

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1633092> since 2017-05-11T12:26:28Z

Published version:

DOI:10.1016/j.phrs.2017.04.009

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in PHARMACOLOGICAL RESEARCH, 120, 2017, 10.1016/j.phrs.2017.04.009.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.phrs.2017.04.009

The publisher's version is available at:

<http://linkinghub.elsevier.com/retrieve/pii/S1043661817301822>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1633092>

1
2
3 **TITLE: TARGETING ENDOTHELIAL METAINFLAMMATION TO COUNTERACT DIABESITY**
4 **CARDIOVASCULAR RISK: CURRENT AND PERSPECTIVE THERAPEUTIC OPTIONS.**
5

6 Authors: Maria Assunta Potenza¹, Carmela Nacci¹, Maria Antonietta De Salvia¹, Luca Sgarra²,
7 Massimo Collino³, Monica Montagnani^{1*}
8

9 *Affiliation(s)*

10 ¹ *Department of Biomedical Sciences and Human Oncology – Pharmacology Section, Medical School –*
11 *University of Bari “Aldo Moro”, Bari, Italy*

12 ² *Department of Emergency and Organ Transplantation – Section of Cardiovascular Diseases, Medical*
13 *School – University of Bari “Aldo Moro”, Bari, Italy*

14 ³ *Department of Drug Science and Technology - University of Turin, Turin, Italy*
15
16
17

18 ***Correspondence to:**

19 Monica Montagnani, MD, PhD
20 Department of Biomedical Sciences and Human Oncology
21 Pharmacology Section, Medical School
22 University of Bari “Aldo Moro”
23 Policlinico, Piazza Giulio Cesare, 11
24 70124 Bari - ITALY
25 e-mail: monica.montagnani@uniba.it
26
27

28
29 **Abstract:** The association of obesity and diabetes, termed “diabesity”, defines a combination of
30 primarily metabolic disorders with insulin resistance as the underlying common pathophysiology.
31 Cardiovascular disorders associated with diabesity represent the leading cause of morbidity and
32 mortality in the Western world. This makes diabesity, with its rising impacts on both health and
33 economics, one of the most challenging biomedical and social threats of present century. **The**
34 **emerging comprehension of the genes whose alteration confers inter-individual differences on risk**
35 **factors for diabetes or obesity, together with the potential role of genetically determined variants on**
36 **mechanisms controlling responsiveness, effectiveness and safety of anti-diabetic therapy underlines**
37 **the need of additional knowledge on molecular mechanisms involved in the pathophysiology of**
38 **diabesity.** Endothelial cell dysfunction, resulting from the unbalanced production of endothelial-
39 derived vascular mediators, is known to be present at the earliest stages of insulin resistance and
40 obesity, and may precede the clinical diagnosis of diabetes by several years. Once considered as a
41 mere consequence of metabolic abnormalities, it is now clear that endothelial dysfunctional activity
42 may play a pivotal role in the progression of diabesity. In the vicious circle where vascular defects
43 and metabolic disturbances worsen and reinforce each other, a low-grade, chronic, and 'cold'
44 inflammation (metaflammation) has been suggested to serve as the pathophysiological link that
45 binds endothelial and metabolic dysfunctions. In this paradigm, it is important to consider how
46 traditional antidiabetic treatments (specifically addressing metabolic dysregulation) may directly
47 impact on inflammatory processes or cardiovascular function. Indeed, not all drugs currently
48 available to treat diabetes possess the same anti-inflammatory potential, or target endothelial cell
49 function equally. Perspective strategies pointing at reducing metaflammation or directly addressing
50 endothelial dysfunction may disclose beneficial consequences on metabolic regulation. This review
51 focuses on existing and potential new approaches ameliorating endothelial dysfunction and vascular
52 inflammation in the context of diabesity.
53

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

55 **Conflict of interest** The authors declare no conflict of interest.
56
57
58
59

60
61
62 **SUMMARY**
63

64
65 **DIABESITY**
66

67 **ENDOTHELIAL DYSFUNCTION AND METAFILAMMATION**
68

- 69 • *Metabolic derangement triggers endothelial dysfunction*
- 70 • *Endothelial dysfunction contributes to metabolic abnormalities*
- 71 • *Inflammatory signaling links endothelial to metabolic impairments*
72

73 **HOW CONVENTIONAL DIABETIC TREATMENTS MAY AMELIORATE ENDOTHELIAL DYSFUNCTION**
74

- 75 • *Metformin*
- 76 • *Thiazolidine-2-4-diones (TZDs)*
- 77 • *Glucagon-like peptide-1 receptor (GLP-1R) agonists*
- 78 • *Dipeptidyl peptidase 4 (DPP4) inhibitors*
79

80
81 **NEW STRATEGIES FOR TREATMENT OF DIABETES AND THEIR IMPACT ON ENDOTHELIAL**
82 **DYSFUNCTION**
83

- 84 • *Anti-inflammatory drugs*
- 85 • *NLRP3 inflammasome inhibitors*
- 86 • *AGE inhibitors*
- 87 • *PKC inhibitors*
- 88 • *VEGF inhibitors*
- 89 • *PARP inhibitors*
- 90 • *ROCK (Rho-associated kinase) inhibitors*
- 91 • *AMPK activators*
- 92 • *Anti-oxidants*
 - 93 - *Vitamin C and Vitamin E*
 - 94 - *Polyphenols*
95

96 **CONCLUSIONS**
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118

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

178
179
180 end-point and, at present, brachial artery flood mediated dilation (FMD) remains the most widely
181 applied non-invasive method. Nevertheless, the importance of endothelium in participating and
182 predicting cardiovascular risk factors encourages further efforts in this direction. In the next
183 paragraphs the potential contribution of endothelial dysfunction to metaflammation and metabolic
184 disturbances of diabetes is briefly discussed.
185

186 **ENDOTHELIAL DYSFUNCTION and METAFLEMMATION** 187

188 With the growing understanding of the functional role played by the endothelium, and the
189 subsequent discovery of several endothelial mediators and their respective mechanism of action, it
190 has become increasingly clear that endothelial abnormalities represent an early sign of both
191 hemodynamic and metabolic disturbances [8]. Indeed, endothelial dysfunction - a condition in which
192 the endothelium loses its physiological properties and shifts toward a vasoconstrictor, pro-
193 thrombotic and pro-inflammatory state - is considered a major contributing factor in the etiology of
194 diabetes-related microvascular diseases such as retinopathy, nephropathy, neuropathy, and impaired
195 wound healing [9,10]. Moreover, endothelial dysfunction precedes the onset of macrovascular
196 complications, mainly represented by atherosclerosis, which in diabetic patients is more rapid and
197 more severe than in control population [10,11].
198

199 Typically, endothelial dysfunction is defined by a reduced availability of nitric oxide (NO), a
200 gaseous mediator with vasodilant, anti-thrombotic and anti-inflammatory properties [12,13]. NO
201 bioavailability depends on several multiple factors, ranging from the efficiency of the producing
202 enzyme endothelial NO synthase (eNOS) to the speed of conversion of NO itself to more stable
203 nitrate/nitrite derivatives. The dynamic, highly regulated physiological production of NO in the
204 endothelium may be disrupted when eNOS protein expression is decreased, when eNOS substrates
205 and/or co-factors are insufficient, when enzymatic activity of eNOS is impaired or uncoupled, or
206 when the production of endothelial mediators with opposing vascular effects is relatively increased
207 [9,14]. The deficiency of NO bioavailability and the increased reactive oxygen species (ROS) and
208 proinflammatory factors are requisite hallmarks for endothelial dysfunction [15].
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236

