

## Incretins in type 2 diabetes mellitus: cardiovascular and anti-atherogenic effects beyond glucose lowering

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### Abstract

Type 2 diabetes mellitus is the most outspreading disease of the western world and it provides cardiovascular disease. During the past decade new drug categories were added to the already existing ones. Perhaps, the most outstanding, as promising, too, are glucagon-like peptide-1 (GLP-1) analogues, which pinpointed at the incretin hormone system, targeting mainly at the postprandial hyperglycemia. It seemed that these novel drugs have beneficial effects on ischemic heart, heart failure, blood pressure, even on lipids and body weight in type 2 diabetics, considering them not only as another glucose lowering agent. A lot of recent studies investigate the potential relationship between GLP-1 and its possible cardioprotective and anti-atherogenic effects in type 2 diabetes and the present review discusses these effects of GLP-1. Hippokratia. 2012; 16 (2): 100-105

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Type 2 diabetes mellitus (T2DM) is mainly a b-cell progressive disease resulting in persistent hyperglycemia and consequently to an increased risk of both microvascular and macrovascular complications<sup>1</sup>. To avoid these complications, current treatment algorithms suggest primarily lifestyle aggressive interventions and early use of medications e.g. metformin, targeting to the tight glucose homeostasis with the less possible adverse effects<sup>2</sup>. Cardiovascular disease remains the most common and most dangerous complication of type 2 diabetes, since diabetic patients, not only have high risk for it, but also poor prognosis for survival after experiencing a cardiovascular event<sup>3</sup>. Hyperglycemia as part of “glucose variability”, is considered as an independent risk factor for cardiovascular complications<sup>4</sup>. During the past few years, novel therapies focused on the incretin hormone system which mainly regulates postprandial glucose levels. Two incretins have been identified: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). They affect glucose homeostasis through several mechanisms, like enhancement of glucose-dependent insulin secretion and slow gastric emptying<sup>5</sup>, without having significant adverse effects such as hypoglycemia and weight gain, like other agents and insulin have<sup>6</sup>. In addition, recent studies have shown that incretins have multiple benefits in type 2 diabetes, apart from its blood lowering action, including cardioprotective features<sup>7</sup> and interesting anti-atherogenic effects, by reducing mainly systolic and in some cases diastolic

blood pressure and improving the overall lipid profile<sup>8</sup>. This review will discuss in detail and focus on the potential cardiovascular and anti-atherogenic effects of GLP-1 analogues, in type 2 diabetes.

### Biology-Secretion-Genetics

The gastrointestinal (GI) system plays a fundamental role in glucose balance, since two GI peptide hormones (the incretins)-GLP-1 and GIP-were found to have predominant glucoregulatory effects<sup>5</sup>. GLP-1, the most important incretin hormone, is produced by L cells together with GLP-2 and peptide tyrosine-tyrosine (PYY), while glucose-dependent insulinotropic polypeptide (GIP) is produced by the K cells in the duodenum and jejunum. The L cells are open-type epithelial enteroendocrine cells, which are mainly located in the mucosa of the distal small intestine and the colon, but can also be found throughout the rest of the small intestine<sup>9</sup>.

The secretion of incretins and especially GLP-1 seems to be stimulated by the ingestion of oral food, particularly of carbohydrates and fat<sup>10</sup>. It is very interesting the fact that GLP-1 is secreted within minutes of nutrient ingestion, and this is specifically how GLP-1 effects glucose homeostasis through the so-called “incretin effect”, that is orally administered glucose has a greater stimulatory effect on insulin secretion than intravenous glucose, in non-diabetic individuals<sup>11-13</sup>. The incretin effect is estimated to cause more than 50% of the total insulin secretion<sup>10</sup>. Furthermore, the stimulation of insulin is regulated in a

glucose dependent manner, since the pancreatic b-cells secrete insulin only when blood glucose reaches approximately a value of 120 mg/dl, avoiding hypoglycemia<sup>14</sup>.

