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REVIEW ARTICLE

Extracellular microRNAs and endothelial hyperglycaemic memory: a therapeutic opportunity?

F. Prattichizzo[1,2], A. Giuliani[2], V. De Nigris[1], G. Pujadas[1], A. Ceka[2], L. La Sala[3], S. Genovese[3], R. Testa[4], A. D. Procopio[2,5], F. Olivieri[2,5] & A. Ceriello[1,3]

[1] *Insititut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain*
[2] *Department of Clinical and Molecular Sciences, DISCLIMO, Università Politecnica delle Marche, Ancona, Italy*
[3] *Department of Cardiovascular and Metabolic Diseases, IRCCS Gruppo Multimedica, Milan, Italy*
[4] *Experimental Models in Clinical Pathology, INRCA-IRCCS National Institute, Ancona, Italy*
[5] *Center of Clinical Pathology and Innovative Therapy, INRCA-IRCCS National Institute, Ancona, Italy*

Type 2 diabetes mellitus (T2DM) is a major cause of cardiovascular (CV) disease. Several large clinical trials have shown that the risk for patients with diabetes of developing CV complications is only partially reduced by early, intensive glycaemic control and lifestyle interventions, and that such complications result from changes in complex, not fully explored networks that contribute to the maintenance of endothelial function. The accumulation of senescent cells and the low-grade, systemic, inflammatory status that accompanies aging (inflammaging) are involved in the development of endothelial dysfunction. Such phenomena are modulated by epigenetic mechanisms, including microRNAs (miRNAs). MiRNAs can modulate virtually all gene transcripts. They can be secreted by living cells and taken up in active form by recipient cells, providing a new communication tool between tissues and organs. MiRNA deregulation has been associated with the development and progression of a number of age-related diseases, including the enduring gene expression changes seen in patients with diabetes. We review recent evidence on miRNA changes in T2DM, focusing on the ability of diabetes-associated miRNAs to modulate endothelial function, inflammaging and cellular senescence. We also discuss the hypothesis that miRNA-containing extracellular vesicles (i.e. exosomes and microvesicles) could be harnessed to restore a 'physiological' signature capable of preventing or delaying the harmful systemic effects of T2DM.

Keywords: antidiabetic drug, cardiovascular disease, diabetes complications, extracellular vesicles, exosomes, glycaemic control, metabolic memory, metformin, microRNAs, type 2 diabetes

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic, multifactorial, metabolic disease caused by a complex interplay among environmental and genetic factors [1]. The number of patients with diabetes is increasing relentlessly in western countries, and is expected to reach 552 million by 2030 [2]. T2DM is a source of disability and morbidity, especially as a result of its vascular complications, which eventually lead to retinopathy, nephropathy, neuropathy, ischaemic heart disease and peripheral vasculopathy [2]. Endothelial dysfunction (ED), chronic, low-grade systemic inflammation and (probably) cellular senescence contribute to the development of severe vascular complications, and have been proposed as key therapeutic targets for T2DM [3–5]. Large clinical trials [6,7] have found that early hyperglycaemia can promote disease progression and late complications, perpetuating ED and vascular damage despite the achievement of improved glycaemic control, a phenomenon that has been called 'metabolic memory' [1]. The term indicates the vascular damage that persists after glucose normalization, whereas the general, long-term, harmful effects of diabetes (i.e. complications other than vascular) have been referred to as the 'legacy effect' [8]. Different cell types are affected by metabolic memory, including endothelial cells (ECs), immune cells, smooth muscle cells and fibroblasts [1]. The lasting molecular changes involving the endothelium could be termed 'endothelial hyperglycaemic memory'. According to recent evidence, oscillating glucose levels may actually be more harmful, and induce more enduring effects on endothelial health, than hyperglycaemia itself [9].

A variety of mechanisms are involved in metabolic memory, including increased production of advanced glycation end products (AGEs), AGE receptor overexpression, increased anion superoxide formation, mitochondrial protein glycation, mitochondrial DNA damage, protein kinase C activation, and polyol pathway and hexosamine flux alterations [1]. However, targeting these changes with new therapies has had limited success in slowing down disease progression and the development of complications [10], indicating that not all the imbalances

Correspondence to: Francesco Prattichizzo, PhD, Insititut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), C/Rosselló, 149-153, 08036, Barcelona, Spain.
E-mail: f.prattichizzo@univpm.it

experienced by people with diabetes can be addressed by therapies addressing single targets. Moreover, even though combined treatment with glucose-lowering and lipid-lowering drugs and antihypertensive medications has greatly improved diabetes management, it cannot prevent the eventual development of vascular complications, especially in patients with long-standing disease [10,11].

Genome-wide association studies, linkage studies, candidate gene association studies and meta-analyses have identified a number of genes involved in susceptibility to both T2DM and its complications [12]. However, genetic testing cannot predict the clinical risk of vascular complications in T2DM with accuracy, suggesting that the cardiovascular (CV) complications of diabetes are only partially attributable to genetic predisposition [12,13].

Recently, epigenetic mechanisms have been hypothesized to be a crucial interface between genetic and environmental factors to explain metabolic memory [11,14–16]. Hyperglycaemia can induce a variety of epigenetic changes that persist for days after normalization of glucose levels [11,14–20], mainly through the involvement of inflammatory genes [17,18]. DNA methylation and post-translational histone modifications (PTHMs) are the most extensively investigated epigenetic mechanisms involved in metabolic memory. Hyperglycaemia can affect the activity of PTHMs and DNA methyltransferases, and changes may become irreversible over time, explaining the long-term harmful effects of metabolic memory [16–20].

Recently, further epigenetic mechanisms have been identified. Non-coding RNAs, including microRNAs (miRNAs), have emerged as key factors in gene expression regulation and are likely to participate in metabolic memory modulation. More than 2000 human miRNAs have been identified to date, making them one of the most abundant classes of epigenetic regulatory molecules [21]. MiRNAs were previously thought to act mainly as negative regulators of gene expression, by binding to the three-untranslated regions of their target protein-coding mRNAs in a sequence-dependent manner [21]; however, a growing body of evidence supports the notion that they are not only post-transcriptional regulators of gene expression, but can directly repress or stimulate target gene transcription by directly binding to promoter regions, a phenomenon that has been called RNA activation [22]. Moreover, miRNAs can target enzymes involved in DNA methylation and miRNA genes which, in turn, are closely regulated at the level of promoter methylation, transcription and processing [23]. Although miRNA modulation in the bloodstream and tissues has been extensively studied in patients with diabetes [24], their involvement in the CV complications of diabetes has only recently been established conclusively [25,26].

In the present paper, we review the latest data on miRNA changes in diabetes, address their potential relevance to the development of CV complications, and highlight the possible relationships among some affected pathways, altered molecular data and the major pathogenic factors (i.e. low-grade inflammation and ED) that are involved in the vascular complications of T2DM. The possibility of erasing metabolic memory by restoring physiological miRNA levels using innovative therapies harnessing the miRNAs contained in microvesicles (MVs) or exosomes is also discussed.

Chronic, Low-grade Inflammatory Phenotype and Type 2 Diabetes

Several age-related conditions, including T2DM and CV diseases, share a chronic, low-grade inflammatory state [3–5,27]. According to a recent, brilliant hypothesis, the build up of cells with a senescence-associated secretory phenotype (SASP) could promote the development of diabetes and its vascular complications [5]. Senescent cells are believed to accumulate during physiological aging, driving the development of age-related diseases through chronic secretion of a variety of (SASP-related) factors that contribute to inflammaging (i.e. the chronic, low-grade, systemic inflammation that accompanies aging) [28]. The SASP is capable of transmitting senescence (via the 'bystander effect') and of exerting harmful effects in a paracrine as well as systemic way. The inflammatory phenotype is characterized by persistent activation of the nuclear factor kappa B (NF-kB) pathway, which induces transcription of a number of genes involved in inflammatory response modulation, including adhesion molecules such as VCAM-1 and cytokines such as interleukin (IL)-6 and tumour necrosis factor (TNF)α [29,30]. These genes are chronically activated in cells from patients with diabetes [14–20]. The role of senescence in the development of the vascular complications of T2DM, and whether their establishment precedes or follows low-grade inflammation and vascular complications are being extensively investigated [5,31]. *In vivo* senescence probably encompasses a spectrum of states ranging from a low to a high secretory phenotype, depending on its inducers (i.e. replication or hyperglycaemia) and cell types, among other factors. Epigenetic modifications leading to chronic inflammation have been described in ECs and immune cells of patients with diabetes even in the absence of replicative senescence biomarkers [14–20]; however, most of the inflammatory mediators involved in the vascular complications of diabetes, which are induced *in vitro* by hyperglycaemia in ECs and immune cells, are the molecules released by cells bearing the SASP (i.e. NF-kB, IL-1, IL-6, TNFα, VCAM-1) [14–20], suggesting a causal role for them in the maintenance of the chronic, systemic inflammation that accompanies diabetes. A comparative analysis of gene (and pseudogene) expression in replicative and hyperglycaemia-induced senescence could shed some light. Hyperglycaemia clearly promotes the acquisition of a proinflammatory cellular phenotype that may be defined as diabetes- (DASP) or hyperglycaemia-associated secretory phenotype (HASP). Moreover, mounting evidence suggests an important role for the inflammasome platform in both T2DM and atherosclerotic disease [32,33]. The NOD-like receptor (NLR)-caspase 1-IL-1β cascade can be activated by endogenous metabolism or injury-derived byproducts called damage-associated pattern molecules, resulting in chronic secretion of inflammatory cytokines [34,35]. Strikingly, the inflammasome controls the transmission of the SASP senescence signal [30]. Besides NLR activation, toll-like receptor (TLR) activation has also been proposed to be involved in

T2DM and its complications, supporting a role for innate immunity, and probably for microbiota, in the diabetic inflammatory milieu [36,37]. Remarkably, all lines of evidence point to the chronic, low-grade inflammation typical of T2DM as a key therapeutic target [3,38,39].

Our group has recently published a pioneering study suggesting that some miRNAs may be part of the secretome of cells bearing the SASP [40–42]. MiRNAs are expressed by all living cells and can actively be released or shed in the bloodstream and taken up in active form by receiving cells, acting as highly efficient systemic communication tools. Easy detection in serum and plasma makes miRNAs emerging, minimally invasive biomarkers of complex processes like age-related diseases, including T2DM and CV diseases [43,44]. MiRNAs can be secreted or released by cells within small membranous vesicles (e.g. exosomes, MVs and apoptotic bodies), or packaged in HDLs or RNA-binding proteins (e.g. Argonaute) [44]. MiRNAs have been shown to be functional mediators capable of coordinating multiple pathways and of modulating virtually all cellular responses to environmental stimuli, according to each individual's genetic make-up. Factors associated with diabetic complications, such as hyperglycaemia, ED, inflammation and senescence, can induce deregulation of epigenetic mechanisms, thus affecting circulating miRNA profiles. As a consequence, the expression of specific genes in receiving cells, especially ECs, fibroblasts, vascular smooth muscle and immune cells, may exhibit extensive changes even in the absence of other adverse stimuli (i.e. return to normoglycaemia), disseminating and possibly amplifying a pathological signature.

MiRNAs Involved in the Pathogenesis of Diabetic Complications and Metabolic Memory

After the initial metabolic insult, the pathways involved in diabetic vascular complications are complex, interlinked and self-perpetuating [10]. It is unlikely that a single druggable pathway can prevent their onset. Targeting a number of pathways to slow down the development of diabetic complications seems to hold greater promise, but has not proved effective so far, probably because the intricate connections among the mechanisms giving rise to such complications create redundancy [10]. Since a single miRNA can target several genes, and multiple miRNAs share common targets, miRNAs are particularly suited to target processes and pathways at the 'network' level [45], and could prove effective in eradicating metabolic memory as well as multifactorial age-related diseases and metabolism-related diseases [46,47]. Moreover, recent findings indicate that circulating miRNAs, either carried by exosomes or bound to HDL/proteins, can provide efficient communication between different tissues and organs, suggesting that they can exert a remote action to regulate gene expression in target cells [44].

A miRNA array approach involving human aortic ECs has found that the expression of some miRNAs (miR-125b, miR-146a-5p and miR-29a-3p) is associated with metabolic memory. Interestingly, these miRNAs are involved in the modulation of proinflammatory pathways and EC dysfunction [48]. The demonstration that direct inhibition of miR-125b expression, or miR-146a-5p upregulation, improves endothelial function, blunting NF-kB signals, suggests that glucose-induced changes in miR-125b and miR-146a-5p are related to long-standing activation of the NF-kB pathway, and help perpetuate metabolic memory. These data strongly suggest that miRNA modifications could have a significant role in metabolic memory. They also provide a miRNA-based explanation for the constitutive activation of the NF-kB pathway, which is considered to be one of the main causes of ED in metabolic memory [14–20]. Importantly, miR-146a is the most extensively investigated inflammation-related miRNA (inflamma-miRNA) and senescence-associated miRNA [49,50]. Under chronic stimulation, it is overexpressed in several cell types, including ECs and white blood cells, restraining inflammation and switching off acute inflammation after removal of the harmful stimulus [49,50]. Altered (increased or decreased) miR-146a expression has been detected in several diabetic tissue and cell types exposed to hyperglycaemia [51–53]. Moreover, it plays an important role in mitochondrial homeostasis [54,55], possibly connecting aging- or hyperglycaemia-induced low-grade inflammation to mitochondrial alterations, which are a hallmark of the complications of diabetes and of cellular senescence [56,57]. Because a single miRNA can influence multiple features (i.e. low-grade inflammation and ED) that are modulated by different pathways in different tissues, some circulating miRNA signatures during aging and in patients with the major age-related diseases suggest that they participate in a complex cross-talk among tissues and organs. Interestingly, miR-146a is found in exosomes, and its content increases after bacterial stimulation, suggesting its systemic spread in some conditions [58,59].

A recent meta-analysis has found 40 circulating miRNAs, including miR-21, miR-29a, miR-34a, miR-103, miR-107, miR-126, miR-132, miR-142-3p, miR-144 and miR-375, that are significantly deregulated in T2DM [43].

MiR-126 is the most extensively studied miRNA in T2DM. Its best characterized biological function is to maintain vascular integrity, which promotes the mobilization of haematopoietic stem/progenitor cells and vascular cell survival [60]. A number of reports have documented that miR-126 is downregulated in plasma/serum, ECs and endothelial progenitor cells of patients with diabetes [42,61–64]. Our group has reported that circulating miR-126 increases both during aging and EC senescence, and that diabetes/hyperglycaemia abolish this trend [42]. The recent demonstration that miR-126 targets insulin receptor substrate (IRS)-1 expression via PI3K/AKT signalling pathways suggests that it is involved in IR modulation [65]. The key tissues regulating glucose homeostasis in response to insulin are liver, skeletal muscle and adipose tissue. IRS-1 is a key insulin signalling protein, whose adipose tissue expression is reduced in humans and animals with T2DM, impairing downstream insulin signalling through the PI3K and AKT pathways, and resulting in reduced insulin-stimulated glucose uptake [66,67]. Moreover, IRS-1 is a target of miR-126 in adipose tissue and hepatocytes [68]. miR-126 downregulation in the diabetic milieu could therefore be a compensatory mechanism counteracting the loss of insulin sensitivity [69].

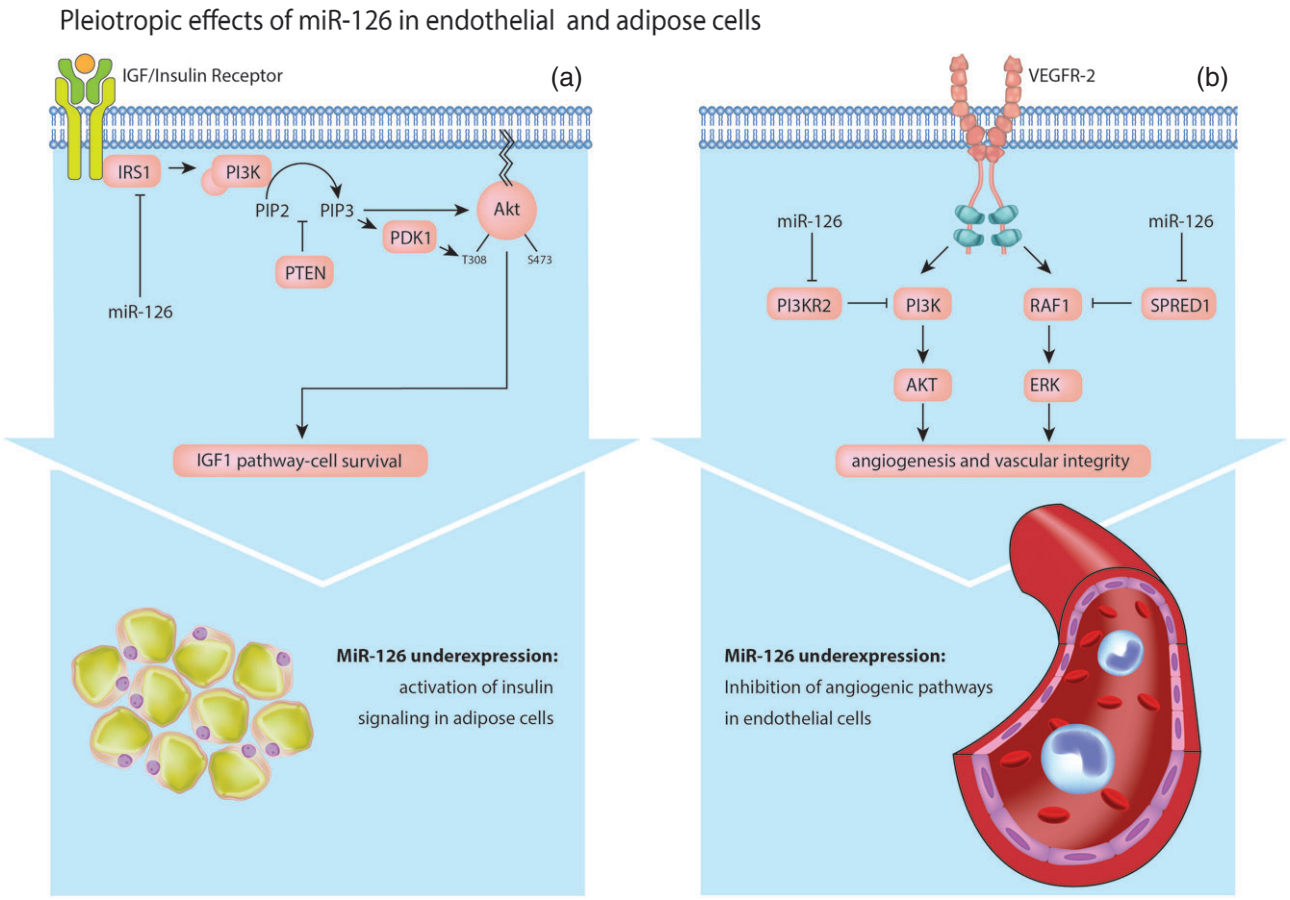

Figure 1. Pleiotropic effects of hyperglycaemia-induced miR-126 underexpression and insulin resistance. Hyperglycaemia-induced underexpression of miR-126 may have favourable effects on adipose cells and hepatocytes in patients with insulin resistance, because miR-126 downregulates insulin receptor substrate (IRS)-1, the key gene signalling insulin activation (a). Reduced miR-126 levels in such a setting could thus increase cell survival chances; however, hyperglycaemia-induced miR-126 underexpression can exert harmful effects on EC, inducing upregulation of SPRED1 and PIK3R2, two of the most effective angiogenic pathway inhibitors (b). In turn, endothelial dysfunction promotes development of diabetic complications.

The reduced intracellular miR-126 levels seen in cells cultured in hyperglycaemic conditions as well as in plasma from patients with T2DM could have a beneficial role in adipose cells and hepatocytes, reducing IR. However, because miR-126 has pleiotropic effects, being also involved in the maintenance of endothelial function, its downregulation in ECs exposed to hyperglycaemic conditions promotes ED (Figure 1). In this scenario, the extracellular exchange of miR-126 could have clinical relevance in T2DM progression. Interestingly, miR-126 has also been detected in circulating exosomes/MVs of endothelial and adipose origin. MiR-126 downregulation has been documented in exosomes from patients with diabetes [42,61–64]; however, circulating miR-126 does not seem to have predictive value for the development of CV complications in subjects with diabetes [51,70], possibly as a result of the large number of factors and tissues contributing to its circulating levels (e.g. age, gender, medications and intrinsic response differences between cell types) [42,51]. Exosomes/MVs of endothelial origin might provide a more accurate source of information about EC health. A large prospective study of patients with stable coronary artery disease has shown that only MVs containing miR-126 predicted a CV event over the following 6 years, whereas circulating levels were uninformative [71]. Similarly, ECs exposed to hyperglycaemia release less miR-126 into the culture medium [42]; this is especially evident in miR-126 found in vesicles, both exosomes and MVs (unpublished data from our laboratory). Convincing data show the possibility of microparticle exchange among ECs, where miR-126 interchange can regulate SPRED-1 expression, and consequently the proliferation status of receiving cells, a mechanism that is blunted in the diabetic environment [62].

Recently, new molecular changes have been described in association with altered miR-126 levels. Unacylated ghrelin protects diabetic mice from peripheral artery disease by restoring miR-126 levels and consequently VCAM-1, SIRT1 and SOD-2 regulation, suggesting that miR-126 could have anti-inflammatory and anti-senescence activity [72]. A huge amount of data stresses the central role of miR-126 in IR and endothelial homeostasis. The development of cell type-specific delivery strategies could turn miR-126 mimics into a therapeutic opportunity.

MiR-21 is extensively studied in cancer, but recently a role for it in aging-induced inflammation [73] and endothelial senescence has also been disclosed [74]. Several reports have

described its downregulation in serum and endothelial progenitor cells from patients with diabetes [61,63,70] and still lower serum levels in patients with diabetic complications [70]. In contrast, a tissue-specific increase in miR-21 has been reported in different hyperglycaemic environments [75–77], including patients with diabetes with proliferative diabetic retinopathy [75], while tissue upregulation has been seen to promote renal fibrosis in diabetic nephropathy [76]. Interestingly, the levels of circulating miR-21 can predict the development of end-stage renal disease [78]. Moreover, it plays a crucial role in cardiac fibrosis and related heart failure in a mouse pressure-overload-induced model [79], an effect that seems to be mediated by miR-21* contained in fibroblast-derived exosomes [80]. Pharmacological inhibition of miR-21* in a mouse model of angiotensin II-induced cardiac hypertrophy has attenuated the disorder [80]; similar findings are emerging for diabetic cardiomyopathy [81]. A list of circulating miRNAs differentially expressed in plasma, serum and blood-derived microparticles from patients with T2DM compared with healthy subjects is reported in Table 1.

MiR-21, miR-126 and miR-146a are three extensively studied miRNAs in relation to T2DM and its vascular complications, as they display altered circulating as well as tissue levels [11,43,51,103]; however, several other miRNAs, including miR-1, miR-16, miR-125b, miR-133, miR-155, miR-206, miR-221, miR-223 and miR-503, have been associated with the vascular complications of diabetes [11,14,51,104] through inflammatory pathway alterations and impairment of endothelial function [11,14,51,104].

According to a recent interesting paper, intensive glycaemic control in streptozotocin-treated mice is unable to reverse the deregulation of a large miRNA panel in the diabetic heart; in particular, 268 of 316 miRNAs remained dysregulated after intensive glycaemic control with insulin for 3 weeks [105], suggesting a strong role for miRNAs in diabetic cardiomyopathy and metabolic memory; informatics analysis then disclosed that the majority of dysregulated miRNAs were involved in inflammation, fibrosis, apoptosis and hypertrophy. An *ex vivo* study of left ventricle biopsies from patients with heart failure has found several deregulated miRNAs (miR-34b/c, miR-199b, miR-210, miR-223 and miR-650) in individuals with diabetes compared with individuals without [106].

Epigenetic therapy has finally moved from the workbench to the clinic [107]. A variety of pharmacological tools have been developed to target miRNA pathways [46] or exploit miRNAs for selective gene therapy [108]. Promising *in vivo* results have been achieved in patients with CV disease, and progress is continuous [46,47,108]. Several experimental strategies have been tested to deliver miRNA mimics or antagonists. Synthetic miRNA or pre-miRNA duplexes, chemically modified to enhance stability and cellular uptake, have been loaded onto different delivery systems, including lipid nanoparticles with surface receptor ligands to improve tissue specificity. Adeno-associated viruses and other viral-based vectors are further well-studied delivery methods [46]. Antisense oligonucleotides complementary to the mature miRNA sequence, or 'antagomiRNAs', were the first miRNA inhibitors to be used in mammals [109]. AntagomiRNAs were subjected to a

Table 1. Circulating microRNAs differentially expressed in patients with type 2 diabetes and control subjects, and sample type.

miRNA	Expression in patients with T2DM versus control subjects	Sample type	Proteins targeted in recipient cells by miRNA transfer
let-7a	Down	Plasma [82]	
let-7f	Down	Plasma [82]	
let-7i	Down	Serum [83]	
miR-100	Down	Whole blood [84]	
miR-124a	Up	Serum [85]	
miR-125b	Down	Plasma [86]	
miR-126	Down	Plasma [86]	SPRED1 [62]
	Down	Plasma [87]	IRS-1 [90]
	Down	Plasma[61]	FGF2 [90]
	Down	Microparticles [88]	
	Down	Circulating microparticle, plasma [62]	
	Down	Serum [89]	
	Down	Plasma [70]	
miR-1303	Up	Serum [91]	
miR-130b	Down	Plasma [86]	
miR-140-5p	Up	Plasma [86]	
miR-142-3p	Up	Plasma [86]	
miR-144	Up	Peripheral blood [92]	
miR-146a	Up	Serum [85]	IRAK1 [59]
	Up	Plasma [93]	TRAF6 [59]
	Down	Serum [83]	NFkB pathway [59]
	Down	Peripheral blood [92]	
	Down	Serum [94]	
miR-150	Up	Peripheral blood [92]	
miR-15a	Down	Plasma [61]	
miR-182	Down	Peripheral blood [92]	
mir-186	Down	Serum [83]	
mir-191	Down	Serum [83]	
	Down	Plasma [61]	
miR-192	Down	Plasma [86]	
	Down	Serum [83]	
	Up	Peripheral blood [92]	
miR-195	Down	Plasma [86]	
miR-197	Down	Plasma [61]	
miR-199a	Up	Plasma [94]	
miR-20b	Down	Plasma [61]	
miR-21	Down	Plasma [61]	
	Down	Plasma [70]	
miR-222	Up	Plasma [86]	ICAM-1 [95]
miR-223	Down	Plasma [61]	
miR-23a	Down	Serum [83]	
miR-23b	Down	Peripheral blood [96]	
miR-24	Down	Plasma [61]	
miR-26a	Down	Microparticles [62]	
miR-27a	Up	Whole blood [97]	
miR-28-3p	Up	Plasma [61]	
miR-29a	Up	Serum [85]	
	Up	Peripheral blood [92]	
miR-29b	Down	Plasma [61]	
miR-30d	Up	Serum [85]	
miR-320a	Down	Plasma [61]	IGF1 [98]
	Up	Peripheral blood [92]	Hsp20 [98]
	Up	Serum exosomes [97]	Est2 [98]

Table 1. Continued

miRNA	Expression in patients with T2DM versus control subjects	Sample type	Proteins targeted in recipient cells by miRNA transfer
miR-326	Up	Plasma [82]	
miR-34a	Up	Serum [85]	
miR-375	Up	Serum [85]	
	Up	Plasma [99]	
miR-423-5p	Down	Plasma [86]	
mir-486	Down	Serum [83]	
	Down	Plasma [61]	
miR-503	Down	Serum [100]	EFNB2 [102]
	Up	Plasma [101]	VEGFA [102]
miR-532-5p	Down	Plasma [86]	
miR-571	Up	Serum [91]	
miR-661	Up	Serum [91]	
miR-770-5p	Up	Serum [91]	
miR-892-5p	Up	Serum [91]	
miR-9	Up	Serum [85]	
mir-96	Down	Serum [83]	

IRS, insulin receptor substrate; miRNA, microRNA; NF-kB, nuclear factor kappa B; T2DM, type 2 diabetes.
Target proteins are reported only for those microRNAs whose transfer has been shown to regulate protein expression levels in recipient cells.

number of chemical adjustments. Cholesterol conjugation via a 20′-O-methyl linkage in the 30 end, phosphorothioate linkage, 20′-O-methyl-modified ribose sugar, 20-′,40′-constrained 20′-O-ethyl-modified nucleotides, 20′-O-methoxyethyl and 20′-fluoro and 20′-fluoro/methoxyethyl are all modifications introduced to improve their pharmacokinetic and pharmacodynamic properties [46]. Finally, locked nucleic acid (LNA)-antagomiRNA technology has successfully been tested in an *in vivo* trial [107]. The ribose moiety of an LNA nucleotide has been modified with an extra bridge connecting the 2′ oxygen and 4′ carbon, conferring higher stability, binding affinity and increased selectivity to complementary RNA [46].

Both miRNA antagonists and miRNA mimics, however, still have some technical, pharmacological and pharmacokinetic problems [46]. MiRNA shuttling by exosomes or MVs is expected to overcome technical difficulties, providing a valuable, practical strategy for efficient delivery of corrective or protective miRNA signatures to target cells. Extracellular vesicles (EVs) are physiological cell-derived nanocarriers that are immunologically inert if purified from a compatible cell source [110]. Moreover, it has been shown that polymeric nanoparticles can be engineered to target certain tissues selectively [111]. In particular, any nanoparticles designed to target the vascular endothelium could provide an attractive drug delivery tool. In this context, EV integrin expression patterns appear as the main determinants of vesicles tropism [112].

MiRNAs and Off-target Effects of Diabetes Medications

Among the medications currently used to treat patients with T2DM, some molecules have shown better results in terms of protection against CV complications. For example, a number of clinical studies have shown that metformin, a hypoglycaemic agent, reduces the risk of myocardial infarction and all-cause mortality compared with other medications [113,114]. The drug's off-target molecular effects are not yet clear, but some interesting data suggest that it can mitigate endothelial senescence both *in vitro* [115] and *in vivo* [116], and that it exerts similar effects on the SASP through NF-kB inhibition in oncogene-induced senescence [117]. Moreover, metformin has proven molecular *in vitro* efficacy against metabolic memory in ECs through SIRT-1 activation [118] and an *in vivo* anti-inflammatory effect in patients with diabetes and atherosclerosis [33,119,120]. These data suggest the existence of shared molecular and epigenetic alterations in diabetes and aging that are probably related to low-grade inflammation; such changes could provide other possible exploitable targets for T2DM treatment (i.e. the sirtuin family) [121,122]. In a recent study, circulating levels of miR-140-5p and miR-222, two of the most extensively studied inflamma-miRNAs that are altered in patients with diabetes, were reduced by 3-month metformin treatment [86]. Interestingly, miR-222 has recently been shown in endothelial microparticles; its transfer can regulate ICAM-1, which is impaired in a hyperglycaemic environment [95]. MiR-222 also has an important role in ED and atherosclerosis progression [123]. Circulating miRNA profiling after human administration could offer key information on the off-target effects of metformin. If its anti-inflammatory/secretory activity is confirmed [33], it can be harnessed to design new drugs or miRNA-based strategies.

At present, antihypertensive medications are among the most effective treatments for ED prescribed to subjects with diabetes [124]. Blood pressure reduction confers the strongest protection against CV events in such patients [124]. Angiotensin-receptor blockers (ARBs) seem to be able to reverse metabolic memory in diabetes [125,126].

Beyond the extensive molecular imbalances induced by high blood pressure on endothelial function and the inflammatory profile [127], a possible role for senescence and the associated epigenetic changes should also be considered. Hyperglycaemia and hypertension are strong individual inducers of senescence [127–130]. It is conceivable that high blood pressure and hyperglycaemia, combined with the characteristic, low-grade chronic inflammation of diabetes, can accelerate the onset of senescence, which would otherwise develop later in life. Remarkably, there seems to be a partial overlap between miRNAs that are deregulated in patients with diabetes and in hypertension because the levels of miR-21 [131], miR-126 [132], miR-146a [133], miR-155 [134], and a long-coding RNA, which functions as a host transcript for miR-221 and miR-222 [135], are affected in either condition, both in the circulation and in tissue. In particular, a disturbed flow can negatively regulate miR-126-5p and abrogate EC proliferation at predilection sites in response to hyperlipidaemic stress through upregulation of Dlk1 expression [136]. Administration of miR-126-5p rescued EC proliferation at predilection sites and limited atherosclerosis; moreover, miR-126 downregulation and the subsequent SPRED-1 increase contribute to right ventricle failure in pulmonary arterial hypertension [137].

Table 2. MicroRNAs expression changes in the bloodstream after treatment with currently used diabetes medications.

Treatment	Experimental procedure	Modulated miRNAs	Sample type	References
Metformin	Three-month metformin treatment in patients with T2DM	↓ miR-140-5p ↓ miR-222 ↓ miR-192	Plasma	[86]
Angiotensin-receptor blocker or angiotensin-converting enzyme inhibitor + statin	Twelve-month combined treatment with atorvastatin and telmisartan or atorvastatin and enalapril in patients with coronary artery disease	↓ miR-146a/b ↓ miR-31 ↓ miR-181a ↓ miR-16 ↓ miR-145	PBMCs Plasma	[133] [139]
Metformin + anti-diabetic agents (dipeptidyl peptidase 4 inhibitors and glynides)	Glucose-lowering treatment followed by clinical re-evaluation at 12 months	↑ let-7a ↑ let-7f	Plasma exosomes	[82]

miRNA, microRNA; PBMC, peripheral blood mononuclear cells; T2DM, type 2 diabetes.

Furthermore, combined treatment with an ARB and a statin has been shown to counteract the effects of both acute hyperglycaemia and acute hyperlipidaemia [138], and to reduce circulating miR-146a/b and TLR4 signalling in patients with coronary artery disease [133]. ARBs appear to be more effective than angiotensin-converting enzyme inhibitors in modulating a panel of TLR4-responsive miRNAs [139]. Statins have a known pleiotropic anti-inflammatory effect [140]. Recently, a role for them has been proposed in telomerase and senescence regulation [140]. Because miR-146a increases during senescence [50,141], attenuating IL-6 release in both fibroblasts and ECs acquiring the SASP, it is conceivable that the anti-inflammatory effect of statins is partly mediated by miR-146a. Moreover, oscillatory shear stress is capable of upregulating miR-21, which in turn targets peroxisome proliferator-activated receptor-α (PPAR-α) in an autoregulatory loop, modulating flow-induced endothelial inflammation [142]. Fenofibrate, the only PPAR-α agonist approved for human use, has shown great potential in diabetic retinopathy [143]. Various mechanisms have been proposed to explain this off-target effect [143]. Fenofibrate also modulates miR-199a and miR-214 [144], which play an important role in retinal neovascularization [145].

It is difficult to establish whether miRNA modulation after drug treatment is direct or mediated by other medication-modified factors; however, since existing anti-diabetic drugs can modulate miRNA levels, the topic deserves further investigation. The literature describing miRNA expression changes after administration of antidiabetic treatment is summarized in Table 2.

Therapeutic Potential of Exosome/Microvesicle-contained miRNAs and Metabolic Memory

One approach to overcome some technical problems of miRNA-based treatment is to use physiological, human-derived, and ready-packaged EVs, either exosomes or MVs. EVs released from donor cells by shedding from the plasma membrane are commonly referred to as MVs, whereas those secreted by multivesicular endosomes are called exosomes [146]. EVs contain mRNA, miRNAs, other non-coding RNAs, and a variety of protein types. EVs can be transferred to recipient cells, where shuttled RNA can be functional [147,148]. The functional relevance of miRNA-containing EV transfer has been described both *in vitro* and *in vivo* [147,148]. EVs, particularly exosomes, have attracted considerable interest for their potential use both as biomarkers and as vehicles for gene (or pseudogene) therapy [147,148]. They are found in the circulation in healthy individuals, and their number rises in several CV conditions associated with inflammation; a growing number of reports have been documenting a role for them in endothelial function regulation [149].

MiR-146a and miR-155 are involved in the vascular complications of diabetes [51,150] and are probably the two best explored inflamma-miRNAs [41,49]. A recent and innovative study has found them in exosomes released from dendritic cells after LPS (a TLR4 ligand) stimulation. In particular, exogenous miRNAs can reprogramme the cellular response to endotoxin, where miR-155 enhances and miR-146a reduces inflammatory gene expression [59]. NLR and TLR pathways play a significant role in the pathogenesis of inflammaging and inflammation-mediated ED [36]. Endogenous TLR ligands activate the TLR pathway, inducing NF-kB activation and promoting inflammation-mediated ED [36,39]. It is conceivable that diabetic adipose tissue is a source of inflammatory exosomes with altered miRNA content. For instance, stimulation of human-isolated adipocytes with LPS induces release of specific miRNAs into the culture medium [151]. Interestingly, secreted miR-155, miR-221 and miR-222, which play a role in the CV complications of diabetes, are shared between inflamed adipocytes and M1 macrophages [151]. Evidence of EVs containing inflamma-miRNAs that can modulate ED is already being published [64,152–155].

It is still unclear whether the EV content closely reflects the cell of origin or whether it may be altered also in the absence of major imbalances in parent tissue [147,148]. Evidence for both options has been provided [156,157]. Some discrepancies probably depend on the size of the EVs examined, as different molecular mechanisms regulate the sorting of molecules into exosomes or MVs [147]. In any case, hyperglycaemia itself can induce epigenetic damage [14–20]; the resulting EVs may thus have an altered content capable of propagating an 'incorrect' signature that modifies the epigenetic set-up in receiving cells

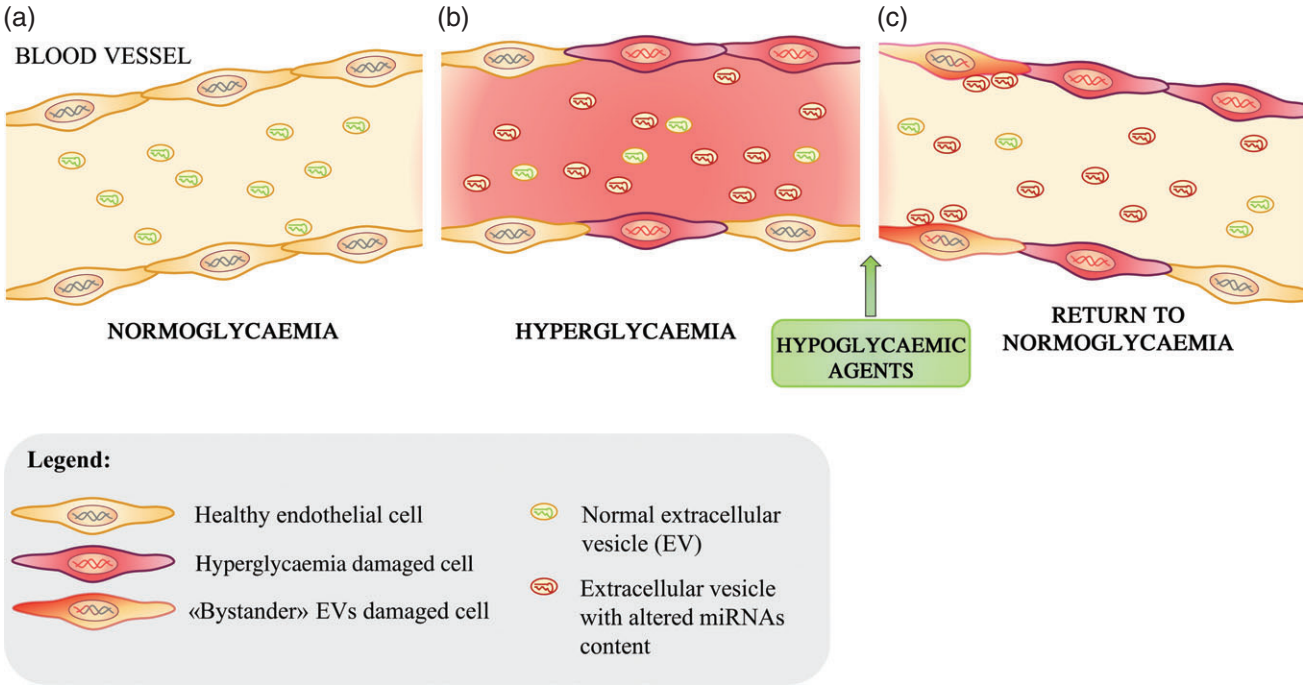

Figure 2. Epigenetic damage transmission. Postulated mechanism. Extracellular vesicles (EVs) contain mRNAs, microRNAs (miRNAs) and other non-coding RNAs, as well as a number of proteins. EVs can be transferred to recipient cells, where shuttled RNA can be functional. The endothelium uses EVs for physiological cell–cell communication (a). Hyperglycaemia can exert semi-permanent epigenetic damage in endothelial cells (b). The resulting EVs may have an altered content capable of propagating an 'incorrect' signature that modifies the epigenetic set-up in receiving cells even after stimulus removal; this would perpetuate the insult despite glucose normalization (c), which can be achieved through hypoglycaemic medications and/or lifestyle interventions.

even after stimulus removal. This would perpetuate the insult despite glucose normalization (Figure 2), a phenomenon that could be defined as 'epigenetic damage transmission'.

Reports of altered EV content in diabetic humans or mice and of hyperglycaemia-challenged ECs, fibroblasts, adipose, immune and pancreatic cells are increasingly frequent [62,82,95,98,102,153,155,158–161]. Intra- and inter-tissue horizontal miRNA transfer through exosomes or MVs appears to be an important phenomenon, especially for the vascular complications of diabetes. Imbalances in the content of pro- or anti-inflammatory miRNAs (i.e. miR-21, miR-146a and miR-155) and pro- or anti-angiogenic miRNAs (i.e. miR-126, miR-320 and miR-503) currently seem to be the most promising exploitable differences [62,95,98,102,153,155,160–162].

Exosomes derived from cardiomyocytes of Goto-Kakizaki rats, a widely used T2DM model, have been seen to increase miR-320 and reduce miR-126 content. Their transfer achieved functional downregulation of target genes (e.g. IGF-1, Hsp20 and Ets2) in recipient ECs, and miR-320 overexpression inhibited endothelial migration and tube formation [98]. Engineered exosomes, enriched with miR-320 antagonists, have already been proposed as a therapeutic option to increase angiogenesis in the diabetic heart [98,160]. Moreover, high glucose induces NF-kB binding to the miR-503 promoter region and upregulates miR-503 expression in ECs. NF-kB further induces shedding of endothelial microparticles carrying miR-503, inducing its transfer from ECs to vascular pericytes; integrin-mediated uptake of miR-503 in recipient pericytes reduces EFNB2 and VEGFA expression, resulting in impaired migration and proliferation [102].

Finally, proof-of-concept showing that miRNA-rich exosomes secreted from fibrocytes can accelerate wound healing in diabetic mice has been provided [162].

Conclusions and Future Prospects

A range of interventions, including lifestyle modification and/or pharmacological treatment, can be harnessed to improve outcomes in patients with diabetes; however, they are not sufficient, alone, to prevent the onset of the long-term disease complications. Epigenetic mechanisms, including DNA methylation, histone modifications and non-coding RNA expression modulation, have tremendously expanded our knowledge of some basic mechanisms of metabolic memory. EVs containing miRNAs are emerging as ideal candidates to provide diagnostic and prognostic information about diabetes and its CV complications. Moreover, exosome/MV-shuttling of miRNAs might provide a novel therapeutic approach to mitigate ED and inflammation in T2DM by trying to avoid or delay the harmful effects of diabetes on CV complications.

How do we go on from here? Further progress requires provision of two sorts of experimental data: (i) extensive comparative characterization of the nucleic acid (mRNA, miRNAs and other non-coding RNAs) and protein content of exosomes/MVs from

diabetic and healthy subjects; and (ii) the demonstration that chronic EV administration (chronic parabiosis) from a diabetic to a healthy mouse and vice versa is sufficient to induce and mitigate the CV complications of diabetes, to confirm the feasibility of 'small balls' therapy.

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Conflict of Interest

None of the authors have a conflict of interest to declare.

F.P., F.O. and A.C. conceived the idea and have been involved in manuscript conception and drafting. A.G., A.C., V.D.N., G.P. and L.L.S. collected the literature, drew the figure and supervised the parts devoted to circulating miRNAs in T2DM and its complications. A.C. and A.D.P. supervised the parts addressing the pharmacological and clinical aspects of T2DM and its complications. S.G. and R.T. supervised the paragraph related to metabolic memory and revised the manuscript critically. All authors have given their final approval of this version to be published. All authors have read and approved the final manuscript.

References

1. Ceriello A. The emerging challenge in diabetes: the "metabolic memory". Vascul Pharmacol 2012; **57**: 133–138.
2. Laakso M, Kuusisto J. Insulin resistance and hyperglycaemia in cardiovascular disease development. Nat Rev Endocrinol 2014; **10**: 293–302.
3. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes 2002; **51**: 1157–1165.
4. Guarner V, Rubio-Ruiz ME. Low-grade systemic inflammation connects aging, metabolic syndrome and cardiovascular disease. Interdiscip Top Gerontol 2015; **40**: 99–106.
5. Palmer AK, Tchkonia T, LeBrasseur NK, Chini EN, Xu M, Kirkland JL. Cellular senescence in type 2 diabetes: a therapeutic opportunity. Diabetes 2015; **64**: 2289–2298.
6. Hill D, Fisher M. The effect of intensive glycaemic control on cardiovascular outcomes. Diabetes Obes Metab 2010; **12**: 641–647.
7. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008; **359**: 1577–1589.
8. Chalmers J, Cooper ME. UKPDS and the legacy effect. N Engl J Med 2008; **359**: 1618–1620.
9. Ceriello A, Esposito K, Piconi L et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008; **57**: 1349–1354.
10. Russell ND, Cooper ME. 50 years forward: mechanisms of hyperglycaemia-driven diabetic complications. Diabetologia 2015; **58**: 1708–1714.
11. Reddy MA, Zhang E, Natarajan R. Epigenetic mechanisms in diabetic complications and metabolic memory. Diabetologia 2015; **58**: 443–455.
12. Meigs JB, Shrader P, Sullivan LM et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008; **359**: 2208–2219.
13. Ahlqvist E, van Zuydam NR, Groop LC, McCarthy MI. The genetics of diabetic complications. Nat Rev Nephrol 2015; **11**: 277–287.
14. Reddy MA, Natarajan R. Epigenetic mechanisms in diabetic vascular complications. Cardiovasc Res 2011; **90**: 421–429.
15. Pirola L, Balcerczyk A, Okabe J, El-Osta A. Epigenetic phenomena linked to diabetic complications. Nat Rev Endocrinol 2010; **6**: 665–675.
16. Wegner M, Neddermann D, Piorunska-Stolzmann M, Jagodzinski PP. Role of epigenetic mechanisms in the development of chronic complications of diabetes. Diabetes Res Clin Pract 2014; **105**: 164–175.
17. Brasacchio D, Okabe J, Tikelli C et al. Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene activating epigenetic marks that coexist on the lysine tail. Diabetes 2009; **58**: 1229–1236.
18. Miao F, Gonzalo IG, Lanting L, Natarajan R. In vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. J Biol Chem 2004; **279**: 18091–18097.
19. Reddy MA, Natarajan R. Role of epigenetic mechanisms in the vascular complications of diabetes. Subcell Biochem 2013; **61**: 435–454.
20. Keating ST, El-Osta A. Epigenetic changes in diabetes. Clin Genet 2013; **84**: 1–10.
21. Baek D, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature 2008; **455**: 64–71.
22. Iorio MV, Piovan C, Croce CM. Interplay between microRNAs and the epigenetic machinery: an intricate network. Biochim Biophys Acta 1799; **2010**: 694–701.
23. Breving K, Esquela-Kerscher A. The complexities of microRNA regulation: mirandering around the rules. Int J Biochem Cell Biol 2010; **42**: 1316–1329.
24. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol 2013; **9**: 513–521.
25. McClelland AD, Kantharidis P. microRNA in the development of diabetic complications. Clin Sci (Lond) 2014; **126**: 95–110.
26. Beltrami C, Angelini TG, Emanueli C. Noncoding RNAs in diabetes vascular complications. J Mol Cell Cardiol 2015; **89**: 42–50.
27. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014; **69**(Suppl. 1): S4–S9.
28. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 2013; **123**: 966–972.
29. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 2010; **5**: 99–118.
30. Acosta JC, Banito A, Wuestefeld T et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 2013; **15**: 978–990.
31. Testa R, Genovese S, Ceriello A. Nutritional imbalances linking cellular senescence and type 2 diabetes mellitus. Curr Opin Clin Nutr Metab Care 2014; **17**: 338–342.
32. Robbins GR, Wen H, Ting JP. Inflammasomes and metabolic disorders: old genes in modern diseases. Mol Cell 2014; **54**: 297–308.
33. Lee HM, Kim JJ, Kim HJ, Shong M, Ku BJ, Jo EK. Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes. Diabetes 2013; **62**: 194–204.
34. Shin JJ, Lee EK, Park TJ, Kim W. Damage-associated molecular patterns and their pathological relevance in diabetes mellitus. Ageing Res Rev 2015; **24**: 66–76.
35. Dixit VD. Nlrp3 inflammasome activation in type 2 diabetes: is it clinically relevant? Diabetes 2013; **62**: 22–24.

36. Prajapati B, Jena PK, Rajput P, Purandhar K, Seshadri S. Understanding and modulating the Toll like Receptors (TLRs) and NOD like Receptors (NLRs) cross talk in type 2 diabetes. Curr Diabetes Rev 2014; **10**: 190–200.

37. Qin J, Li Y, Cai Z et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012; **490**: 55–60.

38. Dregan A, Charlton J, Chowienczyk P, Gulliford MC. Chronic inflammatory disorders and risk of type 2 diabetes mellitus, coronary heart disease, and stroke: a population-based cohort study. Circulation 2014; **130**: 837–844.

39. Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. Nat Rev Drug Discov 2014; **13**: 465–476.

40. Weilner S, Schraml E, Redl H, Grillari-Voglauer R, Grillari J. Secretion of microvesicular miRNAs in cellular and organismal aging. Exp Gerontol 2013; **48**: 626–633.

41. Olivieri F, Albertini MC, Orciani M et al. DNA damage response (DDR) and senescence: shuttled inflamma-miRNAs on the stage of inflamm-aging. Oncotarget 2015; **6**: 35509–35521.

42. Olivieri F, Bonafè M, Spazzafumo L et al. Age- and glycemia-related miR-126-3p levels in plasma and endothelial cells. Aging (Albany NY) 2014; **6**: 771–787.

43. Zhu H, Leung SW. Identification of microRNA biomarkers in type 2 diabetes: a meta-analysis of controlled profiling studies. Diabetologia 2015; **58**: 900–911.

44. Turchinovich A, Weiz L, Burwinkel B. Extracellular miRNAs: the mystery of their origin and function. Trends Biochem Sci 2012; **37**: 460–465.

45. Inukai S, Slack F. MicroRNAs and the genetic network in aging. J Mol Biol 2013; **425**: 3601–3608.

46. Dávalos A, Chroni A. Antisense oligonucleotides, microRNAs, and antibodies. Handb Exp Pharmacol 2015; **224**: 649–689.

47. Hartig SM, Hamilton MP, Bader DA, McGuire SE. The miRNA interactome in metabolic homeostasis. Trends Endocrinol Metab 2015; **26**: 733–745.

48. Zhong X, Liao Y, Chen L et al. The microRNAs in the pathogenesis of metabolic memory. Endocrinology 2015; **156**: 3157–3168.

49. Saba R, Sorensen DL, Booth SA. MicroRNA-146a: a dominant, negative regulator of the innate immune response. Front Immunol 2014; **5**: 578.

50. Olivieri F, Lazzarini R, Babini L et al. Anti-inflammatory effect of ubiquinol-10 on young and senescent endothelial cells via miR-146a modulation. Free Radic Biol Med 2013; **63**: 410–420.

51. Prattichizzo F, Giuliani A, Ceka A et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics 2015; **7**: 56.

52. Alipour MR, Khamaneh AM, Yousefzadeh N, Mohammad-nejad D, Soufi FG. Upregulation of microRNA-146a was not accompanied by downregulation of pro-inflammatory markers in diabetic kidney. Mol Biol Rep 2013; **40**: 6477–6483.

53. Feng B, Chen S, McArthur K et al. miR-146a-Mediated extracellular matrix protein production in chronic Diabetes complications. Diabetes 2011; **60**: 2975–2984.

54. Rippo MR, Olivieri F, Monsurrò V, Prattichizzo F, Albertini MC, Procopio AD. MitomiRs in human inflamm-aging: a hypothesis involving miR-181a, miR-34a and miR-146a. Exp Gerontol 2014; **56**: 154–163.

55. Wang WX, Visavadiya NP, Pandya JD, Nelson PT, Sullivan PG, Springer JE. Mitochondria-associated microRNAs in rat hippocampus following traumatic brain injury. Exp Neurol 2015; **265**: 84–93.

56. Sharma K. Mitochondrial hormesis and diabetic complications. Diabetes 2015; **64**: 663–672.

57. Lanza IR, Zabielski P, Klaus KA et al. Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab 2012; **16**: 777–788.

58. Halkein J, Tabruyn SP, Ricke-Hoch M et al. MicroRNA-146a is a therapeutic target and biomarker for peripartum cardiomyopathy. J Clin Invest 2013; **123**: 2143–2154.

59. Alexander M, Hu R, Runtsch MC et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat Commun 2015; **6**: 7321.

60. Bijkerk R, van Solingen C, de Boer HC et al. Hematopoietic microRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. J Am Soc Nephrol 2014; **25**: 1710–1722.

61. Zampetaki A, Kiechl S, Drozdov I et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010; **107**: 810–817.

62. Jansen F, Yang X, Hoelscher M et al. Endothelial microparticle-mediated transfer of microRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation 2013; **128**: 2026–2038.

63. Meng S, Cao JT, Zhang B, Zhou Q, Shen CX, Wang CQ. Downregulation of microRNA-126 in endothelial progenitor cells from diabetes patients impairs their functional properties, via target gene Spred-1. J Mol Cell Cardiol 2012; **53**: 64–72.

64. Togliatto G, Dentelli P, Gili M et al. Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications. Int J Obes (Lond) 2015; **40**: 102–111.

65. Ryu HS, Park SY, Ma D, Zhang J, Lee W. The induction of microRNA targeting IRS-1 is involved in the development of insulin resistance under conditions of mitochondrial dysfunction in hepatocytes. PLoS One 2011; **6**: e17343.

66. Rondinone CM, Wang LM, Lonnroth P, Wesslau C, Pierce JH, Smith U. Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein forphosphatidylinositol3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A 1997; **94**: 4171–4175.

67. Carvalho E, Jansson PA, Nagaev I, Wenthzel AM, Smith U. Insulin resistance with low cellular IRS-1 expression is also associated with low GLUT4 expression and impaired insulin-stimulated glucose transport. FASEB J 2001; **15**: 1101–1103.

68. Fernandez-Twinn DS, Alfaradhi MZ, Martin-Gronert MS et al. Downregulation of IRS-1 in adipose tissue of offspring of obese mice is programmed cell-autonomously through post-transcriptional mechanisms. Mol Metab 2014; **3**: 325–333.

69. Luan Y, Zuo L, Zhang S, Wang G, Peng T. MicroRNA-126 acts as a tumor suppressor in glioma cells by targeting insulin receptor substrate 1 (IRS-1). Int J Clin Exp Pathol 2015; **8**: 10345–10354.

70. Olivieri F, Spazzafumo L, Bonafè M et al. MiR-21-5p and miR-126a-3p levels in plasma and circulating angiogenic cells: relationship with type 2 diabetes complications. Oncotarget 2015; **6**: 35372–35382.

71. Jansen F, Yang X, Proebsting S et al. MicroRNA expression in circulating microvesicles predicts cardiovascular events in patients with coronary artery disease. J Am Heart Assoc 2014; **3**: e001249.

72. Togliatto G, Trombetta A, Dentelli P et al. Unacylated ghrelin induces oxidative stress resistance in a glucose intolerance and peripheral artery disease mouse model by restoring endothelial cell miR-126 expression. Diabetes 2015; **64**: 1370–1382.

73. Olivieri F, Spazzafumo L, Santini G et al. Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging. Mech Ageing Dev 2012; **133**: 675–685.

74. Dellago H, Preschitz-Kammerhofer B, Terlecki-Zaniewicz L et al. High levels of oncomiR-21 contribute to the senescence-induced growth arrest in normal human cells and its knock-down increases the replicative lifespan. Aging Cell 2013; **12**: 446–458.

75. Qing S, Yuan S, Yun C et al. Serum miRNA biomarkers serve as a fingerprint for proliferative diabetic retinopathy. Cell Physiol Biochem 2014; **34**: 1733–1740.

76. McClelland AD, Herman-Edelstein M, Komers R et al. miR-21 promotes renal fibrosis in diabetic nephropathy by targeting PTEN and SMAD7. Clin Sci (Lond) 2015; **129**: 1237–1249.
77. Dey N, Das F, Mariappan MM et al. MicroRNA-21 orchestrates high glucose-induced signals to TOR complex 1, resulting in renal cell pathology in diabetes. J Biol Chem 2011; **286**: 25586–25603.
78. Pezzolesi MG, Satake E, McDonnell KP, Major M, Smiles AM, Krolewski AS. Circulating TGF-β1-regulated miRNAs and the risk of rapid progression to ESRD in type 1 diabetes. Diabetes 2015; **64**: 3285–3293.
79. Thum T, Gross C, Fiedler J et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008; **456**: 980–984.
80. Bang C, Batkai S, Dangwal S et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014; **124**: 2136–2146.
81. Liu S, Li W, Xu M, Huang H, Wang J, Chen X. Micro-RNA 21 targets dual specific phosphatase 8 to promote collagen synthesis in high glucose-treated primary cardiac fibroblasts. Can J Cardiol 2014; **30**: 1689–1699.
82. Santovito D, De Nardis V, Marcantonio P et al. Plasma exosome microRNA profiling unravels a new potential modulator of adiponectin pathway in diabetes: effect of glycemic control. J Clin Endocrinol Metab 2014; **99**: E1681–E1685.
83. Yang Z, Chen H, Si H et al. Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes. Acta Diabetol 2014; **51**: 823–831.
84. Pek SL, Sum CF, Lin MX et al. Circulating and visceral adipose miR-100 is down-regulated in patients with obesity and type 2 diabetes. Mol Cell Endocrinol 2016; **427**: 112–123.
85. Kong L, Zhu J, Han W et al. Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol 2011; **48**: 61–69.
86. Ortega FJ, Mercader JM, Moreno-Navarrete JM et al. Profiling of circulating microRNAs reveals common microRNAs linked to type 2 diabetes that change with insulin sensitization. Diabetes Care 2014; **37**: 1375–1583.
87. Zhang T, Li L, Shang Q, Lv C, Wang C, Su B. Circulating miR-126 is a potential biomarker to predict the onset of type 2 diabetes mellitus in susceptible individuals. Biochem Biophys Res Commun 2015; **463**: 60–63.
88. Jansen F, Wang H, Przybilla D et al. Vascular endothelial microparticles-incorporated microRNAs are altered in patients with diabetes mellitus. Cardiovasc Diabetol 2016; **15**: 49.
89. Liu Y, Gao G, Yang C et al. The role of circulating microRNA-126 (miR-126): a novel biomarker for screening prediabetes and newly diagnosed type 2 diabetes mellitus. Int J Mol Sci 2014; **15**: 10567–10577.
90. Zhou B, Ma R, Si W et al. MicroRNA-503 targets FGF2 and VEGFA and inhibits tumor angiogenesis and growth. Cancer Lett 2013; **333**: 159–169.
91. Wang C, Wan S, Yang T et al. Increased serum microRNAs are closely associated with the presence of microvascular complications in type 2 diabetes mellitus. Sci Rep 2016; **6**: 20032.
92. Karolina DS, Armugam A, Tavintharan S et al. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. PLoS One 2011; **6**: e22839.
93. Rong Y, Bao W, Shan Z et al. Increased microRNA-146a levels in plasma of patients with newly diagnosed type 2 diabetes mellitus. PLoS One 2013; **8**: e73272.
94. Yan ST, Li CL, Tian H et al. MiR-199a is overexpressed in plasma of type 2 diabetes patients which contributes to type 2 diabetes by targeting GLUT4. Mol Cell Biochem 2014; **397**: 45–51.
95. Jansen F, Yang X, Baumann K et al. Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism. J Cell Mol Med 2015; **19**: 2202–2214.
96. Zhao B, Li H, Liu J et al. MicroRNA-23b targets Ras GTPase-activating protein SH3 domain-binding protein 2 to alleviate fibrosis and albuminuria in diabetic nephropathy. J Am Soc Nephrol 2016; pii: ASN.2015030300. [Epub ahead of print].
97. Karolina DS, Tavintharan S, Armugam A et al. Circulating miRNA profiles in patients with metabolic syndrome. J Clin Endocrinol Metab 2012; **97**: E2271–E2276.
98. Wang X, Huang W, Liu G et al. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 2014; **74**: 139–150.
99. Sun K, Chang X, Yin L et al. Expression and DNA methylation status of microRNA-375 in patients with type 2 diabetes mellitus. Mol Med Rep 2014; **9**: 967–972.
100. Pescador N, Pérez-Barba M, Ibarra JM et al. Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. PLoS One 2013; **8**: e77251.
101. Caporali A, Meloni M, Völlenkle C et al. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. Circulation 2011; **123**: 282–291.
102. Caporali A, Meloni M, Nailor A et al. p75(NTR)-dependent activation of NF-kB regulates microRNA-503 transcription and pericyte-endothelial crosstalk in diabetes after limb ischaemia. Nat Commun 2015; **6**: 8024.
103. Roggli E, Britan A, Gattesco S et al. Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells. Diabetes 2010; **59**: 978–986.
104. Shantikumar S, Caporali A, Emanueli C. Role of microRNAs in diabetes and its cardiovascular complications. Cardiovasc Res 2012; **93**: 583–593.
105. Costantino S, Paneni F, Lüscher TF, Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. Eur Heart J 2015; **37**: 572–576.
106. Greco S, Fasanaro P, Castelvecchio S et al. MicroRNA dysregulation in diabetic ischemic heart failure patients. Diabetes 2012; **61**: 1633–1641.
107. Janssen HL, Reesink HW, Lawitz EJ et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013; **368**: 1685–1694.
108. Santulli G, Wronska A, Uryu K et al. A selective microRNA-based strategy inhibits restenosis while preserving endothelial function. J Clin Invest 2014; **124**: 4102–4114.
109. Krützfeldt J, Rajewsky N, Braich R et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005; **438**: 685–689.
110. O'Loughlin AJ, Woffindale CA, Wood MJ. Exosomes and the emerging field of exosome-based gene therapy. Curr Gene Ther 2012; **12**: 262–274.
111. Leoni G, Neumann PA, Kamaly N et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. J Clin Invest 2015; **125**: 1215–1227.
112. Hoshino A, Costa-Silva B, Shen TL et al. Tumour exosome integrins determine organotropic metastasis. Nature 2015; **527**: 329–335.
113. Bannister CA, Holden SE, Jenkins-Jones S et al. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. Diabetes Obes Metab 2014; **16**: 1165–1173.
114. Morgan CL, Mukherjee J, Jenkins-Jones S, Holden SE, Currie CJ. Association between first-line monotherapy with sulphonylurea versus metformin and risk of all-cause mortality and cardiovascular events: a retrospective, observational study. Diabetes Obes Metab 2014; **16**: 957–962.
115. Arunachalam G, Samuel SM, Marei I, Ding H, Triggle CR. Metformin modulates hyperglycaemia-induced endothelial senescence and apoptosis through SIRT1. Br J Pharmacol 2014; **171**: 523–535.
116. Forouzandeh F, Salazar G, Patrushev N et al. Metformin beyond diabetes: pleiotropic benefits of metformin in attenuation of atherosclerosis. J Am Heart Assoc 2014; **3**: e001202.

117. Moiseeva O, Deschênes-Simard X, St-Germain E et al. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-kB activation. Aging Cell 2013; **12**: 489–498.

118. Zheng Z, Chen H, Li J et al. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. Diabetes 2012; **61**: 217–228.

119. Chakraborty A, Chowdhury S, Bhattacharyya M. Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. Diabetes Res Clin Pract 2011; **93**: 56–62.

120. Stocker DJ, Taylor AJ, Langley RW, Jezior MR, Vigersky RA. A randomized trial of the effects of rosiglitazone and metformin on inflammation and subclinical atherosclerosis in patients with type 2 diabetes. Am Heart J 2007; **153**: 445.e1-6.

121. Paneni F, Volpe M, Lüscher TF, Cosentino F. SIRT1, p66(Shc), and Set7/9 in vascular hyperglycemic memory: bringing all the strands together. Diabetes 2013; **62**: 1800–1807.

122. Balestrieri ML, Rizzo MR, Barbieri M et al. Sirtuin 6 expression and inflammatory activity in diabetic atherosclerotic plaques: effects of incretin treatment. Diabetes 2015; **64**: 1395–1406.

123. Xue Y, Wei Z, Ding H et al. MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1α in the progression of atherosclerosis. Atherosclerosis 2015; **241**: 671–681.

124. Zoungas S, Chalmers J, Neal B et al. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N Engl J Med 2014; **371**: 1392–1406.

125. Ceriello A, Piconi L, Esposito K, Giugliano D. Telmisartan shows an equivalent effect of vitamin C in further improving endothelial dysfunction after glycemia normalization in type 1 diabetes. Diabetes Care 2007; **30**: 1694–1698.

126. Ceriello A, Motz E. Angiotensin-receptor blockers, type 2 diabetes, and renoprotection. N Engl J Med 2002; **346**: 705–707.

127. Harvey A, Montezano AC, Touyz RM. Vascular biology of ageing-Implications in hypertension. J Mol Cell Cardiol 2015; **83**: 112–121.

128. Donato AJ, Morgan RG, Walker AE, Lesniewski LA. Cellular and molecular biology of aging endothelial cells. J Mol Cell Cardiol 2015; **89**: 122–135.

129. Westhoff JH, Hilgers KF, Steinbach MP et al. Hypertension induces somatic cellular senescence in rats and humans by induction of cell cycle inhibitor p16INK4a. Hypertension 2008; **52**: 123–129.

130. Yokoi T, Fukuo K, Yasuda O et al. Apoptosis signal-regulating kinase 1 mediates cellular senescence induced by high glucose in endothelial cells. Diabetes 2006; **55**: 1660–1665.

131. Ling S, Nanhwan M, Qian J et al. Modulation of microRNAs in hypertension-induced arterial remodeling through the β1 and β3-adrenoreceptor pathways. J Mol Cell Cardiol 2013; **65**: 127–136.

132. Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. MicroRNA-9 and microRNA-126 expression levels in patients with essential hypertension: potential markers of target-organ damage. J Am Soc Hypertens 2014; **8**: 368–375.

133. Takahashi Y, Satoh M, Minami Y, Tabuchi T, Itoh T, Nakamura M. Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. Clin Sci (Lond) 2010; **119**: 395–405.

134. Ceolotto G, Papparella I, Bortoluzzi A et al. Interplay between miR-155, AT1R A1166C polymorphism, and AT1R expression in young untreated hypertensives. Am J Hypertens 2011; **24**: 241–246.

135. Leung A, Trac C, Jin W et al. Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells. Circ Res 2013; **113**: 266–278.

136. Schober A, Nazari-Jahantigh M, Wei Y et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014; **20**: 368–376.

137. Potus F, Ruffenach G, Dahou A et al. Downregulation of microRNA-126 contributes to the failing right ventricle in pulmonary arterial hypertension. Circulation 2015; **132**: 932–943.

138. Ceriello A, Assaloni R, Da Ros R et al. Effect of irbesartan on nitrotyrosine generation in non-hypertensive diabetic patients. Diabetologia 2004; **47**: 1535–1540.

139. Satoh M, Takahashi Y, Tabuchi T et al. Circulating Toll-like receptor 4-responsive microRNA panel in patients with coronary artery disease: results from prospective and randomized study of treatment with renin-angiotensin system blockade. Clin Sci (Lond) 2015; **128**: 483–491.

140. Olivieri F, Mazzanti I, Abbatecola AM et al. Telomere/Telomerase system: a new target of statins pleiotropic effect? Curr Vasc Pharmacol 2012; **10**: 216–224.

141. Bhaumik D, Scott GK, Schokrpur S et al. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging (Albany, NY) 2009; **1**: 402–411.

142. Zhou J, Wang KC, Wu W et al. MicroRNA-21 targets peroxisome proliferators-activated receptor-alpha in an autoregulatory loop to modulate flow-induced endothelial inflammation. Proc Natl Acad Sci U S A 2011; **108**: 10355–10360.

143. Noonan JE, Jenkins AJ, Ma JX, Keech AC, Wang JJ, Lamoureux EL. An update on the molecular actions of fenofibrate and its clinical effects on diabetic retinopathy and other microvascular end points in patients with diabetes. Diabetes 2013; **62**: 3968–3975.

144. Li C, Mpollo MS, Gonsalves CS, Tahara SM, Malik P, Kalra VK. Peroxisome proliferator-activated receptor-α-mediated transcription of miR-199a2 attenuates endothelin-1 expression via hypoxia-inducible factor-1α. J Biol Chem 2014; **289**: 36031–36047.

145. Shen J, Yang X, Xie B et al. MicroRNAs regulate ocular neovascularization. Mol Ther 2008; **16**: 1208–1216.

146. Stoorvogel W. Resolving sorting mechanisms into exosomes. Cell Res 2015; **25**: 531–532.

147. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; **200**: 373–383.

148. Turchinovich A, Tonevitsky AG, Cho WC, Burwinkel B. Check and mate to exosomal extracellular miRNA: new lesson from a new approach. Front Mol Biosci 2015; **2**: 11.

149. Fleury A, Martinez MC, Le Lay S. Extracellular vesicles as therapeutic tools in cardiovascular diseases. Front Immunol 2014; **5**: 370.

150. Kato M, Castro NE, Natarajan R. MicroRNAs: potential mediators and biomarkers of diabetic complications. Free Radic Biol Med 2013; **64**: 85–94.

151. Ortega FJ, Moreno M, Mercader JM et al. Inflammation triggers specific microRNA profiles in human adipocytes and macrophages and in their supernatants. Clin Epigenetics 2015; **7**: 49.

152. Lakhter AJ, Sims EK. Emerging roles for extracellular vesicles in diabetes and related metabolic disorders. Mol Endocrinol 2015; **29**: 1535–1548.

153. Hulsmans M, Holvoet P. MicroRNA-containing microvesicles regulating inflammation in association with atherosclerotic disease. Cardiovasc Res 2013; **100**: 7–18.

154. Ferrante SC, Nadler EP, Pillai DK et al. Adipocyte-derived exosomal miRNAs: a novel mechanism for obesity-related disease. Pediatr Res 2015; **77**: 447–454.

155. Guay C, Menoud V, Rome S, Regazzi R. Horizontal transfer of exosomal microRNAs transduce apoptotic signals between pancreatic beta-cells. Cell Commun Signal 2015; **13**: 17.

156. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 2013; **4**: 2980.

157. de Jong OG, Verhaar MC, Chen Y et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. J Extracell Vesicles 2012; **1**: 18396.

158. Mocharla P, Briand S, Giannotti G et al. AngiomiR-126 expression and secretion from circulating CD34(+) and CD14(+) PBMCs: role for proangiogenic effects and alterations in type 2 diabetics. Blood 2013; **121**: 226–236.

159. Chaturvedi P, Kalani A, Medina I, Familtseva A, Tyagi SC. Cardiosome mediated regulation of MMP9 in diabetic heart: role of mir29b and mir455 in exercise. J Cell Mol Med 2015; **19**: 2153–2161.

160. Shantikumar S, Angelini GD, Emanueli C. Diabetes, microRNAs and exosomes: les liaisons dangereuses. J Mol Cell Cardiol 2014; **74**: 196–198.

161. Garcia NA, Ontoria-Oviedo I, González-King H, Diez-Juan A, Sepúlveda P. Glucose starvation in cardiomyocytes enhances exosome secretion and promotes angiogenesis in endothelial cells. PLoS One 2015; **10**: e0138849.

162. Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochem Biophys Res Commun 2015; **467**: 303–309.