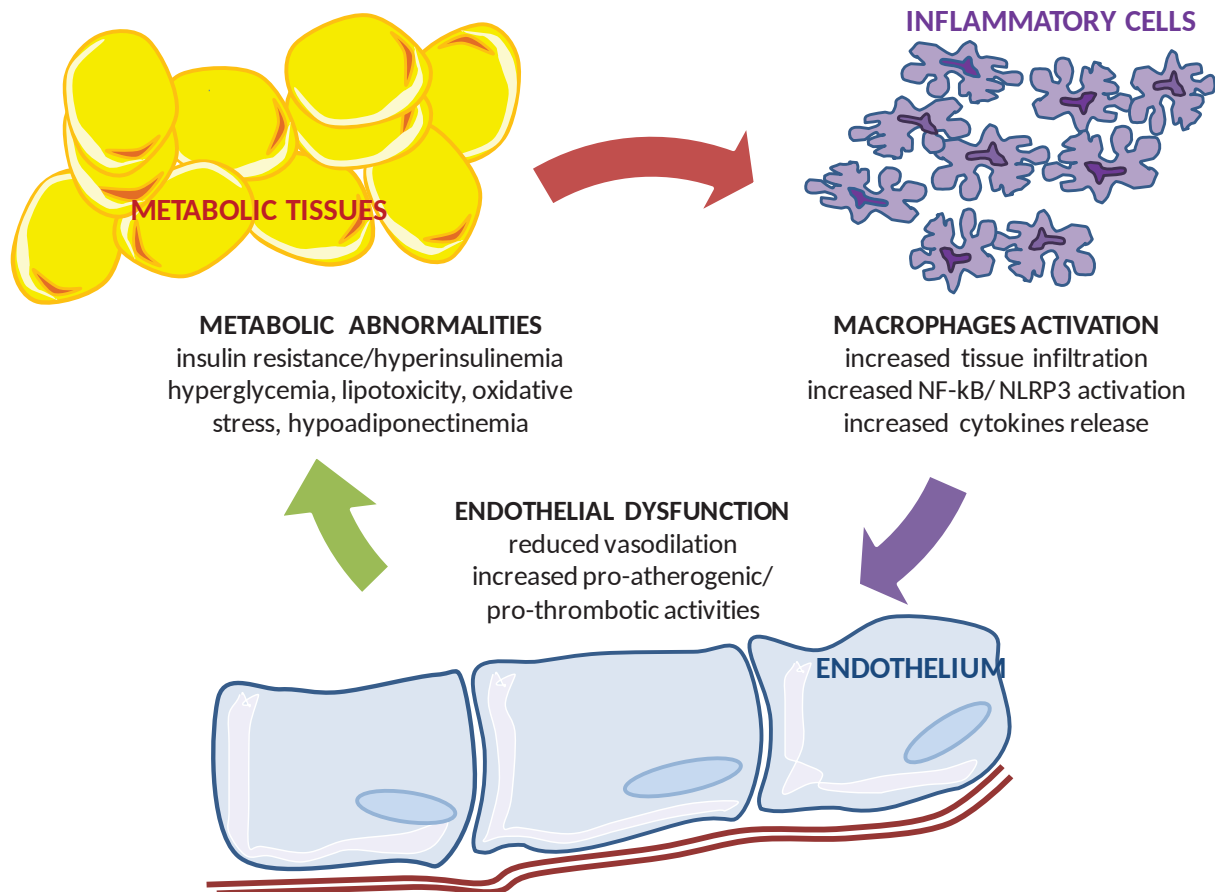


Figure 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes.

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-1 β 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

296
297
298 on hemodynamic homeostasis. Direct vascular effects have been documented, in addition to insulin
299 [21], for gonadal steroids [22], for hormones like leptin [23] and ghrelin [24], and for a number of
300 bioactive proteins secreted by adipose tissue and skeletal muscles termed “adipokines” and
301 “myokines”, respectively [25]. In the context of diabetic endothelial pathophysiology, the
302 cardiovascular properties of insulin and adiponectin (Ad) are perhaps the most relevant in terms of
303 current and perspective therapeutic approaches [26].
304

305 **Insulin** - Biological vascular actions of insulin have been clearly identified and progressively
306 described among the last three decades (comprehensively described in [21]). In addition to systemic
307 hemodynamic effects, related to renal reabsorption of sodium, or stimulation of sympathetic activity,
308 insulin is capable to directly modulate endothelial release of both vasoconstrictor factors such as
309 endothelin-1 (ET-1) and vasodilating mediators including NO. All these activities physiologically
310 contribute to overall metabolic homeostasis [27], since insulin-mediated activation of eNOS and
311 subsequent production of NO enhance local vasodilation, blood flow and nutrient delivery, thereby
312 participating to efficient insulin-mediated glucose uptake on target tissues [28]. Production of NO in
313 response to insulin depends on activation of insulin receptor tyrosine kinase (IR), and involves a
314 signaling cascade leading to phosphorylation of eNOS on Ser¹¹⁷⁷ via a signaling pathway recruiting
315 insulin receptor substrate-1 (IRS-1), phosphatidylinositol (PI) 3-kinase and Akt [29-33].
316 Concomitantly, the insulin-dependent activation of the Ras/MAP kinase signaling stimulates
317 secretion of ET-1 from endothelial cells [34-36], and it is involved in insulin-stimulated expression of
318 adhesion molecules including VCAM-1 and E-selectin [37]. Thus, by activating distinct intracellular
319 signaling pathways, insulin modulates the endothelial production of mediators with opposing
320 vascular effects. It is of particular interest that endothelial signaling pathways related to production
321 of NO and metabolic signaling pathways regulating translocation of the glucose carrier GLUT4 are
322 almost completely overlapping. Common insulin signaling in distinct tissues with metabolic or
323 vascular functions helps to understand why a selective impaired sensitivity in the PI-3K/Akt signaling
324 branch may greatly contribute to the pathophysiology of diabetic vascular complications [19]. **On
325 this respect, the emerging comprehension of the genes whose alteration confers an elevated risk to
326 develop diabetes and insulin resistance might also help to predict the onset of insulin-dependent
327 endothelial dysfunction: for example, polymorphisms in the IRS-1 gene (associated to impaired PI 3K
328 binding and insulin secretion in the beta-cells) [38] might also predispose to defects in insulin-
329 mediated NO production in endothelium. Similarly, genetic defects in the IR gene or in genes
330 encoding downstream signaling proteins may affect vascular actions of insulin as well as metabolic
331 activity in target tissues (extensively reviewed in [3]). In the next years, further analysis of results
332 from the genome-wide association studies (GWAS) will be important to uncover additional gene
333 variants and, hopefully, to turn these information into the identification of prognostic and predictive
334 biomarkers of insulin resistance. In the context of diabetes, this would be of utmost importance
335 since insulin-dependent endothelial dysfunction is known to precede and predict the onset of
336 metabolic abnormalities.**
337

338 **Adiponectin** - Adiponectin (Ad) is a protein hormone produced exclusively by adipose tissues with
339 identified beneficial effects on insulin sensitivity and **lipid metabolism** [39]. In addition, Ad exerts
340 multiple vasoprotective effects via its anti-inflammatory, antioxidant, antiapoptotic, antiatherogenic,
341 vasodilatory, and antithrombotic properties on endothelial cells, monocytes, macrophages,
342 leukocytes, platelets, and vascular smooth muscle cells [40]. **The gene encoding Ad is located in the
343 chromosome region 3q27, a locus mapped for susceptibility to both diabetes and cardiovascular risk
344 factors. Among several single nucleotide polymorphisms (SNPs) examined in the Ad gene, SNPs at
345 positions 45 [41,42] and 276 [43] have been linked to increased risk of cardiovascular events in
346 diabetic individuals. These findings support the influence of Ad genetic variability on cardiovascular
347 protection, especially in patients with diabetes [44]. Circulating Ad may exist as monomers, or form
348 oligomers and multimers. The exact role for each of these multiple Ad conformations is not
349 completely defined, but the high-molecular-weight (HMW) Ad is particularly active and equally able
350 to bind both the AdipoR1 and the AdipoR2 to exert metabolic and vasoprotective effects in target
351
352
353
354**

355
356
357 tissues [45]. On endothelial cells, Ad binding to AdipoR1/2 receptors activates AMP kinase (AMPK),
358 that leads to an increase in eNOS activity and NO production via phosphorylation of eNOS at Ser¹¹⁷⁷
359 and Ser⁶³³ [46]. Other beneficial endothelial effects of Ad include its ability to counteract the
360 expression of adhesion molecules and to suppress the TNF- α -mediated monocyte adhesion [47]. The
361 signaling events linking AdipoR1/2 receptors to activation of AMP kinase/eNOS require, among
362 others, the adaptor protein APPL1 [48], but the exact sequence of receptor downstream signaling
363 cascade has not been completely elucidated. Nevertheless, APPL1 isoform is involved in cellular
364 mechanisms resulting in Ad-suppressed foam cell formation [49], suggesting that the Ad-AdipoR1/2-
365 APPL1 axis may be a potential therapeutic target for preventing endothelial activation and
366 atherosclerotic processes. Interestingly, while production and release of proinflammatory cytokines
367 increases proportionally to adipose mass under obesity, Ad levels are inversely reduced, and
368 hypoadiponectinemia has been considered a significant predictor of endothelial dysfunction in
369 both the peripheral and coronary arteries besides other markers of the metabolic syndrome [50].
370 These clinical observations are supported by experimental findings showing that Ad-deficient animals
371 are more prone to develop neointimal hyperplasia [51], impaired endothelium-dependent
372 vasodilation [52], and high blood pressure [53]. **Accordingly, exogenous administration of Ad has
373 been effective to improve cardiovascular disease in animal models. At present, however, a number
374 of concerns limit the possibility to extend this approach to humans: these include the high circulating
375 levels needed, the unclear role of monomers, oligomers and multimers of Ad, and its moderately
376 short half-life, together with the need to better characterize the extensive posttranslational
377 modifications that might affect its potential beneficial activity in patients. In the meantime,
378 interventions aiming at increasing the endogenous Ad levels or restoring tissue sensitivity to Ad may
379 represent potential alternatives to prevent diabetes-related vascular disorders. On this respect,
380 several nutraceutical compounds have been shown to enhance plasma Ad levels, including green tea
381 extract [54] and resveratrol [55]. Similarly, a number of currently used pharmacological agents such
382 as renin-angiotensin system blockers, bezafibrate and fenofibrate, thiazolidinediones, statins, and
383 nebivolol are able to increase plasma Ad levels [40,56]. Although the contribution of Ad to overall
384 effects of these drugs is not always straightforward, these findings altogether support the possibility
385 to consider circulating Ad levels not only a biomarker of vascular disturbances under diabetes, but
386 also an indicator of the effectiveness for specific treatments. Currently, total serum and urinary Ad
387 levels may be measured by commercially available radioimmunoassay kit or enzyme-linked
388 immunoabsorbent assays. However, laboratory methods still lack appropriate standardization, and
389 the determination of ideally therapeutic Ad levels for each clinical setting requires further efforts
390 [57].**

