Targeting endothelial metaflammation to counteract diabesity cardiovascular risk: Current and perspective therapeutic options

TITLE: TARGETING ENDOTHELIAL METAFLAMMATION TO COUNTERACT DIABESITY CARDIOVASCULAR RISK: CURRENT AND PERSPECTIVE THERAPEUTIC OPTIONS.

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Abstract: The association of obesity and diabetes, termed "diabesity", defines a combination of primarily metabolic disorders with insulin resistance as the underlying common pathophysiology. Cardiovascular disorders associated with diabesity represent the leading cause of morbidity and mortality in the Western world. This makes diabesity, with its rising impacts on both health and economics, one of the most challenging biomedical and social threats of present century. The emerging comprehension of the genes whose alteration confers inter-individual differences on risk factors for diabetes or obesity, together with the potential role of genetically determined variants on mechanisms controlling responsiveness, effectiveness and safety of anti-diabetic therapy underlines the need of additional knowledge on molecular mechanisms involved in the pathophysiology of diabesity. Endothelial cell dysfunction, resulting from the unbalanced production of endothelial-derived vascular mediators, is known to be present at the earliest stages of insulin resistance and obesity, and may precede the clinical diagnosis of diabetes by several years. Once considered as a mere consequence of metabolic abnormalities, it is now clear that endothelial dysfunctional activity may play a pivotal role in the progression of diabesity. In the vicious circle where vascular defects and metabolic disturbances worsen and reinforce each other, a low-grade, chronic, and 'cold' inflammation (metaflammation) has been suggested to serve as the pathophysiological link that binds endothelial and metabolic dysfunctions. In this paradigm, it is important to consider how traditional antidiabetic treatments (specifically addressing metabolic dysregulation) may directly impact on inflammatory processes or cardiovascular function. Indeed, not all drugs currently available to treat diabetes possess the same anti-inflammatory potential, or target endothelial cell function equally. Perspective strategies pointing at reducing metaflammation or directly addressing endothelial dysfunction may disclose beneficial consequences on metabolic regulation. This review focuses on existing and potential new approaches ameliorating endothelial dysfunction and vascular inflammation in the context of diabesity.

Keywords: endothelial dysfunction; diabesity; metaflammation; anti-diabetic drugs

Conflict of interest The authors declare no conflict of interest.

SUMMARY

DIABESITY

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- *VEGF inhibitors*
- *PARP inhibitors*
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- *AMPK activators*
- *Anti-oxidants*
    - *Vitamin C and Vitamin E*
    - *Polyphenols*

CONCLUSIONS

DIABESITY

Diabetes occurring in the context of obesity has been defined "diabesity" [1] and represents a worldwide growing phenomenon affecting both developed and developing countries (https://ec.europa.eu/research/health/pdf/diabesity-conference-report-022012_en.pdf). The social and economic burden of diabesity includes consequences in terms of productivity and life expectancy, costs related to health care once the disease has been diagnosed, and costs connected with long-term complications such as blindness, limb amputation, or kidney and heart diseases.

The association between diabesity and cardiovascular risk is well recognized, and increasing attention has been devoted to the long-term effects of drug treatments on progression and severity of cardiovascular morbidity and mortality. Current therapeutic options involve agents that target elevated blood sugar, impaired insulin resistance, increased blood pressure, and high cholesterol levels. Nevertheless, these abnormalities represent downstream symptoms more than causal agents of diabesity, with the consequence that a significant number of monotherapy treatments lacks efficacy overtime and/or does not adequately control cardiovascular complications [2]. In addition, inappropriate therapies can increase the risk of hypoglycemic episodes, which in turn may trigger cardiovascular acute events.

In the clinical practice, therapeutic recommendations to treat metabolic and cardiovascular disturbances are largely based on standard protocols that address typical dysfunctions in average diabetic and obese patients. Unfortunately, this approach may be unsuccessful or inappropriate in subjects that, for a variety of reasons, are unable to reach the therapeutic goals. One of these reasons is based on the recognition that both obesity and diabetes are multifactorial diseases, resulting from the complex interplay between environmental factors and genetic inheritance. Therefore, diabetic and obese patients may differ in their individual susceptibility to the disease or their response to a specific treatment.

Significant progresses have been made in understanding the variant genes predisposing to these diseases [3]. At present, the high susceptibility makes the predictive value of the gene variants very limited. Nevertheless, novel scientific discoveries at the genomic level are expected to shed light on risk factors for diabetes or obesity, and help to identify subpopulations of patients with specific characteristics.

On the same line, the increased appreciation of the inter-individual differences in response to drugs have highlighted the potential role of genetically determined variants on mechanisms controlling the absorption, bioavailability, tissue responsiveness, effectiveness and safety of current anti-diabetic therapy [4,5]. Although the clinical applicability of these data requires further efforts, the evolving area of pharmacogenomics and pharmacogenetics opens the road to the possibility of a more individual-tailored, personalized medicine [6,7].

Considering the economic impact of diabesity, it is of foremost importance to plan strategies and approaches that may enhance prediction on the onset and course of the disease, and at the same time identify individuals who are most likely to benefit from a specific management strategy. In this complex scenario, the rising number of options to treat diabesity significantly broadens the range of therapeutic opportunities, but concomitantly underlines the need of additional knowledge on molecular mechanisms involved in the pathophysiology of diabesity. A state of chronic, low-grade inflammation in which inflammatory molecules produced by infiltrating macrophages exert pathological changes in all insulin-sensitive tissues has been proposed to bridge the gap between epidemiology and pathobiochemistry of diabesity.

The role played by the endothelium in triggering and/or enhancing metaflammation is particularly intriguing, and sets a new challenge on the development of novel therapies to treat diabesity. In addition, endothelial dysfunction is - to a certain extent- a reversible process, and ameliorated endothelial function is indicative of improved cardiovascular protection. Along with the implications related to its potential role on cardiovascular risk, evaluation of endothelial function might represent a useful biomarker to assess the effectiveness of therapy. Current techniques to measure endothelial function do not fulfill all the essential criteria required for a clinical surrogate

end-point and, at present, brachial artery flood mediated dilation (FMD) remains the most widely applied non-invasive method. Nevertheless, the importance of endothelium in participating and predicting cardiovascular risk factors encourages further efforts in this direction. In the next paragraphs the potential contribution of endothelial dysfunction to metaflammation and metabolic disturbances of diabesity is briefly discussed.

ENDOTHELIAL DYSFUNCTION and METAFLAMMATION

With the growing understanding of the functional role played by the endothelium, and the subsequent discovery of several endothelial mediators and their respective mechanism of action, it has become increasingly clear that endothelial abnormalities represent an early sign of both hemodynamic and metabolic disturbances [8]. Indeed, endothelial dysfunction - a condition in which the endothelium loses its physiological properties and shifts toward a vasoconstrictor, pro-thrombotic and pro-inflammatory state - is considered a major contributing factor in the etiology of diabetes-related microvascular diseases such as retinopathy, nephropathy, neuropathy, and impaired wound healing [9,10]. Moreover, endothelial dysfunction precedes the onset of macrovascular complications, mainly represented by atherosclerosis, which in diabetic patients is more rapid and more severe than in control population [10,11].

Typically, endothelial dysfunction is defined by a reduced availability of nitric oxide (NO), a gaseous mediator with vasodilatant, anti-thrombotic and anti-inflammatory properties [12,13]. NO bioavailability depends on several multiple factors, ranging from the efficiency of the producing enzyme endothelial NO synthase (eNOS) to the speed of conversion of NO itself to more stable nitrate/nitrite derivatives. The dynamic, highly regulated physiological production of NO in the endothelium may be disrupted when eNOS protein expression is decreased, when eNOS substrates and/or co-factors are insufficient, when enzymatic activity of eNOS is impaired or uncoupled, or when the production of endothelial mediators with opposing vascular effects is relatively increased [9,14]. The deficiency of NO bioavailability and the increased reactive oxygen species (ROS) and proinflammatory factors are requisite hallmarks for endothelial dysfunction [15].

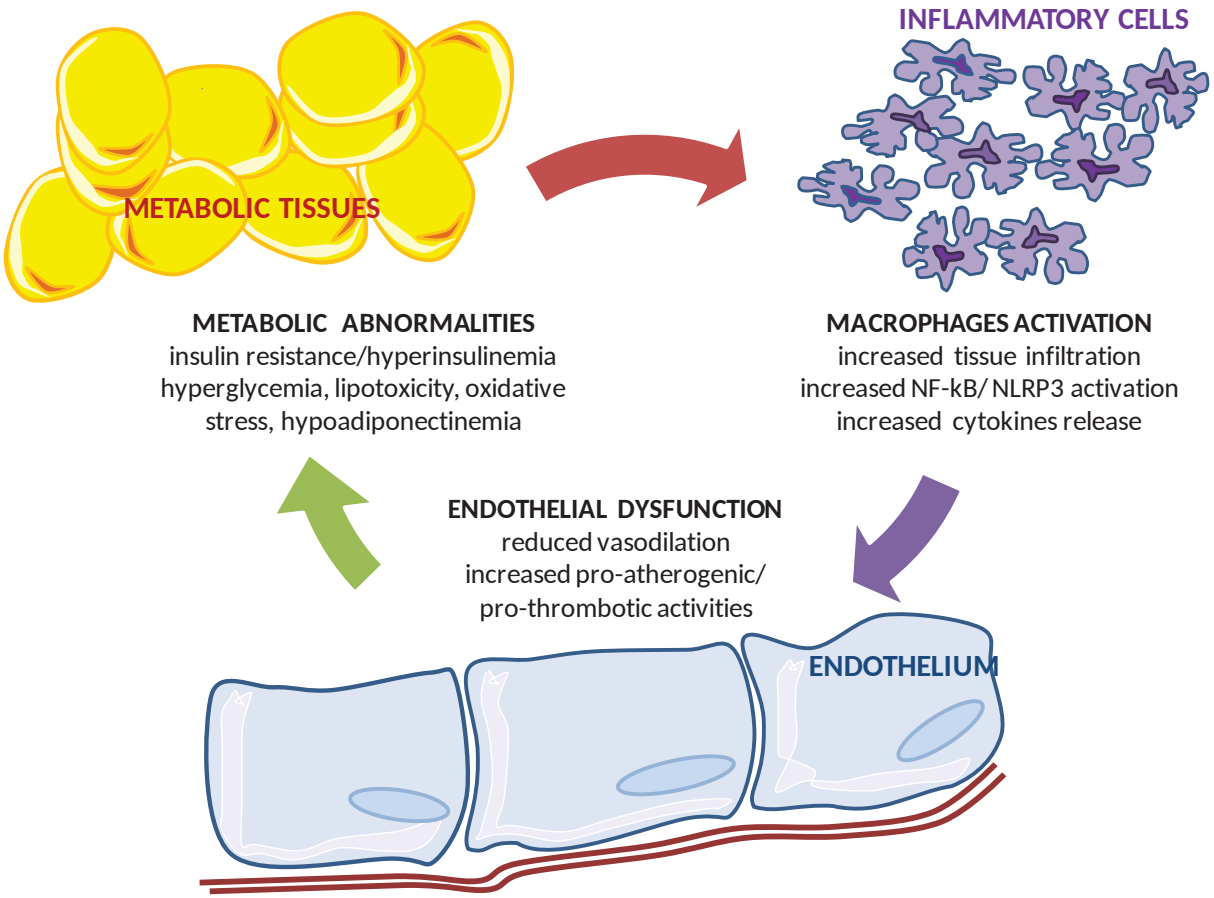

Figure 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes.

In the reciprocal relationship between metabolic abnormalities and vascular dysfunction, a key role has been attributed to inflammation: when endothelial cells undergo inflammatory activation by cytokines such as IL-1β and TNF-α, or by ox-LDL uptake via ox-LDL receptor-1 (LOX-1) this results in the increased expression of selectins and adhesion molecules that promote the adherence of monocytes. In turn, continued release of cytokines, such as MCP-1, by activated endothelial cells not only perpetuate inflammation but also contribute to lipid accumulation within the atheroma and dysregulated activity of underlying vascular smooth muscle cells (see [16] for review). The most significant mechanisms linking metabolic abnormalities, inflammatory signaling and endothelial dysfunction in diabetes are summarized below and schematically depicted in Figure 1.

Metabolic derangement triggers endothelial dysfunction - Metabolic derangements known to occur in diabetes include, among others, hyperglycemia, oxidative stress, excess free fatty acid release, and lipotoxicity, with insulin resistance and compensatory hyperinsulinemia underlying and driving all others. Each of these abnormalities results from complex rearrangement of physiological homeostasis and may impact on endothelial function individually [17,18], and/or participating in a vicious cycle, where every change worsen and reinforce all others [19]. The specific molecular mechanisms by which these disturbances disrupt NO synthesis or degradation, and their overall impact on endothelial function has been deeply investigated over time and extensively debated elsewhere [9,20].

One fundamental notion that helps to explain the tight link between diabetic metabolic abnormalities and vascular impairment is the well-recognized effect of several metabolic mediators

on hemodynamic homeostasis. Direct vascular effects have been documented, in addition to insulin [21], for gonadal steroids [22], for hormones like leptin [23] and ghrelin [24], and for a number of bioactive proteins secreted by adipose tissue and skeletal muscles termed "adipokines" and "myokines", respectively [25]. In the context of diabetic endothelial pathophysiology, the cardiovascular properties of insulin and adiponectin (Ad) are perhaps the most relevant in terms of current and perspective therapeutic approaches [26].

Insulin - Biological vascular actions of insulin have been clearly identified and progressively described among the last three decades (comprehensively described in [21]). In addition to systemic hemodynamic effects, related to renal reabsorption of sodium, or stimulation of sympathetic activity, insulin is capable to directly modulate endothelial release of both vasoconstrictor factors such as endothelin-1 (ET-1) and vasodilating mediators including NO. All these activities physiologically contribute to overall metabolic homeostasis [27], since insulin-mediated activation of eNOS and subsequent production of NO enhance local vasodilation, blood flow and nutrient delivery, thereby participating to efficient insulin-mediated glucose uptake on target tissues [28]. Production of NO in response to insulin depends on activation of insulin receptor tyrosine kinase (IR), and involves a signaling cascade leading to phosphorylation of eNOS on Ser^{1177} via a signaling pathway recruiting insulin receptor substrate-1 (IRS-1), phosphatidylinositol (PI) 3-kinase and Akt [29-33]. Concomitantly, the insulin-dependent activation of the Ras/MAP kinase signaling stimulates secretion of ET-1 from endothelial cells [34-36], and it is involved in insulin-stimulated expression of adhesion molecules including VCAM-1 and E-selectin [37]. Thus, by activating distinct intracellular signaling pathways, insulin modulates the endothelial production of mediators with opposing vascular effects. It is of particular interest that endothelial signaling pathways related to production of NO and metabolic signaling pathways regulating translocation of the glucose carrier GLUT4 are almost completely overlapping. Common insulin signaling in distinct tissues with metabolic or vascular functions helps to understand why a selective impaired sensitivity in the PI-3K/Akt signaling branch may greatly contribute to the pathophysiology of diabetic vascular complications [19]. On this respect, the emerging comprehension of the genes whose alteration confers an elevated risk to develop diabetes and insulin resistance might also help to predict the onset of insulin-dependent endothelial dysfunction: for example, polymorphisms in the IRS-1 gene (associated to impaired PI 3K binding and insulin secretion in the beta-cells) [38] might also predispose to defects in insulin-mediated NO production in endothelium. Similarly, genetic defects in the IR gene or in genes encoding downstream signaling proteins may affect vascular actions of insulin as well as metabolic activity in target tissues (extensively reviewed in [3]). In the next years, further analysis of results from the genome-wide association studies (GWAS) will be important to uncover additional gene variants and, hopefully, to turn these information into the identification of prognostic and predictive biomarkers of insulin resistance. In the context of diabesity, this would be of utmost importance since insulin-dependent endothelial dysfunction is known to precede and predict the onset of metabolic abnormalities.

