The Incretin Axis in Cardiovascular Disease

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In 1964, studies in just 2 subjects offered a simple, salient, and fundamental observation reported in 612 words: Glucose induces a greater insulin response when introduced through the gastrointestinal tract than when injected intravenously (the Figure, A).2 This finding built on studies dating to 1928 that injecting extracts of small intestine into animals lowered their glucose levels. Subsequently, this incretin effect was found to be mediated by glucagon-like peptide-1 (GLP1) and its action on pancreatic GLP1 receptors, in addition to contributions from glucose-dependent insulinotropic polypeptide.3,4 Moreover, the incretin response was found to be impaired in those with type 2 diabetes mellitus (T2D). We now know that the incretin axis also includes the enzyme dipeptidyl peptidase-IV (DPPIV), a serine protease that rapidly degrades GLP1 and other proteins.5 Ultimately, this arc of discovery led to new approved antidiabetic therapies: GLP1 analogs (exenatide, liraglutide) and DPPIV inhibitors (sitagliptin, saxagliptin, and, outside the United States, vildagliptin).4 For both classes of drugs, early preclinical experiments and smaller human studies suggest that targeting the incretin axis might achieve the elusive goal of an antidiabetic agent that improves cardiovascular disease.6,7 In the current issue of Circulation, Shah et al add this evolving story with their report that alogliptin, a DPPIV inhibitor in development, limits atherosclerosis and inflammation in 2 different mouse models. Given the increasing clinical use of approved incretin modulators, current large cardiovascular outcome trials with GLP1 agents and DPPIV inhibitors, and ongoing development of novel agents that target incretin signaling, further consideration of how the incretin axis might intersect the cardiovascular system is well warranted.

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GLP1, along with glucagon and several other proteins, is transcribed off the proglucagon gene.8 Posttranslational processing of GLP1 yields its major active form, which consists of amino acids 7 to 36 (the Figure, B). GLP1 is produced by pancreatic α cells and intestinal L cells, where release is stimulated by nutrients. DPPIV cleaves 2 amino terminal peptides from GLP1 (7—36), producing GLP1 (9—36), which lacks insulinotropic activity but, interestingly, may exert cardiovascular effects, as discussed below.10 The GLP1 receptor is a widely expressed G protein–coupled receptor found in pancreatic islets, the lungs, endothelial cells, vascular smooth muscle cells, and the myocardium. GLP1 receptor activation has multiple effects, including increased cAMP, cAMP response binding element phosphorylation, increased activity of multiple kinases, and increased intracellular calcium. In terms of endocrine effects, GLP1 receptor activation increases insulin secretion, protects pancreatic β cells (more proliferation, less apoptosis), and decreases hepatic glucose output, glucagon release, gastric emptying, and appetite.

Therapeutic manipulation of the incretin axis has required some clever maneuvering, given the voracious action of DPPIV, which keeps the half-life of native GLP1 to ≈2 minutes. The poisonous Gila monster lizard (Heloderma suspectum) circumvents this issue as part of its adaption to its infrequent eating patterns.11 To activate the necessary metabolic responses to its rare meals, the Gila monster’s saliva contains exendin-4, which is resistant to DPPIV action, thus allowing islet cells to awaken from their predominantly inactive state. Exenatide, a 39–amino acid synthetic peptide based on exendin-4, is also DPPIV resistant with a half-life of 2.4 hours and approved use to treat T2D through twice-daily subcutaneous injections.12 Liraglutide resembles human GLP1 but circumvents DPPIV degradation through an amino acid substitution (arginine for lysine at position 34) and insertion of a fatty acid chain to the lysine at position 26, resulting in liraglutide’s association with albumin. With these changes, liraglutide has a 13-hour half-life and is approved for treating T2D through once-daily subcutaneous injections.13 Even longer-acting forms of GLP1 agents (weekly, monthly) are being pursued.14 DPPIV inhibitors include sitagliptin and saxagliptin.15,16 Alogliptin is in late stages of development, as are several other DPPIV agents. Both GLP1 agonists and DPPIV inhibitors lower glucose and decrease A1c levels. The GLP1 effects on appetite and satiety have fostered interest in targeting the incretin axis for cardiometabolic disease because GLP1 agonists can significantly decrease weight while DPPIV agonists are considered weight neutral, both an advantage over the weight gain seen with other antidiabetic agents.17

