Cellular cross-talk between epicardial adipose tissue and myocardium in relation to the pathogenesis of cardiovascular disease

Sam Cherian, Gary D. Lopaschuk, and Eugenia Carvalho

Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; Department of Pediatrics, Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada; and The Portuguese Diabetes Association, Lisbon, Portugal

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Human Adipose Tissue: a General Account

Adipose tissue can be divided into two major types: white adipose tissue (WAT) and brown adipose tissue (BAT), both of which have different physiological roles ascribed to them. Subcutaneous (SAT) and visceral (VAT) are two types of WAT. Although it has been reported that SAT is less metabolically active than VAT, it is an important storage organ, implicated in the accumulation of triacylglycerols (TGs) during periods of excess energy intake, and the supply of free fatty acids (FFAs) during periods of fasting, starvation, or exercise. SAT also serves as a buffer during intake of dietary lipids and thus protects other tissues from lipotoxic effects of these lipids (39). VAT can be omental or mesenteric, and it surrounds various inner organs in humans. The omental fat depot covers the stomach and spleen and extends into the ventral abdomen, while the mesenteric depot is attached to the intestine (10). BAT is found in defined and dispersed areas in the body such as clavicular, supraclavicular, and subscapular regions (10) or as clusters within WAT in different animals (18). The main role of BAT is reported to be nonshivering thermogenesis in mammals, with this role in humans being particularly important in neonates (35).

Fat Depots Around the Heart and Blood Vessels

The terminology used to distinguish fat depots around the heart is often confusing (105), with terms such as intra- and extrapericardial fat being used (70). However, in broad terms, pericardial adipose tissue (PAT) (also known as extrapericardial or intrathoracic), has been defined as the fat depot outside the visceral pericardium and on the external surface of the parietal pericardium, which therefore includes both EAT and PAT (25, 66, 119). EAT has been defined as the intrapericardial fat depot that is located between the myocardium and visceral pericardium, while the storage of TG droplets within the cardiomyocytes have been termed myocardial fat (70). The fat surrounding the vasculature has also been termed perivascular adipose tissue (PVAT), irrespective of location (148). This review focuses mainly on EAT and the myocardial fat.
depots. Readers are directed elsewhere for details on other fat depots (10, 66, 105, 111).

EAT

EAT is the true visceral fat depot around the heart and can generate various bioactive molecules, (Fig. 1 and Table 1). Results of post mortem studies on healthy subjects [without any clinical signs of cardiovascular disease (CVD) or diabetes mellitus] revealed that EAT constitutes ~20% of the total ventricular weight of the human heart (26) while covering 80% of the heart’s surface (63). EAT has the potential to be a good storage depot for fatty acids, thus potentially protecting the heart against high fatty acid levels. It may also serve as a buffer for the arterial circulating fatty acids and may be a local energy source of fatty acids during times of high energy demand by channeling fatty acids to the myocardium (96).

In heart adiposity, epicardial fat increases and extends over the anterior surface of the heart [with more on the right ventricle (RV) than the left ventricle (LV)] and over the LV midway between the apex and base (63). As epicardial fat volume increases, the coronary arteries become encased or lie between the fat and the myocardium. The fat may even penetrate from the subepicardial connective tissue into the connective tissue lying between the muscle bundles and muscle fibers, a condition defined by Smith and Willius (128) as adiposity of the heart. In cases of extreme obesity, fat can completely cover the heart and may extend up to 2 cm or more in thickness (119). In addition, increased LV mass and hypertrophy are associated with increased epicardial fat thickness, which has also been reported to be associated with changes in LV mass and diastolic function (62, 64). A significant correlation between increases in epicardial fat thickness and enlarged atria