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

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

414
415
416 events, however, EPC availability and/or mobilization tends to progressively decrease, and this may
417 accelerate the onset of endothelial dysfunction [69]. Insulin resistance reduces EPCs survival and
418 diminish their capacity for adhesion, endothelial integration, proliferation, and differentiation by
419 multiple mechanisms [70] (see [71,72] for review). These involve, among others, a reduced NO
420 bioavailability associated to increased oxidative stress and impaired PI 3K/Akt signaling, which are
421 found to alter the cytoskeletal structure and decrease mobilization of EPCs in diabetic and obese
422 patients [73-75]. Based on these data, EPCs have been proposed as cellular biomarkers of disease
423 and predictors of cardiovascular outcomes. An updated definition of their role and their biological
424 properties in health and disease has been recently published [76].
425

426 **Inflammatory signaling links endothelial to metabolic impairments** - In diabetes,
427 glucotoxicity and lipotoxicity induce a proinflammatory trait in macrophages residing or invading the
428 adipose tissue and the vasculature [77,78], and are responsible for oxidative and endoplasmic
429 reticulum stress. This in turn elicits the activation of thioredoxin-interacting protein (TXNIP) and the
430 NLR family, pyrin domain containing 3 (NLRP3) inflammasome, which increase the release of active
431 interleukin (IL)-1 β [79,80]. IL-1 β -amplified inflammation increases the expression of various cytokines
432 and chemokines, and favors the recruitment of macrophages in diabetic β -cells, adipose tissue, and
433 blood vessels [19,78]. In turn, the unbalanced activity in endothelium may further enhance
434 leukocytes adhesion, and increased release of inflammatory cells, thereby promoting lipids
435 deposition and facilitating the atherosclerotic plaques formation. In addition, alterations in the gut
436 microbiome along with increased gut leakiness of bacterial wall lipopolysaccharides (endotoxins) may
437 further promote tissue inflammation [81]. Endotoxins, free fatty acids, and cholesterol induce
438 inflammation by activating Toll-like receptor (TLR) pathways and, subsequently, nuclear factor- κ B
439 (NF- κ B)-mediated release of a broad range of cytokines and chemokines including tumor necrosis
440 factor (TNF), IL-1 β , IL-8, and MCP-1 that promote the accumulation of various immune cells in
441 different tissues [77,78].
442

443 **The likelihood that blockade of vascular inflammation and oxidative stress may be effective**
444 **to prevent metabolic disorders is supported by the finding that suppression of inflammatory**
445 **processes in the vasculature prevents the onset of insulin resistance in metabolic target tissues and**
446 **prolongs lifespan [82]. In a transgenic animal model, overexpression of the inhibitory NF- κ B subunit**
447 **I κ B α in endothelium correlates with reduced macrophage infiltration in adipose tissue and decreased**
448 **circulating markers of oxidative stress, concomitantly increasing blood flow, muscle mitochondrial**
449 **content, and locomotor activity [82].** These findings confirm the pivotal role of the transcription
450 factor NF κ B in oxidative stress, vascular dysfunction, and inflammation, and further support the
451 central role of endothelium in obesity-induced insulin resistance.
452

453 On the other hand, proinflammatory cytokines may play important roles in the pathogenesis
454 of both endothelial dysfunction and insulin resistance [83]. Development of insulin resistance has
455 classically been attributed to adipocyte-derived inflammation, resulting in macrophage infiltration
456 and altered secretory profile of adipose tissue depots with increased levels of pro-inflammatory
457 cytokines and decreased Ad synthesis and release [84-86]. The differential role of regional fat depots
458 in synthesis and release of specific mediators and vasoactive substances may help to reconcile the
459 generally accepted 'outside-in' theory of vascular inflammation, which postulates that inflammation
460 begins in adipose tissue and then spreads inward to the vasculature [87], with the 'inside-out'
461 process of vascular inflammation, proposing that the first step is intimal injury, that then extends to
462 the media and adventitia [88,89]. For example, it is plausible that intimate connections between
463 perivascular adipose tissue (PVAT) and other components of the vessel wall result in PVAT being the
464 first adipose depot to sense and respond to signals from circulating bioactive factors that influence
465 activity of endothelial and vascular smooth muscle cells [90-92].
466

467 On this regard, another important inflammatory pathway, the NLRP3 inflammasome, has
468 been proposed to be involved in the vasculoprotective effects exerted by Ad [93]. The NLRP3
469 inflammasome is a large multimeric protein complex mediating the cleavage of inactive
470
471
472

473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531

prointerleukin- (IL-) 1 β 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-1 β and IL-18 overproduction [95-97]. Activation of the endothelial NLRP3 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 NLRP3 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-**glucosidase**. 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-transporter-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

532
533
534 indicate that metformin has a direct action on the endothelium that seem to involve a reduction in
535 oxidative stress secondary to modulation of mitochondrial complex 1, and activation of signaling
536 pathways controlled by the deacetylase Sirtuin 1 (SIRT-1) [114]. In addition, metformin possesses
537 anti-inflammatory effects secondary to inhibition of cytokine-induced activation of NF-κB-mediated
538 pathways in endothelial cells [115], and it has been shown to enhance eNOS activation via an AMPK-
539 dependent signaling [116,117]. Activation of AMPK/eNOS signaling by metformin seems also
540 responsible for the ameliorated angiogenic function of bone marrow-derived EPC [118,119].
541 **Pharmacogenetic studies have suggested that therapeutic responses to metformin may differ inter-**
542 **individually due to some polymorphisms in the genes encoding for organic cation transporters**
543 **(OCTs), responsible for metformin active transport across membranes into the intestinal epithelial**
544 **cells, hepatocytes and renal tubular cells. This may result in metformin intolerance in some subjects,**
545 **while in others might explain the reduced efficacy of this drug in controlling both metabolic and**
546 **vascular disturbances [120].**

547
548 **Thiazolidine-2-4-diones (TZDs)** – These insulin sensitizer agents are exogenous activators of
549 the peroxisome proliferator-activated receptor-γ (PPAR-γ) [121]. TZDs may improve endothelial
550 function, increase forearm blood flow and reduce blood pressure in humans by both direct and
551 indirect mechanisms (reviewed in [122]). In endothelial cells, TZDs-mediated improvement of PI 3-K
552 signaling pathways results in increased protein expression of eNOS [123] and subsequent enhanced
553 production of NO in response to insulin and other mediators acting via PI 3-kinase-dependent
554 pathways [124]. TZDs may also improve eNOS activity and NO bioavailability by decreasing eNOS
555 uncoupling, reducing generation of superoxide anion [125] and diminishing ADMA levels in vessels
556 from diabetic patients [126]. Among TZDs, rosiglitazone has been shown to counteract
557 hyperglycemia-mediated oxidative stress not by a PPARγ activation mechanism, but rather as a
558 consequence of AMPK-dependent **signaling** pathways in endothelium [127]. Activation of AMPK in
559 response to rosiglitazone correlates with inhibition of the DAG/PKC pathway, subsequent reduction
560 of NADPH oxidase activity, and amelioration of oxidative balance [127].

