, \( P < 0.0031 \)). RAMP2 mRNA expression showed a tendency to increase in sWAT obtained from CAD compared with NCAD patients, whereas it was not affected by the coronary status in eWAT. RAMP3 mRNA levels were comparable between sWAT and eWAT in NCAD patients, whereas they increased in WAT obtained from CAD patients compared with NCAD subjects. However, the above mentioned difference reached significance only for sWAT biopsies. Figure 4, top, shows the results of the qRT-PCR analysis of 11β-HSD-1 mRNA in sWAT and eWAT extracts obtained from the 10 NCAD and the 12 CAD patients. The levels of 11β-HSD-1

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**Fig. 2.** Top: brightfield photomicrographs showing representative immunohistochemistry experiment for adrenomedullin (AM; A, E), calcitonin receptor-like receptor (CRLR; B, F), receptor activity-modifying protein-2 (RAMP2; C, G) and RAMP3 (D, H) in eWAT obtained from a non-coronary artery disease (NCAD) subject and AM in eWAT obtained from a CAD patient (I, J). Sections were counterstained with Mayer’s hematoxylin. AM was expressed in blood vessels (A), stromal areas (A, arrows), and isolated stromal cells close to adipocytes (E, arrows), whereas mature adipocytes were not labeled. CRLR, RAMP2, and RAMP3 proteins were expressed in vessel walls (B, C, D, respectively) and in isolated stromal cells close to adipocytes (F, G, H, respectively, arrows). Intensity of labeling of blood vessels (I), and density of AM-positive isolated stromal cells close to adipocytes (J, arrows), were increased in eWAT sections obtained from a CAD patient compared with an NCAD one (A, E). Note: AM-positive cells showing a central round vacuole (J, crossed arrow), reminiscent of a lipidic droplet, thus strongly suggesting a differentiating preadipocyte phenotype, were present in eWAT obtained from a CAD subject. Control experiments (incubation without primary antiserum) resulted in a lack of signal, demonstrating specificity of the sera and of the immunohistochemical procedure (K). Bar, 20 μm. Bottom: 2-channel confocal scanning of representative eWAT section obtained from an NCAD patient showing immunoreactivities for AM (fluorescein labeling, L), CD68 (TRITC labeling, M), and AM + CD68 (N). Corresponding control sections are shown in O–Q. Note: AM immunoreactivity colocalized with CD68 (yellow to orange fluorescence) in small round cells resembling macrophages. Bar, 10 μm.
Our data demonstrate for the first time that AM and its receptors are expressed in eWAT and are regulated by coronary status. We found that AM, CRLR, RAMP2, and RAMP3 proteins were present in blood vessel walls and in the stromal fraction of eWAT, consistent with our previous observations in human abdominal sWAT and vWAT (41). Our finding showing that AM and CD68 immunoreactivities colocalized in some stromal cells strongly suggests that macrophages infiltrated within eWAT can synthesize AM, consistent with the demonstration that macrophages derived from circulating monocytes contain AM mRNA and produce large amounts of AM (34). Immunohistochemical experiments also suggested that preadipocytes, which are part of the stromal cells and are more numerous in eWAT obtained from CAD patients, could be a source of AM. A more straightforward demonstration that preadipocytes synthesize AM could have been investigated by studying AM secretion and/or synthesis during in vitro adipocyte differentiation. For obvious ethical and methodological reasons, we were unable to collect sufficient amounts of eWAT to perform cell culture. However, we (41) previously demonstrated that the kinetics of AM secretion from sWAT preadipocytes undergoing in vitro differentiation was opposite to that of leptin. Also, Li et al. (28) showed that AM mRNA was expressed in 3T3-L1 preadipocytes but was undetectable in adipocytes and that AM immunoreactivity was detected in the culture media of 3T3-L1 preadipocytes and adipocytes, with higher concentrations found in preadipocytes. Taken together, the above-mentioned observations suggest that preadipocytes in eWAT can synthesize AM. In addition, we demonstrated that CRLR and RAMP2 and -3 immunoreactivity was detected in isolated stromal cells close to adipocytes. Interestingly, Isumi et al. (20) have shown that AM acts as a proliferative factor for a preadipocyte cell line (Swiss 3T3 cells), suggesting that, in eWAT, AM could regulate, through an autocrine or paracrine mechanism, preadipocyte multiplication.

We found that AM mRNA levels were increased in both sWAT and eWAT in CAD patients. It has been recently demonstrated that the sWAT and eWAT levels of the mRNAs coding for several adipokines [such as resistin, IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNFα] were higher at the end compared with the beginning of cardiac surgery (25). It is unlikely that anesthesia and surgical stress are involved in the above-mentioned AM mRNA variations, because WAT sampling was done within 15–20 min after the beginning of the surgery in both groups of patients and because the stimulation of AM mRNA by hypoxia in primary cultures of adult rat cardiac myocytes needs at least 6-h exposure (5). The increase of sWAT and eWAT AM mRNA in CAD patients can participate in the enhanced circulating AM levels found in patients with CAD (43). Moreover, our results suggest that eWAT could be a source of coronary AM under pathological conditions. Plasma AM levels in patients with acute myocardial infarction are higher in aorta and coronary sinus than in the general circulation, suggesting a cardiac or epicardial synthesis (46). Because eWAT lacks fascia and shares the same vascularization with the myocardium, as illustrated in Fig. 1, increased eWAT AM synthesis may participate in coronary AM in response to chronic hypoxemia. Because AM has been shown to buffer against the hypertensive, proliferative, oxidant, and apoptosis signals induced by angiotensin II, endothelin-1, and aldosterone in myocardium and to act as an anti-inflammatory and angiogenic factor, thus promoting collateral vessel formation (10, 19, 22), it can be suggested that increased AM synthesis in eWAT of CAD patients can play a cardioprotective role. In addition, it has been demonstrated that AM inhibits lipolysis through NO-mediated β-adrenergic agonist oxidation (12). Interestingly, mean adipocyte surface was increased in sWAT and eWAT obtained from CAD compared with NCAD patients, suggesting that increased AM synthesis in response to CAD is one of the possible mechanisms that can affect adipocyte size. Because more CAD than NCAD patients were treated with β-blockers (9 of 12 vs. 1 of 10, respectively), it can also be suggested that
the increased adipocyte surface found in CAD patient was subsequent to an inhibition of catecholamines-induced lipolysis (13). It has been demonstrated that, in patients with chronic heart failure, treatment with β-blockers increases total body fat mass (26). However, the exact role of β-blockers on eWAT mass clearly needs further investigation.

