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PATHOPHYSIOLOGY OF ISCHEMIC DISORDERS- ISCHEMIA, ADIPOCYTOKINES AND DIABETES MELLITUS: A REVIEW

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases which result from defects in insulin secretion, or action, or both. Three main forms of diabetes are: type 1 diabetes mellitus, type 2 diabetes mellitus and type 3 or gestational diabetes mellitus. Endothelial dysfunction has been demonstrated in type 1 and type 2 diabetes. In type 1 diabetes, this alteration appears temporally linked to vascular disease and is more likely a consequence of the metabolic alterations. In type 2 diabetes, endothelial cell (EC) dysfunction is detectable very early in the course of the disease, even before overt hyperglycemia ensues, and may play a key role in the etiopathology of the vasculopathy associated with this disease. The hypothesis that endothelial dysfunction may be causative of some of the features of the syndrome of insulin resistance, however, deserves further research. Recent studies have provided evidence that adipose tissue may play a crucial role in the development of insulin resistance, type 2 diabetes, and their complications through the secretion of a variety of biologically active molecules (adipocytokines). Leptin affects insulin sensitivity and may participate in the development of hypertension. These adipocytokines may cause the atherosclerotic vascular disease in type 2 diabetes directly or through the development of insulin resistance. Therefore, the leptin-to-adiponectin ratio might serve as an atherogenic index superior to leptin or adiponectin alone. Furthermore, it has been demonstrated that endothelial progenitor cells (EPCs) are reduced in macrovascular diabetes complications. Hence, it might be interesting to study the effect of adipokines mainly leptin on EPCs from healthy individuals and patients with both type 1 and type 2 diabetes mellitus. This might provide opportunity to understand the cellular and intracellular changes in EPCs collected from patients with diabetes, and better knowledge of the molecular basis of diabetic disorders.

Keywords: Ischemia, diabetes mellitus, adipocytokines, pathophysiology, endothelial progenitor cells

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases which results from defects in insulin secretion, or action, or both. There are three main forms of diabetes: type 1 diabetes mellitus, type 2 diabetes mellitus and type 3 or gestational diabetes mellitus, although these three "types" of diabetes are more accurately considered patterns of pancreatic failure rather than single diseases. Type 1 diabetes is an autoimmune disease, and hence, the immune system attacks and destroys the insulin-producing beta cells in the pancreas. The pancreas then produces little or no insulin. Type 2 Diabetes is the most common form of diabetes and is often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities. About 80 percent of people with type 2 diabetes are overweight. When type 2 diabetes is diagnosed, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance. Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a chance of developing type-2 diabetes.

Endothelial dysfunction has been demonstrated in type 1 and type- 2 diabetes. In type 1 diabetes, this alteration appears temporally linked to vascular disease and is more likely a consequence of the metabolic alterations. In type 2 diabetes, endothelial cell (EC) dysfunction is detectable very early in the course of the disease, even before overt hyperglycemia ensues, and may play a key role in the etiopathology of the vasculopathy associated with this disease. The hypothesis that endothelial dysfunction may be causative of some of the features of the syndrome of insulin resistance, however, deserves further research. The evolving role of adipokines in endothelial dysfunction adds a new dimension to our understanding of the relationship between obesity, notably for those with increased abdominal fat, and CVD risks. Further investigations into the molecular links between obesity and atherosclerosis will unravel innovative therapeutic strategies to improve cardiovascular health in people affected by obesity-linked insulin resistance, metabolic syndrome, and Type-2 diabetes.

The dysfunction in endothelial function has been noted in diabetes (Imanishi *et al*., 2005; Fadini *et al*., 2006; Marchetti *et al*., 2006; Marumo *et al*., 2006; Rosso *et al*., 2006; Segal *et al*., 2006; Chen *et al*., 2007; Thum *et al*., 2007; Chang *et al*., 2010; Oikawa *et al*., 2010; Kowluru *et al*., 2011; Leicht *et al*., 2011). Insulin resistance is one of the important risk factors associated with atherosclerosis and diabetes. Insulin resistance often accompanies visceral

fat accumulation (Matsuzawa *et al*., 1999). Studies have provided evidence that adipose tissue may play a crucial role in the development of insulin resistance, type 2 diabetes, and their complications through the secretion of a variety of biologically active molecules (adipocytokines) (Matsuzawa *et al*., 1999). Role of adipokines in β-cell failure of type 2 diabetes has recently been reviewed (Dunmore and Brown, 2013). Leptin is an adipose-specific hormone contributing to the regulation of energy expenditure and food intake (Caro *et al*., 1996). Leptin also affects insulin sensitivity and may participate in the development of hypertension (Shimomura *et al*., 1999). These adipocytokines may cause the atherosclerotic vascular disease in type 2 diabetes directly or through the development of insulin resistance. Adiponectin is a novel adipose-specific collagen-like molecule that belongs to the collectin family (Maeda *et al*., 1996). Adiponectin bound to collagens I, III, and V (major components of the vascular intima) in a solid-phase binding assay and accumulated in the vascular wall when the endothelial barrier was damaged. Furthermore, high levels of eicosonoids, dysfunction in EPCs and diabetes in diabetic patients has recently been found (Issan *et al*., 2013). Another report shows the role of heme oxygenase-adiponectin in bone marrow stem cell transplant into intra-bone cavity preventing type 2 diabetes (Abraham *et al*., 2008).

Obese patients, type 2 diabetic patients, and patients with coronary artery disease show significantly lower levels of plasma adiponectin (Arita *et al*., 1999). It was found that administration of adiponectin decreased the attachment of monocytic cell line THP-1 cells to human aortic endothelial cells (Ouchi *et al*., 1999), which is an early event in atherosclerotic vascular damage. Adiponectin decreases the expression of multiple adhesion molecules, including in endothelial cells via the modulation of NFkappa B signaling (Ouchi *et al*., 1999). Adiponectin also dramatically suppressed the secretion of TNF-alpha from human monocyte-macrophages (Yokota *et al*., 2000). A clear association between high levels of eicosonoids, dysfunction in EPCs and diabetes in diabetic patients with ischemia has been investigated (Issan *et al*., 2013). However, no relationship could be detected between the decrease in the level of EPC and in the level of total adiponectin in blood from patients with type 2 diabetes (Li *et al*., 2011). Another report shows the decrease in circulating endothelial progenitor cells (EPCs) in type 2 diabetes independent of the severity of the hypoadiponectemia (Li *et al*., 2011). Most of these clinical and experimental observations suggest in general that adiponectin plays some protective role against the atherosclerotic vascular change and that the decreased plasma adiponectin in type 2 diabetic patients may contribute to the development of atherosclerotic complications. The mechanism of decreased plasma adiponectin in type 2 diabetes, however, has not yet been clarified.

Obesity promotes the progression of atherosclerosis by inducing multiple cardiovascular and metabolic derangements such as diabetes, hypertension, and dyslipidemia, all of which have high atherogenic potential. Adipose tissue has been considered an important endocrine organ that secretes adipocytokines (Matsuzawa *et al*., 1999). Two major adipocytokines, leptin and adiponectin, are thought to play important roles in the regulation of cardiovascular and metabolic homeostasis. Leptin acts directly on the hypothalamus, thereby regulating food intake and energy expenditure (Friedman and Halaas, 1998). Plasma leptin concentrations are significantly elevated in obese subjects in proportion to the degree of adiposity (Considine *et al*., 1996), suggesting that hyperleptinemia may play a role in the pathogenesis of obesity-related complications. On the other hand, adiponectin increases tissue fat oxidation, leading to reduced levels of fatty acids and tissue triglyceride content, thus increasing insulin sensitivity (Matsuzawa *et al*., 2004).

The investigations in basic and clinical studies during the past two decades have changed the physicians' views about adipocyte pathophysiology especially after the discovery of leptin in 1994, and white adipose tissue then recognized as an endocrine organ secreting adipokines was found to be involved in the pathogenesis of inflammation, endothelial dysfunction, diabetes mellitus, atherosclerosis, chronic kidney disease and other obesity-related pathologic processes. (Adamczak and Wiecek, 2013). Furthermore, it has been found that adiponectin might contribute in increaseng the bioavailability of nitric oxide via reducing superoxide production and inhibiting inflammation and adhesion molecules as well in aorta in type 2 diabetic mice (Lee *et al*., 2012). The view about the adipocyte pathophysiology entirely changed after the discovery of leptin and has now been considered as involved in the pathogenesis of inflammation, endothelial dysfunction, diabetes mellitus, atherosclerosis, chronic kidney disease and other obesity-related pathologic processes. (Adamczak and Wiecek, 2013).Paradoxically, plasma adiponectin concentrations are decreased in obese subjects (Arita *et al*., 1999), suggesting that hypoadiponectinemia is involved in the pathophysiology of obesity. Two recent studies have demonstrated that vascular remodeling and neointimal formation are markedly attenuated in leptin-deficient ob/ob mice and db/db mice with leptin receptor mutation (Stephenson *et al*., 2004), suggesting that leptin may accelerate the development of vascular injury. Conversely, studies with adiponectin-deficient mice have revealed that adiponectin plays a protective role in the development of atherosclerosis. In obese type 2 diabetic patients who are susceptible to atherosclerosis, plasma concentrations of leptin are increased, whereas those of adiponectin are decreased. The leptin-to-adiponectin ratio therefore, might

serve as an atherogenic index superior to leptin or adiponectin alone. Hence, it is significant to design and assess the potential of the leptin-to-adiponectin ratio as a biomarker for atherosclerosis in obese type 2 diabetic patients.

An unexplained paradox puzzles diabetologists, that diabetic patients must face both poor vessel growth in ischemic heart and limbs and increased angiogenesis in retinal complications(Abaci *et al*., 1999). Endothelial progenitor cells (EPCs) are marrow-derived cells involved in adult neovascularization and endothelial homeostasis (Asahara *et al*., 1997). It has been postulated that low EPCs in peripheral blood may have a role in cardiovascular disease, and it was also demonstrated that EPCs are reduced in macrovascular diabetes complications (Hill *et al*., 2003). On the other hand, an excess of EPCs may be involved in pathologic neoangiogenesis of cancer and proliferative retinopathy. Therefore, diabetes complications may be associated with both decreased and increased EPCs. Recently, novel therapeutic approaches have been directed to enrich the EPC pool in ischemic diseases and to block EPC function in proliferative diseases (Abaci *et al*., 1999). EPC dysfunction in diabetes has been found in the mobilization from bone marrow (Fadini *et al*., 2006; Thum *et al*., 2007; Oikawa *et al*., 2010; Kowluru *et al*., 2011), Trafficking (Rosso *et al*., 2006; Segal *et al*., 2006; Leicht *et al*., 2011), Survival (Imanishi *et al*., 2005; Marumo *et al*., 2006; Chang *et al*., 2010) and Homing and Differentiation (Marchetti *et al*., 2006; Chen *et al*., 2007).These approaches in diabetic subjects require cautious evaluation of the implications carried by the paradox and new studies to unravel its causes. Hence, it is essentially required to study the effect of adipokines mainly leptin on EPCs from healthy individuals and patients with both type 1 and type 2 diabetes mellitus. This may provide opportunity to understand the cellular and intracellular changes in EPCs collected from patients with diabetes, and better knowledge of the molecular basis of diabetic disorders.

ENDOTHELIAL CHARACTERIZATION AND SIGNIFICANCE

Considering the importance of blood vessel development on organogenesis, vasculogenesis by EPCs may be an essential cascade for tissue and organ regeneration following pathological damage in various critical diseases. Tissue regeneration for organ recovery in adults has two physiological mechanisms. One is the replacement of differentiated cells by newly generated populations derived from residual cycling stem cells. Hematopoietic cell regeneration is a typical example of this kind of mechanism. Whole hematopoietic lineage cells are derived from a few self-renewal stem cells by regulated differentiation under the influence of appropriate cytokines and/or growth factors.

Another mechanism is the self-repair of differentiated functioning cells, preserving their proliferative activity. Hepatocytes, ECs, smooth muscle cells, keratinocytes, and fibroblasts are considered to possess this ability. After physiological stimulation or injury, factors secreted from surrounding tissues stimulate cell replication and replacement. However, regenerative activity of these fully differentiated cells is still limited because of finite proliferation by senescence and because of their inability to incorporate into remote target sites. Whereas most cells in adult organs are composed of differentiated cells, which express a variety of specific phenotypic genes adapted to each organ's environment, quiescent stem or progenitor cells are maintained locally or in the systemic circulation and are activated by environmental stimuli for physiological and pathological tissue regeneration. In the past decade researchers have defined the stem or progenitor cells from various tissues, including bone marrow, peripheral blood, brain, liver, and reproductive organs, in both adult animals and humans. The isolation and characterization of endothelial progenitor cells from peripheral blood was first reported by Asahara *et al.* (1977). The exact phenotype and lineage of endothelial progenitor cells (EPCs) are still a matter of debate and different expansion protocols are used to obtain them. Experimentally, preplating may be a way to reduce the heterogeneity of the cultivated EPCs, because this excludes rapidly adhering cells such as differentiated monocytic or possible mature endothelial cells. A comprehensive review on the current applications of EPC in cardiovascular and peripheral vascular diseases, show the importance of the therapeutic application of EPCs to patients with ischemic disease, and explains the undergoing processes with the feedback of clinical researches (Asahara *et al*., 2011).

The ex vivo expansion of purified CD14+ mononuclear cells yielded cells with an endothelial characteristic, which incorporated in newly formed blood vessels in vivo (Urbich *et al*., 2003). These data would suggest that myeloid cells can differentiate (or transdifferentiate) to the endothelial lineage. Interestingly, lineage tracking showed that myeloid cells are the hematopoietic stem cell-derived intermediates, which contribute to muscle regeneration,(Camargo *et al*., 2003) suggesting that myeloid intermediates may be part of the repair capacity after injury. Thus, CEPCs appear to be a heterogenous group of cells originating from multiple precursors within the bone marrow and present in different stages of endothelial differentiation in peripheral blood. For this reason, the accurate characterization of EPCs is difficult because many of the cell surface markers used in phenotyping are shared by hematopoietic stem cells and by adult endothelial cells. In culture, EPCs emerge as late (>2 weeks) outgrowth colonies after plating in endothelium specified medium. Typically, the cells are defined on the basis of expression of cell surface markers such as CD34, Flk-1, and CD-133. CD133 (Suuronen *et al*., 2006) mand may be the most

specific marker of endothelial progenitor cells (EPCs), which are thought to be largely confined to the bone marrow milieu.

Recently, tissue-resident stem cells have been isolated from the heart, which are capable to differentiate to the endothelial lineage (Beltrami *et al*., 2003). The cells have high proliferative potential, albeit for a finite number of cell divisions. As the cells differentiate, they acquire endothelial lineage markers, vascular endothelium-cadherin, PECAM-1 (CD31), von Willebrand factor, endothelial nitric oxide synthase (eNOS), and E-selectin, and incorporate acetylated low-density lipoprotein cholesterol. The loss of hematopoietic stem cell marker CD133 expression coincides with EPC differentiation into cells with functional characteristics of adult endothelial cells. These data support the notion that it will be difficult to define the "true" endothelial progenitor cells. In the light of recent findings, we may have to redefine our thinking of endothelial cells as well as of perivascular mural cells (Kopp *et al*., 2006). The relative abundance of CEPC is low in basal conditions. However, the number of circulating cells increases several fold after exogenous stimulation with cytokines and hormones. The applications of the therapeutic application of EPCs to patients with ischemic disease provide information for future studies (Asahara *et al*., 2011).

Mononuclear cells (MNCs)that are unfractionated are cultivated in medium enriched with endothelial specific growth factors such as vascular endothelial growth factor (VEGF). Within days of plating, colonies of adherent cells proliferate rapidly to form a monolayer with the cobblestone morphology typical of endothelium. After 2 weeks, the cells adopt endothelial-like characteristics such as expression of von Willebrand factor, uptake of acetylated low-density lipoprotein cholesterol, and the ability to assemble into vascular tube-like structures . Using this approach, we are able to expand the circulating cells in culture to yield sufficient number for autologous transplantation onto injured blood vessels and prosthetic grafts in rabbits.

ENDOTHELIAL DYSFUNCTION, ADIPOCYTOKINES AND DIABETES MELLITUS

The investigations in basic and clinical studies during the past two decades have changed the physicians' views about adipocyte pathophysiology especially after the discovery of leptin in 1994, and white adipose tissue then recognized as an endocrine organ secreting adipokines was found to be involved in the pathogenesis of inflammation, endothelial dysfunction, diabetes mellitus, atherosclerosis, chronic kidney disease and other obesity-related pathologic processes. (Adamczak and Wiecek, 2013). Adiponectin is found involved in increasing the bioavailability of nitric oxide by reducing superoxide production and inhibiting inflammation and adhesion molecules as well in aorta in type 2 diabetic mice (Lee *et al*., 2012). Diminished capacity of NOS to generate NO has been demonstrated experimentally when endothelial cells (ECs) are exposed either in vitro or in vivo to a diabetic environment. The EC is then a target of the diabetic milieu and endothelial dysfunction is thought to play an important role in the vasculopathy of this disease state. A large body of evidence in humans indicates that endothelial dysfunction is closely associated to microangiopathy and atherosclerosis in both types 1 and 2 diabetes mellitus (Consentino and Luscher, 1998). This association is particularly true in those patients with type 1 diabetes who have either early (microalbuminuria) or late (macroalbuminuria) nephropathy. In these patients, a great variety of markers indicate endothelial dysfunction: poor EC-dependent vasodilation, increased blood levels of von Willebrand factor (vWF), thrombomodulin, selectin, PAI-1, type IV collagen, and t-PA (Consentino and Luscher, 1998).

Once established, EC dysfunction can, in turn, induce alterations in vessels that worsen vasculopathy and progress disease. Of note is that arteries and arterioles are not considered commonly as target tissues/organs of insulin action. However, in recent years a body of evidence has accumulated that supports the hypothesis that vessels are insulin responsive.

It is not clear from the literature whether EC dysfunction is the consequence of the diabetic milieu in type 1 diabetes or a marker of vascular damage. In a experimental model of type 1 diabetes, endothelial function was evaluated directly in rats (Brands and Fitzgerald, 1998) with chronically implanted flow probes. The responses to acetylcholine and sodium nitroprusside were not altered significantly; moreover, neither endothelium mediated vasodilation nor responsiveness to NO was impaired, and hyperglycemia did not directly or significantly impair endothelium-mediated relaxation in this model of insulin-dependent diabetes mellitus. In a recent study (Huvers *et al*., 1999), endothelium-dependent and endothelium-independent vasodilatation, endothelium-dependent hemostatic factors, and vasoconstrictor responses were determined in type 1 diabetic patients during euglycemia with and without microvascular complications. Forearm endothelium-dependent and endothelium-independent vasodilatation and adrenergic responsiveness were unaltered in type 1 diabetic patients with and without microvascular complications. Relative to healthy control subjects, endothelium-dependent vasodilatation was depressed during a repeated ACh challenge (with L-arginine coinfusion) in the diabetic patients without complications or with microalbuminuria. In contrast, this vasodilatation was enhanced in the patients with retinopathy.

Elevation of the endothelium-derived tissue factor plasminogen inhibitor was the most consistent marker of endothelial damage of all the endothelial markers measured in these group of patients but showed no correlation with the presence or absence of microvascular complications. Clarkson *et al*. (Clarkson *et al*., 1996) compared 80 young adults with insulin-dependent diabetes with 80 matched nondiabetic control subjects. Thus, in this study, EC dysfunction was found as an early manifestation of vascular disease but late in the course of type 1 diabetes. In another study (Fasching *et al*., 1996), the plasma fibronectin 30-kDa domain was measured in 44 type 1 diabetic patients and in 20 healthy subjects. A significantly raised mean concentration of a free N-terminal fibronectin 30-kDa domain was found in plasma of diabetic patients with proliferative retinopathy as compared with healthy persons, and a positive correlation was observed between free N-terminal fibronectin and vWF in plasma of all examined subjects (r = 0.62). A similar correlation was present between fibronectin and the degree of albuminuria (r = 0.56). However, no relationship was found between fibronectin and the degree of control of diabetes. Thus, in these two cross-sectional studies, type 1 diabetes has been associated more with the presence of microvascular disease than with the diabetic milieu. The relationship between adipocytokines and disorders has been found in patients with diabetes mellitus type 2 (Uslu *et al*., 2012).

It is the general consensus that the occurrence of EC dysfunction in type 1 diabetes signifies a very high risk of micro- and macroangiopathy and, although the diabetic state predisposes to EC dysfunction in this disease, is not sufficient to cause it. More likely, other agents (genes, environment) are likely to play a role in determining those patients that will develop aggressive angiopathy and hence EC dysfunction. Irrespective of whether EC dysfunction is a cause or a consequence of vascular injury in type 1 diabetes, therapeutic efforts aimed at restoring EC to normal will more likely have an affect on the natural history of vasculopathy in type 1 diabetes.

The role of endothelial dysfunction in type 2 diabetes is more complicated than that for type 1. The effects of aging, hyperlipidemia, hypertension, and other factors add to the complexity of the problem. In contrast to patients with type 1 diabetes, endothelial dysfunction can also occur in patients with type 2 diabetes even when the patients have normal urinary albumin excretion. In fact, markers of endothelial dysfunction are often elevated years before any evidence of microangiopathy becomes evident (Consentino and Luscher, 1998). A major pathophysiological alteration of type 2 diabetes is insulin resistance. As a result, a great research effort has been focused on defining the possible contribution of insulin resistance to endothelial dysfunction. There is a growing body of evidence accumulating to demonstrate the coexistence of insulin resistance and endothelial dysfunction. Insulin-induced vasodilation, which is partially mediated by NO release, is impaired in obese individuals who do not have type 2 diabetes but who display insulin resistance. Moreover, the obese state, a model of human insulin resistance, is associated with high levels of endothelin in plasma. PAI-1 concentrations in blood also are high in patients with otherwise uncomplicated obesity; a drastic decrease in PAI-1 levels has been noted by our laboratory in response to moderate weight loss.

It has been demonstrated that women with previous gestational diabetes have evidence of endothelial dysfunction (Knock *et al*., 1997). In women with polycystic ovary syndrome, high levels of PAI-1 have been found which improve with any therapeutic intervention that improves insulin sensitivity (Anderson *et al*., 1995). Further, evidence suggests that endothelial dysfunction occurs in a concomitant manner with insulin resistance and antedates overt hyperglycemia in patients with type 2 diabetes. Steinberg *et al*. (1997) recently demonstrated that elevated free fatty acid levels in blood (produced in normal individuals by simultaneous infusion of triglycerides and heparin) induced endothelial dysfunction. FFA are classically elevated in obese patients, patients with type 2 diabetes, and, in general, in those individuals who display features of the syndrome of insulin resistance. Thus, data support the hypothesis that the metabolic abnormalities of insulin resistance may lead to endothelial dysfunction. Under normal conditions, the blood is constantly in a balance between a "basal on-going" activation of coagulation and compensatory fibrinolysis. A current hypothesis states that, in patients with endothelial dysfunction, PAI-1 levels are elevated, which in turn inhibits dissolution of fibrin deposits on the luminal side of the vessel wall. Several investigators have suggested that PAI-1 plays a major role in the generation and/or progression of atherosclerosis. Examples of this are type 2 diabetes, upper body obesity, and polycystic ovary syndrome. These disease states also are associated with accelerated atherosclerosis, which supports the hypothesis that high levels of PAI-1 may play a role in initiation and/or progression of macrovascular disease.

It has been proposed that the hyper (pro)-insulinemia of insulin resistance might be implicated as an etiological alteration for the high blood levels of PAI-1. Several in vitro, in vivo, and animal models are strongly supportive of this hypothesis . However, other studies have found that infusion of insulin directly in humans for up to 6 h is not associated with an increase in the blood levels of PAI-1 (Grant *et al*., 1990). Recently, it was found that simulation of the diabetic environment in normal individuals by exogenous insulin infusions, resulting in hyperinsulinemia, hyperglycemia, and hyperlipidemia, was associated with an increase in the blood concentrations of PAI-1 (Calles-Escandon *et al*., 1998).

Adipose tissue is no longer viewed as a passive repository for triacylglycerol storage and a source of free fatty acids (FFAs). As developing preadipocytes differentiate to become mature adipocytes, they acquire the ability to synthesize hundreds of proteins, many of which are released as enzymes, cytokines, growth factors, and hormones involved in overall energy homeostasis (Ahima and Flier, 2000). No relationship could be detected between the decrease in the level of EPC and in the level of total adiponectin in blood from patients with type 2 diabetes (Li *et al*., 2011). However, recently a clear association between high levels of eicosonoids, dysfunction in EPCs and diabetes in diabetic patients with ischemia has been investigated (Issan *et al*., 2013).

Mature adipocytes are widely acknowledged as an active endocrine and paracrine organ secreting an ever-increasing number of mediators that participate in diverse metabolic processes (Ferroni *et al*., 2004). Compounds that influence adipogenesis include lipoprotein lipase, cholesterol ester transfer protein, angiotensinogen, complement factors (adipsin or complement D, adiponectin or C1q, acylation stimulating protein or C3-desArg), interleukin-6 (IL-6), prostaglandins , tumor necrosis factor-alpha (TNF-alpha), a novel protein/cytokine named adipocyte differentiation factor, and, more recently, nitric oxide (NO). These fat-derived molecules act via endocrine, paracrine, autocrine, and/or juxatacrine modes of action to modulate fat depot size and body fat redistribution and ultimately influence the levels of secretory proteins. More recently, adipose tissue is recognized as a rich source of proinflammatory mediators that may directly contribute to vascular injury, insulin resistance, and atherogenesis. These proinflammatory adipocytokines, or adipokines, include TNF-alpha, IL-6, leptin, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, resistin, and, more recently, C-reactive protein (CRP) .

On the other hand, NO and adiponectin confer protection against inflammation and obesity-linked insulin resistance (Kubota *et al*., 2002). It is likely that many more undiscovered fat cell-derived mediators will be causally linked to cardiovascular health, insulin resistance, and diabetes. Adipose tissue serves as a rich source of proinflammatory mediators such as TNF-alpha, IL-6, leptin, plasminogen activator inhibitor (PAI)-1, angiotensinogen, resistin, and C-reactive protein (CRP), which promote endothelial dysfunction, insulin resistance, and, ultimately, atherosclerosis. Other adipocyte products such as nitric oxide (NO) and adiponectin confer protection, but these appear to decrease in amount with increasing levels of obesity. These proinflammatory mediators, released by adipocytes, exert effects on the vasculature promoting the various stages of atherogenesis, namely, endothelial dysfunction, plaque initiation, plaque progression, and plaque rupture. ET-1, endothelin; ATII, angiotensin II; oxLDL, oxidized low-density lipoproteins; MCP-1, monocyte chemoattractant protein 1; SMC, smooth muscle cell; MMP, matrix metalloproteinase; EC, endothelial cell. Circulating adipokine levels are elevated in obese and insulin-resistant states in animals and humans, and intra-abdominal fat appears to produce several of the adipokines in greater amounts than other fat depots (Wajchenberg, 2000).

Weight loss is associated with a decrease in the serum levels of most of these adipokines, with the exception of adiponectin, which is increased (Wajchenberg, 2000). A large number of adipokines also affect insulin action, glucose, and fat metabolism and consequently insulin resistance, which ultimately leads to Type 2 diabetes. Hence, they exert direct as well as indirect influences on the process of atherosclerosis. Atherosclerosis is an inflammatory process that initially begins with endothelial dysfunction. Endothelial dysfunction, a systemic disorder and an early pivotal event in the pathogenesis of atherosclerosis, is characterized by an imbalance between endothelium-dependent vasodilatation and vasoconstriction as well as antithrombotic and prothrombotic factors. NO maintains the vasodilatory property of endothelium and opposes the effects of such vasoconstrictors like endothelin (ET)-1 and angiotensin II (ANG II) (Verma and Andersen, 2002). It inhibits leukocyte and platelet activation and aggregation and, together with prostacyclins, helps to maintain the endothelium as a smooth nonthrombotic barrier. In response to inflammatory triggers, an increase in endothelial adhesion and permeability leads to leukocyte entry and expression in the endothelium of adhesion molecules, namely, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Verma and Andersen, 2002). The expression of P- and E-selectins, which are involved in leukocyte recruitment and rolling, monocyte chemoattractant protein-1 (MCP-1), which promotes leukocyte transmigration, and integrins, which mediate subsequent adherence to the intima, are also increased (Libby *et al*., 2002). Recently, adipocytes have been demonstrated to produce MCP-1 directly (Christiansen *et al*., 2005). The phagocytosis of oxidized low-density lipoprotein (LDL) particles by monocytes leads to the formation of foam cells and the development of fatty streaks and plaques as well as smooth muscle cell proliferation.

Thus endothelial dysfunction is a prominent feature at various stages of atherogenesis, from the development of early subclinical atherosclerotic fatty streaks to atheromatous plaques, to plaque vulnerability and rupture, vasospasm, thrombus formation, and eventually vessel occlusion and infarction, and should be considered a marker of atherosclerosis. Evidence is mounting to suggest that adipokines may directly influence endothelial function through their proinflammatory properties. While the majority of the data are based on in vitro studies, they shed light on the molecular links between obesity, insulin resistance, and endothelial dysfunction. The secretory products of

adipose tissue contribute to the elevated risks of CVD, and these effects appear to be independent of their effects on insulin resistance and diabetes. Emerging data also link leptin to CVD. Leptin is a fat cell-specific hormone that functions as a signaling molecule on the brain to complete the negative feedback loop of the lipostatic theory of weight control (Friedman, 2000). It has been suggested that leptin exerts a paracrine effect on fat cells and that its expression and secretion by fat cells can be induced by IL-6 and inhibited by TNF-alpha, underscoring the potential role of interaction among adipokines on their release by fat cells (Abdel-Hafez *et al*., 2002; Lau *et al*., 2002). Leptin, like CRP, upregulates ET-1 and endothelial NO synthase production in endothelial cells and promotes accumulation of reactive oxygen species (Cooke and Oka, 2002). It stimulates the proliferation and migration of endothelial cells (Park *et al*., 2001) and vascular smooth muscle cells (Artwohl *et al*., 2002). MCP-1 expression in aortic endothelial cells is stimulated by leptin (Yamagishi *et al*., 2001). Furthermore, leptin increases platelet aggregation and arterial thrombosis via a leptin receptor-dependent pathway (Cooke and Oka., 2002), has a direct action on macrophages by increasing the release of monocyte colony-stimulating factor (Loffreda *et al*., 1998), promotes cholesterol accumulation in macrophages under high glucose conditions (O'Rourke *et al*., 2002), and stimulates angiogenesis (Sierra-Honigman *et al*.,2002). Leptin also increases peripheral sympathetic tone, with observations of lower arterial pressures in leptin-deficient mice suggesting a possible role for leptin in hypertension (Boulomie *et al*., 1999; Quehenberger *et al*., 2002).

It has been shown that angiogenic repair of ischemic hindlimbs was impaired in Lepr(db/db) mice, a leptin receptor-deficient model of diabetes, compared with wild-type (WT) C57BL/6 mice, as evaluated by laser Doppler flow and capillary density analyses (Sheikofer *et al*., 2005). To identify molecular targets associated with this disease process, hindlimb cDNA expression profiles were created from adductor muscle of Lepr(db/db) and WT mice before and after hindlimb ischemia using Affymetrix Gene Chip Mouse Expression Set microarrays. The expression patterns of numerous angiogenesis-related proteins were altered in Lepr(db/db) versus WT mice after ischemic injury. These transcripts included neuropilin-1, vascular endothelial growth factor-A, placental growth factor, elastin, and matrix metalloproteinases implicated in blood vessel growth and maintenance of vessel wall integrity. These data illustrate that impaired ischemia-induced neovascularization in type 2 diabetes is associated with the dysregulation of a complex angiogenesis-regulatory network. Adiponectin, also known as adipo Q and Acrp30, is a complement factor (C1q) abundantly expressed in adipocytes that increases fat oxidation and insulin sensitivity (Yamauchi *et al*., 2001). Adiponectin gene expression in human visceral adipose tissue is negatively regulated by glucocorticoids and TNF-alpha and positively by insulin and IGF-1. Adiponectin levels are decreased in obesity and are inversely correlated to insulin-resistant states and high-sensitivity CRP levels. Subjects with coronary heart disease have lower adiponectin levels compared with age- and body mass index-adjusted controls, suggesting that adiponectin, in contrast to other adipokines, confers a protective effect against atherosclerosis. In vitro data and an animal model of premature atherosclerosis, the Apo E-deficient mouse, support this tenet (Okamoto *et al*., 2002). Weight loss in obese subjects and treatment of diabetes with a TZD insulin-sensitizing drug restores plasma adiponectin levels to those of controls (Yang *et al*., 2001). Adiponectin exerts antiatherogenic properties by suppressing the endothelial inflammatory response, inhibiting vascular smooth muscle proliferation, and decreasing VCAM-1 mRNA expression, all of which are associated with endothelial injury and the subsequent development of atherosclerotic lesions (Okamoto *et al*., 2002). Adiponectin has been shown to inhibit the TNF-alpha-induced changes in monocyte adhesion molecule expression and in the endothelial inflammatory response. Adiponectin also suppresses the transformation of macrophages to foam cells. Finally, adiponectin-deficient mice are markedly insulin resistant and demonstrate a twofold greater neointimal proliferation than wild-type mice in response to injury (Kubota *et al*., 2002).

Insulin resistance correlates with the degree of obesity, notably abdominal obesity, and is a strong predictor for the development of Type 2 diabetes. The continuing dual epidemics of obesity and diabetes lend support to the notion that insulin resistance is the causal link between the two prevalent conditions (Mokdad *et al*., 2001). Insulin resistance is a central abnormality of the metabolic syndrome of insulin resistance, or syndrome X, originally hypothesized by Reaven (Reaven, 1988) to describe a constellation of metabolic abnormalities, including hyperglycemia, hyperinsulinemia, hypertension, dyslipidemia with increased triglycerides, and decreased HDL. Metabolic syndrome is strongly associated with endothelial dysfunction and increased atherosclerosis risk. Insulin resistance and adaptive hyperinsulinemia are thought to cause endothelial dysfunction and exert mitogenic influences on vascular smooth muscle cells, in contrast to insulin's vasodilatory effect by promoting NO release under normal physiological conditions.

In addition to hyperinsulinemia, several other factors in the metabolic syndrome have been implicated in endothelial dysfunction. Impaired insulin action in adipose tissue causes elevated rates of lipolysis and augmented FFA release. The increased flux of FFAs not only impairs insulin secretion by pancreatic islet beta-cells and induces

insulin resistance in muscle and the liver by interfering with glucose transport and insulin-mediated glucose uptake but also exerts negative influences on vascular health. FFAs impair vasodilatation and reduce NO bioavailability by attenuating endothelial NO synthase activity and stimulating the production of reactive oxygen species by NADPH oxidase. FFAs are believed to play a major role in increased hepatic gluconeogenesis and overproduction of triglyceride-rich very-low-density lipoproteins, which in turn lead to higher levels of small, dense, atherogenic LDLs and decreased cardioprotective HDLs. The abnormal lipoprotein metabolism that results from the metabolic syndrome negatively influences endothelial function and the atherogenic process.

Insulin resistance and adaptive hyperinsulinemia are thought to cause endothelial dysfunction by promoting endothelial activation and a proatherogenic environment. The adipokines reenforce these detrimental effects. High glucose levels induce endothelial free radical production and activate NF-kappaB and protein kinase C as well as enhancing intracellular advanced glycation end-product formation (Brownlee, 2001). As a result, hyperglycemia enhances nonenzymatic oxidation of lipoproteins, which independently contribute to atherogenesis (Brownlee, 2001). Hyperglycemia also increases superoxide in endothelial cells and oxidative stress, both of which can result in endothelial injury (Marfella, 2001). Hyperglycemia also augments the expression of adipokines, including PAI-1 (Kohler and Grant, 2000), further fueling the vicious cycle of adipokine-related endothelial dysfunction.

Circulating endothelial progenitor cells (EPCs) may be involved in the maintenance of vascular homeostasis and their impairment may be conducive to vascular disease (Wolk *et al*, 2005). An association between adipokines, dysfunction in EPCs and diabetic patients shows interesting and applied pathophysiological interpretations (Abraham *et al*., 2008; Li *et al*., 2011; Issan *et al*., 2013). A comprehensive aspects of the therapeutic application of EPCs to patients with ischemic disease has been described (Asahara *et al*., 2011). In summary, the leptin receptor is present in human EPCs and leptin may affect EPC function, both in physiological and in hyperleptinemic conditions. These findings are relevant to leptin-mediated regulation of vasculogenesis in humans, and the association between hyperleptinemia and obesity with cardiovascular disease.

CONCLUSION

Diabetes is a risk factor for the development of cardiovascular diseases associated with impaired angiogenesis or increased endothelial cell apoptosis. Whereas endothelial dysfunction may be causative of some of the features of the syndrome of insulin resistance. Hence, valuable information might be provided by carrying out apoptotic and EPC activity assays.

The evolving role of adipokines in endothelial dysfunction adds a new dimension to our understanding of the relationship between obesity, notably for those with increased abdominal fat, and CVD risks. Role of adipokines in β-cell failure of type 2 diabetes has recently been explained (Dunmore and Brown, 2013). Further investigations into the molecular links between obesity and atherosclerosis may unravel innovative therapeutic strategies to improve cardiovascular health in people affected by obesity-linked insulin resistance, and type 2 diabetes.

Adipose tissue may play a crucial role in the development of insulin resistance, type 2 diabetes, and their complications through the secretion of a variety of biologically active molecules (adipocytokines). As leptin is an adipose-specific hormone contributing to the regulation of energy expenditure and food intake, it may affect insulin sensitivity and may participate in the development of hypertension. It may cause the atherosclerotic vascular disease in type 2 diabetes directly or through the development of insulin resistance. Hence, it is very significant in searching the role of adipokines in the mentioned disorders. An association between adipokines, dysfunction in EPCs and diabetic patients shows interesting and applied pathophysiological aspects (Abraham *et al*., 2008; Li *et al*., 2011; Issan *et al*., 2013). A comprehensive sketch of the therapeutic application of EPCs to patients with ischemic disease has been explained (Asahara *et al*., 2011).

The clinical and experimental observations suggest that adiponectin plays some protective role against the atherosclerotic vascular change and that the decreased plasma adiponectin in type 2 diabetic patients may contribute to the development of atherosclerotic complications. The mechanism of decreased plasma adiponectin in type 2 diabetes, however, has not yet been clarified. Therefore, further studies in this line might become potential to explore the involvement of vascular disordered conditions.

Leptin may accelerate the development of vascular injury. Studies with adiponectin-deficient mice have revealed that adiponectin plays a protective role in the development of atherosclerosis. Therefore, the leptin-to-adiponectin ratio may serve as an atherogenic index superior to leptin or adiponectin alone. This requires to design and to assess the potential of the leptin-to-adiponectin ratio as a biomarker for atherosclerosis in obese type 2 diabetic patients. Such approach may provide interesting information for the future exploration of the pathogenesis and therapeutic advancement in the area of diabetology.

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