561
562 Anti-inflammatory effects of TZDs on NF-κB/STAT/AP-1 signaling pathways correlate with a
563 marked decrease in the inducible NOS isoform (iNOS) expression, which in turn decreases the
564 amount of reactive oxygen species produced in monocyte/macrophages and in target tissues of
565 metabolic derangements [128-131]. Moreover, both pioglitazone and rosiglitazone reduce plasma
566 levels of TNF-α, leptin, PAI-1 and C-reactive protein, decrease vascular expression of adhesion
567 molecules, and significantly improve circulating levels of Ad [132]. These activities, associated to the
568 reduced expression of matrix metalloproteinases-9 (MMP-9) [133], decreased levels of both FFA and
569 atherogenic LDL cholesterol particles [134], and reduced PAI-1 plasma levels, may contribute to
570 maintain plaque stability, prevent platelet aggregation and inhibit thrombus formation. **The**
571 **significant decrease in systolic blood pressure (SBP) observed in diabetic patients treated with TZDs**
572 **further supports their ability to improve endothelial function [135].**

573
574 Thus, when the first meta-analysis reported a significant increase in myocardial infarction
575 (MI) and in cardiovascular-related risk death (CVD) in patients assuming rosiglitazone [136], the long
576 list of apparently beneficial effects of TZDs on endothelial cells and vascular function generated a
577 rather confusing reaction [122]. Whether the puzzling results were related to the single drug
578 rosiglitazone or to the whole class of TZD is still an incompletely resolved question. **However, single**
579 **nucleotide variations found in PPAR-γ gene might, at least in part, help to explain the different**
580 **therapeutic outcome in patients treated with TZDs [137]. Analogously, variants of the gene encoding**
581 **for CYP2C8 hepatic enzyme may impair rosiglitazone clearance and hence contribute to the degree of**
582 **therapeutic/harmful effects of TZDs in particular subjects [138].** While the European Medicine
583 Agency (EMA) recommended withdraw of all licensed rosiglitazone-containing drugs in 2010, the FDA
584 opted for rosiglitazone use restriction in the US [139]. However, following the rosiglitazone lesson,
585 from 2008 all drugs investigated for diabetes must also undergo specific clinical trials evaluating their
586 cardiovascular safety profile.
587
588
589
590

591
592
593 **Glucagon-like peptide-1 receptor (GLP-1R) agonists** - Incretins are peptides produced by the
594 gastrointestinal system able to enhance insulin secretion in a glucose-dependent manner [140]. The
595 two main human incretins are glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic
596 polypeptide (GIP) whose secretions are impaired in diabetes. GLP-1-mediated effects may be
597 sustained and improved with two strategies currently available: longer-acting GLP-1 receptor
598 agonists resistant to degradation of the dipeptidyl peptidase 4 (DPP4) enzyme, that can provide
599 supraphysiological stimulation of GLP-1 receptor [141]; and inhibitors of DPP4 enzyme, that extends
600 the half-life of endogenous GLP-1 [142].
601

602 The receptor for GLP-1 (GLP-1R), originally identified in pancreatic cells, is also expressed on
603 endothelial cells, cardiomyocytes and coronary smooth muscle cells [143]. Activation of GLP-1R
604 improves myocardial function, improves endothelial function in high risk cardiac patients, and
605 enhances natriuresis, with potential positive implications for systolic blood pressure control [144].
606 Beneficial effects of GLP-1 on endothelial cells are mediated by an increased NO production, and in
607 endothelial cells from human coronary arteries GLP-1R agonists stimulate proliferation via eNOS-,
608 PKA-, and PI3K/Akt-dependent pathways [145]. It has also been shown that GLP-1 exerts a protective
609 effect on atherosclerosis by reducing neointimal formation [146], foam cell formation, and
610 atherosclerotic lesion size [147].
611

612 The GLP-1 receptor agonists (GLP-1Ra) are peptides and, such as insulin, require
613 subcutaneous injection to avoid degradation by gastrointestinal enzymes. Most of the clinical trials,
614 in line with animal studies, have reported a reduction of blood pressure after chronic treatment of
615 GLP-1R agonists [148-151]. This may be explained, at least in part, by the ability of GLP-1 analogs to
616 upregulate anti-oxidative enzymes and inhibit NFkB-mediated inflammatory signaling on endothelial
617 cells [152,153], and to reduce oxidative stress by suppressing MAPK signaling pathways in peripheral
618 lymphocytes of type 2 diabetic patients [154]. GLP-1-associated improvement of several
619 cardiovascular markers suggests that therapies with GLP-1R agonists may have a positive effect on
620 cardiovascular risk factors in patients with diabetes. Nevertheless, their long-term safety profile and
621 the direct clinical benefit to cardiovascular outcomes remain to be determined [155].
622 **Pharmacogenetic studies on this class of drugs are still limited.**
623

624 **Dipeptidyl peptidase 4 (DPP4) inhibitors** - DPP4 is a widely expressed membrane serine
625 exopeptidase involved in degradation of various oligopeptides, including GLP-1. DPP4 is anchored to
626 the cell membrane but, under certain circumstances, may be released in soluble form. Endothelial
627 cells are the main source of soluble DPP4 form, whose expression and enzymatic activity may
628 increase after chronic exposure to high glucose concentrations [156].
629

630 Currently approved DPP4 inhibitors include sitagliptin, saxagliptin, linagliptin and vildagliptin
631 [157]. These small molecular-weight substances inhibit more than 90% of DPP4 activity and can be
632 orally administered. Several studies in animal models support the evidence of DPP4 inhibition in
633 improving endothelial function and blood pressure [158,159]. In isolated aorta rings incubated with
634 DPP4-inhibitor, the relaxant effect of DPP4-inhibitor is GLP-1 independent and results from Akt
635 phosphorylation and eNOS activation with a rapid increase in NO levels [160]. Saxagliptin treatment
636 has been shown to reduce blood pressure levels in the spontaneously hypertensive rats with a
637 concomitant increase of aortic and glomerular NO release and comparable reductions in
638 peroxynitrite levels [161]. There is good evidence that DPP4 inhibition mediates protective effect on
639 myocardial infarction, hypertension and atherosclerosis. Pharmacological treatment with sitagliptin
640 has been able to enhance the expression of cardioprotective proteins and improve heart functional
641 recovery after I/R injury [162]. It is not clear how these potential benefits may be mediated, but one
642 possibility involves the DPP4 inhibitors ability to modulate innate and adaptive immunity by
643 suppressing the NF-kB signaling downstream TNF and IL-6 [143,159], and by inhibiting the NLRP3
644 inflammasome, a multiprotein complex involved in caspase-1 activation and downstream maturation
645 of pro-inflammatory cytokines, TLR4 and IL1 β in human macrophages [163].
646
647
648
649

650
651
652 In contrast to the positive effects in animal experiments, the consequences of treatment with DPP4
653 inhibitors on endothelial functions in humans have not always been consistent. In a double-blind
654 study on patients with diabetes, treatment with vildagliptin for 4 weeks improved forearm blood flow
655 in response to intra-arterially delivered acetylcholine [164]. On the contrary, other studies measuring
656 FMD of the brachial artery have shown diametrically opposite results, suggesting that sitagliptin and
657 alogliptin actually seem to worsen flow-mediated dilation (FMD) when used to treat diabetic patients
658 [165]. At present, clinical evidence supporting the vascular protective effects of gliptins is uncertain,
659 given the relatively short follow-up [166-168]. Future and ongoing studies (CAROLINA,
660 <http://clinicaltrials.gov/show/NCT01243424>; TECOS, <https://clinicaltrials.gov/show/NCT00790205>)
661 should help determine whether DPP4-inhibitors may contribute to improve cardiovascular outcomes
662 in patients with T2DM.
663

664 **Sodium glucose co-transporter 2 (SGLT-2) inhibitors.** The isoform 2 of the sodium glucose
665 transporter (SGLT-2) is located in the proximal convoluted tubule of the kidney and it reabsorbs
666 approximately the 90% of filtered glucose. Inhibition of SGLT-2 represents a novel strategy for
667 achieving glucose control in diabetic patients. It is based on a mechanism of action that targets the
668 kidney to promote urinary glucose excretion and reduce hyperglycemia, and is therefore
669 independent of pancreatic β -cell function or the degree of insulin resistance [169,170].
670

671 Among the SGLT-2 inhibitors, canagliflozin, dapagliflozin, and empagliflozin are currently
672 used. Other gliflozins include ertugliflozin and sotagliflozin and, in Japan, ipragliflozin, tofogliflozin
673 and luseogliflozin. Most of clinical trials of SGLT-2 inhibitors show that patients receiving
674 dapagliflozin, canagliflozin, or empagliflozin as add-on therapy exhibit reductions in systolic blood
675 pressure of approximately 3-5 mmHg versus placebo [171,172]. One possible explanation is that
676 urinary glucose excretion stimulated by inhibition of SGLT-2 causes a diuretic effect responsible for
677 lowering blood pressure levels. However, a recent study on dapagliflozin proposed that SGLT-2
678 inhibitors may possess an additional diuretic-like capacity to lower blood pressure [173]. The
679 improvements in blood pressure, associated to a moderate decrease in body weight [174] induced by
680 treatment with SGLT-2 inhibitors suggest the potential for reducing the risk of cardiovascular events
681 [175,176]. To date, SGLT-2 inhibitors are generally well tolerated, with a favorable safety profile
682 similar to that of placebo. As easily expected, common adverse effects of SGLT-2 inhibitors include
683 genital tract infections and osmotic diuresis. Results from large cardiovascular trials underway for
684 dapagliflozin, canagliflozin and empagliflozin will likely provide greater insights into the effects of
685 SGLT-2 inhibition on cardiovascular outcomes [177].
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708