Adiponectin - Adiponectin (Ad) is a protein hormone produced exclusively by adipose tissues with identified beneficial effects on insulin sensitivity and lipid metabolism [39]. In addition, Ad exerts multiple vasoprotective effects via its anti-inflammatory, antioxidant, antiapoptotic, antiatherogenic, vasodilatory, and antithrombotic properties on endothelial cells, monocytes, macrophages, leukocytes, platelets, and vascular smooth muscle cells [40]. The gene encoding Ad is located in the chromosome region 3q27, a locus mapped for susceptibility to both diabetes and cardiovascular risk factors. Among several single nucleotide polymorphisms (SNPs) examined in the Ad gene, SNPs at positions 45 [41,42] and 276 [43] have been linked to increased risk of cardiovascular events in diabetic individuals. These findings support the influence of Ad genetic variability on cardiovascular protection, especially in patients with diabetes [44]. Circulating Ad may exist as monomers, or form oligomers and multimers. The exact role for each of these multiple Ad conformations is not completely defined, but the high-molecular-weight (HMW) Ad is particularly active and equally able to bind both the AdipoR1 and the AdipoR2 to exert metabolic and vasoprotective effects in target

tissues [45]. On endothelial cells, Ad binding to AdipoR1/2 receptors activates AMP kinase (AMPK), that leads to an increase in eNOS activity and NO production via phosphorylation of eNOS at Ser^{1177} and Ser^{633} [46]. Other beneficial endothelial effects of Ad include its ability to counteract the expression of adhesion molecules and to suppress the TNF-α-mediated monocyte adhesion [47]. The signaling events linking AdipoR1/2 receptors to activation of AMP kinase/eNOS require, among others, the adaptor protein APPL1 [48], but the exact sequence of receptor downstream signaling cascade has not been completely elucidated. Nevertheless, APPL1 isoform is involved in cellular mechanisms resulting in Ad-suppressed foam cell formation [49], suggesting that the Ad-AdipoR1/2-APPL1 axis may be a potential therapeutic target for preventing endothelial activation and atherosclerotic processes. Interestingly, while production and release of proinflammatory cytokines increases proportionally to adipose mass under obesity, Ad levels are inversely reduced, and hypoadiponectinemia has been is considered a significant predictor of endothelial dysfunction in both the peripheral and coronary arteries besides other markers of the metabolic syndrome [50]. These clinical observations are supported by experimental findings showing that Ad-deficient animals are more prone to develop neointimal hyperplasia [51], impaired endothelium-dependent vasodilation [52], and high blood pressure [53]. Accordingly, exogenous administration of Ad has been effective to improve cardiovascular disease in animal models. At present, however, a number of concerns limit the possibility to extend this approach to humans: these include the high circulating levels needed, the unclear role of monomers, oligomers and multimers of Ad, and its moderately short half-life, together with the need to better characterize the extensive posttranslational modifications that might affect its potential beneficial activity in patients. In the meantime, interventions aiming at increasing the endogenous Ad levels or restoring tissue sensitivity to Ad may represent potential alternatives to prevent diabetes-related vascular disorders. On this respect, several nutraceutical compounds have been shown to enhance plasma Ad levels, including green tea extract [54] and resveratrol [55]. Similarly, a number of currently used pharmacological agents such as renin-angiotensin system blockers, bezafibrate and fenofibrate, thiazolidinediones, statins, and nebivolol are able to increase plasma Ad levels [40,56]. Although the contribution of Ad to overall effects of these drugs is not always straightforward, these findings altogether support the possibility to consider circulating Ad levels not only a biomarker of vascular disturbances under diabesity, but also an indicator of the effectiveness for specific treatments. Currently, total serum and urinary Ad levels may be measured by commercially available radioimmunoassay kit or enzyme-linked immunoadsorbent assays. However, laboratory methods still lack appropriate standardization, and the determination of ideally therapeutic Ad levels for each clinical setting requires further efforts [57].

Endothelial dysfunction contributes to metabolic abnormalities - The recognition that metabolic and endothelial physiology are fully integrated and reciprocally controlled suggests that, beside representing the immediate target of metabolic abnormalities, unbalanced endothelial function may in turn actively contribute to disrupted metabolic homeostasis [58]. This has long been hypothesized for angiotensin II [59], and more recently demonstrated for prostanoids [60] and NO itself [61]. The interference of ET-1 on metabolic function has been deeply investigated: ET-1 infusion results in hyperinsulinemia and insulin resistance in vivo [62], and chronically elevated ET-1 levels may contribute to desensitization of metabolic signaling pathways on adipocytes [63]. In addition, ET-1 inhibits adipocyte differentiation, reduces lipoprotein lipase activity, inhibits insulin-stimulated glucose uptake [64], and stimulates lipolysis [65]. ET-1 seems involved in production and secretion of Ad [66]. On this respect, we have recently provided evidence that increased circulating levels of ET-1 in obese children directly contribute to reduced levels of Ad via ET receptor-mediated activation of p42/44 MAPK signaling pathways [67].

The relationship between endothelial dysfunction and metabolic derangement is further complicated by the limited ability of endothelium to regenerate overtime. Physiologically, endothelial cell injury is at least partially mitigated by endogenous reparative processes mediated by bone marrow-derived endothelial progenitor cells (EPC) [68]. Under chronic exposure to damaging

events, however, EPC availability and/or mobilization tends to progressively decrease, and this may accelerate the onset of endothelial dysfunction [69]. Insulin resistance reduces EPCs survival and diminish their capacity for adhesion, endothelial integration, proliferation, and differentiation by multiple mechanisms [70] (see [71,72] for review). These involve, among others, a reduced NO bioavailability associated to increased oxidative stress and impaired PI 3K/Akt signaling, which are found to alter the cytoskeletal structure and decrease mobilization of EPCs in diabetic and obese patients [73-75]. Based on these data, EPCs have been proposed as cellular biomarkers of disease and predictors of cardiovascular outcomes. An updated definition of their role and their biological properties in health and disease has been recently published [76].

Inflammatory signaling links endothelial to metabolic impairments – In diabetes, glucotoxicity and lipotoxicity induce a proinflammatory trait in macrophages residing or invading the adipose tissue and the vasculature [77,78], and are responsible for oxidative and endoplasmic reticulum stress. This in turn elicits the activation of thioredoxin-interacting protein (TXNIP) and the NLR family, pyrin domain containing 3 (NLRP3) inflammasome, which increase the release of active interleukin (IL)-1β [79,80]. IL-1β-amplified inflammation increases the expression of various cytokines and chemokines, and favors the recruitment of macrophages in diabetic β-cells, adipose tissue, and blood vessels [19,78]. In turn, the unbalanced activity in endothelium may further enhance leukocytes adhesion, and increased release of inflammatory cells, thereby promoting lipids deposition and facilitating the atherosclerotic plaques formation. In addition, alterations in the gut microbiome along with increased gut leakiness of bacterial wall lipopolysaccharides (endotoxins) may further promote tissue inflammation [81]. Endotoxins, free fatty acids, and cholesterol induce inflammation by activating Toll-like receptor (TLR) pathways and, subsequently, nuclear factor-κB (NF-κB)-mediated release of a broad range of cytokines and chemokines including tumor necrosis factor (TNF), IL-1β, IL-8, and MCP-1 that promote the accumulation of various immune cells in different tissues [77,78].

The likelihood that blockade of vascular inflammation and oxidative stress may be effective to prevent metabolic disorders is supported by the finding that suppression of inflammatory processes in the vasculature prevents the onset of insulin resistance in metabolic target tissues and prolongs lifespan [82]. In a transgenic animal model, overexpression of the inhibitory NF-kB subunit IkBα in endothelium correlates with reduced macrophage infiltration in adipose tissue and decreased circulating markers of oxidative stress, concomitantly increasing blood flow, muscle mitochondrial content, and locomotor activity [82]. These findings confirm the pivotal role of the transcription factor NFkB in oxidative stress, vascular dysfunction, and inflammation, and further support the central role of endothelium in obesity-induced insulin resistance.

On the other hand, proinflammatory cytokines may play important roles in the pathogenesis of both endothelial dysfunction and insulin resistance [83]. Development of insulin resistance has classically been attributed to adipocyte-derived inflammation, resulting in macrophage infiltration and altered secretory profile of adipose tissue depots with increased levels of pro-inflammatory cytokines and decreased Ad synthesis and release [84-86]. The differential role of regional fat depots in synthesis and release of specific mediators and vasoactive substances may help to reconcile the generally accepted 'outside-in' theory of vascular inflammation, which postulates that inflammation begins in adipose tissue and then spreads inward to the vasculature [87], with the 'inside-out' process of vascular inflammation, proposing that the first step is intimal injury, that then extends to the media and adventitia [88,89]. For example, it is plausible that intimate connections between perivascular adipose tissue (PVAT) and other components of the vessel wall result in PVAT being the first adipose depot to sense and respond to signals from circulating bioactive factors that influence activity of endothelial and vascular smooth muscle cells [90-92].

On this regard, another important inflammatory pathway, the NLRP3 inflammasome, has been proposed to be involved in the vasculoprotective effects exerted by Ad [93]. The NLRP3 inflammasome is a large multimeric protein complex mediating the cleavage of inactive

prointerleukin- (IL-) 1β and IL-18 into their active form [94]. We have recently provided evidence that activation of NLRP3 inflammasome contributes to the development of insulin resistance and diet-induced renal and myocardial dysfunctions, mainly by inducing IL-1β and IL-18 overproduction [95-97]. Activation of the endothelial NLPR3 inflammasome by injurious adipokines such as visfatin may disrupt inter-endothelial junctions and increase paracellular permeability of the endothelium contributing to the early onset of endothelial injury during metabolic disorders [98]. The role of NLRP3 inflammasome in evoking tight junction disruption induced by high glucose has been demonstrated both in vivo and in vitro, resulting in increased release of the high mobility group box protein-1 (HMGB1), which enhances permeability of endothelial monolayers possibly via its autocrine or paracrine action on receptor for advanced glycation end products (RAGE)-mediated pathway [99]. Using established mouse models of coronary arteritis, recent studies have implicated NLRP3 inflammasome as a major intracellular molecular machinery able to switch on the inflammatory responses that contribute to the development of endothelial dysfunctions leading to atherosclerotic acceleration [100, Tomita, 1993 #125,101,102]. Such activation of the endothelial NLPR inflammasome is due to lysosome membrane permeabilization and cathepsin B release and can be suppressed by lysosome stabilization agents [103]. Thus, the Ad-induced inhibition of NLRP3 inflammasome activation, as recently documented [104] may represent an original and pivotal cross-talk mechanism to mitigate endothelial dysfunctions due to metabolic derangements.

HOW CONVENTIONAL DIABETIC TREATMENTS MAY AMELIORATE ENDOTHELIAL DYSFUNCTION

Current therapeutic options to treat type 2 diabetes aim at reducing plasma glycemic levels by increasing insulin pancreatic secretion and/or ameliorating insulin sensitivity in peripheral tissues. Although blood glucose normalization and increased insulin sensitivity may *per se* prevent endothelial dysfunction and reduce low-grade inflammation via indirect interrelated mechanisms, a growing number of clinical studies have been conducted to ascertain the specific effects on endothelial function and inflammatory signaling pathways for most of the drugs used (see [105,106] for review). Beside insulin and its analogues (reviewed in [107]), conventional anti-diabetic drugs include biguanides compound metformin, insulin secretagogues, insulin sensitizers, and inhibitors of the alpha-glycosidase. Incretin analogs such as glucagon-like peptide 1 (GLP-1) agonists and dipeptidyl peptidase-4 enzyme (DPP4) inhibitors represent novel classes of anti-diabetics [108,109]. Finally, sodium-glucose co-trasporter-2 (SGLT-2) inhibitors have been more recently introduced on the market and successfully included among therapeutic options to treat diabetes [110,111].

Although extensive revision of vascular effects for all conventional anti-diabetic drugs is beyond the scope of this review, metformin – despite being the oldest drug used - deserves some attention for its multiple effects on endothelial and vascular cells, and for its potential "anti-inflammatory" activities. In addition, we briefly describe thiazolidinediones, whose specific effects on endothelial function have prompted the current recommendation to explicitly evaluate cardiovascular effects for all compounds investigated for diabetes. The vascular profile of incretins and DPP4 inhibitors, and the profile of SGLT-2 inhibitors in terms of endothelial and vascular function is also introduced (Figure 2).

Metformin - This synthetic dimethyl biguanide has been in clinical use for over 55 years. Despite almost 6 decades of research, the cellular mechanisms that underlie the cardioprotective effects of metformin are not completely understood, but a number of clinical studies report an improved endothelial function associated to increased flow-mediated vasodilatation in patients treated with metformin (reviewed in [112]). On one side, the vascular protective actions of metformin are thought to be secondary to the antihyperglycemic effects of this drug, mediated via activation of adenosine 5′-monophosphate–activated protein kinase (AMPK) and subsequent inhibition of hepatic gluconeogenesis, fatty acid oxidation and insulin sensitizing action in striated muscle and adipose tissue [113]. On the other hand, data from both clinical and bench studies

indicate that metformin has a direct action on the endothelium that seem to involve a reduction in oxidative stress secondary to modulation of mitochondrial complex 1, and activation of signaling pathways controlled by the deacetylase Sirtuin 1 (SIRT-1) [114]. In addition, metformin possesses anti-inflammatory effects secondary to inhibition of cytokine-induced activation of NF-κB-mediated pathways in endothelial cells [115], and it has been shown to enhance eNOS activation via an AMPK-dependent signaling [116,117]. Activation of AMPK/eNOS signaling by metformin seems also responsible for the ameliorated angiogenic function of bone marrow-derived EPC [118,119]. Pharmacogenetic studies have suggested that therapeutic responses to metformin may differ inter-individually due to some polymorphisms in the genes encoding for organic cation transporters (OCTs), responsible for metformin active transport across membranes into the intestinal epithelial cells, hepatocytes and renal tubular cells. This may result in metformin intolerance in some subjects, while in others might explain the reduced efficacy of this drug in controlling both metabolic and vascular disturbances [120].

Thiazolidine-2-4-diones (TZDs) – These insulin sensitizer agents are exogenous activators of the peroxisome proliferator-activated receptor-γ (PPAR-γ) [121]. TZDs may improve endothelial function, increase forearm blood flow and reduce blood pressure in humans by both direct and indirect mechanisms (reviewed in [122]). In endothelial cells, TZDs-mediated improvement of PI 3-K signaling pathways results in increased protein expression of eNOS [123] and subsequent enhanced production of NO in response to insulin and other mediators acting via PI 3-kinase-dependent pathways [124]. TZDs may also improve eNOS activity and NO bioavailability by decreasing eNOS uncoupling, reducing generation of superoxide anion [125] and diminishing ADMA levels in vessels from diabetic patients [126]. Among TZDs, rosiglitazone has been shown to counteract hyperglycemia-mediated oxidative stress not by a PPARγ activation mechanism, but rather as a consequence of AMPK-dependent signaling pathways in endothelium [127]. Activation of AMPK in response to rosiglitazone correlates with inhibition of the DAG/PKC pathway, subsequent reduction of NADPH oxidase activity, and amelioration of oxidative balance [127].

