ABSTRACT
This article provides a conceptual framework for the possible role of (i) periadventitial adipose tissue, and (ii) epicardial adipose tissue in the pathogenesis of cardiovascular disease. Traditional concepts of atherogenesis are focused on luminal surface, where “inside-out” inflammatory events trigger the extravasation of immune cells and the accumulation of lipids, smooth muscle cells and matrix components leading to atherosclerotic plaque formation. However, increasing evidence supports a new concept of an “outside-in” responses, involving periadventitial adipose tissue, herein referred to as tunica adiposa, and epicardial adipose tissue, these two adipose loci functioning as secretory tissues. Thus, a paracrine signals originated from these tissues could be transmitted into both the coronary artery intima and the myocardium. The present review highlights the possibility for tunica adiposa and epicardial adipose tissue to play an important role in an “outside-in” signaling in the development of atherosclerosis and cardiomyopathy. In effect, adipose-targeted pharmacology and non-invasive measures might provide novel clinical insights into cardiovascular adipobiology. Data of adipose-derived adipokines, including neurotrophic factors and neuropeptides, are also presented, raising a hypothesis of neuroendocrine potential of adipose tissue; it may also be instrumental in the pathogenesis of cardiovascular disease.

Key words: adipokines, atherosclerosis, epicardial adipose tissue, neurotrophic factors, neuropeptides, periadventitial adipose tissue

INTRODUCTION
“Ask yourself for each of your thoughts: is it a new one?”
Carl Gustav Jung (1875-1961)

Contemporary human lifestyle related to feeding and physical activity can lead to an increasing accumulation of adipose tissue. Hence the incidence of obesity and related cardiovascular diseases including atherosclerosis, hypertension and metabolic syndrome is increasing dramatically in all countries of the world. The overwhelming influence of these diseases contributes to a decreased quality of life as well as significant economic consequences. Hopefully, there would be great benefits if research could achieve effective prevention and therapy for cardiovascular diseases.

Work over the past several years has revealed that adipose tissue plays a pivotal role in controlling whole-body lipid and glucose homeostasis in both normal and disease state, visceral adipose tissue accumulation and inflammation-related insulin resistance being considered as the common denominators of the development of cardiovascular disease (1-4).

Atherosclerosis is a disease affecting mainly “large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction” (5). In atherogenesis, the response-to-injury hypothesis of Russell Ross proposes that lymphocyte and monocyte extravasation into the intima, and vascular smooth muscle cell proliferation (5) and oversecretion of matrix molecules (6) are the key events in the initiation and development of atherosclerotic plaques. Because advanced intimal lesions lead to luminal loss, resulting in infarction, the intima is considered by many authors the most important vascular area involved in atherogenesis. Recently, growing evidence, however, rises the possibility of adventitial (7-10), peri-
adventitial adipose tissue (PAAT) (11-14) and epicardial adipose tissue (EAT) (15-19) pathways in the cardiovascular injury response.

Here we emphasize that heart-associated adipose tissue represented by PAAT (herein referred to as tunica adipose) and EAT are an additional example of the secretory potential of the heart, the first one being atrial natriuretic peptide secretion initially suggested by 1964 George Palade’s electron microscopic description of “specific granules in atrial muscles” (20). Further, there is at present evidence that the sharing of ligands (growth factors, cytokines, and adipokines) and their receptors constitute a molecular language of neural, immune and adipose cells (21-29). Note that perinodal adipose tissue is a newly recognized feature of the lymph node (30), thus making lymph nodes an excellent example of adipo-immune paracrine interactions. Other examples include orbital adipose tissue in Graves’ thyroid-associated ophthalmopathy (31), mammary gland-associated adipose tissue in breast cancer (32), and artery- and heart-associated adipose tissue in cardiovascular diseases (33-42). Briefly, PAAT and EAT could represent another examples of adipoparacrinology (43). Table 1 presents heart-associated adipose tissue loci.