The factors responsible for the stimulation of WAT AM synthesis in CAD patients are not known. It has been demonstrated in primary cultures of rat ventricular cardiac myocytes that increased AM gene transcription during exposure to hypoxia is primarily mediated by hypoxia-inducible factor-1 (HIF-1; see Ref. 5). Because the human AM gene contains multiple consensus sequences for HIF-1 (19) and because HIF-1 is increased in response to myocardial ischemia (27), it can be suggested that HIF-1 stimulates eWAT AM mRNA in CAD patients. Inflammatory mediators can also play an important role. We demonstrate that AM and CD68 immunoreactivities were colocalized, indicating that the dense macrophagic infiltrates already described in eWAT obtained from CAD patients (31) can produce AM. The above-mentioned hypothesis is consistent with the findings of Ishikawa et al. (18), who demonstrated that AM immunoreactivity was localized in macrophages in coronary plaques obtained from patients with stable or unstable angina. In addition, it is established that TNFα, whose circulating concentrations show significant associations with CAD (4), is a potent stimulator of in vitro AM secretion (5, 19), and therefore could be involved, through a paracrine mechanism in the stimulation of eWAT AM and through an endocrine mechanism in the stimulation of sWAT AM in CAD patients.

Glucocorticoids are potent stimulators of AM mRNA in human coronary vascular smooth muscle cells (8). Our findings that 11β-HSD-1 mRNA levels are increased in eWAT obtained from CAD patients compared with NCAD subjects and that eWAT AM mRNA concentrations in the whole population correlate with those of 11β-HSD-1 suggest either that 11β-HSD-1 and AM genes share common regulatory mechanisms or that 11β-HSD-1-induced local cortisol reactivation participates in the stimulation of eWAT AM mRNA levels. In addition, the recent demonstration that pharmacological blockade of 11β-HSD-1 in macrophages significantly reduces the lipopolysaccharide-induced increase in IL-1β, TNFα, and MCP-1 (17) suggests that the enhanced 11β-HSD-1 expression found in eWAT of CAD patients could also stimulate AM gene expression through a modulation of proinflammatory cytokines originating from macrophages. However, it is important to consider that AM efficiency depends not only on its local or circulating levels but also on the expression of its receptors. Although mRNA levels do not necessarily correlate with protein synthesis, it has been shown in rats that induction of myocardial infarction induced an increase in RAMP2 and RAMP3 in cardiomyocytes, with a parallel enhancement of AM binding and AM-stimulated adenyl cyclase activity (38), and that in vitro overexpression of either CRLR or RAMP2 in cardiomyocytes potentiated the AM signaling response (2). We found that RAMP2 mRNA concentrations were unchanged whereas CRLR mRNA levels were decreased in eWAT, irrespectively of the coronary status, suggesting that AM may be less efficient in eWAT than in sWAT. The mechanisms responsible for the decrease in eWAT CRLR mRNA in eWAT from patients with CAD are not clear. Proinflammatory cytokines can be involved in the above-mentioned phenomenon because TNFα downregulates CRLR mRNA expression in human coronary artery smooth muscle cells (33). It is established that glucocorticoids are potent regulators of membrane receptors (36) and that the 5′-flanking region of the CRLR gene contains several glucocorticoid receptor responsive elements (37). Dexamethasone (a synthetic glucocorticoid) has complex effects on the regulation of CRLR gene expression in vivo. In human coronary vascular smooth muscle cells, dexamethasone has an inhibitory effect after exposure to low to moderate doses and a transient stimulatory action after exposure to high doses (8), whereas it decreased CRLR mRNA expression and protein levels in osteoblastic cells (45). As a consequence, the above-mentioned phenomenon could limit the beneficial effects of increased AM synthesis found in CAD patients. In addition, it is surprising that CRLR mRNA levels were unchanged in eWAT obtained in CAD patients, because the CRLR promoter contains HIF-1 consensus elements and acute hypoxia activates CRLR gene transcription in microvascular endothelial cells (37). We found that RAMP3 mRNA levels were increased in WAT of CAD patients, suggesting that it can be involved in cell mechanisms other than AM signal transduction. Indeed, it is established that RAMP3 promotes the forward trafficking of the calcium-sensing receptor (3), a G protein-coupled receptor involved in the regulation of arterial blood pressure (42). Further experiments are clearly needed to understand the regulation of CRLR and RAMP3 in eWAT.

In conclusion, our data demonstrate that AM and its receptors are expressed in eWAT. Because of the close relationship among eWAT, the coronary vessels, and the myocardium, increased AM synthesis in eWAT during chronic CAD could play a cytoprotective role.

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is increased in both visceral and subcutaneous adipose tissue of obese patients. *Obesity* 14: 794–798, 2006.