Anti-inflammatory effects of TZDs on NF-kB/STAT/AP-1 signaling pathways correlate with a marked decrease in the inducible NOS isoform (iNOS) expression, which in turn decreases the amount of reactive oxygen species produced in monocyte/macrophages and in target tissues of metabolic derangements [128-131]. Moreover, both pioglitazone and rosiglitazone reduce plasma levels of TNF-α, leptin, PAI-1 and C-reactive protein, decrease vascular expression of adhesion molecules, and significantly improve circulating levels of Ad [132]. These activities, associated to the reduced expression of matrix metalloproteases-9 (MMP-9) [133], decreased levels of both FFA and atherogenic LDL cholesterol particles [134], and reduced PAI-1 plasma levels, may contribute to maintain plaque stability, prevent platelet aggregation and inhibit thrombus formation. The significant decrease in systolic blood pressure (SBP) observed in diabetic patients treated with TZDs further supports their ability to improve endothelial function [135].

Thus, when the first meta-analysis reported a significant increase in myocardial infarction (MI) and in cardiovascular-related risk death (CVD) in patients assuming rosiglitazone [136], the long list of apparently beneficial effects of TZDs on endothelial cells and vascular function generated a rather confusing reaction [122]. Whether the puzzling results were related to the single drug rosiglitazone or to the whole class of TZD is still an incompletely resolved question. However, single nucleotide variations found in PPAR-γ gene might, at least in part, help to explain the different therapeutic outcome in patients treated with TZDs [137]. Analogously, variants of the gene encoding for CYP2C8 hepatic enzyme may impair rosiglitazone clearance and hence contribute to the degree of therapeutic/harmful effects of TZDs in particular subjects [138]. While the European Medicine Agency (EMA) recommended withdraw of all licensed rosiglitazone-containing drugs in 2010, the FDA opted for rosiglitazone use restriction in the US [139]. However, following the rosiglitazone lesson, from 2008 all drugs investigated for diabetes must also undergo specific clinical trials evaluating their cardiovascular safety profile.

Glucagon-like peptide-1 receptor (GLP-1R) agonists - Incretins are peptides produced by the gastrointestinal system able to enhance insulin secretion in a glucose-dependent manner [140]. The two main human incretins are glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) whose secretions are impaired in diabetes. GLP-1-mediated effects may be sustained and improved with two strategies currently available: longer-acting GLP-1 receptor agonists resistant to degradation of the dipeptidyl peptidase 4 (DPP4) enzyme, that can provide supraphysiological stimulation of GLP-1 receptor [141]; and inhibitors of DPP4 enzyme, that extends the half-life of endogenous GLP-1 [142].

The receptor for GLP-1 (GLP-1R), originally identified in pancreatic cells, is also expressed on endothelial cells, cardiomyocytes and coronary smooth muscle cells [143]. Activation of GLP-1R improves myocardial function, improves endothelial function in high risk cardiac patients, and enhances natriuresis, with potential positive implications for systolic blood pressure control [144]. Beneficial effects of GLP-1 on endothelial cells are mediated by an increased NO production, and in endothelial cells from human coronary arteries GLP-1R agonists stimulate proliferation via eNOS-, PKA-, and PI3K/Akt-dependent pathways [145]. It has also been shown that GLP-1 exerts a protective effect on atherosclerosis by reducing neointimal formation [146], foam cell formation, and atherosclerotic lesion size [147].

The GLP-1 receptor agonists (GLP-1Ra) are peptides and, such as insulin, require subcutaneous injection to avoid degradation by gastrointestinal enzymes. Most of the clinical trials, in line with animal studies, have reported a reduction of blood pressure after chronic treatment of GLP-1R agonists [148-151]. This may be explained, at least in part, by the ability of GLP-1 analogs to upregulate anti-oxidative enzymes and inhibit NFkB-mediated inflammatory signaling on endothelial cells [152,153], and to reduce oxidative stress by suppressing MAPK signaling pathways in peripheral lymphocytes of type 2 diabetic patients [154]. GLP-1-associated improvement of several cardiovascular markers suggests that therapies with GLP-1R agonists may have a positive effect on cardiovascular risk factors in patients with diabetes. Nevertheless, their long-term safety profile and the direct clinical benefit to cardiovascular outcomes remain to be determined [155]. Pharmacogenetic studies on this class of drugs are still limited.

Dipeptidyl peptidase 4 (DPP4) inhibitors - DPP4 is a widely expressed membrane serine exopeptidase involved in degradation of various oligopeptides, including GLP-1. DPP4 is anchored to the cell membrane but, under certain circumstances, may be released in soluble form. Endothelial cells are the main source of soluble DPP4 form, whose expression and enzymatic activity may increase after chronic exposure to high glucose concentrations [156].

Currently approved DPP4 inhibitors include sitagliptin, saxagliptin, linagliptin and vildagliptin [157]. These small molecular-weight substances inhibit more than 90% of DPP4 activity and can be orally administered. Several studies in animal models support the evidence of DPP4 inhibition in improving endothelial function and blood pressure [158,159]. In isolated aorta rings incubated with DPP4-inhibitor, the relaxant effect of DPP4-inhibitor is GLP-1 independent and results from Akt posphorylation and eNOS activation with a rapid increase in NO levels [160]. Saxagliptin treatment has been shown to reduce blood pressure levels in the spontaneously hypertensive rats with a concomitant increase of aortic and glomerular NO release and comparable reductions in peroxynitrite levels [161]. There is good evidence that DPP4 inhibition mediates protective effect on myocardial infarction, hypertension and atherosclerosis. Pharmacological treatment with sitagliptin has been able to enhance the expression of cardioprotective proteins and improve heart functional recovery after I/R injury [162]. It is not clear how these potential benefits may be mediated, but one possibility involves the DPP4 inhibitors ability to modulate innate and adaptative immunity by suppressing the NF-kB signaling downstream TNF and IL-6 [143,159], and by inhibiting the NLRP3 inflammasome, a multiprotein complex involved in caspase-1 activation and downstream maturation of pro-inflammatory cytokines, TLR4 and IL1β in human macrophages [163].

In contrast to the positive effects in animal experiments, the consequences of treatment with DPP4 inhibitors on endothelial functions in humans have not always been consistent. In a double-blind study on patients with diabetes, treatment with vidagliptin for 4 weeks improved forearm blood flow in response to intra-arterially delivered acetylcholine [164]. On the contrary, other studies measuring FMD of the brachial artery have shown diametrically opposite results, suggesting that sitagliptin and alogliptin actually seem to worsen flow-mediated dilation (FMD) when used to treat diabetic patients [165]. At present, clinical evidence supporting the vascular protective effects of gliptins is uncertain, given the relatively short follow-up [166-168]. Future and ongoing studies (CAROLINA, http://clinicaltrials.gov/show/NCT01243424; TECOS, https://clinicaltrials.gov/show/NCT00790205) should help determine whether DPP4-inhibitors may contribute to improve cardiovascular outcomes in patients with T2DM.

Sodium glucose co-transporter 2 (SGLT-2) inhibitors. The isoform 2 of the sodium glucose transporter (SGLT-2) is located in the proximal convoluted tubule of the kidney and it reabsorbs approximately the 90% of filtered glucose. Inhibition of SGLT-2 represents a novel strategy for achieving glucose control in diabetic patients. It is based on a mechanism of action that targets the kidney to promote urinary glucose excretion and reduce hyperglycemia, and is therefore independent of pancreatic β-cell function or the degree of insulin resistance [169,170].

Among the SGLT-2 inhibitors, canagliflozin, dapagliflozin, and empagliflozin are currently used. Other gliflozins include ertugliflozin and sotagliflozin and, in Japan, ipragliflozin, tofogliflozin and luseogliflozin. Most of clinical trials of SGLT-2 inhibitors show that patients receiving dapagliflozin, canagliflozin, or empagliflozin as add-on therapy exhibit reductions in systolic blood pressure of approximately 3-5 mmHg versus placebo [171,172]. One possible explanation is that urinary glucose excretion stimulated by inhibition of SGLT-2 causes a diuretic effect responsible for lowering blood pressure levels. However, a recent study on dapagliflozin proposed that SGLT-2 inhibitors may possess an additional diuretic-like capacity to lower blood pressure [173]. The improvements in blood pressure, associated to a moderate decrease in body weight [174] induced by treatment with SGLT-2 inhibitors suggest the potential for reducing the risk of cardiovascular events [175,176]. To date, SGLT-2 inhibitors are generally well tolerated, with a favorable safety profile similar to that of placebo. As easily expected, common adverse effects of SGLT-2 inhibitors include genital tract infections and osmotic diuresis. Results from large cardiovascular trials underway for dapagliflozin, canagliflozin and empagliflozin will likely provide greater insights into the effects of SGLT-2 inhibition on cardiovascular outcomes [177].

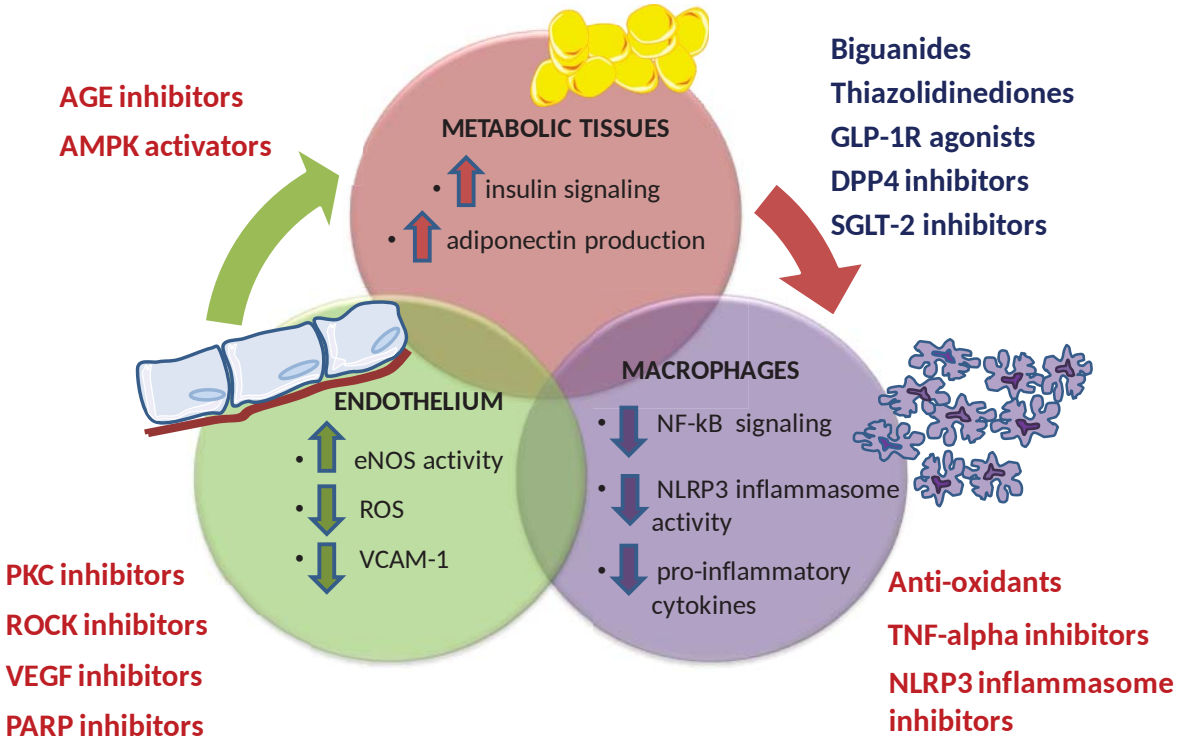

Figure 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction.

NEW STRATEGIES FOR TREATMENT OF DIABETES AND THEIR IMPACT ON ENDOTHELIAL DYSFUNCTION

The mounting understanding of pathophysiological mechanisms concurring to endothelial dysfunction in diabetes has undoubtedly multiplied the number of potential therapeutic targets for preventing or delaying its vascular complications. Among emerging strategies, molecules targeting inflammatory conditions, AGEs formation, oxidative stress or disrupted intracellular metabolic signalings may independently improve various aspects of diabetic endothelial dysfunction and are foreseen as add-on treatments. For the majority of these new molecules their possible use in clinical practice is still far from being achieved; nevertheless, they may represent the rationale alternative/additional approaches to treat or prevent vascular complications of diabetes (Figure 2).

Anti-inflammatory drugs - Increasing evidence suggests that metaflammation plays an important role in the pathogenesis of diabetes-related vascular complications, therefore suggesting that targeting inflammation may ameliorate diabetes, preventing its progression and delaying vascular complications. This concept is supported by the notion that current drugs including insulins, statins and metformin may improve diabetic symptoms by alleviating systemic and tissue-specific inflammation [178]. Since the effects of immunomodulatory treatments are not limited to tissues involved in disease pathophysiology and might have unwarranted side effects, the added value of using specific immunomodulatory treatments needs to be confirmed. Nevertheless, for some of these drugs including anti-TNFα antibody infliximab, or anti-IL1β receptor antagonist anakinra, a number of experimental and observational findings suggest their possible role on endothelial protection [92,179,180]. At present, well-designed clinical studies are still missing.

NLRP3 inflammasome inhibitors - Dietary phytochemicals, mainly flavanols, have been demonstrated to exert their vascular beneficial effects by multiple mechanisms (see below paragraph

on ***Polyphenols***). However, most recent evidence from the literature demonstrates that inhibition of the NLRP3 inflammasome may significantly contribute to the beneficial effects exerted by few of them [181]. For instance, the B-type procyanidins (PCB) have been found to suppress the activity of NLRP3 inflammasome on endothelium via the inhibition of AP-1 pathway, a transcriptional machinery involved in endothelial production of pro-inflammatory adhesion molecules and chemokines [182]. Similarly, corosolic acid, a natural triterpenoid with antioxidative activity, is supposed to protect endothelial homeostasis by suppressing NLRP3 inflammasome activation and preventing mitochondrial damage [183]. The epigallocatechin-3-gallate (EGCG), the most abundant active polyphenolic component of green tea, is capable of inhibiting NLRP3 inflammasome via enhancing the Nrf2 antioxidant pathway [184]. Moreover, the protective effects of astragaloside IV and cycloastragenol – both contained in Astragalus membranaceus Moench (Fabaceae) - against endoplasmic reticulum stress-induced apoptosis are thought to be mediated via NLRP3 inflammasome inhibition in endothelial cells [176,185]. NLRP3 inflammasome inhibition has also been proposed as new pivotal mechanism contributing to endothelial protective function of rutin, a flavonoid that can be obtained from different dietary sources [186]. Another group of natural anthocyanin derived from purple sweet potato, named purple sweet potato color (PSPC), seems to attenuate atherosclerotic progress in an insulin-resistant mice model by suppressing premature senescence of endothelium throughout inhibition of NLRP3 inflammasome [187].

Despite these interesting experimental findings, so far, the selective mechanism(s) underlying their inflammasome-suppressing effects remain largely unclear. Thus, the described activities could be due to interferences up- or downstream of inflammasome activation, and the availability of selective NLRP3 inflammasome inhibitors is an essential prerequisite to include the NLRP3 inflammasome in the list of potential pharmacological target for endothelial protection. At present, efficacious NLRP3 inflammasome inhibitors are still under development. We recently contributed to characterize the cardiovascular effect of the small molecule INF4E, one of the few compounds that has been demonstrated to directly target the NLRP3 inflammasome and inhibit the ATPase activity of NLRP3 required for its activation [97]. Similar cardiovascular protective effects have been recently documented by using another small molecule which prevents the formation of the NLRP3 inflammasome complex in cardiomiocytes, thus ameliorating cardiac function after ischemia/reperfusion injury [188]. Although there are clear indications that these compounds exert their cardioprotective effects against myocardial ischemia/reperfusion injury via a specific effect on NLRP3 inflammasome, the exact mechanism of action has still to be clarified and further insights are needed to demonstrate potential direct endothelial effects. However, the ability of members of the NLRP3 inflammasome protein complex to target molecular and cellular pathways involved in both metabolic and cardiovascular diseases suggest that selective pharmacological modulation of NLRP3 inflammasome has the potential to exert synergistic effects in the control of metabolic disorders and related cardiovascular complications. The prospective clinical relevance of this strategy is also supported by recent investigations on the influence of genetic variability in inflammasome on long-term cardiovascular complications in diabetic patients. A few studies have reported a relationship between NLRP3 genetic polymorphisms and development of type 2 diabetes [189,190] and, most notably, a specific polymorphic NLRP3 allele has been reported to be associated with increased risk for development of macrovascular complications in subjects with long-term diabetes [191].

