A.S.O. "S.Croce e Carle"
Cuneo

S.C. Endocrinologia, Diabetologia e Metabolismo
S.S. Malattie Metaboliche e Diabetologia
S.C. Nefrologia

DIABETE IN OSPEDALE:
il paziente con insufficienza renale

Sabato, 25 Gennaio 2014

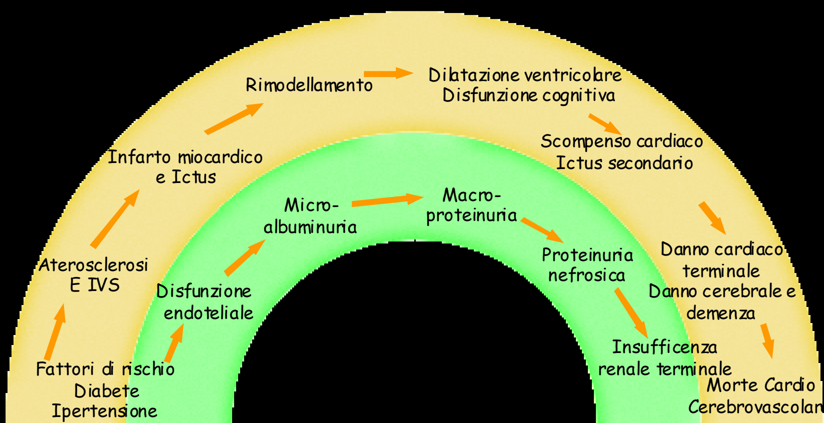
Centro Incontri della Provincia
Corso Dante, n. 41
12100 CUNEO

EFFETTI " RENALI " DEI NUOVI FARMACI IPOGLICEMIZZANTI

Silvio Settembrini

ASL Napoli 1

Servizio di Endocrinologia Diabetologia e Malattie Metaboliche - DS 26
Unita' di Nefro-Diabetologia , UOC di Nefrologia e Dialisi
Ospedale dei Pellegrini - Napoli



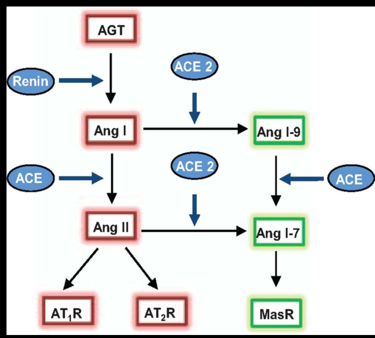
Obiettivo: prevenire, arrestare, ridurre il danno d'organo che è lo step intermedio tra esposizione ai fattori di rischio ed eventi

Quale è il "link" tra
sindrome cardio-metabolica e danno renale ?

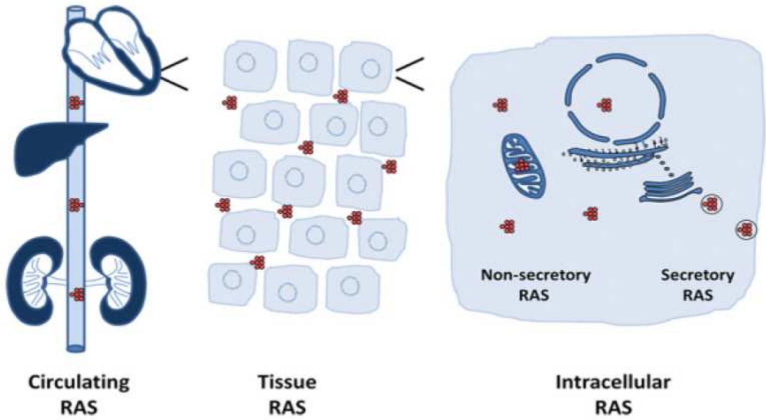
- Ipertensione arteriosa ?
- Diabete mellito di tipo 2 ?
- Stato proinfiammatorio (citochine ?)
- Dislipidemia ?
- Attivazione del sistema RAS e più in generale fattori emodinamici ?
- La disfunzione endoteliale ?
- L'insulinoresistenza ?

Quale terapia per prevenire CKD ?

- E' possibile prevenire CKD ?
- E' possibile stratificare il rischio di CKD in un paziente con sindrome metabolica ?
- Quale è la terapia adeguata ?
- Quale tempistica per iniziare una terapia adeguata ?
- Quale è il marker, se esiste, per monitorare l'efficacia del nostro intervento terapeutico ?
- Vi sono dei farmaci che ci possono indicare delle nuove opportunità terapeutiche ?



RENAL RAAS



The circulating, tissue and intracellular RASs

Intrarenal renin–angiotensin system in regulation of glomerular function

L. Gabriel Navar

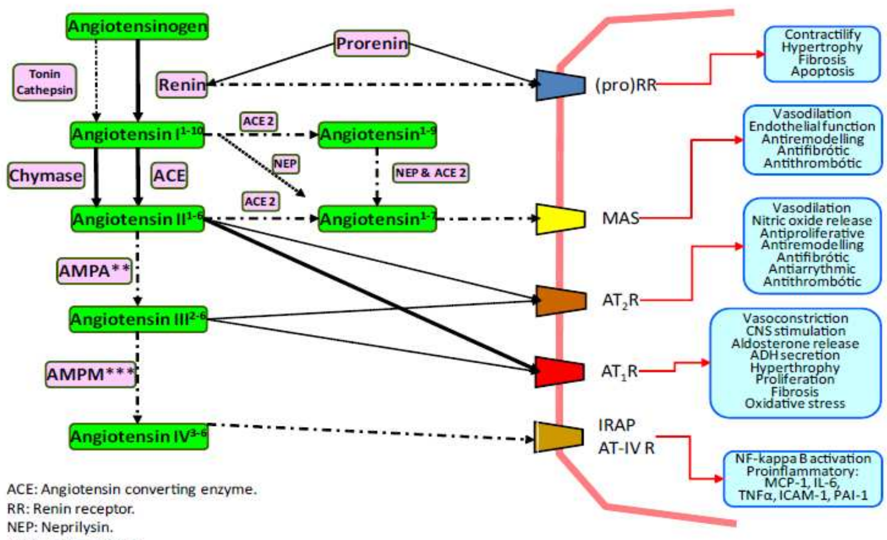
Purpose of review
The purpose of this review is to provide an update on the current knowledge regarding the role of the intrarenal rennin–angiotensin system (RAS) in the regulation of glomerular function including glomerular dynamics and filtration rate, glomerular permeability and structural alterations during chronic increases in intrarenal angiotensin (Ang) II.

Recent findings
Recent studies have continued to delineate the complex interactions among the various RAS components that participate in regulating glomerular function. Although Ang II acting on AT1 receptors remains as the predominant influence on glomerular dynamics, some of these effects are indirectly mediated by Ang II modulating the sensitivity of the macula densa tubuloglomerular feedback mechanism as well as the more recently described feedback mechanism from the connecting tubule. Interestingly, the actions of Ang II on these systems cause opposite effects on glomerular function demonstrating the complexities associated with the influences of Ang II on glomerular function. When chronically elevated, Ang II also stimulates and/or interacts with other factors, including reactive oxygen species, cytokines and growth factors and other hormones or paracrine agents, to elicit structural alterations.

Summary
Recent studies have provided further evidence for the presence of many components of the RAS in glomerular structures, which supports the importance of locally produced angiotensin peptides to regulate glomerular haemodynamics, filtration rate and macromolecular permeability and contribute to fibrosis and glomerular injury when inappropriately augmented.

Curr Opin Nephrol Hypertens 2014, 23:38–45

Renin-Angiotensin-Aldosterone System



ACE: Angiotensin converting enzyme.
RR: Renin receptor.
NEP: Neprilysin.
MAS: MAS receptor.
AMPA: Aminopeptidase A.
AMPM: Aminopeptidase M.
IRAP: insulin-regulated aminopeptidase

Cascade of intratubular RAAS in Ang II dependent hypertension

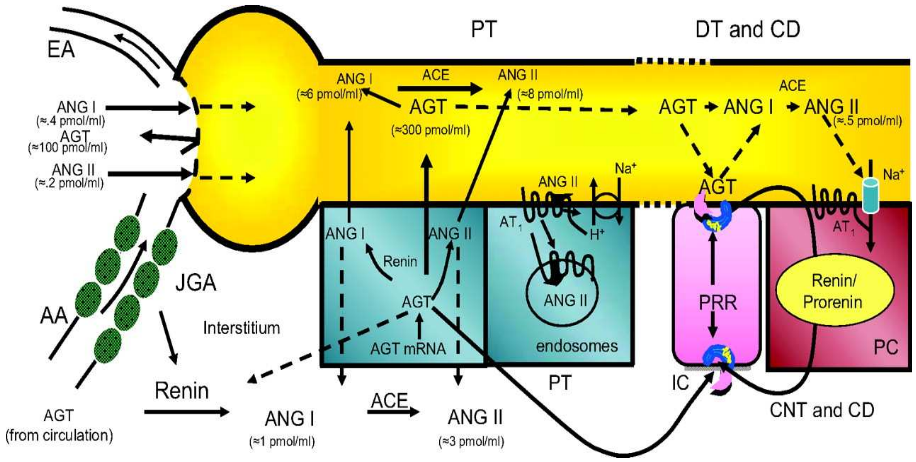


Figure 3. Cascade of intratubular RAS in Ang II–dependent hypertension. In Ang II–dependent hypertension, the kidney maintains intrarenal Ang II formation, enhanced proximal tubule AGT formation and spillover into distal nephron segments coupled with enhancement of CD renin and stimulation of tubular ACE (refer to text for relevant references). PT indicates proximal tubule; IC, intercalated cell; PC, principal cell; AA, afferent arteriole; EA, efferent arteriole.

EXTRA nephron

Nephron Extra 2013;3:36–49
DOI: 10.1159/000351044
Published online: April 27, 2013

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Minireview

Renoprotection and the Bardoxolone Methyl Story – Is This the Right Way Forward? A Novel View of Renoprotection in CKD Trials: A New Classification Scheme for Renoprotective Agents

Macaulay Onuigbo

College of Medicine, Mayo Clinic, Rochester, Minn., and Mayo Health System Practice-Based Research Network, and Department of Nephrology, Mayo Clinic Health System, Eau Claire, Wisc., USA

Nephron Extra 2013;3:36–49
DOI: 10.1159/000351044
Published online: April 27, 2013

Minireview

Renoprotection and the Bardoxolone Methyl Story – Is This the Right Way Forward? A Novel View of Renoprotection in CKD Trials: A New Classification Scheme for Renoprotective Agents

Limitations of Current Renoprotection Paradigms Using Angiotensin Inhibition

Additionally, we feel obligated to ask another more loaded question: Given the fact that, despite decades of widespread and extensive utilization of ACE inhibitors and ARBs in the USA and around the world, we have continued to experience progression of CKD to ESRD, with some authorities declaring the existence of a continuing global ESRD pandemic in recent years [2–7], is it not time to re-strategize on the current concepts of renoprotection? In a 2010 issue of the *International Journal of Clinical Practice*, we had asked a similarly loaded rhetorical question: Is renoprotection with RAAS blockade a failed paradigm? [3]. The next section is a critical reappraisal of the limitations of the current concept of renoprotection that is solely predicated on angiotensin blockade. This concept is scrutinized against the backdrop of the plausible existence of potentially multiple, disparately different and independent putative pathways and/or mechanisms that are mechanistically responsible for both the initiation and/or propagation of CKD to ESRD [2–4, 39].

nephron EXTRA

Nephron Extra 2013;3:36–49

DOI: 10.1159/000351044
Published online: April 27, 2013

Minireview

Renoprotection and the Bardoxolone Methyl Story – Is This the Right Way Forward? A Novel View of Renoprotection in CKD Trials: A New Classification Scheme for Renoprotective Agents

Putative Pathogenetic Mechanisms for CKD Progression in Diabetic and Non-Diabetic Nephropathy – Can a Single Agent Truly and Consistently Deliver Renoprotection?

Putative Pathogenetic Mechanisms for CKD Progression in Diabetic and Non-Diabetic Nephropathy – Can a Single Agent Truly and Consistently Deliver Renoprotection?

Second, a critical and dispassionate review of the available literature in this regard will lead to the conclusion that the culprit pathogenetic molecule(s) or mechanistic factor(s) responsible for the initiation and propagation of diabetic and/or non-diabetic nephropathy, and subsequent CKD to ESRD progression, remain unverified, unconfirmed, uncertain, and possibly unknown [39, 48–70]. Undeniably, several independent and often conflicting lines of evidence in the literature, from both human and experimental studies, suggest that a variety of presumed pathogenetic culprit mechanisms and factors, such as oxidative stress, inflammation, underlying genetic predispositions and different chemical molecules capable of directly causing AKI, could be responsible for CKD to ESRD progression [39, 48–70]. The following is a listing of some of these reported mechanisms or factors:

- several predisposing genetic abnormalities including variations of the non-muscle myosin heavy chain 9 gene (MYH9) on chromosome 22 and variants at chromosome 6q24–27 among African-Americans [48–51];
- oxidative stress combined with a paradoxical hypoxic renal environment conditioned by an underlying genetic predisposition (see above) [50, 52];
- the production of advanced glycosylation end products and the interaction of these end products on the multiligand receptor of the immunoglobulin superfamily receptor for advanced glycation end products [53, 54];
- intrarenal angiotensin II and/or renin production [55];
- inflammation [56];
- lipid toxicity [57–59];
- podocyte injury and apoptosis [60, 61];
- cytokine/chemokine/growth factor release causing renal injury [62, 63];
- asymmetric dimethylarginine [64];
- uric acid in CKD progression continues to attract increasing global attention [65–70].

Nephron Extra 2013;3:36–49
DOI: 10.1159/000351044

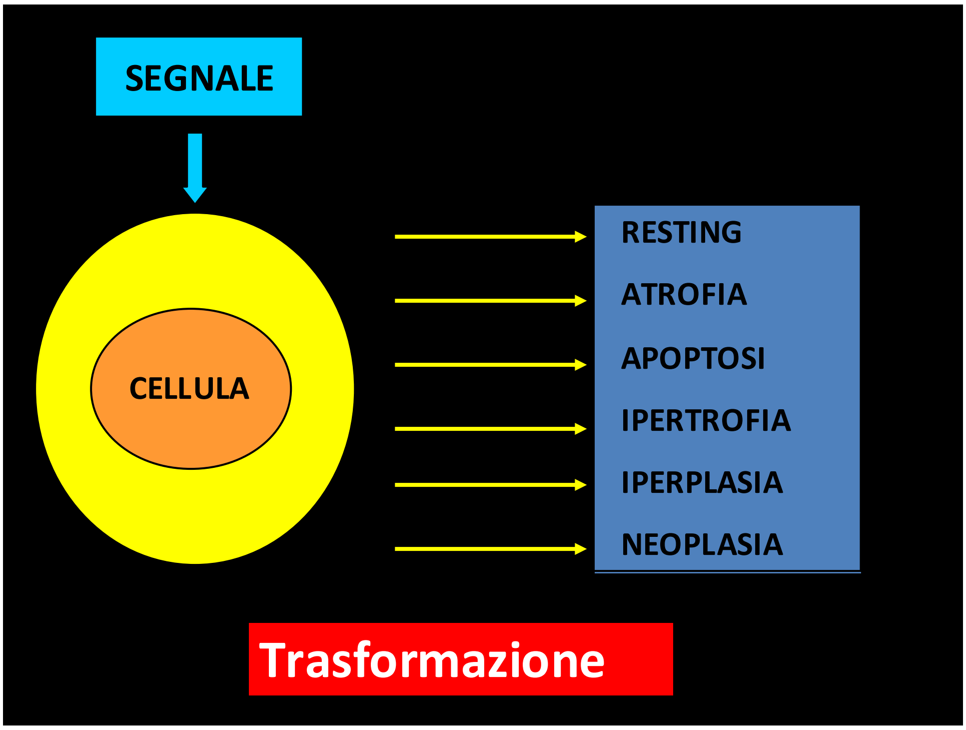
The Search for Novel Renoprotective Agents – A Case for More Effective Renoprotective Agents Capable of Simultaneously Attenuating Multiple Pathogenetic Pathways Involved in CKD to ESRD Progression: A Proposed New Classification Scheme for Renoprotective Agents

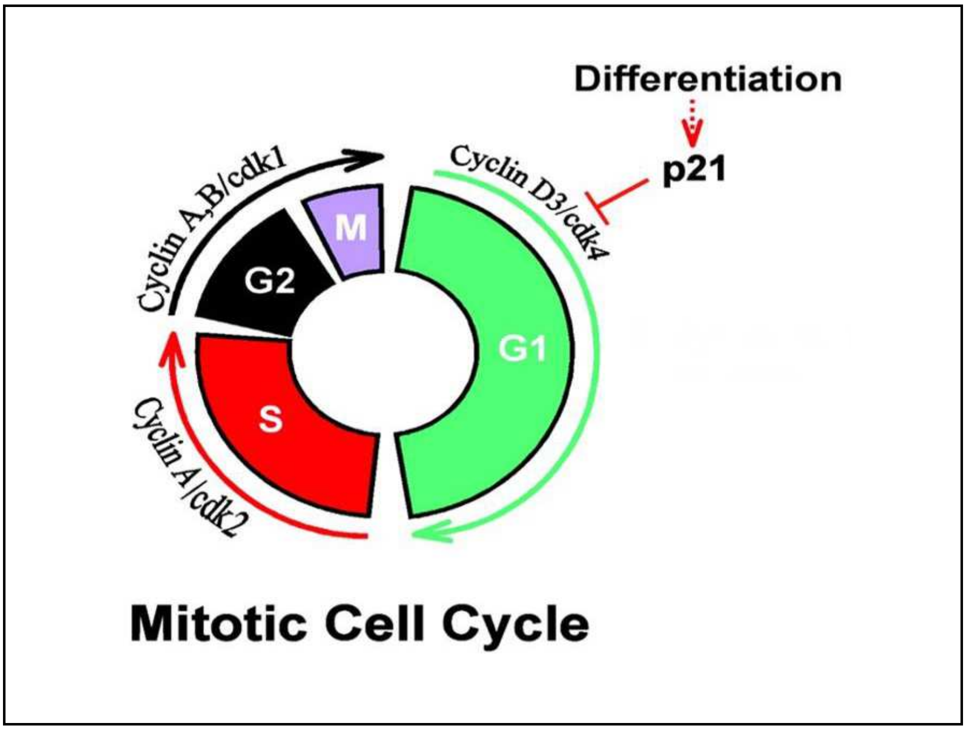
Conclusions

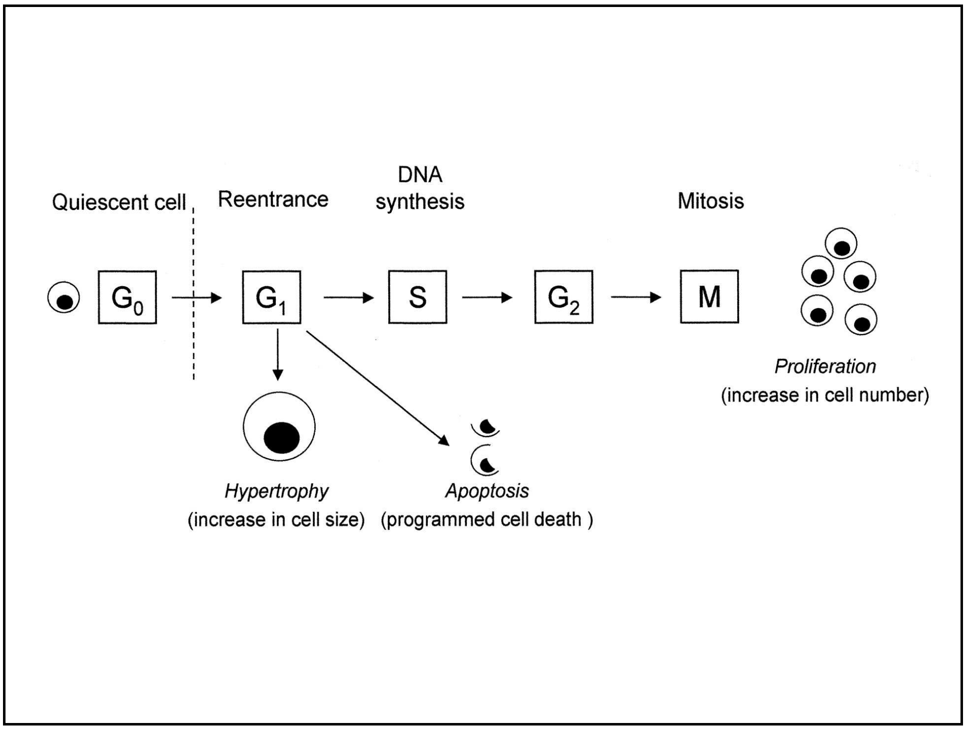
CKD, which used to be called chronic kidney failure, and its progression to ESRD requiring renal replacement therapy remain a major health problem worldwide, accounting for huge and increasing health care costs all around the world, both in developed countries and in the poorer developing countries [88–95]. Although current renoprotection paradigms are focused mainly on the blockade and antagonism of the renin-angiotensin system, we hypothesize that it is mandatory that new therapeutic modalities capable of simultaneously attenuating multiple and independent pathophysiological mechanisms and pathways, the so-called MPBs, must be developed and introduced into clinical medicine, and in quick order [39]. This critical mission is urgent, especially if we are to make any significant progress in our current efforts to slow down CKD to ESRD progression and to begin to retard the pace of the growing and costly global ESRD pandemic [39, 88].

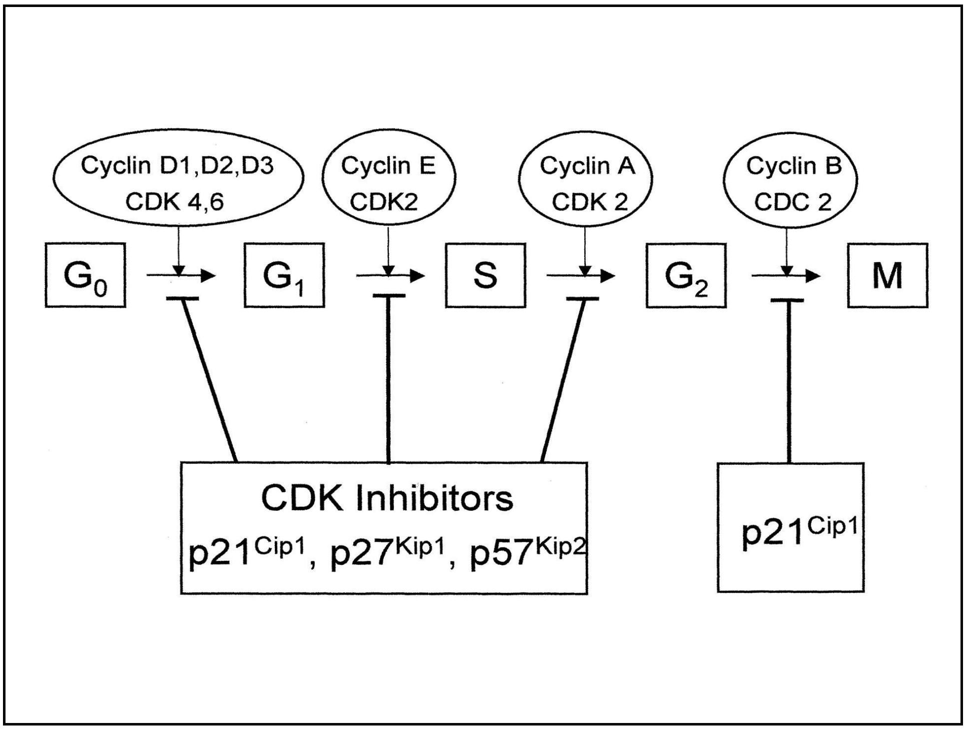
39 Onuigbo MA, Onuigbo N: Chronic Kidney Disease and RAAS Blockade: A New View of Renoprotection. London, Lambert Academic Publishing GmbH & Co. KG, 2011.

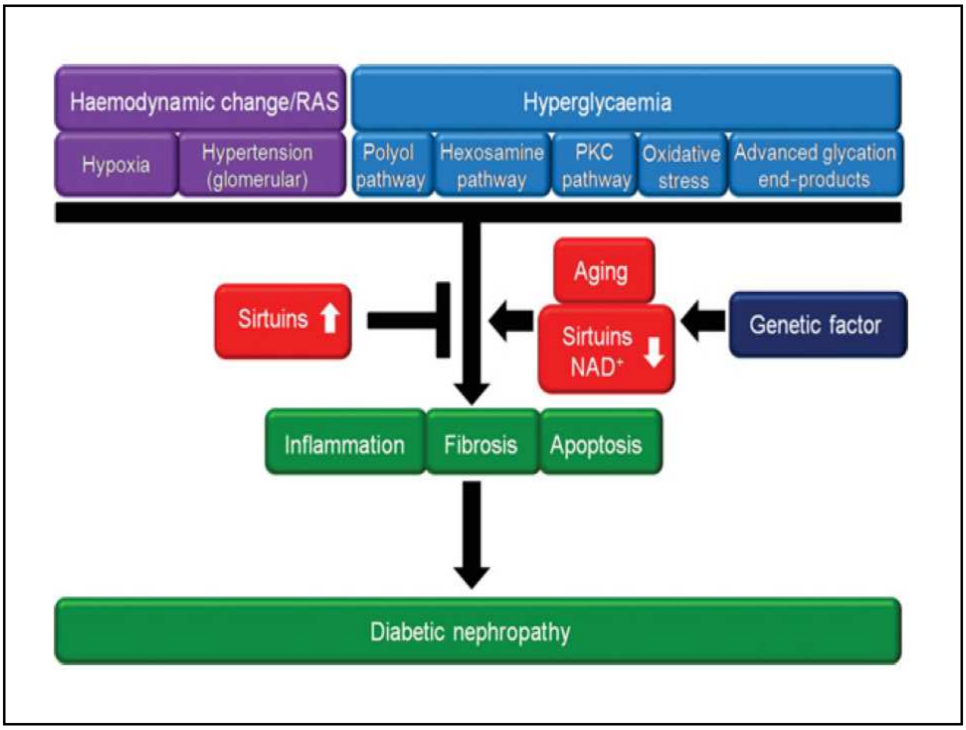
88 Shah SV: Progress toward novel treatments for chronic kidney disease. J Ren Nutr 2010;20(5 suppl):S122–S126.

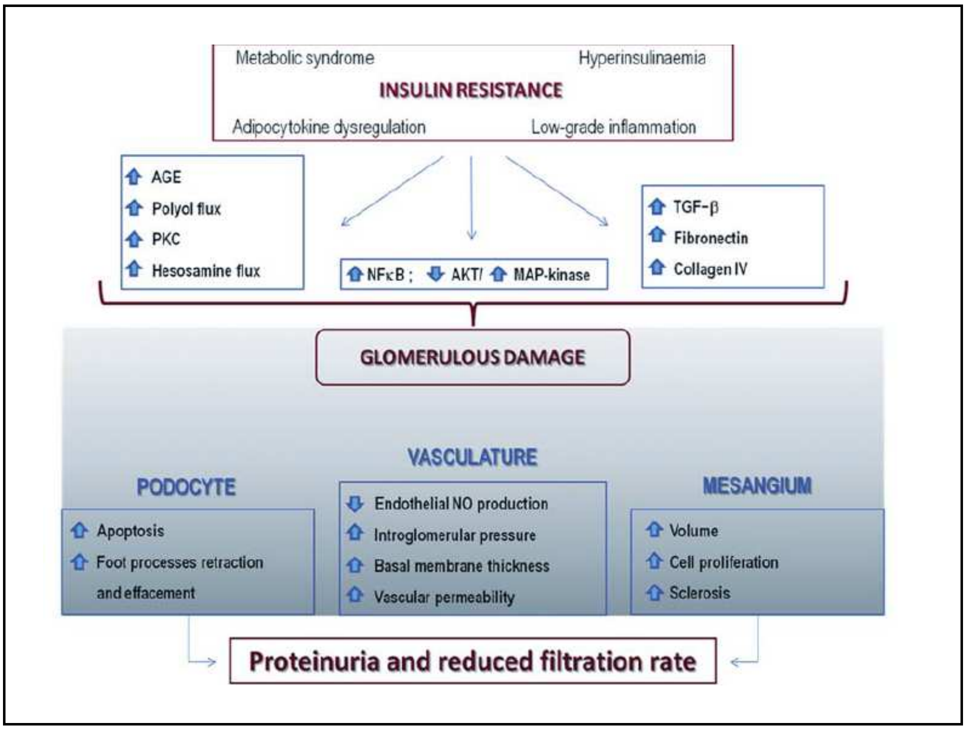

SEGNALE
CELLULA
RESTING
ATROFIA
APOPTOSI
IPERTROFIA
IPERPLASIA
NEOPLASIA
Trasformazione

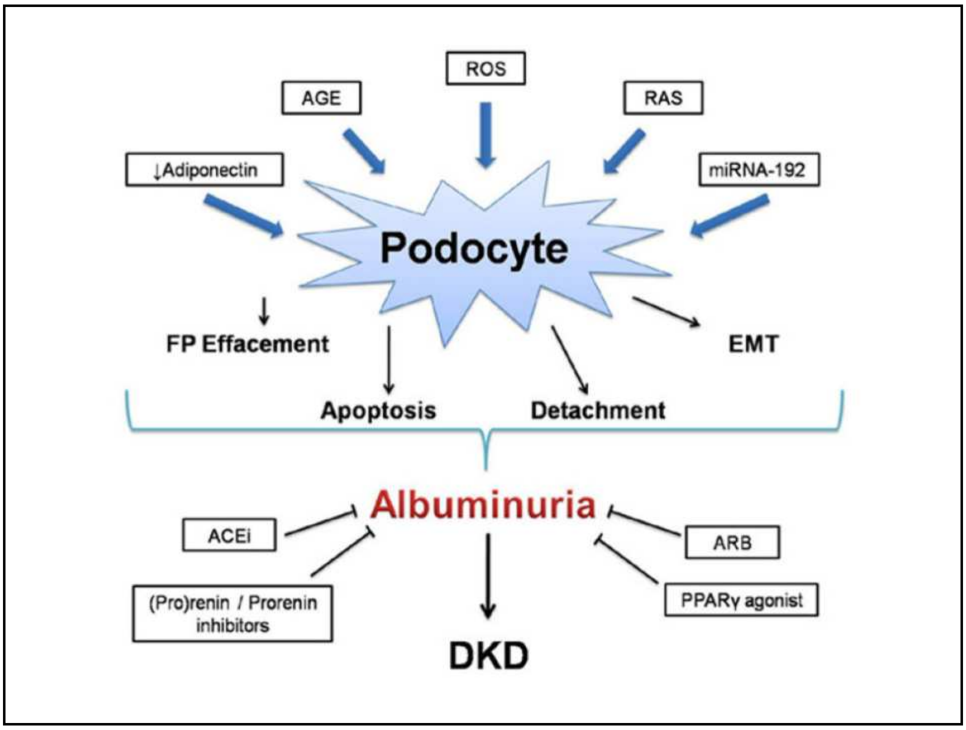

Differentiation
p21
Cyclin A,B/cdk1
Cyclin D3/cdk4
M
G2
G1
S
Cyclin A/cdk2
Mitotic Cell Cycle

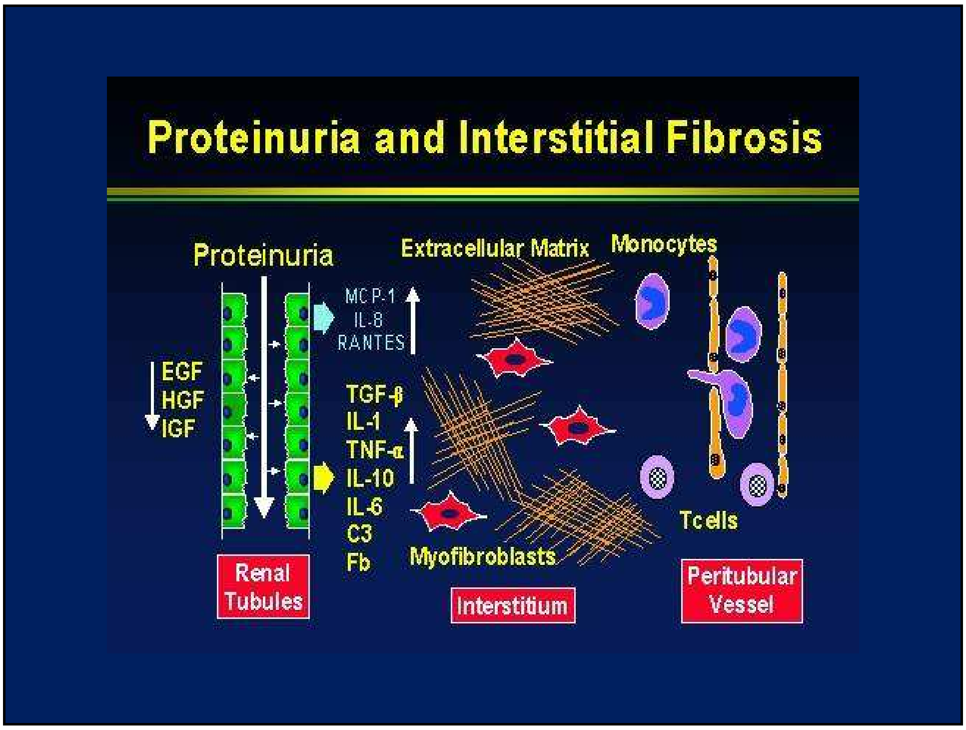

Quiescent cell
Reentrance
DNA synthesis
Mitosis
G_0
G_1
S
G_2
M
Proliferation
(increase in cell number)
Hypertrophy
(increase in cell size)
Apoptosis
(programmed cell death)


Cyclin D1,D2,D3
CDK 4,6
Cyclin E
CDK2
Cyclin A
CDK 2
Cyclin B
CDC 2
G_0
G_1
S
G_2
M
CDK Inhibitors
p21^{Cip1}, p27^{Kip1}, p57^{Kip2}
p21^{Cip1}

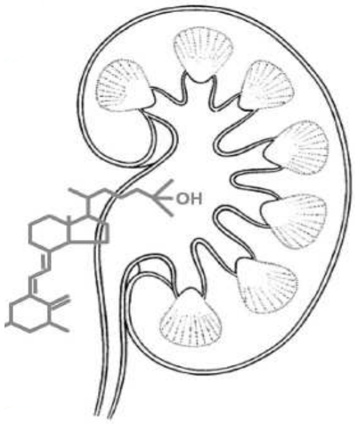

Haemodynamic change/RAS
Hyperglycaemia
Hypoxia
Hypertension (glomerular)
Polyol pathway
Hexosamine pathway
PKC pathway
Oxidative stress
Advanced glycation end-products
Aging
Sirtuins
Sirtuins NAD+
Genetic factor
Inflammation
Fibrosis
Apoptosis
Diabetic nephropathy


Metabolic syndrome
Hyperinsulinaemia
INSULIN RESISTANCE
Adipocytokine dysregulation
Low-grade inflammation
AGE
Polyol flux
PKC
Hesosamine flux
NFκB ; AKT/ MAP-kinase
TGF-β
Fibronectin
Collagen IV
GLOMERULOUS DAMAGE
VASCULATURE
PODOCYTE
MESANGIUM
Apoptosis
Foot processes retraction and effacement
Endothelial NO production
Introglomerular pressure
Basal membrane thickness
Vascular permeability
Volume
Cell proliferation
Sclerosis
Proteinuria and reduced filtration rate


ROS
AGE
RAS
↓Adiponectin
miRNA-192
Podocyte
FP Effacement
EMT
Apoptosis
Detachment
Albuminuria
ACEi
ARB
(Pro)renin / Prorenin inhibitors
PPARγ agonist
DKD

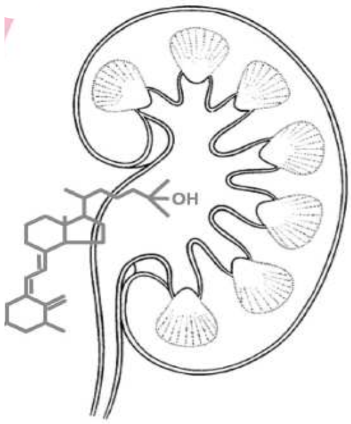

Proteinuria and Interstitial Fibrosis
Proteinuria
Extracellular Matrix
Monocytes
MCP-1
IL-8
RANTES
EGF
HGF
IGF
TGF-β
IL-1
TNF-α
IL-10
IL-6
C3
Fb
T cells
Myofibroblasts
Renal Tubules
Interstitium
Peritubular Vessel

The Kidney as an Endocrine Organ



The Kidney as an Endocrine Organ

but…. also as **ENDOCRINE TARGET**



The insulin receptor and the kidney

Lorna J. Hale and Richard J.M. Coward

Purpose of review
In recent years, it has become clear that the insulin receptor is important in a variety of renal cell types. It is through this transmembrane receptor that insulin, and to a lesser extent insulin-like growth factor, hormones bind and can control important cellular functions. This review will summarize the advances in our understanding of the role of the insulin receptor and insulin signalling in the glomeruli and tubules of the kidney.

Recent findings
The insulin receptor is important for podocyte function and when lost results in a number of features resembling diabetic nephropathy. Exciting recent data also highlight the importance of mammalian target of rapamycin in nutrient sensing and protein biosynthesis in the podocyte, which may also be regulated by the insulin receptor. The insulin receptor has also been shown to perform an important role in the distal regions of the renal tubules, regulating sodium excretion and blood pressure control here.

Summary
The insulin receptor is crucial for renal function in glomeruli and tubules. When signalling is diminished here, as may occur in insulin-resistant states, it may be responsible for a number of important renal complications including albuminuric glomerular disease and hypertension.

Curr Opin Nephrol Hypertens 2013, 22:100–106

Cell Metabolism
Article

Cell Metabolism *12*, 329–340, October 6, 2010

Insulin Signaling to the Glomerular Podocyte Is Critical for Normal Kidney Function

Gavin I. Welsh,[1] Lorna J. Hale,[1] Vera Eremina,[5] Marie Jeansson,[5] Yoshiro Maezawa,[5] Rachel Lennon,[1] Deborah A. Pons,[1] Rachel J. Owen,[3] Simon C. Satchell,[1] Mervyn J. Miles,[3] Christopher J. Caunt,[4] Craig A. McArdle,[4] Hermann Pavenstädt,[6] Jeremy M. Tavaré,[2] Andrew M. Herzenberg,[7] C. Ronald Kahn,[8] Peter W. Mathieson,[1] Susan E. Quaggin,[5] Moin A. Saleem,[1] and Richard J.M. Coward[1,5,*]
[1]Academic Renal Unit
[2]School of Biochemistry
[3]School of Physics
[4]Department of Molecular Pharmacology
School of Clinical Sciences, University of Bristol, Bristol BS8 1TH, UK
[5]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario M5G 1X5, Canada
[6]Department of Internal Medicine D, Nephrology and Hypertension, University Clinics Muenster, Muenster 48149, Germany
[7]Department of Pathology, University Health Network and University of Toronto, Toronto, Ontario M5G 2C4, Canada
[8]Joslin Diabetes Center and Harvard Medical School, Boston, MA 02215, USA
*Correspondence: richard.coward@bristol.ac.uk
DOI 10.1016/j.cmet.2010.08.015

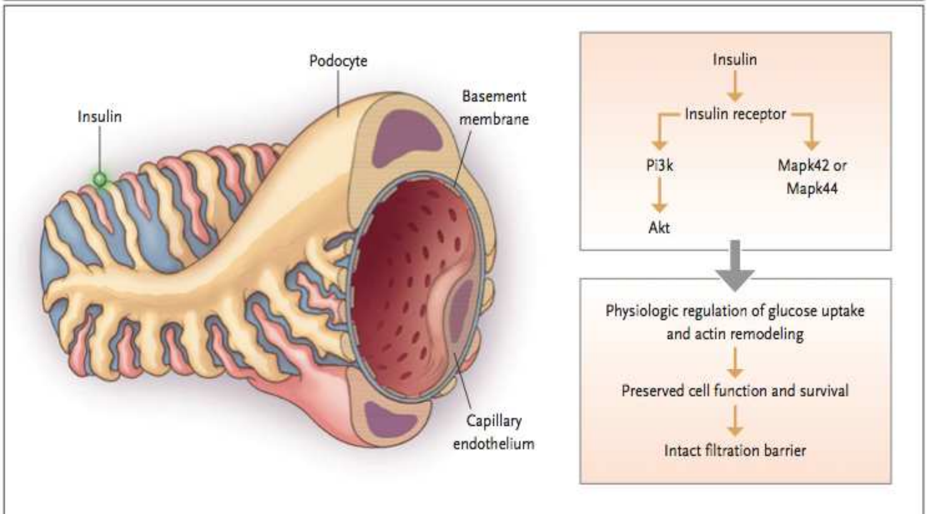


Figure 1. The Insulin-Sensing Podocyte.

A recent study by Welsh and colleagues[1] indicates that, in podocytes of wild-type mice, insulin activation of the insulin receptor results in the phosphorylation of Akt (the v-akt murine thymoma viral oncogene homologue 1 protein) and Mapk42 or Mapk44 (mitogen-activated protein kinase 42 or 44), resulting in physiologic remodeling of the actin cytoskeleton and preservation of cell function and survival. In podocytes of mice lacking the insulin receptor, insulin signaling through Akt and Mapk42 or Mapk44 is abrogated and results in the effacement of foot processes, thickening of the glomerular basement membrane, and cell malfunction or death, leading to proteinuria.

Podocytes as a Target of Insulin

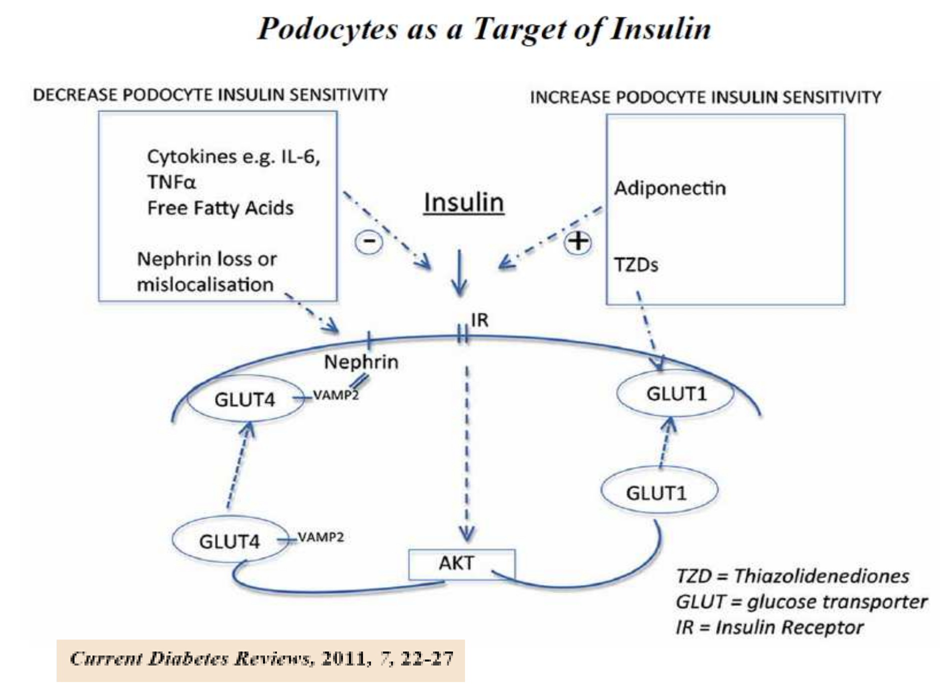
Richard J.M. Coward and Moin A. Saleem*

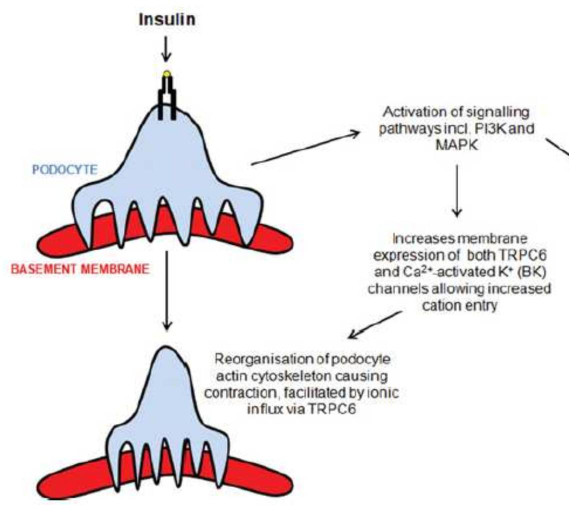
Academic and Children's Renal Unit, Southmead Hospital, Bristol BS10 5NB, UK

Abstract: Diabetic nephropathy (DN) presents with a gradual breakdown of the glomerular filtration barrier to protein, culminating in widespread glomerular damage and renal failure. The podocyte is the central cell of the glomerular filtration barrier, and possesses unique architectural and signaling properties guided by the expression of key podocyte specific proteins. How these cellular features are damaged by the diabetic milieu is unclear, but what is becoming increasingly clear is that damage to the podocyte is a central event in DN. Here we present accumulating evidence that insulin action itself is important in podocyte biology, and may be deranged in the pathomechanism of early DN. This introduces a rationale for therapeutic intervention to improve podocyte insulin sensitivity early in the presentation of DN.

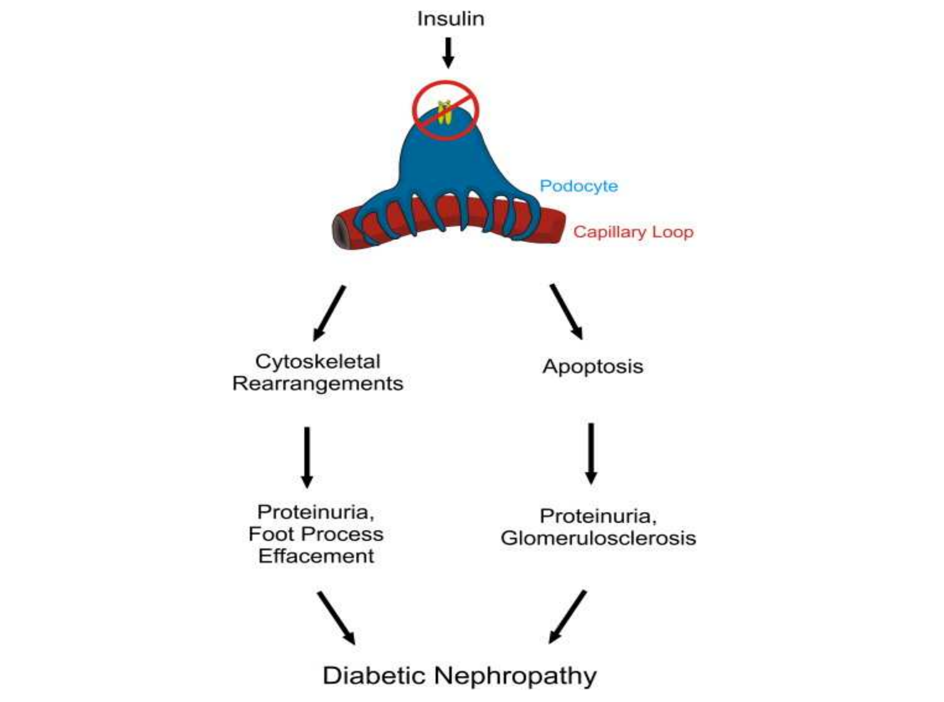
Keywords: Podocytes, Diabetic nephropathy, Insulin, Glomerular filtration barrier.

Current Diabetes Reviews, 2011, *7*, 22-27





Post-prandial actions of insulin in the podocyte
Insulin is released into the circulation by the pancreas and then reaches the podocyte by passing through the GFB. It is small (6 kDa), so freely passes through here. It then causes the podocyte changes depicted in a short time frame of less than 15 min.

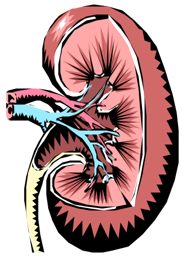


The Kidney's Many Contributions to Glucose Homeostasis

The Kidney's Many Contributions to Glucose Homeostasis
Insulin clearance
Gluconeogenesis
Clearance of proinsulin and C-peptide, insulin-like growth factors, glucagon, growth hormone, and the adipokines, leptin and adiponectin
Glucose reabsorption
Glucose excretion

The Liver and the Kidney Both Contribute to Glucose Production

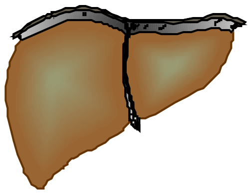
Gluconeogenesis 20–25%*



Glucose production ~70 g/day

Gluconeogenesis 25–30%*

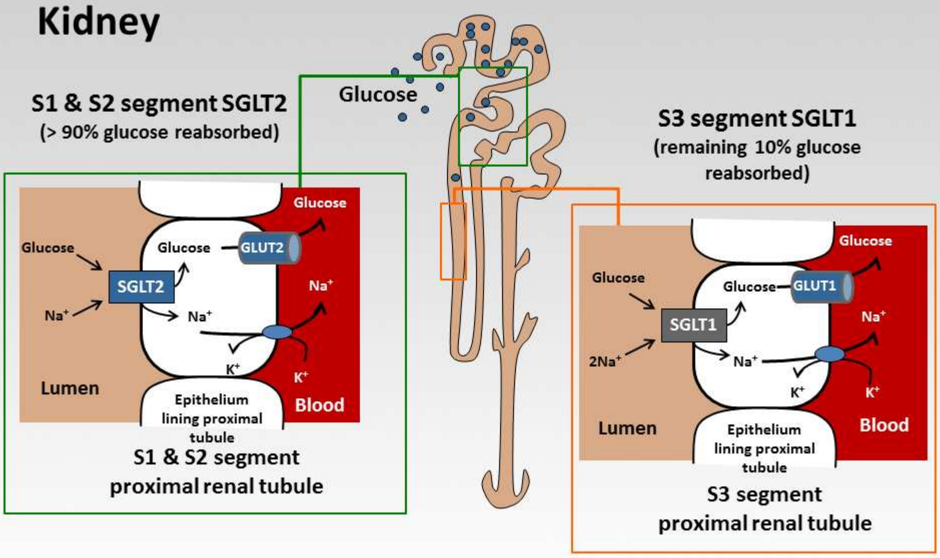
Glycogenolysis 45–50%*



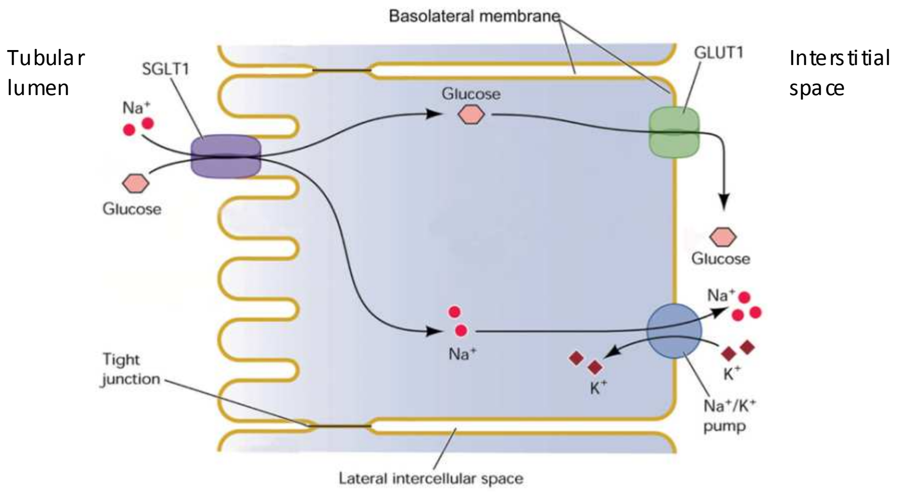
*post-absorptive state

Gerich JE. *Diabet Med* 2010;27:136–42.

Location of Sodium Glucose Transporters in the Kidney



SGLT1 and GLUT1 Mechanism of Transport



Adapted from Wright EM, et al. Physiology 2004;19:370–6.

The Kidney Filters and Reabsorbs a Large Proportion of the Glucose Present in the Body

Net ~0 g/day

Glucose input ~250 g/day:
- Dietary intake ~180 g/day
- Glucose production ~70 g/day
  – Gluconeogenesis
  – Glycogenolysis

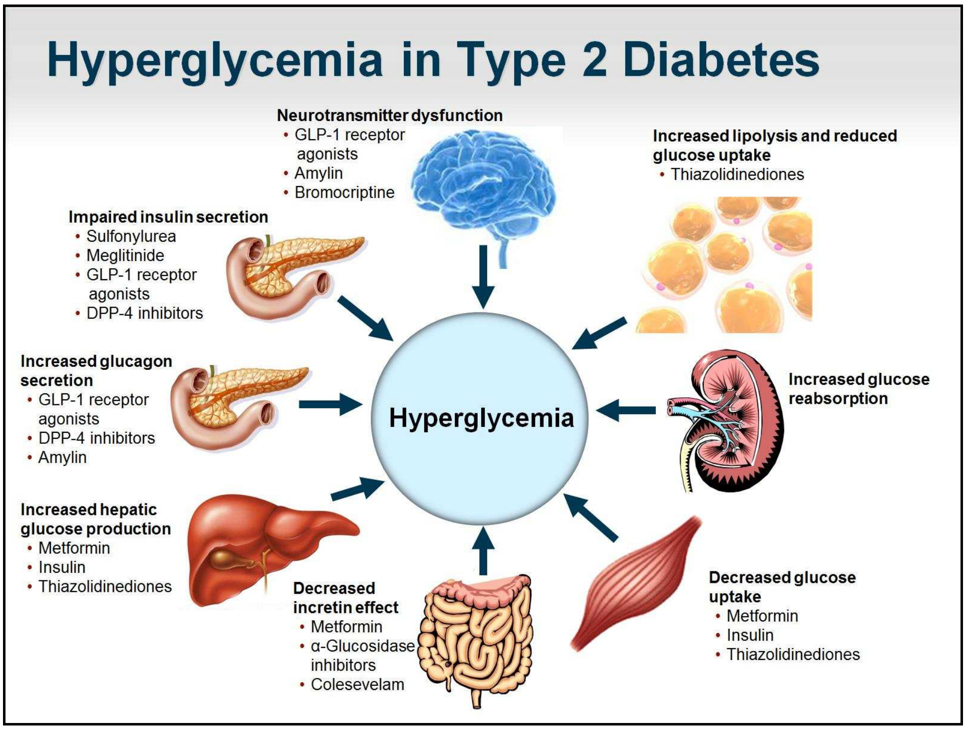
Glucose utilization ~250 g/day:
- Brain ~125 g/day
- Rest of body ~125 g/day

Glucose pool in body ~450 g

Glucose filtered ~180 g/day

Glucose reabsorbed ~180 g/day

Wright EM, et al. *J Int Med* 2007;261:32–43.



Glucose Transporters and the Rationale for SGLT2 Inhibition in Diabetes Mellitus

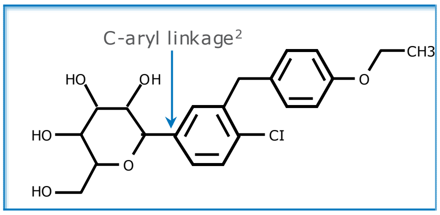
Dapagliflozin

- **A highly potent and selective SGLT2 inhibitor[1]**
- **Stability :**

C-aryl glycoside less susceptible to O-glucosidase degradation[1]

Prolonged half-life (~17 hours)[3]

Main metabolite is inactive, eliminated in urine[4]



Human transporters	Dapagliflozin EC_{50}, mean (nM ± SEM)[1,5]	Dapagliflozin K_i (nM ± SEM)[6]
SGLT2	1.12 ± 0.065	0.2 ± 0.06
SGLT1	1391 ± 7	610 ± 180
Selectivity for SGLT2:SGLT1	1200	3000

EC_{50}, concentration required for half-maximal response; Ki, dissociation constant; SEM, standard error of the mean.

1. Meng W, *et al. J Med Chem* 2008;51:1145–9; 2. Washburn W. *J Med Chem* 2009;52:1785–94;
3. Komoroski B, *et al. Clin Pharmacol Ther* 2009;85:520–6;
5. Han S, *et al. Diabetes* 2008;57:1723–9; 6. Bellamine A, *et al. Diabetes* 2011;60:A271.

original article

Diabetes, Obesity and Metabolism 15: 853–862, 2013.

ORIGINAL ARTICLE

Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes

H. J. Lambers Heerspink[1], D. de Zeeuw[1], L. Wie[3], B. Leslie[2] & J. List[2]

[1] *Department of Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*
[2] *Global Clinical Research, Bristol-Meyer-Squibb, Princeton, NJ, USA*
[3] *Global Biometric Sciences, Bristol-Meyer-Squibb, Hopewell, NJ, USA*

Aims: Sodium–glucose co-transporter 2 (SGLT2) reabsorbs glucose and sodium in the renal proximal tubule. Dapagliflozin, an SGLT2 inhibitor, targets hyperglycaemia in type 2 diabetes by increasing renal glucose excretion. To investigate whether the parallel occurring sodium loss would have diuretic-like physiologic effects, we compared dapagliflozin and hydrochlorothiazide (HCTZ) effects on 24-h blood pressure (BP), body weight, plasma volume and glomerular filtration rate (GFR).

Methods: In this randomized, placebo-controlled, double-blind trial, 75 subjects with type 2 diabetes were assigned placebo, dapagliflozin 10 mg/day, or HCTZ 25 mg/day. Changes from baseline BP, body weight, plasma volume and GFR were assessed after 12 weeks of treatment.

Results: Subjects' mean age was 56 years, type 2 diabetes mellitus (T2DM) duration 6.3 years, and haemoglobin A1c (HbA1c) 7.5%. Treatment with placebo, dapagliflozin or HCTZ resulted in changes from baseline in 24-h ambulatory mean systolic blood pressure (SBP) of −0.9 (95%CI −4.2, +2.4), −3.3 (95%CI −6.8, +0.2), and −6.6 (95%CI −9.9, −3.2) mmHg, respectively at week 12, adjusted for baseline SBP. Body weight decreased with dapagliflozin and HCTZ. In a sub-study plasma volume appeared to decrease with dapagliflozin but did not change with placebo or HCTZ treatment. Dapagliflozin induced a greater reduction in GFR (−10.8%; 95%CI −14.6, −6.7) relative to placebo (−2.9%; 95% CI −6.9, +1.2) or HCTZ (−3.4%; 95%CI −7.3, +0.6).

Conclusions: Dapagliflozin-induced SGLT2 inhibition for 12 weeks is associated with reductions in 24-h BP, body weight, GFR and possibly plasma volume. Cumulatively, these effects suggest that dapagliflozin may have a diuretic-like capacity to lower BP in addition to beneficial effects on glycaemic control.

Keywords: blood pressure, dapagliflozin, HbA1c, renal function, type 2 diabetes

In Type 2 Diabetes Mellitus, Counterproductive Increases in SGLT2 Upregulation and Glucose Reabsorption Occur

*p<0.05; † from human exfoliated proximal tubular epithelial cells (HEPTECs)

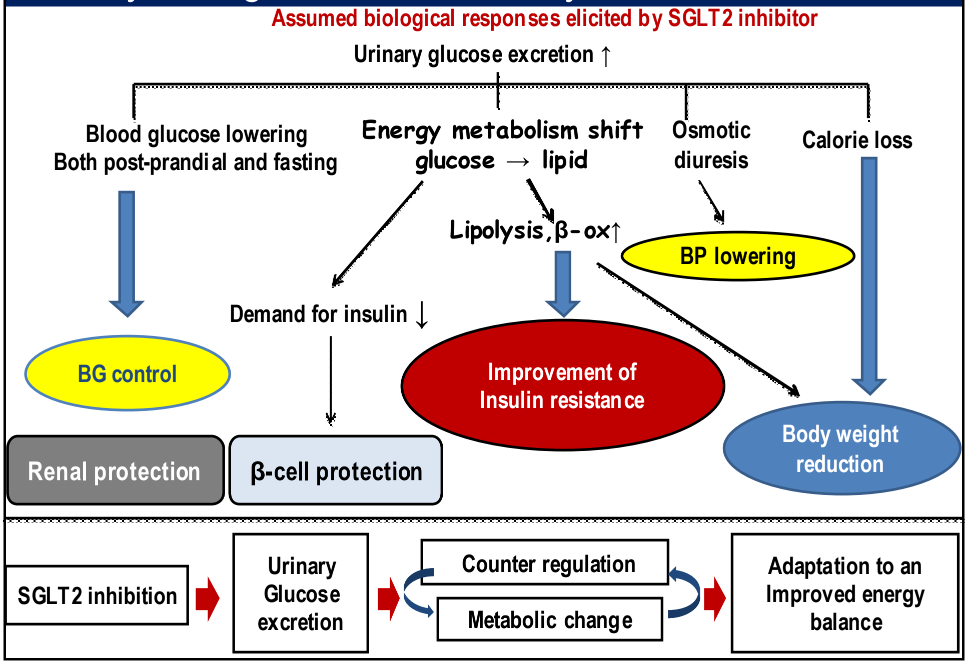
Rahmoune H, et al. *Diabetes* 2005;54:3427–34.

Dapagliflozin: Glucosuric and Metabolic Effects :

- 300 / 400 Kcal for 80 – 100 gr. of glucose loss

Glucosuria	↑ 52-85 g/day
FPG	↓ 16-30 mg/dL
PPG	↓ 23-29 mg/dL
Body weight	↓ 2.2-3.2 kg (↓ 2.5%-3.4%)
Urine volume	↑ 107-470 mL/day

Beyond glucose control by SGLT2 inhibitor



OPEN ACCESS Freely available online PLOS ONE

Effects of SGLT2 Inhibition in Human Kidney Proximal Tubular Cells—Renoprotection in Diabetic Nephropathy

Usha Panchapakesan[1]*, Kate Pegg[1], Simon Gross[1], Muralikrishna Gangadharan Komala[1], Harshini Mudaliar[1], Josephine Forbes[2], Carol Pollock[1], Amanda Mather[1]

1 Department of Medicine, The University of Sydney, Renal Research Group, Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, New South Wales, Australia, 2 Glycation and Diabetes Research Group, Mater Medical Research Institute, South Brisbane Qld, Australia

Abstract

Sodium/glucose cotransporter 2 (SGLT2) inhibitors are oral hypoglycemic agents used to treat patients with diabetes mellitus. SGLT2 inhibitors block reabsorption of filtered glucose by inhibiting SGLT2, the primary glucose transporter in the proximal tubular cell (PTC), leading to glycosuria and lowering of serum glucose. We examined the renoprotective effects of the SGLT2 inhibitor empagliflozin to determine whether blocking glucose entry into the kidney PTCs reduced the inflammatory and fibrotic responses of the cell to high glucose. We used an *in vitro* model of human PTCs. HK2 cells (human kidney PTC line) were exposed to control 5 mM, high glucose (HG) 30 mM or the profibrotic cytokine transforming growth factor beta (TGFβ1; 0.5 ng/ml) in the presence and absence of empagliflozin for up to 72 h. SGLT1 and 2 expression and various inflammatory/fibrotic markers were assessed. A chromatin immunoprecipitation assay was used to determine the binding of phosphorylated smad3 to the promoter region of the SGLT2 gene. Our data showed that TGFβ1 but not HG increased SGLT2 expression and this occurred via phosphorylated smad3. HG induced expression of Toll-like receptor-4, increased nuclear deoxyribonucleic acid binding for nuclear factor kappa B (NF-κB) and activator protein 1, induced collagen IV expression as well as interleukin-6 secretion all of which were attenuated with empagliflozin. Empagliflozin did not reduce high mobility group box protein 1 induced NF-κB suggesting that its effect is specifically related to a reduction in glycotoxicity. SGLT1 and GLUT2 expression was not significantly altered with HG or empagliflozin. In conclusion, empagliflozin reduces HG induced inflammatory and fibrotic markers by blocking glucose transport and did not induce a compensatory increase in SGLT1/GLUT2 expression. Although HG itself does not regulate SGLT2 expression in our model, TGFβ increases SGLT2 expression through phosphorylated smad3.

PLOS ONE | www.plosone.org February 2013 | Volume 8 | Issue 2 | e54442

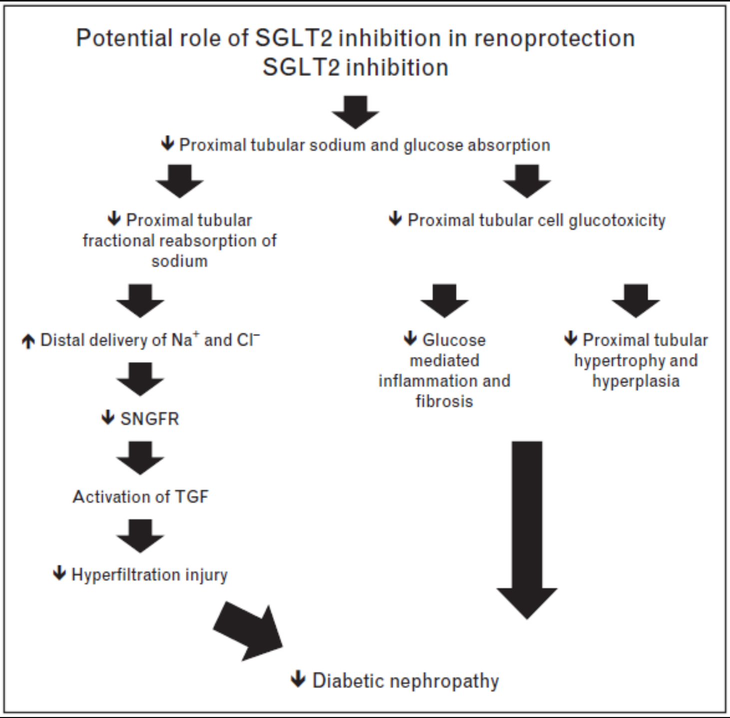
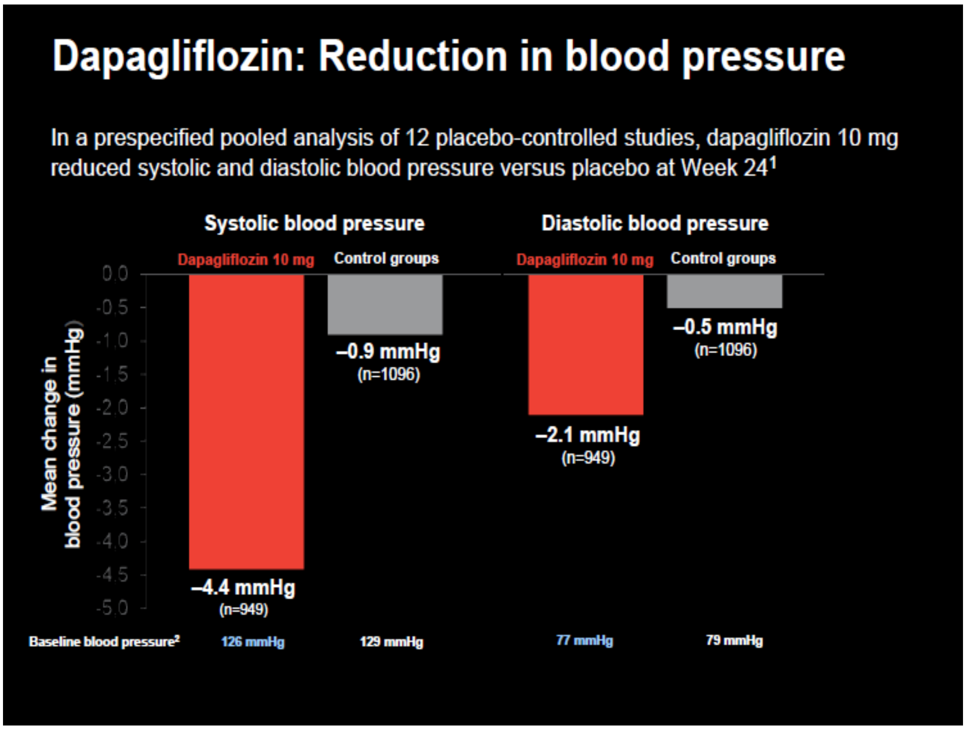


Diagram showing the relationship between Na^+/glucose reabsorption and single-nephron glomerular filtration rate (SNGFR) in diabetes with and without sodium–glucose linked cotransporter-2 (SGLT2) inhibition. (a) Diabetes. (1) Increased glucose in the glomerular ultrafiltrate leads to augmented glucose delivery to the proximal tubule. (2) As SGLT1 and 2 co-transport Na^+ with glucose, Na^+ delivery to the macula densa ([Na,Cl,K]MD) will be diminished. (3) This reduces the tubuloglomerular feedback (TGF) signal thereby increasing SNGFR. (b) Diabetes+SGLT2 inhibition. (1) By reducing glucose reabsorption, SGLT2 inhibition also reduces Na^+ reabsorption in the proximal tubule. (2) Na^+ delivery to the macula densa will therefore be increased, augmenting the TGF signal, and (3) reducing SNGFR.

Response of renal proximal tubular cell line to glucose *in vitro*. (a) Following 72 h of growth arrest, 25 mmol/l glucose was added to the apical compartment of proximal tubular cells, while 5 mmol/l glucose was added to the basolateral compartment. Intracellular glucose (bar graph columns) was measured after lysing cells in ice cold sterile water, showing rapid (1 h) elevation in intracellular glucose. Apical supernatant glucose concentration (red) fell with time, while the basolateral glucose concentration (blue) rose, consistent with glucose transport from the apical aspect, through the cell and into the basolateral (interstitial) compartment. (b) In response to high glucose (25 mmol/l) exposure on their apical aspect, proximal tubular epithelial cells secrete fibronectin basolaterally where it can accumulate to cause peritubular basement membrane thickening and ultimately interstitial fibrosis. By contrast, 25 mmol/l L-glucose, the non-metabolizable stereoisomer of biological D-glucose, was without effect, as was the nonmetabolizable, alpha-methyl glucose.



Review Article

Diabetes is predominantly an intestinal disease

Debmalya Sanyal

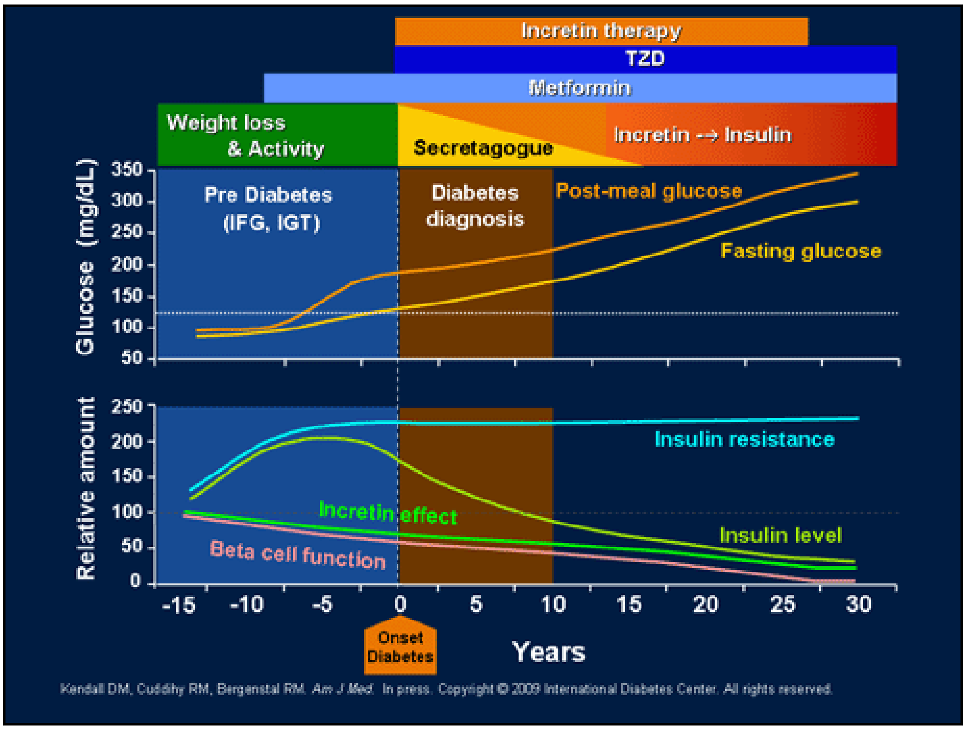
KPC Medical College, Kolkata, West Bengal, India

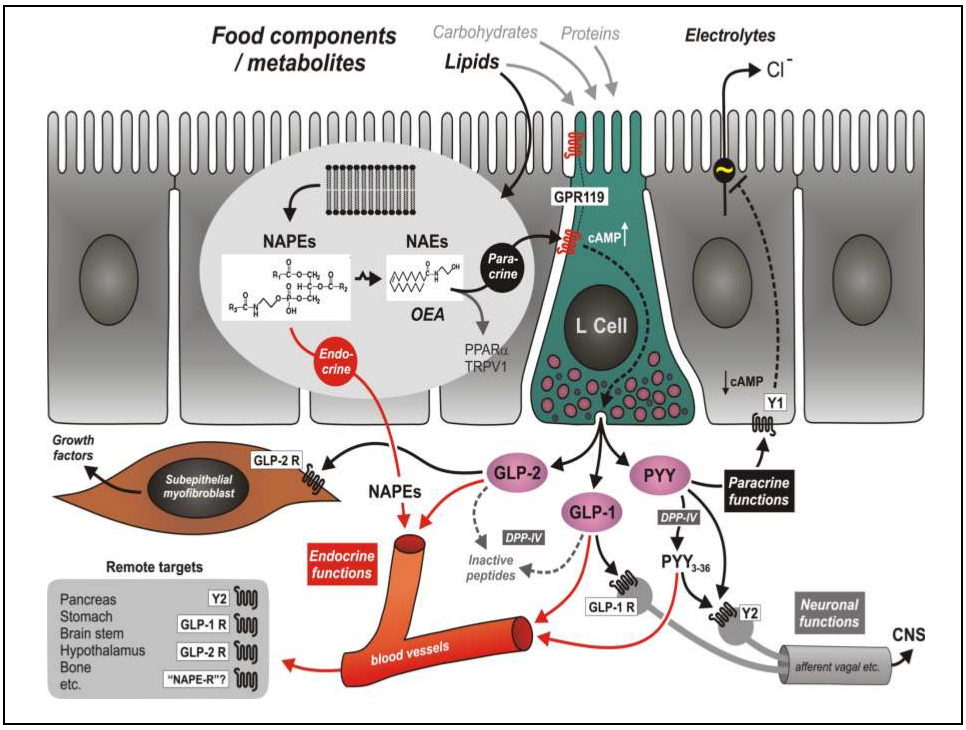
ABSTRACT

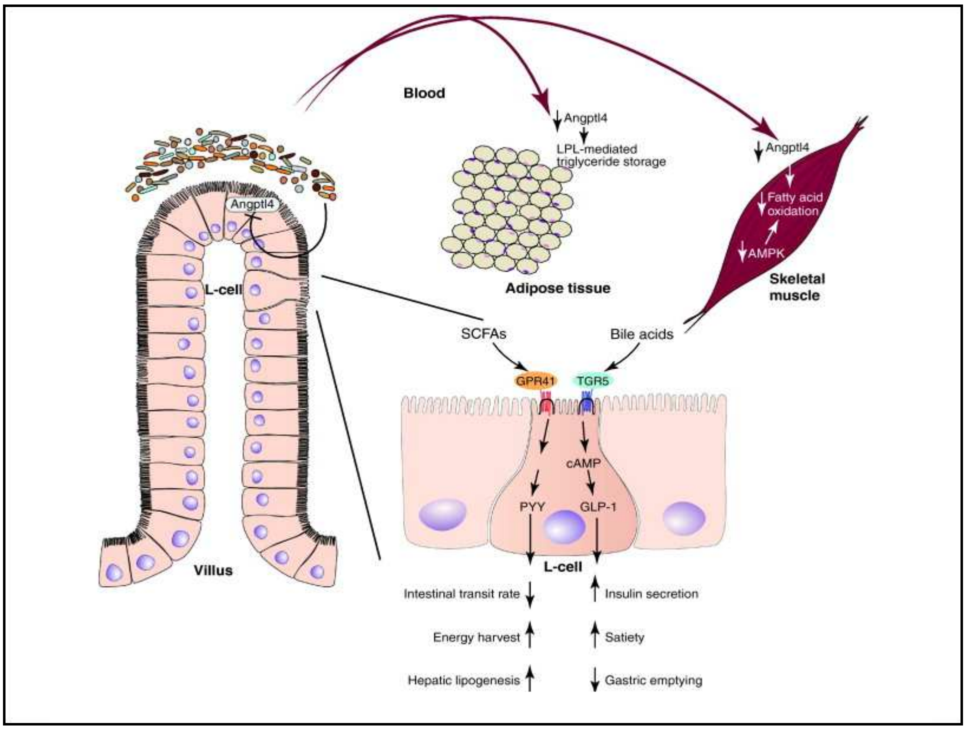
Diabetes mellitus (DM) is a chronic, progressive, medically incurable disease and is poorly controlled in a vast majority, in spite of tremendous advancements in pharmacotherapy. Altered gut microbiome can predict diabetes. There is strong and consistent evidence regarding role of the gut and many gut hormones like incretins in energy and glucose homeostasis. Incretin group of agents including glucagon-like peptide (GLP-1) receptor agonists and dipeptidyl peptidase IV (DPP-IV) inhibitors are efficacious therapeutic agents in diabetes treatment. A growing body of evidence, however, appears to indicate that type 2 DM (T2DM) may be an operable intestinal illness—a novel revolutionary concept about an old disease. This may facilitate research that can better clarify our understanding of the etiology of the disease and provide a new opportunity to develop new and more effective therapies. Future research should focus on an approach to bypass the bypass, that is, to replace the gastric bypass by equally effective but less invasive treatments for majority of diabetics.

Key words: Bariatric surgery, gut microbiota, type 2 diabetes, gastric inhibitory polypeptide, GLP-1

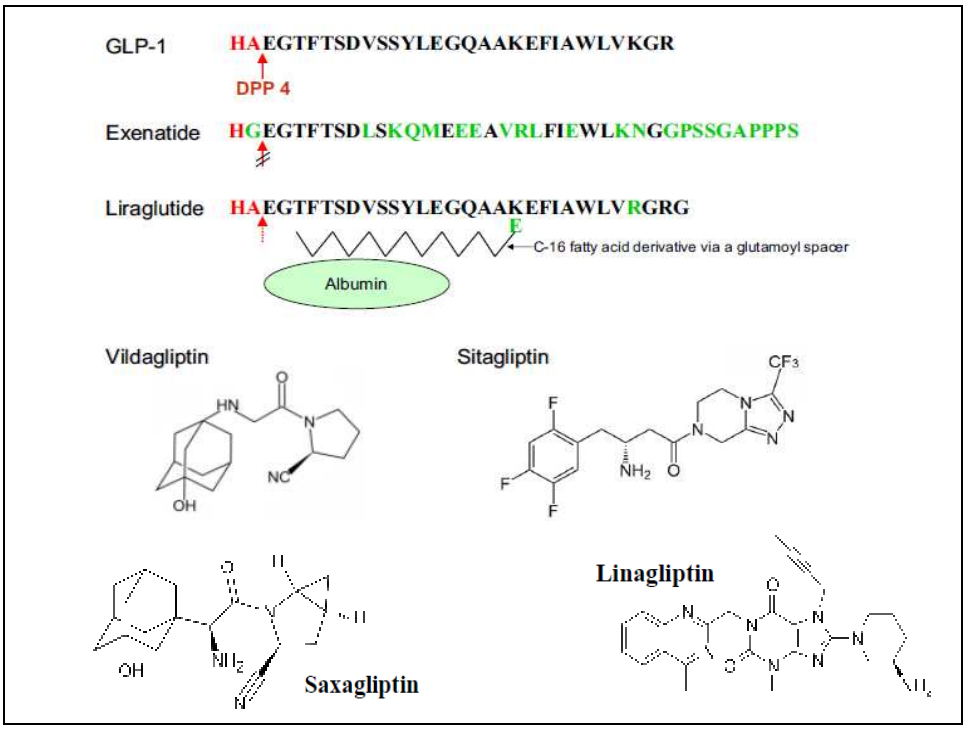
Indian Journal of Endocrinology and Metabolism / 2013 / Vol 17 / Supplement 1


Incretin therapy
TZD
Metformin
Weight loss & Activity
Secretagogue
Incretin --> Insulin
Glucose (mg/dL)
350
300
250
200
150
100
50
Pre Diabetes (IFG, IGT)
Diabetes diagnosis
Post-meal glucose
Fasting glucose
Relative amount
250
200
150
100
50
0
Insulin resistance
Incretin effect
Insulin level
Beta cell function
-15
-10
-5
0
5
10
15
20
25
30
Onset Diabetes
Years
Kendall DM, Cuddihy RM, Bergenstal RM. Am J Med. In press. Copyright © 2009 International Diabetes Center. All rights reserved.


Food components / metabolites
Carbohydrates
Proteins
Lipids
Electrolytes
Cl-
GPR119
cAMP
NAPEs
NAEs
Para-crine
OEA
PPARα
TRPV1
Endo-crine
L Cell
cAMP
Y1
Growth factors
Subepithelial myofibroblast
GLP-2 R
NAPEs
GLP-2
GLP-1
PYY
Paracrine functions
DPP-IV
DPP-IV
Inactive peptides
PYY3-36
Endocrine functions
GLP-1 R
Y2
Neuronal functions
CNS
afferent vagal etc.
blood vessels
Remote targets
Pancreas
Stomach
Brain stem
Hypothalamus
Bone
etc.
Y2
GLP-1 R
GLP-2 R
"NAPE-R"?



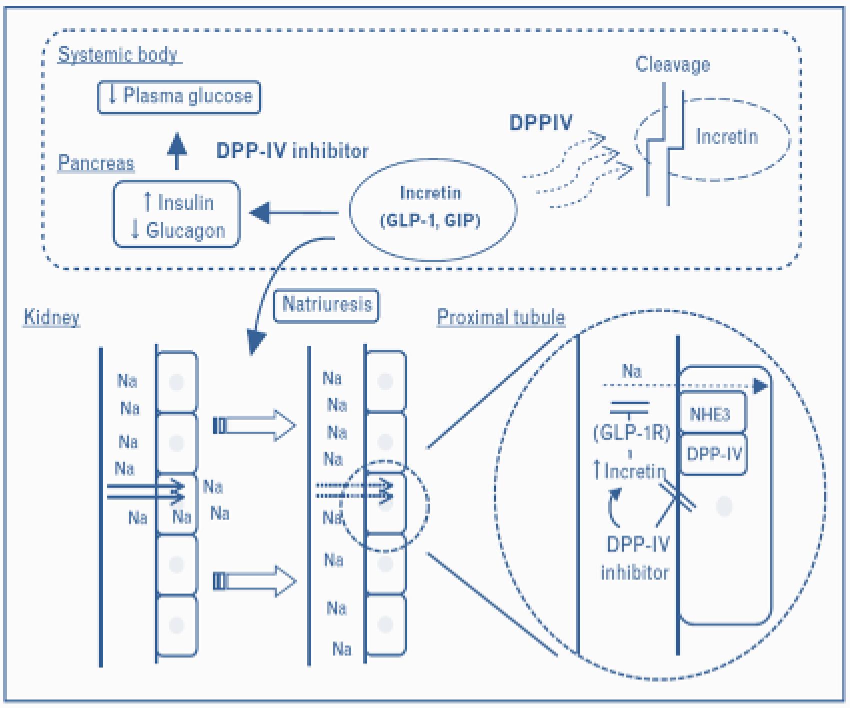
Blood
Angptl4
LPL-mediated triglyceride storage
Adipose tissue
Angptl4
Fatty acid oxidation
AMPK
Skeletal muscle
Angptl4
L-cell
Villus
SCFAs
Bile acids
GPR41
TGR5
cAMP
PYY
GLP-1
L-cell
Intestinal transit rate
Energy harvest
Hepatic lipogenesis
Insulin secretion
Satiety
Gastric emptying



GLP-1 HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR
DPP 4
Exenatide HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS
Liraglutide HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG
E
C-16 fatty acid derivative via a glutamoyl spacer
Albumin
Vildagliptin
Sitagliptin
Saxagliptin
Linagliptin

Effetti del GLP-1 sul sodio e sull'omeostasi idrica

- **GLP-1R è espresso sulle cellule tubulari prossimali**
- **Gli effetti del GLP-1 sono mediati dal GLP-1Rec. nel rene:**
  - Aumento della diuresi
  - Aumento dell'escrezione di sodio, cloro e calcio
  - Riduzione della escrezione di H+



Am J Physiol Renal Physiol 301: F355–F363, 2011.
First published May 18, 2011; doi:10.1152/ajprenal.00729.2010.

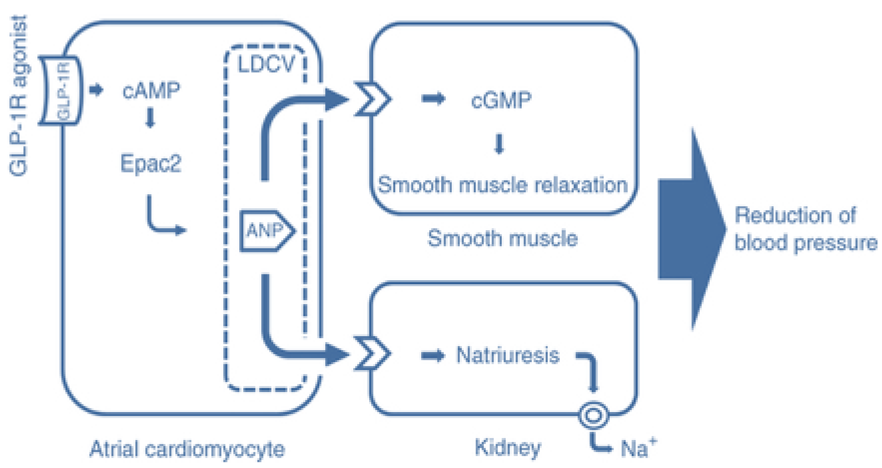
Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1

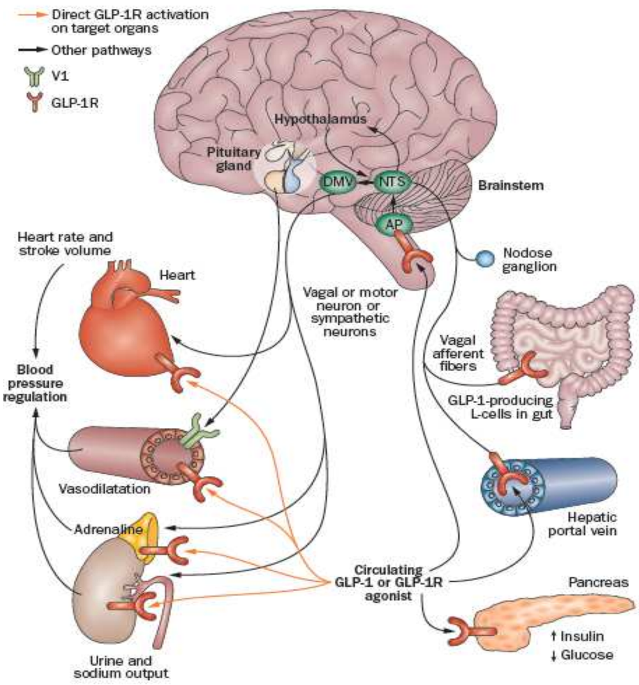
Renato O. Crajoinas,[1] Felipe T. Oricchio,[1] Thaissa D. Pessoa,[2] Bruna P. M. Pacheco,[1] Lucília M. A. Lessa,[2] Gerhard Malnic,[2] and Adriana C. C. Girardi[1]

[1]*Heart Institute (InCor) Medical School and* [2]*Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

In summary, the results of the present study suggest that binding of GLP-1 to its receptor GLP-1R activates the cAMP/PKA signaling pathway, leading, in turn, to phosphorylation of the PKA consensus sites at the NHE3 COOH-terminal region. Subsequently, inhibition of NHE3-mediated Na^+/H^+ exchange in proximal tubule decreases sodium, bicarbonate, and water reabsorption. The same signaling cascade might be triggered to affect the vascular renal resistance leading to an increase in renal blood flow and GFR.

we demonstrate that the beneficial effects of the incretin agents on renal function are mediated by an increase of GFR and RPF and by a decrease of proximal tubular NHE3 function. Our findings further support the view that the GLP-1-based therapeutics may also have a potential clinical usefulness in hypertension and other disorders of sodium retention.





Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
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Effects of Glucagon-Like Peptide-1 in Patients With Acute Myocardial Infarction and Left Ventricular Dysfunction After Successful Reperfusion

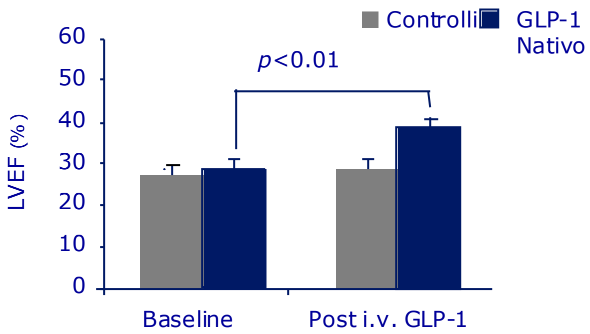
Lazaros A. Nikolaidis, MD; Sunil Mankad, MD; George G. Sokos, DO; Glen Miske, DO; Ankur Shah, MD; Dariush Elahi, PhD; Richard P. Shannon, MD

Background—Glucose-insulin-potassium infusions are beneficial in uncomplicated patients with acute myocardial infarction (AMI) but are of unproven efficacy in AMI with left ventricular (LV) dysfunction because of volume requirements associated with glucose infusion. Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin with both insulinotropic and insulinomimetic properties that stimulate glucose uptake without the requirements for concomitant glucose infusion.

Methods and Results—We investigated the safety and efficacy of a 72-hour infusion of GLP-1 (1.5 pmol/kg per minute) added to background therapy in 10 patients with AMI and LV ejection fraction (EF) <40% after successful primary angioplasty compared with 11 control patients. Echocardiograms were obtained after reperfusion and after the completion of the GLP-1 infusion. Baseline demographics and background therapy were similar, and both groups had severe LV dysfunction at baseline (LVEF=29±2%). GLP-1 significantly improved LVEF (from 29±2% to 39±2%, $P<0.01$), global wall motion score indexes ($1.94\pm0.11\rightarrow1.63\pm0.09$, $P<0.01$), and regional wall motion score indexes ($2.53\pm0.08\rightarrow2.02\pm0.11$, $P<0.01$) compared with control subjects. The benefits of GLP-1 were independent of AMI location or history of diabetes. GLP-1 was well tolerated, with only transient gastrointestinal effects.

Conclusions—When added to standard therapy, GLP-1 infusion improved regional and global LV function in patients with AMI and severe systolic dysfunction after successful primary angioplasty. (***Circulation*. 2004;109:962-965.**)

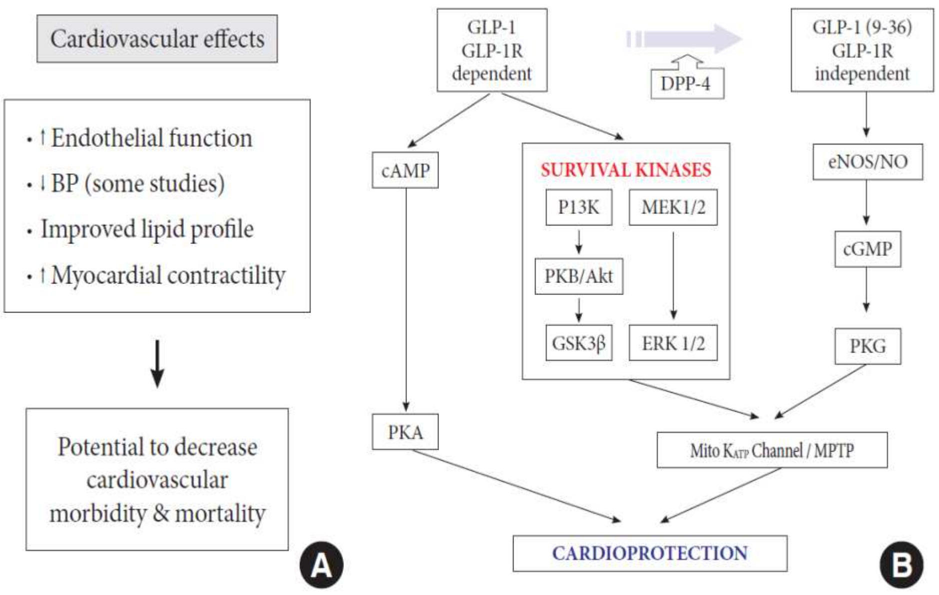
IL GLP-1 nativo migliora la funzione ventricolare sinistra nei pazienti ad alto rischio cardiaco



- **Effetti dell'infusione di GLP-1 per 72 ore in pazienti con infarto miocardico acuto e ad alto rischio di insufficienza cardiaca post-infartuale, dopo efficace riperfusione (angioplastica)**

Dati espressi come media±SE; post i.v. GLP-1, post infusione endovenosa di GLP-1 per 72 ore

Nikolaidis et al. Circulation 2004;109:962–5; Moller et al. Am Heart J 2006;151:419–25.



European Heart Journal
doi:10.1093/eurheartj/ehr309

CLINICAL RESEARCH

Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction

Jacob Lønborg[1]*, Niels Vejlstrup[1], Henning Kelbæk[1], Hans Erik Bøtker[2], Won Yong Kim[2], Anders B. Mathiasen[1], Erik Jørgensen[1], Steffen Helqvist[1], Kari Saunamäki[1], Peter Clemmensen[1], Lene Holmvang[1], Leif Thuesen[2], Lars Romer Krusell[2], Jan S. Jensen[3], Lars Køber[1], Marek Treiman[4], Jens Juul Holst[4], and Thomas Engstrøm[1]

[1]Department of Cardiology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark; [2]Department of Cardiology, Skejby, Aarhus, Denmark; [3]Department of Cardiology, Gentofte Hospital, Copenhagen, Denmark; and [4]Department of Biomedical Sciences and The Danish National Foundation Research Centre for Heart Arrhythmia, University of Copenhagen, Denmark

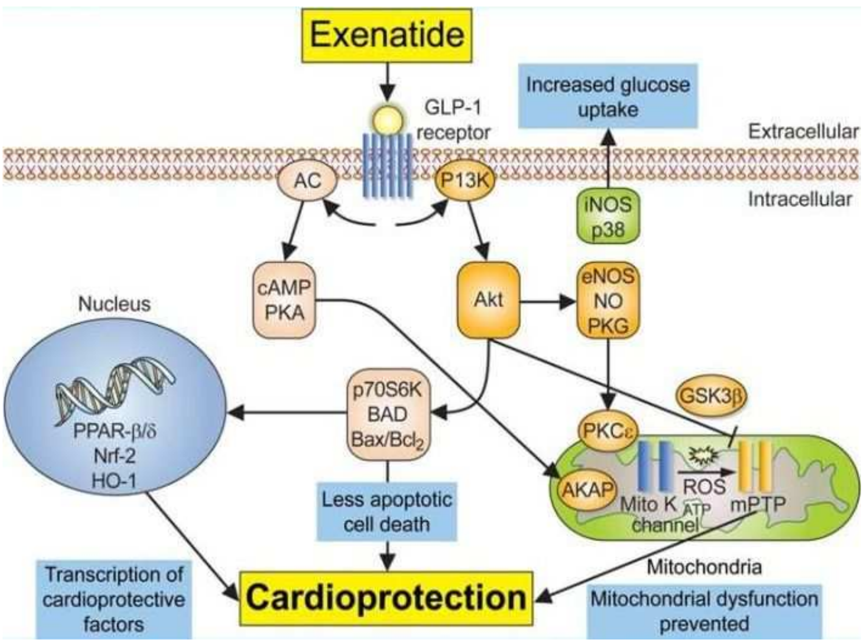
Received 30 May 2011; revised 18 July 2011; accepted 3 August 2011

Aims Exenatide, a glucagon-like-peptide-1 analogue, increases myocardial salvage in experimental settings with coronary occlusion and subsequent reperfusion. We evaluated the cardioprotective effect of exenatide at the time of reperfusion in patients with ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI).

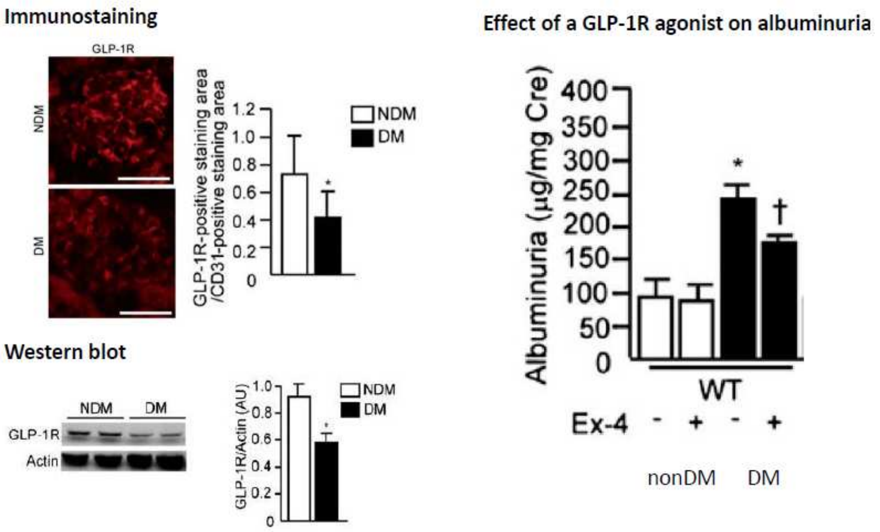
Methods and results A total of 172 patients with STEMI and Thrombolysis in Myocardial Infarction flow 0/1 were randomly assigned to exenatide or placebo (saline) intravenously. Study treatment was commenced 15 min before intervention and maintained for 6 h after the procedure. The primary endpoint was salvage index calculated from myocardial area at risk (AAR), measured in the acute phase, and final infarct size measured 90 ± 21 days after pPCI by cardiac magnetic resonance (CMR). In 105 patients evaluated with CMR, a significantly larger salvage index was found in the exenatide group than in the placebo group (0.71 ± 0.13 vs. 0.62 ± 0.16; $P = 0.003$). Infarct size in relation to AAR was also smaller in the exenatide group (0.30 ± 0.15 vs. 0.39 ± 0.15; $P = 0.003$). In a regression analysis, there was a significant correlation between the infarct size and the AAR for both treatment groups and an analysis of covariance showed that datapoints in the exenatide group lay significantly lower than for the placebo group ($P = 0.011$). There was a trend towards smaller absolute infarct size in the exenatide group (13 ± 9 vs. 17 ± 14 g; $P = 0.11$). No difference was observed in left ventricular function or 30-day clinical events. No adverse effects of exenatide were observed.

Conclusion In patients with STEMI undergoing pPCI, administration of exenatide at the time of reperfusion increases myocardial salvage.

Keywords Reperfusion injury • Exenatide • Acute myocardial infarction • Cardiac magnetic • Resonance • Primary percutaneous coronary intervention



The GLP-1 Receptor is Present in the Kidney and decreases albuminuria in diabetic mice.



Mima A,...., King GL. Diabetes 61:2967–2979, 2012

http://www.kidney-international.org

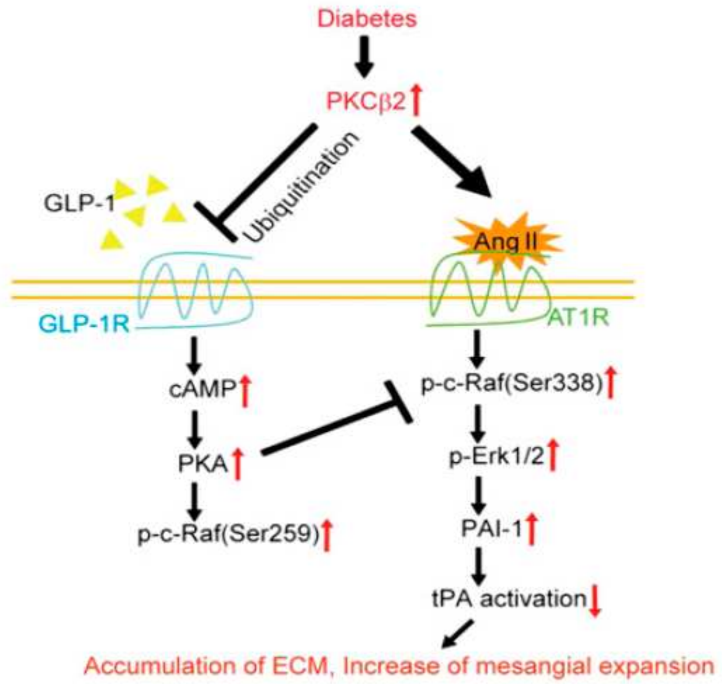
basic research

The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential

Hiroki Fujita[1], Tsukasa Morii[1], Hiromi Fujishima[1], Takehiro Sato[1], Tatsunori Shimizu[1], Mihoko Hosoba[1], Katsushi Tsukiyama[1], Takuma Narita[1], Takamune Takahashi[2], Daniel J. Drucker[3,4], Yutaka Seino[5] and Yuichiro Yamada[1]

[1]*Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University Graduate School of Medicine, Akita, Japan;* [2]*Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, Tennessee, USA;* [3]*Department of Medicine, University of Toronto, Toronto, Ontario, Canada;* [4]*The Lunenfeld-Tanenbaum Research Institute, Mt Sinai Hospital, Toronto, Ontario, Canada and* [5]*Kansai Electric Power Hospital, Osaka, Japan*

Kidney International advance online publication, 23 October 2013; doi:10.1038/ki.2013.427



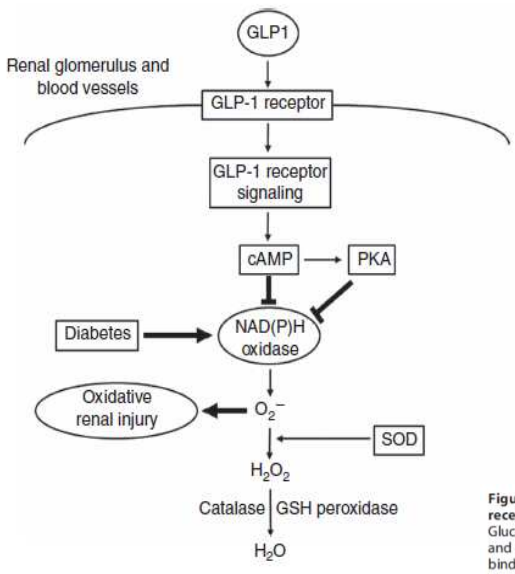


Figure 10 | Proposed glucagon-like peptide-1 receptor-dependent signal-transduction pathway in the kidney. Glucagon-like peptide-1 receptors are expressed in the glomerulus and blood vessels in the kidney. After glucagon-like peptide (GLP-1) binds to its receptor on glomerular capillary and vascular walls, glucagon-like peptide-1R signals upregulate the production of a major second messenger cAMP and consequently activate protein kinase A (PKA). The upregulated cAMP and PKA are expected to contribute to the amelioration of oxidative renal injury via inhibition of a major source of superoxide anion ($O_2^{\bullet -}$), NAD(P)H oxidase, activated under chronic diabetic condition. GSH, glutathione; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase.

Journal of Diabetes

Journal of Diabetes •• (2013) ••–••

REVIEW ARTICLE

Dipeptidyl peptidase-4 inhibitors: Multitarget drugs, not only antidiabetes drugs

Yunjuan ZHAO, Lin YANG, and Zhiguang ZHOU

Diabetes Center, Institute of Metabolism and Endocrinology, The Second Xiangya Hospital and Key Laboratory of Diabetes Immunology, Ministry of Education, Central South University, Changsha, China

Correspondence
Zhiguang Zhou, Middle Ren-Min Road No.139, Changsha, Hunan 410011, China.
Tel: +86 731 85292154
Fax: +86 731 85267220
Email: zhouzg@hotmail.com

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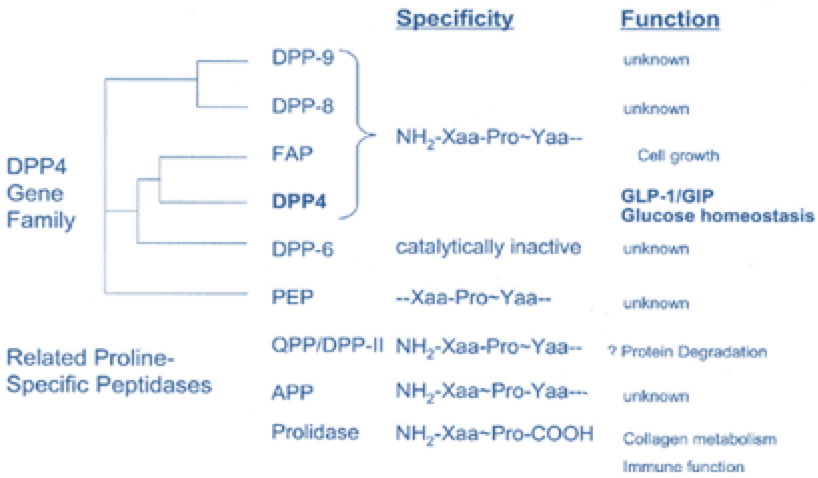
doi: 10.1111/1753-0407.12063

Abstract

Dipeptidyl peptidase (DPP)-4 inhibitors are a new class of antidiabetic agents that reduce blood glucose by preventing the degradation of the endogenous incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. Protection by DPP-4 inhibitors of β-cell function has been demonstrated in patients with type 2 diabetes. Because DPP-4 is an enzyme widely expressed in humans, DPP-4 inhibitors are speculated to be multitarget agents. However, other potential therapeutic benefits of DPP-4 inhibitors remain unknown. Recently, some therapeutic effects of DPP-4 inhibitors, such as immune regulation, cardiovascular protection, and anti-inflammatory effects, have been observed. This article provides a systematic and comprehensive review of current research into the newly found effects and mechanism of action of DPP-4 inhibitors in a therapeutic context.

Keywords: anti-inflammatory, cardiovascular protection, dipeptidyl peptidase-4 inhibitors, immunomodulatory.

The DPP4 Protease Family



Native DPPIV is a ubiquitous type II transmembrane glycoprotein and a serine protease of the S9 prolyl-oligopeptidase family. *In vivo*, it is synthesized with a signal peptide which functions as the membrane anchoring domain.[1,2] There is an 88% sequence homology between the human and porcine kidney enzymes.[3]

Both the human and porcine kidney enzymes exist as homodimers with a subunit molecular mass of ~30 kDa. The high mannose 100 kDa DPPIV precursor is processed in the Golgi to yield a 124 kDa heavily N- and O-linked mature glycoprotein.[4] It is then sorted to the apical membrane through the concerted action of both N- and O-linked glycans and association with lipid microdomains.[5] The porcine enzyme contains 18.3% carbohydrates, of which the glycan composition is 0.9% fucose, 3.4% mannose, 5.1% galactose, 8.2% glucosamine, and 0.7% sialic acid.[1,2]

References

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2. Matter, K., and Hauri, H. P., *Biochemistry*, **30**, 1916-1923 (1991).
3. Wilhelm, A., *et al.*, *J. Biol. Chem.*, **279**, 43330-43335 (2004).

CD26/DPPIV is a multifunctional molecule

CD26/DPPIV is a 110-kDa cell surface glycoprotein that belongs to the serine protease family. It is expressed on a variety of tissues including T lymphocytes, endothelial and epithelial cells. It is composed of a short cytoplasmic domain, a transmembrane region, and an extracellular domain with dipeptidyl peptidase activity which selectively removes the N-terminal dipeptide from peptides with proline or alanine in the second position (Tanaka et al., 1992). Possible substrates of DPPIV include several critical cytokines and chemokines. Activity of RANTES (regulated on activation, normal T cell expressed and secreted) is altered by the enzymatic cleavage of DPPIV, as CD26/DPPIV enzymatic cleavage of RANTES affects important activities such as those implicated in monocyte chemotaxis and HIV-1 infection (Proost et al., 1998). Other important chemokines that appear to be substrates of the enzymatic activity of DPPIV include eotaxin, macrophage- derived chemokine (MDC), interferon inducible chemokines, and other chemokines involved with the inhibition of HIV infection (Proost et al., 1999).

Proost P., De Meester I., Schols D., Struyf S., Lambeir A.-M., Wuyts A., Opdenakker G., De Clercq E., Scharpe S. and Van Damme J. (1998). Amino-terminal truncation of chemokines by CD26/dipeptidyl-peptidase IV: conversion of RANTES into a potent inhibitor of monocyte chemotaxis and HIV-1-infection. J. Biol. Chem. 273, 7222-7227.

Proost P., Struyf S., Schols D., Opdenakker G., Sozzani S., Allavena P., Mantovani A., Augustyns K., Bal G., Haemers A., Lambeir A.-M., Scharpe S., Van Damme J. and De Meester I. (1999). Truncation of macrophage-derived chemokine by CD26/dipeptidyl-peptidase IV beyond its predicted cleavage site affects chemotactic activity and CC chemokine receptor 4 interaction. J. Biol. Chem. 274, 3988-3993.

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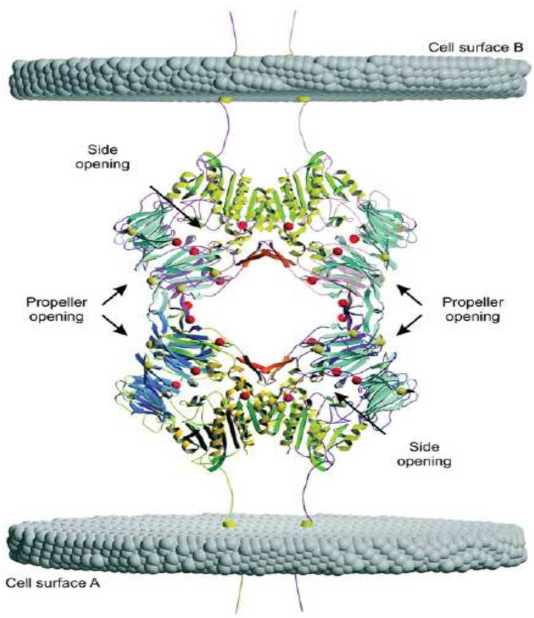
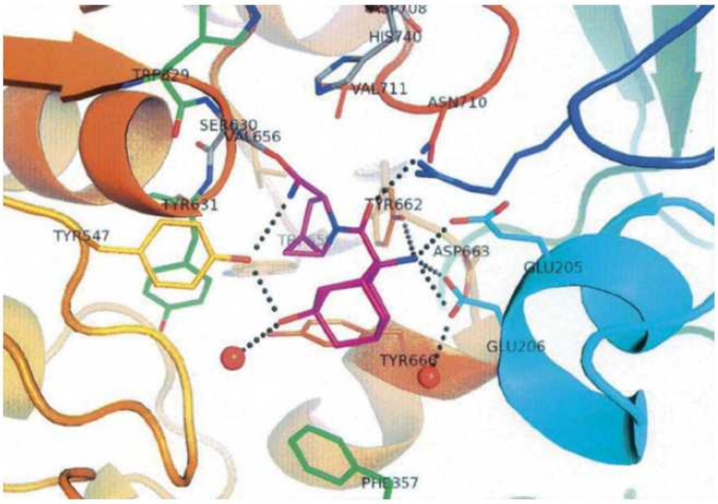
Tanaka T., Kameoka J., Yaron A., Schlossman S.F. and Morimoto C. (1993). The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity. Proc. Natl. Acad. Sci. USA 90, 4586-4590.

Ohnuma K., Munakata Y., Ishii T., Iwata S., Kobayashi S., Hosono O., Kawasaki H., Dang N.H. and Morimoto C. (2001). Soluble CD26/dipeptidyl peptidase IV induces T cell proliferation through CD86 up-regulation on APCs. J. Immunol. 167, 6745-6755.



Schematic representation of the structure of DPP-4



DPP-4 active site with active-site residues

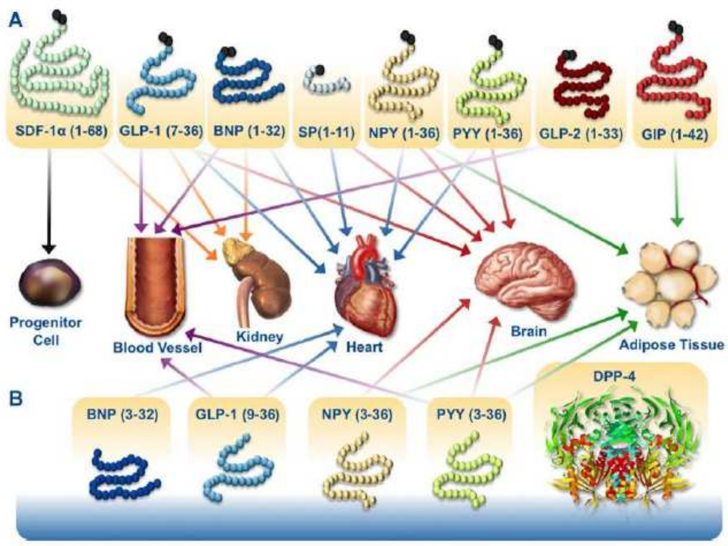
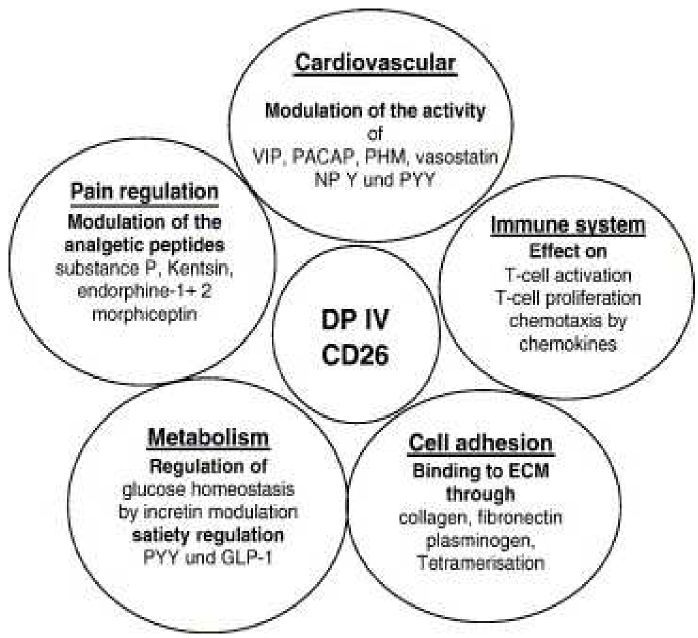


Figure 5. DPP-4 substrates that directly or indirectly regulate cardiovascular function. Multiple DPP-4 substrates have been identified that act on multiple peripheral tissues that influence the cardiovascular system. For a summary of these direct effects on target tissues, refer to Table 1. SP, Substance P.

Endocrine Reviews **33: 187–215, 2012**


Cardiovascular
Modulation of the activity
of
VIP, PACAP, PHM, vasostatin
NP Y und PYY
Pain regulation
Modulation of the
analgetic peptides
substance P, Kentsin,
endorphine-1+ 2
morphiceptin
DP IV
CD26
Immune system
Effect on
T-cell activation
T-cell proliferation
chemotaxis by
chemokines
Metabolism
Regulation of
glucose homeostasis
by incretin modulation
satiety regulation
PYY und GLP-1
Cell adhesion
Binding to ECM
through
collagen, fibronectin
plasminogen,
Tetramerisation

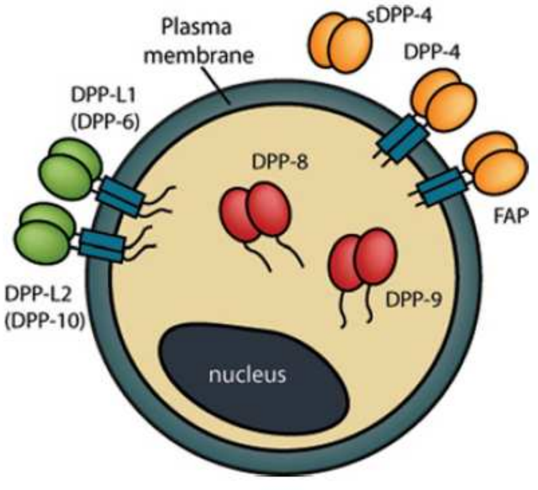
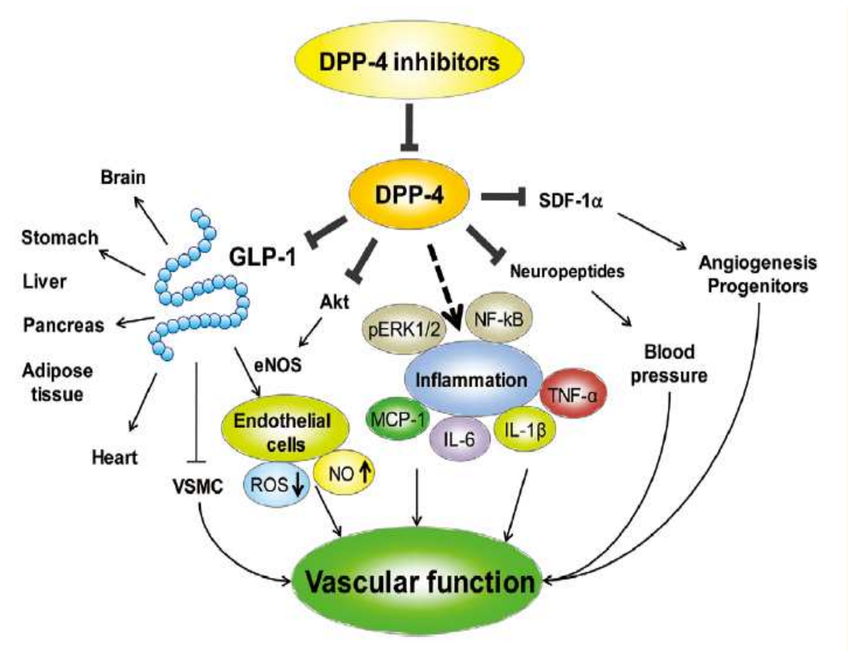
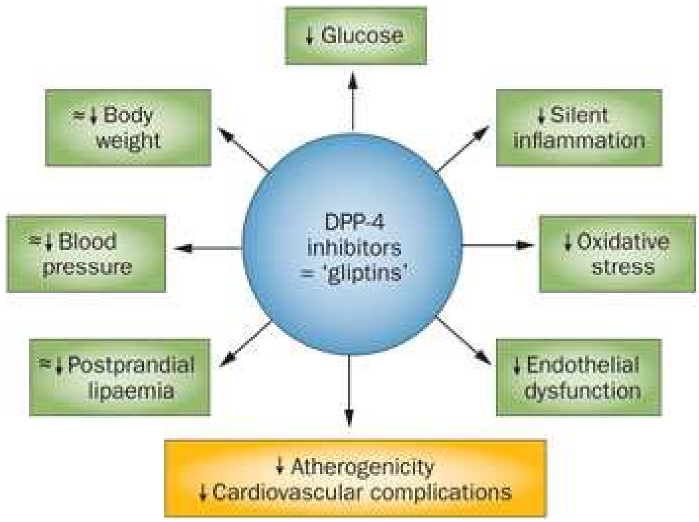


Figure 4 **Cellular localization of the six members of the *DPP-4* gene family**

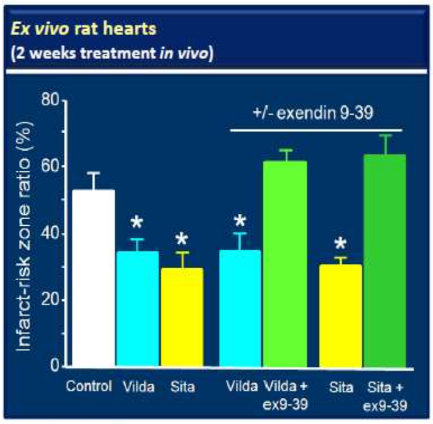
sDPP-4, soluble form of DPP-4.



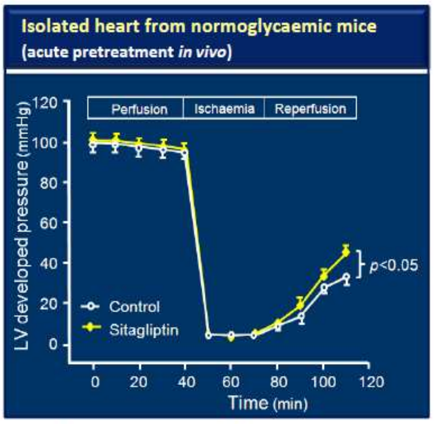
DPP-4 inhibitors
DPP-4
SDF-1α
Angiogenesis
Progenitors
Brain
Stomach
Liver
Pancreas
Adipose
tissue
Heart
GLP-1
Akt
eNOS
Neuropeptides
pERK1/2
NF-kB
Inflammation
TNF-α
MCP-1
IL-6
IL-1β
Blood
pressure
Endothelial
cells
ROS
NO
VSMC
Vascular function



DPP-4 Inhibition Reduces Infarct Size and Improves Cardiac Function After Ischaemia/Reperfusion Injury in Rodents



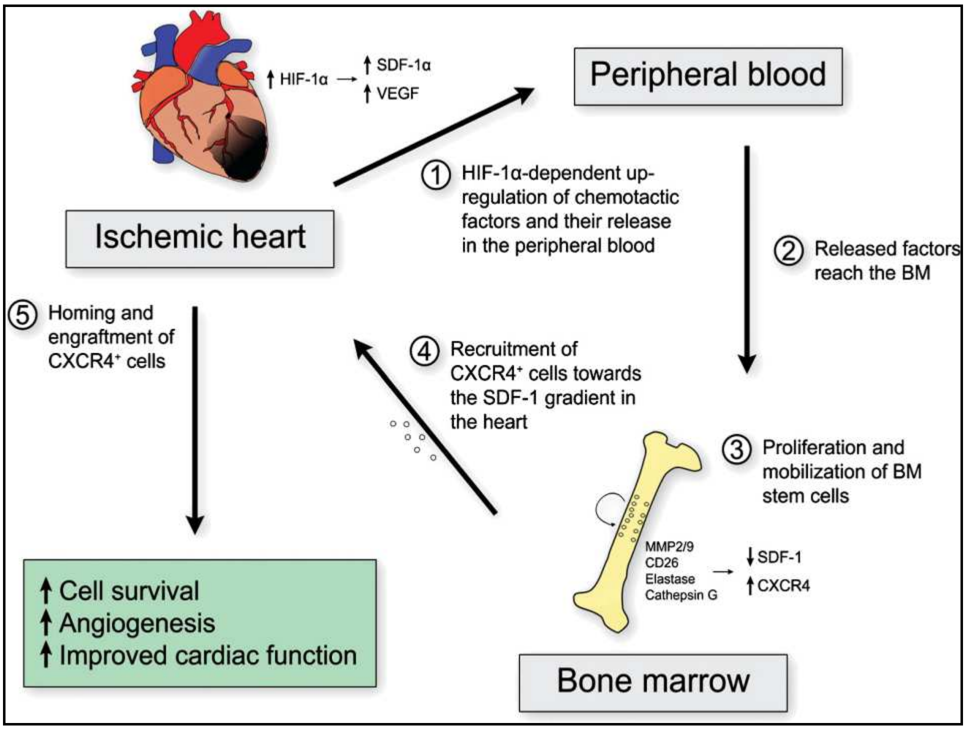
Vilda = vildagliptin
Sita = sitagliptin
Exendin 9-39 = a GLP-1 receptor antagonist

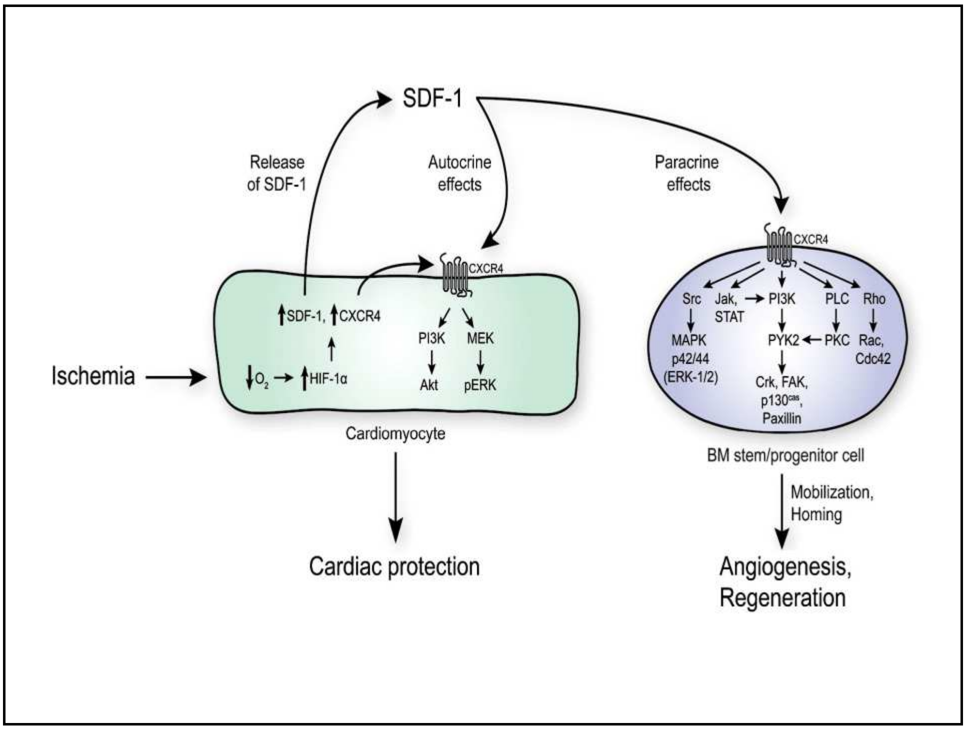


Sitagliptin improved functional recovery after ischemia/reperfusion injury ex vivo in normoglycemic mice.

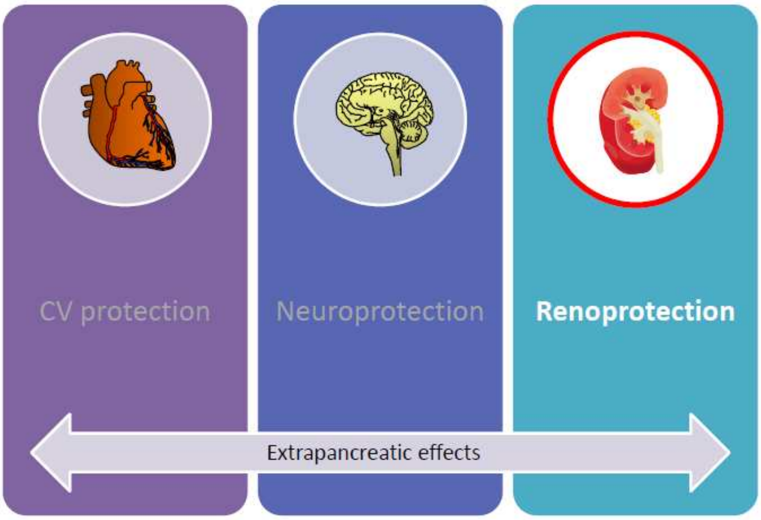
Hausenloy et al, Heart (2010), abstract FC1

Sauvé et al; Diabetes 2010


HIF-1α → SDF-1α
VEGF
Peripheral blood
Ischemic heart
1 HIF-1α-dependent up-regulation of chemotactic factors and their release in the peripheral blood
2 Released factors reach the BM
5 Homing and engraftment of CXCR4+ cells
4 Recruitment of CXCR4+ cells towards the SDF-1 gradient in the heart
3 Proliferation and mobilization of BM stem cells
MMP2/9
CD26
Elastase
Cathepsin G
SDF-1
CXCR4
Cell survival
Angiogenesis
Improved cardiac function
Bone marrow

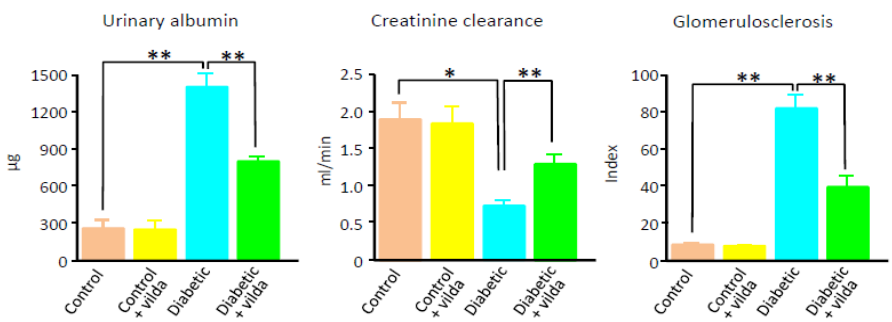

SDF-1
Release of SDF-1
Autocrine effects
Paracrine effects
CXCR4
SDF-1, CXCR4
PI3K
MEK
Akt
pERK
Ischemia
O2 → HIF-1α
Cardiomyocyte
Cardiac protection
Src
Jak, STAT
PI3K
PLC
Rho
MAPK p42/44 (ERK-1/2)
PYK2
PKC
Rac, Cdc42
Crk, FAK, p130cas, Paxillin
BM stem/progenitor cell
Mobilization, Homing
Angiogenesis, Regeneration

What is there beyond glycemic control?



DPP-4 Inhibition Has Renoprotective Effects in Rats with Streptozotocin-Induced Diabetes

Vildagliptin increased active GLP-1 levels, which probably prevented oxidative DNA damage and renal cell apoptosis.



Liu WJ, Xie SH, Liu YN, Kim W, Jin HY, Park SK, Shao YM, Park TS. J Pharmacol Exp Ther.2012 Feb;340(2):248-55

Vilda; vildagliptin (8 mg/kg/d)

Endocrine Journal The Japan Endocrine Society

RAPID COMMUNICATION

Advance Publication
doi: 10.1507/endocrj. K10E-382
January 2011

Sitagliptin reduces albuminuria in patients with type 2 diabetes

Sachiko Hattori

Endocrinology and Metabolism, Tsunemi-cho Clinic, Ashikaga 326-0022, Japan

Abstract. We investigated the inhibitory effect of sitagliptin on albuminuria in patients with type 2 diabetes. Thirty-six patients (19 men and 17 women) whose HbA1c was higher than 6.5% (NGSP) despite receiving education on diet and exercise and medical treatment for at least 6 months at our clinic were enrolled into this study and were successfully followed over 6 months of sitagliptin treatment. Sitagliptin (50 mg/day) treatment significantly lowered both systolic and diastolic blood pressures, fasting blood glucose and postprandial blood glucose, HbA1c, and glycated albumin at 3 months and 6 months. Significant reductions in highly sensitive C-reactive protein and soluble vascular cell adhesion molecule 1 were also observed at 6 months. Urinary albumin excretion (measured as urinary albumin-to-creatinine ratio (ACR: mg/g Cr)) did not change in the 6 months before sitagliptin treatment (ΔACR: 2.3 ± 19.9) and decreased in the 6 months after sitagliptin treatment (ΔACR: -20.6 ± 24.6); these differences were statistically significant. At 6 months, the ACR decreased from 11.6 ± 8.4 to 4.5 ± 5.0 in 13 patients with normoalbuminuria (ACR<30), from 98.4 ± 79 to 24.9 ± 20 in 15 patients with microalbuminuria (30<ACR<300), and from 1263 ± 492 to 561 ± 89 in 8 patients with macroalbuminuria (ACR>300). Thus, the present findings strongly suggest that sitagliptin reduces albuminuria without lowering the estimated glomerular filtration rate, most likely depending on known factors such as blood sugar reduction, blood pressure reduction, and inflammation reduction, as well as yet undetermined factors caused by an increase in active glucagon-like peptide-1.

Chen *et al. Journal of Translational Medicine* 2013, **11**:270
http://www.translational-medicine.com/content/11/1/270

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

Exendin-4 and sitagliptin protect kidney from ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction

Yen-Ta Chen[1], Tzu-Hsien Tsai[2], Chih-Chau Yang[3], Cheuk-Kwan Sun[4], Li-Teh Chang[5], Hung-Hwa Chen[6], Chia-Lo Chang[6], Pei-Hsun Sung[2], Yen-Yi Zhen[2], Steve Leu[7], Hsueh-Wen Chang[8], Yung-Lung Chen[2] and Hon-Kan Yip[2,7*]

Abstract

Background: This study tested the hypothesis that exendin-4 and sitagliptin can effectively protect kidney from acute ischemia-reperfusion (IR) injury.

Methods: Adult SD-rats ($n = 48$) equally divided into group 1 (sham control), group 2 (IR injury), group 3 [IR + sitagliptin 600 mg/kg at post-IR 1, 24, 48 hr)], and group 4 [IR + exendin-4 10 μm/kg at 1 hr after procedure] were sacrificed after 24 and 72 hrs ($n = 6$ at each time from each group) following clamping of bilateral renal pedicles for 60 minutes (groups 2–4).

Results: Serum creatinine level and urine protein to creatinine ratio were highest in group 2 and lowest in group 1 (all $p < 0.001$) without notable differences between groups 3 and 4. Kidney injury score, expressions of inflammatory biomarkers at mRNA (MMP-9, TNF-α, IL-1β, PAI-1), protein (TNF-α, NF-κB and VCAM-1), and cellular (CD68+) levels in injured kidneys at 24 and 72 hr showed an identical pattern compared to that of creatinine level in all groups (all $p < 0.0001$). Expressions of oxidized protein, reactive oxygen species (NOX-1, NOX-2), apoptosis (Bax, caspase-3 and PARP), and DNA damage marker (γH2AX+) of IR kidney at 24 and 72 hrs exhibited a pattern similar to that of inflammatory mediators among all groups (all $p < 0.01$). Renal expression of glucagon-like peptide-1 receptor, and anti-oxidant biomarkers at cellular (GPx, GR) and protein (NQO-1, HO-1, GPx) levels at 24 and 72 hr were lowest in group 1, significantly lower in group 2 than in groups 3 and 4 (all $p < 0.01$).

Conclusion: Exendin-4 and sitagliptin provided significant protection for the kidneys against acute IR injury.

Keywords: Exendin-4, Sitagliptin, Acute ischemia-reperfusion injury, Inflammation, Oxidative stress

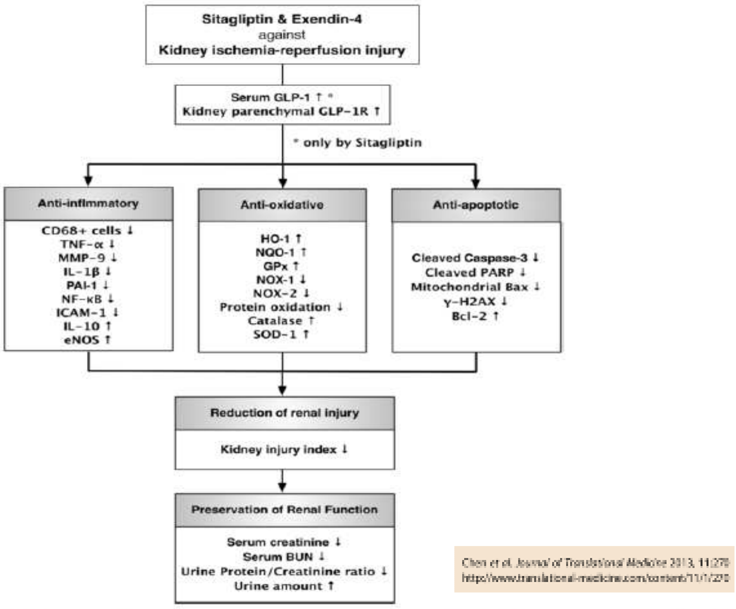


Figure 14 Proposed mechanisms underlying the positive therapeutic effects of sitagliptin and exendin-4 on kidney ischemia-reperfusion (IR) injury. GLP-1R = glucagon-like peptide-1 receptor; TNF = tumor necrotic factor; MMP = matrix metalloproteinase; IL = interleukin; PAI = plasminogen activator inhibitor; NF = nuclear factor; ICAM = intercellular adhesion molecule; eNOS = endothelial nitric oxide synthase; HO = heme oxygenase; NQO = NAD(P)H quinone oxidoreductase; GPx = glutathione peroxidase; NOX = ADPH oxidase; SOD = superoxide dismutase; PARP = poly(ADP-ribose) polymerase; BUN = blood urine nitrogen.

ORIGINAL

Advance Publication
doi: 10.1507/endocrj. EJ13-0305

DPP-4 inhibition with alogliptin on top of angiotensin II type 1 receptor blockade ameliorates albuminuria via up-regulation of SDF-1α in type 2 diabetic patients with incipient nephropathy

Hiroki Fujita[1)], Hisanori Taniai[2)], Hiroko Murayama[3)], Haruyo Ohshiro[3)], Hikaru Hayashi[2)], Seiko Sato[2)], Nyuko Kikuchi[3)], Taiga Komatsu[3)], Koga Komatsu[3)], Kanji Komatsu[3)], Takuma Narita[1)] and Yuichiro Yamada[1)]

[1)] *Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University Graduate School of Medicine, Akita 010-8543, Japan*
[2)] *Division of Internal Medicine, Honjo Daiichi Hospital, Yurihonjo 015-8567, Japan*
[3)] *Division of Gastroenterology, Honjo Daiichi Hospital, Yurihonjo 015-8567, Japan*

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Correspondence to: Hiroki Fujita, M.D., Ph.D., Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. E-mail: hirofuji@gipc.akita-u.ac.jp

ORIGINAL

Advance Publication
doi: 10.1507/endocrj. EJ13-0305

DPP-4 inhibition with alogliptin on top of angiotensin II type 1 receptor blockade ameliorates albuminuria via up-regulation of SDF-1α in type 2 diabetic patients with incipient nephropathy

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[2)] *Division of Internal Medicine, Honjo Daiichi Hospital, Yurihonjo 015-8567, Japan*
[3)] *Division of Gastroenterology, Honjo Daiichi Hospital, Yurihonjo 015-8567, Japan*

Abstract. Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new class of anti-diabetic drug which exerts its glucose-lowering action by suppressing the degradation of a gut incretin hormone glucagon-like peptide-1 (GLP-1). To elucidate whether treatment with stronger DPP-4 inhibitor on top of angiotensin II type 1 receptor blocker (ARB) provides greater renal protective effects, we performed a crossover study with two DPP-4 inhibitors, sitagliptin and alogliptin, in twelve type 2 diabetic patients with incipient nephropathy taking ARBs. This study consisted of three treatment periods: sitagliptin 50 mg/day for 4 weeks (first period), alogliptin 25 mg/day for 4 weeks (second period), and sitagliptin 50 mg/day for 4 weeks (third period). Significant changes in body mass index, blood pressure, serum lipids, serum creatinine, estimated glomerular filtration rate, and HbA1c were not observed among the three treatment periods. Reduced urinary levels of albumin and an oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG), increased urinary cAMP levels, and elevated plasma levels of stromal cell-derived factor-1α (SDF-1α) which is a physiological substrate of DPP-4 were observed after the switch from sitagliptin to a stronger DPP-4 inhibitor alogliptin. Given a large body of evidence indicating anti-oxidative action of cAMP and up-regulation of cellular cAMP production by SDF-1α, the present results suggest that more powerful DPP-4 inhibition on top of angiotensin II type 1 receptor blockade would offer additional protection against early-stage diabetic nephropathy beyond that attributed to glycemic control, via reduction of renal oxidative stress by SDF-1α-cAMP pathway activation.

DPP-4 inhibitor and diabetic nephropathy

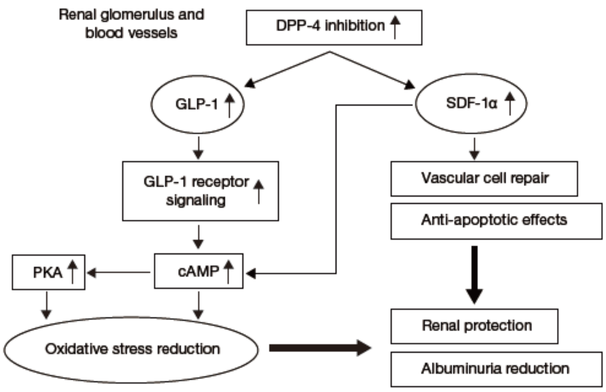

Fig. 3 Proposed DPP-4 inhibition signal transduction pathway in kidney

Effects of DPP-4 Inhibitors on the Heart in a Rat Model of Uremic Cardiomyopathy

Lyubov Chaykovska[1,2], Karoline von Websky[1,2], Jan Rahnenführer[1,2], Markus Alter[1,2,3], Susi Heiden[1,2], Holger Fuchs[4], Frank Runge[4], Thomas Klein[4], Berthold Hocher[1,2]*

1 Charité - Universitätsmedizin Berlin, Center for Cardiovascular Research, Institute for Pharmacology and Toxicology, Berlin, Germany, 2 Institute of Nutritional Science, University of Potsdam, Potsdam-Nuthetal, Germany, 3 Charité - Universitätsmedizin Berlin, Medizinische Klinik für Endokrinologie und Nephrologie, Berlin, Germany, 4 Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

Abstract

Background: Uremic cardiomyopathy contributes substantially to mortality in chronic kidney disease (CKD) patients. Glucagon-like peptide-1 (GLP-1) may improve cardiac function, but is mainly degraded by dipeptidyl peptidase-4 (DPP-4).

Methodology/Principal Findings: In a rat model of chronic renal failure, 5/6-nephrectomized [5/6N] rats were treated orally with DPP-4 inhibitors (linagliptin, sitagliptin, alogliptin) or placebo once daily for 4 days from 8 weeks after surgery, to identify the most appropriate treatment for cardiac dysfunction associated with CKD. Linagliptin showed no significant change in blood level AUC(0-∞) in 5/6N rats, but sitagliptin and alogliptin had significantly higher AUC(0-∞) values; 41% and 28% ($p = 0.0001$ and $p = 0.0324$), respectively. No correlation of markers of renal tubular and glomerular function with AUC was observed for linagliptin, which required no dose adjustment in uremic rats. Linagliptin 7 µmol/kg caused a 2-fold increase in GLP-1 (AUC 201.0 ng/l*h) in 5/6N rats compared with sham-treated rats (AUC 108.6 ng/l*h) ($p = 0.01$). The mRNA levels of heart tissue fibrosis markers were all significantly increased in 5/6N vs control rats and reduced/normalized by linagliptin.

Conclusions/Significance: DPP-4 inhibition increases plasma GLP-1 levels, particularly in uremia, and reduces expression of cardiac mRNA levels of matrix proteins and B-type natriuretic peptides (BNP). Linagliptin may offer a unique approach for treating uremic cardiomyopathy in CKD patients, with no need for dose-adjustment.

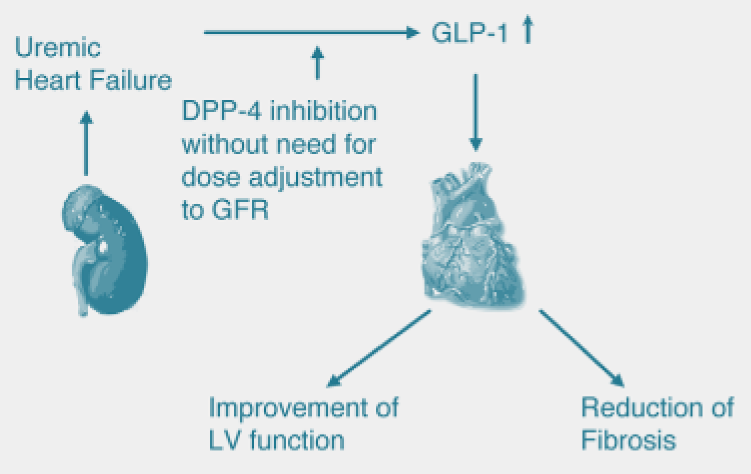

Figure 4. Influence of DPP-4 inhibition on cardiac impairment in the setting of uremia.
doi:10.1371/journal.pone.0027861.g004

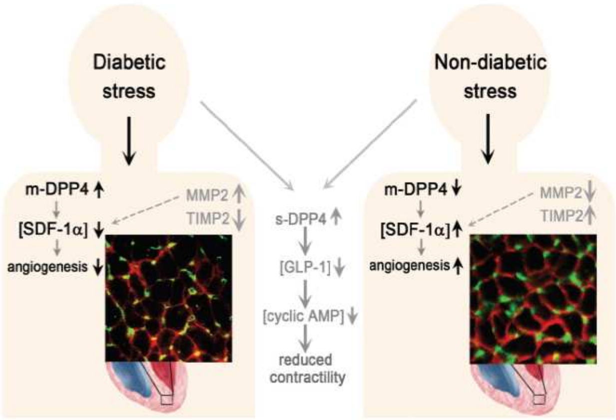

Figure 8. A suggested working model. Dipeptidyl peptidase-4 (DPP4) activity modulates cardiac dysfunction via membrane-bound form of DPP4 (m-DPP4)/stromal-derived factor-1α (SDF-1α)–dependent and matrix metalloproteinase-2 (MMP2)/tissue inhibitor of metalloproteinase-2 (TIMP2)–dependent mechanisms. Circulating DPP4 activity determines the glucagon-like peptide-1 (GLP-1) level, which may modulate left ventricular (LV) contractility.

CardioRenal Medicine

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www.karger.com/crm

Original Paper

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

Annayya Aroor[a, f] Susan McKarns[d, e] Ravi Nistala[b, f] Vincent DeMarco[c] Michael Gardner[a] Mariana Garcia-Touza[a] Adam Whaley-Connell[b, f] James R. Sowers[a, c, f]

Divisions of [a]Endocrinology, Diabetes and Metabolism and [b]Nephrology and Hypertension, Department of Internal Medicine, and Departments of [c]Medical Pharmacology and Physiology, [d]Surgery and [e]Molecular Microbiology and Immunology, University of Missouri Columbia School of Medicine, and [f]Harry S. Truman Memorial Veterans Hospital, Columbia, Mo., USA

DPP-4 enzymatic activity is very high in the kidney.

DPP4 activity (nmol min^{-1} g $tissue^{-1}$)	
Kidney	1460·8 ± 54·9
Liver	119·7 ± 9·6
Pancreas	11·2 ± 0·8
Epididymal fat	19·7 ± 2·8

In F344 rats

Kirino Y et al. Journal of Endocrinology (2009) 200, 53–61

Diabetes and Vascular Disease Research
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Original Article

Diabetes & Vascular Disease Research
9(4) 301–308

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DOI: 10.1177/1479164111434318
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Dipeptidyl peptidase-IV is a potential molecular biomarker in diabetic kidney disease

Ai-li Sun[1], Jing-ti Deng[2], Guang-ju Guan[1], Shi-hong Chen[1], Yuan-tao Liu[1], Jing Cheng[1], Zhen-wei Li[3], Xiang-hua Zhuang[1], Fu-dun Sun[1] and Hao-ping Deng[4]

Abstract

The present study was designed to identify the changes in microvesicle-dipeptidyl peptidase-IV (DPP IV) levels in human urine and serum, and to determine whether there were correlations with the severity of diabetic kidney disease (DKD). A total of 127 patients with type 2 diabetes mellitus (T2DM) were divided into three groups according to the urinary albumin/ creatinine ratio (UACR): microalbuminuria group (n = 50); macroalbuminuria group (n = 34) and normoalbuminuria group (n = 43), and 34 age- and sex-matched non-diabetic healthy subjects were selected as controls. Microvesicle-bound DPP IV and free urinary DPP IV were separated by a filtra-centrifugation method. The total microvesicles were captured by a specific monoclonal antibody, AD-1. DPP IV activity was determined by measuring the cleavage of chromogenic free 4-nitroaniline from Gly-Pro-p-nitroanilide at 405 nm with an ELISA plate reader. DPP IV protein levels were determined by ELISA and Western blot. Our results showed that the microvesicle-bound type was the major form of DPP IV in urine; the urinary microvesicle-DPP IV excretion of each T2DM group was significantly higher compared with controls. The urinary microvesicle-DPP IV level was positively correlated with UACR in patients with T2DM. These findings suggest that the urinary level of microvesicle-bound DPP IV is associated with the severity of DKD.

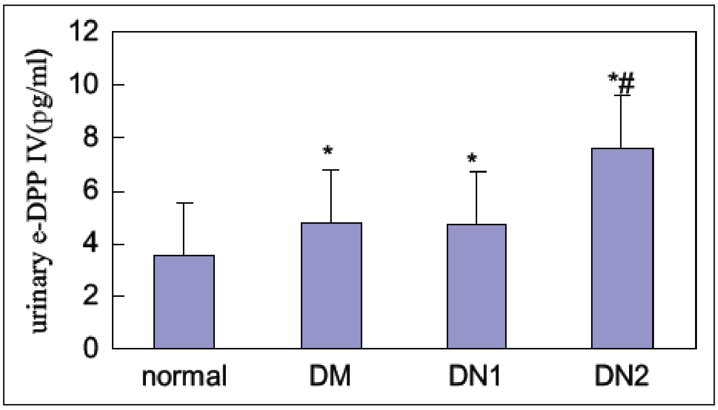

Figure 2. The urinary excretion of microvesicle-dipeptidyl peptidase-IV (m-DPP IV) in T2DM patients with normoalbuminuria (DM), microalbuminuria (DN1), macroalbuminuria (DN2) and healthy controls. *$p < 0.01$ vs. control group *# $p < 0.01$ vs. DM or DN1 group.

Figure 5. Electron micrograph of urinary microvesicles.

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Lysosomal enzymes are decreased in the kidney of diabetic rats

Giovani B. Peres [a], Maria A. Juliano [b], Manuel J. Simões [c], Yara M. Michelacci [a,*]

[a] Departamento de Bioquímica, Escola Paulista de Medicina, UNIFESP, São Paulo, SP, Brazil
[b] Departamento de Biofísica, Escola Paulista de Medicina, UNIFESP, São Paulo, SP, Brazil
[c] Departamento de Morfologia, Escola Paulista de Medicina, UNIFESP, São Paulo, SP, Brazil

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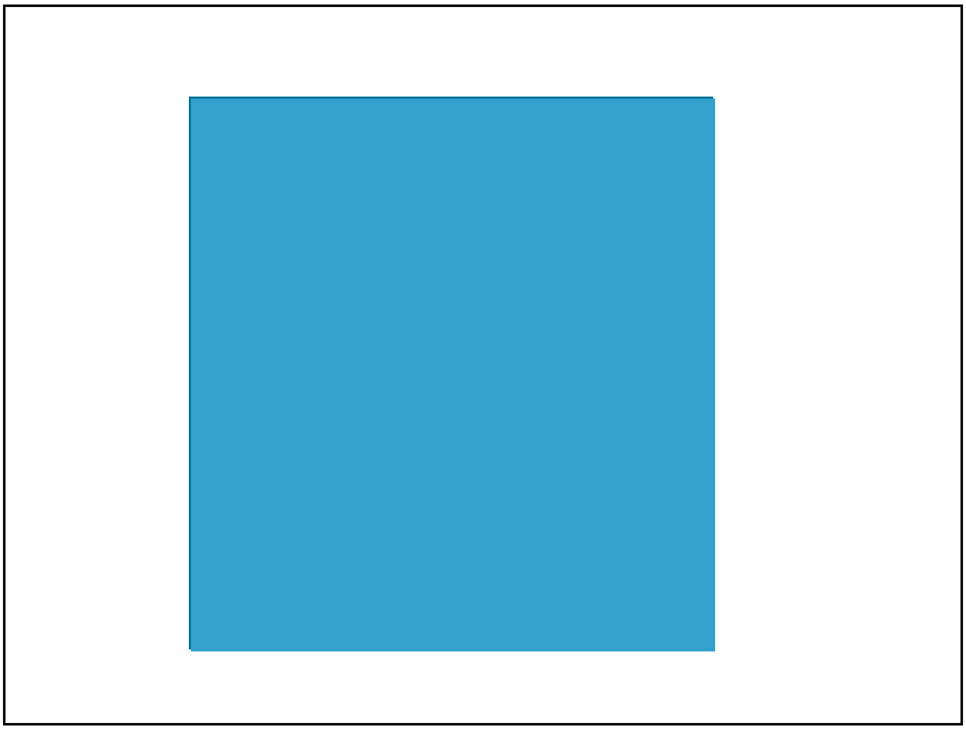
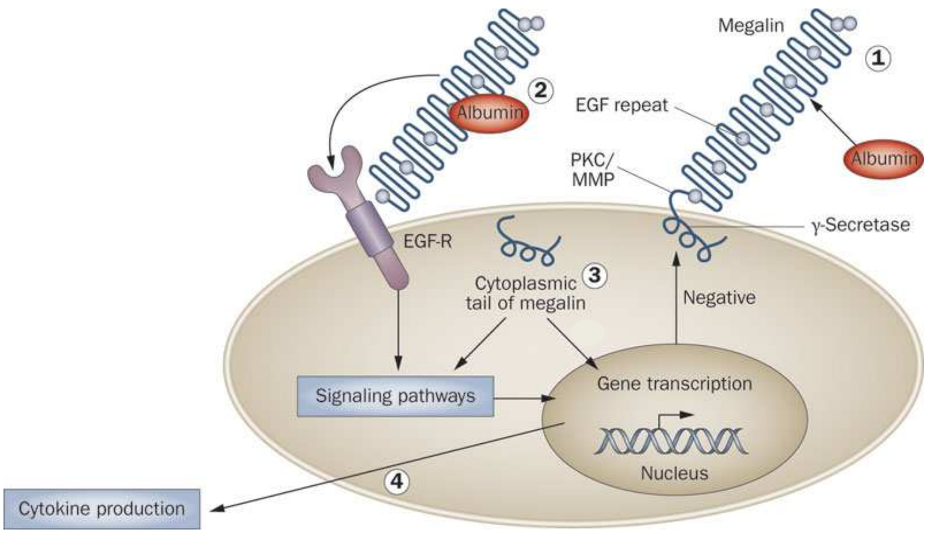
Tubular cells

ABSTRACT

The objective of the present study was to investigate the expression and activities of lysosomal enzymes that act upon proteins and sulfated polysaccharides in diabetic rat kidney. Cathepsins, glycosidases and sulfatases were studied on the 10th (DM-10) and on the 30th (DM-30) day of streptozotocin-induced diabetes mellitus (DM). The activity of cathepsin B, the main kidney cysteine protease, was decreased both in DM-10 and DM-30. Gel filtration chromatography of urinary proteins has shown the prevalence of low molecular weight peptides in normal and DM-10 urine, in contrast to the prevalence of high molecular weight peptides and intact proteins in DM-30. These results show that the decrease in lysosomal proteases could explain, at least in part, the increased albuminuria detected by radial immunodiffusion (RID), due to the excretion of less degraded or intact albumin. Concerning sulfated polysaccharides, the activities of β-glucuronidase, N-acetyl-β-D-glucosaminidase, and N-acetyl-β-D-galactosaminidase were also decreased in DM-30, while aryl sulfatases did not vary. Increased toluidine blue metachromatic staining of the tissue suggests that the lower activities of glycosidases could lead to intracellular deposition of partially digested molecules, and this could explain the decreased urinary excretion and increased tissue buildup of these molecules. The main morphological changes observed in kidney were proximal convoluted tubules with thinner walls and thinner brush border. Immunohistochemistry revealed that most of cathepsin B was located in the brush border of proximal tubular cells, highlighting the involvement of proximal convoluted tubules in diabetic nephropathy.




Megalin
1
2
Albumin
EGF repeat
Albumin
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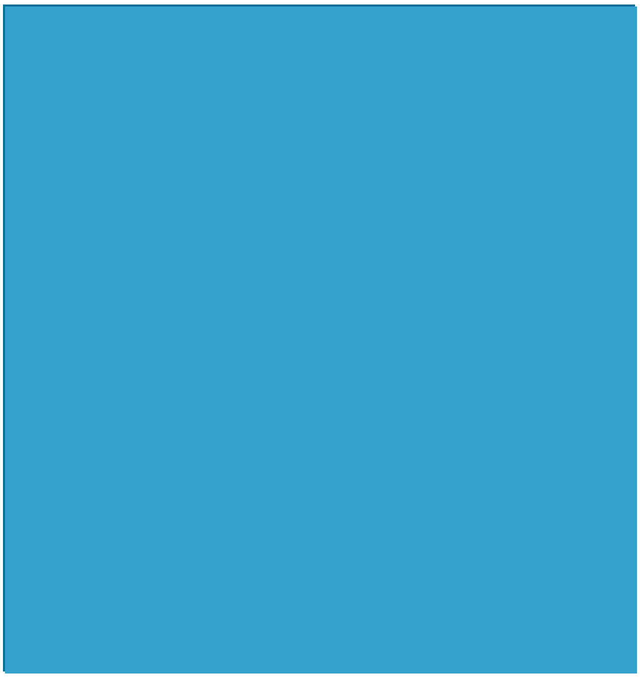
Cytokine production



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Dipeptidyl peptidase IV activity in commercial solutions of human serum albumin ☆

David Bar-Or [a,b,c,d,*], Denetta S. Slone [e], Charles W. Mains [d,f], Leonard T. Rael [a,b]

[a] Trauma Research Laboratory, Swedish Medical Center, Englewood, CO 80113, USA
[b] Ampio Pharmaceuticals, Greenwood Village, CO 80111, USA
[c] Emergency Department, Swedish Medical Center, Englewood, CO 80113, USA
[d] Rocky Vista University, Parker, CO 80134, USA
[e] Trauma Services, Swedish Medical Center, Englewood, CO 80113, USA
[f] Trauma Services, St. Anthony Central Hospital, Lakewood, CO 80228, USA

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Cohn fractionation
Inflammation

ABSTRACT

Due to the heterogeneous nature of commercial human serum albumin (cHSA), other components, such as the protease dipeptidyl peptidase IV (DPP-IV), possibly contribute to the therapeutic effect of cHSA. Here, we provide evidence for the first time that DPP-IV activity contributes to the formation of aspartate–alanine diketopiperazine (DA-DKP), a known immunomodulatory molecule from the N terminus of human albumin. cHSA was assayed for DPP-IV activity using a specific DPP-IV substrate and inhibitor. DPP-IV activity was assayed at 37 and 60 °C because cHSA solutions are pasteurized at 60 °C. DPP-IV activity in cHSA was compared with other sources of albumin such as a recombinant albumin (rHSA). In addition, the production of DA-DKP was measured by negative electrospray ionization/liquid chromatography mass spectrometry (ESI^-/LCMS). Significant levels of DPP-IV activity were present in cHSA. This activity was abolished using a specific DPP-IV inhibitor. Fully 70 to 80% DPP-IV activity remained at 60 °C compared with the 37 °C incubate. No DPP-IV activity was present in rHSA, suggesting that DPP-IV activity is present only in HSA produced using the Cohn fractionation process. The formation of DA-DKP at 60 °C was observed with the DPP-IV inhibitor significantly decreasing this formation. DPP-IV activity in cHSA results in the production of DA-DKP, which could account for some of the clinical effects of cHSA.

CURRENT OPINION

Physiology and pathophysiology of incretins in the kidney

Karoline von Websky[a,b], Christoph Reichetzeder[a,b], and Berthold Hocher[a]

Purpose of review
Incretin-based therapy with glucagon-like peptide-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors is considered a promising therapeutic option for type 2 diabetes mellitus. Cumulative evidence, mainly from preclinical animal studies, reveals that incretin-based therapies also may elicit beneficial effects on kidney function. This review gives an overview of the physiology, pathophysiology, and pharmacology of the renal incretin system.

Recent findings
Activation of GLP-1R in the kidney leads to diuretic and natriuretic effects, possibly through direct actions on renal tubular cells and sodium transporters. Moreover, there is evidence that incretin-based therapy reduces albuminuria, glomerulosclerosis, oxidative stress, and fibrosis in the kidney, partially through GLP-1R-independent pathways. Molecular mechanisms by which incretins exert their renal effects are understood incompletely, thus further studies are needed.

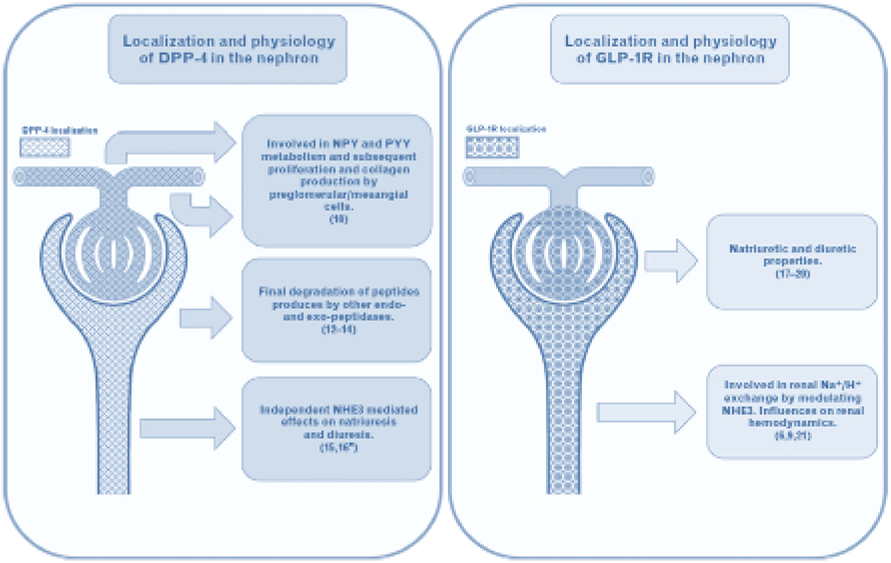
Summary
The GLP-1R and DPP-4 are expressed in the kidney in various species. The kidney plays an important role in the excretion of incretin metabolites and most GLP-1R agonists and DPP-4 inhibitors, thus special attention is required when applying incretin-based therapy in renal impairment. Preclinical observations suggest direct renoprotective effects of incretin-based therapies in the setting of hypertension and other disorders of sodium retention, as well as in diabetic and nondiabetic nephropathy. Clinical studies are needed in order to confirm translational relevance from preclinical findings for treatment options of renal diseases.

Keywords
DDP-4 inhibition, diabetes, diabetic nephropathy, GLP-1 receptor, hypertension, incretins, kidney, renal impairment

Curr Opin Nephrol Hypertens 2014, 23:000–000
DOI:10.1097/01.mnh.0000437542.77175.a0

CURRENT OPINION

Physiology and pathophysiology of incretins in the kidney



Curr Opin Nephrol Hypertens 2014, 23:000–000
DOI:10.1097/01.mnh.0000437542.77175.a0

Biochem. J. (1976) **155**, 169–182
Printed in Great Britain

Dipeptidyl Peptidase IV, a Kidney Brush-Border Serine Peptidase

By A. JOHN KENNY, ANDREW G. BOOTH, STEPHEN G. GEORGE,*
JEAN INGRAM, DOUGLAS KERSHAW, EDWARD J. WOOD and A. RHOEN YOUNG
Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.

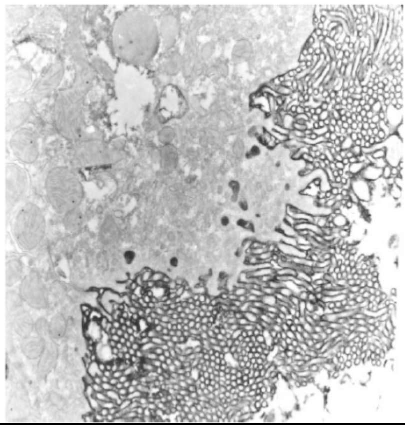
(*Received* 28 *January* 1976)

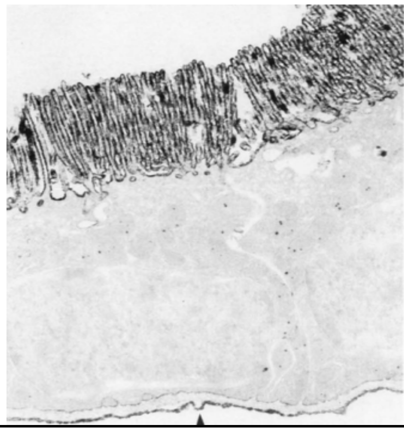
Dipeptidyl peptidase IV, an enzyme that releases dipeptides from substrates with *N*-terminal sequences of the forms X-Pro-Y or X-Ala-Y, was purified 300-fold from pig kidney cortex. The kidney is the main source of the enzyme, where it is one of the major microvillus-membrane proteins. Several other tissues contained demonstrable activity against the usual assay substrate glycylproline 2-naphthylamide. In the small intestine this activity was greatly enriched in the microvillus fraction. In all tissues examined, the activity was extremely sensitive to inhibition by di-isopropyl phosphorofluoridate (Dip-F), but relatively resistant to inhibition by phenylmethylsulphonyl fluoride. It is a serine proteinase which may be covalently labelled with [^{32}P]Dip-F, and is the only enzyme of this class in the microvillus membrane. The apparent subunit mol.wt. estimated by sodium dodecylsulphate/polyacrylamide-gel electrophoresis and by titration with [^{32}P]Dip-F was 130000. Gel-filtration and sedimentation-equilibrium methods gave values in the region of 280000, which is consistent with a dimeric structure, a conclusion supported by electron micrographs of the purified enzyme. Among other well-characterized serine proteinases, this enzyme is unique in its membrane location and its large subunit size. Investigation of the mode of attack of the peptidase on oligopeptides revealed that it could hydrolyse certain *N*-blocked peptides, e.g. Z-Gly-Pro-Leu-Gly-Pro. In this respect it is acting as an endopeptidase and as such may merit reclassification and renaming as microvillus-membrane serine peptidase.

Biochem. J. (1976) **155**, 169–182
Printed in Great Britain

Dipeptidyl Peptidase IV, a Kidney Brush-Border Serine Peptidase

Two other roles are conceivable: the microvillus membrane might be consumed during pinocytosis of proteins and peptides, and contribute to pinocytic vesicles an internal surface rich in hydrolytic activity, which could later reinforce the lysosomal proteinases





Biochem. J. (1979) 179, 379–395
Printed in Great Britain

Proteins of the Kidney Microvillar Membrane

THE AMPHIPATHIC FORM OF DIPEPTIDYL PEPTIDASE IV

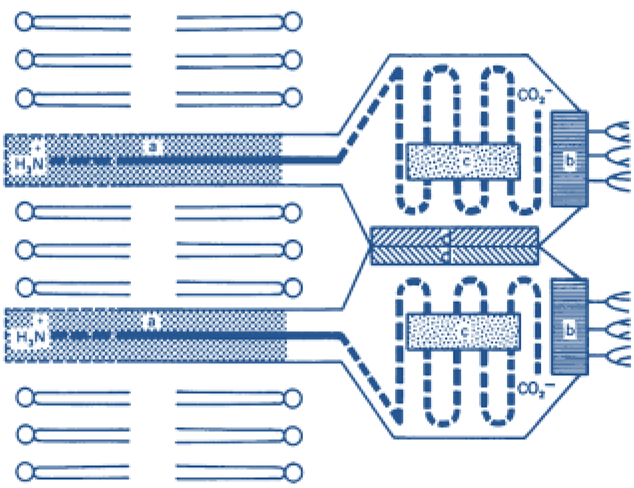


Fig. 2. *Model showing topology of dipeptidyl peptidase IV*

The model assumes that the enzyme has a dimeric structure in the membrane. The uncertainty concerning the extent of penetration of the anchor across the membrane is indicated by the broken lines near the internal surface of the membrane. Four domains on each subunit are represented schematically: (a) a surface participating in association of the two monomers; (b) a glycosylated region: (c) a catalytic site containing the reactive serine residue and; (d) a hydrophobic domain, at or near the *N*-terminus, anchoring the protein to the lipid bilayer. The model is not to scale; the anchor comprises no more than 2–3 % of the polypeptide chain.

0022-1554/96/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 44, No. 5, pp. 445–461, 1996
Printed in U.S.A.

Original Article

Organ Distribution of Aminopeptidase A and Dipeptidyl Peptidase IV in Normal Mice[1]

STEF MENTZEL,[2] HENRI B. P. M. DIJKMAN, JACCO P. H. F. VAN SON, ROBERT A. P. KOENE, and KAREL J. M. ASSMANN
Department of Pathology (SM, HBPMD, JPHFvS, KJMA) and Department of Medicine, Division of Nephrology (RAPK), University Hospital Nijmegen, Nijmegen, The Netherlands.

Received for publication August 31, 1995 and in revised form December 1, 1995; accepted December 10, 1995 (5A3762).

Table 1. *Organ distribution of APA and DPP IV*[a]

Organ	Distribution		APA	DPP IV
Kidney	Glomerulus		+ + +	+ + +
	Proximal tubules BB microvilli	S1	+ + +	+ +
		S2	+ + +	+ +
		S3	+	+ +
	Distal tubules		–	–
	Loops of Henle		–	+
	Peritubular capillaries		+ / + +	–
	Pars media arteries		+ +	–
	Juxtaglomerular granular cells		+	–
	Capsule		–	+ +

The Journal of Biological Chemistry
Vol. 276, No. 49, Issue of December 7, pp. 46671–46677, 2001
Printed in U.S.A.

Association of Na^+-H^+ Exchanger Isoform NHE3 and Dipeptidyl Peptidase IV in the Renal Proximal Tubule*

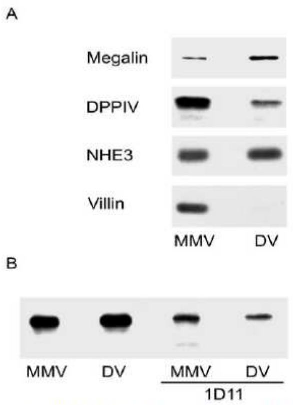
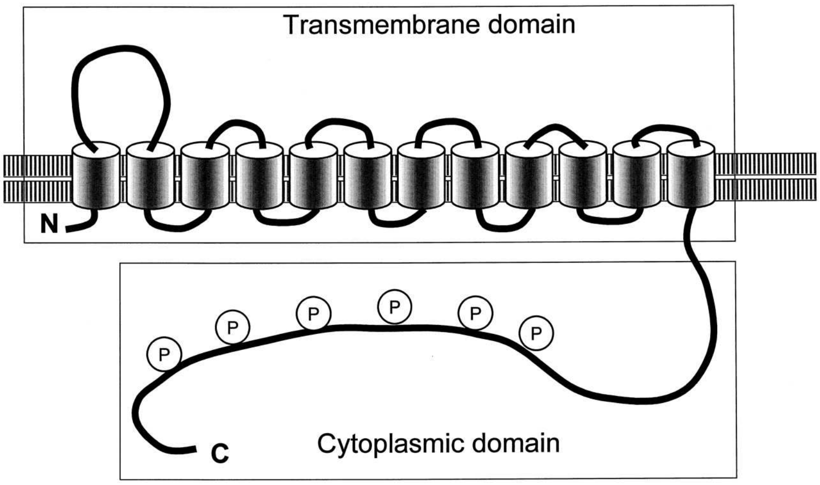
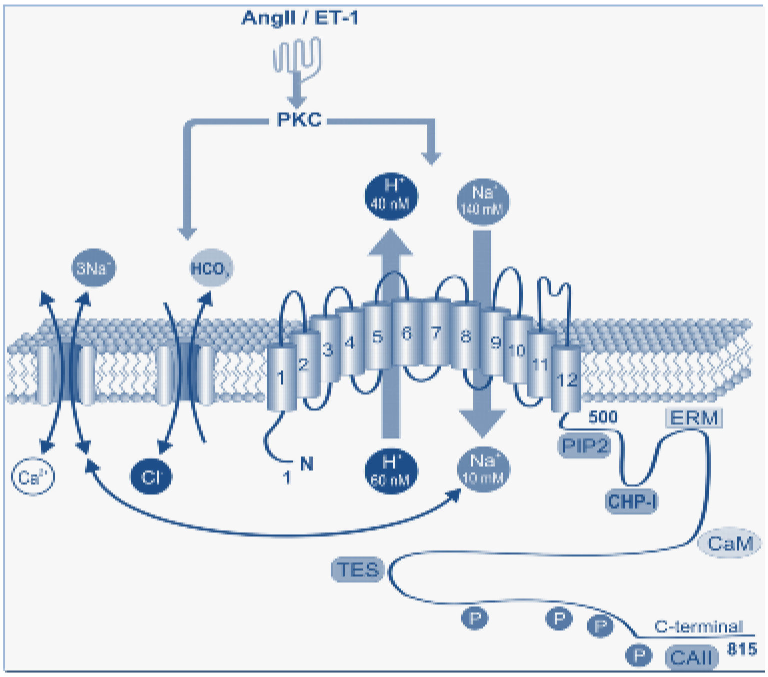


FIG. 6. **Distribution of the NHE3-DPPIV complex in the microdomains of rabbit renal brush border.** *A*, equal quantities (20 μg of protein) of microvillus membrane vesicles (*MMV*) prepared by divalent cation aggregation and dense vesicles (*DV*) prepared by density fractionation of postmitochondrial microsomes, were analyzed by immunoblotting. Blots were probed with antibodies to megalin (mAb 10A3), DPPIV (mAb 1D11), NHE3 (goat polyclonal Ab) and villin (mAb). *B*, equivalent quantities (100 μg) of solubilized microvillus membrane vesicles (*MMV*) and dense vesicles (*DV*) were immunoprecipitated with anti-DPPIV mAb 1D11 (1D11 IP). The immune complexes, as well as samples of the starting material, were prepared for immunoblotting, and the blot was probed with a polyclonal goat antibody to NHE3.

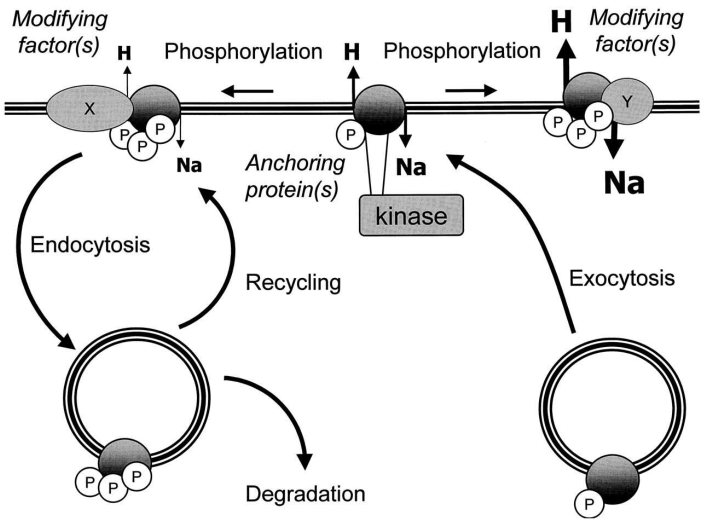
Structure of mammalian Na/H exchangers



MOE O W J ASN 1999;10:2412-2425



Changes in NHE-3 activity without change in NHE-3 antigen.



MOE O W J ASN 1999;10:2412-2425

JASN

Am J Physiol Cell Physiol 302: C1569–C1587, 2012.
First published March 28, 2012; doi:10.1152/ajpcell.00017.2012.

Review

Deciphering the mechanisms of the Na^+/H^+ exchanger-3 regulation in organ dysfunction

Role of NHE3 in the Pathophysiology of the Diabetic Kidney

The increase in NHE3 activity in diabetic kidneys was further confirmed in several studies using cell culture models. Ambuhl and colleagues demonstrated in opossum kidney (OK) cells, a well-characterized proximal tubule cell line, that treatment with high glucose (to mimic hyperglycemia) results in stimulation of NHE3 activity accompanied by increases in both NHE3 protein and transcript expression (5). Similar results were found in human proximal tubular cells (hPTC) exposed to high glucose (181). High glucose treatment also leads to an increase in expression of the serum glucocorticoid-regulated kinase-1 (SGK1) in hPTC (181); activation of *Sgk1* gene expression was also confirmed in STZ-diabetic rats (181). In summary, activation of SGK1 is a good candidate signaling pathway to regulate NHE3 activation in diabetic nephropathy.

Am J Physiol Cell Physiol 302: C1569–C1587, 2012.
First published March 28, 2012; doi:10.1152/ajpcell.00017.2012.

Review

Deciphering the mechanisms of the Na^+/H^+ exchanger-3 regulation in organ dysfunction

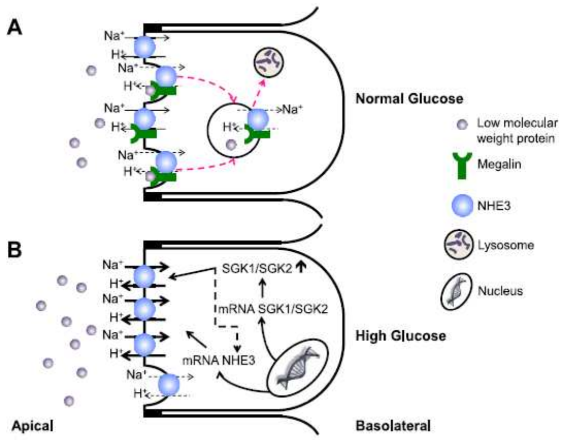


Fig. 2. Model of how NHE3 could participate in the reabsorption of filtered proteins and how its transport function could be increased in diabetic nephropathy. *A*: under physiological conditions, NHE3 interacts with megalin, which is responsible for receptor-mediated endocytosis of low-molecular-weight proteins such as albumin. NHE3 may be recruited in endocytic vesicles in complex with megalin and promote albumin uptake. The complex may be degraded in the lysosomes. *B*: the proteinuria associated with diabetic nephropathy may be due in part to a reduction of albumin uptake at the tubule level. This decrease in albumin endocytosis and the rise of intratubular albumin concentration are proposed to be due to a reduction in megalin expression. Furthermore, NHE3 expression level is increased in models of diabetic nephropathy. Possible molecular mechanisms of activation in NHE3 transport function are presented. Lower level of megalin expression would result in a decrease in the amount of megalin in complex with NHE3 and the megalin-dependent NHE3 endocytosis. The increase in intratubular albumin concentration stimulates NHE3 expression. Furthermore, serum glucocorticoid-regulated kinase-1 (SGK1) expression is increased in animal models of diabetic nephropathy and cells treated with high glucose to mimic hyperglycemia. SGK is proposed as one of the signaling pathways activated to stimulate NHE3 transport function in diabetic nephropathy. This model requires further experimental validation. Dashed lines are used to indicate NHE3 transport activity or signaling pathways that remain to be fully defined.

Am J Physiol Renal Physiol 297: F1647–F1655, 2009.
First published September 23, 2009; doi:10.1152/ajprenal.00082.2009.

Regulation of Na^+/H^+ exchanger NHE3 by glucagon-like peptide 1 receptor agonist exendin-4 in renal proximal tubule cells

Luciene R. Carraro-Lacroix,[1,3] Gerhard Malnic,[1] and Adriana C. C. Girardi[1,2]

[1]Department of Physiology and Biophysics, Institute of Biomedical Sciences; [2]Heart Institute (InCor), Medical School, University of São Paulo, and [3]Department of Physiology, Federal University of São Paulo, São Paulo, Brazil

In summary, we have found that the GLP-1R agonist exendin-4 reduces NHE3 activity in renal proximal tubule cells. This effect is mediated via PKA and EPAC signaling pathways and is associated with increased levels of NHE3 phosphorylation. The inhibition of DPPIV may enhance the renal effects of GLP-1, and might explain why DPPIV inhibitors decrease NHE3-mediated $NaHCO_3$ reabsorption in the rat renal proximal tubule.

Am J Physiol Renal Physiol 294: F414–F422, 2008.
First published December 12, 2007; doi:10.1152/ajprenal.00174.2007.

Dipeptidyl peptidase IV inhibition downregulates Na^+-H^+ exchanger NHE3 in rat renal proximal tubule

Adriana Castello Costa Girardi, Lívia Emy Fukuda, Luciana Venturini Rossoni, Gerhard Malnic, and Nancy Amaral Rebouças

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

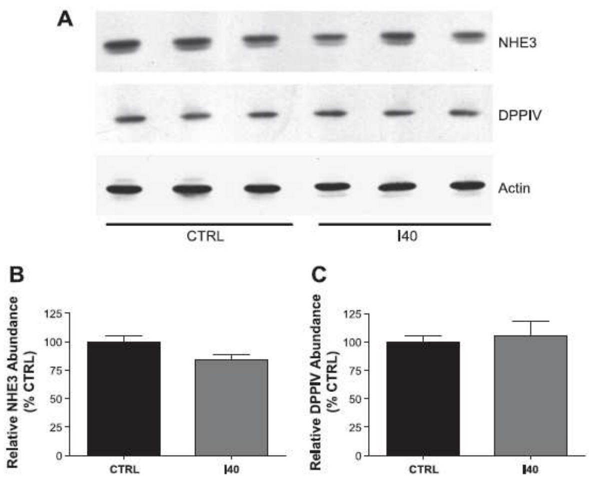


Fig. 5. Effect of DPPIV inhibition on NHE3 and DPPIV protein expression levels in rat renal cortex. *A*: 50 μg of renal cortical membranes isolated from rats treated with I40 or the structurally related noninhibitory compound Lys[Z(NO_2)]-OH (control) were subjected to SDS-PAGE, transferred to a PVDF membrane, and analyzed by immunoblotting. Blots were probed successively with antibodies to NHE3, DPPIV, and actin. Densitometry values for NHE3 (*B*) and DPPIV (*C*) were normalized with actin and plotted as a bar graph. Results are expressed as % of control. Values are means ± SE; n = 6/group.

The Journal of Experimental Biology 212, 1630-1637
Published by The Company of Biologists 2009
doi:10.1242/jeb.027375

Review

Tethering, recycling and activation of the epithelial sodium–proton exchanger, NHE3

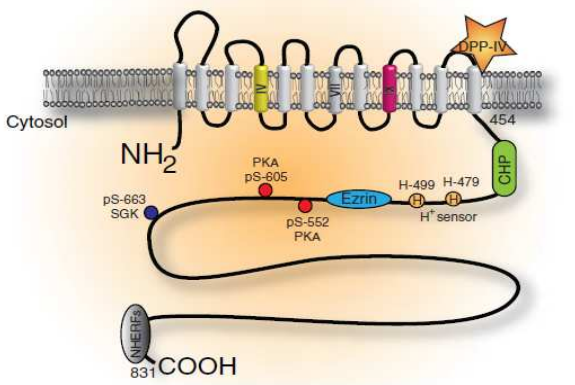


Fig. 1. The structure of NHE3 is divided into 12 transmembrane domains (residues 1–454) and a large cytosolic C-terminal domain (residues 455–831). The transmembrane domains implicated in ion transport are depicted in yellow and the domain responsible for inhibition by amiloride is depicted in pink (transmembrane domain IX). The diagram also indicates the putative binding sites for CHP (calcineurin homologous protein), ezrin and the NHERFs (sodium–hydrogen exchanger regulatory factor), the proton modifier sites (H^+ sensor) and the sites that are phosphorylated by either protein kinase A (PKA) and serum and glucocorticoid kinase (SGK). DPP, dipeptidyl peptidase.

Diabetes Care Publish Ahead of Print, published online September 11, 2013

Clinical Care/Education/Nutrition/Psychosocial Research

ORIGINAL ARTICLE

Linagliptin Lowers Albuminuria on Top of Recommended Standard Treatment in Patients With Type 2 Diabetes and Renal Dysfunction

Per-Henrik Groop, md, dmsc[1,2,3]
Mark E. Cooper, mbbs, phd[3]
Vlado Perkovic, mbbs, phd[4]
Angela Emser, phd[5]
Hans-Juergen Woerle, md[5]
Maximilian von Eynatten, md[5]

OBJECTIVE—Preclinical data suggest that linagliptin, a dipeptidyl peptidase-4 inhibitor, may lower urinary albumin excretion. The ability of linagliptin to lower albuminuria on top of renin-angiotensin-aldosterone system (RAAS) inhibition in humans was analyzed by pooling data from four similarly designed, 24-week, randomized, double-blind, placebo-controlled, phase III trials.

RESEARCH DESIGN AND METHODS—A pooled analysis of four completed studies identified 217 subjects with type 2 diabetes and prevalent albuminuria (defined as a urinary albumin-to-creatinine ratio [UACR] of 30–3,000 mg/g creatinine) while receiving stable doses of RAAS inhibitors. Participants were randomized to either linagliptin 5 mg/day (n = 162) or placebo (n = 55). The primary end point was the percentage change in geometric mean UACR from baseline to week 24.

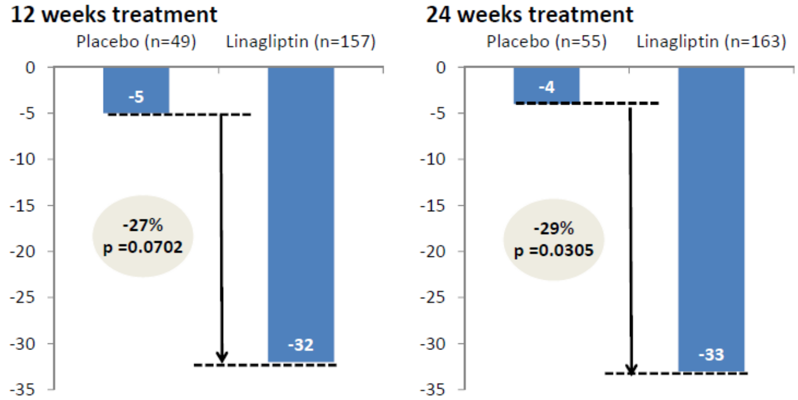
RESULTS—UACR at week 24 was reduced by 32% (95% CI −42 to −21; $P < 0.05$) with linagliptin compared with 6% (95% CI −27 to +23) with placebo, with a between-group difference of 28% (95% CI −47 to −2; $P = 0.0357$). The between-group difference in the change in HbA_{1c} from baseline to week 24 was −0.61% (−6.7 mmol/mol) in favor of linagliptin (95% CI −0.88 to −0.34% [−9.6 to −3.7 mmol/mol]; $P < 0.0001$). The albuminuria-lowering effect of linagliptin, however, was not influenced by race or HbA_{1c} and systolic blood pressure (SBP) values at baseline or after treatment.

CONCLUSIONS—Linagliptin administered in addition to stable RAAS inhibitors led to a significant reduction in albuminuria in patients with type 2 diabetes and renal dysfunction. This observation was independent of changes in glucose level or SBP. Further research to prospectively investigate the renal effects of linagliptin is underway.

Linagliptin Lowers Albuminuria on Top of Recommended Standard Treatment for Diabetic Nephropathy

Linagliptin lowered albuminuria in patients with diabetic nephropathy

Adjusted mean change in albuminuria from baseline



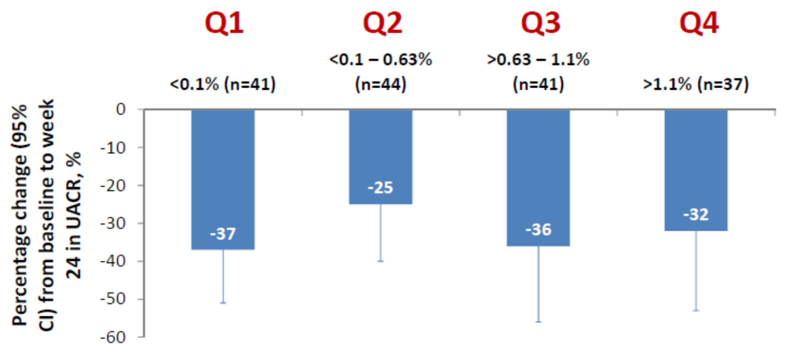
95% of the overall albuminurea lowering effect occurred as early as 12 weeks

*Based on adjusted (log [baseline UACR] and trial effect) geometric mean ratio (Week 12 or 24/baseline). †Relative change was calculated as the ratio of the percentage change in the linagliptin group divided by the percentage change in the placebo group
Source: Groop PH, et al. ADA 2012 Poster: 953-P

Linagliptin Lowers Albuminuria on Top of Recommended Standard Treatment for Diabetic Nephropathy

Linagliptin lowers UACR independent of its glucose lowering effect.

There was no correlation between changes in HbA1c and UACR from baseline to Week 24 (Pearson's r=0.073, n=218)



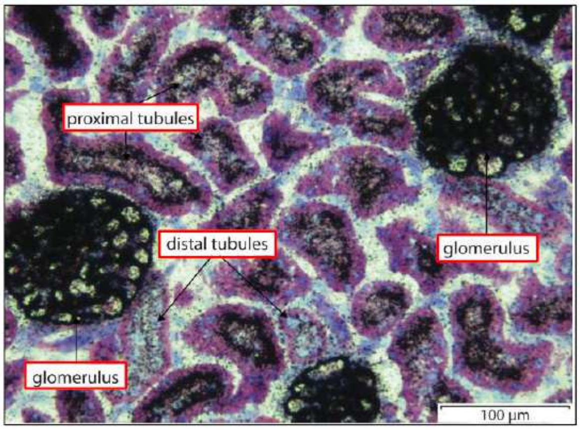
Adjusted mean change in UACR (%) from baseline at week 24 by quartiles of HbA1c change

Significant change in UACR (%) v. baseline after 24 weeks, Analysis includes all patients from the treated set with a baseline and ≥1 on-treatment value for both HbA1c and UACR
Groop PH, et al. ADA 2012 Poster: 953-P

Linagliptin with high tissue penetration property is present not only in glomerulus also in tubules

Microautoradiograms from rat kidney sampled at 3 h after intravenous injection of 7.4 µg/kg [^{3}H] Linagliptin

Presence of Linagliptin is indicated by black area (radioactivity)



Greischel A, et al. *Drug Metab Dispos.*. 2010;38(9):1443-1448.

Comparison of the Effects of Telmisartan and Linagliptin in Rats With 5/6N

Hocher , B.
EASD 2013
143 , S 66

	Telmisartan	Linagliptin
Blood pressure	↓↓↓	↓
Interstital fibrosis	no effect	↓
Albumin excretion/ACR	↓↓	↓
Glomerular size	no effect	↓
Osteopontin, VEGF-A, N-GAL	↓	no effect
Leptin, RANTES	↑	no effect
Phospho-SMAD2		↓
Urinary DPP-4 excretion	no effect	↓
Active GLP-1	no effect	↑↑

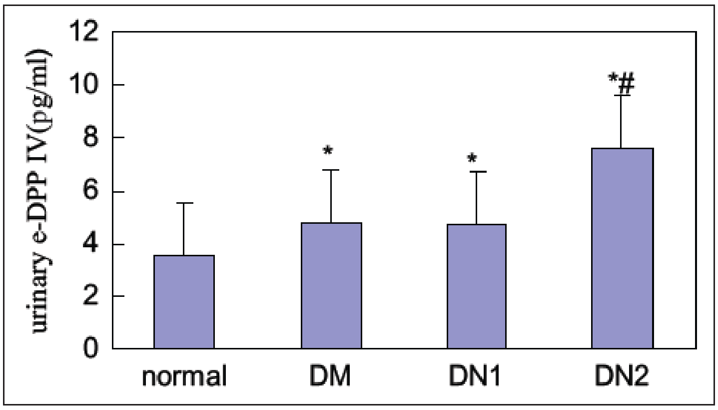


Figure 2. The urinary excretion of microvesicle-dipeptidyl peptidase-IV (m-DPP IV) in T2DM patients with normoalbuminuria (DM), microalbuminuria (DN1), macroalbuminuria (DN2) and healthy controls. *$p < 0.01$ vs. control group *# $p < 0.01$ vs. DM or DN1 group.

Linagliptin Nephro-protection
by exploring renal DPP-IV enzyme inhibition
Pharmacodinamics Implications

L I N E D Study

Grazie per l'attenzione !

S.S.