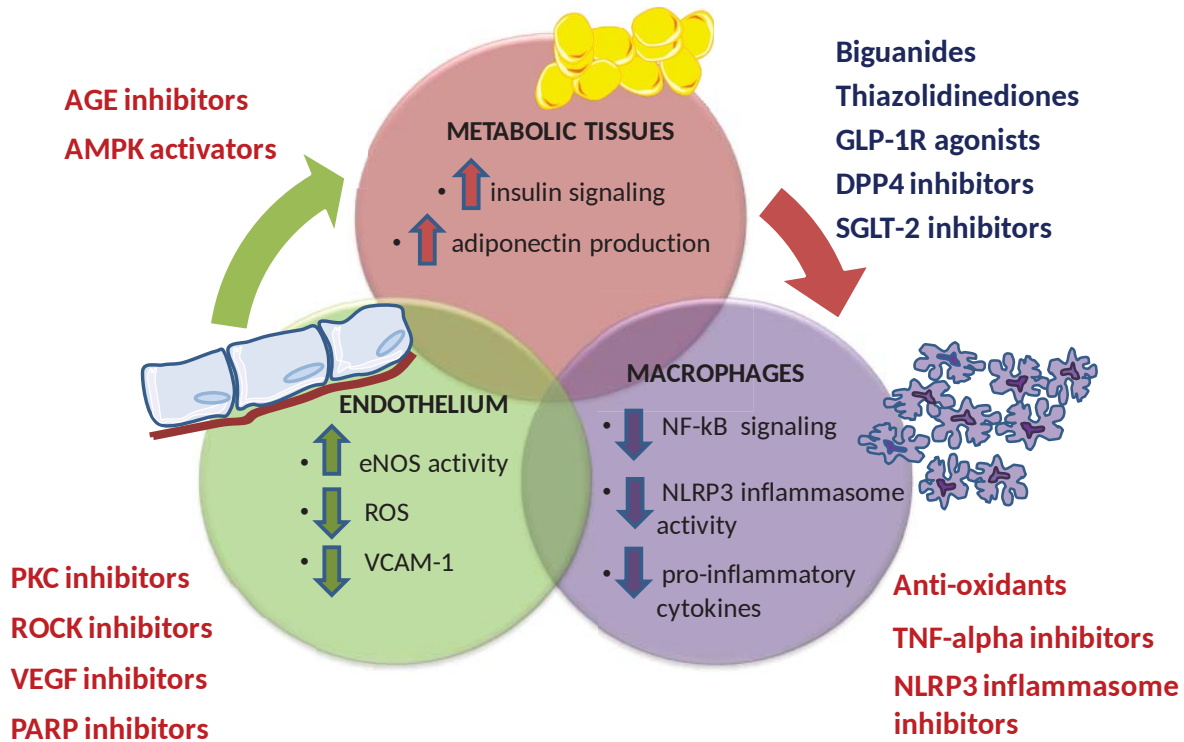


Figure 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction.

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-TNF α antibody infliximab, or anti-IL1 β 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

768
769
770 on **Polyphenols**). However, most recent evidence from the literature demonstrates that inhibition of
771 the NLRP3 inflammasome may significantly contribute to the beneficial effects exerted by few of
772 them [181]. For instance, the B-type procyanidins (PCB) have been found to suppress the activity of
773 NLRP3 inflammasome on endothelium via the inhibition of AP-1 pathway, a transcriptional
774 machinery involved in endothelial production of pro-inflammatory adhesion molecules and
775 chemokines [182]. Similarly, corosolic acid, a natural triterpenoid with antioxidative activity, is
776 supposed to protect endothelial homeostasis by suppressing NLRP3 inflammasome activation and
777 preventing mitochondrial damage [183]. The epigallocatechin-3-gallate (EGCG), the most abundant
778 active polyphenolic component of green tea, is capable of inhibiting NLRP3 inflammasome via
779 enhancing the Nrf2 antioxidant pathway [184]. Moreover, the protective effects of astragaloside IV
780 and cycloastragenol - both contained in *Astragalus membranaceus* Moench (Fabaceae) - against
781 endoplasmic reticulum stress-induced apoptosis are thought to be mediated via NLRP3
782 inflammasome inhibition in endothelial cells [176,185]. NLRP3 inflammasome inhibition has also
783 been proposed as new pivotal mechanism contributing to endothelial protective function of rutin, a
784 flavonoid that can be **obtained from** different dietary sources [186]. Another group of natural
785 anthocyanin derived from purple sweet potato, named purple sweet potato color (PSPC), seems to
786 attenuate atherosclerotic progress in an insulin-resistant mice model by suppressing premature
787 senescence of endothelium throughout inhibition of NLRP3 inflammasome [187].
788

789 Despite these interesting experimental findings, so far, the selective mechanism(s)
790 underlying their inflammasome-suppressing effects remain largely unclear. Thus, the described
791 activities could be due to interferences up- or downstream of inflammasome activation, and the
792 availability of selective NLRP3 inflammasome inhibitors is an essential prerequisite to include the
793 NLRP3 inflammasome in the list of potential pharmacological target for endothelial protection. At
794 present, efficacious NLRP3 inflammasome inhibitors are still under development. We recently
795 contributed to characterize the cardiovascular effect of the small molecule INF4E, one of the few
796 compounds that has been demonstrated to directly target the NLRP3 inflammasome and inhibit the
797 ATPase activity of NLRP3 required for its activation [97]. Similar cardiovascular protective effects
798 have been recently documented by using another small molecule which prevents the formation of
799 the NLRP3 inflammasome complex in cardiomyocytes, thus ameliorating cardiac function after
800 ischemia/reperfusion injury [188]. Although there are clear indications that these compounds exert
801 their cardioprotective effects against myocardial ischemia/reperfusion injury **via** a specific effect on
802 NLRP3 inflammasome, the exact mechanism of action has still to be clarified and further insights are
803 needed to demonstrate potential direct endothelial effects. **However, the ability of members of the**
804 **NLRP3 inflammasome protein complex to target molecular and cellular pathways involved in both**
805 **metabolic and cardiovascular diseases suggest that selective pharmacological modulation of NLRP3**
806 **inflammasome has the potential to exert synergistic effects in the control of metabolic disorders and**
807 **related cardiovascular complications. The prospective clinical relevance of this strategy is also**
808 **supported by recent investigations on the influence of genetic variability in inflammasome on long-**
809 **term cardiovascular complications in diabetic patients. A few studies have reported a relationship**
810 **between NLRP3 genetic polymorphisms and development of type 2 diabetes [189,190] and, most**
811 **notably, a specific polymorphic NLRP3 allele has been reported to be associated with increased risk**
812 **for development of macrovascular complications in subjects with long-term diabetes [191].**
813
814

815 **AGE inhibitors** - The renowned deleterious effects of AGEs on endothelial function and
816 vessel structure has prompted numerous studies on molecules with **promising blocking** effects on
817 AGE formation and AGE-receptor (RAGE)-mediated activity, or acting to prevent or disrupt AGE-
818 protein cross-links (reviewed in [192-194]). Aminoguanidine is able to inhibit the formation of AGEs
819 by interaction with and quenching of dicarbonyl compounds. Despite the reported *in vivo* ability to
820 attenuate the formation of diabetes-induced AGEs and consequently reduce the extent of cross-
821 linking of connective tissue proteins in the arterial wall [195], the unfavorable risk/benefit ratio
822 discourages the use of aminoguanidine in the clinical setting [196, 197]. Another compound,
823 alagebrium chloride (ALT-711), which cleaves **AGEs** and protein cross-links thereby facilitating **AGEs**
824
825
826

827
828
829 clearance, appears to have a more encouraging safety profile; however, protective effects on
830 endothelial function have been not consistent in patients with isolated systolic hypertension [198],
831 atherosclerosis [199], or chronic heart failure [200]. **Although** B vitamins (pyridoxamine, thiamine
832 and its derivative benfotiamine) and pyridoxamine analogue ALT-946 may improve endothelial
833 dysfunction [201] and prevent AGE-related complications through inhibition of AGE-dependent
834 oxidative damage [202], some side effects on kidney function and creatinine levels [203,204]
835 together with **uncertainty** on overall beneficial effects makes the clinical utility of these drugs still
836 controversial [205].
837

838 **PKC inhibitors** - Impaired endothelium-dependent vasodilation secondary to hyperglycemia
839 may be significantly improved by administration of PKC- β inhibitors [206]. Based on this observation
840 ruboxistaurin, a selective PKC- β inhibitor, has been proposed to improve retinal blood flow
841 distribution and decrease macular edema in diabetic patients [207,208]. In addition, treatment with
842 this molecule has been show to ameliorate diabetic peripheral neuropathy without significant
843 adverse effects [209]. Promising results were obtained in two combined phase III clinical trials
844 **assessing** the ability of ruboxistaurin to reduce visual loss of 50% above standard care [210]. More
845 recently, ruboxistaurin has been proposed as a potential treatment for reducing atherosclerotic
846 **plaques** in diabetic patients. Since PKC activation promotes endothelial dysfunction by de-regulating
847 IL-18/IL-18BP pathway, leading to increased VCAM-1 expression, monocyte/macrophage adhesion,
848 and accelerated atherosclerotic plaque formation, inhibition of PKC by ruboxistaurin may represent a
849 potential new mechanism to ameliorate endothelial dysfunction in diabetic patients [211].
850

851 **VEGF inhibitors** - Activation of tyrosine kinase receptors VEGFR-1 and VEGFR-2 [212] by
852 increased levels of VEGF is associated with neovascularization in proliferative diabetic retinopathy as
853 well as diabetic macular edema (DME) [213]. **Strategies to inhibit the action of VEGF have to consider**
854 **that VEGF activity is vital in processes such as angiogenesis in the myocardium and wound healing**
855 **[214]. Therefore, although systemic anti-VEGF treatments are approved and used for other clinical**
856 **conditions, their serious side effects in the context of diabetes must be carefully evaluated [215]. On**
857 **the other hand, intraocular administration of VEGF inhibitors such as ranibizumab, bevacizumab,**
858 **pegaptanib, and aflibercept has been approved for treatment of diabetic retinopathy.** Ranibizumab is
859 a recombinant antigen-binding Fab fragment of humanized anti-VEGF monoclonal antibody with a
860 high ability to penetrate through the retina. At present, ranibizumab is the only compound approved
861 for the treatment of visual loss due to DME [216]. Extended pharmacovigilance studies, however, are
862 required to confirm the long-term ocular and systemic safety of ranibizumab treatment in patients
863 with DME [217]. Bevacizumab, initially developed for intravenous treatment of metastatic colorectal
864 cancer, has been adapted for off-label use in an intraocular administration [218] and it seems
865 effective in decreasing retinal, disc and iris neovascularization in diabetic patients with proliferative
866 retinopathy and macular edema [219] [220,221]. Debates remained in the past years on whether
867 bevacizumab is superior to ranibizumab in terms of potency, efficacy and safety (reviewed in [221]).
868 Results from the Diabetic Retinopathy Clinical Research (DRCR) phase II and Bevacizumab Or Laser
869 Therapy (BOLT) **studies** showed favorable effects of intravitreal bevacizumab administration for the
870 treatment of diabetic ocular neovascularization [222,223].
871

872 **PARP inhibitors** - The overactivation of the nuclear enzyme poly (ADP ribose) polymerase 1
873 (PARP-1) is implicated in acute endothelial dysfunction of diabetic vasculature [224,225]. Therefore,
874 pharmacological inhibition of PARP-1 appears a potential strategy to approach diabetic vascular
875 complications [226]. Experimental studies indicate a potential beneficial role of PARP-inhibition in
876 diabetic retinopathy [227] and in coronary arteriole dysfunction of db/db mice [228]. The therapeutic
877 effects of PARP-1 inhibitors are currently evaluated in clinical studies as potential candidates in
878 cancer or cardiovascular **diseases** including cardiovascular complications of diabetes [229].
879

880 **ROCK (Rho-associated kinase) inhibitors** - The RhoA/ROCK signaling pathway mediates
881 vascular smooth muscle contraction, downregulates eNOS gene expression and reduces protein
882 kinase B/Akt activation, therefore decreasing eNOS phosphorylation and catalytic activity [230].
883
884
885

886
887
888
889
890
891
892
893
894
895
896
897
898
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].

899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
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 (Ser⁵¹⁵ and Ser⁵¹⁸) 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 riboside (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].**

927
928
929
930
931
932
933
934
935
936
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]).

937
938
939
940
941
942
943
944
-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

945
946
947 vasodilation in diabetic patients [253-256]. In part, these frustrating results may be explained
948 by the non-selective scavenging properties of vitamin C and E, which probably interfere with
949 physiological important **signaling** mediated by ROS. Recent experimental studies have ascribed
950 the protective effects of vitamin E to reduced oxidative stress and apoptosis in experimental
951 diabetic cardiomyopathy and to decreased Ox-LDL mediated oxidative stress and vascular
952 muscle cell proliferation in aortic wall [257,258]. Clinical studies suggest that a combination of
953 vitamin C and insulin or a simultaneous infusion of GLP-1 and vitamin C may help to normalize
954 endothelial dysfunction and reduce both oxidative stress and inflammation in diabetic patients
955 [259,260], but the antioxidant potential of vitamin C and E in type 2 diabetic complications is
956 still controversial [261-263].

958 - **Polyphenols** - Polyphenols contained in fruits, vegetables, and beverages are well known for
959 their anti-oxidant properties [264]. **Scientific interest in polyphenols as therapeutic agents is**
960 **constantly increasing, and** results from several experimental studies suggest that these
961 compounds may improve endothelial function by multiple mechanisms **related, but not**
962 **limited, to the ability to increase eNOS expression and prostacyclin production, or to inhibit ET-**
963 **1 and endothelial NADPH oxidase activity, or via more complex intercellular activities that**
964 **reduce matrix metalloproteinase (MMP) activation, inhibit vascular cells migration and**
965 **proliferation, and modulate angiogenesis.** Polyphenols contained in cocoa, purple grape juice,
966 red wine, black and green tea, coffee and berry have also shown the ability to acutely and
967 chronically inhibiting platelet activation and aggregation. Moreover, for flavanols and
968 flavonols, prevention of vascular injury has been proposed to involve counterregulation of
969 AGE-mediated toxicity, low density lipoprotein oxidation, or inhibition of inflammatory
970 responses (see [265,266] for complete review). Recent studies indicate that resveratrol is able
971 to reverse the effects of hyperglycemia on mitochondrial function in endothelial cells, exerting
972 protective actions in the early diabetes-associated endothelial dysfunction [267]. **However,**
973 **many studies reporting biological effects of food polyphenols are limited by the insufficient**
974 **elucidation of the molecular, cellular, and physiological mechanisms underlying their effects.**
975 **Obstacles in this field may include non-specific effects of polyphenols with pleiotropic**
976 **activities, and complex interference among distinct active principles from the same food or**
977 **beverage. In addition, based upon the structure of the particular polyphenol and the cellular**
978 **redox context, most of these molecules may disclose both anti-oxidant and/or pro-oxidant**
979 **properties. Finally, effects of polyphenols are highly dependent on cell type, stress conditions,**
980 **and concentrations reached at the site of action. Thus, the clinical applicability of effects**
981 **observed *in vitro* must be proven, since absorption through the gut may significantly reduce**
982 **bioavailability and tissue concentration *in vivo*.** Clinical studies addressing these issues and
983 demonstrating the potential benefits of polyphenols are still awaited.

984 985 986 987 **CONCLUSIONS**

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

1004
1005
1006 improving metabolic homeostasis and inflammatory reaction, may effectively delay the progression
1007 of endothelial dysfunction and reduce the risk of cardiovascular events in the majority of diabetic
1008 patients. Concomitantly, the inter-individual susceptibility to diabetes and obesity, as well as the
1009 recognition of individual response to pharmacotherapy due to polymorphisms in genes encoding
1010 drug-metabolizing enzymes, transporters, receptors and signal transduction molecules is opening
1011 new roads to comprehend the variability existing in clinical outcomes of drugs used in diabetes.
1012 Overall, the choice of treatment for patients with diabetes should take into account the specific
1013 characteristics of the patient, the disease and the medication, aiming to a true personalized
1014 medicine. In this respect, the identification of intracellular signaling pathways and soluble mediators
1015 acting on metabolic, inflammatory and vascular cells has significantly broaden the spectrum of
1016 therapeutic opportunities to treat micro- and macrovascular complications of diabetes. Despite
1017 clinical substantiation is still far away, the current awareness of the key role played by the
1018 endothelium and its influence on vascular inflammation may represent an important step to develop
1019 promising new strategies to improve vascular dysfunction in diabetes.
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062

1063
1064
1065
1066
1067 **FIGURE LEGENDS**
1068

1069 **Figure 1.** The vicious circle linking metabolic abnormalities and inflammatory signaling to
1070 endothelial dysfunction in diabetes.
1071

1072
1073 **Figure 2.** Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial
1074 vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities,
1075 inflammatory response and endothelial dysfunction.
1076

1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180

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. *Pharmacogenomics 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.

1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239

- 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-Mamaeighani 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.

- 1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
- 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 Szmítko 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 adipovascular 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.

- 1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
- 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 hypo adiponectinemia 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.

1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416

- 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, Ebrahimiyan 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.