AGE inhibitors – The renowned deleterious effects of AGEs on endothelial function and vessel structure has prompted numerous studies on molecules with promising blocking effects on AGE formation and AGE-receptor (RAGE)-mediated activity, or acting to prevent or disrupt AGE–protein cross-links (reviewed in [192-194]). Aminoguanidine is able to inhibit the formation of AGEs by interaction with and quenching of dicarbonyl compounds. Despite the reported *in vivo* ability to attenuate the formation of diabetes-induced AGEs and consequently reduce the extent of cross-linking of connective tissue proteins in the arterial wall [195], the unfavorable risk/benefit ratio discourages the use of aminoguanidine in the clinical setting [196, 197]. Another compound, alagebrium chloride (ALT-711), which cleaves AGEs and protein cross-links thereby facilitating AGEs

clearance, appears to have a more encouraging safety profile; however, protective effects on endothelial function have been not consistent in patients with isolated systolic hypertension [198], atherosclerosis [199], or chronic heart failure [200]. Although B vitamins (pyridoxamine, thiamine and its derivative benfotiamine) and pyridoxamine analogue ALT-946 may improve endothelial dysfunction [201] and prevent AGE-related complications through inhibition of AGE-dependent oxidative damage [202], some side effects on kidney function and creatinine levels [203,204] together with uncertainty on overall beneficial effects makes the clinical utility of these drugs still controversial [205].

PKC inhibitors – Impaired endothelium-dependent vasodilation secondary to hyperglycemia may be significantly improved by administration of PKC-β inhibitors [206]. Based on this observation ruboxistaurin, a selective PKC-β inhibitor, has been proposed to improve retinal blood flow distribution and decrease macular edema in diabetic patients [207,208]. In addition, treatment with this molecule has been show to ameliorate diabetic peripheral neuropathy without significant adverse effects [209]. Promising results were obtained in two combined phase III clinical trials assessing the ability of ruboxistaurin to reduce visual loss of 50% above standard care [210]. More recently, ruboxistaurin has been proposed as a potential treatment for reducing atherosclerotic plaques in diabetic patients. Since PKC activation promotes endothelial dysfunction by de-regulating IL-18/IL-18BP pathway, leading to increased VCAM-1 expression, monocyte/macrophage adhesion, and accelerated atherosclerotic plaque formation, inhibition of PKC by ruboxistaurin may represent a potential new mechanism to ameliorate endothelial dysfunction in diabetic patients [211].

VEGF inhibitors – Activation of tyrosine kinase receptors VEGFR-1 and VEGFR-2 [212] by increased levels of VEGF is associated with neovascularization in proliferative diabetic retinopathy as well as diabetic macular edema (DME) [213]. Strategies to inhibit the action of VEGF have to consider that VEGF activity is vital in processes such as angiogenesis in the myocardium and wound healing [214]. Therefore, although systemic anti-VEGF treatments are approved and used for other clinical conditions, their serious side effects in the context of diabetes must be carefully evaluated [215]. On the other hand, intraocular administration of VEGF inhibitors such as ranibizumab, bevacizumab, pegaptanib, and aflibercept has been approved for treatment of diabetic retinopathy. Ranibizumab is a recombinant antigen-binding Fab fragment of humanized anti-VEGF monoclonal antibody with a high ability to penetrate through the retina. At present, ranibizumab is the only compound approved for the treatment of visual loss due to DME [216]. Extended pharmacovigilance studies, however, are required to confirm the long-term ocular and systemic safety of ranibizumab treatment in patients with DME [217]. Bevacizumab, initially developed for intravenous treatment of metastatic colorectal cancer, has been adapted for off-label use in an intraocular administration [218] and it seems effective in decreasing retinal, disc and iris neovascularization in diabetic patients with proliferative retinopathy and macular edema [219] [220,221]. Debates remained in the past years on whether bevacizumab is superior to ranibizumab in terms of potency, efficacy and safety (reviewed in [221]). Results from the Diabetic Retinopathy Clinical Research (DRCR) phase II and Bevacizumab Or Laser Therapy (BOLT) studies showed favorable effects of intravitreal bevacizumab administration for the treatment of diabetic ocular neovascularization [222,223].

PARP inhibitors - The overactivation of the nuclear enzyme poly (ADP ribose) polymerase 1 (PARP-1) is implicated in acute endothelial dysfunction of diabetic vasculature [224,225]. Therefore, pharmacological inhibition of PARP-1 appears a potential strategy to approach diabetic vascular complications [226]. Experimental studies indicate a potential beneficial role of PARP-inhibition in diabetic retinopathy [227] and in coronary arteriole dysfunction of db/db mice [228]. The therapeutic effects of PARP-1 inhibitors are currently evaluated in clinical studies as potential candidates in cancer or cardiovascular diseases including cardiovascular complications of diabetes [229].

ROCK (Rho-associated kinase) inhibitors - The RhoA/ROCK signaling pathway mediates vascular smooth muscle contraction, downregulates eNOS gene expression and reduces protein kinase B/Akt activation, therefore decreasing eNOS phosphorylation and catalytic activity [230].

Elevated levels of peroxynitrites have been recently associated to an increased RhoA activity, largely responsible for vascular dysfunction in experimental diabetes [231]. On the same line, diabetes-induced endothelial aortic dysfunction is improved in ROCK knockout mice [232]. ROCK inhibitors such as Y-27632 and fasudil have shown promising therapeutic advantages on cardiovascular diseases including atherosclerosis, pulmonary and systemic hypertension and chronic heart failure [233,234], as well as significant beneficial effects on diabetic endothelial dysfunction of retinal [235], coronary [236] and intrarenal arteries [237]. In addition, fasudil has been able to limit TNF-alpha-mediated ICAM-1 expression and eNOS dephosphorylation in diabetic microvasculature [238]. Newer ROCK inhibitors with higher specificity among ROCK isoforms might represent potential therapeutic approaches to treat vascular complications associated to diabetes [239,240].

AMPK activators – AMPK is a serine/threonine protein kinase which plays a major role in regulating cellular and metabolic homeostasis, insulin sensitivity and mitochondrial function [241]. AMPK, activated in response to a variety of metabolic mediators, is known to regulate endothelial function and eNOS activity [242] and may contribute to ameliorate vascular endothelial function by suppressing diabetes-enhanced degradation of GTP-cyclohydrolase [243]. AMPK also suppresses inflammation, and very recently it has been reported that pharmacological activation of AMPK inhibits the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway by phosphorylating two residues (Ser^{515} and Ser^{518}) within the Src homology 2 domain of JAK1 [244]. Decreased AMPK activity has been found associated to endothelial dysfunction, apoptosis and altered lipid metabolism in aortic endothelium of obese rats [245]. Various natural compounds such as resveratrol, berberine and α-lipoic acid are able to activate AMPK *in vivo* and *in vitro*, resulting in beneficial vascular effects [246]. Numerous pharmacological agents currently used for the treatment of diabetes including biguanides, thiazolidinediones and GLP-1R agonists are able to indirectly activate AMPK [247]. Other pharmacological AMPK activators such as A-769662 and 5-Aminoimidazole-4-carboxamide roboside (AICAR) already tested in animal studies, are unlikely to be used in patients with diabetes or metabolic syndrome due to poor bioavailability and short half-life [246]. AMPK-activated signaling is involved in cardiac and vascular protective effects of Ad [46,248], whose reduced levels under diabetes are improved by numerous natural products such as fish oil, linoleic acid, green tea extracts and pharmacological agents including statins, renin-angiotensin system blockers, PPAR-α agonists and PPAR-γ agonists [40]. Despite the well-known beneficial effects of Ad, no exogenous Ad-based drugs have been developed so far [249]. In part, this may be due to concerns (see paragraph on ***Adiponectin***) related, for example, to the proteic nature of Ad that renders its oral administration ineffective, or to the unclear role of several multimeric Ad isoforms. Thus, therapeutic strategies aimed to increase endogenous Ad levels or activity are currently pursued to prevent diabetes-linked cardiovascular complications. Recently, orally active AdipoR1 and AdipoR2 agonists have been tested in animal models with promising results [250].

Anti-oxidants - Anti-oxidants is a general term to indicate a heterogeneous group of synthetic or natural substances which may potentially counteract oxidative stress by direct radical scavenging or indirect upregulation of endogenous enzymes and cytoprotective proteins. Anti-oxidant molecules may protect from endothelial dysfunction by re-coupling eNOS activity, reduce superoxide production, increase the activity of superoxide scavenging enzymes, or decrease vascular NAD(P)H oxidase activity [251,252]. Novel antioxidant therapies aiming to restore production of endothelial-derived NO or to act as endogenous antioxidant enzymes may represent a more targeted strategy focusing specifically on the mechanisms implicated in diabetes-induced endothelial dysfunction (reviewed in [240].

-Vitamin C and Vitamin E - Despite high expectation, administration of traditional antioxidants such as ascorbic acid (vitamin C) or tocopherol (vitamin E) have provided disappointing outcomes in clinical studies. Oral treatments with vitamin C or vitamin E have given controversial results on postprandial endothelial dysfunction and amelioration of forearm

vasodilation in diabetic patients [253-256]. In part, these frustrating results may be explained by the non-selective scavenging properties of vitamin C and E, which probably interfere with physiological important signaling mediated by ROS. Recent experimental studies have ascribed the protective effects of vitamin E to reduced oxidative stress and apoptosis in experimental diabetic cardiomyopathy and to decreased Ox-LDL mediated oxidative stress and vascular muscle cell proliferation in aortic wall [257,258]. Clinical studies suggest that a combination of vitamin C and insulin or a simultaneous infusion of GLP-1 and vitamin C may help to normalize endothelial dysfunction and reduce both oxidative stress and inflammation in diabetic patients [259,260], but the antioxidant potential of vitamin C and E in type 2 diabetic complications is still controversial [261-263].

- ***Polyphenols*** - Polyphenols contained in fruits, vegetables, and beverages are well known for their anti-oxidant properties [264]. Scientific interest in polyphenols as therapeutic agents is constantly increasing, and results from several experimental studies suggest that these compounds may improve endothelial function by multiple mechanisms related, but not limited, to the ability to increase eNOS expression and prostacyclin production, or to inhibit ET-1 and endothelial NADPH oxidase activity, or via more complex intercellular activities that reduce matrix metalloproteinase (MMP) activation, inhibit vascular cells migration and proliferation, and modulate angiogenesis. Polyphenols contained in cocoa, purple grape juice, red wine, black and green tea, coffee and berry have also shown the ability to acutely and chronically inhibiting platelet activation and aggregation. Moreover, for flavanols and flavonols, prevention of vascular injury has been proposed to involve counterregulation of AGE-mediated toxicity, low density lipoprotein oxidation, or inhibition of inflammatory responses (see [265,266] for complete review). Recent studies indicate that resveratrol is able to reverse the effects of hyperglycemia on mitochondrial function in endothelial cells, exerting protective actions in the early diabetes-associated endothelial dysfunction [267]. However, many studies reporting biological effects of food polyphenols are limited by the insufficient elucidation of the molecular, cellular, and physiological mechanisms underlying their effects. Obstacles in this field may include non-specific effects of polyphenols with pleiotropic activities, and complex interference among distinct active principles from the same food or beverage. In addition, based upon the structure of the particular polyphenol and the cellular redox context, most of these molecules may disclose both anti-oxidant and/or pro-oxidant properties. Finally, effects of polyphenols are highly dependent on cell type, stress conditions, and concentrations reached at the site of action. Thus, the clinical applicability of effects observed *in vitro* must be proven, since absorption through the gut may significantly reduce bioavailability and tissue concentration *in vivo*. Clinical studies addressing these issues and demonstrating the potential benefits of polyphenols are still awaited.

CONCLUSIONS

In the clinical practice, management of diabetes, obesity and cardiovascular risk factors is not always integrated. Nevertheless, the close association between metabolic disorders and a cluster of cardiovascular disturbances underscores the importance of a jointed approach to prevention and treatment. Experimental and clinical studies have progressively increased our understanding on molecular mechanisms underlying the tight and reciprocal cross-talk between hemodynamic and metabolic regulation. The role played by the endothelium on cardiovascular risks associated to diabetes and obesity may offer an integrative point of view: not only endothelial dysfunction might represent a potential unifying target of current and perspective treatments, but also provide a potential tool to assess the effectiveness of therapy. In this review, we have described the large body of data associating endothelial dysfunction to the pathogenesis of vascular complications in diabesity, focusing on inflammatory signaling as a common pathogenetic mechanism. Based on current evidence, treatments aiming at reducing glucotoxicity and lipotoxicity, simultaneously

improving metabolic homeostasis and inflammatory reaction, may effectively delay the progression of endothelial dysfunction and reduce the risk of cardiovascular events in the majority of diabetic patients. Concomitantly, the inter-individual susceptibility to diabetes and obesity, as well as the recognition of individual response to pharmacotherapy due to polymorphisms in genes encoding drug-metabolizing enzymes, transporters, receptors and signal transduction molecules is opening new roads to comprehend the variability existing in clinical outcomes of drugs used in diabesity. Overall, the choice of treatment for patients with diabesity should take into account the specific characteristics of the patient, the disease and the medication, aiming to a true personalized medicine. In this respect, the identification of intracellular signaling pathways and soluble mediators acting on metabolic, inflammatory and vascular cells has significantly broaden the spectrum of therapeutic opportunities to treat micro- and macrovascular complications of diabetes. Despite clinical substantiation is still far away, the current awareness of the key role played by the endothelium and its influence on vascular inflammation may represent an important step to develop promising new strategies to improve vascular dysfunction in diabesity.

FIGURE LEGENDS

Figure 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes.

Figure 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction.

REFERENCES

1 Astrup A, Finer N. Redefining type 2 diabetes: 'diabesity' or 'obesity dependent diabetes mellitus'? Obes Rev 2000;1:57-59.

2 Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med 2008;358:580-591.

3 Brunetti A, Chiefari E, Foti D. Recent advances in the molecular genetics of type 2 diabetes mellitus. World J Diabetes 2014;5:128-140.

4 Manolopoulos VG, Ragia G, Tavridou A. Pharmacogenomics of oral antidiabetic medications: Current data and pharmacoepigenomic perspective. Pharmacogenomics 2011;12:1161-1191.

5 Singh S, Usman K, Banerjee M. Pharmacogenetic studies update in type 2 diabetes mellitus. World J Diabetes 2016;7:302-315.

6 Glauber HS, Rishe N, Karnieli E. Introduction to personalized medicine in diabetes mellitus. Rambam Maimonides Med J 2014;5:e0002.

7 Dawed AY, Zhou K, Pearson ER. Pharmacogenetics in type 2 diabetes: Influence on response to oral hypoglycemic agents. Pharmgenomics Pers Med 2016;9:17-29.

8 Vanhoutte PM, Shimokawa H, Feletou M, Tang EH. Endothelial dysfunction and vascular disease - a 30th anniversary update. Acta Physiol (Oxf) 2017;219:22-96.

9 Potenza MA, Gagliardi S, Nacci C, Carratu MR, Montagnani M. Endothelial dysfunction in diabetes: From mechanisms to therapeutic targets. Curr Med Chem 2009;16:94-112.

10 Shi Y, Vanhoutte PM. Macro- and microvascular endothelial dysfunction in diabetes. J Diabetes 2017

11 Boyle PJ. Diabetes mellitus and macrovascular disease: Mechanisms and mediators. Am J Med 2007;120:S12-17.

12 Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-376.