The two main active isotypes of GLP-1 include GLP-1(7–37) and GLP-1(7–36). The form of GLP-1(7–36) is the predominant one, because it accounts for about 80% of the total active amide<sup>15</sup>. After secretion into the circulation active GLP-1(7-36) is rapidly inactivated by the enzyme dipeptidyl peptidase 4 (DPP-4), an ubiquitous serine protease, to its metabolite GLP-1(9-36), after the removal of an N-terminal dipeptide<sup>16</sup>. Surprisingly, even if GLP-1 (9-36) is the major circulating form of GLP-1, seems to be biologically inactive to insulin regulation<sup>17</sup>. However, there are controversial studies about its possible effects on glucose homeostasis and its biological role remains unclear<sup>18,19</sup>.

Both incretins, GLP-1 and GIP, express their insulin-regulatory actions through specific G-protein coupled receptors (GLP-1R)<sup>5,20,21</sup>. In pancreatic tissue, GLP-1 receptors are expressed on alpha, beta and delta cells, while GIP receptors are expressed mainly on beta cells<sup>5,10</sup>. Both GIP and GLP-1 bind to other target tissues. GLP-1 receptors have been found in lungs, heart, GI tract, gastric glands, central and peripheral nervous system and more recently in aorta<sup>22</sup>, whereas GIP receptors are expressed in adipose tissue and the CNS<sup>5</sup>.

The GLP-1R was first found in rat pancreatic tissue<sup>21</sup>. It was also showed that human pancreatic GLP-1R is almost 90 % homologous to the rat GLP-1R, and that its gene is localized to chromosome 6p21<sup>23</sup>. When GLP-1 binds to its receptor in a certain domain with high affinity<sup>24</sup>, it leads to the production and further biochemically evolution of cyclic AMP through the activation of factors such as adenylate cyclase and protein kinase A<sup>25</sup>. Finally, this complex mechanism secretes insulin via calcium channel activation<sup>26</sup>. Stimulation of GLP-1R not only activates rapid production of insulin, but it also enhances  $\beta$ -cell proliferation and delays their apoptosis and leads to long-term insulin synthesis<sup>5,27-29</sup>.

### GLP-1 and cardioprotection

The observation that exogenous GLP-1 restores blood glucose to almost normal levels in patients with type 2 diabetes mellitus<sup>30</sup>, had finally resulted to novel anti-diabetic treatment with incretins, that is long-acting GLP-1 receptor agonists, such as exenatide and liraglutide<sup>31</sup>. Even though the main physiological function of GLP-1 deals with glycemic regulation, it seems that incretins and specifically GLP-1 might have additional cardioprotective actions<sup>32</sup>. Furthermore, as it was noticed above, the fact that GLP-1Rs have been found in many others extra pancreatic tissues, such as the heart and vasculature<sup>7,33,34</sup>, also suggests that GLP-1 may play an important role in the cardiovascular system.

### GLP-1 and vascular function

It is well known that endothelial dysfunction is common among patients with type 2 diabetes, even in the

existence of normal coronary arteries<sup>35</sup> and metabolic syndrome as an entity<sup>36</sup>. Furthermore, insulin resistance alone may be associated with coronary endothelial dysfunction<sup>37</sup>, making it a very important factor in the physical course of diabetes. Several studies supported the improvement of the endothelium by antidiabetic medications such as thiazolidinediones<sup>37</sup> and metformin<sup>38</sup>. Of course, in diabetes the cause of endothelial dysfunction seems to include different independent factors like hypertension, dyslipidemia, microalbuminuria and others<sup>36</sup>.

GLP-1, in turn, influences endothelial function<sup>39-44</sup>. It was already referred that the presence of GLP-1 in a variety of different organs, including vascular smooth muscle cells, cardiac endothelial cells and microvascular endothelium<sup>3,5</sup>. In animal studies it has been proved that GLP-1 also improves endothelial function in targeted rat vessels<sup>39-42</sup>. Apart from animals, GLP-1 was investigated in both patients with type 2 diabetes mellitus, and healthy non-diabetic individuals and improvement of endothelial blood-flow vasodilation was confirmed, too<sup>43,44</sup>.

The mechanism through which GLP-1 gets vascular function better is not so clear. Studies supported that GLP-1R are the main reason for it<sup>41</sup>, while others involve endothelium, nitric oxide<sup>7,40</sup>, or other biochemical pathways<sup>41,42</sup>.

Despite the fact that the exact etiology of GLP-1 beneficial actions to the endothelial function is not so well defined, the results of these actions to the cardiovascular system of the diabetic patients seem to be very protective<sup>43</sup>.

### GLP-1 and the ischemic heart

Perhaps, the most outstanding and promising feature of GLP-1, apart of course of its glucose regulatory value, is heart-protection from ischemia, since it is already known that diabetes is considered as a coronary risk equivalent in type 2 diabetic patients<sup>45</sup> and tight glycemic control improves the outcome in hospitalized patients after having acute myocardial infarction<sup>46</sup>.

Most experimental studies in animals demonstrated that GLP-1 analogues evolves wall motion recovery after reperfusion<sup>47</sup>, enhances recovery of post-ischemic contractile dysfunction<sup>48</sup>, reduces infarct size and improves recovery of both systolic and diastolic function<sup>49,50</sup>, while just one study showed that GLP-1 analogue (liraglutide) has a neutral effect on myocardial infarct size in a porcine ischemia reperfusion model<sup>51</sup>. In contrast, other studies on diabetic mice, concluded that administration of liraglutide, has been associated with infarct size reduction and outcome better than metformin, of heart protection and survival<sup>52</sup>. Further studies in humans, showed a cardiac function improvement, expressed through ejection fraction and wall motion kinetics after treatment with GLP-1 and, furthermore, associated with shorter hospitalization time and less intensive treatment after coronary artery bypass<sup>53,54</sup>. It is possible that the main cause for all these cardio-protective actions is the myocardial GLP-1R<sup>49,52,55</sup>, even if similar actions of GLP-1 are present in

the absence of its receptor<sup>7</sup>.

Independently of the precise cause of anti-ischemic features of GLP-1, this is a very interesting field to study even more.

### GLP-1 and blood pressure

Hypertension is common among patients with diabetes<sup>56,57</sup>. Some studies in animals came to a not very promising result with incretin mimetics, since treatment with GLP-1 in normal rodents led to increase of blood pressure and heart rate in a dose dependent way<sup>32,58-60</sup>. It seems that this phenomenon has to do with direct actions GLP-1 provides, having CNS central action<sup>57,60,61</sup> and increasing vascular resistance<sup>60</sup>. However, other studies in bigger animals showed neutral effects about the anti-hypertensive action of GLP-1<sup>62,63</sup>. These conflicting results could be explained from the fact that stimulation of GLP-1R has both vasoconstrictive<sup>60</sup>, and vasodilative<sup>41</sup> effects in different arteries. In humans, GLP-1 administration had no effect at all in blood pressure or heart rate<sup>64-67</sup>. Moreover, in other studies, GLP-1 appeared to have an interesting biphasic reaction in blood pressure, the first hypertensive phase followed by a more prolonged hypotensive period<sup>59,68</sup>. We should always keep in mind that animals, especially little rodents, have different physiology from humans and this reflects in the different way they react from GLP-1 administration.

The results of other trials have been more optimistic in diabetic patients. Treatment with either exenatide<sup>69-71</sup> or liraglutide<sup>72</sup> was associated with lowering of systolic and diastolic blood pressure with no variation in heart rate, but the latter finding may be due to the overall improvement of other risk factors e.g. blood glucose and weight, and needs to be studied even more. More specifically the 82 week extension study showed a significant decrease (approximately 4mmHg) in systolic blood pressure<sup>73</sup> while another study reported that both exenatide and exenatide-lar (a once weekly form of exenatide) resulted in a significant decrease in both systolic and diastolic blood pressure (around -4.0 mmHg, and -1.7 mmHg respectively)<sup>74</sup>. The LEAD (Liraglutide Effect Action in Diabetes) programme<sup>75-80</sup> found a slight beneficial effect, in all six studies, on systolic blood pressure (almost 2.5 mmHg), which was reassured in a meta-analysis of the LEAD program<sup>81</sup>.

### GLP-1 and heart failure

There is compelling evidence that diabetes mellitus increases the risk of heart failure, independently of co-existence of hypertension and ischemic disease and the term diabetic cardiomyopathy denotes ventricular dysfunction<sup>82</sup>.

In animal trials, GLP-1 seems to have a beneficial effect on ventricular diastolic function, since it was found that rodents lacking GLP-1R have worse ventricular activity<sup>83</sup>. Furthermore, the administration of GLP-1 improves, in animals, too, heart failure<sup>84</sup>.

In human trials long-term administration of GLP-1 with New York Heart Association class III-IV heart failure showed a noticeable improvement in ejection fraction and general functional status in patients<sup>66</sup>. Another study with few patients, all having diabetes mellitus and heart failure, did not end to a statistical significant result, although a slight improvement in overall cardiac function was noticed, after short-term administration of GLP-1<sup>65</sup>.

The role of inactive metabolite GLP-1 (9-36) was studied and some evidence in animals support its benefit in cardiac function<sup>85</sup>. It is understandable that a lot more studies should be required, before the definite establishment of the beneficial effects of GLP-1 in heart failure.

### GLP-1 and body weight and lipids

It has been proved that treatment of obesity in type 2 diabetic patients is associated with reduction in cardiovascular risk<sup>86</sup>.

Some studies concluded that GLP-1 reduces weight both peripherally by minimizing peristaltic gastric motility<sup>87</sup> and creating a central anorectic effect<sup>88</sup>.

More or less recent studies demonstrated results that favored weight loss after GLP-1 administration in patients with type 2 diabetes. Exenatide reduced body weight both as a single agent and in combination with other anti-diabetic drugs<sup>89-92</sup>. More recently, exenatide LAR, was compared to exenatide in a 30 week study and a similar weight reduction (approximately 4Kg) was found in both forms, though exenatide LAR resulted in a better glycemic control<sup>74</sup>.

LEAD program demonstrated same results for liraglutide<sup>75-80</sup> regarding body weight either as monotherapy, or in combination with different types of anti-diabetic agents.

It should also be considered that all these studies that supported the weight-reducing feature if GLP-1 agonists, were scheduled to investigate primarily the glucose-lowering effect of these agents. It would be very interesting to see a more targeted trial studying the direct relation between GLP-1 and weight loss. However, the fact that weight loss occurred in healthy non-diabetic patients, too, seems very promising<sup>93,94</sup>.

Since, it is well established that modification of lipid abnormalities in diabetic patients, younger and older, leads to cardiovascular benefits, some studies of GLP-1 medications looked also after lipid levels as secondary endpoints<sup>95,96</sup>.

There are studies that support the beneficial effect of GLP-1 agonists on lipids. Specially exenatide was found to improve both triglycerides and cholesterol fragments (total, LDL and HDL), in overweight subjects with type 2 diabetes mellitus<sup>70,97</sup>. Retrospectively, liraglutide has shown an improvement in triglyceride status<sup>98</sup>. GLP-1 was even associated with improvement in post-prandial lipemia<sup>99</sup>. Further longitudinal and prospective studies, are needed to investigate this very interesting feature of GLP-1. A recent meta-analysis of the 6 trials from the LEAD program, showed a slight but statistically signifi-

cant decrease in total cholesterol, LDL, HDL and triglycerides after 6 months of therapy with liraglutide<sup>100</sup>.

### Conclusion

It is clear that GLP-1 receptor agonists are a very useful weapon in our daily fight with diabetic state. It seems that beyond its undoubtful hypoglycemic effects, they have more pleiotropic actions regarding cardiovascular and anti-atherogenic effects. Ongoing and future studies, may prove, these actions of GLP-1, establishing them even as an effective first or second-line therapy in type 2 diabetic patients.

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