Where does this incretin axis intersect the cardiovascular system? Considerable evidence incriminates the dysfunctional adipocyte and excess adiposity—in the liver, around visceral organs, and/or in the skeletal musculature—as culprits in both T2D and its atherosclerotic complications.18 The adipocyte is a dynamic cell that releases a host of systemic mediators, including cytokines, chemokines, coagulation factors, adipokines, and regulators of blood pressure and vascular tone.19 Inflammatory cells may also nest within adipose tissue, influencing local and systemic responses from that position.20 Some of these mediators may influence not only the vasculature but also myocardial ischemia, infarction, and contractility. Thus, the weight loss associated with GLP1...
Figure. A, The incretin effect. The well-documented phenomenon of oral glucose eliciting a higher insulin response than intravenous glucose at identical plasma levels of glucose is known as the incretin effect. The left (plasma glucose levels) and right (insulin levels) graphs represent concurrent windows of time (after Perley and Kipnis). B, The incretin axis in cardiovascular responses. Glucagon-like peptide-1 (GLP1; major active form, amino acids 7–37) and glucose-dependent insulinotropic polypeptide (not shown) are incretin hormones released in response to nutrients. GLP1 signals via GLP1 receptor. In pancreatic β cells, GLP1 promotes insulin release, promotes proliferation, and decreases apoptosis. In the central nervous system (CNS), GLP1 increases satiety, thus promoting weight loss. GLP1 metabolic actions of weight loss and preserved β cell function could potentially limit atherosclerosis and inflammation. GLP1 may also act through GLP1 receptors expressed in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), cardiomyocytes, and monocytes/macrophages (MPs). Some of the molecular mechanisms invoked as being involved in these cellular settings with subsequent clinical responses are shown. The protease dipeptidyl peptidase-IV (DPPIV) removes 2 amino acids from GLP1, producing GLP1(9–37), which exerts no insulin effects but may have some vascular and/or cardiac effects. DPPIV action on other non-GLP1 proteins could also modulate inflammatory and/or cardiovascular (CV) responses. Whether these actions have positive (+) or negative (−) effects remains unknown. GLP1 mimetics and DPPIV inhibitors are therapeutic agents that modulate the incretin axis. Whether these responses improve cardiac function, decrease atherosclerosis, and/or decrease cardiovascular events is under study. PPARα indicates peroxisome proliferator-activated receptor-α; GSK3β, glycogen synthase kinase; Nrf-2, nuclear factor E2-related factor 2; EPC, endothelial progenitor cell; eNOS, endothelial nitric oxide synthetase; ADA, adenosine deaminase; PKA, protein kinase A; PDGF, platelet-derived growth factor; SDF-1, stromal derived factor-1; ERK1/2, extracellular signal-regulated kinase-1; PI3K, phosphatidylinositol 3-kinase; LV, left ventricular; CHF, congestive heart failure; and VCAM, vascular cell adhesion molecule.
agonists might be predicted to decrease cardiovascular risk and to influence myocardial function (the Figure, B).

Incretins and their modulation by GLP1 agonists and DPPIV inhibitors may also modify cardiovascular responses directly, independently of adiposity, through GLP1 receptor signaling in relevant cells (the Figure, B). Some of the first and most studied actions of incretin effects relate to the heart.21 GLP1 agonists reportedly limit myocardial infarct size in mice independently of body weight changes, with regulation of multiple signals relevant in cardiomyocyte biology.22 Some of these cardiac effects persist in the GLP1 receptor–deficient mouse and were seen with GLP1(9 to 37), the product of DPPIV action thought to be inactive when it comes to insulin release.23 Others report that GLP1 limits myocardial stunning and improves left ventricular function in dogs. Early studies in humans support these effects.24-26 Fields and colleagues21 and Nikolaidis et al26 found that GLP1 agonist treatment of myocardial infarction patients after angioplasty was associated with improved ejection fraction, lower in-hospital mortality, and shorter length of stay, whereas others find that incretin affects chronic heart failure. Similarly, DPPIV is also implicated in myocardial responses.27 These myocardial benefits with incretin modulation may derive from favorable shifts in fuel use by an ischemic, postischemic, or failing cardiomyocyte.

GLP1 receptor expression in endothelial cells, vascular smooth muscle cells, monocytes, macrophages, and lymphocytes also raises the prospect for direct effects on atherosclerosis and inflammation (the Figure, B). Likewise, DPPIV is present in vascular cells and inflammatory cells. In endothelial cells, both GLP1 agonists and DPPIV inhibitors can inhibit tumor necrosis factor α–mediated induction of plasminogen activator inhibitor and adhesion molecule expression, resulting in decreased monocyte adhesion and atherosclerosis in rodents.6,7,28 Recurring themes reported in the effects of GLP1 and DPPIV inhibitors are changes in nuclear factor-κB and nitric oxide production.29 Regulation of $K_{\text{ATP}}$ channels and cAMP levels may also be involved. Manipulating the incretin axis may improve vasoreactivity in both rodents and humans. Intriguingly, as suggested for the myocardium, both native GLP1 and the cleaved GLP1(9 to 36) still improved vasodilatation in mice lacking the GLP1 receptor, suggesting either the presence of alternative GLP1 receptors in the vasculature or receptor-independent effects. Both GLP1 agents and DPPIV inhibitors modestly but significantly reduce systolic blood pressure. These agents may also increase slightly in heart rate, an effect that remains poorly understood.

The incretin axis is also involved in inflammation.6 In monocytes/macrophages, GLP1 agonists and DPPIV inhibitors repress cAMP and protein kinase A effects, leading to decreased inflammatory cell accumulation in the arterial wall. Importantly, DPPIV has many protein substrates other than GLP1 that are implicated in both promoting and limiting inflammation. Other DPPIV targets include those associated with stem cell survival and proliferation like stromal derived factor-1, which could underlie the increased endothelial progenitor cells reported with DPPIV inhibitors. New approaches like proteomics/peptidomics are uncovering novel DPPIV substrates that could provide additional insight into DPPIV inhibitor effects.30 Deeper understanding of DPPIV biology, the actions of GLP1(9 to 37), and GLP1 receptor–dependent versus –independent effects will be key in understanding the cardiovascular effects of incretins and how GLP1 agonists may differ or even synergize with DPPIV inhibitors.

In the current issue of Circulation, Shah et al offer new data on how DPPIV activity and inhibition influence adiposity, inflammation, and atherosclerosis.8 Low-density lipoprotein receptor–deficient mice fed a high-fat diet manifest marked increases in DPPIV activity; when this is inhibited by the DPPIV inhibitor alogliptin, less atherosclerosis was seen, along with improved insulin sensitivity, lower blood pressure, decreased visceral adiposity, fewer macrophages in the aortic wall, and shifts in adipose tissue macrophages toward less inflammatory subtypes. The authors point to decreased monocyte chemotaxis as a key contributor to the effects of DPPIV inhibition. Along these lines, these investigators show that an adenosine receptor 2A antagonist blocked the repression by alogliptin of tumor necrosis factor α–induced chemotaxis and inhibited the effects of alogliptin on Rac1, a cellular regulator of chemotaxis. These responses connect to data that DPPIV and adenosine deaminase associate with and alter local concentrations of adenosine, which can activate Rac1 and promote chemotaxis. Indeed, another name for DPPIV is adenosine deaminase complexing protein (also CD26).31 Administering the GLP1(9–37) metabolite did not replicate the adenosine deaminase/Rac1 effects.

Given the multiple changes induced by alogliptin in the models studied by Shah et al, including decreased blood pressure, visceral adiposity, inflammatory gene expression, and hyperglycemia, it remains unclear whether changes in adenosine deaminase and Rac1 underlie the decreased atherosclerosis seen. Perhaps the beneficial effects evident with alogliptin in these models can be best taken as further evidence of the relevance of the incretin axis in inflammation and atherosclerosis and perhaps offering encouragement to those who think that the best chance for decreasing cardiovascular events in T2D rests in improving the multiple abnormalities found in T2D, including adiposity and insulin resistance. Of course, similar arguments were made with other antidiabetic agents, including peroxisome proliferator-activated receptor-γ agonists; discussions continue as to whether this concept is supported by the positive secondary end point of pioglitazone in the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROActive) or dismissed by the pattern of increased risk for rosiglitazone in meta-analyses.

Ultimately, although preclinical studies like the article by Shah et al yield new mechanistic insight and additional data supporting the pursuit of cardiovascular benefits through incretin modulation, the relevant answers must come from human clinical trials. Fortunately, such data regarding the impact of the incretin axis on cardiovascular disease should be forthcoming, driven by the Food and Drug Administration’s cardiovascular safety hurdles for new antidiabetic drug approval and the recognized need and potential for a diabetes drug that decreases cardiovascular events. Multiple clinical
cardiovascular trials with DPPIV inhibitors and GLP1 agonists are underway (Table).4 In terms of the agent studied by Shah et al in this issue of Circulation, A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate Cardiovascular Outcomes Following Treatment With Alogliptin in Addition to Standard of Care in Subjects With T2D and Acute Coronary Syndrome (EXAMINE) will compare the impact of alogliptin and usual treatment in terms of first occurrence of myocardial infarction, and nonfatal stroke in 5400 post–acute coronary syndrome patients with diabetes mellitus. It will be of considerable interest to see whether the data from clinical cardiovascular trials reveal that the early preclinical evidence of anti-inflammatory, anti-atherosclerotic, anti-obesity, and/or myoprotective effects through the incretin axis proved relevant to clinical outcomes in humans. If so, 50 years ago, when investigators noted different responses after administering glucose into veins versus the gut, critical observations were being made on how pathways originating in the gut can alter outcomes not in the veins but in the arterial system, where the pathological consequences of T2D occur.

### Disclosures

Dr Plutzky serves as consultant for Amylin Pharmaceuticals, Bristol Myers Squibb, Novo Nordisk, Roche, and Takeda Pharmaceuticals.

### References


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