13 Gimbrone MA, Jr. Vascular endothelium: An integrator of pathophysiologic stimuli in atherosclerosis. Am J Cardiol 1995;75:67B-70B.

14 Marasciulo FL, Montagnani M, Potenza MA. Endothelin-1: The yin and yang on vascular function. Curr Med Chem 2006;13:1655-1665.

15 Ritchie RH, Drummond GR, Sobey CG, De Silva TM, Kemp-Harper BK. The opposing roles of no and oxidative stress in cardiovascular disease. Pharmacol Res 2016

16 Zhang C. The role of inflammatory cytokines in endothelial dysfunction. Basic Res Cardiol 2008;103:398-406.

17 King GL. The role of hyperglycaemia and hyperinsulinaemia in causing vascular dysfunction in diabetes. Ann Med 1996;28:427-432.

18 Li H, Horke S, Forstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 2014;237:208-219.

19 Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: Molecular and pathophysiological mechanisms. Circulation 2006;113:1888-1904.

20 Potenza MA, Addabbo F, Montagnani M. Vascular actions of insulin with implications for endothelial dysfunction. Am J Physiol Endocrinol Metab 2009;297:E568-577.

21 Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463-491.

22 Miller VM, Duckles SP. Vascular actions of estrogens: Functional implications. Pharmacol Rev 2008;60:210-241.

23 Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: Response to therapeutic interventions. Circulation 2008;117:3238-3249.

24 Zhang G, Yin X, Qi Y, Pendyala L, Chen J, Hou D, Tang C. Ghrelin and cardiovascular diseases. Curr Cardiol Rev 2010;6:62-70.

25 Raschke S, Eckel J. Adipo-myokines: Two sides of the same coin--mediators of inflammation and mediators of exercise. Mediators Inflamm 2013;2013:320724.
26 Zhao L, Fu Z, Liu Z. Adiponectin and insulin cross talk: The microvascular connection. Trends Cardiovasc Med 2014;24:319-324.
27 Montagnani M, Quon MJ. Insulin action in vascular endothelium: Potential mechanisms linking insulin resistance with hypertension. Diabetes Obes Metab 2000;2:285-292.
28 Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 1995;96:786-792.
29 Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ. Roles for insulin receptor, pi3-kinase, and akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. Circulation 2000;101:1539-1545.
30 Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. J Clin Invest 1996;98:894-898.
31 Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of enos is independent of ca++ but requires phosphorylation by akt at ser1179. J Biol Chem 2001;276:30392-30398.
32 Montagnani M, Ravichandran LV, Chen H, Esposito DL, Quon MJ. Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1 are required for insulin-stimulated production of nitric oxide in endothelial cells. Mol Endocrinol 2002;16:1931-1942.
33 Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by akt- dependent phosphorylation. Nature 1999;399:601-605.
34 Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, Montagnani M. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between no and et-1 production. Am J Physiol Heart Circ Physiol 2005;289:H813-822.
35 Eringa EC, Stehouwer CD, van Nieuw Amerongen GP, Ouwehand L, Westerhof N, Sipkema P. Vasoconstrictor effects of insulin in skeletal muscle arterioles are mediated by erk1/2 activation in endothelium. Am J Physiol Heart Circ Physiol 2004;287:H2043-2048.
36 Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, Panza JA. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. Circulation 1999;100:820-825.
37 Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. J Biol Chem 2002;277:1794-1799.
38 Sesti G, Marini MA, Cardellini M, Sciacqua A, Frontoni S, Andreozzi F, Irace C, Lauro D, Gnasso A, Federici M, Perticone F, Lauro R. The arg972 variant in insulin receptor substrate-1 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. Diabetes Care 2004;27:1394-1398.
39 Ruan H, Dong LQ. Adiponectin signaling and function in insulin target tissues. J Mol Cell Biol 2016;8:101-109.
40 Ebrahimi-Mamaeghani M, Mohammadi S, Arefhosseini SR, Fallah P, Bazi Z. Adiponectin as a potential biomarker of vascular disease. Vasc Health Risk Manag 2015;11:55-70.
41 Lacquemant C, Froguel P, Lobbens S, Izzo P, Dina C, Ruiz J. The adiponectin gene snp+45 is associated with coronary artery disease in type 2 (non-insulin-dependent) diabetes mellitus. Diabet Med 2004;21:776-781.
42 Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Hussain T. Adiponectin gene variants and the risk of coronary artery disease in patients with type 2 diabetes. Mol Biol Rep 2011;38:3703-3708.
43 Bacci S, Menzaghi C, Ercolino T, Ma X, Rauseo A, Salvemini L, Vigna C, Fanelli R, Di Mario U, Doria A, Trischitta V. The +276 g/t single nucleotide polymorphism of the adiponectin gene is associated with coronary artery disease in type 2 diabetic patients. Diabetes Care 2004;27:2015-2020.

44 Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 2007;56:1198-1209.
45 Yamauchi T, Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. Int J Obes (Lond) 2008;32 Suppl 7:S13-18.
46 Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 2003;278:45021-45026.
47 Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: Adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473-2476.
48 Cheng KK, Lam KS, Wang Y, Huang Y, Carling D, Wu D, Wong C, Xu A. Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by appl1 in endothelial cells. Diabetes 2007;56:1387-1394.
49 Tian L, Luo N, Zhu X, Chung BH, Garvey WT, Fu Y. Adiponectin-adipor1/2-appl1 signaling axis suppresses human foam cell formation: Differential ability of adipor1 and adipor2 to regulate inflammatory cytokine responses. Atherosclerosis 2012;221:66-75.
50 Szmitko PE, Teoh H, Stewart DJ, Verma S. Adiponectin and cardiovascular disease: State of the art? Am J Physiol Heart Circ Physiol 2007;292:H1655-1663.
51 Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem 2002;277:37487-37491.
52 Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N, Tagawa T, Ueda S, Shimomura I, Funahashi T, Matsuzawa Y. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. J Clin Endocrinol Metab 2003;88:3236-3240.
53 Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003;42:231-234.
54 Hsu CH, Tsai TH, Kao YH, Hwang KC, Tseng TY, Chou P. Effect of green tea extract on obese women: A randomized, double-blind, placebo-controlled clinical trial. Clin Nutr 2008;27:363-370.
55 Wang A, Liu M, Liu X, Dong LQ, Glickman RD, Slaga TJ, Zhou Z, Liu F. Up-regulation of adiponectin by resveratrol: The essential roles of the akt/foxo1 and amp-activated protein kinase signaling pathways and dsba-l. J Biol Chem 2011;286:60-66.
56 Zhu W, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: Molecular mechanisms and potential therapeutic intervention. Clin Sci (Lond) 2008;114:361-374.
57 Fisman EZ, Tenenbaum A. Adiponectin: A manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? Cardiovasc Diabetol 2014;13:103.
58 Ciccone MM, Faienza MF, Altomare M, Nacci C, Montagnani M, Valente F, Cortese F, Gesualdo M, Zito A, Mancarella R, Leogrande D, Viola D, Scicchitano P, Giordano P. Endothelial and metabolic function interactions in overweight/obese children. J Atheroscler Thromb 2016;23:950-959.
59 Saint-Marc P, Kozak LP, Ailhaud G, Darimont C, Negrel R. Angiotensin ii as a trophic factor of white adipose tissue: Stimulation of adipose cell formation. Endocrinology 2001;142:487-492.
60 Mohite A, Chillar A, So SP, Cervantes V, Ruan KH. Novel mechanism of the vascular protector prostacyclin: Regulating microrna expression. Biochemistry 2011;50:1691-1699.
61 Sansbury BE, Cummins TD, Tang Y, Hellmann J, Holden CR, Harbeson MA, Chen Y, Patel RP, Spite M, Bhatnagar A, Hil BG. Overexpression of endothelial nitric oxide synthase prevents diet-induced obesity and regulates adipocyte phenotype. Circ Res 2012;111:1176-1189.
62 Wilkes JJ, Hevener A, Olefsky J. Chronic endothelin-1 treatment leads to insulin resistance in vivo. Diabetes 2003;52:1904-1909.

63 Ishibashi KI, Imamura T, Sharma PM, Huang J, Ugi S, Olefsky JM. Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3t3-l1 adipocytes. J Clin Invest 2001;107:1193-1202.
64 Usui I, Imamura T, Babendure JL, Satoh H, Lu JC, Hupfeld CJ, Olefsky JM. G protein-coupled receptor kinase 2 mediates endothelin-1-induced insulin resistance via the inhibition of both galphaq/11 and insulin receptor substrate-1 pathways in 3t3-l1 adipocytes. Mol Endocrinol 2005;19:2760-2768.
65 Juan CC, Chang CL, Lai YH, Ho LT. Endothelin-1 induces lipolysis in 3t3-l1 adipocytes. Am J Physiol Endocrinol Metab 2005;288:E1146-1152.
66 Bedi D, Clarke KJ, Dennis JC, Zhong Q, Brunson BL, Morrison EE, Judd RL. Endothelin-1 inhibits adiponectin secretion through a phosphatidylinositol 4,5-bisphosphate/actin-dependent mechanism. Biochem Biophys Res Commun 2006;345:332-339.
67 Nacci C, Leo V, De Benedictis L, Carratu MR, Bartolomeo N, Altomare M, Giordano P, Faienza MF, Montagnani M. Elevated endothelin-1 (et-1) levels may contribute to hypoadiponectinemia in childhood obesity. J Clin Endocrinol Metab 2013;98:E683-693.
68 Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-967.
69 Kim KA, Shin YJ, Kim JH, Lee H, Noh SY, Jang SH, Bae ON. Dysfunction of endothelial progenitor cells under diabetic conditions and its underlying mechanisms. Arch Pharm Res 2012;35:223-234.
70 Awad O, Jiao C, Ma N, Dunnwald M, Schatteman GC. Obese diabetic mouse environment differentially affects primitive and monocytic endothelial cell progenitors. Stem Cells 2005;23:575-583.
71 Cubbon RM, Rajwani A, Wheatcroft SB. The impact of insulin resistance on endothelial function, progenitor cells and repair. Diab Vasc Dis Res 2007;4:103-111.
72 Menegazzo L, Albiero M, Avogaro A, Fadini GP. Endothelial progenitor cells in diabetes mellitus. Biofactors 2012;38:194-202.
73 Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type ii diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002;106:2781-2786.
74 Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A. Circulating cd34+ cells, metabolic syndrome, and cardiovascular risk. Eur Heart J 2006;27:2247-2255.
75 Segal MS, Shah R, Afzal A, Perrault CM, Chang K, Schuler A, Beem E, Shaw LC, Li Calzi S, Harrison JK, Tran-Son-Tay R, Grant MB. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. Diabetes 2006;55:102-109.
76 Madonna R, De Caterina R. Circulating endothelial progenitor cells: Do they live up to their name? Vascul Pharmacol 2015;67-69:2-5.
77 Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, Liu-Bryan R, Glass CK, Neels JG, Olefsky JM. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via toll-like receptors 2 and 4 and jnk-dependent pathways. J Biol Chem 2007;282:35279-35292.
78 Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The nlrp3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011;17:179-188.
79 Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11:136-140.
80 Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 2009;27:519-550.

81 Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470-1481.
82 Hasegawa Y, Saito T, Ogihara T, Ishigaki Y, Yamada T, Imai J, Uno K, Gao J, Kaneko K, Shimosawa T, Asano T, Fujita T, Oka Y, Katagiri H. Blockade of the nuclear factor-kappab pathway in the endothelium prevents insulin resistance and prolongs life spans. Circulation 2012;125:1122-1133.
83 Tesauro M, Rizza S, Iantorno M, Campia U, Cardillo C, Lauro D, Leo R, Turriziani M, Cocciolillo GC, Fusco A, Panza JA, Scuteri A, Federici M, Lauro R, Quon MJ. Vascular, metabolic, and inflammatory abnormalities in normoglycemic offspring of patients with type 2 diabetes mellitus. Metabolism 2007;56:413-419.
84 Chatterjee TK, Stoll LL, Denning GM, Harrelson A, Blomkalns AL, Idelman G, Rothenberg FG, Neltner B, Romig-Martin SA, Dickson EW, Rudich S, Weintraub NL. Proinflammatory phenotype of perivascular adipocytes: Influence of high-fat feeding. Circ Res 2009;104:541-549.
85 Marchesi C, Ebrahimian T, Angulo O, Paradis P, Schiffrin EL. Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. Hypertension 2009;54:1384-1392.
86 Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, Laing I, Yates AP, Pemberton PW, Malik RA, Heagerty AM. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 2009;119:1661-1670.
87 Britton KA, Fox CS. Perivascular adipose tissue and vascular disease. Clin Lipidol 2011;6:79-91.
88 Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-1143.
89 Moreno PR, Purushothaman KR, Fuster V, O'Connor WN. Intimomedial interface damage and adventitial inflammation is increased beneath disrupted atherosclerosis in the aorta: Implications for plaque vulnerability. Circulation 2002;105:2504-2511.
90 Eringa EC, Bakker W, van Hinsbergh VW. Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. Vascul Pharmacol 2012;56:204-209.
91 Szasz T, Bomfim GF, Webb RC. The influence of perivascular adipose tissue on vascular homeostasis. Vasc Health Risk Manag 2013;9:105-116.
92 Nacci C, Leo V, De Benedictis L, Potenza MA, Sgarra L, De Salvia MA, Quon MJ, Montagnani M. Infliximab therapy restores adiponectin expression in perivascular adipose tissue and improves endothelial nitric oxide-mediated vasodilation in mice with type 1 diabetes. Vascul Pharmacol 2016;87:83-91.
93 Ehsan M, Singh KK, Lovren F, Pan Y, Quan A, Mantella LE, Sandhu P, Teoh H, Al-Omran M, Verma S. Adiponectin limits monocytic microparticle-induced endothelial activation by modulation of the ampk, akt and nfkappab signaling pathways. Atherosclerosis 2016;245:1-11.
94 Benetti E, Chiazza F, Patel NS, Collino M. The nlrp3 inflammasome as a novel player of the intercellular crosstalk in metabolic disorders. Mediators Inflamm 2013;2013:678627.
95 Collino M, Benetti E, Rogazzo M, Mastrocola R, Yaqoob MM, Aragno M, Thiemermann C, Fantozzi R. Reversal of the deleterious effects of chronic dietary hfcs-55 intake by ppar-delta agonism correlates with impaired nlrp3 inflammasome activation. Biochem Pharmacol 2013;85:257-264.
96 Chiazza F, Couturier-Maillard A, Benetti E, Mastrocola R, Nigro D, Cutrin JC, Serpe L, Aragno M, Fantozzi R, Ryffel B, Thiemermann C, Collino M. Targeting the nlrp3 inflammasome to reduce diet-induced metabolic abnormalities in mice. Mol Med 2015
97 Mastrocola R, Penna C, Tullio F, Femmino S, Nigro D, Chiazza F, Serpe L, Collotta D, Alloatti G, Cocco M, Bertinaria M, Pagliaro P, Aragno M, Collino M. Pharmacological inhibition of nlrp3 inflammasome attenuates myocardial ischemia/reperfusion injury by activation of risk and mitochondrial pathways. Oxid Med Cell Longev 2016;2016:5271251.