- 1417
1418
1419 98 Chen Y, Pitzer AL, Li X, Li PL, Wang L, Zhang Y. Instigation of endothelial nlrp3 inflammasome by
1420 adipokine visfatin promotes inter-endothelial junction disruption: Role of hmgb1. *J Cell Mol Med*
1421 2015;19:2715-2727.
1422 99 Wang L, Chen Y, Li X, Zhang Y, Gulbins E. Enhancement of endothelial permeability by free fatty
1423 acid through lysosomal cathepsin b-mediated nlrp3 inflammasome activation. *Oncotarget*
1424 2016;7:73229-73241.
1425 100 Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, Fishbein MC, Lehman TJ, Arditi M.
1426 Interleukin-1beta is crucial for the induction of coronary artery inflammation in a mouse model
1427 of kawasaki disease. *Circulation* 2012;125:1542-1550.
1428 101 Chen S, Lee Y, Crother TR, Fishbein M, Zhang W, Yilmaz A, Shimada K, Schulte DJ, Lehman TJ,
1429 Shah PK, Arditi M. Marked acceleration of atherosclerosis after lactobacillus casei-induced
1430 coronary arteritis in a mouse model of kawasaki disease. *Arterioscler Thromb Vasc Biol*
1431 2012;32:e60-71.
1432 102 Tomita S, Myones BL, Shulman ST. In vitro correlates of the I. Casei animal model of kawasaki
1433 disease. *J Rheumatol* 1993;20:362-367.
1434 103 Chen Y, Li X, Boini KM, Pitzer AL, Gulbins E, Zhang Y, Li PL. Endothelial nlrp3 inflammasome
1435 activation associated with lysosomal destabilization during coronary arteritis. *Biochim Biophys*
1436 *Acta* 2015;1853:396-408.
1437 104 De Boer AA, Monk JM, Liddle DM, Hutchinson AL, Power KA, Ma DW, Robinson LE. Fish-oil-
1438 derived n-3 polyunsaturated fatty acids reduce nlrp3 inflammasome activity and obesity-related
1439 inflammatory cross-talk between adipocytes and cd11b(+) macrophages. *J Nutr Biochem*
1440 2016;34:61-72.
1441 105 Hamilton SJ, Chew GT, Watts GF. Therapeutic regulation of endothelial dysfunction in type 2
1442 diabetes mellitus. *Diab Vasc Dis Res* 2007;4:89-102.
1443 106 Singh TP, Vangaveti VN, Malabu UH. Dipeptidyl peptidase-4 inhibitors and their potential role in
1444 the management of atherosclerosis-a review. *Diabetes Metab Syndr* 2015
1445 107 Cahn A, Miccoli R, Dardano A, Del Prato S. New forms of insulin and insulin therapies for the
1446 treatment of type 2 diabetes. *Lancet Diabetes Endocrinol* 2015
1447 108 Knop FK, Vilsboll T, Holst JJ. Incretin-based therapy of type 2 diabetes mellitus. *Curr Protein Pept*
1448 *Sci* 2009;10:46-55.
1449 109 Cernea S, Raz I. Therapy in the early stage: Incretins. *Diabetes Care* 2011;34 Suppl 2:S264-271.
1450 110 Kawalec P, Mikrut A, Lopuch S. The safety of dipeptidyl peptidase-4 (dpp-4) inhibitors or sodium-
1451 glucose cotransporter 2 (sglt-2) inhibitors added to metformin background therapy in patients
1452 with type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Metab Res Rev*
1453 2014;30:269-283.
1454 111 Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, Espadero RM, Woerle HJ, Broedl
1455 UC, Johansen OE. Sglit-2 inhibitors and cardiovascular risk: Proposed pathways and review of
1456 ongoing outcome trials. *Diab Vasc Dis Res* 2015;12:90-100.
1457 112 Triggie CR, Ding H. Metformin is not just an antihyperglycaemic drug but also has protective
1458 effects on the vascular endothelium. *Acta Physiol (Oxf)* 2017;219:138-151.
1459 113 Kinaan M, Ding H, Triggie CR. Metformin: An old drug for the treatment of diabetes but a new
1460 drug for the protection of the endothelium. *Med Princ Pract* 2015;24:401-415.
1461 114 Arunachalam G, Samuel SM, Marei I, Ding H, Triggie CR. Metformin modulates hyperglycaemia-
1462 induced endothelial senescence and apoptosis through sirt1. *Br J Pharmacol* 2014;171:523-535.
1463 115 Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor kappaB
1464 activation via amp-activated protein kinase activation in vascular endothelial cells. *Hypertension*
1465 2006;47:1183-1188.
1466 116 Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the amp-activated kinase by antidiabetes drug
1467 metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock
1468 protein 90 and endothelial nitric oxide synthase. *Diabetes* 2006;55:496-505.
1469
1470
1471
1472
1473
1474
1475

1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534

- 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.

- 1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
- 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, Doroshenko 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, Sjolholm 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 tnfr-alpha-

- 1594 induced oxidative stress and inflammation in endothelial cells. *Atherosclerosis* 2012;221:375-
1595 382.
- 1596
1597
1598 153 Krasner NM, Ido Y, Ruderman NB, Cacicedo JM. Glucagon-like peptide-1 (glp-1) analog
1599 liraglutide inhibits endothelial cell inflammation through a calcium and ampk dependent
1600 mechanism. *PLoS One* 2014;9:e97554.
- 1601
1602 154 He L, Wong CK, Cheung KK, Yau HC, Fu A, Zhao HL, Leung KM, Kong AP, Wong GW, Chan PK, Xu
1603 G, Chan JC. Anti-inflammatory effects of exendin-4, a glucagon-like peptide-1 analog, on human
1604 peripheral lymphocytes in patients with type 2 diabetes. *J Diabetes Investig* 2013;4:382-392.
- 1605 155 Prasad-Reddy L, Isaacs D. A clinical review of glp-1 receptor agonists: Efficacy and safety in
1606 diabetes and beyond. *Drugs Context* 2015;4:212283.
- 1607 156 Pala L, Pezzatini A, Dicembrini I, Ciani S, Gelmini S, Vannelli BG, Cresci B, Mannucci E, Rotella CM.
1608 Different modulation of dipeptidyl peptidase-4 activity between microvascular and
1609 macrovascular human endothelial cells. *Acta Diabetol* 2012;49 Suppl 1:S59-63.
- 1610 157 Deacon CF, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and
1611 sulphonylureas. *Diabetes Obes Metab* 2016;18:333-347.
- 1612 158 Matsubara J, Sugiyama S, Sugamura K, Nakamura T, Fujiwara Y, Akiyama E, Kurokawa H, Nozaki
1613 T, Ohba K, Konishi M, Maeda H, Izumiya Y, Kaikita K, Sumida H, Jinnouchi H, Matsui K, Kim-
1614 Mitsuyama S, Takeya M, Ogawa H. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin,
1615 improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein e-
1616 deficient mice. *J Am Coll Cardiol* 2012;59:265-276.
- 1617 159 Aroor AR, Sowers JR, Bender SB, Nistala R, Garro M, Mugerfeld I, Hayden MR, Johnson MS,
1618 Salam M, Whaley-Connell A, Demarco VG. Dipeptidylpeptidase inhibition is associated with
1619 improvement in blood pressure and diastolic function in insulin-resistant male Zucker obese
1620 rats. *Endocrinology* 2013;154:2501-2513.
- 1621 160 Shah Z, Pineda C, Kampfrath T, Maiseyeu A, Ying Z, Racoma I, Deulius J, Xu X, Sun Q, Moffatt-
1622 Bruce S, Villamena F, Rajagopalan S. Acute dpp-4 inhibition modulates vascular tone through
1623 glp-1 independent pathways. *Vascul Pharmacol* 2011;55:2-9.
- 1624 161 Mason RP, Jacob RF, Kubant R, Ciszewski A, Corbalan JJ, Malinski T. Dipeptidyl peptidase-4
1625 inhibition with saxagliptin enhanced nitric oxide release and reduced blood pressure and sicam-
1626 1 levels in hypertensive rats. *J Cardiovasc Pharmacol* 2012;60:467-473.
- 1627 162 Sauve M, Ban K, Momen MA, Zhou YQ, Henkelman RM, Husain M, Drucker DJ. Genetic deletion
1628 or pharmacological inhibition of dipeptidyl peptidase-4 improves cardiovascular outcomes after
1629 myocardial infarction in mice. *Diabetes* 2010;59:1063-1073.
- 1630 163 Dai Y, Dai D, Wang X, Ding Z, Mehta JL. Dpp-4 inhibitors repress nlrp3 inflammasome and
1631 interleukin-1beta via glp-1 receptor in macrophages through protein kinase c pathway.
1632 *Cardiovasc Drugs Ther* 2014;28:425-432.
- 1633 164 van Poppel PC, Netea MG, Smits P, Tack CJ. Vildagliptin improves endothelium-dependent
1634 vasodilatation in type 2 diabetes. *Diabetes Care* 2011;34:2072-2077.
- 1635 165 Ayaori M, Iwakami N, Uto-Kondo H, Sato H, Sasaki M, Komatsu T, Iizuka M, Takiguchi S, Yakushiji
1636 E, Nakaya K, Yogo M, Ogura M, Takase B, Murakami T, Ikewaki K. Dipeptidyl peptidase-4
1637 inhibitors attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2
1638 diabetic patients. *J Am Heart Assoc* 2013;2:e003277.
- 1639 166 Savarese G, Perrone-Filardi P, D'Amore C, Vitale C, Trimarco B, Pani L, Rosano GM.
1640 Cardiovascular effects of dipeptidyl peptidase-4 inhibitors in diabetic patients: A meta-analysis.
1641 *Int J Cardiol* 2015;181:239-244.
- 1642 167 Ahmed HA, May DW, Fagan SC, Segar L. Vascular protection with dipeptidyl peptidase-iv
1643 inhibitors in diabetes: Experimental and clinical therapeutics. *Pharmacotherapy* 2015;35:277-
1644 297.
- 1645 168 Abbas AS, Dehbi HM, Ray KK. Cardiovascular and non-cardiovascular safety of dipeptidyl
1646 peptidase-4 inhibition: A meta-analysis of randomized controlled cardiovascular outcome trials.
1647 *Diabetes Obes Metab* 2016;18:295-299.
- 1648
1649
1650
1651
1652

1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711

- 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.

- 1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
- 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, Wuertth 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, Spitalowitz 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.

1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829

- 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 c beta 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, Gerald 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.

- 1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
- 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

1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947

- 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 kappa b 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 Antoniadou 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, Chowienzyk 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.

1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006

- 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, Glud 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 Sarmiento 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, Chindusting 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.

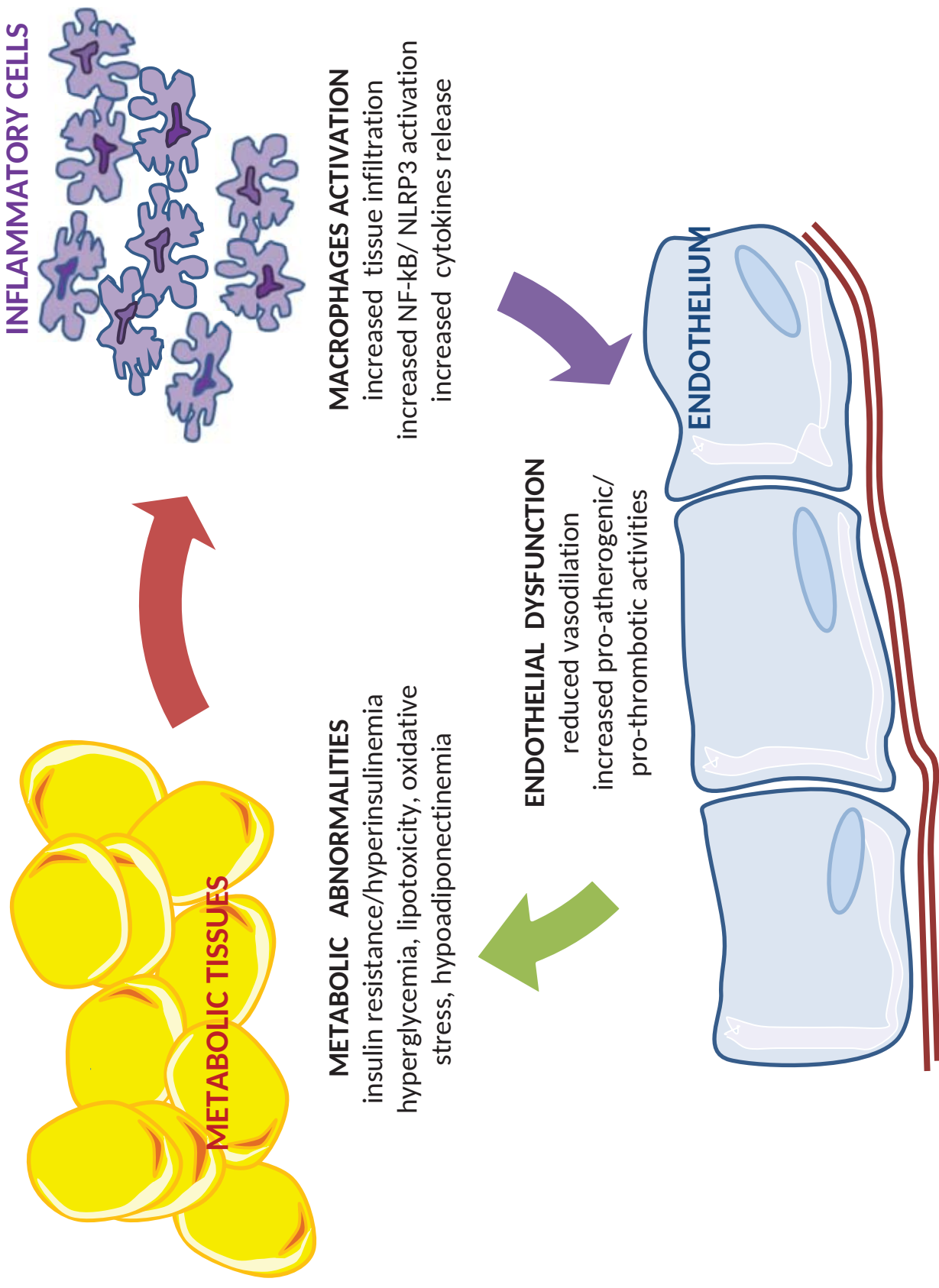


FIGURE 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes

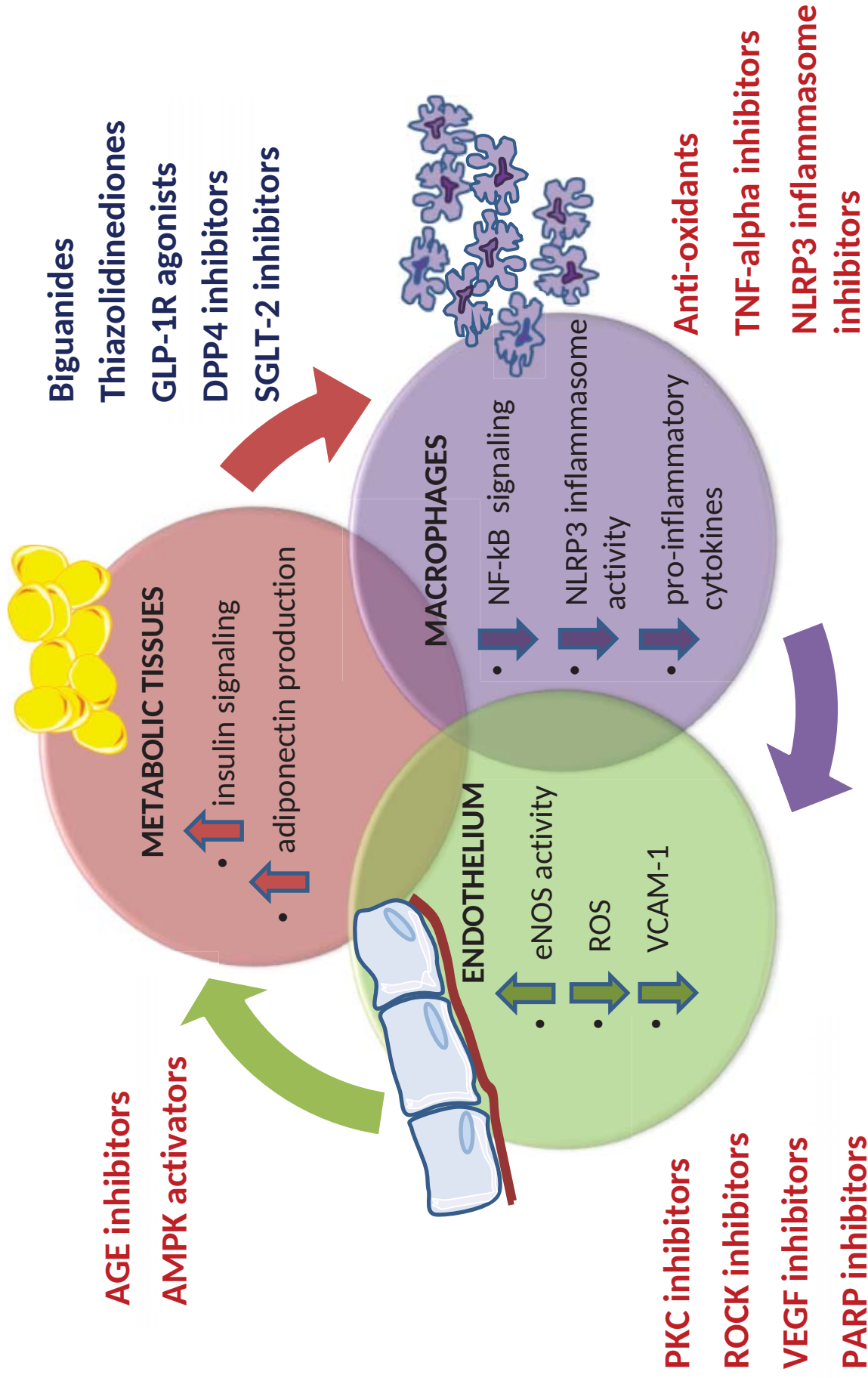


FIGURE 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction