

**Original Paper**

# DPP-4 Inhibitors as Therapeutic Modulators of Immune Cell Function and Associated Cardiovascular and Renal Insulin Resistance in Obesity and Diabetes

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**Key Words**

DPP-4 · Cardiorenal syndrome · Obesity · Diabetes · Insulin resistance

**Abstract**

The prevalence of obesity and diabetes continues to rise in the United States and worldwide. These findings parallel the expansion of childhood obesity and diabetes. Obesity is a central component of the cardiorenal metabolic syndrome (CRS) which increases the risk for cardiovascular disease (CVD) and chronic kidney disease (CKD). The hallmark of obesity, CRS, and early type 2 diabetes is insulin resistance, a result of decreased insulin metabolic signaling due, in part, to enhanced serine phosphorylation and/or proteasome-mediated degradation of the insulin receptor substrate. Cardiovascular and renal insulin resistance significantly contributes to endothelial dysfunction, impaired cardiac diastolic and vascular relaxation, glomerular injury, and tubular dysfunction. In this context, multiple factors including oxidative stress, increased inflammation, and inappropriate activation of the renin-angiotensin-aldosterone and the sympathetic nervous system contribute to overweight- and obesity-induced systemic and tissue insulin resistance. One common link between obesity and the development of insulin resistance appears to be a low-grade inflammatory response resulting from dysfunctional innate and adaptive immunity. In this regard, there has been recent work on the role of dipeptidyl peptidase-4 (DPP-4) in modulating innate and adaptive immunity. The direct effects of DPP-4 on immune cells and the indirect effects through GLP-1-dependent and -independent pathways suggest effects of DPP-4 inhibition may have beneficial effects beyond glycemic control in improving CVD and renal outcomes. Accordingly, this review addresses new insights into the role of DPP-4 in immune modulation and the potential beneficial effects of DPP-4 inhibitors in insulin resistance and associated CVD and CKD prevention.

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## Impact of Obesity and Diabetes on Cardiovascular and Chronic Kidney Disease

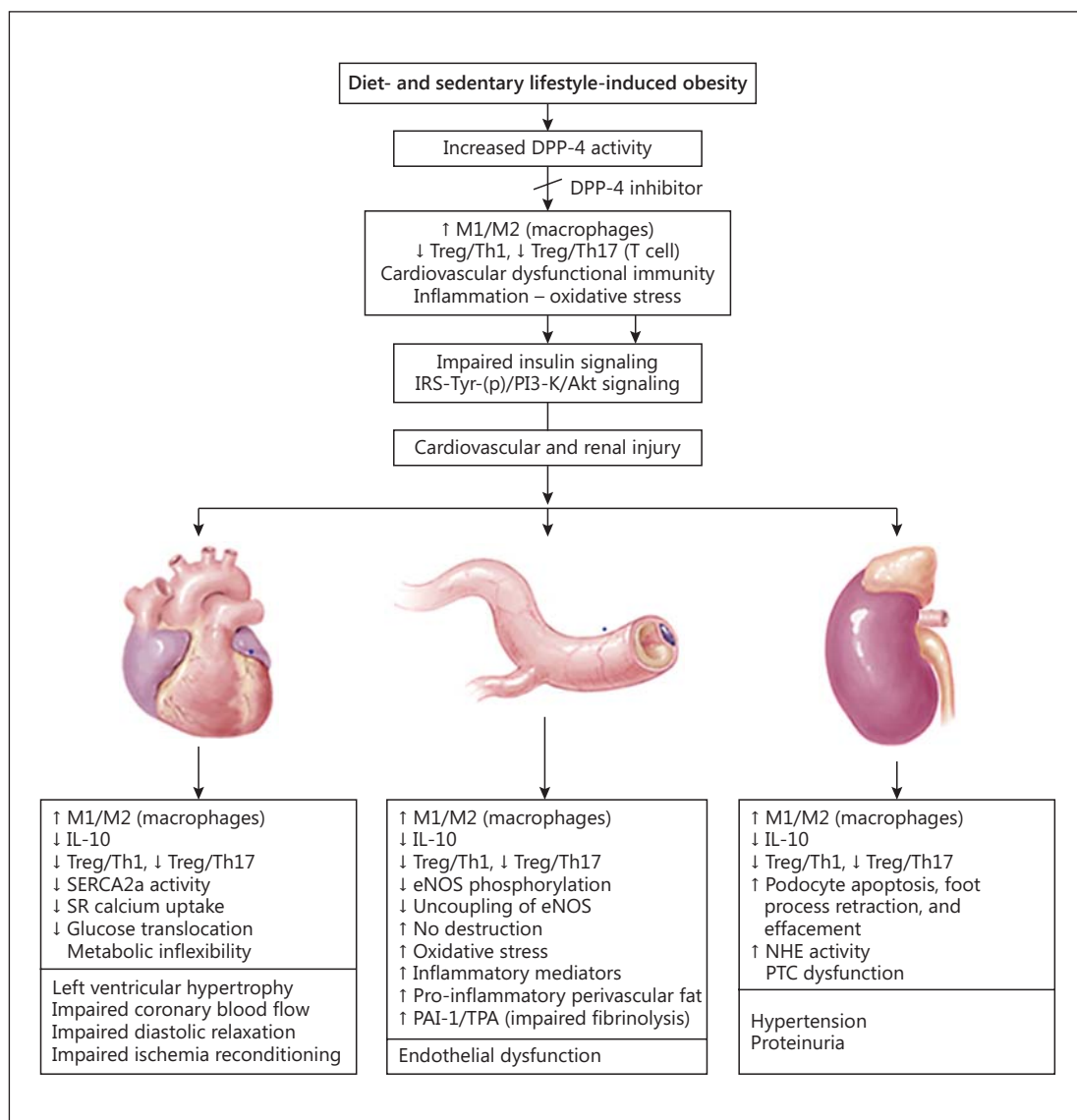
Overweight and obesity occur in more than 72 million American adults [1]. This epidemic is associated with increased cardiovascular disease (CVD) and chronic kidney disease (CKD) [2–4]. In addition, childhood-adolescent overweight and obesity are emerging major global public health concerns [5–7]. This emerging pandemic of childhood-adolescent obesity is largely thought to be triggered by the same sociologic/environmental factors which include a high fructose and fat intake and a sedentary lifestyle [7–9]. The presence of a constellation of interactive CVD and CKD risk factors, including overweight/obesity, hypertension, insulin resistance, metabolic dyslipidemia, hypertension, microalbuminuria, and renal function, contribute to the cardiorenal metabolic syndrome (CRS) in both children and adults [1, 6, 10]. These abnormalities are often present at an early age, long before clinical manifestations of CVD and CKD. Overweight and obesity contribute to the increasing prevalence of heart failure, especially that characterized by impaired diastolic function. There is also increasing evidence that excessive fat mass contributes to the development and progression of CKD independent of hypertension and diabetes mellitus [6, 10, 11]. Obesity, CRS, and CKD epidemics in the United States have paralleled the substantially increased consumption of high-fructose corn syrup which has increased dramatically in the past three decades [12, 13].

## Insulin Resistance and Increased Risk of CVD and CKD in Obesity and Diabetes

A common underlying mechanism that contributes to the progression of CVD and kidney injury is insulin resistance (fig. 1). Although heart failure may be attributed to the presence of associated conditions such as hypertension and coronary heart disease, the recognition of cardiac diastolic dysfunction in the absence of coronary heart disease and hypertension in obesity raises the intriguing notion that insulin resistance has a profound effect on cardiac function, especially on diastolic relaxation [14–16]. Microalbuminuria is a well-established early risk marker for vascular endothelial dysfunction, early CVD, and CKD in non-diabetic as well as diabetic patients. In this regard, insulin resistance may precede, facilitate, and predict microalbuminuria [17–23].

## Insulin Metabolic Signaling in the Heart, Vasculature, and Kidney and Impairment in the CRS

Insulin signaling occurs through two different pathways: the phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (PKB) (Akt) signaling pathway eliciting mainly metabolic responses and the mitogen-activated protein kinase (MAPK) signaling pathway eliciting growth responses [24–33]. The major converging point contributing to insulin resistance is the docking protein insulin receptor substrate (IRS). The phosphorylation of serine residues of IRS by protein kinase C (PKC), c-Jun kinase (JNK), and ribosomal p70 S6 kinase promotes phosphorylation of serine residues on IRS-1, which triggers proteasome-dependent degradation and attenuates IRS-1 tyrosine phosphorylation, association with p85 subunit of PI3-K, and downstream metabolic signaling [26–33]. Phosphorylation of tyrosine residues in the IRS results in the engagement of Src homology 2 (SH2) domain-binding motifs for SH2 domain signaling molecules, including PI3-K and Grb-2. When SH2 domains of the p85 regulatory subunit of PI3-K bind to the tyrosine-phosphorylated motifs on IRS, this activates the p110 catalytic subunit to generate phosphatidylinositol 3,4,5,



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**Fig. 1.** Role of DPP-4 in dietary obesity-mediated dysfunctional immunity and associated cardiovascular and renal insulin resistance.

(PI(3,4,5)P3). This molecule then binds to the pleckstrin homology domain in 3-phosphoinositide-dependent protein kinase-1 (PDK-1), resulting in its phosphorylation and the activation of other downstream kinases, including PKB (Akt) and atypical PKC isoforms, which mediate a number of metabolic actions including glucose transporter-4 (GLUT-4) translocation to the membrane, leading to glucose uptake in myocardial tissue and skeletal muscle as well as nitric oxide (NO) production in blood vessels. Binding of tyrosine-phosphorylated IRS-1 or Shc to the SH2 domain of Grb-2 activates MAPK to augment insulin growth effects and associated myocardial remodeling, hypertrophy, and cardiac fibrosis [26, 33].

## Cardiovascular and Renal Insulin Resistance and Organ Damage

The earliest manifestation of impaired cardiac insulin metabolic signaling is impaired diastolic relaxation related to impaired calcium handling, changes in substrate metabolism, mitochondrial dysfunction, inflammation, and oxidative stress resulting in cardiac fibrosis and reduced calcium uptake into the sarcoplasmic reticulum [27–33]. Endothelial dysfunction is also an important link between insulin resistance and cardiac diastolic dysfunction. Insulin resistance in endothelial cells results in impaired endothelial nitric oxide synthase (eNOS) activation and reduced bioavailable NO, leading to decreased substrate delivery, metabolic inflexibility, and cardiac fibrosis. Impaired insulin signaling in the kidney can lead to the glomerular and tubular dysfunction that is seen in obesity and early diabetes [28–33].

## Innate and Adaptive Immunity Influences Insulin Metabolism in Cardiac, Vascular, and Renal Disease (CRS)

An increased content of resident and infiltrating monocytes and macrophages is characteristic of adipose tissue in obese humans and mice. Macrophages are quite versatile and are thought to be key contributors to the chronic inflammation, insulin resistance, and cardiovascular dysfunction that accompany obesity-induced diabetes [34–36]. Upon activation, partly in response to immunological stimuli from the local microenvironment as well as systemic circulation, macrophages polarize into classical (M1) or alternative (M2) phenotypes. Interferon (IFN)- $\gamma$  and lipopolysaccharides (LPS) induce macrophages to differentiate into the M1 phenotype characterized by strong microbicidal and inflammatory activity. Alternatively, T helper 2 (Th2) cytokines (e.g. IL-4 and IL-13) convert macrophages to the M2 phenotype which are characterized by immunosuppressive and anti-inflammatory features [34–38]. M1 macrophages express elevated levels of surface CD11c and produce high levels of pro-inflammatory cytokines, IL-1 $\beta$ , and iNOS. In contrast, M2 macrophages are phenotypically characterized by their elevated expression of YM1, arginase-1, and IL-10 as well as their capacity to downregulate NADPH oxidase and oxidative stress [37–41]. In this regard, increased accumulation of pro-inflammatory M1 macrophages decreases insulin sensitivity [38–40]. In addition to macrophages, T cells also accumulate in adipose tissues in obesity with a similar polarization profile [39, 40]. Th1 are pro-inflammatory IL-2- and IFN- $\gamma$ -producing cells that promote tissue M1 infiltration and insulin resistance [38–40]. Activated Th1 cells also contribute to oxidative stress [39]. As mentioned earlier, IL-4-, IL-5-, and IL-13-producing Th2 cells play a key role in driving M2 macrophage polarization. T regulatory cells (Tregs) are a unique population of T cells which play a crucial role in the maintenance of self-tolerance and suppression of potentially pro-inflammatory T cells. A decrease in CD4+CD25+ Tregs and their ratio to Th17 and Th1 cells are typically observed in the adipose tissue of diabetic mice. This imbalance may have an effect on M1/M2 polarization and contribute to the insulin resistance and cardiovascular complications of diabetes.

The transcription factor forkhead/winged helix transcription factor 3 (FoxP3) is considered to be an essential factor for the proper development, maintenance, and function of cardiorenal protective CD4+CD25+ Tregs [42–48]. Tregs are immunosuppressive in insulin adipocytes [41–43]. The secretion of IL-10 has been identified as one mechanism by which FoxP3+ Tregs suppress NADPH oxidase in vascular tissue [43]. In contrast to Tregs, Th17 T cells promote inflammation in adipose tissue, thus the balance of Tregs versus Th17 cells is critical for maintaining functional immune homeostasis [49, 50]. Recently, cross-regulation of macrophage polarization and Treg function has been demonstrated. M2 macrophages have

also been shown to promote induction of Tregs [47], which regulate the induction of (with anti-inflammatory) M2 polarization in macrophages [46].

An understanding of the role of the immune system in regulating angiotensin II (Ang II) and aldosterone-induced cardiac dysfunction, vascular injury, and hypertension is emerging. For example, the adoptive transfer of CD4+CD25+ Tregs protects against Ang II-induced cardiovascular injury and renal dysfunction by limiting inflammation [48]. Recently, a pro-inflammatory cytokine produced by Th17 cells has been implicated in antagonizing the effects of Tregs [49]. Chronic infusion of Ang II and aldosterone results in increased production of IL-17 and decreased accumulation of Tregs [50]. We opine that an imbalance between inflammatory Th17 and anti-inflammatory Tregs may be one of the determinants of systemic and tissue insulin resistance and CRS induced by Ang II and aldosterone.

### **Role of DPP-4/DPP-4 Inhibition in Improvement of Insulin Sensitivity and CRS: Beyond Glycemic Control and Inhibition of Glucagon-Like Peptide-1 Degradation**

The gut-derived incretin hormones glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) play an important role in post-prandial and long-term glucose homeostasis by enhancing glucose-stimulated insulin secretion and suppressing glucagon release [51, 52]. The exopeptidase dipeptidyl peptidase-4 (DPP-4) rapidly degrades circulating GLP-1 and GIP, and DPP-4 inhibitors which decrease incretin degradation are valuable oral drugs in the treatment of type 2 diabetes. The effects of DPP-4 inhibitors extend beyond glycemic control, and in this review we explore the cardiovascular and renal benefits [51–55]. GLP-1 has been found to be cardioprotective in experimental models of heart failure and myocardial infarction [53, 54]. Since DPP-4 is not specific for GLP-1, it has the potential to mediate a wide range of pleiotropic effects independent of GLP-1 [55]. DPP-4 substrates other than GLP-1 include a chemokine, known as stromal cell-derived factor (SDF-1) alpha [56]. With respect to potential benefits of SDF-1, endothelial progenitor cells (EPCs) derived from the bone marrow are known to promote vascular repair and neoangiogenesis. EPCs differentiate into mature endothelial cells and assist in the reconstruction of the vasculature. Importantly, one of the regulators of EPCs is SDF-1 alpha, which actually stimulates their mobilization. Since SDF-1 alpha is a known substrate for DPP-4, inhibition of DPP-4 will increase SDF-1 alpha concentrations, potentially enhancing the delivery of EPCs to injured vascular sites [55–57].

The role of GLP-1 in lowering blood pressure has been reported in studies in both animals and humans [52, 56–58]. Sitagliptin has been shown to decrease blood pressure in hypertensive humans as well as hypertensive and diabetic rats. Direct vasodilator effects of DPP-4 inhibitors have also been described in vascular rings of the varied DPP-4 inhibitors tested; linagliptin was the most potent compound, followed by alogliptin and vildagliptin [52]. The vasorelaxant effects of alogliptin and linagliptin have been shown to involve the NO/cGMP pathway. In this regard, interactions of Ang II and DPP-4/GLP-1 signaling have been proposed as one of the mechanisms for a blood pressure-lowering effect of DPP-4 inhibition [58]. There is accumulating data to support the notion that Ang II binding to the Ang II type 1 receptor (AT1R) decreases adenylyl cyclase activity, inhibits cyclic-AMP formation, and stimulates type 3 sodium-hydrogen transport (NHE3) activity and transcellular Na<sup>+</sup> transport in kidney proximal tubule cells [59]. In this context, proximal tubular AT1R signaling is enhanced with prolonged exposure to insulin (24 h). This, in turn, generates a stronger increase in NHE3 activity compared to Ang II alone [60]. Recent work has been directed at exploring vesicle trafficking of NHE3, supporting the concept that NHE3 exists in a physical complex with DPP-4 in brush-border membranes isolated from proximal tubule cells. The DPP-4 and NHE3

complex has been localized to the microvillar domain, where NHE3 normally functions in Na<sup>+</sup> uptake and intracellular pH (pHi) recovery [61, 62]. However, DPP-4 activity in kidney tissue is increased by excess dietary fat and sucrose in rodent models [56, 63]. In addition to DPP-4, the receptor for GLP-1 (GLP-1r) has been identified in the proximal tubule and has been shown to have actions on proximal tubule Na<sup>+</sup> excretion. In a series of studies, GLP-1 infusion and GLP-1r agonism with exendin-4 have been shown to induce a natriuretic response in humans and rodent models. In this regard, direct infusion of GLP-1 has been shown to reduce NHE3-dependent pHi recovery through a PKA-dependent mechanism. Inhibition of DPP-4 has also been shown to reduce proximal tubular NHE3 activity and associated proximal tubular sodium uptake [61, 62].

### **Immunomodulatory Role of DPP-4 and Inhibition as a Target to Improve Systemic and Tissue Insulin Metabolic Signaling**

The immune modulatory role of DPP-4 in cardiovascular inflammation has been minimally investigated. Because of the widespread expression of the DPP-4 enzyme in T cells and macrophages, the role of DPP-4 inhibitors in the modulation of innate and adaptive immunity is an area of emerging importance [64–68]. In this regard, decreased accumulation of inflammatory M1 macrophages and increased levels of M2 macrophages seen in adipose tissue or atherosclerotic lesions following DPP-4 inhibitor treatment are intriguing [69, 70]. These observations raise the possibility that observed improvements in inflammation may occur because of attenuated DPP-4-mediated polarization of macrophages (decreases in M1 macrophages). Since DPP-4 activity in serum and tissues is markedly increased in obesity in animal models and humans [56, 63], inhibition of DPP-4 offers a novel strategy for suppression of low-grade inflammation and associated tissue insulin resistance. Moreover, recent studies demonstrating that M2 macrophages promote Treg induction [47] and DPP-4 inhibitors enhance M2 polarization [69, 70] have suggested the possibility that DPP-4 inhibitory therapy may improve cardiac and coronary artery insulin metabolic signaling and associated heart and coronary artery relaxation and renal damage. Therefore, the mutual effects of DPP-4 inhibition/GLP-1 signaling opposing Ang II/aldosterone effects may contribute to the beneficial modulation of immune responses in the CRS. In this respect, GLP-1 has been shown to modulate macrophage polarization and Treg expansion [71], and AT1R antagonist increases GLP-1r expression [72]. DPP-4 inhibition results in Treg expansion [73], whereas Ang II infusion or aldosterone infusion result in depletion of Tregs [43, 73]. The administration of DPP-4 inhibitors or Treg adoptive transfer suppresses oxidative stress in cardiovascular and renal tissues and raises the possibility of therapeutic targeting of immune function by combined administration of a DPP-4 inhibitor and an Ang II/mineralocorticoid receptor antagonist to improve insulin resistance and cardiovascular and renal function. In this regard, beneficial effects of such a combination have been shown to improve islet cell regeneration and diabetic nephropathy [74, 75]. In addition, a combination strategy of a DPP-4 inhibitor and adoptive transfer of Tregs may further improve insulin sensitivity (fig. 1) [76, 77].

In conclusion, a disrupted M1/M2 (or imbalance of the Th17/Th1/Tregs) homeostatic balance may define a critical link between nutrient excess and insulin resistance. One of the important regulatory pathways for this immunomodulation is crosstalk between the RAAS system and DPP-4 signaling. Drugs targeting these interactions offer promising therapy for the integrated control of glycemia and cardiovascular and renal outcomes of insulin resistance. In this regard, beneficial effects of combination of angiotensin receptor antagonists and DPP-4 inhibitors in improving islet cell regeneration and diabetic nephropathy have been reported [74, 75].

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## Disclosure Statement

The authors have nothing to disclose.

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