### Table 1. Heart-associated adipose tissue loci*

<table>
<thead>
<tr>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium/subepicardium</td>
<td></td>
</tr>
<tr>
<td>Pericardium</td>
<td></td>
</tr>
<tr>
<td>Atrial septum</td>
<td></td>
</tr>
<tr>
<td>Coronary arteries (proximal segments)</td>
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</tr>
</tbody>
</table>

* Heart adiposity should be distinguished from obesity-related lipotoxic cardiomyopathy, in which excessive fat accumulates inside cardiomyocytes (19).

### SECERTORY NATURE OF HEART-ASSOCIATED ADIPOSE TISSUE

The white adipocyte has long been recognized as the main producer of triglycerides during feeding, which are stored in highly concentrated form in a single large-sized lipid droplet. Whereas during fasting, the triglycerides are hydrolyzed and released into the blood circulation as free fatty acids (and glycerol), which are transported to other tissue to be oxidized in mitochondria as an energy source. Note that circulating fatty acids mediate insulin resistance (in skeletal muscle) in obesity and related diseases (1). By contrast, a high content of mitochondria (which produces the brown color) and of the uncoupling protein-1 allows the brown adipocyte to generate heat, thus being specialized primarily for the regulation of non-shivering thermogenesis.

Arguably, the most momentous changes that have occurred in the field of adipose tissue study have been the discovery and elucidation of its endocrine and paracrine function. Initial insights into this new biology of adipose tissue resulted from the discovery of leptin in 1994. The secretory potential of adipose tissue is executed by adipocytes as well as non-adipocyte cell types such as tissue matrix cells, stromovascular cells and associated macrophages, mast cell and lymphocytes (43-51). All these cells secrete a large number of proteins designated adipokines (Table 2). Adipokines play important roles in the pathogenesis of a various diseases beyond obesity. Accordingly, adipobiology of disease has emerged as a novel field of studies, which has enjoyed explosive growth in the past 10 years in basic, translational and clinical medicine (11-13,43-56).

We argue that each intracellular step of adiposecretion, including synthesis, sorting, storage, and exocytosis, as well as adipokine receptors, may provide novel targets in adipopharmacology of cardiovascular disease (25-27,45,49).

### Table 2. A selected list of adipose tissue-derived mediators, as related to cardiovascular disease

#### Pro-inflammatory adipokines
- C-reactive protein, serum amyloid A, haptoglobin, leptin, IL-1, IL-6, IL-18, MCP-1/CCL2, IL-1ra/CCL3,  (tumor necrosis factor-α, plasminogen activator inhibitor-1, NGF, angiotensin II)

#### Anti-inflammatory and metabolic adipokines
- Adiponectin*, IL-1 receptor antagonist*, IL-10*, leukemia inhibitory factor (LIF)*, metallothionein 1-3, ciliary neurotrophic factor, NGF*, BDNF*, transforming growth factor-β, cardiac natriuretic peptide, adrenomedullin*, angiotensin-like protein-4*

#### Vasodilators
- Adipocyte-derived relaxing factor, nitric oxide (NO)*, adiponectin*, cardiac natriuretic peptide, adrenomedullin*

#### Vasoconstrictors
- Superoxide anion, angiotensin II, endothelin-1

#### Other mediators
- Complement-C3c, TNF-related protein-1 (CTRP1), kisspeptide, estrogens*, free fatty acids, prostaglandins

* At present, antiatherogenic and/or metabolotropic effects of these mediators are reported (114 for adrenomedullin; 115 for ILF; 116 for adrenomedullin-like protein 4; references for other mediators are indicated in the text). For stimulation of aldosterone production by CTRP1 (117), for receptor-mediated actions of free fatty acids (118); for metallothioneins (51,119-121); for angiotensin-1 (122); for NGF/VEGF (123,124); for kisspeptide-1 (125); for NGF, BDNF and other metabolotropic factors (103,112,126)

A long standing paradigm holds that the vascular wall consists of three concentric tissue coats (tunicae): intima, media, and adventitia. Almost every systemic blood vessel, including arterioles (13), are surrounded by PAAT, which had been mainly considered as a mechanical support for vascular wall. Note that PAAT and EAT, also pericardial adipose tissue (57-59) and atrial septum-associate adipose tissue (60), are visceral adipose depot, which have not been studied thoroughly as visceral abdominal adipose tissue and subcutaneous
One aspect of the role of tunica adiposa is whether it facilitates or inhibits the process of atherogenesis. From human coronary atherosclerosis we know that the proximal coronary segments, particularly the left anterior descending (LAD) branch, are surrounded by subEAT and are atherosclerosis-prone as compared to the distal, intramyocardial, adipose coat-free, atherosclerosis-resistant coronaries (33,61). Reminding Carl Jung’s concept of cryptomnesia expressed in the motto of present article, it must be mentioned that in 1933, Smith and Willius (cited by 19,59) reported their results obtained from autopsies on 136 obese subjects and suggested a functional relationship between the EAT and the LAD coronary artery. The authors found that “in most instances, a definite relationship between the excess of epicardial fat and the degree of general obesity occurred.” Another important reason for subEAT to serve as an example of (a large-sized) tunica adiposa is the close association of the coronary vasculogenesis with epicardium-derived mesenchymal cells. These cells invade the subepicardial matrix and differentiate into coronary vascular smooth muscle and endothelial cells (64). Last not least, segments of coronaries lacking PAAT and thus being bridged by myocardial fibers are protected against the development of atherosclerosis (see 19,33). However, when EAT and probably PAAT are totally absent in congenital generalized lipodystrophy, coronary atherosclerosis can still occur. Thus, we should remind ourselves that “a little fat is good” (cited by 12) as well as “the fatter, the better” (65).

Further, in 1962 Schwartz (cited by 7) wrote with respect to the presence of adventitial mononuclear cell infiltration of atherosclerotic vessels: “it is perhaps surprising that such prominent cellular accumulation should have received so little attention... Nevertheless, since cellular infiltration of the adventitia shows such a constant relationship to the presence and degree of plaque formation, it should not be disregarded.” Today, this statement may also address PAAT (tunica adiposa). Thus, PAAT may indeed represent one of many paths leading to atherosclerosis (66).
NEUROTROPHINS AS ADIPOKINES

Within the vascular wall, smooth muscle cells comprise the primary target of the sympathetic neurons and, respectively, serve as the main source of nerve growth factor (NGF) (61-63 and references therein). Recent evidence shows that plasma and tissue levels of the neurotrophins NGF and/or brain-derived neurotrophic factor (BDNF), which is also produced by adipose tissue (67), are decreased in various cardiovascular diseases (61-63,68-71, cf. 72,73). Noteworthy, also decreased are the plasma levels of adiponectin (17,74,75), an "old" antithromogenic (48) and a novel vasorelaxing (53) adipokine.

Given the key role of inflammation and fibrosis in the development of atherosclerotic lesions, what role, for example, might tunica adiposa and EAT play in the process of atherogenesis? The expansion of adipose tissue seen in obesity is associated with an imbalanced secretion represented by an enhanced release of inflammatory adipokines and by decreased release of antiinflammatory adipokines (Table 2). Such an “enemy-or-friend” (36), “bystander or player” (38,39) or “double role” (40,41) secretory capacity of PAAT requires specific pharmacological manipulation, which aims at boosting the production and/or receptor sensitivity of anti-inflammatory/metabolotropic adipokines. Further, both leptin (76,77) and NGF (78-81) accelerate skin wound healing and thus raise a pressing question of whether this may also be the case with atherosclerotic vascular wound (see 5 to remind Russell Ross’s paradigm of atherogenesis). Note that there is an increased tissue levels of NGF in diabetic skin wound (79,80) as well as ischemic cardiac tissue (82), whereas exogenous administration of NGF improves the healing process in both tissues. Likewise, treatment with NGF (83) or adiponectin (84) resulted in a significant up-regulation of IL-10, an antiinflammatory cytokine. These data may also be relevant to a potential therapeutic effect of both NGF and adiponectin in atherosclerosis, an inflammatory, IL-10-deficient disease (5).

Clearly, the importance of local and systemic secretory involvement of both tunica adipose and EAT requires further evaluation in cardiovascular pathology. Understanding the paracrine signaling issued from PAAT and EAT might eventually lead to therapeutic and preventive strategies that will improve human vascular and metabolic health (85,86).

NEUROPEPTIDES AS ADIPOKINES

As neurotrophins, a large number of neuropeptides are also produced by adipose cells and exert extraneuronal effects, including on glucose, lipid and energy metabolism. Examples include substance P (87), neuropeptide tyrosine (NPY) (88-90) and other neuropeptides (91-96). In the same vein, most hypothalamic and pituitary neuropeptides, hormones and releasing factors, termed “adipotrophins” (97), express their receptors in adipose tissue, creating hypothalamic-pituitary-adipose axis (98-100). Further, (i) amino acid neurotransmitters such as glutamate and gamma-aminobutyric acid and their receptors are expressed in adipose tissue (101), and (ii) multipotential stem cells are associated with perivascular adipose tissue (102).

Taken together, the adipose-expression of neuropeptides/receptors and neurotrophic factors such as NGF (103-105), BDNF (67,105) and ciliary neurotrophic factor (103 and references therein) raises a possibility of adipose tissue’s neuroendocrine potential, which may also be implicated in the pathogenesis of cardiovascular disease.

Nevertheless, one thing stays much clear: in basic cardiovascular research, we should no longer cut neither adventitia, nor tunica adiposa and EAT, but keep them attached and in place, and subject to thorough examination. “Non-touch harvesting technique” in coronary artery by-pass surgery (106) is a clinical example of such an adipoprotective approach (see 107-110). Further, non-invasive measures at present are focused on vascular functions and structures such as flow-mediated vasodilatation and intima-media thickness, whereas measurements of the thickness of adventitia and adipose are neglected; indeed, measurements of EAT have been recently performed in clinical practice (15,111). In future, echographic, MRI and other non-invasive assessments of these cardiovascular coats may form a rational for identifying high-risk population susceptible to atherosclerosis, and monitor vascular wall changes during follow-up studies and therapeutic trials.

POST SCRIPTUM

In humans, white adipose tissue is partitioned into a few large depots, including visceral and subcutaneous location, and many small depots associated with heart, blood vessels, lymph nodes, ovaries, eyes, kidneys, adrenal glands, also located in liver, skeletal muscles and mammary glands (30-33,45). Accordingly, Homo obesus (112) is currently viewed as a disorder triggering the pathogenesis of a variety of cardiometabolic, liver, vascular, lung, and neurodegenerative (e.g. Alzheimer’s) diseases. Adipotopography (fat mapping) is an emerging subfield of adipobiology dealing with localization and amount of adipose tissue in the human’s body (113). Thus people may express TOFI (Thin Outside, Fat Inside), TOTI or other phenotypes (Table 3).

TOFI was described by Dr Jimmy Bell, head of the Molecular Imaging Group at Hammersmith Hospital, London, UK. It can be visualized by using current imaging technologies such as echography, computed tomography, magnetic resonance imaging, and proton magnetic resonance spectroscopy. A predictive message of adipotopography is that "being thin does not automatically mean you are..."
not fat”, quoting Dr Bell. The concept of TOFI holds that small adipose depots, when enlarged and activated (by inflammatory, overnutritional or other stimuli), may exert disease-promoting actions over adipose tissue-associated organ(s). Thus, the traditional diagnostic significance of BMI, as well as other anthropometric criteria (waist and hip circumference alike), should be re-evaluated in obesity and related diseases. Importantly, dieting is enough to keep one being thin outside, whereas physical activity prevents the accumulation of internal fat, thus one can be thin inside. In conclusion, TOFI is a Trojan Horse inside the human’s body, a pathological phenomenon, whereas TOTI is a healthy adipose phenotype. Briefly, slim or obese, get your fat map.

Table 3. Adipotopography (fat mapping): variations+

<table>
<thead>
<tr>
<th>TOFI**</th>
<th>thin outside, fat inside</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTI*****</td>
<td>thin outside, thin inside</td>
</tr>
<tr>
<td>FOFI*</td>
<td>fat outside, fat inside</td>
</tr>
<tr>
<td>TOTI**</td>
<td>fat outside, thin inside</td>
</tr>
</tbody>
</table>

+ The number of asterisks indicates the quality of cardiometabolic health, as related to adipose tissue. Hence, stay TOTI. From 113.

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