98 Chen Y, Pitzer AL, Li X, Li PL, Wang L, Zhang Y. Instigation of endothelial nlrp3 inflammasome by adipokine visfatin promotes inter-endothelial junction disruption: Role of hmgb1. J Cell Mol Med 2015;19:2715-2727.
99 Wang L, Chen Y, Li X, Zhang Y, Gulbins E. Enhancement of endothelial permeability by free fatty acid through lysosomal cathepsin b-mediated nlrp3 inflammasome activation. Oncotarget 2016;7:73229-73241.
100 Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, Fishbein MC, Lehman TJ, Arditi M. Interleukin-1beta is crucial for the induction of coronary artery inflammation in a mouse model of kawasaki disease. Circulation 2012;125:1542-1550.
101 Chen S, Lee Y, Crother TR, Fishbein M, Zhang W, Yilmaz A, Shimada K, Schulte DJ, Lehman TJ, Shah PK, Arditi M. Marked acceleration of atherosclerosis after lactobacillus casei-induced coronary arteritis in a mouse model of kawasaki disease. Arterioscler Thromb Vasc Biol 2012;32:e60-71.
102 Tomita S, Myones BL, Shulman ST. In vitro correlates of the l. Casei animal model of kawasaki disease. J Rheumatol 1993;20:362-367.
103 Chen Y, Li X, Boini KM, Pitzer AL, Gulbins E, Zhang Y, Li PL. Endothelial nlrp3 inflammasome activation associated with lysosomal destabilization during coronary arteritis. Biochim Biophys Acta 2015;1853:396-408.
104 De Boer AA, Monk JM, Liddle DM, Hutchinson AL, Power KA, Ma DW, Robinson LE. Fish-oil-derived n-3 polyunsaturated fatty acids reduce nlrp3 inflammasome activity and obesity-related inflammatory cross-talk between adipocytes and cd11b(+) macrophages. J Nutr Biochem 2016;34:61-72.
105 Hamilton SJ, Chew GT, Watts GF. Therapeutic regulation of endothelial dysfunction in type 2 diabetes mellitus. Diab Vasc Dis Res 2007;4:89-102.
106 Singh TP, Vangaveti VN, Malabu UH. Dipeptidyl peptidase-4 inhibitors and their potential role in the management of atherosclerosis-a review. Diabetes Metab Syndr 2015
107 Cahn A, Miccoli R, Dardano A, Del Prato S. New forms of insulin and insulin therapies for the treatment of type 2 diabetes. Lancet Diabetes Endocrinol 2015
108 Knop FK, Vilsboll T, Holst JJ. Incretin-based therapy of type 2 diabetes mellitus. Curr Protein Pept Sci 2009;10:46-55.
109 Cernea S, Raz I. Therapy in the early stage: Incretins. Diabetes Care 2011;34 Suppl 2:S264-271.
110 Kawalec P, Mikrut A, Lopuch S. The safety of dipeptidyl peptidase-4 (dpp-4) inhibitors or sodium-glucose cotransporter 2 (sglt-2) inhibitors added to metformin background therapy in patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes Metab Res Rev 2014;30:269-283.
111 Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, Espadero RM, Woerle HJ, Broedl UC, Johansen OE. Sglt-2 inhibitors and cardiovascular risk: Proposed pathways and review of ongoing outcome trials. Diab Vasc Dis Res 2015;12:90-100.
112 Triggle CR, Ding H. Metformin is not just an antihyperglycaemic drug but also has protective effects on the vascular endothelium. Acta Physiol (Oxf) 2017;219:138-151.
113 Kinaan M, Ding H, Triggle CR. Metformin: An old drug for the treatment of diabetes but a new drug for the protection of the endothelium. Med Princ Pract 2015;24:401-415.
114 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.
115 Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor kappab activation via amp-activated protein kinase activation in vascular endothelial cells. Hypertension 2006;47:1183-1188.
116 Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the amp-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. Diabetes 2006;55:496-505.

117 Ghosh S, Lakshmanan AP, Hwang MJ, Kubba H, Mushannen A, Triggle CR, Ding H. Metformin improves endothelial function in aortic tissue and microvascular endothelial cells subjected to diabetic hyperglycaemic conditions. Biochem Pharmacol 2015;98:412-421.
118 Li WD, Du XL, Qian AM, Hu N, Kong LS, Wei S, Li CL, Li XQ. Metformin regulates differentiation of bone marrow-derived endothelial progenitor cells via multiple mechanisms. Biochem Biophys Res Commun 2015;465:803-809.
119 Yu JW, Deng YP, Han X, Ren GF, Cai J, Jiang GJ. Metformin improves the angiogenic functions of endothelial progenitor cells via activating ampk/enos pathway in diabetic mice. Cardiovasc Diabetol 2016;15:88.
120 Pearson ER. Personalized medicine in diabetes: The role of 'omics' and biomarkers. Diabet Med 2016;33:712-717.
121 Hauner H. The mode of action of thiazolidinediones. Diabetes Metab Res Rev 2002;18 Suppl 2:S10-15.
122 Sgarra L, Addabbo F, Potenza MA, Montagnani M. Determinants of evolving metabolic and cardiovascular benefit/risk profiles of rosiglitazone therapy during the natural history of diabetes: Molecular mechanisms in the context of integrated pathophysiology. Am J Physiol Endocrinol Metab 2012;302:E1171-1182.
123 Kim YB, Ciaraldi TP, Kong A, Kim D, Chu N, Mohideen P, Mudaliar S, Henry RR, Kahn BB. Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110beta protein levels in skeletal muscle of type 2 diabetic subjects. Diabetes 2002;51:443-448.
124 Formoso G, Chen H, Kim JA, Montagnani M, Consoli A, Quon MJ. Dehydroepiandrosterone mimics acute actions of insulin to stimulate production of both nitric oxide and endothelin 1 via distinct phosphatidylinositol 3-kinase- and mitogen-activated protein kinase-dependent pathways in vascular endothelium. Mol Endocrinol 2006;20:1153-1163.
125 Bagi Z, Koller A, Kaley G. Ppargamma activation, by reducing oxidative stress, increases no bioavailability in coronary arterioles of mice with type 2 diabetes. Am J Physiol Heart Circ Physiol 2004;286:H742-748.
126 Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. Jama 2002;287:1420-1426.
127 Ceolotto G, Gallo A, Papparella I, Franco L, Murphy E, Iori E, Pagnin E, Fadini GP, Albiero M, Semplicini A, Avogaro A. Rosiglitazone reduces glucose-induced oxidative stress mediated by nad(p)h oxidase via ampk-dependent mechanism. Arterioscler Thromb Vasc Biol 2007;27:2627-2633.
128 Collino M, Aragno M, Castiglia S, Miglio G, Tomasinelli C, Boccuzzi G, Thiemermann C, Fantozzi R. Pioglitazone improves lipid and insulin levels in overweight rats on a high cholesterol and fructose diet by decreasing hepatic inflammation. Br J Pharmacol 2010;160:1892-1902.
129 Collino M, Aragno M, Mastrocola R, Gallicchio M, Rosa AC, Dianzani C, Danni O, Thiemermann C, Fantozzi R. Modulation of the oxidative stress and inflammatory response by ppar-gamma agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. Eur J Pharmacol 2006;530:70-80.
130 Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK. Expression of the peroxisome proliferator-activated receptor gamma (ppargamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1998;95:7614-7619.
131 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998;391:79-82.
132 Wakino S, Law RE, Hsueh WA. Vascular protective effects by activation of nuclear receptor ppargamma. J Diabetes Complications 2002;16:46-49.

133 Sidhu JS, Kaposzta Z, Markus HS, Kaski JC. Effect of rosiglitazone on common carotid intima-media thickness progression in coronary artery disease patients without diabetes mellitus. Arterioscler Thromb Vasc Biol 2004;24:930-934.
134 Bailey CJ. Treating insulin resistance in type 2 diabetes with metformin and thiazolidinediones. Diabetes Obes Metab 2005;7:675-691.
135 Raji A, Seely EW, Bekins SA, Williams GH, Simonson DC. Rosiglitazone improves insulin sensitivity and lowers blood pressure in hypertensive patients. Diabetes Care 2003;26:172-178.
136 Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007;356:2457-2471.
137 Kang ES, Park SY, Kim HJ, Kim CS, Ahn CW, Cha BS, Lim SK, Nam CM, Lee HC. Effects of pro12ala polymorphism of peroxisome proliferator-activated receptor gamma2 gene on rosiglitazone response in type 2 diabetes. Clin Pharmacol Ther 2005;78:202-208.
138 Kirchheiner J, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshyenko O, Jetter A, Stehle S, Tsahuridu M, Meineke I, Brockmoller J, Fuhr U. Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to cyp2c8 genotype. Clin Pharmacol Ther 2006;80:657-667.
139 Rosen CJ. Revisiting the rosiglitazone story--lessons learned. N Engl J Med 2010;363:803-806.
140 Cho YM, Fujita Y, Kieffer TJ. Glucagon-like peptide-1: Glucose homeostasis and beyond. Annu Rev Physiol 2013;76:535-559.
141 Madsbad S. Review of head-to-head comparisons of glucagon-like peptide-1 receptor agonists. Diabetes Obes Metab 2016;18:317-332.
142 Zhong J, Gong Q, Goud A, Srinivasamaharaj S, Rajagopalan S. Recent advances in dipeptidyl-peptidase-4 inhibition therapy: Lessons from the bench and clinical trials. J Diabetes Res 2015;2015:606031.
143 Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. Endocr Rev 2012;33:187-215.
144 Lovshin J, Cherney D. Glp-1r agonists and endothelial dysfunction: More than just glucose lowering? Diabetes 2015;64:2319-2321.
145 Erdogdu O, Nathanson D, Sjoholm A, Nystrom T, Zhang Q. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through enos-, pka- and pi3k/akt-dependent pathways and requires glp-1 receptor. Mol Cell Endocrinol 2010;325:26-35.
146 Goto H, Nomiyama T, Mita T, Yasunari E, Azuma K, Komiya K, Arakawa M, Jin WL, Kanazawa A, Kawamori R, Fujitani Y, Hirose T, Watada H. Exendin-4, a glucagon-like peptide-1 receptor agonist, reduces intimal thickening after vascular injury. Biochem Biophys Res Commun 2011;405:79-84.
147 Nagashima M, Watanabe T, Terasaki M, Tomoyasu M, Nohtomi K, Kim-Kaneyama J, Miyazaki A, Hirano T. Native incretins prevent the development of atherosclerotic lesions in apolipoprotein e knockout mice. Diabetologia 2011;54:2649-2659.
148 Hirata K, Kume S, Araki S, Sakaguchi M, Chin-Kanasaki M, Isshiki K, Sugimoto T, Nishiyama A, Koya D, Haneda M, Kashiwagi A, Uzu T. Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model. Biochem Biophys Res Commun 2009;380:44-49.
149 Horton ES, Silberman C, Davis KL, Berria R. Weight loss, glycemic control, and changes in cardiovascular biomarkers in patients with type 2 diabetes receiving incretin therapies or insulin in a large cohort database. Diabetes Care 2010;33:1759-1765.
150 Buse JB, Drucker DJ, Taylor KL, Kim T, Walsh B, Hu H, Wilhelm K, Trautmann M, Shen LZ, Porter LE. Duration-1: Exenatide once weekly produces sustained glycemic control and weight loss over 52 weeks. Diabetes Care 2010;33:1255-1261.
151 Grimm M, Han J, Weaver C, Griffin P, Schulteis CT, Dong H, Malloy J. Efficacy, safety, and tolerability of exenatide once weekly in patients with type 2 diabetes mellitus: An integrated analysis of the duration trials. Postgrad Med 2013;125:47-57.
152 Shiraki A, Oyama J, Komoda H, Asaka M, Komatsu A, Sakuma M, Kodama K, Sakamoto Y, Kotooka N, Hirase T, Node K. The glucagon-like peptide 1 analog liraglutide reduces tnf-alpha-

induced oxidative stress and inflammation in endothelial cells. Atherosclerosis 2012;221:375-382.
153 Krasner NM, Ido Y, Ruderman NB, Cacicedo JM. Glucagon-like peptide-1 (glp-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and ampk dependent mechanism. PLoS One 2014;9:e97554.
154 He L, Wong CK, Cheung KK, Yau HC, Fu A, Zhao HL, Leung KM, Kong AP, Wong GW, Chan PK, Xu G, Chan JC. Anti-inflammatory effects of exendin-4, a glucagon-like peptide-1 analog, on human peripheral lymphocytes in patients with type 2 diabetes. J Diabetes Investig 2013;4:382-392.
155 Prasad-Reddy L, Isaacs D. A clinical review of glp-1 receptor agonists: Efficacy and safety in diabetes and beyond. Drugs Context 2015;4:212283.
156 Pala L, Pezzatini A, Dicembrini I, Ciani S, Gelmini S, Vannelli BG, Cresci B, Mannucci E, Rotella CM. Different modulation of dipeptidyl peptidase-4 activity between microvascular and macrovascular human endothelial cells. Acta Diabetol 2012;49 Suppl 1:S59-63.
157 Deacon CF, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. Diabetes Obes Metab 2016;18:333-347.
158 Matsubara J, Sugiyama S, Sugamura K, Nakamura T, Fujiwara Y, Akiyama E, Kurokawa H, Nozaki T, Ohba K, Konishi M, Maeda H, Izumiya Y, Kaikita K, Sumida H, Jinnouchi H, Matsui K, Kim-Mitsuyama S, Takeya M, Ogawa H. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein e-deficient mice. J Am Coll Cardiol 2012;59:265-276.
159 Aroor AR, Sowers JR, Bender SB, Nistala R, Garro M, Mugerfeld I, Hayden MR, Johnson MS, Salam M, Whaley-Connell A, Demarco VG. Dipeptidylpeptidase inhibition is associated with improvement in blood pressure and diastolic function in insulin-resistant male zucker obese rats. Endocrinology 2013;154:2501-2513.
160 Shah Z, Pineda C, Kampfrath T, Maiseyeu A, Ying Z, Racoma I, Deiuliis J, Xu X, Sun Q, Moffatt-Bruce S, Villamena F, Rajagopalan S. Acute dpp-4 inhibition modulates vascular tone through glp-1 independent pathways. Vascul Pharmacol 2011;55:2-9.
161 Mason RP, Jacob RF, Kubant R, Ciszewski A, Corbalan JJ, Malinski T. Dipeptidyl peptidase-4 inhibition with saxagliptin enhanced nitric oxide release and reduced blood pressure and sicam-1 levels in hypertensive rats. J Cardiovasc Pharmacol 2012;60:467-473.
162 Sauve M, Ban K, Momen MA, Zhou YQ, Henkelman RM, Husain M, Drucker DJ. Genetic deletion or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes after myocardial infarction in mice. Diabetes 2010;59:1063-1073.
163 Dai Y, Dai D, Wang X, Ding Z, Mehta JL. Dpp-4 inhibitors repress nlrp3 inflammasome and interleukin-1beta via glp-1 receptor in macrophages through protein kinase c pathway. Cardiovasc Drugs Ther 2014;28:425-432.
164 van Poppel PC, Netea MG, Smits P, Tack CJ. Vildagliptin improves endothelium-dependent vasodilatation in type 2 diabetes. Diabetes Care 2011;34:2072-2077.
165 Ayaori M, Iwakami N, Uto-Kondo H, Sato H, Sasaki M, Komatsu T, Iizuka M, Takiguchi S, Yakushiji E, Nakaya K, Yogo M, Ogura M, Takase B, Murakami T, Ikewaki K. Dipeptidyl peptidase-4 inhibitors attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2 diabetic patients. J Am Heart Assoc 2013;2:e003277.
166 Savarese G, Perrone-Filardi P, D'Amore C, Vitale C, Trimarco B, Pani L, Rosano GM. Cardiovascular effects of dipeptidyl peptidase-4 inhibitors in diabetic patients: A meta-analysis. Int J Cardiol 2015;181:239-244.
167 Ahmed HA, May DW, Fagan SC, Segar L. Vascular protection with dipeptidyl peptidase-iv inhibitors in diabetes: Experimental and clinical therapeutics. Pharmacotherapy 2015;35:277-297.
168 Abbas AS, Dehbi HM, Ray KK. Cardiovascular and non-cardiovascular safety of dipeptidyl peptidase-4 inhibition: A meta-analysis of randomized controlled cardiovascular outcome trials. Diabetes Obes Metab 2016;18:295-299.

169 Abdul-Ghani MA, DeFronzo RA. Inhibition of renal glucose reabsorption: A novel strategy for achieving glucose control in type 2 diabetes mellitus. Endocr Pract 2008;14:782-790.
170 Chao EC. Sglt-2 inhibitors: A new mechanism for glycemic control. Clinical Diabetes 2014;32:4-11.
171 Zhang M, Zhang L, Wu B, Song H, An Z, Li S. Dapagliflozin treatment for type 2 diabetes: A systematic review and meta-analysis of randomized controlled trials. Diabetes Metab Res Rev 2013;30:204-221.
172 Kovacs CS, Seshiah V, Merker L, Christiansen AV, Roux F, Salsali A, Kim G, Stella P, Woerle HJ, Broedl UC. Empagliflozin as add-on therapy to pioglitazone with or without metformin in patients with type 2 diabetes mellitus. Clin Ther 2015
173 Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15:853-862.
174 Cefalu WT, Leiter LA, Yoon KH, Arias P, Niskanen L, Xie J, Balis DA, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin versus glimepiride in patients with type 2 diabetes inadequately controlled with metformin (cantata-su): 52 week results from a randomised, double-blind, phase 3 non-inferiority trial. Lancet 2013;382:941-950.
175 Ptaszynska A, Hardy E, Johnsson E, Parikh S, List J. Effects of dapagliflozin on cardiovascular risk factors. Postgrad Med 2013;125:181-189.
176 Dziuba J, Alperin P, Racketa J, Iloeje U, Goswami D, Hardy E, Perlstein I, Grossman HL, Cohen M. Modeling effects of sglt-2 inhibitor dapagliflozin treatment versus standard diabetes therapy on cardiovascular and microvascular outcomes. Diabetes Obes Metab 2014;16:628-635.
177 Monami M, Dicembrini I, Mannucci E. Effects of sglt-2 inhibitors on mortality and cardiovascular events: A comprehensive meta-analysis of randomized controlled trials. Acta Diabetol 2015
178 Pollack RM, Donath MY, LeRoith D, Leibowitz G. Anti-inflammatory agents in the treatment of diabetes and its vascular complications. Diabetes Care 2016;39 Suppl 2:S244-252.
179 O'Neill F, Charakida M, Topham E, McLoughlin E, Patel N, Sutill E, Kay CW, D'Aiuto F, Landmesser U, Taylor PC, Deanfield J. Anti-inflammatory treatment improves high-density lipoprotein function in rheumatoid arthritis. Heart 2016
180 Vallejo S, Palacios E, Romacho T, Villalobos L, Peiro C, Sanchez-Ferrer CF. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. Cardiovasc Diabetol 2014;13:158.
181 Tozser J, Benko S. Natural compounds as regulators of nlrp3 inflammasome-mediated il-1beta production. Mediators Inflamm 2016;2016:5460302.
182 Yang H, Xiao L, Yuan Y, Luo X, Jiang M, Ni J, Wang N. Procyanidin b2 inhibits nlrp3 inflammasome activation in human vascular endothelial cells. Biochem Pharmacol 2014;92:599-606.
183 Li Y, Zhou ZH, Chen MH, Yang J, Leng J, Cao GS, Xin GZ, Liu LF, Kou JP, Liu BL, Li P, Wen XD. Inhibition of mitochondrial fission and nox2 expression prevent nlrp3 inflammasome activation in the endothelium: The role of corosolic acid action in the amelioration of endothelial dysfunction. Antioxid Redox Signal 2016;24:893-908.
184 Tsai PY, Ka SM, Chang JM, Chen HC, Shui HA, Li CY, Hua KF, Chang WL, Huang JJ, Yang SS, Chen A. Epigallocatechin-3-gallate prevents lupus nephritis development in mice via enhancing the nrf2 antioxidant pathway and inhibiting nlrp3 inflammasome activation. Free Radic Biol Med 2011;51:744-754.
185 Zhao Y, Li Q, Zhao W, Li J, Sun Y, Liu K, Liu B, Zhang N. Astragaloside iv and cycloastragenol are equally effective in inhibition of endoplasmic reticulum stress-associated txnip/nlrp3 inflammasome activation in the endothelium. J Ethnopharmacol 2015;169:210-218.
186 Wang W, Wu QH, Sui Y, Wang Y, Qiu X. Rutin protects endothelial dysfunction by disturbing nox4 and ros-sensitive nlrp3 inflammasome. Biomed Pharmacother 2016;86:32-40.
187 Sun C, Fan S, Wang X, Lu J, Zhang Z, Wu D, Shan Q, Zheng Y. Purple sweet potato color inhibits endothelial premature senescence by blocking the nlrp3 inflammasome. J Nutr Biochem 2015;26:1029-1040.

188 Marchetti C, Toldo S, Chojnacki J, Mezzaroma E, Liu K, Salloum FN, Nordio A, Carbone S, Mauro AG, Das A, Zalavadia AA, Halquist MS, Federici M, Van Tassell BW, Zhang S, Abbate A. Pharmacologic inhibition of the nlrp3 inflammasome preserves cardiac function after ischemic and nonischemic injury in the mouse. J Cardiovasc Pharmacol 2015;66:1-8.
189 Wang S, Fang F, Jin WB, Wang X, Zheng XS. Investigation into the association between nlrp3 gene polymorphisms and susceptibility to type 2 diabetes mellitus. Genet Mol Res 2015;14:17447-17452.
190 Zheng Y, Zhang D, Zhang L, Fu M, Zeng Y, Russell R. Variants of nlrp3 gene are associated with insulin resistance in chinese han population with type-2 diabetes. Gene 2013;530:151-154.
191 Klen J, Goricar K, Janez A, Dolzan V. Nlrp3 inflammasome polymorphism and macrovascular complications in type 2 diabetes patients. J Diabetes Res 2015;2015:616747.
192 Sourris KC, Harcourt BE, Forbes JM. A new perspective on therapeutic inhibition of advanced glycation in diabetic microvascular complications: Common downstream endpoints achieved through disparate therapeutic approaches? Am J Nephrol 2009;30:323-335.
193 Schalkwijk CG, Miyata T. Early- and advanced non-enzymatic glycation in diabetic vascular complications: The search for therapeutics. Amino Acids 2010;42:1193-1204.
194 Nagai R, Murray DB, Metz TO, Baynes JW. Chelation: A fundamental mechanism of action of age inhibitors, age breakers, and other inhibitors of diabetes complications. Diabetes 2012;61:549-559.
195 Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 1986;232:1629-1632.
196 Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am J Nephrol 2004;24:32-40.
197 Turgut F, Bolton WK. Potential new therapeutic agents for diabetic kidney disease. Am J Kidney Dis 2010;55:928-940.
198 Zieman SJ, Melenovsky V, Clattenburg L, Corretti MC, Capriotti A, Gerstenblith G, Kass DA. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. J Hypertens 2007;25:577-583.
199 Little WC, Zile MR, Kitzman DW, Hundley WG, O'Brien TX, Degroof RC. The effect of alagebrium chloride (alt-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. J Card Fail 2005;11:191-195.
200 Hartog JW, Willemsen S, van Veldhuisen DJ, Posma JL, van Wijk LM, Hummel YM, Hillege HL, Voors AA. Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. Eur J Heart Fail 2011;13:899-908.
201 Stirban A, Negrean M, Stratmann B, Gawlowski T, Horstmann T, Gotting C, Kleesiek K, Mueller-Roesel M, Koschinsky T, Uribarri J, Vlassara H, Tschoepe D. Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. Diabetes Care 2006;29:2064-2071.
202 Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med 2003;9:294-299.
203 Williams ME, Bolton WK, Khalifah RG, Degenhardt TP, Schotzinger RJ, McGill JB. Effects of pyridoxamine in combined phase 2 studies of patients with type 1 and type 2 diabetes and overt nephropathy. Am J Nephrol 2007;27:605-614.
204 Lewis EJ, Greene T, Spitalewiz S, Blumenthal S, Berl T, Hunsicker LG, Pohl MA, Rohde RD, Raz I, Yerushalmy Y, Yagil Y, Herskovits T, Atkins RC, Reutens AT, Packham DK, Lewis JB. Pyridorin in type 2 diabetic nephropathy. J Am Soc Nephrol 2011;23:131-136.

205 Montagnani M. Diabetic cardiomyopathy: How much does it depend on age? Br J Pharmacol 2008;154:725-726.
206 Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Creager MA. Inhibition of protein kinase cbeta prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. Circ Res 2002;90:107-111.
207 Strom C, Sander B, Klemp K, Aiello LP, Lund-Andersen H, Larsen M. Effect of ruboxistaurin on blood-retinal barrier permeability in relation to severity of leakage in diabetic macular edema. Invest Ophthalmol Vis Sci 2005;46:3855-3858.
208 Aiello LP, Clermont A, Arora V, Davis MD, Sheetz MJ, Bursell SE. Inhibition of pkc beta by oral administration of ruboxistaurin is well tolerated and ameliorates diabetes-induced retinal hemodynamic abnormalities in patients. Invest Ophthalmol Vis Sci 2006;47:86-92.
209 Vinik AI, Bril V, Kempler P, Litchy WJ, Tesfaye S, Price KL, Bastyr EJ, 3rd. Treatment of symptomatic diabetic peripheral neuropathy with the protein kinase c beta-inhibitor ruboxistaurin mesylate during a 1-year, randomized, placebo-controlled, double-blind clinical trial. Clin Ther 2005;27:1164-1180.
210 Sheetz MJ, Aiello LP, Davis MD, Danis R, Bek T, Cunha-Vaz J, Shahri N, Berg PH. The effect of the oral pkc beta inhibitor ruboxistaurin on vision loss in two phase 3 studies. Invest Ophthalmol Vis Sci 2013;54:1750-1757.
211 Durpes MC, Morin C, Paquin-Veillet J, Beland R, Pare M, Guimond MO, Rekhter M, King GL, Geraldes P. Pkc-beta activation inhibits il-18-binding protein causing endothelial dysfunction and diabetic atherosclerosis. Cardiovasc Res 2015;106:303-313.
212 Ferrara N. Vascular endothelial growth factor: Basic science and clinical progress. Endocr Rev 2004;25:581-611.
213 Behl T, Kotwani A. Exploring the various aspects of the pathological role of vascular endothelial growth factor (vegf) in diabetic retinopathy. Pharmacol Res 2015;99:137-148.
214 van Wijngaarden P, Coster DJ, Williams KA. Inhibitors of ocular neovascularization: Promises and potential problems. Jama 2005;293:1509-1513.
215 Strain WD, Cos X, Prunte C. Considerations for management of patients with diabetic macular edema: Optimizing treatment outcomes and minimizing safety concerns through interdisciplinary collaboration. Diabetes Res Clin Pract 2017;126:1-9.
216 Massin P, Bandello F, Garweg JG, Hansen LL, Harding SP, Larsen M, Mitchell P, Sharp D, Wolf-Schnurrbusch UE, Gekkieva M, Weichselberger A, Wolf S. Safety and efficacy of ranibizumab in diabetic macular edema (resolve study): A 12-month, randomized, controlled, double-masked, multicenter phase ii study. Diabetes Care 2010;33:2399-2405.
217 Bandello F, De Benedetto U, Knutsson KA, Parodi MB, Cascavilla ML, Iacono P. Ranibizumab in the treatment of patients with visual impairment due to diabetic macular edema. Clin Ophthalmol 2011;5:1303-1308.
218 Rich RM, Rosenfeld PJ, Puliafito CA, Dubovy SR, Davis JL, Flynn HW, Jr., Gonzalez S, Feuer WJ, Lin RC, Lalwani GA, Nguyen JK, Kumar G. Short-term safety and efficacy of intravitreal bevacizumab (avastin) for neovascular age-related macular degeneration. Retina 2006;26:495-511.
219 Mason JO, 3rd, Nixon PA, White MF. Intravitreal injection of bevacizumab (avastin) as adjunctive treatment of proliferative diabetic retinopathy. Am J Ophthalmol 2006;142:685-688.
220 Friedlander SM, Welch RM. Vanishing disc neovascularization following intravitreal bevacizumab (avastin) injection. Arch Ophthalmol 2006;124:1365.
221 Zhang XY, Guo XF, Zhang SD, He JN, Sun CY, Zou Y, Bi HS, Qu Y. Comparison of bevacizumab and ranibizumab in age-related macular degeneration: A systematic review and meta-analysis. Int J Ophthalmol 2014;7:355-364.
222 Ababneh OH, Yousef YA, Gharaibeh AM, Abu Ameerh MA, Abu-Yaghi NE, Al Bdour MD. Intravitreal bevacizumab in the treatment of diabetic ocular neovascularization. Retina 2013;33:748-755.
223 Stefanini FR, Arevalo JF, Maia M. Bevacizumab for the management of diabetic macular edema. World J Diabetes 2013;4:19-26.

224 Soriano FG, Virag L, Szabo C. Diabetic endothelial dysfunction: Role of reactive oxygen and nitrogen species production and poly(adp-ribose) polymerase activation. J Mol Med 2001;79:437-448.
225 Soriano FG, Pacher P, Mabley J, Liaudet L, Szabo C. Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(adp-ribose) polymerase. Circ Res 2001;89:684-691.
226 Pacher P, Szabo C. Role of poly(adp-ribose) polymerase 1 (parp-1) in cardiovascular diseases: The therapeutic potential of parp inhibitors. Cardiovasc Drug Rev 2007;25:235-260.
227 Mohammad G, Siddiquei MM, Abu El-Asrar AM. Poly (adp-ribose) polymerase mediates diabetes-induced retinal neuropathy. Mediators Inflamm 2013;2013:510451.
228 Choi SK, Galan M, Kassan M, Partyka M, Trebak M, Matrougui K. Poly(adp-ribose) polymerase 1 inhibition improves coronary arteriole function in type 2 diabetes mellitus. Hypertension 2012;59:1060-1068.
229 Singh SS, Sarma JA, Narasu L, Dayam R, Xu S, Neamati N. A review on parp1 inhibitors: Pharmacophore modeling, virtual and biological screening studies to identify novel parp1 inhibitors. Curr Top Med Chem 2014;14:2020-2030.
230 Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho gtpase/rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase b/akt in human endothelial cells. Mol Cell Biol 2002;22:8467-8477.
231 Ali TK, Al-Gayyar MM, Matragoon S, Pillai BA, Abdelsaid MA, Nussbaum JJ, El-Remessy AB. Diabetes-induced peroxynitrite impairs the balance of pro-nerve growth factor and nerve growth factor, and causes neurovascular injury. Diabetologia 2011;54:657-668.
232 Yao L, Chandra S, Toque HA, Bhatta A, Rojas M, Caldwell RB, Caldwell RW. Prevention of diabetes-induced arginase activation and vascular dysfunction by rho kinase (rock) knockout. Cardiovasc Res 2013;97:509-519.
233 Mallat Z, Gojova A, Sauzeau V, Brun V, Silvestre JS, Esposito B, Merval R, Groux H, Loirand G, Tedgui A. Rho-associated protein kinase contributes to early atherosclerotic lesion formation in mice. Circ Res 2003;93:884-888.
234 Fukumoto Y, Matoba T, Ito A, Tanaka H, Kishi T, Hayashidani S, Abe K, Takeshita A, Shimokawa H. Acute vasodilator effects of a rho-kinase inhibitor, fasudil, in patients with severe pulmonary hypertension. Heart 2005;91:391-392.
235 Arita R, Hata Y, Nakao S, Kita T, Miura M, Kawahara S, Zandi S, Almulki L, Tayyari F, Shimokawa H, Hafezi-Moghadam A, Ishibashi T. Rho kinase inhibition by fasudil ameliorates diabetes-induced microvascular damage. Diabetes 2009;58:215-226.
236 Pearson JT, Jenkins MJ, Edgley AJ, Sonobe T, Joshi M, Waddingham MT, Fujii Y, Schwenke DO, Tsuchimochi H, Yoshimoto M, Umetani K, Kelly DJ, Shirai M. Acute rho-kinase inhibition improves coronary dysfunction in vivo, in the early diabetic microcirculation. Cardiovasc Diabetol 2013;12:111.
237 Yin H, Ru H, Yu L, Kang Y, Lin G, Liu C, Sun L, Shi L, Sun Q. Targeting of rho kinase ameliorates impairment of diabetic endothelial function in intrarenal artery. Int J Mol Sci 2013;14:20282-20298.
238 Arita R, Nakao S, Kita T, Kawahara S, Asato R, Yoshida S, Enaida H, Hafezi-Moghadam A, Ishibashi T. A key role for rock in tnf-alpha-mediated diabetic microvascular damage. Invest Ophthalmol Vis Sci 2013;54:2373-2383.
239 Satoh K, Fukumoto Y, Shimokawa H. Rho-kinase: Important new therapeutic target in cardiovascular diseases. Am J Physiol Heart Circ Physiol 2011;301:H287-296.
240 Sharma A, Bernatchez PN, de Haan JB. Targeting endothelial dysfunction in vascular complications associated with diabetes. Int J Vasc Med 2012;2012:750126.
241 Gasparrini M, Giampieri F, Alvarez Suarez JM, Mazzoni L, Forbes Hernandez TY, Quiles JL, Bullon P, Battino M. Ampk as a new attractive therapeutic target for disease prevention: The role of dietary compounds. Curr Drug Targets 2015

242 Zou MH, Wu Y. Amp-activated protein kinase activation as a strategy for protecting vascular endothelial function. Clin Exp Pharmacol Physiol 2008;35:535-545.
243 Wang C, Li L, Zhang ZG, Fan D, Zhu Y, Wu LL. Globular adiponectin inhibits angiotensin ii-induced nuclear factor kappab activation through amp-activated protein kinase in cardiac hypertrophy. J Cell Physiol 2009;222:149-155.
244 Rutherford C, Speirs C, Williams JJ, Ewart MA, Mancini SJ, Hawley SA, Delles C, Viollet B, Costa-Pereira AP, Baillie GS, Salt IP, Palmer TM. Phosphorylation of janus kinase 1 (jak1) by amp-activated protein kinase (ampk) links energy sensing to anti-inflammatory signaling. Sci Signal 2016;9:ra109.
245 Lee WJ, Lee IK, Kim HS, Kim YM, Koh EH, Won JC, Han SM, Kim MS, Jo I, Oh GT, Park IS, Youn JH, Park SW, Lee KU, Park JY. Alpha-lipoic acid prevents endothelial dysfunction in obese rats via activation of amp-activated protein kinase. Arterioscler Thromb Vasc Biol 2005;25:2488-2494.
246 Fogarty S, Hardie DG. Development of protein kinase activators: Ampk as a target in metabolic disorders and cancer. Biochim Biophys Acta 2010;1804:581-591.
247 Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. Ampk activation: A therapeutic target for type 2 diabetes? Diabetes Metab Syndr Obes 2014;7:241-253.
248 Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, Funahashi T, Ouchi N, Walsh K. Adiponectin protects against myocardial ischemia-reperfusion injury through ampk- and cox-2-dependent mechanisms. Nat Med 2005;11:1096-1103.
249 Hossain MM, Mukheem A, Kamarul T. The prevention and treatment of hypoadiponectinemia-associated human diseases by up-regulation of plasma adiponectin. Life Sci 2015;135:55-67.
250 Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, Yamaguchi M, Tanabe H, Kimura-Someya T, Shirouzu M, Ogata H, Tokuyama K, Ueki K, Nagano T, Tanaka A, Yokoyama S, Kadowaki T. A small-molecule adipor agonist for type 2 diabetes and short life in obesity. Nature 2013;503:493-499.
251 Iacobazzi D, Mangialardi G, Gubernator M, Hofner M, Wielscher M, Vierlinger K, Reni C, Oikawa A, Spinetti G, Vono R, Sangalli E, Montagnani M, Madeddu P. Increased antioxidant defense mechanism in human adventitia-derived progenitor cells is associated with therapeutic benefit in ischemia. Antioxid Redox Signal 2014;21:1591-1604.
252 Ceriello A, Testa R, Genovese S. Clinical implications of oxidative stress and potential role of natural antioxidants in diabetic vascular complications. Nutr Metab Cardiovasc Dis 2016;26:285-292.
253 Anderson RA, Evans LM, Ellis GR, Khan N, Morris K, Jackson SK, Rees A, Lewis MJ, Frenneaux MP. Prolonged deterioration of endothelial dysfunction in response to postprandial lipaemia is attenuated by vitamin c in type 2 diabetes. Diabet Med 2006;23:258-264.
254 Antoniades C, Tousoulis D, Tountas C, Tentolouris C, Toutouza M, Vasiliadou C, Tsioufis C, Toutouzas P, Stefanadis C. Vascular endothelium and inflammatory process, in patients with combined type 2 diabetes mellitus and coronary atherosclerosis: The effects of vitamin c. Diabet Med 2004;21:552-558.
255 Chen H, Karne RJ, Hall G, Campia U, Panza JA, Cannon RO, 3rd, Wang Y, Katz A, Levine M, Quon MJ. High-dose oral vitamin c partially replenishes vitamin c levels in patients with type 2 diabetes and low vitamin c levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 2006;290:H137-145.
256 Darko D, Dornhorst A, Kelly FJ, Ritter JM, Chowienczyk PJ. Lack of effect of oral vitamin c on blood pressure, oxidative stress and endothelial function in type ii diabetes. Clin Sci (Lond) 2002;103:339-344.
257 Shirpoor A, Norouzi L, Nemati S, Khadem Ansari MH. Protective effect of vitamin e against diabetes-induced oxidized ldl and aorta cell wall proliferation in rat. Iran Biomed J 2015;19:117-123.
258 Shirpoor A, Salami S, Khadem-Ansari MH, Ilkhanizadeh B, Pakdel FG, Khademvatani K. Cardioprotective effect of vitamin e: Rescues of diabetes-induced cardiac malfunction, oxidative stress, and apoptosis in rat. J Diabetes Complications 2009;23:310-316.

259 Ceriello A, Kumar S, Piconi L, Esposito K, Giugliano D. Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type 1 diabetes. Diabetes Care 2007;30:649-654.
260 Ceriello A, Novials A, Ortega E, Canivell S, La Sala L, Pujadas G, Bucciarelli L, Rondinelli M, Genovese S. Vitamin c further improves the protective effect of glucagon-like peptide-1 on acute hypoglycemia-induced oxidative stress, inflammation, and endothelial dysfunction in type 1 diabetes. Diabetes Care 2013;36:4104-4108.
261 Bjelakovic G, Nikolova D, Gluud C. Antioxidant supplements and mortality. Curr Opin Clin Nutr Metab Care 2014;17:40-44.
262 Fuchs D, Sperner-Unterweger B. Can intake of extra antioxidants delay the development and progression of atherosclerosis? Atherosclerosis 2013;226:43-44.
263 Sarmento RA, Silva FM, Sbruzzi G, Schaan BD, Almeida JC. Antioxidant micronutrients and cardiovascular risk in patients with diabetes: A systematic review. Arq Bras Cardiol 2013;101:240-248.
264 Goszcz K, Duthie GG, Stewart D, Leslie SJ, Megson IL. Bioactive polyphenols and cardiovascular disease: Chemical antagonists, pharmacological agents or xenobiotics that drive an adaptive response? Br J Pharmacol 2017
265 Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, El Bedoui J, Chataigneau M, Schini-Kerth VB. Vascular protection by dietary polyphenols. Eur J Pharmacol 2004;500:299-313.
266 Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JP. Polyphenols and human health: Prevention of disease and mechanisms of action. Nutrients 2010;2:1106-1131.
267 Joshi MS, Williams D, Horlock D, Samarasinghe T, Andrews KL, Jefferis AM, Berger PJ, Chin-Dusting JP, Kaye DM. Role of mitochondrial dysfunction in hyperglycaemia-induced coronary microvascular dysfunction: Protective role of resveratrol. Diab Vasc Dis Res 2015;12:208-216.

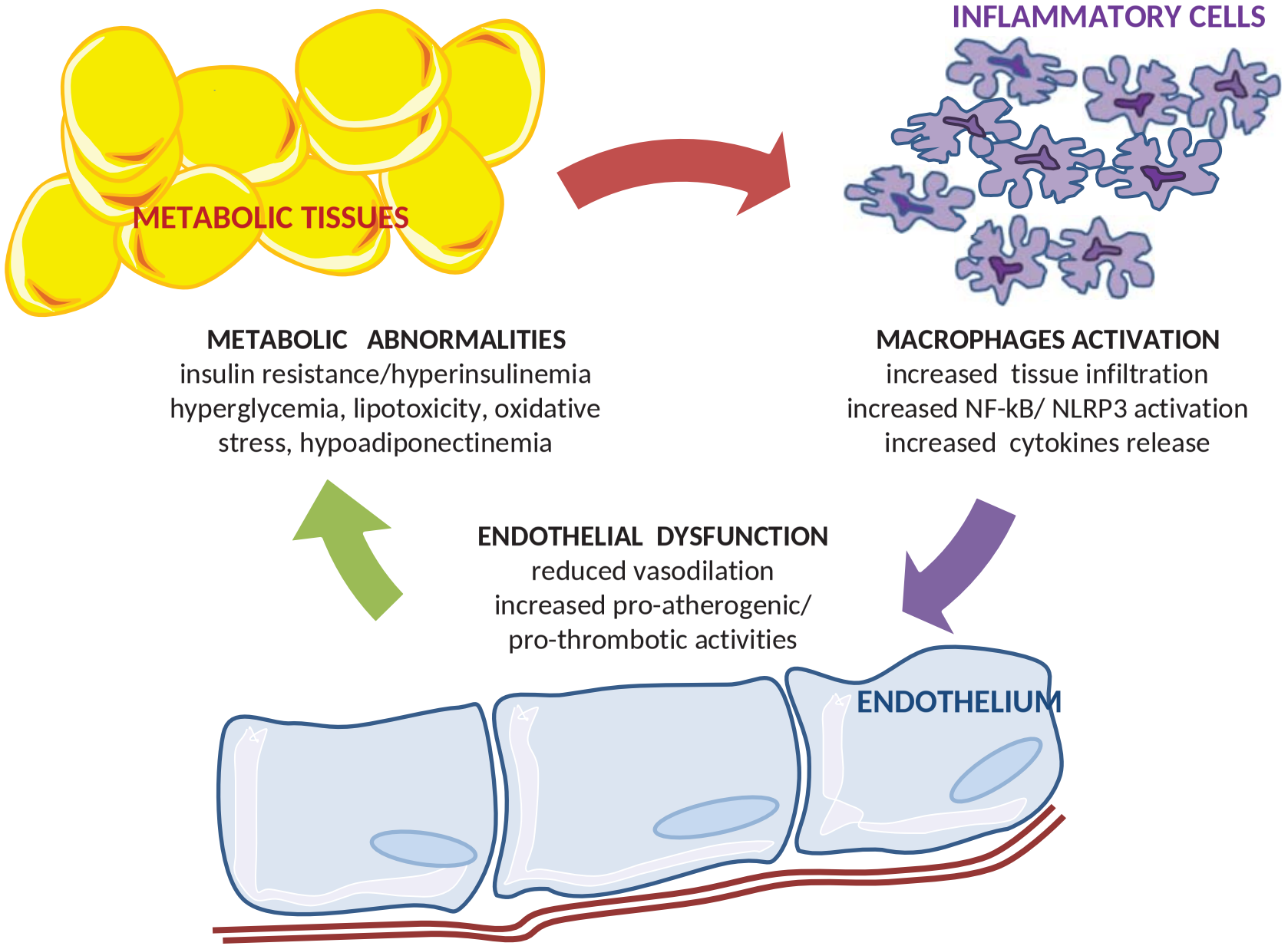

FIGURE 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabesity

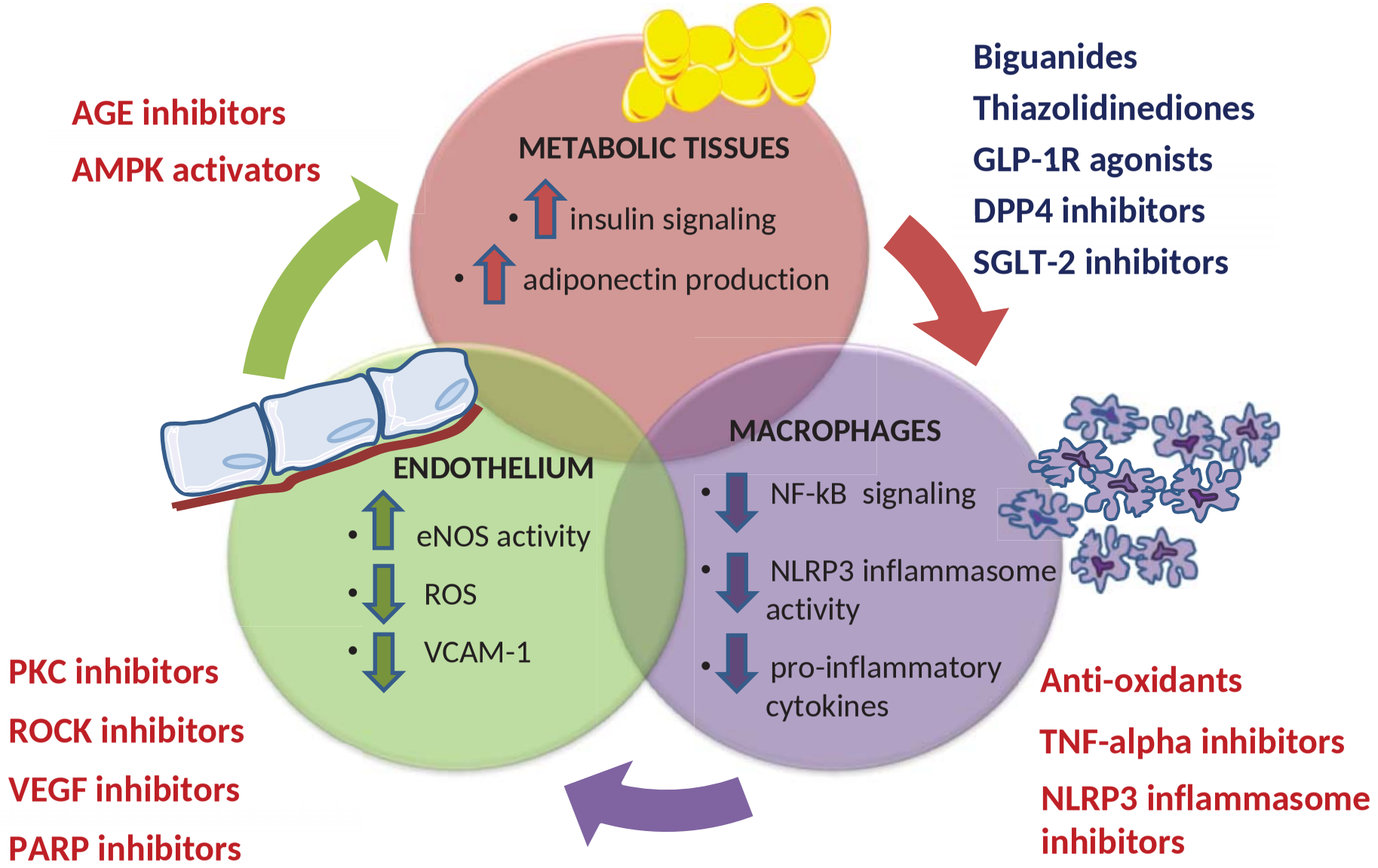

FIGURE 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction