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Diabetes & Metabolism

Diabetes and the Heart

A symposium organized by ALFEDIAM
(Association de Langue Française pour l'Etude du Diabète et des Maladies Métaboliques)
and SFC (Société Française de Cardiologie). Paris, December 7th, 2007

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Editorial

Diabetes and the Heart

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The French Society of Cardiology (SFC) and the French-speaking Society of Diabetology (ALFEDIAM) co-organize this year a scientific meeting dedicated to the heart in diabetes mellitus. Since myocardial ischaemia in patients with diabetes is frequently discussed, the aim of this 2007 scientific meeting was to give information mainly on cardiomyocyte metabolism, myocardial dysfunction, arterial calcification and novel pathophysiological mechanisms of macrovascular disease in diabetes. In this special issue of *Diabetes & Metabolism* are reported the main presentations at this conference.

There is now a large body of evidence supporting the existence of "diabetic cardiomyopathy". In their well documented paper, *D. Feuvray et al.* explain the role of increased fatty acids in disturbing cardiomyocyte metabolism leading to cardiac dysfunction. The mechanisms involved include opening of the cell membrane K_{ATP} channel, increased lipid peroxidation and apoptosis. *B. Vergès et al* report data showing that Nt-ProBNP is, in patients with diabetes, a very strong marker of the poor short-term prognosis after myocardial infarction in patients with diabetes. Nt-proBNP seems to reflect the integration of different risk markers for adverse outcomes (death, heart failure) following myocardial infarction with high informative value.

Recently more attention was given to vascular calcification which is frequent in patients with diabetes. New data on the pathophysiology of vascular calcification are reported in the paper from *ZA Massy et al*. The presence of vascular calcification could be explained by an imbalance between osteoblast-like and osteoclast-like cell activities in the arterial wall. Many longitudinal studies have demonstrated the predictive value of arterial stiffness, beyond and above classical risk factors for cardiovascular disease. *P Boutouyrie* describe new techniques for assessing arterial stiffness including measurements of aortic pulse wave velocity and central pressure.

Circulating microparticles derived from apoptotic cells are early markers of vascular dysfunction and may be involved in vascular complications. In their paper, *AS Leroyer et al* indicate that microparticles levels are increased in patient with diabetes. They present data showing that microparticles, due to their pro-inflammatory properties, could be involved in the development of vascular complications in diabetes by promoting thrombosis, endothelial dysfunction and angiogenesis. During the past years, a lot of interest has risen on Endothelial Progenitor Cells (EPC), which are involved in re-endothelialisation of injured vessels and formation of new vessels in ischemic tissue. *JB Silvestre*, in his article, shows data indicating that diabetes reduces the availability and the functions of EPC, leading to diminish the EPC-induced post-ischemic vessel growth.

The role of chronic hyperglycaemia in the development and progression of macrovascular disease is discussed by *S Hadjadj et al* in the light of the intervention studies performed in patients with diabetes. The available data indicate that there is probably no specific HbA_{1c} threshold for macrovascular disease. The on-going trials, such as ADVANCE and ACCORD should give additional information to clarify this point.

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Diabetes-related metabolic perturbations in cardiac myocyte

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Abstract

Although the pathogenesis of diabetic cardiomyopathy is poorly understood, recent evidence implicates perturbations in cardiac energy metabolism. Whereas mitochondrial fatty acid oxidation is the chief energy source for the normal postnatal mammalian heart, the relative contribution of glucose utilization pathways is significant, allowing the plasticity necessary for steady ATP production in the context of diverse physiologic and dietary conditions. In the uncontrolled diabetic state, because of the combined effects of insulin resistance and high circulating fatty acids, cardiac myocytes use fatty acids almost exclusively to support ATP synthesis. Studies using various diabetic rodent models have shown a direct relationship between the chronic drive on myocardial fatty acid metabolism and the development of cardiomyopathy including ventricular hypertrophy and dysfunction. Fatty acids also play a critical role in triggering the development of cellular insulin resistance through derangements in insulin signalling cascade. There are similarities in cardiac dysfunction in animal models and human type 2 diabetes and/or obesity. For instance, obese young women showed increased cardiac fatty acid utilization measured by positron emission tomography and increased myocardial oxygen consumption with reduced cardiac efficiency. Furthermore, accumulation of triglycerides within cardiac myocytes was an early metabolic marker that was associated with increased left ventricular mass. Moreover, data indicate that alterations in cardiac energetics occur early in the pathophysiology of type 2 diabetes and are correlated negatively with the fasting plasma free fatty acid concentrations.

Résumé

Perturbations métaboliques des cardiomyocytes liées au diabète

Nombre de données récentes, obtenues chez l'animal et chez l'homme, indiquent que le diabète est associé à des altérations du métabolisme énergétique des myocytes cardiaques. Bien que l'oxydation mitochondriale des acides gras à longue chaîne représente normalement la source principale d'énergie pour le myocarde, la contribution des voies d'utilisation intracellulaire du glucose est significative et elle permet, notamment, l'adaptabilité nécessaire de la production d'ATP aux diverses conditions physiologiques et nutritionnelles. Au cours du diabète, l'entrée des acides gras dans les myocytes cardiaques est majorée, d'une part en raison de leur plus grande disponibilité, et, d'autre part, de la réduction de l'utilisation du glucose qui accompagne l'insulino-résistance. L'oxydation très largement prédominante des acides gras est associée à une augmentation de consommation d'oxygène du myocarde et à une accumulation cellulaire de dérivés lipidiques. Les altérations métaboliques des cardiomyocytes au cours du diabète peuvent elles-mêmes être à l'origine d'anomalies de la structure (remodelage ventriculaire) et de la fonction ventriculaires cardiaques.

Keywords: Diabetes; Cardiac energy metabolism; Fatty acid utilization; Ventricular dysfunction; Review

Mots clés : Diabète ; Métabolisme du myocyte cardiaque ; Acides gras ; Dysfonction ventriculaire ; Revue

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1. Introduction

Cardiovascular complications are now the leading cause of diabetes mellitus related morbidity and mortality [1]. It is generally appreciated that the major cardiac complications of diabetes mellitus include the large conduit arteries, epicardial coronary arteries, and the microvasculature. What is less appreciated and still considered somewhat controversial is the concept that diabetes affects cardiac structure and function independent of blood pressure or coronary artery disease. There are now considerable experimental, pathological, epidemiological, and clinical data to support the existence of "diabetic cardiomyopathy" [2], a concept initially described 35 years ago [3]. Since Rubler et al [3] first suggested the existence of "diabetic cardiomyopathy" based on postmortem findings in only four adult patients, the definition of diabetic cardiomyopathy has been greatly redefined and there is now general agreement on the type of heart disease associated with diabetes. Diabetes is now well recognized as a risk factor for the development of heart failure. Men with diabetes are more than twice as likely to have heart failure than those without the disease, and diabetic women have a five-fold increased risk [4]. Heart failure can affect either systolic function or diastolic function, or both. Another, often unappreciated, but important feature of diabetic heart disease, particularly in type 2 diabetes, is a disproportionate increase in left ventricular mass [5,6]. Specifically, the Framingham Heart Study first identified associations of diabetes with higher left ventricular wall thickness and mass in women but not in men [7]. However a more recent report, the Strong Heart Study, confirmed and extended this observation by demonstrating associations of left ventricular absolute and relative wall thickness, as well as left ventricular mass in absolute terms and indexed for measures of body size with diabetes in both men and women [6]. Thus diabetes is associated with an increased risk of left ventricular hypertrophy and left ventricular dysfunction.

In this short review, we provide an update on our current understanding of the complexities of diabetic cardiomyopathy with a special emphasis on the relationship between the metabolic perturbations that accompany diabetes and lead to a cardiomyopathic phenotype. An understanding of the cellular effects of these metabolic disturbances on cardiomyocytes should be useful in predicting the structural and functional cardiac consequences.

2. Cardiomyocyte metabolic disturbances in diabetes

Under normal conditions, myocardial energy substrate preference varies in a dynamic manner to fulfill the tremendous energy needs of the postnatal mammalian heart. Whereas the fetal heart relies primarily on glucose and lactate, the capacity for mitochondrial fatty acid oxidation increases markedly after birth, providing the adult heart the option of using glucose or fatty acids to meet energy demands depending on dietary and physiologic conditions [8] (Fig. 1). In diabetes, the capacity for cardiac energy substrate switches becomes constrained due to the importance of insulin in the control of myocardial glucose uptake and utilization. Type 1 diabetes differs principally from type 2 diabetes in that it is unaccompanied by a period of hyperinsulinemia and is characterized by early- as opposed to late-onset hyperglycemia. Another characteristic metabolic disturbance evident in diabetic states is hyperlipidemia, usually in the form of increased triglycerides and nonesterified fatty acids. In the uncontrolled diabetic state, because of the combined effects of myocyte insulin resistance and high circulating fatty acids [9], cardiomyocytes use fatty acids almost exclusively to support ATP synthesis.

Cardiac fatty acid utilization pathways are controlled, in part, at the gene regulatory level. Nuclear receptor transcription factors are particularly well-suited for regulating the cardiac metabolic gene program [10]. In recent

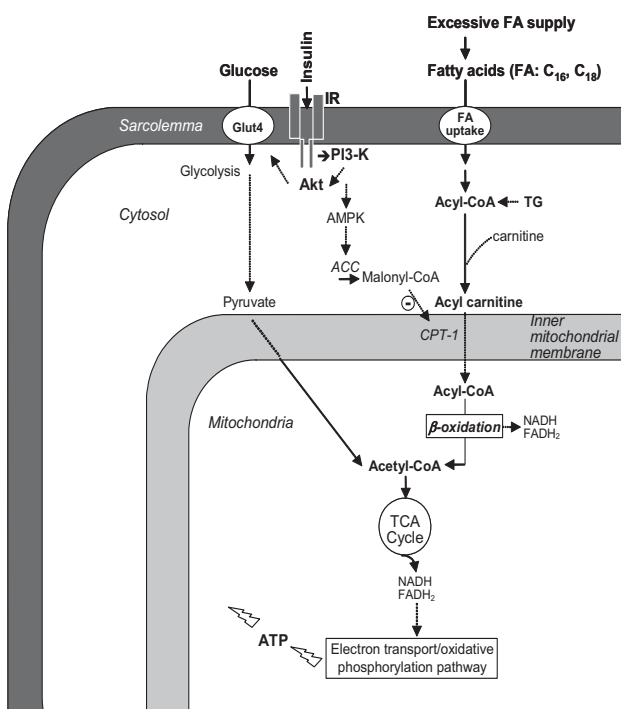

Fig. 1. Schematic diagram showing cardiac myocyte energy metabolism. FA and glucose oxidation are the main ATP producing pathways. In the uncontrolled diabetic state, because of the combined effects of insulin resistance and high circulating fatty acids, cardiomyocytes use fatty acids almost exclusively to support ATP synthesis. FA indicates fatty acid; TG, triglycerides; Acyl-CoA, long-chain acyl-CoA esters; Acyl carnitine, long-chain acyl carnitine; IR, insulin receptor; PI3-K, phosphatidylinositol 3-kinase; Akt, also known as protein kinase B; AMPK, 5'-AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyl transferase-1; TCA, tricarboxylic acid.

years, a number of receptors identified without prior insight to their ligands have been shown to respond to dietary-derived lipid intermediates, including long-chain fatty acids [11,12]. Because the heart must adapt to continuously changing energy demands but has limited capacity for storing fatty acids or glucose, myocardial energy substrate flux must be tightly matched with demand. Ligand-activated nuclear receptors are poised to rapidly respond to fluctuating energy substrate levels. The peroxisome proliferator-activated receptors (PPARs), as fatty acid-activated nuclear receptors, are now recognized as key regulators of cardiac fatty acid metabolism. PPARα isoform is thought to be the primary transcriptional regulator of fat metabolism in tissues with high fatty acid oxidation rates such as the heart. Evidence for PPARα as a key regulator of cardiac energy metabolism has been provided by the PPARα "knockout" (PPARα $^{-/-}$) mouse studies [13,14]. Constitutive expression of genes involved in fatty acid uptake, mitochondrial transport and beta-oxidation are decreased in hearts of PPARα $^{-/-}$ mice [14,15]. Correspondingly, myocardial long-chain fatty acid uptake and oxidation rates are diminished in PPARα $^{-/-}$ hearts [15,16]. Conversely, cardiac-specific PPARα overexpression (MHC- PPARα mice) activates expression of fatty acid utilization genes [17]. Studies using various diabetic rodent models have shown diabetes-related cardiomyocyte metabolic dysregulation. Leptin-deficient obese (ob/ob) mice and Zucker fatty rats exhibit triglyceride accumulation and increased expression of genes involved in lipid uptake and triglyceride synthesis [18,19]. In both models, ventricular mass was increased. Functionally, decreased contractile function observed in Zucker obese rats was attributed to lipotoxic effects of the accumulated species [20]. These studies implicate defective PPARα signalling in these models, causing a *mismatch* between fatty acid uptake and metabolism [20]. The MHC-PPARα mouse model has demonstrated a direct relationship between the chronic drive on myocardial fatty acid metabolism and the development of cardiomyopathy [17]. The expression of genes involved in cardiac fatty acid uptake and oxidation pathways was increased whereas those involved in glucose transport and utilization was repressed. In addition, MHC-PPARα mouse hearts exhibit signature of diabetic cardiomyopathy including ventricular hypertrophy and dysfunction that is exacerbated with high fat feeding [21].

Work from a number of sources [22,23,24,25] supports the notion that fatty acids play a critical role in triggering the development of cellular insulin resistance through derangements in insulin signalling cascade. Insulin signalling is mediated by complex multiple pathways characterized by spatial and temporal aspects [26,27,28]. Insulin binding to the insulin receptor (IR) stimulates the tyrosine kinase activity of IR leading to IR autophosphorylation and to the subsequent phosphorylation of IR substrate (IRS-1/2). Recent studies have suggested that local accumulation of fat metabolites inside skeletal muscle, such as accumulation of fatty acyl-CoA, induces the activation of atypical protein kinase C (PKC) -theta, a serine/threonine kinase that phosphorylates and subsequently activates IκB kinase [29]. IκB kinase phosphorylates serine residues on IRS-1, inhibiting its ability to bind SH2 domains of the p85 regulatory subunit of the lipid kinase phosphatidylinositol 3-kinase (PI3-K), impairing insulin signal transduction. As a consequence, the recruitment of Glut4 transporters (Glut4 translocation) to the plasma membrane, and therefore glucose uptake, is compromised. Although this mechanism is active in skeletal muscle and adipose tissue, it has been less clear whether similar mechanisms are apparent in cardiac muscle [9] although cellular accumulation of long-chain fatty acyl derivatives has been shown to occur [30].

Another role for increased fatty acid concentrations is the attenuation of insulin regulation of 5'-AMP-activated protein kinase (AMPK) [31]. AMPK is a heterotrimeric enzyme [32] that acts as a key "metabolic switch" in the heart in the control of glucose and fatty oxidation. AMPK also phosphorylates and inactivates key enzymes involved in ATP-consuming pathways. In the heart, AMPK stimulates fatty acid oxidation by inactivating acetyl-CoA carboxylase and so decreasing the concentration of malonyl-CoA which inhibits the entry and the subsequent oxidation of long-chain fatty acids into the mitochondria [33,34]. Interestingly, it has been shown that AMPK activation is antagonized by insulin [35]. The anti-AMPK effect of insulin is wortmannin-sensitive, like most short-term effects of insulin, suggesting that it is mediated by PI3-kinase. Therefore, the metabolic consequences of the interaction between insulin and AMPK is normally to increase malonyl-CoA concentration and consequently to limit fatty acid oxidation while facilitating glucose oxidation. This may be blunted by the presence of high plasma fatty acid levels in diabetes [36].

3. Relationship between metabolic disturbances and the development of diabetic cardiomyopathy phenotype

An important question raised relates to the mechanistic link(s) between altered myocardial energy metabolism and cardiac dysfunction in the diabetic heart. In the context of high-level fatty acid uptake [9], lipid intermediates accumulate within cardiac myocytes [30]. Recent evidence [37] suggests that increases in long-chain acyl coenzyme A (CoA) esters and fatty acids directly link metabolism to K_{ATP} channels in the heart. Studies on isolated guinea-pig ventricular myocytes have shown [37] that long-chain acyl-CoA esters facilitate opening of K_{ATP} channels by reducing ATP sensitivity. The effects of

acyl-CoA esters on K_{ATP} channels in cardiac myocytes may be functionally important because long-chain fatty acids, particularly C_{16} and C_{18} fatty acids, serve as the main metabolic substrates of the heart, especially for the diabetic heart. The metabolizable form of these fatty acids is that of acyl-CoA esters, which are synthesized at the outer mitochondrial membrane via acyl-CoA synthetase, imported into the mitochondria, and subsequently metabolized via beta-oxidation. However, since excessive long-chain acyl-CoA ester levels are present in the diabetic heart [30] it is tempting to speculate that this may favour opening of the cell membrane K_{ATP} channel. The resulting shortening of the action potential would lead to a reduction in transsarcolemmal Ca^{2+} influx and subsequently to a reduction in myocardial contractility [37].

Alternatively or together, reliance on fatty acid oxidation for ATP production, which results in higher mitochondrial oxygen consumption costs compared with glucose oxidation, could also contribute to ventricular dysfunction [38]. Theoretical calculations of the yield of ATP per oxygen atom consumed (P/O) show that fatty acids are a less efficient fuel when compared to glucose. In other words, more oxygen is required for ATP production when hearts are metabolizing fatty acids compared to glucose utilization. However, the theoretical difference in cardiac efficiency based on P/O ratios for fat and glucose metabolism is greater than expected when lipid utilization is increased [39]. This discrepancy feeds the argument that fatty acids can induce uncoupling of mitochondria, perhaps by upregulation of the uncoupling protein UCP3 expression and activity. Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner membrane of mitochondria. They belong to the family of anion mitochondrial carriers including adenine nucleotide transporters. The term "uncoupling protein" was originally used for UCP1, which is uniquely present in mitochondria of brown adipocytes [40]. UCP1 catalyzes a highly regulated proton leak, converting energy stored within the mitochondrial proton electrochemical potential gradient to heat. This uncouples fuel oxidation from conversion of ADP to ATP [41]. The most likely action of cardiac UCP3 is to regulate fatty acid oxidation [41]. UCP3 expression was shown to be up-regulated in type 1 diabetic rat hearts [42,43] which have elevated rates of fatty acid oxidation. Basal UCP3 expression was also elevated in Zucker hearts [44]. Interestingly, UCP3 is also a PPARα target [43]. Whether changes in UCP3 gene expression in diabetic hearts result in changes in protein expression or activity or ultimately lead to altered cardiac function had to be determined [43]. A very recent study [45] of mitochondrial energetics in hearts of leptin receptor-mutant (db/db) diabetic obese mice has demonstrated that mitochondrial uncoupling is indeed mediated by activation of uncoupling proteins independently of changes in expression levels. This likely occurs on the basis of increased delivery of the reducing equivalents $FADH_2$ and NADH from beta-oxidation to the electron transport chain, thereby increasing reactive oxygen species production that activates uncoupling proteins.

Finally, the increased intracellular accumulation of fatty acids may contribute to cardiomyocyte death under circumstances in which fatty acid cell uptake overstep beta-oxidation capacity. This has been shown in hearts of Zucker diabetic fatty rats in which endogenous triglyceride levels are high and their hydrolysis to fatty acyl (palmitoyl) -CoA provides increased substrate for ceramide synthesis which, in turn, causes apoptosis [46]. In this study, troglitazone therapy lowered myocardial triglyceride, prevented cardiomyocyte apoptosis and reduced contractile function abnormalities such as reduction in fractional shortening. Under these circumstances, increased fatty acids are said to cause lipotoxicity. Although lipotoxicity has been implicated in the reduction in pancreatic beta-cell reserves, the relevance of these findings in the human diabetic myocardium remains to be established [9]. However, lipotoxicity is not solely dependent on triglyceride stores and lipid deposition; increases in fatty acid oxidation can also contribute. Mitochondria can be a substantial source of reactive oxygen species [47]. Zucker hearts show increases in lipid peroxidation [48]; antioxidant defenses are up-regulated [49] but clearly this up-regulation is insufficient to protect the heart from lipid peroxidation. Lipid peroxidation could be occurring through several mechanisms [45,48], increased production of reactive oxygen species as a result of excessive fatty acid oxidation and an elevated mitochondrial membrane potential, or increased peroxidation due to the presence of a large quantity of lipid which is able to undergo peroxidation. Lipid peroxidation in the Zucker rat heart was proportional to the concentration of myocardial lipid [48]. The role of mitochondria-derived reactive oxygen species in models with confirmed increases in cardiac fatty acid oxidation has yet to be determined [50].

Thus, metabolic abnormalities in the diabetic heart with over-reliance on fatty acid utilization play a central role not only in inducing cardiomyocyte insulin resistance but also in affecting myocardial contractility.

As noted before, diabetes is a strong risk factor for the development of heart failure, and left ventricular hypertrophy has been detected in a significant proportion of type 2 diabetic patients [5,6]. Up to now, the metabolic trigger (s) and cellular signalling pathways associated with altered myocardial structure remain incompletely understood. The role of insulin and of hyperinsulinemia has been reviewed recently [9] and will not be developed here. In short, can hyperinsulinemia cause cardiomyocyte hypertrophy if the cellular actions of insulin are attenuated? Acutely, insulin stimulates growth through the same PI3K-Akt pathway by which it mediates glucose uptake. Then downstream effectors are activated that govern the

hypertrophic process [51,52,53]. However, these effects may be mitigated when insulin signalling pathway (through the PI3K-Akt signalling) is impaired. Other possibilities include stimulation of both the MAP kinase signalling pathway and excessive prenylation of Ras and Rho proteins, as it has been proposed for the proatherogenic action of compensatory hyperinsulinemia in the vascular wall [52].

Another actor that has emerged recently as playing a role in the development of left ventricular myocyte hypertrophy under some circumstances in diabetes is the sarcolemmal Na^+/H^+ exchanger. The Na^+/H^+ exchanger (NHE isoform 1) contributes significantly to the integrated control of intracellular pH (pH_i) in myocardial cells [54] and therefore directly links cardiomyocyte metabolic state to ionic (especially intracellular Na^+) homeostasis. Several studies have indicated that chronic inhibition of NHE1 favourably interferes with the development of cardiac hypertrophy [55,56]. Most recent results [57] have demonstrated an increase in NHE1 activity in cardiac left ventricular myocytes of the GK rat model of type 2 diabetes. This is accompanied by a phenotype of hypertrophy that can be prevented by chronic treatment with a selective pharmacological NHE1 inhibitor. The metabolic trigger for NHE1 activation, which is extremely sensitive to intracellular proton concentration, is a small decrease in pH_i. It was found there that elevated NHE1 activity is able to stimulate the Akt pathway and ultimately lead to cardiomyocyte hypertrophy. These data may contribute to shedding light on the central role that NHE1 may play in favouring LV hypertrophy in type 2 diabetic patients with impaired myocardial perfusion [58,59], and therefore mild myocardial ischaemia, in some circumstances.

4. Conclusion

Taken together these findings highlight the complexity of alterations in myocardial cell metabolism that may be associated with a multifactorial disease such as diabetes, especially type 2 diabetes. Target tissues become resistant to the effects of insulin and fatty acids likely play a critical role in the development of cellular insulin resistance. Certainly, there are similarities in cardiac dysfunction in animal models and human type 2 diabetes and/or obesity. For instance, obese young women showed increased cardiac fatty acid utilization measured by positron emission tomography and increased myocardial oxygen consumption with reduced cardiac efficiency [38]. Furthermore, accumulation of triglycerides within cardiomyocytes (Fig. 2) was an early metabolic marker that was associated with increased left ventricular mass [60]. Similar results have been obtained with heart tissue from heart failure patients undergoing cardiac transplantation [61]. The highest levels of lipid staining were observed in patients with diabetes and obesity. In this situation, intramyocardial lipid deposition was associated with an up-regulation of PPARα regulated genes, as well as with an increase in myosin heavy chain (MHC) -beta expression. Cardiac overexpression of MHC-beta results in decreased systolic function [62]. These changes are very similar to those observed in the Zucker diabetic fatty rat heart with contractile dysfunction. Finally, cardiac high energy phosphate metabolites measured at rest in type 2 diabetic patients using ^{31}P nuclear magnetic resonance spectroscopy, have revealed a decrease in phosphocreatine to ATP ratios [63]. These data also indicate that alterations in cardiac energetics occur early in the pathophysiology of type 2 diabetes and are correlated negatively with the fasting plasma free fatty acid concentrations. All these findings suggest that chronic manipulation of myocardial metabolic

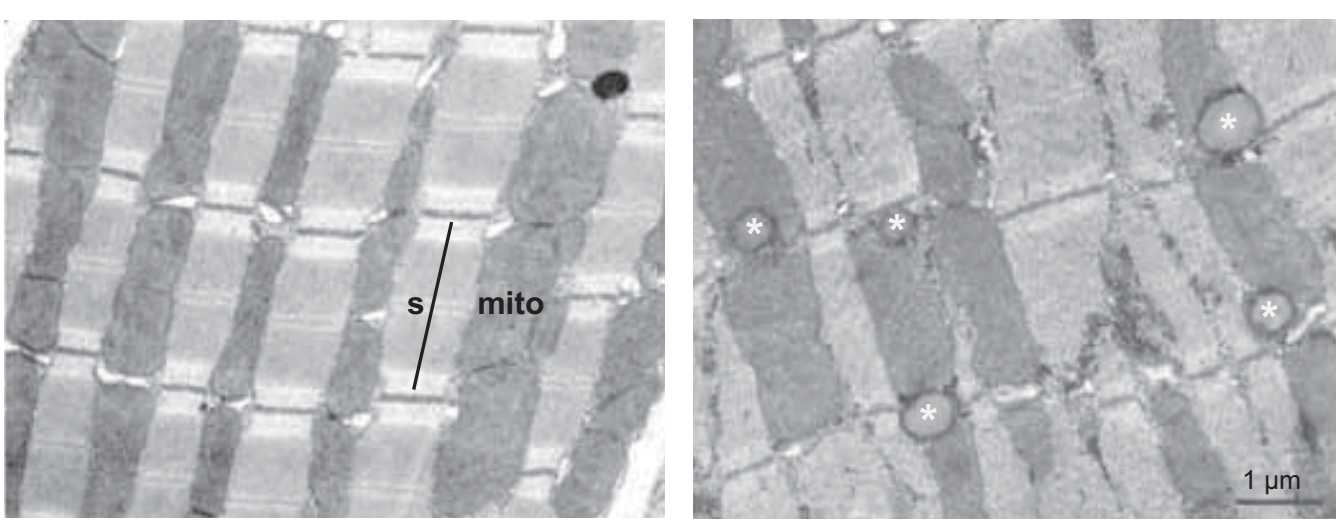

Fig. 2. Representative electron microscopy images obtained from ultra-thin sections of fixed ventricular tissue of a normal (left) and an age-matched (streptozotocin-induced) diabetic rat heart (right). Note the presence of numerous lipid droplets (*, i.e. TG accumulation) inside the cardiac myocyte of the diabetic rat heart (right). S, sarcomere; mito, mitochondria.

substrate aimed at improving the coupling between fatty acid delivery and oxidation in cardiomyocytes, may prevent or slow the progression of left ventricular dysfunction in hearts of diabetic patients.

No potential conflict of interest revelant to this article was reported.

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Plasma N-terminal Pro-Brain Natriuretic Peptide (Nt-proBNP) level and prognosis after myocardial infarction in diabetes.

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Abstract

Plasma N-terminal Pro-Brain Natriuretic Peptide (Nt-proBNP) level has been shown to provide valuable prognostic information on short and long-term mortality in patients with acute Myocardial Infarction, in the general population. Increased plasma Nt-proBNP levels have been found in Type 2 diabetic patients with vascular complications or with hypertension. In a large prospective study performed in 560 patients hospitalized for acute Myocardial Infarction (RICO), we found that median Nt-proBNP levels were significantly higher in the 199 diabetic patients compared to the 361 non-diabetic patients (245 (81-77) vs. 130 (49-199) pmol/L, P<0.0001). This difference remained highly significant after adjustment for confounding factors and we have been able to show that diabetes, per se, was a strong and independent factor for increased plasma Nt-proBNP levels, in this population. In the prospective RICO survey, we have found, in multivariable analysis, that diabetes was an independent factor for in-hospital mortality (OR: 1.79 [1.45-2.20]; *P* =0.0064) and cardiogenic shock (OR: 1.45(1.22-1.72); *P* =0.0364) when the variable Nt-proBNP level was not introduced into the model, but was less significantly associated with mortality (OR: 1.73 (1.39-2.16); *P* =0.0107) and no longer associated with cardiogenic shock when Nt-proBNP was in the model. This data suggest that increased plasma Nt-proBNP may be one of the links between diabetes and poor short-term prognosis after Myocardial Infarction and provides highly valuable prognostic information on in-hospital outcome in diabetic patients.

Résumé

Concentrations plasmatiques de Nt-proBNP et pronostic après infarctus du myocarde au cours du diabète

Dans la population générale, le taux plasmatique de N-terminal Pro-Brain Natriuretic Peptide (Nt-proBNP) est un indice du pronostic à court et long terme après infarctus du myocarde. Des taux augmentés de Nt-proBNP ont été rapportés chez des patients diabétiques de type 2 avec atteinte cardiovasculaire ou hypertension artérielle. Dans une étude prospective de grande taille réalisée cher 560 patients hospitalisés pour infarctus du myocarde (RICO), nous avons observé que le taux médian de Nt-proBNP était significativement plus élevé chez les 199 patients diabétiques que chez les 361 patients non diabétiques (245 (81-77) vs. 130 (49-199) pmol/L, *P* <0,0001). Cette différence demeurait hautement significative après ajustement pour les facteurs confondants et nous avons pu montrer que le diabète, *per se*, était, dans cette population, un puissant facteur indépendant de l'augmentation des taux plasmatiques de Nt-proBNP. Dans cette même étude RICO, nous avons montré qu'en analyse multivariée, le diabète était un facteur indépendant de mortalité hospitalière (OR: 1,79 [1,45-2,20]; P=0,0064) et de choc cardiogénique (OR: 1,45(1,22-1,72); *P* =0,0364), lorsque la variable Nt-proBNP n'était pas prise en compte. Mais, lorsque la variable Nt-proBNP était introduite dans le modèle statistique, l'association indépendante entre le diabète et le choc cardiogénique n'était plus retrouvée et celle entre le diabète et la mortalité hospitalière était moins importante (OR: 1,73 (1,39-2,16); *P*=0,0107). Ces données

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montrent que le Nt-proBNP est un excellent indice du pronostic à court terme après infarctus du myocarde, chez les patients diabétiques et qu'il pourrait être un des liens entre le diabète et le mauvais pronostic après nécrose myocardique.

Keywords: Diabetes; Myocardial infarction; Nt-proBNP; BNP; Ischemia

Mots clés : Diabète ; Infarctus du myocarde ; Nt-proBNP ; BNP ; Ischémie

Brain Natriuretic Peptide (BNP) and N-terminal Pro-Brain Natriuretic Peptide (Nt-proBNP) are secreted from cardiomyocytes in response to increased wall stress [1-4]. BNP is produced as a 108 aminoacid prohormone, proBNP, which is enzymatically cleaved into the 32 amino-acid BNP and the N-terminal part of the prohormone, Nt-proBNP [4]. Levels of BNP and Nt-proBNP correlate with left ventricular dilatation, remodeling and dysfunction in patients after acute Myocardial Infarction [5, 6]. In patients with acute Myocardial Infarction, the increase in Nt-proBNP is greater than in BNP [7] and has a higher discriminative value for early cardiac dysfunction than BNP, suggesting it may be a more sensitive marker of left ventricular dysfunction [8-9]. Plasma Nt-proBNP level has been shown to provide valuable prognostic information on short and long-term mortality in patients with acute Myocardial Infarction [5, 9]. It has recently been shown that Nt-proBNP was independently associated with the risk of sudden death in patients with heart failure [10].

1. Nt-proBNP in Type 2 diabetes

levels have been found increased in Type 2 diabetic patients with vascular complications [11]. In this study, coronary heart disease and nephropathy (defined as an albumin/creatinine ratio above 2 mg/mmol) were each independently associated with elevated values of Nt-proBNP. In a study performed in 60 Type 2 diabetic patients without albuminuria, mean Nt-proBNP level was significantly higher among diabetic hypertensive patients compared with both diabetic normotensive patients and controls but no difference was found between the diabetic normotensive patients and the controls [12]. In this study, diabetic patients with concentric and eccentric hypertrophy or left atrial enlargement had significantly higher Nt-proBNP levels compared with the control group and the increased plasma level of Nt-proBNP observed in hypertensive, normoalbuminuric patients with Type 2 diabetes was related to left ventricular hypertrophy and increased left atrial and ventricular diameters [12]. An echocardiographic study has shown that plasma Nt-proBNP level was elevated in type 2 diabetic patients with normal ejection fraction but with diastolic dysfunction [13]. In a cross-sectional study, it has been shown that median plasma Nt-proBNP was increased in diabetic patients without overt cardiovascular disease suggesting a higher prevalence of asymptomatic left ventricular dysfunction [14]. In a study performed in 560 patients hospitalized for acute Myocardial Infarction, we found that median Nt-proBNP levels were significantly higher in the 199 diabetic patients compared to the 361 non-diabetic patients (245 (81-77) vs. 130 (49-199) pmol/L, P<0.0001) [15]. This difference remained highly significant after adjustment for age, female gender, creatinine clearance, left ventricular ejection fraction (LVEF), plasma peak troponin, anterior wall necrosis and hypertension [15]. We performed a multivariable linear regression analysis to analyze the association between plasma Nt-proBNP and several variables known to be associated with Nt-proBNP. The variables introduced into the model were those which were associated with Nt-proBNP with a p value < 0.20, in the univariate analysis: creatinine clearance, plasma peak troponin, LVEF, age, gender, diabetes, hypertension, anterior wall necrosis and ST segment elevation Myocardial Infarction (STEMI). The multivariable analysis showed that Nt-proBNP was negatively associated with creatinine clearance (P<0.0001) and LVEF (P<0.0001) and positively associated with plasma peak troponin level (P<0.0001), age (P=0.0016), diabetes (P=0.0045) and female gender (P=0.0104), but neither with hypertension, anterior wall necrosis nor STEMI [16]. When multivariable regression analysis was performed in the subgroup of diabetic patients with Myocardial Infarction, Nt-proBNP was negatively associated with creatinine clearance (P=0.0004) and LVEF (P=0.0003) and positively associated with peak plasma troponin level (P=0.0002), mean fasting blood glucose (P=0.0281) and female gender (P=0.0375) [15].

Increased plasma levels of Nt-proBNP have been reported in diabetic patients without overt cardiovascular disease [14, 16] and with acute coronary syndrome [17]. However, no adjustment for LVEF, an important determinant of plasma Nt-proBNP have been performed in these studies. In our study, we found a significant increase in plasma Nt-proBNP in diabetic patients compared to non-diabetic patients independently of possible cofounders such as age, sex, LVEF, creatinine clearance, BMI, hypertension, plasma troponin level and anterior wall location. Our data suggest that diabetes, per se, is a strong and independent factor for plasma Nt-proBNP after Myocardial Infarction. Interestingly, we found a 88% increase in Nt-proBNP median value in diabetic patients after Myocardial Infarction when only a 20% increase was observed by Magnusson et al in diabetic patients without overt cardiovascular disease [14], suggesting a strong influence of diabetes on plasma Nt-proBNP level in acute coronary events.

2. Nt-proBNP and short term prognosis after Myocardial Infarction, in Type 2 diabetes

RICO survey, a registry of patients hospitalized for acute Myocardial Infarction in one eastern region of France, were studied prospectively. Among the 560 patients, 199 (35%) were diabetic. During the hospital stay, mortality was significantly higher in diabetic patients than in non-diabetic patients (15.6% vs. 3.3%, P<0.0001). A significant 2.2 fold increase in cardiogenic shock was observed in the diabetic group compared to the non-diabetic group (17.6% vs. 7.7%, P=0.0004). No significant differences were noted between diabetic and non-diabetic patients for recurrent Myocardial Infarction (12.1% vs. 8.3%, P=0.15) or ventricular arrhythmia (12.1% vs. 8.9%, P=0.23) (Fig. 1). Plasma Nt-proBNP levels were significantly higher in patients who died at hospital (800 (147-3915) vs. 143 (55-357) pmol/L P<0.0001) and in those who suffered a cardiogenic shock during in-hospital stay (680 (164-1577) vs. 137 (53-336) pmol/L, P<0.0001) (Fig. 2).

In multivariate analysis, cardiogenic shock was associated with systolic blood pressure (mm Hg)(OR: 0.96(0.95-0.97); P<0.0001), creatinine clearance <60 ml/min (OR: 1.54 (1.30-1.82); P=0.0125) and diabetes (OR: 1.45(1.22-1.72); P=0.0364), when Nt-proBNP was not introduced into the model. When Nt-proBNP was introduced into the model, cardiogenic shock was associated with Nt-proBNP (OR: 2.22 (1.92-2.58); P<0.0001), systolic blood pressure (OR: 0.96(0.95-0.97); P<0.0001) but no longer with diabetes (Table 1).

In multivariable analysis, diabetes was an independent factor for mortality (p=0.0064) when the variable Nt-proBNP level was not introduced into the model, but was less significantly associated with mortality (P=0.0107), when Nt-proBNP was in the model (Table 2).

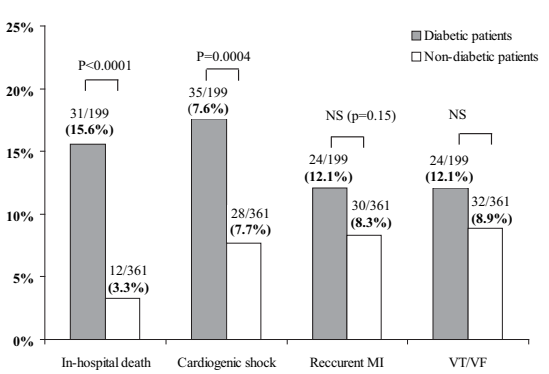

Fig. 1: Incidence of in-hospital events (death, cardiogenic shock, recurrent MI, VT/VF) in MI patients with or without diabetes.
VT/VF: Ventricular Tachycardia/ Ventricular Fibrillation.

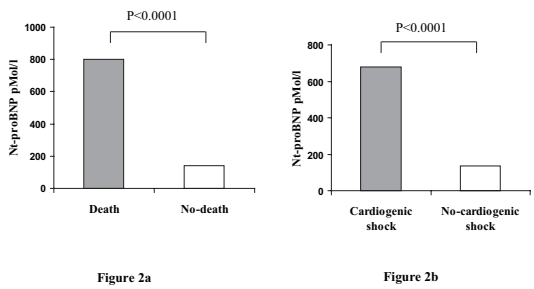

Fig. 2:
Fig. Nt-proBNP plasma levels in patients who died during in-hospital stay and in patients who did not. Data expressed as median (25th-75th).
Fig. 2b: Nt-proBNP plasma levels in patients who suffered cardiogenic shock during in-hospital stay and in patients who did not. Data expressed as median (25th-75th).

Our findings are consistent with the previous data showing an increased incidence of cardiogenic shock and in-hospital mortality after Myocardial Infarction, in diabetic patients [18, 19]. Moreover, we found a strong association between plasma Nt-proBNP level and the level of risk for death or cardiogenic shock after Myocardial Infarction, in patients with diabetes. This is a major finding of our study, as it suggests that increased plasma Nt-proBNP may be one of the links between diabetes and the increased risk for cardiogenic shock after Myocardial Infarction. Indeed, in multivariable analysis, diabetes is a significant independent factor for cardiogenic shock when the variable Nt-proBNP is not introduced into the model but is no more associated with increased risk of cardiogenic shock when Nt-proBNP is introduced into the model. This result supports the hypothesis that the increased risk of both cardiogenic shock and in-hospital mortality after Myocardial Infarction in diabetic patients is linked to elevated Nt-proBNP levels.

Plasma Nt-proBNP reflects not only the size of the myocardial necrosis but also the extent of ischemic territory [9, 20]. Indeed, plasma Nt-proBNP is increased in patients with acute coronary syndrome, even in the absence of necrosis [9, 21]. Plasma Nt-proBNP elevation is also associated with renal impairment, hypertension and systolic dysfunction [5, 9, 16, 17] and seems to reflect the integral of different risk markers for adverse outcomes following Myocardial Infarction with a high informative value.

Several pathophysiological mechanisms might explain the increase in plasma Nt-proBNP after Myocardial Infarction, in diabetic patients. Diabetic patients, even those who are asymptomatic for cardiovascular disease, have frequent and early echographic abnormalities including increased

Table 1
Predictor of cardiogenic shock by multivariable logistic regression analysis.

Model 1 (without Nt-proBNP)	Coef.	SD	Wald	OR [95%] CI	P
Systolic Blood Pressure (mm Hg)	-0.04	0.007	31.70	0.96 [0.95-0.97]	<0.0001
Creatinine clearance <60 ml/min (vs ≥ 60 ml/min)	0.43	0.17	6.24	1.54 [1.30-1.82]	0.0125
Diabetes (vs no diabetes)	0.37	0.17	4.38	1.45 [1.22-1.72]	0.0364

Non significant variables (removed from the model): age (p=0.50), Diastolic Blood pressure (P=0.96) and history of MI (vs no history of MI) (p=0.10).

Model 2 (with Nt-proBNP)	Coef.	SD	Wald	OR [95%] CI	P
Nt-proBNP (log) (pMol/L)	0.80	0.15	26.94	2.22 [1.92-2.58]	<0.0001
Systolic Blood Pressure (mm Hg)	-0.037	0.007	25.38	0.96 [0.95-0.97]	<0.0001

Non significant variables (removed from the model): age (p=0.10), Diastolic Blood pressure (p=0.86), history of MI (vs. no history of MI) (0.52), creatinine clearance <60 ml/min (vs. ≥ 60 ml/min) (0.40) and Diabetes (vs. no diabetes)(0.47).

MI: Myocardial infarction

Table 2
Predictor of mortality by multivariable logistic regression analysis.

Model 1 (without Nt-proBNP)	**Coef.**	**SD**	**Wald**	**OR [95%] CI**	**P**
Creatinine clearance <60 ml/min (vs ≥ 60 ml/min)	1.07	0.23	21.97	2.91 [2.31- 3.66)	<0.0001
Systolic Blood Pressure (mm Hg)	-0.02	0.007	9.18	0.98 [0.97-0.99]	0.0024
Diabetes (vs no diabetes)	0.58	0.21	7.44	1.79 [1.45-2.20]	0.0064
Heart rate at admission (log) (10 pulses/min)	0.360	0.18	3.98	1.43 (1.19-1.71)	0.0461

Non significant variables (removed from the model): age (0.96), Diastolic Blood pressure (P=0.56), history of MI (vs no history of MI) (0.64), anterior wall location (vs other location) (0.89), female gender (vs male) (0.30) and STEMI (vs non STEMI) (0.54).

Model 2 (with Nt-proBNP)	**Coef.**	**SD**	**Wald**	**OR [95%] CI**	**p**
Creatinine clearance <60 ml/min (vs ≥ 60 ml/min)	0.82	0.25	10.93	2.27 [1.77- 2.91)	0.0009
Nt-proBNP (log) (pMol/L)	0.42	0.15	7.18	1.52 [1.31- 1.77]	0.0073
Diabetes (vs no diabetes)	0.55	0.22	6.51	1.73 (1.39-2.16)	0.0107
Systolic Blood Pressure (mm Hg)	-0.017	0.007	5.75	0.98 [0.97-0.99]	0.0165

Non significant variables (removed from the model): age (0.48), Diastolic Blood pressure (P=0.35), history of MI (vs no history of MI) (0.33), anterior wall location (vs other location) (0.96), female gender (vs male) (0.58), STEMI (vs non STEMI) (0.57) and heart rate at admission (log) (pulse/min) (0.10).

MI: Myocardial infarction; STEMI: ST segment Elevation MI

myocardial stiffness, impaired left ventricular compliance and diastolic dysfunction [22, 23]. ATP deficiency may be responsible for the early myocardial dysfunction observed in diabetes. Indeed, diabetic patients have an intracellular glucose deficiency leading to impaired production of ATP, which does not allow adequate Na+ /K+ -ATPase and Ca2+ -ATPase functions. This modification of ion pumps leads to impaired relaxation in the myocardium [24], which could account for the increased Nt-proBNP secretion. Such metabolic features for increased Nt-proBNP in diabetes could also explain the independent association between fasting glycaemia and Nt-proBNP levels found in our study. Moreover, the increased collagen content observed in hearts from diabetic patients is responsible for increased myocardial stiffness and may participate to the elevation of plasma Nt-proBNP in diabetes [25]. In addition, the elevated plasma Nt-proBNP levels, observed in diabetic patients after Myocardial Infarction, may also be explained by the more severe ischemia, compared to non-diabetic patients, even with a similar infarct size. Indeed, autopsic data have shown a lower capillary density in the myocardium of diabetic patients who died from Myocardial Infarction, which could partly explain the severity of ischemia [26]. Furthermore, endothelium dysfunction, which has been reported in diabetic patients, could also be involved in the extent of ischemia [27].

Nt-proBNP and long term prognosis after Myocardial Infarction, in Type 2 diabetes

So far, no specific study analyzing the relationship between plasma Nt-proBNP and long-term prognosis after Myocardial Infarction has been performed. However, two studies have examined the prognostic value of Nt-proBNP in patients with Type 2 diabetes [28, 29]. Gaede et al. have investigated the association between plasma Nt-proBNP and cardiovascular disease in the 160 microalbuminuric type 2 diabetic patients enrolled in the Steno 2 study [28]. In this study, plasma Nt-proBNP being above the median was associated with an increased risk of cardiovascular disease (primary endpoint including cardiovascular mortality, non fatal myocardial infarction, non fatal stroke, PCI, CABG, vascular surgery and amputations) during the 7.8 year follow-up with an unadjusted hazard ratio of 4.4 (95% CI 2.3-8.4, P<0.0001) and hazard ratio of 3.6 (1.7-7.5, P=0.001) after adjustment for other cardiovascular risk factors [28]. In a prospective 15.5 year follow-up study, Tarnow et al. examined the relationship between baseline plasma Nt-proBNP level and cardiovascular mortality [29]. All-cause mortality was increased in patients with Nt-proBNP in the second and third tertiles (hazard ratios [95% CI] compared with the first tertile, 1.70 [1.08-2.67] and 5.19 [3.43-7.88], P<0.001). This association persisted after adjustment for urinary albumin excretion rate, glomerular filtration rate and conventional cardiovascular risk factors for the third tertile (covariate adjusted hazard ratios 2.54 [1.56-4.14], P<0.001). This increased mortality was attributable to more cardiovascular deaths. Thus, it seems that, in patients with Type 2 diabetes, Nt-proBNP is a strong predictor of cardiovascular disease and mortality, independently of urinary albumin secretion rate and conventional cardiovascular risk factors.

No potential conflict of interest revelant to this article was reported.

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The pathophysiology of vascular calcification: are osteoclast-like cells the missing link?

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Abstract

There is increasing evidence to suggest that the initiation of vascular calcification is an active process involving vascular smooth muscle cell (VSMC) apoptosis and trans-differentiation into calcifying cells. This active process results in the deposition of an osteogenic extracellular matrix and may be exacerbated by a reduction in the levels of one or more native calcification inhibitors (such as fetuin A and pyrophosphate). Here, we present data which strongly suggest that the regression of vascular calcification might also be an active cellular process involving osteoclast-like cells. However, the presence of osteoclast like cells in the vascular wall is rather limited. To explain this rarity of osteoclast-like cells, we recently observed that the same factors, which promote the trans-differentiation of VSMCs into osteoblast-like cells are also capable of inhibiting the *in vitro* differentiation of monocytes/macrophages into osteoclast-like cells. An imbalance between osteoblast-like and osteoclast-like cell activities would therefore favour the occurrence of a pathological calcification process in vessel walls. Our new data are strongly evocative of a vascular remodelling process similar to that observed in bone tissue. To confirm this hypothesis, strategies for activating osteoclasts in the vascular wall (with a view to preventing or reversing vascular calcifications) are required.

Résumé

Physiopathologie des calcifications cardiovasculaires: les cellules osteoclast-like sont-elles le chaînon manquant ?

Il est désormais bien établi que le processus de calcification vasculaire est un processus actif impliquant l'apoptose des ostéoblastes ainsi que leur transdifférentiation en cellules calcifiantes. Ce processus conduit au dépôt d'une matrice extracellulaire de type ostéogénique et pourrait être aggravé par la baisse de la quantité d'inhibiteurs de calcification tels la fétuine A et/ou le pyrophosphate. Dans cette revue, nous discutons du fait que la régression des calcifications vasculaires pourrait également être un processus cellulaire actif faisant intervenir les cellules ostéoclastiques. Néanmoins, la présence de cellules ostéoclase-like a été difficilement démontrée dans les parois vasculaires jusqu'ici. Récemment, nous avons mis en évidence que les mêmes facteurs qui induisent la minéralisation de cellules musculaires lisses inhibent la différenciation des précurseurs monocytaires-macrophagiques en ostéoclast-like. Ainsi, ces données pourraient expliquer la faible présence d'ostéoclast-like dans les parois vasculaires. Un déséquilibre entre l'activité des cellules ostéoblastiques-like et ostéoclastiques-like pourrait favoriser la survenue de calcifications dans les parois vasculaires pathologiques. Ces nouveaux éléments suggèrent fortement la possibilité d'un remodelage au niveau vasculaire, semblable à celui observé dans le tissu osseux. Des stratégies visant

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Keywords: Osteoclast; Osteoblast; Monocyte/macrophage; Vascular smooth muscle cells; Inorganic phosphate; Vascular calcification; Review; Urémic toxins

Mots clés : Ostéoclaste ; Ostéoblaste ; Monocyte/macrophage ; Cellules musculaires lisses ; Phosphate inorganique ; Calcification vasculaire ; Revue générale ; Toxines urémiques

1. Introduction

Cardiovascular calcification is frequent in the general population, in diabetic patients, and in patients with chronic kidney disease and is associated with an increased cardiovascular risk.[1-11] Cardiovascular calcification in the vascular walls is accompanied by the deposition of a mineralized osteogenic protein matrix. These calcium deposits are essentially localized in the media of the vascular wall but are also found in subintimal atherosclerotic plaques [12]. Both types of deposit are often observed simultaneously in humans and probably share common cellular and molecular mechanisms in their genesis [13].

2. The physiological development of bone calcification

ted cell-mediated process in bone tissue [14]. Bone derives from intramembranous and/or endochondral cartilage ossification. In the latter process longitudinal bars of cartilage matrix become calcified and thus form the growth plate of cortical bones. Hypertrophic chondrocyte death in this zone means that the extracellular chondroid matrix becomes impregnated with calcium salts. Moreover hypertrophic chondrocytes release matrix vesicles - small membrane-bound bodies equipped with sophisticated cellular tools for creating a microenvironment that favours the nucleation of hydroxyapatite crystals (initiation step). This initial calcification process is followed by (i) the invasion of cartilage cells from the blood vessels (ii) the metaphysis/destruction of cartilage and (iii) the formation of bone along the remaining walls of the precalcified cartilage. The bone matrix produced by osteoblasts is subsequently mineralized by the coordinated interaction of several mineralizing-regulating proteins (i.e. the nucleation and crystal growth steps). Bone mineralization is determined partly by the osteoblasts'ability to remove pyrophosphate (a physiological inhibitor of mineralization) from their surrounding bone matrix via tissue non-specific alkaline phosphatase (Tnap) activity and the presence of a fibrillar (type I) collagen-rich network in the bone matrix [15]. Although the genes coding for Tnap and fibrillar collagen are not osteoblast-specific osteoblasts in bone and odontoblasts in teeth are the only cell types in which they are co-expressed. In the final (regression) step minerals are resorbed by osteoclasts - the only cell type responsible for bone tissue degradation. Mature osteoclasts are large multinuclear cells with a characteristic tartrate-resistant acid phosphatase (TRAP) activity. The regulation of bone resorption involves two major processes: recruitment of new osteoclasts from haematopoietic precursors (monocyte/macrophage cell lineages) and the activation & survival of mature osteoclasts. Hence bone degradation is a multistep process which includes cell adhesion to the bone surface cell polarization and formation of a sub-osteoclastic bone-resorbing compartment where bone degradation occurs [16]. The imbalance of bone remodelling in favour of osteoclast hyperactivity leads to the acceleration of bone demineralization which is not compensated by an increase in osteoblast activity and thus causes osteoporosis [17].

3. The pathophysiological development of vascular calcification

There is increasing evidence to suggest that matrix deposition in the vascular walls results from an active cellular process leading to the accumulation of osteogenic extracellular material and which is initiated (at least in part) by the materials activation of an apoptotic process and the release of apoptotic bodies and matrix vesicles [18]. This active process may be exacerbated by a reduction in the levels of one or more native calcification inhibitors (such as fetuin A pyrophosphate matrix GLA protein osteopontin and osteoprotegerin) [19-22] and/or by a partial defect in phagocytosis [23].

This process also appears to be intimately related to VSMC trans-differentiation into vascular calcifying cells. To date several factors have been shown to promote *in vitro*

ding inorganic phosphate, inflammation, and oxidation products. These factors act through a common mechanism involving core binding factor a1 (Cbfa1) [24, 25], a transcription factor which is specific for the osteoblastic phenotype. Similar changes in the VSMC phenotype have been observed in animal models [24, 26] and human biopsy specimens [25]. However, the recent demonstration of the association of chondrocyte-like cells with media calcification in both rat and human arteries indicates that in addition to VSMC trans-differentiation, a process resembling endo-

chondral bone formation is a second mechanism by which vascular calcification may occur [27]. It is still unclear to what extent the formation of calcifying vascular cells is due to trans-differentiation of VSMCs resident in the local media or cell recruitment from the adventitia after the transformation of pericytes by Wnt signalling [28].

Osteoclast differentiation is tightly coupled to the presence of cell-cell contacts between the osteoclast precursors and osteoblasts in the bone tissue (the receptor activator of NF-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin system). In the calcified vascular wall the presence of (i) monocyte/macrophage cell types that are able to differentiate directly into osteoclasts [29] and (ii) VSMCs that have an osteoblast-like phenotype and secrete factors involved in osteoclast differentiation (such as RANKL macrophage colony-stimulating factor (M-CSF) or pro-inflammatory cytokines) strongly suggests that osteoclastogenesis might occur [13; 30; 31]. The coexistence of these two differentiated cell types led us to hypothesize a vascular remodelling process similar to that observed in bone tissue. Hence an imbalance between the two processes in favour of the osteoblast-like phenotype could promote calcification [32; 33].

Preliminary data support the presence of cells with an osteoclast-like phenotype in the calcified arterial wall [31 34]. Moreover Bas et al. demonstrated that calcitriol-induced vascular tissue calcification in rats was partially reverted shortly after withdrawal of calcitriol treatment and that this process was associated with the presence of activated macrophages in the media [35]. Furthermore in a recent study bone-marrow-derived mature allogenic osteoclasts have been seen to interact with calcified aortic elastin and reduce its mineral content in the absence of detectable elastin degradation [36]. Taken as a whole these data indicate that an active cellular process may be involved in the regression of vascular calcification and that osteoclast-like cells may play a central role in this process.

4. The potential role of osteoclast-like cells in vascular calcification

to those observed in patients with CKD inorganic phosphate (one of the most important uraemic toxins inducing cardiovascular calcifications) significantly and dose-dependently decreased in vitro osteoclast monocyte/macrophage progenitor differentiation into osteoclast-like cells [37]. Inorganic phosphate was shown to affect RANKL-induced signalling mainly via the down-regulation of the RANKL-induced JNK Akt and NF-κB activation pathways. We also recently obtained similar results with other uraemic toxins such as oxidized LDL (data not published). Other researchers have shown that calcifying vascular cells restrict osteoclast differentiation via modulation of the release of matrix proteins (such as osteopontin) and soluble factors (such as osteoprotegerin and interleukin 18) [38]. It therefore appears reasonable to assume that a reduction in the activity of osteoclast-like cells in the arterial wall could be involved in the emergence and persistence of vascular calci-

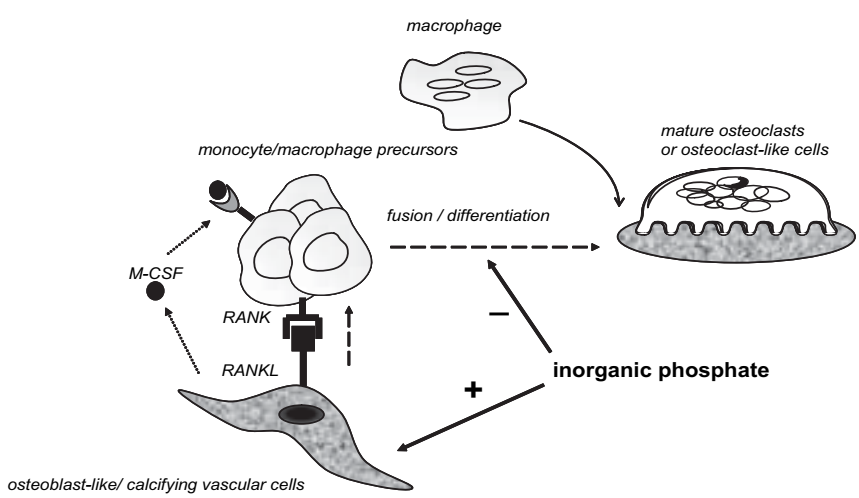

Fig. 1: Inorganic phosphate not only promotes the trans-differentiation of vascular smooth muscle cells into calcifying osteoblast-like cells but also inhibits the differentiation of monocytes/macrophages into mature osteoclast-like cells and thus blocks calcified matrix resorption.
RANK: receptor activator of NF-kB, RANKL: receptor activator of NF-kB ligand; M-CSF: macrophage colony-stimulating factor.

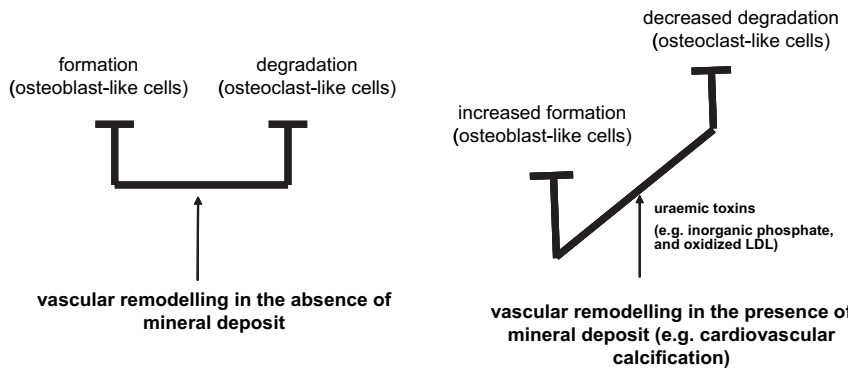

Fig. 2: A hypothetical model of vascular calcification as a consequence of an imbalance in vascular remodelling; an imbalance between osteoblast-like cell activities and osteoclast-like cell activities in favour of the former could promote a pathological calcification process in the vessel walls.

fication. Indeed this type of phenomenon could be comparable to that involved in the development of osteoporosis where an imbalance of bone remodelling in favour of hyperosteoclastogenesis is not compensated by an increase in osteoblast activity and thus results in a severe decrease in bone mineral density and the loss of bone micro-architecture at the trabecular level. This imbalance between osteoblast-like and osteoclast-like cells activities could explain the pathological calcification process that can be observed in vessel walls (Fig. 2).

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New techniques for assessing arterial stiffness

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Abstract

Arterial stiffness is now included in the guidelines of the European Society of Hypertension. In this paper, we review the evidence for the predictive value of arterial stiffness. More than 11 longitudinal trials have proven the predictive value of aortic stiffness measured through carotid to femoral pulse wave velocity, beyond and above classical risk factors. Such evidence is scarcer for central pressure and local arterial stiffness. If we add this evidence to the easiness of performing such measure, carotid to femoral pulse wave velocity is the reference technique for assessing arterial stiffness. Its place in the investigation of patients remains to be precised.

Résumé

Nouvelles techniques de mesure de la rigidité artérielle

La rigidité artérielle est à présent incluse dans les recommandations de l'European Society of Hypertension. Dans cet article, nous passons en revue les preuves de la valeur prédictive de la rigidité artérielle. Plus de 11 études longitudinales ont apporté la preuve de la valeur prédictive de la rigidité aortique par la mesure de la vélocité de l'onde de pouls carotido-fémorale, à côté et au-delà des facteurs de risque cardiovasculaire classiques. De telles preuves sont plus rares pour la pression centrale et la rigidité artérielle locale. Si nous ajoutons à ces preuves la facilité à réaliser une telle mesure, la vélocité de l'onde de pouls carotido-fémorale est la méthode de référence pour mesurer la rigidité artérielle. Sa place dans l'exploration des patients reste à préciser.

Keywords: Artery stiffness; Aorta; Cardiovascular risk; Epidemiology; Mortality; Central pressure

Mots clés : Rigidité artérielle ; Aorte ; Risque cardiovasculaire ; Épidémiologie ; Mortalité ; Pression centrale.

Measurement of arterial stiffness is increasingly popular for assessing target organ damage and cardiovascular risk. In the present paper, we will review the different techniques available and their relative interest.

1. Measurement of aortic pulse wave velocity

We published recently a expert consensus document on arterial stiffness [1]. In this document, over 11 longitudinal studies were listed demonstrating that a simple measure of aortic stiffness through carotid-femoral pulse wave velocity (CF-PWV) (Fig. 1) yielded prognostic values beyond and above traditional risk factors. Other arterial measurements can be used as surrogates for arterial stiffness. Among them, central pulse pressure is interesting since it may be a better estimate of the true pressure acting on target organ damage [2]. The difference between central and peripheral blood pressure is related to arterial stiffness and pressure wave reflection. It may be interesting to substitute

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central blood pressure to peripheral blood pressure, since it has been demonstrated that drugs may have a differential effect on central blood pressure, but not on peripheral [3]. Here again, the level of evidence for the predictive value is lower than for aortic stiffness.

The added value of CF-PWV above and beyond traditional risk factors was demonstrated by two separate studies. The first was performed in 1045 hypertensive patients, with longitudinal follow-up of 5.9 years for CV events (fatal or non-fatal) [4]. The increase in coronary heart disease with tertiles of CF-PWV was steeper for patients belonging to the first and second tertiles of Framingham risk score (FRS). The area under the receiver-operating characteristic curve (AUC) of CF-PWV decreased from the lowest to the highest tertile of FRS (area under the receiver-operating characteristic curve: from 0.65±0.07 to 0.53±0.04; P=0.01). In the group of low to medium risk patients, FRS and CF-PWV had similar predictive value (AUC = 0.65±0.07 and 0.63±0.08, respectively), and when combined, the predictive increased since the area under the receiver operating curve rose to 0.76±0.09 (unpublished data).

More recently, Mattace-Raso et al. published the predictive value of aortic stiffness in the elderly from a general population [5]. In this study, aortic stiffness predicted CV outcome after adjustment on classical risk factors, intima media thickness, wave reflection and pulse pressure. The AUC of the fully adjusted model (including all classical CV risk factors and intima media thickness was 0.70. Including PWV induced a further significant increase in AUC of 2%, $p<0.01$. Interestingly enough, for all published studies about the predictive value of aortic stiffness, patients were subsequently treated for their treatable risk factors. This means that the predictive value of arterial stiffness is independent of common CV drug therapy. This may also explain why once detected and treated, classical risk factors cease to be strong risk factors for further events.

Albeit not new, the techniques have now reached a development allowing easy, reproducible measure by either doctors, nurses or technicians. The device in use may offer simultaneous (Complior, Artech medical, Pantin, France) (Fig. 1), or successive measure (Sphygmocor, Atcor, Sydney, Australia) after synchronization on ECG. Both techniques offer the same quality of measure, however, only the Complior can be used if the patient has arythmia.

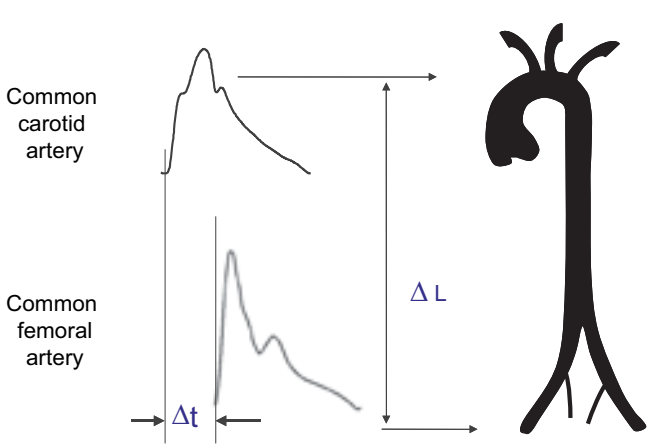

Figure 1: Measurement of carotid-femoral pulse wave velocity with the foot to foot method

2. Measurement of central pressure

From these results, it can be said that aortic stiffness is a strong independent predictor of CV outcome, providing a level of information equal to classical CV risk factors, and whose value is additive to it. Measurement of central pressure is an important advance in the assessment of large artery properties. Central pressure differs markedly from peripheral blood pressure (i.e. the one measured at the site of brachial artery), mostly because of wave reflection. Indeed, when blood is ejected by the left ventricle, the pressure wave is propagating toward the periphery at a finite speed (the pulse wave velocity), and reflects on reflecting points to return toward the heart. Depending on the place of reflecting points and the pulse wave velocity, the reflected wave occurs at different time during the heart cycle. When BP is measured at the periphery of the vasculature, the reflecting sites are closer to the point of measurement of BP, and thus reflection occurs soon after the rise in BP and adds to it. This is the case for the brachial artery. For sites close to the heart, the pressure wave has to travel to and from the reflecting sites and this takes time. If pulse wave velocity and the heart period are slow, the reflected wave has a chance to reach the heart after the termination of ejection. In this case, the reflected wave does not add with the ejection wave, and blood pressure is not further increased. With aging and high blood pressure, the pulse wave velocity is faster, and the reflected wave comes earlier within the ejection, and adds to the ejection pressure wave, causing amplification (Fig. 2). These phenomenon explain why brachial systolic (and pulse) pressure are larger than central systolic and pulse pressure, and why this amplification of SBP and PP along the arterial tree is decreased with aging and high blood pressure.

Central blood pressure is important in many ways. This is the blood pressure seen by the left ventricle, the kidneys and the brain. To that extent, this is the only blood pressure value which should be considered for target organ damage. Second, reflection patterns directly influence LV work and perfusion. Last, when studying local arterial stiffness, local blood pressure is mandatory to derive correct stiffness values, and for central large arteries such as the aorta and common carotid artery, it has to be measured locally.

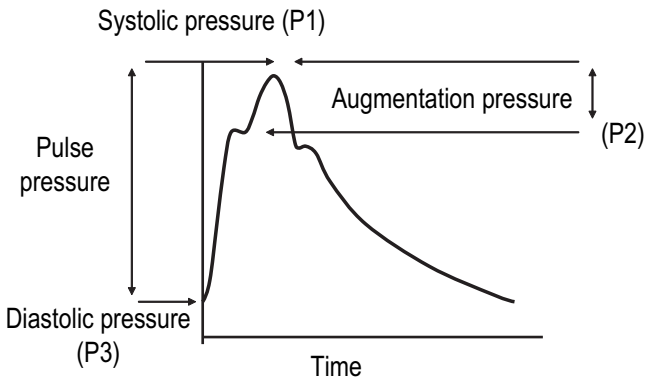

Figure 2: Carotid pressure waveform is recorded by applanation tonometry. The height of the late systolic peak (P1) above the inflection (P2) defines the augmentation pressure, and the ratio of augmentation pressure to PP defines the augmentation index (AIx, in percent).

3. Methods to measure central blood pressure and wave reflection

3.1. Invasive methods

The most direct way to measure central blood pressure is invasive during left catheterization. Blood pressure could be measured either from fluid filled catheter (albeit with reduced frequency response) or from microtip catheter. The pioneering works of O'ROurke, Kelly, Belthram, Yin etc. were done using this technology [6,7]. This is still applicable for clinical research in cath labs, where a simple additional pressure measurement can yield strong prognostic value. If central BP measurements are to be performed during catheterization, some cautions have to be taken to remove air bubbles from the fluid filled systems, and to avoid excessive motion of the catheter tip, because dynamic overshoot of the pressure (because of blood flow or impact of the catheter on aortic walls) may occur. Because of their cost, microtip catheters are not used in routine. Pulse wave analysis is performed in a identical way as described later in this chapter.

3.2. Non invasive assessment

All techniques are derived from applanation tonometry [8]. Just to set it easy first, applanation tonometry takes advantage of a well established theorem saying that when a segment of a pipe is flattened, the transmural pressure is equal to the endovascular pressure. This technique was first used for ophthalmology, to derive not invasively intraocular pressure. Applied to arteries, it necessitates to apply a pressure sensor through the skin and applanate a superficial artery by applying a downward pressure sufficient to flatten the artery (not too large, because otherwise the blood pressure regimen is too disturbed), and to have a "hard" floor against which to perform applanation. This can be easy for the radial and the femoral artery (the radius and pelvis bones allow applanation). This is more difficult for the brachial artery, because of the interposition of tendons and for the carotid artery because of the interposition of muscles and the presence of body fat behind the artery, but measures are still feasible. Carotid artery pressure is the best direct estimate of central pressure. However, some amplification occurs between carotid and the aorta, which may need further mathematics.

If the pressure sensor is correctly calibrated, the pressure recorded through the applanation tonometry is equal to the pressure inside the vessel, at a constant. Indeed, the mean value of applanation pressure is determined by the pressure applied by the hand of the investigator. This has to be corrected by rescaling the applanation pressure around the mean pressure of the subject, which is constant in the large arteries (Fig. 2).

Although this technique was validated and used by some investigators [9,10], it was criticized because of its operator dependency. It was proposed more recently to rescale central pressure using diastolic blood pressure in addition to mean blood pressure. Pauca showed that the mean to diastolic difference was rather constant throughout the body [11]. Therefore, using a simple correction, this mean-diastolic pressure could be used as scaling factor. For doing so, it is simply needed to have reliable measures of brachial mean and DBP [12]. This brings us to the tricky problem of MBP assessment. Most often, people use oscillatory methods to derive BP values. These validated device directly measure SBP and MBP, and derive DBP from on board, unpublished algorithms. Therefore, these values cannot be taken as granted.

Recently, Van Bortel and coworkers showed that the most correct way to measure central blood pressure is first to measure as accurately as possible SBP and DBP at the brachial artery level, then to perform brachial artery applanation using SBP and DBP as scaling values [13]. Then, MBP is measured from the integration of the brachial pressure wave, and this MBP value will be used to calibrate carotid pressure, together with the DBP after carotid applanation is performed. Although this technique is at evidence the best, this is not always feasible. Therefore, most of the investigators do the same calibration procedure, but using the radial pressure instead of the brachial. In this case, some systematic error occurs, by not taking into account the brachial-radial amplification.

4. Estimation of aortic pressure

As we said before, carotid pressure is the best direct measurement of central pressure, although some amplification occurs between the aorta and the carotid. To overcome this problem, M O'Rourke and coworkers proposed to use a generalized transfer function to synthesize aortic pressure wave [14]. To put is simple, the transfer function is the key for translating a distorted signal into the original signal, by applying the inverse of the cross-correlation function of the

original signal to the distorted one. This is widely used in the industry, transmission, adaptive optics, Hi-Fi etc, when the factors of distortion are predictable. In the present case, O'Rourke et al measured simultaneously in 14 patients and subjects, both the radial pressure wave and the aortic pressure wave. They deduced the transfer function from the cross-correlation of the two waves. They showed that this transfer function was rather constant across a wide range of persons, both in body size, age, sex and BP values. Thus this "generalised transfer function" is proposed to assess aortic pressure from radial pressure. It is to be noted that the scaling from mean and diastolic is performed, also for this technique. The generalization of the transfer function has been challenged, but no reliable alternative exists up to now for non invasive aortic pressure measurements.

4.1. Wave reflection quantification

From carotid and aortic pressure waves, it is possible to measure the amplification index, an estimate of wave reflection. This index is the ratio between the amplitude of the reflected wave (determined between the shoulder and the maximum of the systolic arm of the pressure wave) and pulse pressure (Fig. 2).

5. Available devices

A recent consensus document, [15] makes a good synthesis on the validity of different devices. For the moment, the Sphygmocor device® (Artech medical, Sydney Australia) is the gold standard for central pressure measurement. It represents more than 100 entries in Medline, for 2 publications for its closest competitor. It has been used in very large population samples [16] together with large clinical trials [17]. (The Sphygmocor device takes advantage of the transfer function to calculate central pressure from radial pressure waves. Under certain circumstances, it allows the evaluation of endothelial function (pressure wave response to salbutamol [18]). By successively measuring carotid and femoral pulse waves, synchronized on the EKG R-wave, this device allows the measurement of pulse wave velocity, a measure of aortic stiffness [19].

6. Measurement of local arterial stiffness and intima media thickness

Local arterial stiffness of superficial arteries can be determined using ultrasound devices. Carotid stiffness may be of particular interest, since in that artery atherosclerosis is frequent. All types of classical, bi-dimensional vascular ultrasound systems can be used to determine diameter at diastole and stroke changes in diameter, but most of them are limited in the precision of measurements because they generally use a video-image analysis. At present some researchers also measure local arterial stiffness of deep arteries like the aorta using cine magnetic resonance imaging (MRI). However, most of pathophysiological and pharmacological studies have used echo-tracking techniques (Fig. 3).

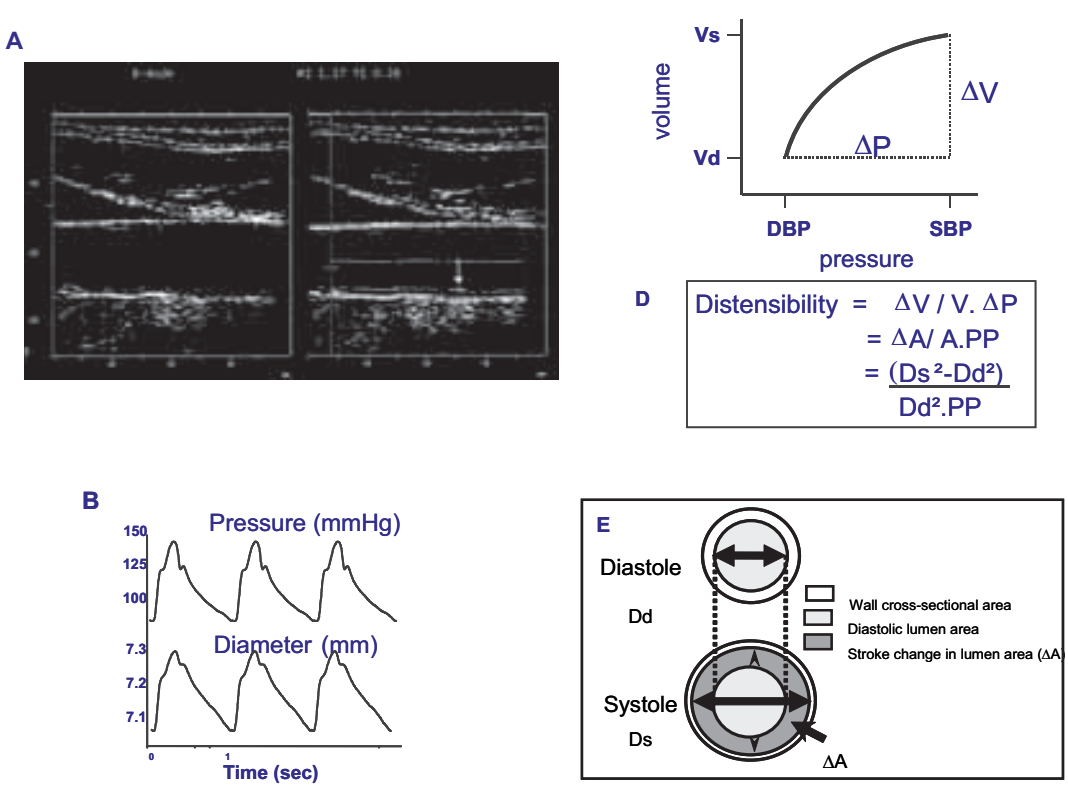

Figure 3: Local arterial distensibility. A: high definition echotracking device measuring diameter, stroke change in diameter and intima-media thickness. B: Simultaneous recording of stroke changes in BP and diameter. C: Pressure-diameter curve. D: Calculation of distensibility. E: Schematic representation of the stroke change (DA) in lumen cross-sectional area (LCSA).

A major advantage is that local arterial stiffness is directly determined, from the change in pressure driving the change in volume, i.e. without using any model of the circulation. However, because it requires a high degree of technical expertise, and takes longer than measuring PWV, local measurement of arterial stiffness is only really indicated for mechanistic analyses in pathophysiology, pharmacology and therapeutics, rather than for epidemiological studies.

Echotracking devices were developed to measure diameter in end-diastole and stroke change in diameter with a very high precision, 6 to 10 time higher than with video-image systems. Now, the latter development of echotracking device (Artlab system, Esaote, Maastricht, NL) allow the measurement along a segment of artery (4 cm, 128 lines) with high definition measurement of diameter and intima-media thickness (precision 35µm and 17 µm, respectively) in real time, with hand held probe. Moreover, it is possible to measure distension at 14 different sites, with a resolution of 1.7 µm. This device allowed us to discover new behaviour of the arterial wall, with local heterogeneity in stiffness [20], and complex patterns of distension profile (bending stress) at the level of small plaques [21].

Local pressure is usually obtained by applanation tonometry of the vessel in question and calibration of the waveform to brachial mean and diastolic pressures obtained by integration of the brachial or radial waveform [12], or automatic calculation using transfer function processing (Sphygmocor, AtCor, Sydney Australia) [22,12]. All the superficial arteries are suitable for the geometrical investigation, and particularly the common carotid, common femoral and brachial arteries. Various indices used to describe the elastic properties of blood vessels, non-invasively obtained with ultrasound measurements may be obtained, expressing the complexity of the mechanical behaviour of these organs (Fig. 3).

Carotid stiffness was predictive of CV events in a small number of patients with ESRD [23] or following renal transplantation [24], but had no independent predictive value in a larger number of patients with manifest arterial disease [25,26]. This may be due to methodological reasons: the use of local (carotid) PP in positive studies and brachial PP in negative studies. In addition, upper and lower limb territories, due to their particular pathophysiology may not reflect aortic, cerebral and coronary artery damage[27].

7. Conclusion

Arterial stiffness measurement is now part of assessment of hypertensive patients. It is likely that many other clinical conditions will require such measures to be performed. Given the variety of techniques available, it is mandatory to state which deserve recommendation or not. Now it is clear that carotid to femoral pulse wave velocity is the most validated technique, and should be used in preference to any other. Local stiffness is to be used in specialized labs, for clinical research, because it is too complex to use and interprete. Central pressure is a proxy for arterial stiffness. Although used in a large clinical trial, the CAFÉ study and in several outcome studies, it is difficult to see how central pressure may improve the classification of patients because of the dependence on peripheral blood pressure.

Now what is awaited is evidence of a better prevention of events when arterial stiffness is used in treatment decision. The only available evidence is the paper from Guerin in end stage renal disease patients, showing that arterial stiffness response to graded intervention is predictive of the outcome [28]. Such data need to be established in patients at lesser risk.

No potential conflict of interest revelant to this article was reported.

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Microparticles and type 2 diabetes

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Abstract

Cell activation or apoptosis leads to plasma membrane blebbing and microparticles (MPs) release in the extracellular space. MPs are submicron membrane vesicles, which harbour a panel of oxidized phospholipids and proteins specific to the cells they derived from. MPs are found in the circulating blood of healthy volunteers. MPs levels are increased in many diseases, including cardiovascular diseases with high thrombotic risk. Exposure of negatively charged phospholipids and tissue factor confers a procoagulant potential to MPs. Elevation of plasma MPs levels, particularly those of endothelial origin, reflects cellular injury and appears now as a surrogate marker of vascular dysfunction. Recent studies demonstrate an elevation of circulating levels of MPs in diabetes. MPs could also be involved in the development of vascular complications in diabetes for they stimulate pro-inflammatory responses in target cells and promote thrombosis, endothelial dysfunction and angiogenesis. Thus, these studies provide new insight in the pathogenesis and treatment of vascular complications of diabetes.

Résumé

Microparticules dans le diabète de type 2

L'activation cellulaire et l'apoptose conduisent au bourgeonnement de la membrane plasmique et à la libération de microparticules (MPs) dans l'espace extracellulaire. Ces MPs sont de petites vésicules membranaires qui expriment toute une gamme de phospholipides oxydés et de protéines caractéristiques de la cellule d'origine. Les phospholipides chargés négativement et le facteur tissulaire portés par certaines MPs leur confèrent un pouvoir procoagulant. Une élévation du taux de MPs dans le sang circulant est donc le reflet d'une atteinte cellulaire et témoigne bien souvent d'une pathologie vasculaire ou thrombotique. De plus, les MPs sont de véritables vecteurs biologiques capables d'induire selon leur composition, une réponse de type pro-inflammatoire dans le compartiment vasculaire. Les études récentes menées sur les MPs et le diabète ont apporté de nouvelles données sur la pathogenèse du diabète. Le diabète de type 2 est en effet associé à une élévation des taux de MPs plasmatiques qui pourraient agir sur les étapes cruciales du diabète et de ses complications: les MPs induisent et entretiennent l'inflammation locale, la coagulation, la dysfonction endothéliale et l'angiogenèse dans le contexte du diabète et de ses complications vasculaires. Les MPs apparaissent donc comme de nouveaux éléments à prendre en considération dans les stratégies thérapeutiques employées contre le diabète.

Key-words: Microparticles; Diabetes; Apoptosis; Vascular injury; Review

Mots clés : Microparticules ; Diabète ; Apoptose ; Complications vasculaires ; Revue générale

Cell activation or apoptosis leads to plasma membrane blebbing and the release of microparticles (MPs) in the extracellular space. MPs are submicron membrane vesicles, which express a panel of phospholipides and proteins specific to the cells they derived from. MPs are found in the circulating blood of healthy volunteers. MPs levels are

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increased in many diseases, particularly cardiovascular diseases with high thrombotic risk. Exposure of negatively charged phospholipids and tissue factor confers a procoagulant potential to MPs. Elevation of plasma MPs levels, particularly those of endothelial origin, reflects cellular injury and appears now as a surrogate marker of vascular dysfunction. MPs are also biologically active and stimulate pro-inflammatory responses in target cells. The development of vasculopathies in diabetes involves multifactorial processes including pathological activation of vascular cells. Release of microparticles by activated cells has been reported for the first time in diabetes in 2002 [1]. Consequently, MPs appears as a new prognostic potential of type 2 diabetes, particularly interesting in the early detection of vascular complications in this disease (Fig. 1).

1. Formation and characteristics of microparticles

The current knowledge on MPs formation derives mainly from experiments on isolated or cultured cells. However, the mediators and the mechanisms involved in *in vivo* MPs formation and shedding remain mostly unknown.

MPs formation is associated with the loss of membrane asymmetry, a characteristic of quiescent cells. This leads to exposure of phosphatidylserine on the outer leaflet as a consequence of the calcium-dependent activation of scramblase and floppase and the inhibition of flippase activities [2-4]. Phosphatidyserine exposure is not always followed by the release of MPs, which may be regulated by the level of intracellular calcium. Moreover, MPs formation and shedding necessitate modifications in cell structural architecture involving disruption of cytoskeleton proteins organization. Cell activation or apoptosis could induce these modifications. For example, platelets release MPs following activation by either thrombin, ADP plus collagen, the complement complex C5b-9, the calcium ionophore A23187, and by high shear stress [5-8]. MPs released from apoptotic cells may be different in lipid and protein composition from membrane vesicles shed following cell activation and could possibly have different patho-physiological effects [9]. Blebbing of the cellular membrane occurs rapidly after cells enter the apoptotic process. Blebs formation depends upon actin cytoskeleton and actin-myosin contraction, which is regulated by caspase3-induced Rho Kinase 1 activation [10,11]. Rho kinase activation is required for re-localization of DNA fragments from the nuclear region to membrane blebs, suggesting that MPs from apoptotic cells may contain nuclear material [11,12]. An important parameter that determines the biological effects of MPs is their protein and lipid composition, and not only their circulating numbers. The experimental evidence so far available indicates that the protein and lipid composition of MPs may vary depending upon the cell they originate from and the type of stimulus involved in their formation. Proteomics analyses have revealed thet the spectrum of proteins found in MPs released *in vitro* from cultured cells is influenced in part by the type of stimulus used to trigger cell vesiculation [13].

2. Detection and measurements of microparticles

Plasma MPs can be detected and their cellular origin characterized using capture assays or flow cytometry. The flow cytometry analysis permit, in addition of characterization, the quantification of MPs by mean of using calibrator beads of defined concentration. A crucial point in circulating MPs analysis concerns the pre-analytical steps involved in blood sampling and platelet-free plasma preparation [14]. Both methods of MPs analysis rely on antibody detection of specific cellular markers and annexin V binding of phosphatidylserine. Although the general understanding is that MPs express phosphatidylserine, which is detected by annexin V labeling, some plasma MPs analyzed by flow cytometry express specific markers of their cellular origin, but do not bind annexin V even in the presence of high calcium concentrations. For instance, in patients with sickle cell disease, circulating endothelial MPs were either positive or negative for annexin V [15]. In a similar way, we recently observed that very few circulating platelet-derived CD41+ MP bind annexin V in some patients with end-stage renal failure [16]. These observations suggest that phosphatidylserine, even if exposed on MPs, could be already engaged in some other molecular interactions and thus unable to bind annexin V, or that other pathway(s) may be involved in their formation and release, but this remains to be demonstrated.

3. MPs: bystanders and effectors

It is now well established that, in addition to their biomarker potential in cardiovascular diseases, MPs may also transfer bioactive molecules to other cells or MPs acting as true diffusible vectors in the transcellular exchange of biological information [17]. For all these reasons, it has been suggested that they can play an important role as a messenger linking thrombosis and inflammation through interaction with other cell types. However, it is still debated whether they play a causal role in the pathogenesis of cardiovascular disease or whether they are a consequence of the disease [18].

4. MPs as markers in diabetes

Circulating levels of MPs are augmented in most cardiovascular diseases when comparing a patient population with a matched group of healthy subjects. The general consensus is that plasma levels of MPs reflect an equilibrium between their release and their removal from the circulation by phagocytes. The observation that circulating MPs are increased following acute myocardial infarction raises the question

whether these MPs might come from the ruptured plaque [19]. This possibility is highly unlikely because of the different pattern of cellular origin between plaque and plasma MPs [20]. The major MPs populations present in the atherosclerotic plaque originate from macrophages, erythrocytes and smooth muscle cells, but not from platelets while circulating MPs derived for a significant part from platelets and are not of smooth muscle cell origin. In addition, although MPs are much more abundant in atherosclerotic plaques than in plasma and account for the procoagulant activity of the lipid core, at least a dozen of large lesions (such as those found in human carotid arteries) would have to rupture simultaneously to fully account for circulating levels of MPs in these patients [20]. Consequently, MPs found in plasma reflect local injury of either vascular or circulating cells. Elevated levels of MPs originating from platelets, monocyte or endothelial cells were found in type 2 diabetes [1, 21-25], as observed also in patients with other cardiovascular diseases [26].

Several recent studies point out that circulating levels of endothelial MPs associate with impaired endothelial function in patients with cardiovascular diseases and in diabetic patients with coronary artery diseases [16, 26-27]. Moreover, endothelial MPs levels predict the presence of coronary artery disease in diabetic patients, whereas other markers of endothelial injury such as soluble ICAM have no pronostic value [27]. These data support the concept that measurement of endothelial MPs could be useful for identifying diabetic patients with increased risk of cardiovascular disease (Fig.). However, the potential mechanisms involved in increased cell vesiculation in diabetes remain unknown. No information is available regarding the effect of glucose, insulin or that of AGE-proteins. Elevated levels of endothelial and platelet MPs found in type 2 diabetes correlated with increased levels of anti-oxidized LDL antibodies in plasma of type 2 diabetic patients, suggesting that oxidized LDL could contribute to endothelial membrane vesiculation [28]. Oxidative stress in diabetes may not be the only trigger of MPs generation in diabetes. Indeed, elevated levels of remnant lipoproteins in type 2 diabetic patients are associated with plasma platelet MPs, suggesting that reducing elevated lipoproteins with lipid-lowering therapy may be an effective strategy to prevent MPs associated-thrombogenic vascular complications in type 2 diabetes. All together, these data demonstrated the clinical significance of MPs detection and characterization in type 2 diabetes

5. MP: biological effectors in diabetes

Most of the experimental evidence available so far indicates that MPs can influence diverse biological functions. However, one should be cautious in interpreting data from studies with MPs generated *in vitro* or from cultured cells, as they may not be fully representative of those patients *in vivo*. The pattern of proteins found on MPs , as well as the level of oxidized phospholipids are likely to influence their effects on target cells [29].

Circulating MPs bear tissue factor at their surface and account for « blood borne tissue factor » [30]. They are involved in the formation of tissue factor-platelet hybrids, a critical phenomenon in thrombus propagation, following tissue factor transfer from leukocytes MPs to platelet membranes [30]. This property may not be restricted to leukocyte-derived MPs as the presence of tissue factor on platelet-erythrocyte- and hematopoietic cell- derived MPs leads also to thrombus propagation *in vivo* [31,32]. Type 2 diabetes is characterized by the presence of an altered platelet metabolism that may contribute to the pathogenesis of atherothrombotic complications of diabetes [33]. An increased level of circulating MPs has been suggested to be one of the procoagulant determinants in patients with type 2 diabetes [34]. Hypercoagulable state of diabetes coud be initiated or maintained by elevated levels of tissue factor positive platelet MPs. Moreover, increased levels of insulin and glucose increase tissue factor procoagulant activity [35], suggesting that high concentrations of tissue factor exposed by MPs present in diabetes are highly pro-thrombogenic (Fig.). These high levels of MPs observed in patients with type 2 diabetes may be related to enhanced reactive oxygen species generation and lipid peroxydation [33, 36].

Circulating MPs also impair the release of nitric oxide from vascular endothelial cells. This was observed on isolated arteries exposed *in vitro* to circulating concentrations of MPs from patients with acute coronary syndromes, end stage renal failure or preeclampsia, but not with MPs from healthy subjects [16, 37, 38] (Fig. 1). The endothelial dysfunction caused by circulating human MPs appears to be mediated by MPs of endothelial origin and is associated with an impaired release of NO but no alteration in endothelial NO synthase expression. Endothelial MPs circulating in diabetic patients were also associated with vascular dysfunction *in vivo* [39]. Indeed, type 2 diabetic patients exhibit impaired postprandial flow-mediated dilatation, which is correlated to increases in circulating endothelial MPs. Taken together, these data suggest that consumption of high-fat meals promotes endothelial injury [39,40]. Endothelial dysfunction is a crucial step in the pathogenesis of atherosclerosis and could link diabetes, atherogenesis and hypercoagulability. This interpretation could explain in diabetic patients, the further increase of endothelial MPs when coronary artery disease is present [21,27].

It is now well established that MPs play a crucial role in inflammation. They are able to deliver arachidonic acid leading to an increased expression of endothelial cyclooxygenase type 2 [41]. Platelet MPs also stimulate endothelial cells in vitro to release cytokines and express adhesion molecules [42]. In addition, platelet MPs can directly interact with activated vascular endothelial cells by increasing leukocytes/monocytes arrest following transcellular delivery of the chemokine RANTES [43].

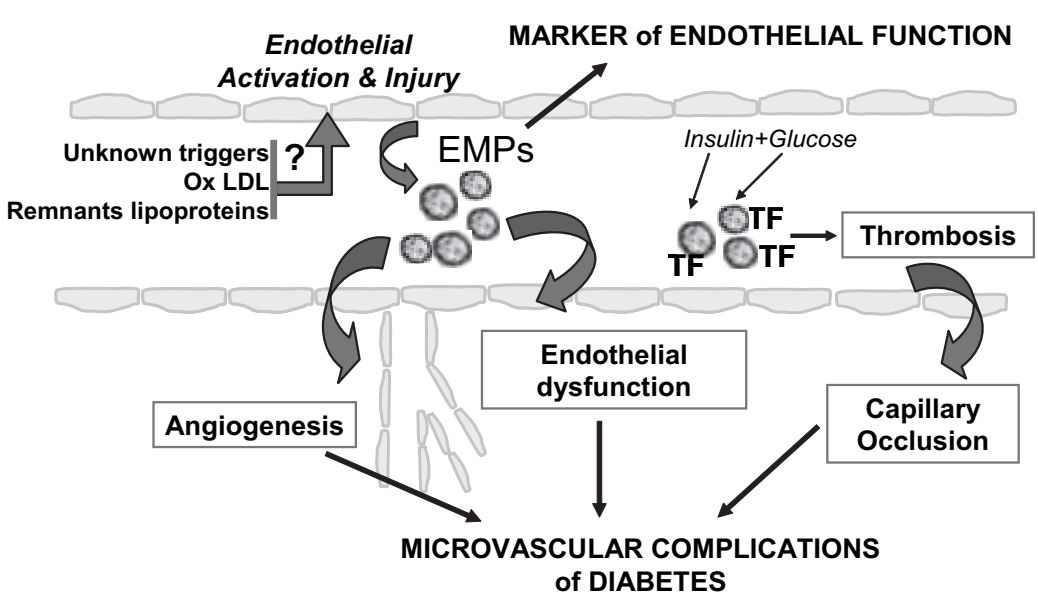
Finally, endothelial, platelet and tumor cell-derived MPs appear to be able to stimulate angiogenesis, an effect mediated by reactive oxygen species, metalloproteinases, growth factors such as VEGF or sphingomyelin [44-48]. In addition, Ogata and coll. showed in diabetic retinopathy that plasma levels of monocyte-derived MPs are significantly higher in patients with areas of capillary occlusion than in patients without areas of capillary occlusion, suggesting that MPs could be involved in the progression of diabetes complications such as retinopathy. Furthermore, MPs are more abundant in vitrous fluid from diabetic when compared to non-diabetic patients and induce endothelial cell proliferation, underlining the potential role of vitreous MPs in proliferative diabetic retinopathy [49] (Fig. 1).

6. Microparticles and prevention of diabetes

The recognition of a role of MPs may not only be important for our understanding of the pathogenesis of diabetes but may also have implications for the prevention and treatment of this disease. Some currently used therapies are known to affect MPs generation. For example, abciximab, a glycoprotein IIb/IIIa receptor antagonist, almost completely blocks platelet vesiculation *in vitro* [50,51], thus providing an alternate mechanism of MPs formation. Furthermore, MPs release from tumor necrosis factor-α-activated endothelial cells is suppressed by fluvastatin [52], whereas combination therapy with losartan and simvastatin [53], as well as antioxidative therapy such as vitamin C [36], is capable of decreasing the number of circulating monocyte-derived MPs. The major class of molecules used in diabetes, which are angiotensin II receptor blockers such as losartan or valsartan, have been shown to have beneficial effect on the angiopathy of hypertension and hyperglycemia and also on levels of microparticles in type 2 diabetic patients. For instance, angiotensin II receptor antagonists inhibit monocyte-derived MPs generation and decrease monocyte MPs levels in type 2 diabetic patients, suggesting that angiotensin II is intimely related to vascular changes that occur in type 2 diabetes mellitus [53-54]. Calcium antagonists, known to improve endothelial function in patients with hypercholesterolaemia by enhancing NO activity, and to increase endothelial NO bioavailability by antioxidant mechanisms, had also benecicial effets on MPs generation. For instance, type 2 diabetic patients treated with nifepidine showed a reduced level of platelet-, monocyte- and endothelial-cell derived MPs [55]. Benidipine administration also decreased concentrations of monocyte and endothelial MPs in hypertensive patients with type 2 diabetes [56]. Administration of probucol and triclopidine to hyperlipidemic patients with type 2 diabetes reduced monocyte and platelet- derived MPs [57]. All together, these results demonstrated the potentiel effectiveness of calcium antagonist therapy in type 2 diabetes. Furthermore, treatment of type 2 diabetic patients with statins reduced the exposure of glycoprotein IIb/IIIa on platelet-derived MPs by inhibiting platelet activation without affecting lipid levels [58]. These observations underlies pleiotropic effects of statins in the regulation of MPs formation in type 2 diabetes an dsuggest that combination of a statin and an angiotensin II receptor blocker might be valuable in reducing MPs effects in patients with type 2 diabetes.

In conclusion, recent studies on microparticles and diabetes provided new insight in the pathogenesis of diabetes. The increased levels of MPs in diabetes may be involved in crucial events leading to disease development and its complications as they promote inflammation, coagulation, endothelial dysfunction and angiogenesis.

Elevated levels of endothelial MPs were found in type 2 diabetes, but mechanisms leading to their release in diabetes are unknown. Accumulation of remnant lipoproteins and oxLDL promote endothelial injury and are possible triggers



EMPs = Endothelial MPs
TF = Tissue Factor
oxLDL = oxidized Low Density Lipoproteins

Fig. 1:
Microparticles and diabetes

tion in this setting. MPs could be involved in the progression of microvascular complications of diabetes. Circulating MPs could amplify endothelial dysfunction in diabetes by impairing NO release (Fig.1). MPs express tissue factor, and tissue factor activity rises with increased levels of insulin and glucose observed in type 2 diabetes. Consequently, MPs could contribute to the hypercoagulable state of diabetes and possibly to capillary occlusion as seen in the retina. Finally, MPs could promote also angiogenesis, such as observed in diabetic retinopathy.

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Vascular progenitor cells and diabetes: role in postischemic neovascularisation

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Abstract

Advances in the field of vascular biology lead to the identification of endothelial progenitor cells (EPC) and to the development of EPC-based cell therapy to induce new vessel formation in ischemic tissues and to accelerate re-endothelialisation of injured vessels in human and various animals models. However, recent studies have shown that age and other risk factors for cardiovascular diseases, such as diabetes, reduce the availability of EPC and impair their function to varying degrees, leading to reduction in postischemic vessel growth. This review focus on the cellular and molecular mechanisms governing EPC-related functions and analyzes the impact of diabetes in this setting.

Résumé

Cellules progénitrices endothéliales et diabète : rôle dans la néovascularisation post-ischémique

Les progrès dans le domaine de la biologie vasculaire ont conduit à l'identification des cellules progénitrices endothéliales (EPC), et au développement de traitements fondés sur les EPC pour induire la formation de néo-vaisseaux dans les tissus ischémiques et accélérer la re-endothélialisation des tissus endommagés chez l'homme et dans différents modèles animaux. Cependant, des études récentes ont montré que le vieillissement et les facteurs de risque cardiovasculaire, comme le diabète, réduisaient la disponibilité en EPC et altéraient la fonction de celles-ci à des degrés variables, conduisant de ce fait à une diminution de la croissance vasculaire post-ischémique. Cette revue générale est centrée sur les mécanismes cellulaires et moléculaires de régulation des fonctions liées aux EPC, et analyse l'impact du diabète dans ce contexte.

Keywords: Vasculogenesis; Stem cells; Ischemia; Diabetes

Mots-clés : Vasculogénèse ; Cellules souches ; Ischémie ; Diabète

Three principal processes, vasculogenesis, angiogenesis and arteriogenesis characterize tissue repair and remodeling occuring in acute and chronic ischemic vascular diseases, and represent the final targets of therapeutic angiogenesis aimed at providing an alternative treatment strategy for patients with lower limb ischemia and coronary artery diseases. Advances in the field of vascular biology lead to the identification of endothelial progenitor cells (EPC) [1] and to the development of EPC-based cell therapy to induce new vessel formation in ischemic tissues and to accelerate re-endothelialisation of injured vessels in human and various animals models.

1. Postischemic vasculogenesis

In the past decade, stem or progenitor cells have been identified in various tissues, including bone marrow, peri-

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pheral blood, umbilical cord blood, brain, heart, liver and adipose tissue [1-3]. Among these stem cells, EPC have been isolated and characterized as progenitor cells with proliferation capacity and potential to differentiate into endothelial lineage cells. EPC are thought to originate fom a common hemangioblast precursor in the bone marrow [2,4]. However, myeloid/monocyte lineage cells ($CD14^+$) can also differentiate into cells with EPC characteristics [5,6]. In addition, some tissues harbor stem cells that may differentiate into various lineages including endothelial cells [3,7]. Typically, these cells are defined on the basis of expression of cell surface markers such as CD34, Flk-1 and CD-133 [1,8]. Recently, $CD34^-/CD133^+$ EPC subpopulation has been identified as a precursor of classical $CD34^+/CD133^+$ EPC with potent vasoregeneratrice capacities [9]. Both in vitro and in vivo, EPC are able to differentiate into cells expressing endothelial lineage markers: VE-cadherin, endothelial nitric oxide synthase, von Willebrand factor, PECAM-1 (CD31), uptake of Dil acetylated LDL and binding of lectin. Their high proliferation rate also distinguishes EPC from mature endothelial cells shed from the vessel wall. EPC are then successfully ex vivo expanded with the use of human peripheral blood mononuclear cells.

EPC appear to be a heterogenous group of cells originating from multiple precursors and present in different stages of endothelial differentiation in peripheral blood. In support of this view, cultured total mononuclear cells from human peripheral blood have been shown to differentiate into both early and late EPC. Early EPC express monocytic lineage markers, display spindle shape, show peak growth at 2 to 3 weeks and die at 4 weeks. Late EPC with cobblestone shape appear late at 2 to 3 weeks, show exponential growth at 4 to 8 weeks, and live up to 12 weeks. Late EPC are different from early EPC in their vasculogenic properties and the expression of VE-cadherin, Flt-1, KDR, and CD45 [10]. It is therefore likely that different cells share similar EPC capacity or that appropriate marker(s) defining the effective subpopulation of cells are still missing.

Several lines of evidence suggest that local tissue injury alters the vascular endothelium to arrest EPC in regions where regeneration of endothelium is needed [11]. For example, in the setting of ischemia, the recruitment of CXCR4-positive EPC to regenerating tissues is mediated by hypoxic gradients via hypoxia-induced factor-1-induced expression of stroma-derived factor-1 (SDF-1) [12]. Bone marrow mononuclear cells or EPC of heterozygous CXCR4 (+/-) mice display reduced CXCR4 expression and disclose impaired in vivo capacity to enhance recovery of ischemic blood flow in nude mice [13]. Local hypoxia also releases a soluble factor, or multiple soluble factors, that act as a chemoattractant for circulating EPC. One likely candidate is VEGF, which has previously been shown to be locally elevated in response to hypoxia, and to promote SDF-1 induction in perivascular tissue [14]. Alternatively, chemokines and platelets may also trigger EPC capture at sites of vascular lesions [15-17]. The arrest of EPC in injuried microvessels is then mediated by cell-surface vascular adhesion molecules and selectins, such as beta2 integrins or L-selectin [18-20]. After their recruitment within the target tissue, EPC may exert their beneficial effects.

However, a major critical point is the identification of cellular mechanisms governing EPC vasoregeneratrice capacities. EPC have been shown to incoporate into blood vessel and physically contribute to vascular endothelium. However, the relative contribution of circulating EPC to adult organ and tumor vasculature is highly variable and may range from a minor [21-23] to a major contribution [24]. The experimental animal models and the method of EPC isolation may have contributed to these different numbers. Hence, whereas both types of EPC show comparable in vivo vasculogenic capacity, late EPC incorporate more readily into human umbilical vein endothelial cells monolayer, and forme capillary tube better than early EPC [10]. In addition, the gradient of hypoxia directs EPC to coalesce into independent vascular structures to restore tissue perfusion in the ischemic region. However, the extent of incorporation is directly proportional to the degree of tissue ischemia [25].

Alternatively, EPC may serve as building blocks for neovascularisation. The plasticity of EPC could be insufficient to ensure their differentiation into mature endothelial cells but may explain their ability to mimic the activities of endothelial cells and to participate in processes such as neovascularisation. This ability has been termed 'vasculogenic mimicry'in certain types of cancer cell and may also exist for EPC [26]. Finally, primary role of progenitor cells may be to deliver angiogenic growth factors to pathological tissues and contribute to neovascularisation and tissue/vessel remodeling by paracrine effects. EPC secrete the angiogenic growth factors: vascular endothelial growth factor (VEGF), hepatocyte growth factor, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor [6,27]. In addition, EPC also release proteases, such as cathepsin L, and promote a concomitant increase in matrix degradation that enables endothelial cell migration and vascular remodelling [28].

2. Diabetes and postischemic vasculogenesis

However, recent studies have shown that age and other risk factors for cardiovascular diseases reduce the availability of EPC and impair their function to varying degrees, leading to reduction in postischemic vessel growth. For example, patients with coronary artery disease showed reduced levels and functional impairment of EPC, which correlated with risk factors for coronary artery disease [29]. Patients with peripheral obstructive arterial diseases (PAD) may also have lower angiogenic potential because of decreased expression of EPC specific molecules in their marrow and blood [30].

Diabetes is a major risk factor for coronary and peripheral artery diseases. Diabetes has been shown to impair endogenous neovascularisation of ischemic tissues. This impairment in new blood vessel formation may result from reduced expression of VEGF and cytokine supplementation achieved by intramuscular adeno-VEGF gene transfer restores neovascularisation in a mouse model of diabetes [31,32,33]. The cellular response of monocytes to VEGF-A is attenuated in diabetic patients because of a downstream signal transduction defect suggesting that abrogation in monocytes migration may be involved in the problem of impaired collateral formation in diabetic patients [34]. Hyperglycemia is also associated with a marked accumulation of advanced glycation end products (AGE). Plasma AGE levels were strongly elevated in diabetic mice when compared with control mice. Treatment with aminoguanidine reduced AGE plasma levels and completely normalized ischemia-induced angiogenesis in diabetic mice. This effect is probably mediated by restoration of matrix degradation processes that are disturbed as a result of AGE accumulation [35].

Diabetes may also hamper EPC-related functions. Hence, EPC decrease is related to PAD severity and that EPC function is altered in diabetic subjects with PAD, strengthening the pathogenetic role of EPC dysregulation in diabetic vasculopathy [36].

Similarly, type I and II diabetes, are associated with reduced EPC numbers and angiogenicity [25,37,38]. Diabetes also decreased the ability of adherent bone marrow-derived mononuclear cells (BM-MNCs) to differentiate into endothelial progenitor cells. Treatment with NAC, apocynin, or p38MAPK inhibitor up-regulated the number of endothelial progenitor cell colonies derived from diabetic BM-MNCs. In the ischemic hindlimb model, injection of diabetic BM-MNCs isolated from NAC-treated or gp91 (phox) -deficient diabetic mice increased neovascularisation by approximately 1.5-fold greater than untreated diabetic BM-MNCs. Thus, inhibition of NADPH oxidase-derived reactive oxygen species overproduction improves the angiogenic and vasculogenic processes and restores postischemic neovascularisation in type 1 diabetic mice [39]. Glucose-mediated EPC dysfunction was protein kinase C dependent, associated with reduced intracellular BH (4) (tetrahydrobiopterin) concentrations, and reversible after exogenous BH (4) treatment. Subsequently, eNOS was uncoupled resulting in eNOS-mediated O (2) (-) production and impairment of EPC function in diabetic patients [40].

Alternatively additional factors may be involved in the diabetes-induced EPC dysfunction. Notably, thrombospondin-1 mRNA expression is significantly up-regulated in diabetic EPC, in relation with the decreased EPC adhesion activity in vitro and in vivo [41]. Diabetic mice showed impaired phosphorylation of BM eNOS, decreased circulating EPCs, and diminished SDF-1alpha expression in cutaneous wounds leading to impaired EPC homing in diabetes [42]. Finally, activation of the Akt/p53/p21 signaling pathway and accelerated onset of senescence are also detectable in EPC from diabetic patients. Diabetic EPC depleted of endogenous p53 do not undergo to senescence-growth arrest and acquire the ability to form tube-like structures in vitro, identifying the activation of the p53 signaling pathway as a crucial event that can contribute to the impaired neovascularisation in diabetes [43].

Therefore, diabetes reduce the availability of EPC and impair their function to varying degrees, leading to reduction in postischemic vessel growth. In addition, the reduction in EPC pro-angiogenic effect associated with diabetes may limit their therapeutic usefulness in these patients population. Furthermore, the relative scarcity of circulating EPC and their finite proliferative potential limits the ability to expand these cells in sufficient numbers for some therapeutic applications. Strategies to improve homing, survival and therapeutic potential of EPC need to be developed to improve therapeutic effect and counteract EPC dysfunction in diabetic patients.

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To what extent should we lower HbA_{1c} in diabetic subjects?

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Abstract

Current recommendations regarding glycemic control suggest that HbA_{1c} should be lower than 6.5%. This is supported by data regarding microvascular disease, namely retinopathy rather than nephropathy. The question is not completely solved regarding cardiovascular diseases, where a strategy of very low HbA_{1c} ("the lower the better") is expected to be effective. Some ongoing studies will help to answer these unsolved questions.

Résumé

Jusqu'où faut-il baisser l'hémoglobine glyquée des sujets diabétiques ?

Les recommandations concernant le contrôle de la glycémie suggèrent que l'hémoglobine glyquée doit être inférieure à 6,5%. Ces recommandations s'appuient sur les études sur la microangiopathie notamment la rétinopathie plutôt que sur la néphropathi.e. La question n'est pas entièrement résolue pour les maladies cardiovasculaires, où une stratégie visant un niveau très bas d'HbA_{1c} (« plus c'est bas, meilleur c'est ») pourrait s'avérer efficace. Des études en cours permettront de répondre à ces questions non résolues.

Keywords: Diabetes; Metabolic control; HbA_{1c}; glycemic goal; Complications; ADVANCE Study

Mots-clés : Diabète sucré ; Équilibre métabolique ; HbA_{1c} ; Objectif glycémique ; Complications, ADVANCE Study

Type 2 diabetic patients are characterized by a chronic Hyperglycemia that contributes to the development and progression of micro- and macrovascular complications. In a large population-based cohort of French type 2 diabetic patients, there was a proportion of patients with cardiovascular diseases, such as myocardial infarction and peripheral arterial disease, in 10% and 19% respectively [1]. The proportion of diabetic patients with retinopathy or kidney disease was 33% and 30% respectively, with a vast majority of patients yielding complications such as non proliferative retinal disease and microalbuminuria [1]. Many prospective studies suggest a link between glycemic control and chronic complications in diabetes [2,3]. Interestingly the data on type 1 diabetes are of great importance. Type 1 diabetic complications are more directly related to glycemic control than in type 2 diabetes, as hypertension, lipid abnormalities, obesity and insulin resistance could be involved in the development and /or progression of diabetic complications. The data from the WESDR study support the use of conclusion drawn from type 1 diabetic patients in type 2 diabetic subjects [4].

The question is currently asked to which extent we should lower HbA_{1c} in diabetic patients, particularly in type 2 diabetes. Two different strategies can be considered: - one

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could be summarized as "the lower the better"- the other one could be summarized as "a specific target, a specific threshold".

Is a "threshold for HbA_{1c}" recommendable? Many recommendations suggest to reach some specific threshold for HbA_{1c} to lower the incidence of chronic complications. Some very recent American publications recommended to consider a threshold for HbA_{1c} as low as feasible, at least targeting a threshold at 7% [5]. French recommendations released by the HAS consider several HbA_{1c} thresholds according to the time to diagnosis of type 2 diabetes and to the use of medications, ranging from 6 to 7% [6]. However, the use of a threshold is particularly relevant if the relationship between a disease and a variable is not linear. The data on the relationship between blood glucose and diabetes, focusing on retinopathy support such a non linear relationship [7,8]. However, the relationship between cardiovascular events and blood glucose seems roughly linear [9]. In this case the use of a specific threshold is debatable as it has less scientific relevance since no "real" threshold exists. Nonetheless it may be very useful for clinicians in a real-life approach as it allows to avoid some glycemic control leeway keeping in mind that a lower value than the targeted threshold is not deleterious if safely obtained.

1. Micro vascular complications

The epidemiological data linking background diabetic retinopathy according to glycemic threshold are available for several populations [8]. There is a general agreement showing that the split in deciles according to the distribution of fasting plasma glucose or of post oral glucose challenge plasma glucose or HbA_{1c} leads to rather homogenous results regarding the risk of microaneurysms. They show that HbA_{1c} threshold near 6.0% is associated with the development of diabetic retinopathy[8]. These data were further replicated in a Japanese population also showing a glycemic threshold at approximately 5.7% [7].

These data suggest that the strategy "a specific target, a specific threshold" can be applied here with a threshold for HbA_{1c} below 6.0% to specifically target retinal complications.

Regarding renal disease, the development of microalbuminuria in a cohort of type 1 diabetic patients showed a non-linear relationship between glycemia and the development of increased urinary albumin excretion [10]. Namely, the threshold for microalbuminuria increased as soon as 8% of HbA_{1c} was reached [10]. These data were in disagreement with the findings by Bojestig et al showing in a Swedish cohort that the intensive glycemic control in type 1 diabetic patients, with HbA_{1c} well below the 8% threshold, was effective to lower microalbuminuria [11].

Orchard et al examined the question of HbA_{1c} threshold using a different approach. These authors proposed to take glycemic exposure into account i.e. time x excess in HbA_{1c}. Interestingly, some results suggest a linear relationship between glycemic exposure and micro-vascular disease [12,13].

Considering more advanced renal involvement, the paper by Fiorretto et al showing that a long term (10 years) normal glycemia – obtained by pancreas graft - was associated with a correction of renal histological parameters, is very important. This paper indeed shows that some fibrous lesions in the kidney are reversible at the price of a long-term strict normal glucose value, obtained with pancreas graft [14].

Altogether these data suggest that a threshold of 6.0% might be adequate to prevent the development of diabetic micro-vascular events. Interventional studies showed that an improved HbA_{1c} leads to fewer micro-vascular events, in large-scale studies on type 1 diabetes, such as in the DCCT-EDIC [15], or in British [16], or Japanese [17] type 2 diabetic patients. However, the question is not solved for renal involvement, as conflicting results on microalbuminuria are available. Glycemic control at a threshold lower than 6.0% seems reasonable. Whether it is sufficient to reverse severe kidney lesions is difficult to assess. However, some studies suggest that a very low Hb1Ac should be targeted.

2. Macrovascular disease

Conversely to micro-vascular risk, many epidemiological data from non-diabetic populations suggest that there is a continuous relationship between glycemic control and cardiovascular risk. Coutinho et al have used a meta-regression analysis to assess the risk of macrovascular disease according to fasting or postprandial blood glucose. They found that the risk for coronary artery disease was present at very low glucose concentration, probably beginning at 72 mg/dl, even after exclusion of diabetic people [9].

In the EPIC Norfolk study, using a non diabetic large-scale cohort, HbA_{1c} at non diabetic levels, was associated with a regular increase in the risk for cardiovascular events or cardiovascular mortality [18].

Data are convergent in type 2 diabetic patients showing a beneficial effect of improving HbA_{1c} without clearly identified threshold, in observational studies: in a Danish cohort of type 2 diabetic patients, a change in 1% in HbA_{1c} was associated with a 20% increased risk for all-cause mortality [19]. Accordingly, HbA_{1c} was a strong risk factor for lower limb amputation (which is a composite of neuropathy, reflecting microvascular disease, and atherosclerosis) in a Finnish cohort [20]. Accordingly the use of the whole cohort of the UKPDS as an epidemiological study also supported the concept of a linear effect of HbA_{1c} on cardiovascular risk, beginning below HbA_{1c} 6.5% [21].

The data from interventional studies also support the improvement of HbA_{1c} without any clear threshold. In the UKPDS, comparing two treatment strategies (intensive vs conventional blood glucose control), the 0.9% difference

during the long-term period was not sufficient to be associated with a decreased risk in cardiovascular events [16]. There was no effect of "intensive glycemic control" compared to "standard care" regarding stroke and a borderline non-significant effect on myocardial infarction was noticed [16].

At variance, in the DCCT EDIC study, there was a significant reduction of approximately 40% in pre-specified macrovascular endpoints in those patients submitted to an intensified glycemic control (reaching a HbA_{1c} level at approximately 7% for 6.5 years) versus those people in the conventional group (remaining at approximately 9% for 6.5 years), even if both groups merged to a HbA_{1c} concentration at 8% during the epidemiological follow-up period (approximately 13 years) [22].

These data suggest that there is probably no specific threshold for macrovascular disease. Altogether these data suggest that for macrovascular endpoints "the lower the better" strategy could be used.

3. Ongoing clinical trials

What is expected from clinical trials targeting blood glucose control? Mainly a strong evidence that improving HbA_{1c} decreases cardiovascular events, which is not supported by any clinical trial. Indeed improving HbA_{1c} did not significantly impact the macrovascular endpoints during the DCCT trial [23], or the PROACTIVE study [24], and the multifactorial intervention in the STENO 2 trial was not specific enough to draw conclusions on glycemic control [25]. Several studies are currently examining this question and are going to deliver their results soon. The ongoing ADVANCE study will thus probably have a chance to evidence a beneficial effect of intensive glycemic balance ($HbA_{1c} < 6.5\%$) only if a sufficient contrast in glycemic control is obtained. In this study, 2 glycemic control strategies are compared with microvascular and macrovascular endpoints: in the intensive group HbA_{1c} target is below 6.5% and the expected difference with the conventional group is 1% [26]. The comparison of the results of the ADVANCE study with the ACCORD study will soon help to clarify our view of the relationship between HbA_{1c} and cardiovascular events in diabetes. In the ACCORD study, 2 glycemic control strategies are compared with macrovascular endpoints: in the intensive group HbA_{1c} target is below 6.0% and 7.0-7.9% in the conventional group [27].

Interestingly, only the so-far published studies showing a large difference in the glycemic control were able to show positive results on macrovascular events such as the DIGAMI 1 study [28]. However, those study with an insufficient glycemic contrast (such as DIGAMI 2 [29], or the CREATE-ECLA studies [30]) failed to show an association between glycemic intervention and cardiovascular endpoints.

4. Conclusion

It must be mentioned that the glucose-centered point of view is important for physicians involved in diabetes care, as it helps to recommend targets of blood glucose control. However, the PROACTIVE or the STENO 2 are good examples of intervention targeting blood glucose that proved to be beneficial on macrovascular endpoints when a conjunction of a beneficial effects was encountered on several risk factors such as HbA_{1c}, lipids, blood pressure. Any effort should thus be made to improve glycemic control in conjunction of improvement of other cardiovascular risk factors.

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Conflicts of interest:

S. Hadjadj: Dr. Samy Hadjadj is participating as a member of scientific committees and speaker at the Servier laboratory satellite symposium ALFEDIAM Advance. He took part in the Advance study supported and promoted by Servier laboratory and is member of a national or international scientific council or committee of MSD laboratories.

P.-J. Saulnier: none.

F. Torremocha: none.

L. Labbé: none.

R. Maréchaud: Professor R. Maréchaud is participating as a member of scientific committees and a speaker for the GSK, Lilly, Novo-Nordisk, Sanofi-Aventis, and Takeda laboratories.

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