Life Sciences 166 (2016) 121–130

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Diabetic nephropathy and endothelial dysfunction: Current and future therapies, and emerging of vascular imaging for preclinical renal-kinetic study

Wilson KC Leung, L Gao, Parco M Siu, Christopher WK Lai *

Department of Health Technology and Informatics, Hong Kong Polytechnic University, Hung Hom, HKSAR, China

ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 13 October 2016
Accepted 14 October 2016
Available online 17 October 2016

Keywords:
Diabetic nephropathy
Endothelial dysfunction
Therapy
Molecular mechanism
Multispectral optoacoustic tomography

ABSTRACT

An explosion in global epidemic of type 2 diabetes mellitus poses major rise in cases with vascular endothelial dysfunction ranging from micro- (retinopathy, nephropathy and neuropathy) to macro-vascular (atherosclerosis and cardiomyopathy) conditions. Functional destruction of endothelium is regarded as an early event that lays the groundwork for the development of renal microangiopathy and subsequent clinical manifestation of nephropathic symptoms. Recent research has shed some light on the molecular mechanisms of type 2 diabetes-associated comorbidity of endothelial dysfunction and nephropathy. Stemming from currently proposed endothelium-centered therapeutic strategies for diabetic nephropathy, this review highlighted some most exploited pathways that involve the intricate coordination of vasodilators, vasoconstrictors and vaso-modulatory molecules in the pathogenesis of diabetic nephropathy. We also emphasized the emerging roles of oxidative and epigenetic modifications of microvasculature as our prospective therapeutics for diabetic renal diseases. Finally, this review in particular addressed the potential use of multispectral optoacoustic tomography in real-time, minimally-invasive vascular imaging of small experimental animals for preclinical renal-kinetic drug trials.

Contents

* Corresponding author at: Department of Health Technology and Informatics, Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China.
E-mail address: chris.lai@polyu.edu.hk (C.W.K. Lai).

http://dx.doi.org/10.1016/j.lfs.2016.10.015

1. Introduction

Before the construction of this review article, we conducted an electronic database search through PubMed access to the MEDLINE database on life sciences and biomedical issues (Fig. 1). This search was inspired by a drastic increase in the number of journal articles on the subject matter of type 2 diabetes (129,504) in the MEDLINE database; approximately two-third of the publications (83,071/129,504) were yielded for the recent 10 years, and more than half of them (49,934/83,071) were found for the recent 5 years by 31 May 2016. Based on our research interest, we searched through PubMed/MEDLINE on the keywords "type 2 diabetes", "endothelial dysfunction" and "nephropathy" for the recent 5 years, and initially came up with a list of 85 full-text journal articles (Table S1), of which 45 irrelevant articles were excluded based on our contextualized interpretations of the titles and abstracts. The remaining 40 journal articles were used as the framework for constructing this review article, and only research articles (27/40) were summarized in Table S2 (including research type, subject characteristics and significant findings).

The aim of this review was to summarize some common signaling pathways of vasodilators, vasoconstrictors and vaso-modulators from currently proposed endothelium-centered therapies for diabetic nephropathy, and hence highlight some key potential cellular mechanisms for our future therapeutic development. This review also discussed the potential uses of cutting-edge optoacoustic imaging tools in the real-time monitoring of renal cortex/pelvis hemodynamics of small laboratory animals for preclinical drug trials.

2. Diabetes mellitus

Diabetes encompasses a spectrum of vascular complications in the micro- (eye, kidney and nerve) and the macro-vasculature (heart and brain) that are considerably responsible for the recently high morbidity and mortality. Epidemiological studies demonstrated the global incidence of diabetes had drastically increased from 108 million in 1980 to 422 million in 2014, which was shown to be obese-associated [1]. Diabetes and higher-than-optimal blood glucose also caused 3.7 million deaths in 2012, of which nearly half of them died before age of 70

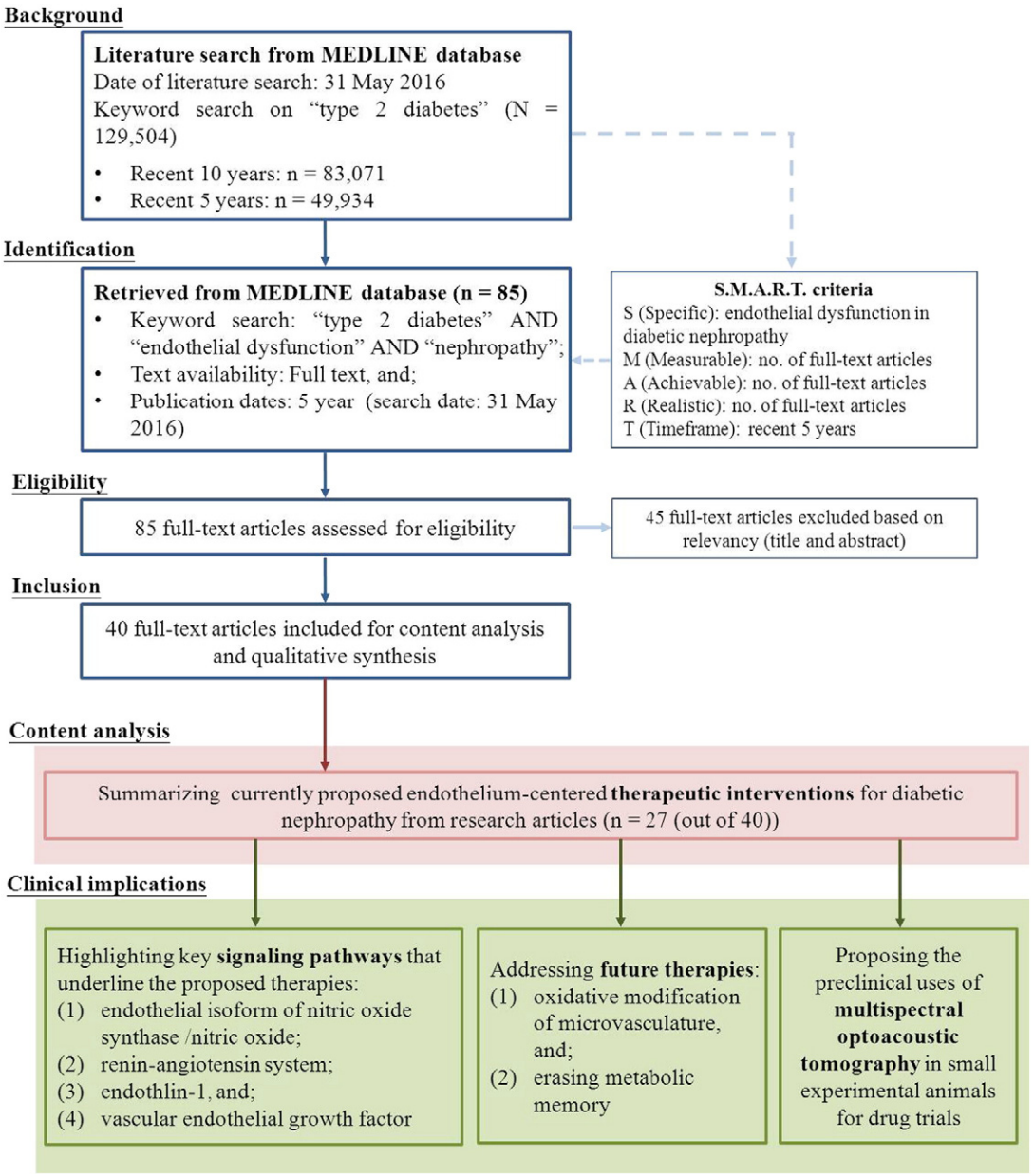

Fig. 1. Schematic workflow outlining the article selection process for the construction of this review article.

years. Intriguingly, it was estimated the mortality and disease burden of diabetes will grow at an unprecedented rate and project to be the 7th leading cause of mortality in 2030 [2]. The aetiology of type 2 diabetes mellitus (T2DM) is multifactorial; the causes of the disease are largely attributed to a complex interplay of genetic and environmental factors. Previous studies revealed the lifetime risk of a child of affected parents suffering from T2DM was 3 to 4 times higher than the general population and he/she usually developed diabetes at younger ages [3]. In addition, behavioral risk factors such as physical inactivity and unhealthy diets leading to overweight or obese problems usually precede the onset of prediabetes or overt diabetes [1].

3. High blood glucose-induced endothelial dysfunction

Hyperglycemia represents the pathologic hallmark of diabetes mellitus, and has been implicated in the onset and progression of endothelial dysfunction. Growing evidence demonstrated hyperglycemia triggers excessive reactive oxygen species (ROS) production, entailing oxidative tissue damages [4–6] and hence engaged in the development and progression of various diseases including cardiovascular diseases [7], nonalcoholic fatty liver disease [8], renal dysfunction [9], retinopathy [10,11] and cancers [12]. In endothelium, high glucose-stimulated ROS overproduction was shown to play a crucial role in endothelial cell senescence [13–16], which is an early sign of vascular complications in diabetes [17,18]. The production of ROS also uncouples the endothelial isoform of nitric oxide synthase (e-NOS) leading to perturbations to or reductions in nitric oxide (NO) bioavailability, which impair endothelium-dependent vasodilatation [19]. More intriguingly, this e-NOS uncoupling further augments superoxide radical production, and hence deteriorates vascular endothelial functionality. The overview of e-NOS/NO signaling and ROS production was summarized in Fig. 2.

4. Renal pathophysiology in T2DM

The primary function of kidney is to maintain constant plasma volume, salt concentrations, pH value and waste levels of extracellular fluids (plasma and interstitial fluid) inside our body [20,21]. The two major layers of membrane in the glomerular capsule provide some filtration barriers to shield plasma proteins, of which albumin does normally enter the filtrate, but only <1% is excreted in the urine. In T2DM, hyperglycemia-induced defects in renal capillary dilatation, podocyte loss and oxidative tubular injury to nephron (i.e. loss of reabsorption) might shed some light on the proposition of albuminuria or proteinuria as a gold diagnostic measure of chronic kidney disease. Elevated presence of albumin in the filtrate causes excessive tubular reabsorption that hence results into inflammatory and fibrotic responses, and progressive loss of renal functions [22]. Numerous recent studies [23–29] define the presence of microalbuminuria as an individual persistently having (≥3 months) (1) urinary albumin excretion ≥30 mg per day, (2) urinary albumin concentration > 20 μg/L, or (3) urinary albumin to creatinine ratio of 30–300 mg/g or >3 mg/mmol. However, some diabetic patients in advanced kidney disease stages did not clinically present microalbuminuria [30]. A recent experimental study also revealed in preclinical murine model, neither peak albuminuria nor albuminuria at 4 weeks after adriamycin-induced nephropathy was significantly correlated with histologic glomerular scarring [31]. These conflicting observations urge a revolutionary reform of the classical staging system of chronic kidney disease upon albuminuria into directly mapping impaired glomerular filtration to renal dysfunction, which can be minimal-invasively measured by current optoacoustic imaging systems in a real-time manner (to be discussed).

5. Current approaches to therapy for diabetic nephropathy and their underlying cellular mechanisms

Histologic presence of nodular glomerulosclerosis usually precedes the disease progression of diabetic nephropathy due to an accumulation of matrix materials, which contribute to glomerular basement membrane thickening, and hence increase renal blood flow and glomerular capillary pressure [32]. Since subtle changes in the vascular tone (vasoconstriction and vasodilation) of the glomerular afferent arterioles (i.e. microcirculation: <100 μm in diameter) adversely impact the blood

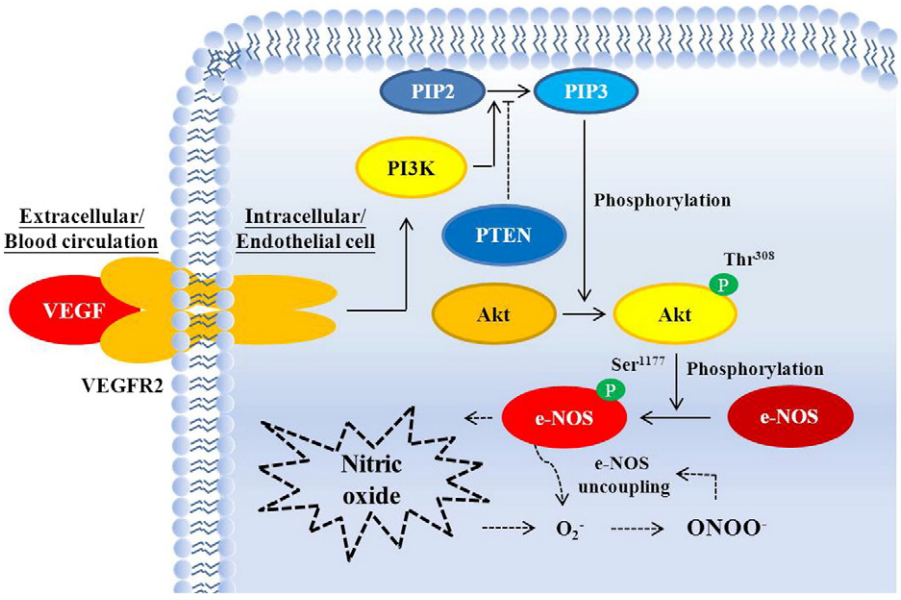

Fig. 2. In endothelial cells, VEGF signaling modulates e-NOS/NO-mediated vasodilation and ROS production. VEGF signaling is induced by the binding of VEGF ligands to their cognate membrane-bound receptors (VEGFR2), upon which the PI3K/Akt pathway is activated. The activated p-Akt^{Thr308} phosphorylates the serine-1177 residues on e-NOS, and triggers an increased production of NO. High-glucose-induced ROS overproduction uncouples e-NOS and hence leads to further O_2^- production, which reacts with NO to form $ONOO^-$. The $ONOO^-$ formation further uncouples e-NOS to augment O_2^- production, which induces substantial oxidative damages and impairs endothelial functions. **e-NOS**, endothelial isoform of nitric oxide synthase; **O_2^-**, superoxide molecules; **$ONOO^-$**, peroxynitrite molecules; **ROS**, reactive oxygen species; **VEGF**, vascular endothelial growth factor; **VEGFR2**, vascular endothelial growth factor receptor 2.

flow and exacerbate diabetic nephropathy, a stringent governing of vasodilators, vasoconstrictors and vaso-modulatory molecules is critically important in preserving renal functional integrity. Based on the findings from preclinical drug trials in laboratory animals and clinical trials in T2DM patients, we herein highlighted 4 potential signaling pathways that govern the vascular endothelial tone as the scientific basis of currently proposed drug therapies for diabetic nephropathy: (1) e-NOS/NO, (2) renin-angiotensin, (3) endothelin-1 (ET-1), and (4) vascular endothelial growth factor (VEGF).

5.1. Endothelial isoform of nitric oxide synthase/nitric oxide signaling

Induced by e-NOS, NO (an endothelium-derived relaxation factor) serves as a paracrine regulator, which diffuses across into vascular smooth muscle cells of blood vessels, triggers a cascade of signal transductions in cytosolic guanylylcyclase/cyclic GMP axis, and subsequently leads to vascular smooth muscle relaxation [19,33,34]. This regulatory molecule plays a central role in the regulation of blood flow and blood pressure that are intricately involved in the development of micro- and macro-vascular diseases. Recent studies addressed T2DM patients with chronic renal impairment displayed higher plasma levels of asymmetric dimethylarginine [35], which is an endogenous inhibitor of NOS. In addition, a significantly lower NOS activity was observed in T2DM patients with end-stage renal disease, whom the NOS activity was positively correlated with serum creatinine clearance (as a measure of renal function) [36]. Experimental evidence addressed the therapeutic potentials of **22-oxacalcitriol** (a vitamin D_3 analog) to improve endothelium-dependent flow-mediated dilatation (FMD) of femoral artery via augmented e-NOS expression in Sprague-Dawley (SD) fatty rats (with blood glucose levels >250 mg/dL), and ameliorate e-NOS uncoupling in high-glucose-treated cultured endothelial cells [37]. Preclinical drug trials of a nitric oxide-potentiating vasodilatory agent, namely **nebivolol**, also demonstrated beneficial effects on normalizing blood pressure, lipid profile, glomerular filtration rate and proteinuria in Zucker diabetic fatty (ZDF) rats, whom the renal or serum expressions of oxidative stress and inflammatory biomarkers including transforming growth factor-β1 and plasminogen activator inhibitor-1 were down-regulated [38].

5.2. Renin-angiotensin system

Under normal physiologic circumstances, declines in the blood volume and blood pressure of kidneys trigger juxtaglomerular secretion of enzyme renin in the formation of angiotensin I from the enzymatic cleavage of angiotensinogen [20,21]. Angiotensin-converting enzyme hence converts angiotensin I into angiotensin II by removal of two C-terminal amino acid residues to facilitate vasoconstriction. Experimental data demonstrated effective blockade of angiotensin-converting enzyme was a potential therapeutic target to relieve glomerular intracapillary pressure and subsequent glomerulosclerotic burden; combinatorial intervention of **ramipril** (angiotensin-converting enzyme inhibitor) and **sitaxsentan** (endothelin A-receptor antagonist) in ZDF rats versus non-diabetic lean controls improved proteinuria and glomerulosclerosis, and relinquished interstitial nephritis [39]. This observation was concordant with the findings in a nonrandomized clinical trial of **ramipril** in T2DM patients with stage-1 chronic kidney disease and proteinuria (>0.5 g/day) in improving proteinuria and FMD of brachial artery [40]. The changes in proteinuria and FMD were shown to inversely correlate with serum fibroblast growth factor (FGF)-23 [40], of which increased levels were suggested to be an independent predictor of chronic kidney progression in T2DM patients [41].

5.3. Circulating vasoactive peptide: endothelin-1

In the kidney, glomerular endothelial cells, mesangial cells and podocytes are capable of producing ET-1 [42], which targets endothelin A- and B-receptors (ET_A and ET_B) of vascular smooth muscle cells and ET_B of endothelial cells to facilitate vasoconstriction of renal vessels [43]. Within vascular smooth muscle cells, ET-1 receptor activation results into the orchestration of a multitude of cellular signaling pathways including MAPK, PI3-K and protein kinase B [44]. Increased circulating levels of ET-1 are commonly found in T2DM patients [45–47], and were shown to impair insulin sensitivity in apparently healthy human subjects in a hyperinsulinemic euglycemic study with co-infusion of ET-1 precursor and/or ET_A- or ET_B-receptor blockade [48]. Previous cross-sectional association studies in T2DM patients demonstrated elevated levels of plasma or urinary ET-1 were significantly correlated with the presence of microalbuminuria/macroalbuminuria and hypertension [49–52]. As aforementioned, combined therapy of **sitaxsentan** (ET_A-receptor antagonist) and **ramipril** (angiotensin-converting enzyme inhibitor) in ZDF rats was shown to improve proteinuria, glomerulosclerosis and interstitial inflammation [39]. A recent double blind, randomized, placebo-controlled clinical trial in T2DM patients with microalbuminuria (urinary albumin to creatinine ratio > 3 mg/mmol) also revealed that a dual endothelin receptor antagonist, namely **bosentan**, increased reactive hyperaemia index (as a measure of microvascular endothelial function) [28]. Experimental evidence from a recent preclinical study on **nebivolol** (a nitric oxide-potentiating vasodilatory agent) in ZDF rats also revealed a significant reduction in serum ET-1 levels [38], indicating a therapeutic crosstalk between endothelial vasodilators and vasoconstrictors of this agent.

5.4. Vascular endothelial growth factor: an endothelial cell-specific mitogen

Capillary losses over the progression of chronic kidney disease to end-stage renal disease are clinically manifested [53]; activation of VEGF, a potent angiogenic factor, may be another therapeutic target for alleviating diabetic nephropathy. Several cross-sectional or longitudinal studies in T2DM patients proposed urinary or serum VEGF levels as independent predictors of the presence of microalbuminuria [25,54] and chronic kidney disease progression [41]. Genetically-engineered constitutively-expressing or doxycycline-inducible **VEGF-A_{165}b** transgenic mice were shown to resist renal impairments upon streptozotocin (STZ) administration (a toxic agent targeting insulin-secreting beta cells of pancreas) as evidenced by reduced histologic features of glomerular abnormalities and preserved morphological integrity of glomerular endothelial glycocalyx versus non-genetically-modified controls [55]. Concurrently, injections of recombinant human **VEGF-A_{165}b** into podocytes of another mouse strain or ectopic expression of **VEGF-A_{165}b** in cultured primary podocytes and endothelial cells exhibited similar functions in protecting against STZ- or high-glucose-induced nephrotoxicity and endothelial dysfunction, respectively. A randomized clinical trial of **pioglitazone** and **rosiglitazone** (peroxisome proliferator-activated receptors-gamma agonists) in the treatment of metabolic syndrome of T2DM patients also accidentally discovered these agonists might hold additional vascular benefits in terms of their induction of angiogenesis markers (VEGF, interleukin-8 and angiogenin) [56]. Another possible angiogenic agent, namely **thymosin β4** (a thromboxane inhibitor), was largely implicated in diabetic retinol neovascularization [57], and was also shown to improve histopathologic changes of kidneys in diabetic KK Cg-Ay mice [58]. Paradoxically, **sulodexide**, an antithrombotic drug, was demonstrated to reduce urinary albumin to creatinine ratio, and suppress renal expressions of pro-fibrotic molecules and phospho-specific p38 MAPK possibly through inhibition of VEGF signaling in Otsuka-Long-Evans-Tokushima-Fatty T2DM rats [59]. This discrepancy can be well explained by dose-dependent impacts of VEGF in either being a friend or foe of diabetic nephropathy; an excessive amount of VEGF was suggested to be largely detrimental to renal endothelial functions in STZ-induced diabetic SD rats and diabetic e-NOS gene knockout mice [60]. Thus, VEGF-targeted intervention could have variable impacts on diabetic microangiopathy.

6. Future approaches to therapy

6.1. Oxidative modification of microvasculature

The pathologic complexes of diabetic vascular complications are simply ascribed to uncontrollable blood glucose levels that stimulate ROS overproduction. In the kidney, excessive ROS production triggers oxidative damages to glomerular basement membrane, subsequently leading to loss of filtration surface and impairment in urinary albumin homeostasis [24]. In endothelial cells, ROS over-production diminishes NO bioavailability, either through oxidative modification of NO (in the formation of peroxynitrite molecules) or direct interaction with e-NOS [19]. This e-NOS uncoupling further increases ROS production and impairs endothelial functionality. Given the angiogenic mechanisms of VEGF ultimately converge on Akt/e-NOS/NO signaling (Fig. 2), ROS-induced distortion in e-NOS/NO system (i.e. downstream effectors) might block the VEGF-stimulated vascular impacts in kidney. In addition, ROS-induced decline in NO may augment the serum levels of ET-1 (a vasoconstrictor) [38], which might exacerbate diabetic nephropathy.

NF-E2-related factor-2 (Nrf2) is recognized as a master guardian of lifespan that acts through targeting antioxidant response element to transactivate a multitude of phase II genes including heme oxygenase-1 (HO-1), whose protein products are tightly involved into ROS detoxification and elimination via conjugative stabilizing reactions or by augmenting cellular antioxidant capacity [61]. HO-1, which is ubiquitously expressed in eukaryotes, is an inducible protein in response to oxidative stress, and catalyzes the degradation of excessive heme into biliverdin (Fig. 3) [62]. The formation of biliverdin releases carbon monoxide and ferrous (Fe^{2+}), and in the presence of biliverdin reductase, it is therefore converted into bilirubin, which scavenges and counteracts ROS. Abrogation of Nrf2/HO-1 signaling was largely implicated in acute kidney injury [62], cardiac dysfunction [63], and cerebral ischemia [64]. Recent pharmacological research in natural products highlighted many bioactive compounds possess antioxidant properties via Nrf2/HO-1 signaling. Lycopene (a pharmacologically active compound abundantly found in many fruits and tomatoes) was shown to protect Wistar rats from cisplatin-induced nephrotoxicity via up-regulating the renal-cellular presence of nuclear Nrf2 and expression of HO-1 [65]. Curcumin, a powdered rhizome of *Curcuma longa* Linn, resisted the progression of cerebral ischemia as evidenced by its effects on the reduction of neurologic deficits, cerebral infarction and brain volume content via over-expressing Nrf2 and HO-1 protein in middle cerebral artery-occluded SD rats [64]. Grounded on the interconnected "Yin/Yang theory" of Traditional Chinese medicines, insufficient blood flow ("Yin/Qi") to organs will impair their functional integrities ("Yang") [66]; given (1) the blood circulation to the kidneys accounts for nearly one-fourth of the total cardiac output [22], (2) a classical traditional Chinese medicinal prescription of *Rheum rhabarbarum* and *Salvia miltiorrhiza* has been proven to be very efficacious and safe in treating chronic kidney diseases [67–69], (3) **Danshensu**, a naturally-occurring aqueous phenolic extract from *Salvia militorrhiza*, is pharmacologically recognized to facilitate blood circulation and get rid of blood stasis, (4) the predominant biodistribution of **Danshensu** was found in the kidney after intraperitoneal injections into mice [70], and (5) its well-recognized antioxidant roles in cardiac vasculature via acting on Akt/Nrf2/HO-1 pathway were largely implicated [71–74], we strongly believe a potential biological role of **Danshensu** in alleviating hyperglycemic oxidative stress in renal and endothelial cellular compartments. However, from our PubMed/MEDLINE search, no study has been found regarding the therapeutic potentials of **Danshensu** in diabetic nephropathy, where this research area deserves further investigations. The mechanistic crosstalk between Nrf2/HO-1 and Akt/e-NOS/NO signaling cascades, and the proposed mechanisms by which **Danshensu** intercepts hyperglycemic ROS production were depicted in Fig. 4.

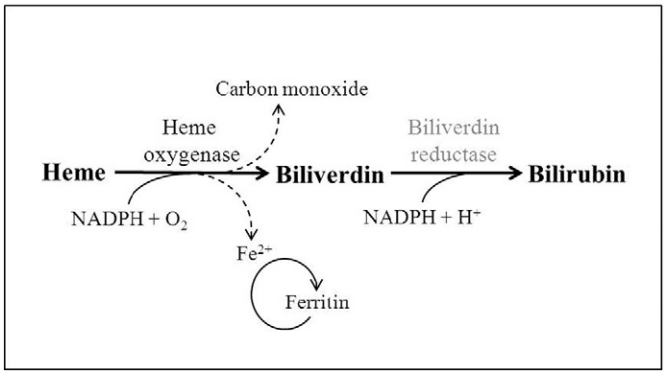

Fig. 3. Heme oxygenase catalyzes the conversion of heme into bilirubin, which counteracts reactive oxygen species. Heme oxygenase degrades heme into biliverdin through which carbon monoxide and ferrous (Fe^{2+}) are released. Biliverdin is thus converted into bilirubin in the presence of biliverdin reductase.

6.2. Erasing metabolic memory: an emerging role of epigenetics in diabetic nephropathy

The existence of metabolic memory is defined as a phenomenon that the effects of long-term or transient blood glucose changes persist long in macro- and micro-vasculatures even after attaining glycemic control in diabetes [75]. This "memory" was initially observed in the Diabetes Control and Complications Trial conducted by the United States National Institute of Diabetes and Digestive and Kidney Diseases that type 1 diabetes under intensive glycemic control had a lower incidence of vascular complications including nephropathy and neuropathy versus those with conventional therapy (although both groups ultimately achieved similar levels of glycated hemoglobin (HbA1c; a standard diagnostic measure of diabetes mellitus [1])) [76], implicating the effects of hyperglycemia on inducing vascular complications last long and even cannot be completely reversed once the vascular endothelial cells had prior exposure to high blood glucose. Some recent studies highlighted diabetogenic signals, in particular high blood glucose, stimulated the phenotypic alterations of vascular endothelial cells without changes in DNA sequences [77]. This pre-established renal vascular complications were thus shown to be largely attributed to hyperglycemic epigenetic histone/DNA modifications of numerous protein-coding genes [75] including forkhead box protein O1 (a gluconeogenic gene) [78], osteopontin (a commonly up-regulated gene in diabetic nephropathy) [79], signal transducer and activator of transcription 1 (a pro-inflammatory molecule) [80], of which most epigenetic methylation/acetylation signatures were highlighted on increased activating histone marks H3Kac, H3K4me1, H3K4me3 and H3K36me2, and decreased inactivating histone mark of H3K27me3.

Besides, non-coding RNA-mediated gene silencing or activation is currently considered as one of the epigenetic mechanisms that regulates endothelial cell phenotypic changes in response to high glucose in diabetes; hyperglycemia was suggested to alter endothelial microRNA (miR) and long non-coding RNA (lncRNA) expressions, where VEGF-targeted miR-320 was up-regulated in myocardial microvascular endothelial cells of type 2 diabetic Goto-Kakizaki rats [81], and anti-angiogenic miR-503 [82] and pro-inflammatory lncRNA metastasis-associated lung adenocarcinoma transcript 1 [83] were up-regulated in high-glucose-treated cultured endothelial cells. Since endothelial cell-derived plasma miRs were able to govern the vascular motile phenotypes of both endothelial and vascular smooth muscle cells [77], further understanding on their

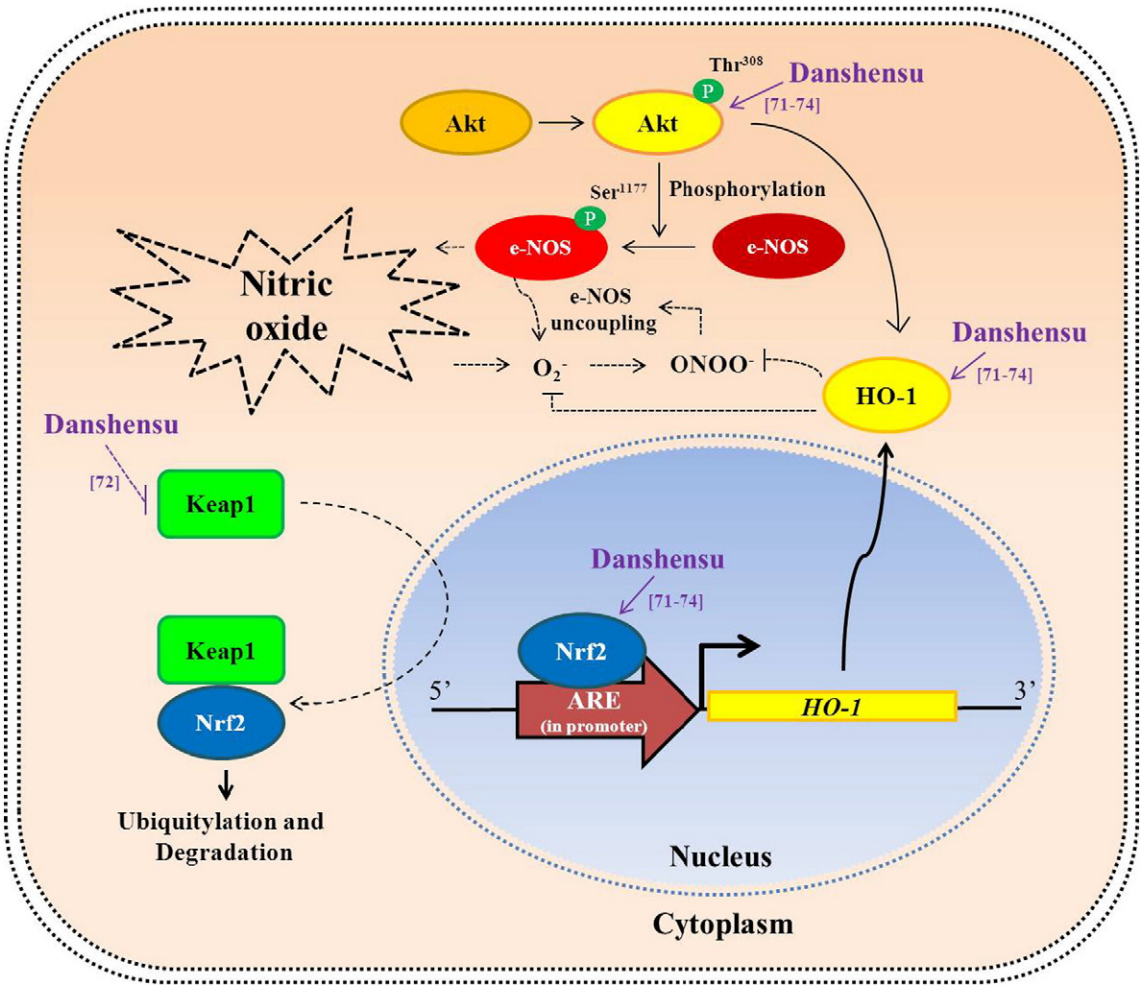

Fig. 4. Schematic representation of PI3K/Akt, Nrf2/HO-1 and e-NOS/NO signaling crosstalk. Keap1 transiently shuttles between nucleus and cytoplasm in cells, and probes Nrf2 to induce the ubiquitylation and proteasomal degradation of Nrf2. Once dissociated from Keap1, Nrf2 translocates into the nucleus, and targets consensus ARE regions to trigger transactivation of HO-1 expression, which therefore impedes ROS production. Danshensu was proposed to interfere ROS production via activations on Nrf2 and HO-1, and increased phosphorylation of Akt. **ARE**, antioxidant response element; **e-NOS**, endothelial isoform of nitric oxide synthase; **HO-1**, heme oxygenase-1; **Keap1**, Kelch-like ECH-associated protein 1; **Nrf2**, NF-E2-related factor-2; $\mathbf{O_2^-}$, superoxide molecules; $\mathbf{ONOO^-}$, peroxynitrite molecules; **ROS**, reactive oxygen species.

regulatory mechanisms could aid our identification of molecular target(s) for therapeutics.

7. Real-time optoacoustic vascular imaging of renal cortex/pelvis of small experimental animals

Since many findings from experimental studies on new drug candidates in tissue cultured cells turn out to be invalid in preclinical animal models and human clinical trials [27,84], macroscopic optic imaging attempted to offer an avenue for characterizing pharmacodynamics and biodistributions of small drug molecules. Nevertheless, a very long image acquisition time usually complicated the use of conventional small-animal optoacoustic techniques for preclinical drug trials [85]. This problem is largely attributed to the fact that large-scale image averaging was required to compensate the very weak signals acquired from low penetration power. In addition, these systems are not appropriate for real-time whole-body imaging of small experimental animals, hence most probably failing to capture some critical moments of relevant physiologic parameters.

Recent breakthrough in the dimension of macroscopic optic imaging has brought a variety of leading advantages from basic biology to preclinical practice; multispectral optoacoustic tomography (MSOT) has afforded very rapid high-radiant-power interlock system in the near-infrared region passing through several millimeters into centimeters of tissue to generate ultrasound signals, which substantially surpass light-scattering interference of tissue in the formation of high-contrast multiple spatial images [86]. It also carries high-throughput capability for quantitative differentiation of target tissues in video-rate mode to avoid image acquisition delay over time. Through multispectral unmixing algorithms, this system can pinpoint some regions of interest in the target tissues and allow multiple detections of signals at various wavelengths simultaneously, thereby accurately decomposing the biodistribution of relevant intrinsic and exogenous chromophores from non-specific background noises. With function-specific exogenous chromophores (e.g. indocyanine green, IRDye 800CW carboxylate, MMPSense 680), functional characterizations of drug candidates on tissues of interest will be optimized. Previous studies demonstrated FITC-sinistrin and IRDye 800CW carboxylate clearance were shown to significantly feature glomerular damage in adriamycin-administered mice [31]. MSOT was also able to detect atherosclerotic activity with aids of a protease-activatable fluorescent probe (MMPSense 680), and the conclusions drawn were in line with that from standard epi-fluorescent cryosection imaging, in situ zymography and immunohistochemistry of elevated activity of various matrix metalloproteases (MMPs) [87].

Besides, MSOT was capable of demonstrating a real-time tumoral expression and inhibition of MMP activities in a subcutaneous tumor-bearing mouse model [88].

Using the same imaging protocol that was validated and adopted in Scarfe group's study on SCID mice [31], our team was able to demonstrate the renal cortex/pelvis hemodynamics using isoflurane- anesthetic diabetic/obese *db*/*db* C57BL/6 mouse model [This preliminary study was approved by the Animal Subjects Ethics Sub-committee of the Hong Kong Polytechnic University (15-16/14-HTI-R-OTHERS)]. The two kinetic curves against time (min) displayed the near-infrared fluorescent IRDye 800CW carboxylate (10 nmol, intravenously injected) transition from renal cortex into pelvis, and the time difference between the signal peaks of the two curves ($T_{MAX-2} - T_{MAX-1}$) was used to compute kidney perfusion time (as a measure of glomerular filtration rate) (Fig. 5). Given the drug candidates potentiate renoprotective functions against diabetogenic nephrotoxicity, the time difference between T_{MAX-1} and T_{MAX-2} will be shortened compared with non-treated group. Fig. 6 illustrated the video-rate, real-time MSOT monitoring of kidney perfusion of the diabetic/obese *db*/*db* mouse.

Due to highly vascularized infrastructure of kidneys, the specificity and sensitivity of MSOT open a novel avenue for studies of renal-kinetic drug candidates in small experimental animals. Without sacrificing the animals as the endpoint, the minimally invasive characteristic of MSOT also anticipates the significance of experimental animal welfare and ethics (replacement, reduction and refinement) to pilot drug efficacy and safety in laboratory animals prior to conducting sizable clinical trials in human.

8. Conclusion

In summary, the published journal articles for the recent 5 years demonstrated a concurrent therapeutic direction for diabetic nephropathy towards targeting vasodilators, vasoconstrictors and vaso-modulatory molecules, where the signaling aberrations underlying the pathogenesis of vascular endothelial dysfunction lay the groundwork for the current therapeutic designs. The hyperglycemic induction of oxidative stress and epigenetic changes in kidneys offers new insight of our future drug studies and experimental trials, and our proposed optoacoustic imaging system provides feasibility of highly penetrating into target organs and processing extremely high-contrast vascular images of preclinical animal models for experimental drug trials that the data are valid and reliably re-produced.

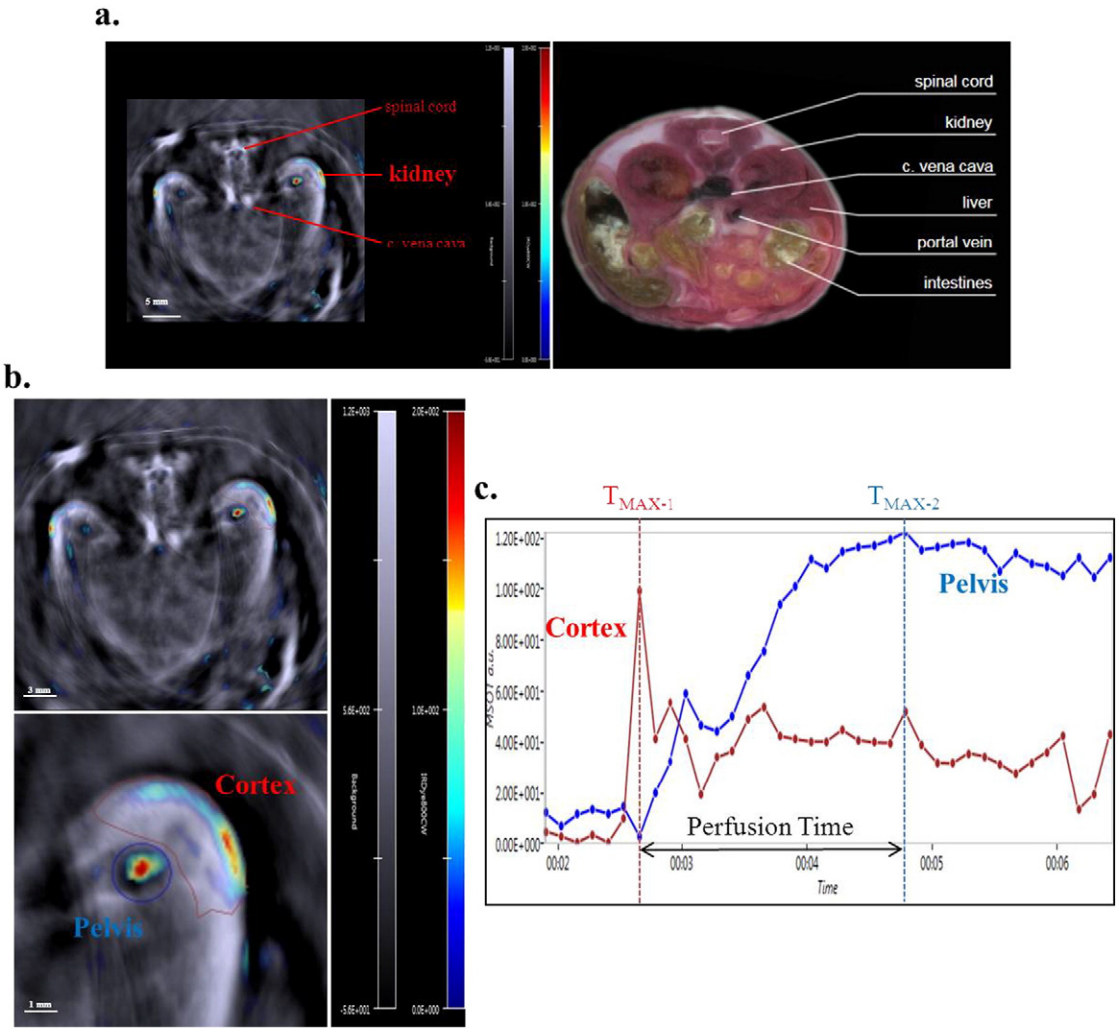

Fig. 5. Cross-sectional optoacoustic image of a diabetic/obese *db*/*db* C57BL/6 mouse. (a) The cross-sectional optoacoustic image aligned with the corresponding cryoslice at the same position. (b) The optoacoustic images illustrated the near-infrared fluorescent IRDye 800CW carboxylate transition from renal cortex to pelvis, and the regions of interest were selected (red, renal cortex; blue, renal pelvis). (c) The two kinetic curves of the selected regions demonstrated the fluorescent signal intensities against time (min) and also indicated two time points with maximal signals (T_{MAX-1} and T_{MAX-2}). The kidney perfusion time was thus computed by subtracting T_{MAX-1} from T_{MAX-2} (arrow).

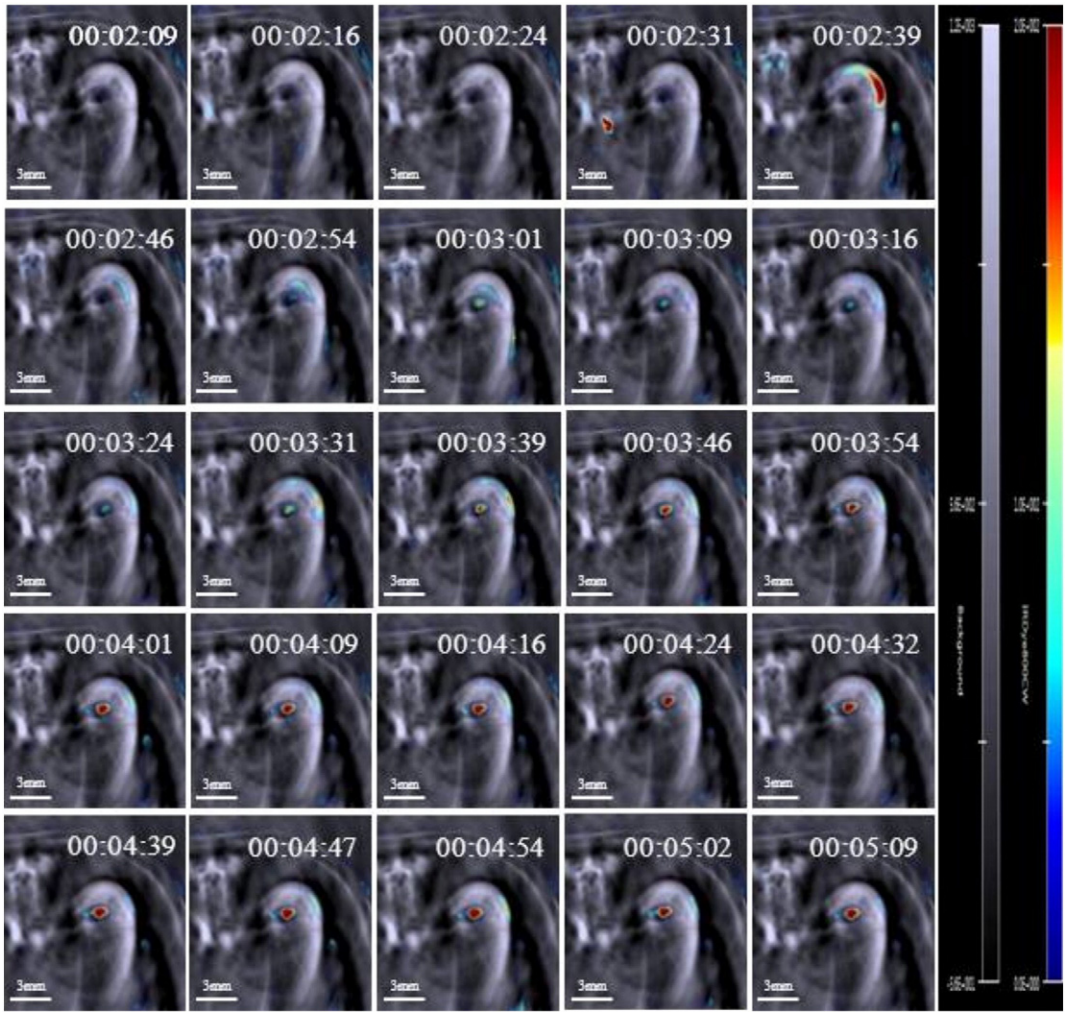

Fig. 6. Video-rate optoacoustic imaging of IRDye 800CW carboxylate perfusion from renal cortex into pelvis of a diabetic/obese *db*/*db* C57BL/6 mouse at various times.

Declaration of conflicting interests

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2016.10.015.

References

[1] World Health Organization, Global Report on Diabetes, World Health Organization, Geneva, 2016.
[2] C.D. Mathers, D. Loncar, Projections of global mortality and burden of disease from 2002 to 2030, PLoS Med. 3 (2006), e442.
[3] G.A.H. GA, Type 2 Diabetes: Prediction and Prevention, Wiley-Blackwell, Hoboken, 2007.
[4] U. Karunakaran, K.G. Park, A systematic review of oxidative stress and safety of antioxidants in diabetes: focus on islets and their defense, Diabetes Metab. J. 37 (2013) 106–112.
[5] B. Li, S. Liu, L. Miao, L. Cai, Prevention of diabetic complications by activation of Nrf2: diabetic cardiomyopathy and nephropathy, Exp. Diabetes Res. 2012 (2012) 216512.
[6] M.A. Modak, P.B. Parab, S.S. Ghaskadbi, Tissue specific oxidative stress profile in relation to glycaemic regulation in mice, Diabetes Metab. Res. Rev. 30 (2014) 31–41.
[7] T.V. Fiorentino, A. Prioletta, P. Zuo, F. Folli, Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases, Curr. Pharm. Des. 19 (2013) 5695–5703.
[8] G. Paradies, V. Paradies, F.M. Ruggiero, G. Petrosillo, Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease, World J. Gastroenterol. 20 (2014) 14205–14218.
[9] P. Rai, T. Singh, R. Lederman, A. Chawla, D. Kumar, K. Cheng, G. Valecha, P.W. Mathieson, M.A. Saleem, A. Malhotra, P.C. Singhal, Hyperglycemia enhances kidney cell injury in HIVAN through down-regulation of vitamin D receptors, Cell. Signal. 27 (2015) 460–469.
[10] B. Kumar, A. Kowluru, R.A. Kowluru, Lipotoxicity augments glucotoxicity-induced mitochondrial damage in the development of diabetic retinopathy, Invest. Ophthalmol. Vis. Sci. 56 (2015) 2985–2992.
[11] Y. Wu, L. Tang, B. Chen, Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives, Oxidative Med. Cell. Longev. 2014 (2014) 752387.
[12] L. Sciacca, R. Vigneri, A. Tumminia, F. Frasca, S. Squatrito, L. Frittitta, P. Vigneri, Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients, Nutr. Metab. Cardiovasc. Dis. 23 (2013) 808–815.
[13] T. Hayashi, K. Kotani, T. Yamaguchi, K. Taguchi, M. Iida, K. Ina, M. Maeda, M. Kuzuya, Y. Hattori, L.J. Ignarro, Endothelial cellular senescence is inhibited by liver X receptor activation with an additional mechanism for its atheroprotection in diabetes, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 1168–1173.
[14] T. Hayashi, H. Matsui-Hirai, A. Miyazaki-Akita, A. Fukatsu, J. Funami, Q.F. Ding, S. Kamalanathan, Y. Hattori, L.J. Ignarro, A. Iguchi, Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17018–17023.
[15] T. Hayashi, T. Yamaguchi, Y. Sakakibara, K. Taguchi, M. Maeda, M. Kuzuya, Y. Hattori, eNOS-dependent antisenscence effect of a calcium channel blocker in human endothelial cells, PLoS One 9 (2014), e88391.
[16] H. Matsui-Hirai, T. Hayashi, S. Yamamoto, K. Ina, M. Maeda, H. Kotani, A. Iguchi, L.J. Ignarro, Y. Hattori, Dose-dependent modulatory effects of insulin on glucose-induced endothelial senescence in vitro and in vivo: a relationship between telomeres and nitric oxide, J. Pharmacol. Exp. Ther. 337 (2011) 591–599.
[17] M. Christ, J. Bauersachs, C. Liebetrau, M. Heck, A. Günther, M. Wehling, Glucose increases endothelial-dependent superoxide formation in coronary arteries by NAD(P)H oxidase activation: attenuation by the 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitor, Diabetes 51 (2002) 2648–2652.
[18] C.M. Sena, A.M. Pereira, R. Seiça, Endothelial dysfunction—a major mediator of diabetic vascular disease, Biochim. Biophys. Acta 1832 (2013) 2216–2231.

[19] W.K. Lai, M.Y. Kan, Homocysteine-induced endothelial dysfunction, Ann. Nutr. Metab. 67 (2015) 1–12.
[20] I.F.S. IF, Human Physiology, thirteenth ed McGraw-Hill, New York, 2012.
[21] F.H. Martini, E.F. Bartholomew, Essentials of Anatomy and Physiology, sixth ed. Pearson, London, 2013.
[22] H. Birn, E.I. Christensen, Renal albumin absorption in physiology and pathology, Kidney Int. 69 (2006) 440–449.
[23] R.J. Martens, R.M. Henry, A.J. Houben, C.J. van der Kallen, A.A. Kroon, C.G. Schalkwijk, M.T. Schram, S.J. Sep, N.C. Schaper, P.C. Dagnelie, D.M. Muris, E.H. Gronenschild, F.M. van der Sande, K.M. Leunissen, J.P. Kooman, C.D. Stehouwer, Capillary rarefaction associates with albuminuria: the Maastricht study, J. Am. Soc. Nephrol. (2016) (pii ASN.2015111219).
[24] A.M. Mackawy, A.A. Khan, M.S. Badawy, Association of the endothelial nitric oxide synthase gene G894T polymorphism with the risk of diabetic nephropathy in Qassim region, Saudi Arabia—A pilot study, Meta. Gene. 2 (2014) 392–402.
[25] L. Petrica, A. Vlad, G. Gluhovschi, F. Gadalean, V. Dumitrascu, C. Gluhovschi, S. Velciov, F. Bob, D. Vlad, R. Popescu, O. Milas, S. Ursoniu, Proximal tubule dysfunction is associated with podocyte damage biomarkers nephrin and vascular endothelial growth factor in type 2 diabetes mellitus patients: a cross-sectional study, PLoS One 9 (2014), e112538.
[26] A. Rafnsson, M. Bäck, Urinary leukotriene E4 is associated with renal function but not with endothelial function in type 2 diabetes, Dis. Markers 35 (2013) 475–480.
[27] A. Alkhalaf, N. Kleefstra, K.H. Groenier, H.J. Bilo, R.O. Gans, P. Heeringa, J.L. Scheijen, C.G. Schalkwijk, G.J. Navis, S.J. Bakker, Effect of benfotiamine on advanced glycation endproducts and markers of endothelial dysfunction and inflammation in diabetic nephropathy, PLoS One 7 (2012), e40427.
[28] A. Rafnsson, F. Böhm, M. Settergren, A. Gonon, K. Brismar, J. Pernow, The endothelin receptor antagonist bosentan improves peripheral endothelial function in patients with type 2 diabetes mellitus and microalbuminuria: a randomized trial, Diabetologia 55 (2012) 600–607.
[29] A.K. Røndbjerg, E. Omerovic, H. Vestergaard, YKL-40 levels are independently associated with albuminuria in type 2 diabetes, Cardiovasc. Diabetol. 10 (2010) 54.
[30] S.Y. Lee, M.E. Choi, Urinary biomarkers for early diabetic nephropathy: beyond albuminuria, Pediatr. Nephrol. 30 (2015) 1063–1075.
[31] L. Scarfe, A. Rak-Raszewska, S. Geraci, D. Darssan, J. Sharkey, J. Huang, N.C. Burton, D. Mason, P. Ranjzad, S. Kenny, N. Gretz, R. Lévy, P.B. Kevin, M. García-Fiñana, A.S. Woolf, P. Murray, B. Wilm, Measures of kidney function by minimally invasive techniques correlate with histological glomerular damage in SCID mice with adriamycin-induced nephropathy, Sci. Rep. 5 (2015) 13601.
[32] R. Bilous, R. Donnelly, Handbook of Diabetes, fourth ed. Wiley-Blackwell, Hoboken, 2010.
[33] J. Deanfield, A. Donald, C. Ferri, C. Giannattasio, J. Halcox, S. Halligan, A. Lerman, G. Mancia, J.J. Oliver, A.C. Pessina, D. Rizzoni, G.P. Rossi, A. Salvetti, E.L. Schiffrin, S. Taddei, D.J. Webb, Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension, Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension, J. Hypertens. 23 (2005) 7–17.
[34] E. Stankevicius, E. Kevelaitis, E. Vainorius, U. Simonsen, Role of nitric oxide and other endothelium-derived factors, Medicina (Kaunas) 39 (2003) 333–341.
[35] M. Marra, F. Marchegiani, A. Ceriello, C. Sirolla, M. Boemi, C. Franceschi, L. Spazzafumo, I. Testa, A.R. Bonfigli, M. Cucchi, R. Testa, Chronic renal impairment and DDAH2-1151 A/C polymorphism determine ADMA levels in type 2 diabetic subjects, Nephrol. Dial. Transplant. 28 (2013) 964–971.
[36] S.S. El-Din Bessa, S.M. Hamdy, Impact of nitric oxide synthase Glu298Asp polymorphism on the development of end-stage renal disease in type 2 diabetic Egyptian patients, Ren. Fail. 33 (2011) 878–884.
[37] M. Hirata, K. Serizawa, K. Aizawa, K. Yogo, Y. Tashiro, S. Takeda, Y. Moriguchi, K. Endo, M. Fukagawa, 22-Oxacalcitriol prevents progression of endothelial dysfunction through antioxidative effects in rats with type 2 diabetes and early-stage nephropathy, Nephrol. Dial. Transplant. 28 (2013) 1166–1174.
[38] J.E. Toblli, G. Cao, J.F. Giani, M.C. Muñoz, M. Angerosa, F.P. Dominici, Long-term treatment with nebivolol attenuates renal damage in Zucker diabetic fatty rats, J. Hypertens. 29 (2011) 1613–1623.
[39] C. Zoja, S. Cattaneo, F. Fiordaliso, V. Lionetti, V. Zambelli, M. Salio, D. Corna, C. Pagani, D. Rottoli, C. Bisighini, G. Remuzzi, A. Benigni, Distinct cardiac and renal effects of ETA receptor antagonist and ACE inhibitor in experimental type 2 diabetes, Am. J. Physiol. Renal Physiol. 301 (2011) F1114–F1123.
[40] M.I. Yilmaz, A. Sonmez, M. Saglam, Y.G. Kurt, H.U. Unal, M. Karaman, M. Gok, H. Cetinkaya, T. Eyileten, Y. Oguz, A. Vural, F. Mallamaci, C. Zoccali, Ramipril lowers plasma FGF-23 in patients with diabetic nephropathy, Am. J. Nephrol. 40 (2014) 208–214.
[41] R. Agarwal, K.L. Duffin, D.A. Laska, J.R. Voelker, M.D. Breyer, P.G. Mitchell, A prospective study of multiple protein biomarkers to predict progression in diabetic chronic kidney disease, Nephrol. Dial. Transplant. 29 (2014) 2293–2302.
[42] D.E. Kohan, Endothelins in the normal and diseased kidney, Am. J. Kidney Dis. 29 (1997) 2–26.
[43] K.E. Meyers, C. Sethna, Endothelin antagonists in hypertension and kidney disease, Pediatr. Nephrol. 28 (2013) 711–720.
[44] A. Bouallegue, G.B. Daou, A.K. Srivastava, Endothelin-1-induced signaling pathways in vascular smooth muscle cells, Curr. Vasc. Pharmacol. 5 (2007) 45–52.
[45] K.J. Mather, B. Mirzamohammadi, A. Lteif, H.O. Steinberg, A.D. Baron, Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes, Diabetes 51 (2002) 3517–3523.
[46] K. Takahashi, M.A. Ghatei, H.C. Lam, D.J. O'Halloran, S.R. Bloom, Elevated plasma endothelin in patients with diabetes mellitus, Diabetologia 33 (1990) 306–310.
[47] M.C. Verhaar, F.E. Strachan, D.E. Newby, N.L. Cruden, H.A. Koomans, T.J. Rabelink, D.J. Webb, Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade, Circulation 97 (1998) 752–756.
[48] G. Ahlborg, J. Lindström, Insulin sensitivity and big ET-1 conversion to ET-1 after ETA- or ETB-receptor blockade in humans, J. Appl. Physiol. 93 (2002) (1985) 2112–2121.
[49] G. Ak, S. Buyukberber, A. Sevinc, H.M. Turk, M. Ates, R. Sari, H. Savli, A. Cigli, The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with type 2 diabetes mellitus, J. Diabetes Complicat. 15 (2001) 150–157.
[50] G. De Mattia, M. Cassone-Faldetta, C. Bellini, M.C. Bravi, O. Laurenti, R. Baldoncini, A. Santucci, C. Ferri, Role of plasma and urinary endothelin-1 in early diabetic and hypertensive nephropathy, Am. J. Hypertens. 11 (1998) 983–988.
[51] Y.J. Lee, S.J. Shin, J.H. Tsai, Increased urinary endothelin-1-like immunoreactivity excretion in NIDDM patients with albuminuria, Diabetes Care 17 (1994) 263–266.
[52] C.M. Zanatta, F. Gerchman, L. Burttet, G. Nabinger, M.C. Jacques-Silva, L.H. Canani, J.L. Gross, Endothelin-1 levels and albuminuria in patients with type 2 diabetes mellitus, Diabetes Res. Clin. Pract. 80 (2008) 299–304.
[53] K. Doi, E. Noiri, T. Fujita, Role of vascular endothelial growth factor in kidney disease, Curr. Vasc. Pharmacol. 8 (2010) 122–128.
[54] I.M. Kacso, C.I. Bondor, G. Kacso, Soluble serum Klotho in diabetic nephropathy: relationship to VEGF-A, Clin. Biochem. 45 (2012) 1415–1420.
[55] S. Oltean, Y. Qiu, J.K. Ferguson, M. Stevens, C. Neal, A. Russell, A. Kaura, K.P. Arkill, K. Harris, C. Symonds, K. Lacey, L. Wijeyaratne, M. Gammons, E. Wylie, R.P. Hulse, C. Alsop, G. Cope, G. Damodaran, K.B. Betteridge, R. Ramnath, S.C. Satchell, R.R. Foster, K. Ballmer-Hofer, L.F. Donaldson, J. Barratt, H.J. Baelde, S.J. Harper, D.O. Bates, A.H. Salmon, Vascular endothelial growth factor-A165b is protective and restores endothelial glycocalyx in diabetic nephropathy, J. Am. Soc. Nephrol. 26 (2015) 1889–1904.
[56] S.K. Vijay, M. Mishra, H. Kumar, K. Tripathi, Effect of pioglitazone and rosiglitazone on mediators of endothelial dysfunction, markers of angiogenesis and inflammatory cytokines in type-2 diabetes, Acta Diabetol. 46 (2009) 27–33.
[57] J.Y. Wang, Q. Lu, Y. Tao, Y.R. Jiang, J.B. Jonas, Intraocular expression of thymosin β4 in proliferative diabetic retinopathy, Acta Ophthalmol. 89 (2011) e396–e403.
[58] J. Zhu, L.P. Su, Y. Zhou, L. Ye, K.O. Lee, J.H. Ma, Thymosin β4 attenuates early diabetic nephropathy in a mouse model of type 2 diabetes mellitus, Am. J. Ther. 22 (2015) 141–146.
[59] J.J. Cha, Y.S. Kang, Y.Y. Hyun, S.Y. Han, Y.H. Jee, K.H. Han, J.Y. Han, D.R. Cha, Sulodexide improves renal function through reduction of vascular endothelial growth factor in type 2 diabetic rats, Life Sci. 92 (2013) 1118–1124.
[60] T. Nakagawa, W. Sato, T. Kosugi, R.J. Johnson, Uncoupling of VEGF with endothelial NO as a potential mechanism for abnormal angiogenesis in the diabetic nephropathy, J. Diabetes Res. 2013 (2013) 184539.
[61] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (2009) 13291–13295.
[62] K.A. Nath, Heme oxygenase-1 and acute kidney injury, Curr. Opin. Nephrol. Hypertens. 23 (2014) 17–24.
[63] L. Wang, X. Zhang, J.Y. Chan, L. Shan, G. Cui, Q. Cui, Y. Wang, J. Li, H. Chen, Q. Zhang, P. Yu, Y. Han, Y. Wang, S.M. Lee, A novel danshensu derivative prevents cardiac dysfunction and improves the chemotherapeutic efficacy of doxorubicin in breast cancer cells, J. Cell. Biochem. 117 (2016) 94–105.
[64] C. Yang, X. Zhang, H. Fan, Y. Liu, Curcumin upregulates transcription factor Nrf2, HO-1 expression and protects rat brains against focal ischemia, Brain Res. 1282 (2009) 133–141.
[65] K. Sahin, M. Tuzcu, N. Sahin, S. Ali, O. Kucuk, Nrf2/HO-1 signaling pathway may be the prime target for chemoprevention of cisplatin-induced nephrotoxicity by lycopene, Food Chem. Toxicol. 48 (2010) 2670–2674.
[66] P.K. Leong, H.S. Wong, J. Chen, K.M. Ko, Yang/Qi invigoration: an herbal therapy for chronic fatigue syndrome with yang deficiency? Evid. Based Complement. Alternat. Med. 2015 (2015) 945901.
[67] Y. Guan, X.X. Wu, J.L. Duan, Y. Yin, C. Guo, G. Wei, Y.H. Wang, Y.R. Zhu, Y. Weng, M.M. Xi, A.D. Wen, Effects and mechanism of combination of Rhein and danshensu in the treatment of chronic kidney disease, Am. J. Chin. Med. 43 (2015) 1381–1400.
[68] J.A. Luo, Y.M. Peng, Y.C. Xia, Y. Lei, Therapeutic effects of Chinese drugs on early renal damage of rats caused by fish bile, Zhongguo Zhong Xi Yi Jie He Za Zhi 13 (1993) 98–99 (Article in Chinese).
[69] H.H. Ngai, W.H. Sit, J.M. Wan, The nephroprotective effects of the herbal medicine preparation, WH30+, on the chemical-induced acute and chronic renal failure in rats, Am. J. Chin. Med. 33 (2005) 491–500.
[70] J. Wang, Z. Ma, Z. Hong, J. Song, Tissue distribution in mice of danshensu from sodium danshensu and Salvia miltiorrhizainjection, Zhongguo Zhong Yao Za Zhi 36 (2011) 1516–1518 (Article in Chinese).
[71] H. Li, F. Song, L.R. Duan, J.J. Sheng, Y.H. Xie, Q. Yang, Y. Chen, Q.Q. Dong, B.L. Zhang, S.W. Wang, Paeonol and danshensu combination attenuates apoptosis in myocardial infarcted rats by inhibiting oxidative stress: roles of Nrf2/HO-1 and PI3K/Akt pathway, Sci. Rep. 6 (2016) 23693.
[72] G. Cui, L. Shan, M. Hung, S. Lei, I. Choi, Z. Zhang, P. Yu, P. Hoi, Y. Wang, S.M. Lee, A novel danshensu derivative confers cardioprotection via PI3K/Akt and Nrf2 pathways, Int. J. Cardiol. 168 (2013) 1349–1359.
[73] T. Hu, G. Wei, M. Xi, J. Yan, X. Wu, Y. Wang, Y. Zhu, C. Wang, A. Wen, Synergistic cardioprotective effects of danshensu and hydroxysafflor yellow A against myocardial ischemia-reperfusion injury are mediated through the Akt/Nrf2/HO-1 pathway, Int. J. Mol. Med. 38 (2016) 83–94.

[74] J. Yu, L. Wang, M. Akinyi, Y. Li, Z. Duan, Y. Zhu, G. Fan, Danshensu protects isolated heart against ischemia reperfusion injury through activation of Akt/ERK1/2/Nrf2 signaling, Int. J. Clin. Exp. Med. 8 (2015) 14793–14804.
[75] M.A. Reddy, E. Zhang, R. Natarajan, Epigenetic mechanisms in diabetic complications and metabolic memory, Diabetologia 58 (2015) 443–455.
[76] Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus, JAMA 287 (2002) 2563–2569.
[77] A.C. Roberts, K.E. Porter, Cellular and molecular mechanisms of endothelial dysfunction in diabetes, Diab. Vasc. Dis. Res. 10 (2013) 472–482.
[78] S. Kumar, H. Pamulapati, K. Tikoo, Fatty acid induced metabolic memory involves alterations in renal histone H3K36me2 and H3K27me3, Mol. Cell. Endocrinol. 422 (2016) 233–242.
[79] M. Cai, P. Bompada, D. Atac, M. Laakso, L. Groop, Y. De Marinis, Epigenetic regulation of glucose-stimulated osteopontin (OPN) expression in diabetic kidney, Biochem. Biophys. Res. Commun. 469 (2016) 108–113.
[80] F. Miao, Z. Chen, S. Genuth, A. Paterson, L. Zhang, X. Wu, S.M. Li, P. Cleary, A. Riggs, D.M. Harlan, G. Lorenzi, O. Kolterman, W. Sun, J.M. Lachin, R. Natarajan, DCCT/EDIC Research Group, Evaluating the role of epigenetic histone modifications in the metabolic memory of type 1 diabetes, Diabetes 63 (2014) 1748–1762.
[81] X.H. Wang, R.Z. Qian, W. Zhang, S.F. Chen, H.M. Jin, R.M. Hu, MicroRNA-320 expression in myocardial microvascular endothelial cells and its relationship with insulin-like growth factor-1 in type 2 diabetic rats, Clin. Exp. Pharmacol. Physiol. 36 (2009) 181–188.
[82] A. Caporali, M. Meloni, C. Völlenkle, D. Bonci, G.B. Sala-Newby, R. Addis, G. Spinetti, S. Losa, R. Masson, A.H. Baker, R. Agami, C. le Sage, G. Condorelli, P. Madeddu, F. Martelli, C. Emanueli, Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia, Circulation 123 (2011) 282–291.
[83] P. Puthanveetil, S. Chen, B. Feng, A. Gautam, S. Chakrabarti, Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells, J. Cell. Mol. Med. 19 (2015) 1418–1425.
[84] T.K. Thethi, M.A. Bajwa, H. Ghanim, C. Jo, M. Weir, A.B. Goldfine, G. Umpierrez, C. Desouza, P. Dandona, Y. Fang-Hollingsworth, V. Raghavan, V.A. Fonseca, Effect of paricalcitol on endothelial function and inflammation in type 2 diabetes and chronic kidney disease, J. Diabetes Complicat. 29 (2015) 433–437.
[85] R. Ma, A. Taruttis, V. Ntziachristos, D. Razansky, Multispectral optoacoustic tomography (MSOT) scanner for whole-body small animal imaging, Opt. Express 17 (2009) 21414–21426.
[86] iTheraMedical, MSOT Principleaccessed 16.06.08) http://www.ithera-medical.com/technology/msot-principle.html2015.
[87] D. Razansky, N.J. Harlaar, J.L. Hillebrands, A. Taruttis, E. Herzog, C.J. Zeebregts, G.M. van Dam, V. Ntziachristos, Multispectral optoacoustic tomography of matrix metalloproteinase activity in vulnerable human carotid plaques, Mol. Imaging Biol. 14 (2012) 277–285.
[88] L. Zhu, J. Xie, M. Swierczewska, F. Zhang, Q. Quan, Y. Ma, X. Fang, K. Kim, S. Lee, X. Chen, Real-time video imaging of protease expression in vivo, Theranostics 1 (2011) 18–27.