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# Dipeptidyl peptidase IV (DPP IV) and related molecules in type 2 diabetes

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## 1. ABSTRACT

Dipeptidyl peptidase IV (DPP IV) is a widely distributed physiological enzyme that can be found solubilized in blood, or membrane-anchored in tissues. DPP IV and related dipeptidase enzymes cleave a wide range of physiological peptides and have been associated with several disease processes including Crohn's disease, chronic liver disease, osteoporosis, multiple sclerosis, eating disorders, rheumatoid arthritis, cancer, and of direct relevance to this review, type 2 diabetes. Here, we place particular emphasis on two peptide substrates of DPP IV with insulin-releasing and antidiabetic actions namely, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The rationale for inhibiting DPP IV activity in type 2 diabetes is that it decreases peptide cleavage and thereby enhances endogenous incretin hormone activity. A multitude of novel DPP IV inhibitor compounds have now been developed and tested. Here we examine the information available on DPP IV and related enzymes, review recent preclinical and clinical data for DPP IV inhibitors, and assess their clinical significance.

## 2. INTRODUCTION

Two primary defects in the pathogenesis of type 2 diabetes are a relative loss of insulin secretion from pancreatic beta cells and a decreased sensitivity of liver and peripheral tissues to insulin (1). Drug treatments for type 2 diabetes have centred therefore on enhancing insulin secretion and action. In the case of sulphonylureas (e.g. glibenclamide) and meglitinides (e.g. nateglinide) insulin secretion is increased from the pancreas (2). The biguanides (e.g. metformin) and thiazolidinediones (e.g. pioglitazone) improve the body's sensitivity to insulin (3-5). Additionally, synthetic insulin and insulin analogues can be administered when oral drugs are no longer sufficient to provide adequate blood glucose control.

Insulin secretagogues and in particular the sulphonylureas suffer from a lack of glucose-dependency which can lead to episodes of hypoglycaemia (6). Also, as the disease progresses and beta-cell function declines, several years of use often lead to declining drug effectiveness. With the prevalence of type 2 diabetes reaching epidemic proportions (predicted to be about 350

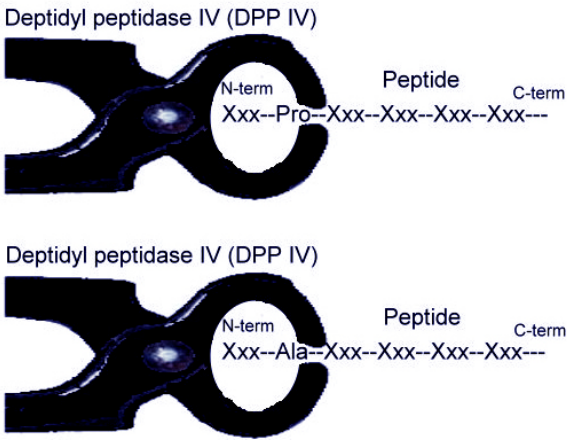


**Figure 1.** N-terminal cleavage activity and substrate specificity of dipeptidyl peptidase IV (DPP IV). DPP IV cleaves dipeptides from the N-terminus of peptides and polypeptides which have either a proline or an alanine residue in the penultimate position (examples of which can be found in Table 1). Xaa represents one of the 20 proteinogenic amino acids.

million by 2025, (7)) new antidiabetic drug treatments are urgently required. Incretin hormones are peptides secreted from endocrine cells in the small intestine which stimulate significant insulin secretion at physiological concentrations in a glucose-dependent manner (8-12). The two principal incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP). Expanding knowledge of the incretin hormones and their physiological inactivation by dipeptidyl peptidase IV (DPP IV) has lead to two new classes of antidiabetic drugs, incretin analogues/mimetics and DPP IV inhibitors/gliptins. In this article we focus on the enzyme DPP IV and the progress made towards the development of DPP IV inhibitors.

## 3. DPP IV AND RELATED ENZYMES

### 3.1. DPP IV (EC 3.4.14.5)

DPP IV is classified as a serine protease by virtue of the classical consensus motif Gly-Xxx-Ser-Xxx-Gly, which is $Gly^{628}$-$Trp^{629}$-$Ser^{630}$-$Tyr^{631}$-$Gly^{632}$ in the case of human DPP IV (13). DPP IV was one of the earliest identified prolyl peptidases and over the years DPP IV has become one of the most intensively studied of its class (14). The proteolytic activity of DPP IV is relatively selective, cleaving only peptide bonds following proline or alanine amino acid residues located penultimate to the N-terminus (14; See Figure 1) (this also commonly occurs at sites following serine (See Table 1)). DPP IV is also the lymphocyte cell surface protein CD26 discussed in detail in other reviews (15). Structural studies with soluble human DPP IV reveal that the inhibitor diprotin A covalently bonds to $Ser^{630}$ in the catalytic triad, irreversibly blocking the active site (13). Physiologically the role of DPP IV is wide ranging since it is capable of interacting with a number of diverse proteins such as collagen (16), fibronectin (17), adenosine deaminase (18), tyrosine phosphatase CD45 (19), and a plethora of regulatory peptides across a range of physiological systems (20-22). Table 1 lists many physiological peptide substrates (or potential substrates) of DPP IV showing N-terminal regions targeted by the enzyme.

DPP IV is expressed by endothelia and epithelia in most tissues, including bone marrow, kidney, intestine, pancreas, liver, lymphocytes, placenta, uterus, prostate and skin (14). Levels and expression and activity of DPP IV in certain tissues and blood plasma are known to vary significantly following the onset of disease, injury or inflammation (reviewed elsewhere 14, 22). Peptide substrates of DPP IV have such extensive physiological implications, that most body systems are likely to be affected including nervous, endocrine, neuroendocrine, immune, vascular, digestive, skeletal and reproductive systems (see substrates in Table 1). Among the most commonly recognized substrates of DPP IV are several chemokines that affect the immune system: (RANTES, eotaxin, IP-10, MCP-1, MCP-2, MCP-3, SDF-1α, SDF-1β, GCP-2 and MDC, see Table 1 (20); several neuropeptides: substance P, bradykinin, peptide YY (PYY), neuropeptide Y (NPY), and pituitary adenylate cyclase activating peptide (PACAP) (20); and glucagon, the counter-regulatory hormone of insulin (23). As we shall see later in this review, much attention has been focused towards the incretin hormones GLP-1 and GIP as substrates of DPP IV. Considered briefly below are other physiological proteases related to DPP IV which possess similar post-proline aminopeptidase activity.

### 3.2. Dipeptidyl peptidase II (DPP II, also known as DPP 7, or quiescent cell proline dipeptidase (QPP) (E.C. 3.4.14.2)

Evidence in recent years has suggested that three earlier identified proteases DPP II, DPP 7 and QPP are in fact the same enzyme (24-26). Discovered in 1968 by the extraction of Lys-Ala- hydrolytic activity from the anterior pituitary, DPP II appears to be widely distributed across a range of mammalian tissues (24, 27). The identification of physiological substrates of DPP II has been hindered by low purification yields from tissue and a lack of information regarding the molecular and catalytic properties. Although there are no totally selective DPP II inhibitors available, compounds such as Ala-ψ[CS-N]-Pyrr and Ala-ψ[CS-N]-Thia have more selectivity towards DPP II than DPP IV (28).

### 3.3. Dipeptidyl peptidase 8 (DPP 8) and dipeptidyl peptidase 9 (DPP 9)

DPP 8 and DPP 9 are relative newcomers to the DPP IV enzyme family and their inadvertent inhibition appears to be responsible for at least some of the toxic side-effects of DPP IV inhibitors including alopecia, thrombocytopenia, anaemia, enlarged spleen, and multiple histological pathologies, including skin lesions and premature mortality in animals (29, 30). DPP 8 and 9 are monomeric soluble cytoplasmic enzymes sharing approximately 50% sequence similarity with human DPP IV (14). Like DPP IV they are widely distributed across human tissues and although they have not yet been

**Table 1.** Physiological regulatory peptides identified as substrates of DPP IV

| Physiological System | Peptide | N-terminus |
|---|---|---|
| Nutrient metabolism and glucose homeostasis | GLP-1 (7-36)amide | His-Ala-Glu- |
| | GIP (1-42) | Tyr-Ala-Asp- |
| | GLP-1 (7-37) | His-Ala-Glu- |
| | Glucagon | His-Ser-Gln- |
| Digestive system | GLP-2 (1-33) | His-Ala-Asp- |
| | Trypsinogen | Phe-Pro-Thr- |
| | Trypsinogen pro-peptide | Phe-Pro-Thr- |
| | Gastrin releasing peptide (GRP) | Val-Pro-Leu- |
| | Pro-colipase | Val-Pro-Asp- |
| | Enterostatin | Val-Pro-Asp- |
| | β-Casomorphin | Tyr-Pro-Phe- |
| | Aprotinin | Arg-Pro-Asp- |
| Growth and development | Insulin-like growth factor-1 (IGF-1) | Gly-Pro-Glu- |
| | Growth hormone releasing factor (GHRF) | Tyr-Ala-Glu- |
| | Growth hormone-releasing hormone (GRH (1-29)) | Tyr-Ala-Asp- |
| | GRH (1-44) | Tyr-Ala-Asp- |
| Neuroendocrine system | PACAP (1-27) | His-Ser-Asp- |
| | PACAP (1-38) | His-Ser-Asp- |
| Nervous system | Substance P | Arg-Pro-Lys- |
| | Neuropeptide Y | Tyr-Pro-Ser- |
| | Peptide YY (1-36) | Tyr-Pro-Ile |
| | Enkephalins | Tyr-Pro-Val- |
| | Corticotropin-like intermediate lobe peptide | Arg-Pro-Val- |
| | Endomorphin-2 | Tyr-Pro-Phe- |
| Vascular system | Bradykinin | Arg-Pro-Pro- |
| Reproductive system | Human chorionic gonadotrophin α (hCGα) | Ala-Pro-Asp- |
| | Leutinising hormone α chain (LHα) | Phe-Pro-Asn- |
| | Prolactin | Thr-Pro-Val- |
| Immune system | Interleukin-2 | Ala-Pro-Thr- |
| | Interleukin-1β | Ala-Pro-Val- |
| | $\alpha_1$-Microglobulin | Gly-Pro-Val- |
| | RANTES | Ser-Pro-Tyr- |
| | Granulocyte chemtactic protein-2 (GCP-2) | Gly-Pro-Val- |
| | Stromal cell-derived factor-1α (SDF-1α) | Lys-Pro-Val- |
| | SDF-1β | Lys-Pro-Val- |
| | Macrophage-derived chemokine (MDC) | Gly-Pro-Tyr- |
| | Monocyte chemotactic protein-1 (MCP-1) | Glu-Pro-Asp- |
| | MCP-2 | Glu-Pro-Asp- |
| | MCP-3 | Glu-Pro-Val- |
| | Eotaxin | Gly-Pro-Ala- |
| | Interferon-γ-inducible protein-10 (IP-10) | Val-Pro-Leu- |
| Endocrine system | Thyrotropin α | Phe-Pro-Asp- |
| | Vasostatin-1 | Leu-Pro-Val- |
| Other | Peptide histidine methionine | His-Ala-Asp- |
| | Tyr-Melanostatin | Tyr-Pro-Leu- |

assigned any particular biological function, the undesirable consequences of unselective DPP IV inhibition may provide important clues. Their post-proline aminopeptidase activity has been evidenced by the hydrolysis of H-Ala-Pro- and H-Gly-Pro derived substrates (31). Selective inhibitors of DPP 8 and DPP 9 are already available and have been used to characterise DPP8/9 activity in human leukocytes (32).

### 3.4. Fibroblast Activation Protein (FAP)

The DPP IV-like activity of FAP has been confirmed by the rapid cleavage of an Ala-Pro-NH $F_3$ Mec substrate (33). FAP has 52% sequence similarity to DPP IV and has been linked with liver injury and chronic liver disease (31, 33). FAP is not as widely expressed as DPP IV and has been identified in serum and pancreatic alpha cells. The active site which carries out N-terminal dipeptide cleavage also possesses collagenolytic activity degrading gelatine and type 1 collagen (34). Selective fluorescent probes have been developed to detect and differentiate between the proteolytic activities of FAP and DPP IV (35).

## 4. THE INCRETIN HORMONES, DPP IV AND DIABETES

### 4.1. The incretins

Although the concept of targeting DPP IV in type 2 diabetes is relatively recent, the origin can be traced back to early work establishing the importance of the gut in regulating post-prandial glucose homeostasis (36, 37). The gut contributes neural and endocrine signals that account for the enhanced physiological insulin response after a meal, a signalling pathway known as the enteroinsular axis (36, 37). GLP-1 and GIP secreted from intestinal L- and K-cells, respectively, account for most of the enteroinsular (or "incretin") effect (38-42). A list of well characterised

actions of GLP-1 and GIP relevant to type 2 diabetes can be found in Table 2. The glucose-dependent nature of their insulinotropic activity provides a clear advantage to enhance postprandial insulin secretion and reduce the risk of interprandial hypoglycaemia (8-10). Insulin biosynthesis is also increased. Furthermore, GLP-1 and GIP possess extrapancreatic mechanisms which contribute to limit hyperglycaemia, e.g. reducing hepatic insulin extraction (43, 44), reported 'insulin-like' effects on skeletal muscle, liver and adipose tissue (45-49), and a reduction of gastric acid secretion or gastric emptying (50-52). Incretin hormones have additional potential benefits over other insulin-releasing drugs through improved islet morphology and protective and proliferative effects on the pancreatic beta-cell (53-59). Such properties might help to counter the characteristic age-related decline of beta-cell mass in diabetes. Although these properties are evident in animal models, substantiating effects of incretin hormones on beta-cell morphology in humans remains problematic. Finally, an important yet occasionally overlooked effect is the suppression of glucagon secretion by GLP-1 (60).

**4.2. Inactivation of the incretins by DPP IV**

Mentlein and co-workers were amongst the first to demonstrate degradation of GLP-1 and GIP by DPP IV *in vitro* (61). Using DPP IV purified from human placenta they observed the enzymatic removal of N-terminal dipeptides $His^{7}$-$Ala^{8}$ and $Tyr^{1}$-$Ala^{2}$ from GLP-1 and GIP, respectively. More significantly, they observed that this degradation also took place when GLP-1 and GIP were incubated in human serum (61). It was subsequently confirmed that DPP IV-mediated metabolism of GLP-1 and GIP did indeed occur *in vivo* (40, 62). The action of DPP IV *in vivo* reduces the half-life of GLP-1 and GIP to <2 min (40, 62, 63). Since the predominant and active forms of incretin hormones are GLP-1(7-36)amide and GIP(1-42), degradation by DPP IV leads to major degradation fragments GLP-1(9-36)amide and GIP(3-42), respectively.

The activities and binding characteristics of these fragments have been elucidated. Since GLP-1(9-36)amide and GIP(3-42) are both non-insulinotropic peptides, it was initially suggested that these were relatively inert and inactive metabolites (64, 65). Although the affinity of GLP-1(9-36)amide for the GLP-1 receptor is 100-fold lower than the parent molecule it appears to act as a weak receptor antagonist (64, 66, 67). While GLP-1(9-36)amide does not antagonise the insulinotropic activity of GLP-1(7-36)amide *in vivo*, there is evidence that this metabolite possesses weak antihyperglycaemic activity through a mechanism not involving insulin secretion (68). This concept currently remains controversial due to conflicting findings in mice, pigs and humans (68-70).

The receptor affinity of GIP fairs comparatively better following truncation by DPP IV. The binding affinity of GIP(3-42) is approximately 4-fold lower than that of GIP(1-42) (71). *In vitro* studies have demonstrated that GIP(3-42) antagonises the GIP receptor (72, 73). However, *in vivo* studies have been conflicting (70-73). A recent study confirmed that GIP(3-42) antagonises GIP-stimulated cAMP production and insulin secretion, but found that *in vivo* it does not behave as an antagonist at physiological concentrations (71). However, pharmacological doses of GIP(3-42) (25 nmol/kg) administered to obese diabetic *(ob/ob)* mice once daily for 14 days enhanced insulin sensitivity and improved glycaemic control (70). This appears to involve extrapancreatic mechanisms which lead to improved insulin sensitivity. During this study neither GIP(3-42) or GLP-1(9-36)amide affected body weight, food intake, pancreatic insulin content or islet morphology (70).

**4.3. Overcoming DPP IV mediated incretin inactivation**

As knowledge of incretin hormone inactivation expanded greater emphasis was placed on ways to overcome this problem. The pharmacological strategies adopted have led to the development of two fundamentally new ways to treat type 2 diabetes. The first approach has involved generating GLP-1 and GIP agonists resistant to the action of DPP IV (reviewed elsewhere (74)). Production and testing of numerous modified forms of the incretin hormones have generated effective DPP IV-resistant analogues of GLP-1 and GIP (74). In human subjects GLP-1 analogues have demonstrated sustained improvements in glycaemic control in type 2 diabetes. To date one GLP-1 agonist, exendin (exenatide/Byetta), has been clinically approved, and another, liraglutide (NN2211) is in phase III clinical trials (75). A second and more recently adopted approach, which is the focus of this review, has been the development of DPP IV inhibitors (22, 76, 77). As illustrated in Figure 2 the concept of DPP IV inhibitors is to enhance endogenous incretin activity by preventing the rapid inactivation of incretin hormones. The preclinical and clinical data for DPP IV inhibitors (or 'gliptins' as they are termed) is reviewed later in this article.

**4.4. Rodent models lacking DPP IV activity**

The generation of rodent models lacking functional DPP IV has brought major advances in our understanding of this enzyme's role in metabolism, and has strengthened the rationale for developing specific inhibitors of DPP IV (78-81). Of particular note is the fact that mice lacking DPP IV activity have significantly reduced glycaemic excursions, greater levels of glucose-stimulated insulin, while the degradation of both GLP-1 and GIP is reduced (78). Similarly, DPP IV-deficient rats have improved glucose tolerance, enhanced insulin release and higher levels of active GLP-1 (79). Evidence gathered from these rodent models underpins the role of DPP IV in regulating incretin activity and consequently glucose homeostasis.

It is especially interesting that DPP IV 'knockout' mice are relatively resistant to the development of glucose intolerance and diabetes following 20 weeks on a high fat diet (80). These mice exhibited reduced food intake and enhanced metabolic energy expenditure and did not develop obesity (80). This has been substantiated by similar observations in DPP IV-deficient Fischer rats (81). Furthermore, DPP IV 'knockout' appears to confer protection from the diabetogenic effects of modest amounts of the beta-cell toxin streptozotocin (80).

**Table 2.** Characterised actions of GLP-1 and GIP relevant to type 2 diabetes

| | GLP-1 | | GIP | |
|---|---|---|---|---|
| | Effect | Reference | Effect | Ref |
| Released in response to a mixed meal | √ | 111, 112 | √ | 111, 112 |
| Lower blood glucose | √ | 113 | √ | 114, 115 |
| Glucose-dependent stimulation of insulin secretion | √ | 8 | √ | 9, 10 |
| Suppress glucagon secretion | √ | 60 | - | - |
| Extrapancreatic glucose-lowering actions | √ | 45-47 | √ | 48, 49 |
| Extend beta cell mass and survival | √ | 53-56 | √ | 57-59 |
| Suppress gastric acid secretion | - | - | √ | 52 |
| Inhibition of gastric emptying | √ | 50, 51 | - | - |
| Inhibition of hepatic insulin extraction | √ | 43 | √ | 44 |
| Enhance satiety | √ | 116 | - | - |
| Reduce body weight | √ | 117 | - | - |

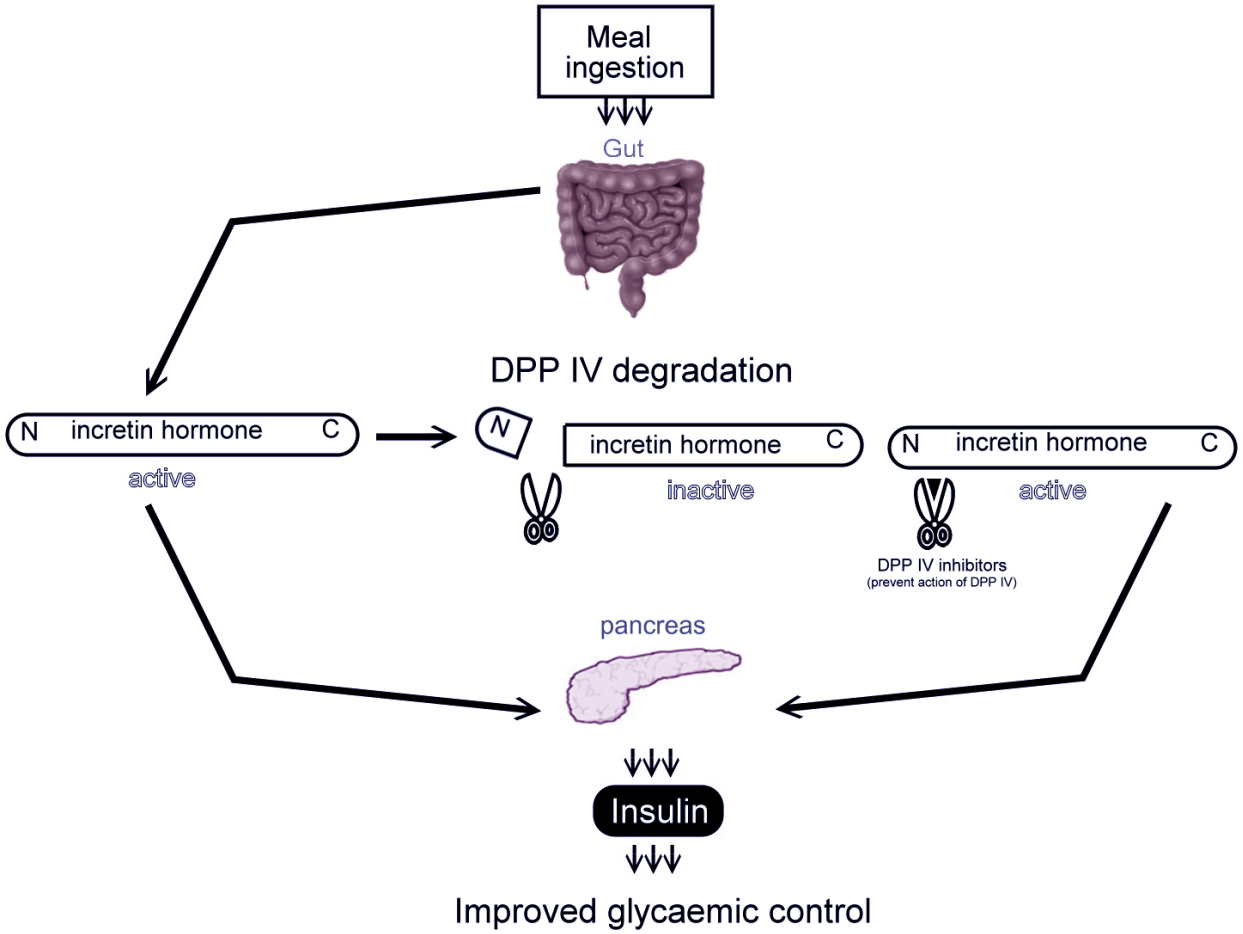


**Figure 2.** Incretin hormone inactivation and DPP IV inhibitor mode of action. The incretin hormones (GLP-1 and GIP) are released from the intestine following meal ingestion. Incretins circulate to the pancreas where they stimulate insulin-release leading to a lowering of plasma glucose concentrations. However, enzymatic cleavage by ubiquitous DPP IV renders them non-insulinotropic. DPP IV inhibitors prevent processing by DPP IV and therefore enhance endogenous incretin hormone activity.

Although, animal models lacking DPP IV activity are viable and appear relatively normal, recent evidence is emerging of some neurological, immunological and inflammatory alterations (82-87). Mice lacking DPP IV have shortened latencies to nociceptive stimuli, perhaps due to observed higher plasma levels of substance P (82). In the context of experimental asthma, rats lacking DPP IV demonstrate decreased T-cell recruitment associated with significantly reduced ovalbumin-specific IgE-titres (83). Furthermore, marked changes in the cytokine responses of interleukins and tumour necrosis factor have been observed (84; 85). DPP IV deficient rats are more susceptible to angiooedema caused by ACE inhibitor administration (86) and it has been reported that DPP IV inhibition in some species (e.g. dogs and monkeys) cause gastrointestinal disturbances and skin lesions, although these remain to be confirmed (31). Finally, the severity of antigen-induced arthritis is increased in DPP IV-deficient mice which may be due to increased levels of circulating active stromal cell-derived factor-1 (87).

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