Fig. 1. Schematic representation of the possible paracrine and vasocrine secretion of adipokines from epicardial adipose tissue (EAT; adipocytes and vascular stromal cells) and the overload of free fatty acids (FFAs) leading to triglyceride (TG) accumulation and other cellular events that take place in cardiomyocytes under metabolic/obese state. Direct and bold arrow lines represent paracrine secretion of adipokines to arterial wall layers and myocardium. Dotted and bold arrow lines represent the vasocrine release of adipokines to arterial wall layers and the lumen. Inflammatory changes in adipocytes (hypertrophy and hyperplasia) and infiltration of macrophages, lymphocytes, monocytes, and mast cells are shown. The paracrine loop involving adipocyte-derived FFA release or FFA overload from circulation and macrophage- and adipocyte-derived inflammatory cytokines could lead to myocardial steatosis (lipotoxic cardiomyopathy). Increased myocardial lipid accumulation and various cellular abnormalities caused by byproducts of lipid metabolism [decreased FA oxidation-ROS, ceramide generation, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and altered gene expression] might cause cardiac dysfunction or heart failure (cardiomyopathy, cardiac fibrosis, ventricular hypertrophy, systolic abnormality). L, lumen; I, intima; M, media; A, adventitia; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1.
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CABG, coronary artery bypass grafting. See text for other definitions.
and impaired RV and LV diastolic filling in morbidly obese subjects has been reported (65).

**EAT Volume and Adjacent Adipose Tissue in Relation to CVD**

A significant amount of evidence attests to the association of EAT with coronary atherosclerosis in patients with coronary artery disease (CAD) (28, 29, 43, 44, 94, 134, 149). The association of EAT and the early stages of atherosclerosis and plaque formation has also been established (3, 82, 134). Studies have shown that there is an independent and strong correlation between EAT volume and the presence of coronary artery occlusions (83, 134). However, other studies have not confirmed these results but rather have observed an equal EAT volume in areas of both calcified and noncalcified plaque (44, 83). A recent study by Iwasaki et al. (75) hypothesized that EAT volume increases steeply in patients with significant coronary artery stenosis and in those with severe coronary artery calcification. To support this hypothesis, they measured EAT volume by multidetector compound tomography and showed that EAT volume was associated with coronary atherosclerosis. Clinical studies have demonstrated a relationship between EAT and other adjacent adipose tissue depots, including coronary PAT thickness to coronary atherosclerosis (30, 43, 89, 95). Furthermore, despite the small fraction of EAT compared with the total amount of body fat, EAT may still be responsible, and may outweigh the local effects of VAT at the level of the heart, in an endocrine/paracrine fashion (Fig. 1).

EAT is an abundant source of resistin, interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, and fatty acid binding proteins, among others, that are proinflammatory and can act directly on the cardiomyocyte, as shown on Fig. 1 and summarized in Table 1 (28, 99, 119). This local molecular cross-talk between EAT and the heart can be the genesis of some of the dysfunctions implicated in atherosclerosis and in the pathogenesis of cardiovascular disorders associated with obesity and diabetes (28, 99, 119).

It is important to note that there are segments of the coronary artery that are free from atherosclerosis and that this has been attributed to the lack of adipose tissue in such areas, thereby not producing proinflammatory stimuli. As shown by CT studies, there is increased atherosclerosis in segments of coronary arteries proximal to myocardial bridges, where adipose tissue is present, whereas the segments overlying the myocardial bridge are free of atherosclerosis (72, 73, 76, 77, 150). This further validates the role of adipose tissue and its secretory products in cardiovascular pathophysiology (Table 1).

**Obesity and the Pathophysiology of Adipose Tissue**

Obesity contributes significantly to morbidity and mortality and is a critical component of, and a major underlying driver for, the metabolic syndrome (82). Metabolic syndrome refers to a constellation of risk factors that can occur together (such as visceral obesity, hypertension, dyslipidemia, insulin resistance, and impaired glucose tolerance) that can lead to CAD (45, 102). Many of the risk factors for CAD may therefore result directly or indirectly from obesity, which in turn is connected to a malfunction of adipose tissue (10). Adipose tissue is composed of various cells types, such as preadipocytes, mature adipocytes, and the stromal vascular fraction (SVF), which includes macrophages, blood cells, and endothelial cells (106). Compared with the adipocytes in SAT, adipocytes in VAT are reported to be more endocrinologically or lipolytically active and may contribute to more FFAs in plasma (51, 103, 144). It has been reported that in obese humans most of the inflammatory cytokines are released by non-fat cells [stromal vascular matrix (SVM)], whereas the chemokines, MCP-1, macrophage migration inhibitory factors, nerve growth factors, and serum amyloid A1 and A2 proteins are secreted by adipocytes (125).

Recent studies have indicated that adipose tissue in obesity is characterized by increased infiltration of macrophages, lymphocytes, and mast cells (14, 27, 126, 145), suggesting that they may be an important source of inflammation. This inflammation may further lead to a state of altered adipokine balance and uncontrolled release of FFAs along with inflammatory cytokines (13, 131). Adipokines like MCP-1 secreted by hypertrophic adipocytes are chemoattractants that aid in monocyte/macrophage infiltration (14, 98). Expanding adipocytes are able to recruit circulating lymphocytes and monocytes through MCP-1 (145, 147). The recruited monocytes enter the SVM by passing through the endothelium of adipose tissue capillaries.

In obesity, adipose tissue inflammation and adipose tissue hypertrophy (increase in cell size) are two closely linked processes (10) that aid in the recruitment of T-cells and macrophages and can contribute to insulin resistance (56). In addition, adipocyte hyperplasia (increased cell number), macrophage infiltration, endothelial cell activation, and fibrosis are characteristics of adipose tissue inflammation in the obese state (12, 36, 124, 126).

Inflammatory changes in adipocytes and macrophages can be controlled by several intracellular signaling pathways, including the mitogen-activated protein (MAP) kinase pathway (131, 132). The MAP kinases [extracellular signal-regulated kinases (ERK), Jun NH2-terminal kinase (JNK) and p38 MAP kinase] are activated by TNF-α in adipocytes (78, 118). Among these, ERK and JNK are involved in TNF-α-induced lipolysis (118, 130). Maury et al. (97) suggested that the paracrine cross-talk between the macrophage and the adipocyte could result in adipocyte hyperresponsiveness to TNF-α with subsequent hyperactivation of the NF-κB pathway in obese subjects. Taken together, a paracrine loop involving adipocyte-derived FFA release and macrophage-derived TNF-α release establishes a vicious cycle that contributes largely to the inflammatory changes observed in obese adipose tissue.

**EAT Physiology, Myocardial Metabolism, and Lipid Accumulation**

The heart has a constant and high demand for energy to sustain its contractile function, which is met primarily by the β-oxidation of long-chain fatty acids. The control of fatty acid β-oxidation is a complex process, and alterations in fatty acid β-oxidation can contribute to cardiac pathologies (92). Under normal physiological conditions, the myocardium mainly metabolizes FFAs from the coronary arterial blood. FFA oxidation account for about 50–70% of the energy production of the heart. In this respect, EAT could act as a buffer zone, protecting the heart against excessive high levels of FFAs while providing for the local energy demands of the
myocardium (59). Recent studies have reported the expression of a marker protein for brown fat, the uncoupling protein-1 (UCP1) in EAT, suggesting that human epicardial fat might function in the same way as brown fat (i.e., playing a significant role in thermogenesis in the myocardium) (34, 120). In addition, Chechi et al. (21) have recently reported that the higher UCP1 expression in EAT compared with other fat depots may indicate the presence of brown adipocytes in this tissue. However, further studies are needed to provide conclusive evidence of uncoupling of the electron transport chain in the mitochondria of EAT cells. Compared with other visceral fat depots, EAT has a greater capacity for release and uptake of FFAs and a lower rate of glucose utilization. However, the transport of FFAs from the EAT to myocardium is a relatively unexplored area and needs further investigation. The present hypothesis is that FFAs could diffuse bidirectionally in interstitial fluid across concentration gradients (59, 109, 119). Human EAT expresses fatty acid-binding protein-4 under pathological conditions, and this might play a significant role in the intracellular transport of FFAs from EAT to the myocardium (59, 143).

Under normal physiological conditions, EAT and pericardial fat show great flexibility in the storage or release of fatty acids compared with other fat depots, which may help to fulfill the energy needs of the arterial wall and heart muscle and help to avoid lipotoxicity (Fig. 1). However, in the obese or metabolic diseased state, these tissues expand, resulting in an increase in macrophage infiltration and T-cell accumulation (55, 126). This may further reduce the production of protective mediators in favor of detrimental ones from adipocytes (70). Increased lipid accumulation into cardiac myocytes, a condition termed “cardiac steatosis” or “cardiac lipotoxicity”, has been proposed to be an important cause of cardiac dysfunction. A growing number of animal studies have demonstrated the toxic consequences of lipid overload accompanying obesity, such as insulin resistance and diabetes, that can lead to cardiomyocyte apoptosis, cardiac fibrosis, and/or impairment in the contractile function of the heart (1, 2, 8, 24, 154). The insights gained from such model studies are now being extended to human studies (85, 115). Proton magnetic resonance spectroscopy (1H-MRS) is a new, sensitive, noninvasive technique for measuring human myocardial and hepatic TG content (85, 101, 114, 133, 137).

Myocardial lipid accumulation has been linked to hyperglycemia including impaired glucose tolerance (IGT) and diabetes mellitus (69, 101). McGavock et al. (101) evaluated whether cardiac steatosis precedes the onset of cardiomyopathy in patients with impaired glucose tolerance and type 2 diabetes mellitus (T2DM). These authors concluded that in humans impaired glucose tolerance is accompanied by cardiac steatosis, which precedes the onset of T2DM and LV systolic dysfunction. This suggests that excess lipid storage in human cardiac myocytes is an early manifestation of the pathogenesis of T2DM, which is evident even in the absence of heart failure. The association of insulin resistance and impaired LV function have also been investigated, with studies showing a causal role of insulin resistance in the development of cardiomyopathy (6, 9, 54, 84, 141). Diet-induced obesity (DIO) results in a profound impairment in insulin-stimulated glucose metabolism in mouse hearts (136, 151–153). Decreased insulin-stimulated glucose oxidation in the mouse heart, and a switch to a greater reliance on fatty acid oxidation as a source of energy, occurs very early in the the development of obesity (136, 151–153). This development of insulin resistance in the heart correlates with the accumulation of diacylglycerol (DAG) in the heart and not long-chain acyl-CoA, ceramides, or TG accumulation (136, 153). The accumulation of DAG in the heart during the development of insulin resistance is also associated with a translocation of protein kinase Cα to the sarcoplasmic membrane and phosphorylation of p70S6K, consistent, with increased DAG in the heart mediating insulin resistance via protein kinase Cα activation.

Studies conducted in T2DM patients have shown that 3 days of caloric restriction can lead to an increase in TG content and a decrease in LV diastolic function (49). However, a prolonged (16 wk) calorie restriction study showed contrasting results, where myocardial TG levels decrease and LV diastolic function improves in parallel with normalization of glucose tolerance (50). It has also been shown that therapeutic administration of metformin (or thiazolidinediones) is able to improve cardiac function without changing TG content or cardiac metabolism despite a decrease in cardiac work by metformin (138). These studies may suggest that dietary interventions could be used for the evaluation of the cardiac TG pool, with an increase of cardiac TG content occurring following fasting or calorie restriction in lean subjects.

In addition, results from several other studies have also provided insights into a number of candidate molecular pathways that mediate cardiac lipotoxicity. Decreased mitochondrial coupling (15), oxidative capacity, endoplasmic reticulum (ER) stress (11), altered membrane composition and function, and altered gene expression through enhanced ligand delivery, are some of the candidates (146, 127). Furthermore, increased lipid overload in the heart may lead to intracellular accumulation of reactive oxygen species (ROS), which subsequently can induce ER stress and cell death (11). It should be noted that, although increased myocardial TG does not have a direct influence on cardiac function, TG increases seen in diabetic and obese individuals may be used as a biomarker for scoring underlying cardiac defects (146). Animal studies have also provided evidence that activation of transcriptional pathways that regulate the expression of fatty acid oxidation enzymes might induce lipotoxicity and cardiac dysfunction (37, 100, 129).

Studies using T2DM and obesity experimental models have demonstrated that excessive plasma FFAs levels lead to accumulation of myocardial TG (114, 122, 135). Therefore, the level of circulating FFAs may be an important determinant of human myocardial fat content (TG storage) (70). To date, studies concerning cardiac steatosis mechanisms using animal models (diabetic rodents) largely reveal that increased cardiac fatty acid uptake is secondary to both elevated plasma nonesterified fatty acid concentrations and increased protein levels of the fatty acid transporters (72). Increased uptake of long-chain fatty acids into the heart can result in an increase in the concentration of intracellular TG, DAG, and ceramides (53). In addition, excessive fatty acid uptake that exceeds the ability of the mitochondria to oxidize the fatty acids, can cause elevated production of ROS leading to mitochondrial dysfunction as well as contractile dysfunction (17).

A recent study by Chokshi et al. (23) provided some insights into the cardinal features in metabolic syndrome associated
with heart failure and myocardial insulin resistance. By distinguishing the neutral lipid species (such as TG) from the toxic lipid intermediates (such as DAG and ceramides) in the myocardium of patients with end-stage heart failure, Chokshi et al. demonstrated a decrease in TGs and an increase in the lipotoxic DAGs and ceramide species compared with nonfailing control subjects. They postulated that the accumulation of these toxic lipid intermediates could play an active role via increased activation of protein kinase C in the development of impaired insulin signaling that was associated with chronic heart failure. These data are consistent with the role of DAG in mediating myocardial insulin resistance in obesity (153). Therefore, an increase in fatty acid uptake in excess of fatty acid oxidation rates could result in a diversion of the lipid storage pools to the intermediate lipotoxic species such as DAG and ceramide. An alternative theory explains heart failure as a state of sustained lipolysis driven by the chronic activation of adrenergic and natriuretic peptide systems, which have both been implicated in the increased hydrolysis of glycerolipids by adipocyte TG lipase and hormone-sensitive lipase in adipocytes (112).

In short, the mechanistic view is that, after uptake by cardiomyocytes, fatty acids are conjugated with CoA to form long-chain acyl-CoA. The fatty acid moieties of long-chain acyl-CoA are then transferred to carnitine to form long-chain acylcarnitine, which is then transported into the mitochondria for oxidation and energy release for cellular function. However, excessive fatty acid uptake in relation to oxidative requirements can cause the buildup of intramyocellular TGs for oxidation and energy release for cellular function. How- ever, fatty acid uptake in relation to oxidative requirements can cause the buildup of intramyocellular TGs (cardiac steatosis). Also, the byproducts of lipid metabolism or the fatty acid intermediates such as DAG and ceramides could generate ROS, which can result in the modulation of sarco(endo)plasmic reticulum Ca2+-ATPase, an early contributor of diastolic dysfunction, myocardial fibrosis, and hypertrophy in the insulin-resistant myocardium (70, 116). As oxidation becomes saturated, the TG accumulation initially may serve as a buffer against the toxic lipid species, but progressive exhaustion of storage capacity could foster the buildup of fatty acid intermediates (long-chain acyl-CoA, DAG, and ceramide) in the cytoplasm, contributing to lipotoxicity (70). In addition, a host of other molecular mediators, such as nitric oxide, ligands of PPAR nuclear receptor, and adipokines (including leptin), have been reported to promote cardiac lipotoxicity (146). The possible cross-talk between adipokine secretion by EAT (Table 1) and associated inflammatory processes, cardiac lipotoxicity, and the subsequent changes that lead to cardiomyocyte dysfunctioning and heart failure are represented schematically in Fig. 1.

Despite a considerable amount of literature on cardiac lipotoxicity, it is still unclear whether cardiac steatosis is the prime cause or the consequence of heart failure. This is because of the lack of well-developed human studies. However, at present, the available evidence suggests that lipid overstorage in cardiac myocytes contributes at least to an early manifestation in the pathogenesis of T2DM and LV dysfunction (70, 101, 115, 116).

**Cellular Cross-Talk and Vasocrine/Paracrine Interactions Between the Myocardium and the EAT**

Several studies have highlighted the central visceral or peripheral adipose tissues (VAT and SAT) as unique, pathogenic fat depots (33, 144, 145). Abdominal VAT is considered the largest visceral fat depot in the human body, with more than 10 times the volume of pericardial fat, and is significantly correlated with CVD risk factors and systemic markers of inflammation (38, 108, 117, 139). In contrast, EAT accounts for only a small fraction of total adipose tissue. An important question that presently remains unclear is how such a small fraction of EAT compared with VAT (especially of SAT and abdominal VAT) could augment or contribute to obesity or to the pathophysiology of CVD. At present, there are two possible mechanisms of interaction (vasocrine/paracrine) between the EAT and the myocardium that might help to explain the cellular cross-talk between these tissues.

The anatomical and functional contiguity of EAT to heart muscle (myocardium) and branches of coronary arteries suggests that vasocrine or paracrine signaling is plausible between adipokines and FFAs diffusing from EAT into the underlying myocardium (59, 119). In addition, migration of cells between these adjacent structures may also be possible (70), causing even small quantities of adipokines to have large pathophysiological effects on the surrounding tissues (Fig. 1) (142). It is postulated that adipokines secreted from epicardial adipocytes and stromal and vascular cells could diffuse in interstitial fluid across the adventia, media, and intima and then could interact with vasa vasorum as well as endothelial and vascular smooth muscle cells of the coronary arteries, as depicted in Fig. 1 and Table 1 (59). An alternate vasocrine signaling pathway whereby adipokines and FFAs released from EAT could directly enter the vasa vasorum and then be transported downstream into the arterial wall cannot be ruled out (Fig. 1). The issue of specific vasocrine or paracrine interactions is yet unclear (59, 119) and requires further research. However, comparative studies involving different adipose tissue depots such as EAT, SAT, and abdominal VAT and the expression and secretion of potential adipokytokine from these tissues might be helpful in determining the role played by each of these tissues in the pathophysiology of metabolic syndrome including obesity and the development of CVD. Therefore, in the following section, we will dissect and discuss some of the recent research findings concerning the potential adipocytokine production from the EAT, the possible cross-talk with the underlying myocardium, and their contributory role to the development of atherosclerosis.

**EAT, Adipokytokines, and Their Role in Atherosclerosis**

A host of proinflammatory and proatherogenic adipocyte factors (including TNF-α, IL-6, MCP-1, nerve growth factor,
resistin, leptin, visfatin, and others) have been investigated in relation to coronary atherosclerosis (Table 1) (32, 60, 99, 109, 119, 121) and have been found to participate in the various stages of atherogenesis (ranging from endothelial dysfunction to plaque destabilization and rupture) (110). The increased visceral adiposity and nutrient stress seen in the metabolic syndrome often disturb the adipocytokine secretion, leading to a chronic inflammatory state, and contribute to the increase in macrophage infiltration into adipose tissue (40, 46). A recent study in mice links nutrient stress to cardiac inflammation causing suppression of glucose metabolism (80). Histopathological studies from EAT in patients with CAD reveal dense inflammatory infiltrates within connective tissue septa, which includes inflammatory cells of diverse origin as well as lymphocytes (CD3⁺), macrophages (CD68⁺), and mast cells (5, 99, 125). Adipocytokines from EAT have been shown to promote atheromatous plaque formation in the intima layer by passing into the myocardium via the vasa vasorum (20, 140), a network of small blood vessels that supply conduit vessels (105).

Baker et al. (4) compared adipokine and cytokine expression levels in EAT from patients with CAD and in omental adipose tissue of patients without CAD. Their study revealed greater expression of inflammatory cytokines such as IL-6 and plasminogen activator inhibitor (PAI)-1 in EAT from CAD patients compared with omental adipose tissue from patients without CAD. In concordance with these data, the results of a recent study demonstrate that TNF-α and leptin gene expression increase prominently in the EAT, PAT, and SAT, whereas adiponectin gene expression decreases significantly in EAT and PAT in metabolic syndrome patients with CAD (compared with the control group) (36). Langheim et al. (88) compared the expression and protein secretion of several EAT adipokines of acute coronary syndrome (ACS) male patients with those of matched stable CAD patients and controls with angiographically normal coronary arteries. The EAT of ACS patients showed significantly higher gene expression and protein secretion of resistin than patients with stable CAD and was associated with increased in vitro endothelial cell permeability. IL-6, PAI-1, and MCP-1 genes were also significantly overexpressed in ACS compared with the control group but not compared with patients with stable CAD. Similarly, adipokines generated in EAT around atherosclerotic coronaries have been hypothesized to interact with vasa vasorum, smooth muscle cells, endothelium, and cellular components of the plaque by diffusion in interstitial fluid across adventia, media, and intima or due to cellular components released from EAT directly to vasa vasorum and transported downstream to arterial wall (Fig. 1) (55, 100, 148).

Another study by Cheng et al. (22) evaluated the potential differences in the tissue expression levels of adipocytokines from the two anatomically distinct fat depots (epicardial and abdominal adipose tissues) in patients with and without CAD. Their findings revealed that tissue levels of TNF-α, IL-6, leptin, and visfatin were significantly higher in CAD patients relative to control subjects (Table 1). In addition, abdominal adipose tissue levels of these four cytokines were significantly higher than levels in the EAT of CAD patients. Furthermore, compared with control subjects, tissue levels of adiponectin were significantly reduced in CAD patients, with significantly lower levels of abdominal than EAT depots, demonstrating that abdominal adiposity may play a crucial and significant role (also in considering the relatively greater fat biomass of abdominal adipose tissue) in the pathogenesis of coronary atherosclerosis. However, it cannot be ruled out that, although abdominal adiposity contributes significantly to adipocytokine levels, the EAT may exert direct effects on coronary atherosclerosis via their paracrine function. In fact, abdominal adiposity measurements by magnetic resonance imaging and EAT mass by ecocardiography have both been correlated with cardiovascular risks (57, 61, 66).

The effects of obesity and the impact that adipokines may have on cardiac energy metabolism and insulin signaling has already been discussed in recent reviews (91, 92). Shibasaki et al. (126) also investigated the relationship between adipokines, adipocytokines, and vasoactive peptides expressed by EAT and SAT in patients with and without CAD. The mRNA levels of inflammatory cytokines, adipokines, neurohumoral factors and their receptors were shown to increase in EAT independently of plasma levels of these mediators. The expressions of IL-6, IL-1β, MCP-1, and TNF-α were also significantly higher in EAT than in SAT. Interestingly, IL-6, IL-1β, MCP-1, natriuretic peptide receptor C (NPR-C), adrenomedullin, and leptin expressions in EAT from patients with CAD are much higher than those without CAD (126). However, adiponectin, PPARγ, and NPR-A expression showed similar trends in both groups of patients. The higher expression of these mediators in EAT of the CAD group did not reflect the plasma levels of these markers between the CAD and non CAD groups, since both groups showed similar pattern of plasma gene expression with regard to IL-6, IL-1β, MCP-1, and TNF-α (126). The authors are of the opinion that inflammatory cytokines produced by EAT may act locally as paracrine atherogenic factors, rather than as circulating factors. In addition, other studies have shown that adipocytes interact with atrial natriuretic peptide, brain natriuretic peptide, and adrenomedullin (68, 123). The mRNA level of NPR-A was found to be lower in EAT than in SAT, although there was no difference in mRNA expression of adrenomedullin or NPR-C between these two types of adipose tissue.

However, some studies on gene expression have shown decreased levels of inflammatory adipocytokine secretion from EAT. For example, Fain et al. (34) conducted a study to evaluate the hypothesis that EAT would exhibit greater expression of inflammatory mRNAs than subcutaneous fat or SAT and that EAT mRNAs would be expressed at higher levels than in omental fat, as represented in Table 1. While gene expression of about 20% of 45 proteins studied was higher in EAT than in subcutaneous fat, none of these were found to be inflammatory cytokines. In fact, the levels of gene expression for inflammatory adipokines, such as, TNF-α and IL-6, as well as that of adiponectin, were lower in EAT than in subcutaneous fat, whereas IL-1β, MCP-1, PAI-1, and the macrophage marker CD68 showed similar expression levels in both EAT and subcutaneous fat tissues (34). Furthermore, a recent transcriptome study evaluated what genes were differentially regulated in EAT, mediastinal (MAT), and SAT and reported that 23 and 73 genes were differentially upregulated in EAT compared with MAT and SAT, respectively (41). That study further reported that 94 genes were downregulated in EAT compared with SAT, although none were downregulated in EAT compared with MAT. Despite these similarities, two genes involved in...
CVDs, namely the adenosine A1 receptor (ADORA1) and prostaglandin D2 synthase (PTGDS), were differentially upregulated in EAT compared with MAT (41). The differential upregulation of these genes suggests a possible cardioprotective role for EAT. Interestingly, activation of ADORA1 seems to have an important role in cardioprotection from ischemic damage, while PTGDS overexpression may prevent cardiac injuries due to its anti-inflammatory properties (71, 113). These results suggest that EAT is potentially different from MAT, indicating perhaps a more direct involvement of EAT in the myocardium.

To provide information regarding the modulatory role of EAT in myocardial homeostasis and atherosclerosis, Kourliouros et al. (81) examined the local and systemic release of the proinflammatory IL-6 and the anti-inflammatory adiponectin in the development of postoperative atrial fibrillation (AF) by analyzing their levels in both plasma and EAT. They showed that patients who remained in sinus rhythm (SR) had a higher adiponectin release from EAT cultures than their AF counterparts. Although baseline serum levels of IL-6 were found to be lower in SR patients, the data did not show any statistical association with the development of AF. Furthermore, a negative, but significant, association (coefficient $= -0.045, P = 0.71$) of EAT adiponectin with increasing age was reported. The study confirms an association between EAT adiponectin and SR following cardiac surgery and also shows that systemic biomarkers provide very limited information in the prediction of postoperative AF. Adrenomedulin had been previously been implicated as a potent angiogenic and anti-inflammatory molecule due to its vasodilatory and antioxidant properties (47, 48, 74).

A recent microchip array study has shown that 271 genes encoding proteins were overexpressed in EAT compared with SAT, with the highest expression being secretory type 2 phospholipase A2 (sPLA2-IIA) (31). In addition, the expression and secretion of these markers were found to be higher in CAD patients than in patients without CAD (31). Overall, an imbalance between the harmful and protective factors secreted by EAT might be a crucial determinant in the development of cardiac pathologies.

It has been shown that adipocytokines interact with each other and that adiponectin counteracts the proinflammatory effects of TNF-$\alpha$ on the arterial wall (90, 104). Leptin and TNF-$\alpha$ also seem to regulate each other’s production (67). Sacks et al. (121) has shown that pioglitazone treatment of T2DM patients with CAD is associated with a reduction of proinflammatory and anti-inflammatory genes (IL-1$\beta$, IL-1Ra, and IL-10) in EAT and that a selective increase in PPAR$\gamma$ is observed in SAT. Although EAT has been reported to be a source of both proinflammatory and anti-inflammatory markers, mounting evidence suggests that local secretion of proinflammatory cytokines from EAT are more predominant and could downregulate the protective and anti-inflammatory cytokines along with adiponectin and adrenomedullin in severe CAD (4, 22, 42, 63, 99, 126).

Although the increased production of proinflammatory adipokines by EAT is related to CAD, to our knowledge there are no studies that show a direct relation between these biomarkers from EAT and cardiac function except for a few reported studies showing a correlation between fat depots such as SAT and cardiac function (86, 87, 93). The role of resistin in cardiac contractility and hypertrophy has been tested in rat cardiomyocytes (79). Overexpression of resistin (isolated from a rat heart cDNA library and subcloned into the pShuttle vector) in adult cultured cardiomyocytes significantly alters myocyte function by depressing cell contractility, as well as decreasing contraction and relaxation velocities (79). Some recent studies on EAT adipokine expression in human pathological states are presented in Table 1.

As indicated so far, many studies show that EAT is a cardiovascular risk factor locally contributing to CAD. However, fewer studies have clearly shown that preexisting CAD contributes to the inflammation in EAT, even though endothelial dysfunction appears to be an important player in the development of atherosclerosis. Recent studies show that increased intracellular NF-$\kappa$B activity is a very important mechanism by which oxidative stress mediates vascular endothelial dysfunction in aging and obesity (52, 107). Thus, endothelial dysfunction could play an equally important role in the increased risk of CVD, with aging being a major risk factor. Furthermore, a large number of studies have so far mostly explored the gene expression levels of various biomarkers of inflammation with regard to EAT. Thus, additional protein expression studies would be valuable to correlate protein changes of these markers with their possible implication in CVD.

**Conclusions and Perspectives**

Visceral fat obesity as a critical component of (and a major underlying driver for) the metabolic syndrome has been a subject of intense study in recent years. Under physiological and metabolic high energy demand conditions, EAT can act as an energy and heat supplier to the myocardium and provide protection to coronary arteries. However, under pathological states, EAT may be an adverse lipotoxic, prothrombotic, and proinflammatory organ. It is becoming increasingly clear that in metabolic disease states EAT-mediated adipokine production plays an important role in CVD, including cardiomyopathy. Although recent animal studies were able to provide important clues, the underlying pathophysiological mechanisms have not been fully elucidated. In addition, although a large number of studies have explored the gene expression levels of various biomarkers of inflammation with regard to EAT, further studies are needed that look particularly at protein expression levels of these markers and their implications in CVD.

Many questions are yet to be answered concerning the causes and correlates of CVD, including cardiac failure associated with the metabolic syndrome. Some of the important questions that need further investigation include: 1) What are the mechanisms behind the increased accumulation of the lipotoxic intermediates in the myocardium and its role in heart failure? 2) How and to what extent does the altered expression of adipokines from adjacent adipose depots like EAT affect myocardial function, as well as its relation to myocardial dysfunction? 3) As demonstrated in a recent study by Chokshi et al. (23), can the LV mechanical assist device support alone improve systemic insulin resistance in the myocardium and the energetics of the RV in patients with advanced heart failure?

In conclusion, more work is needed that involves well-developed translational human studies in order to better under-
REFERENCES


