

Revisión



Glucagon like peptide agonist and cardiovascular disease in type 2 diabetes

GUILLERMO DIEUZEIDE MD PhD.

University of Buenos Aires

Division of Internal Medicine/Service of Endocrinology and Diabetes

Hospital Ntra Sra del Carmen .

Integral Center of Attention in Diabetes, Endocrinology and Metabolism Chacabuco (CAIDEM)

INFORMACIÓN DEL ARTÍCULO

Historia del artículo:

Recibido el 18 de febrero de 2018

Aceptado el 24 de mayo de 2018

The first decade of the 21st century was marked by the end of the “gluco-centric paradigm” in relation to cardiovascular outcomes in cardiovascular diabetes.

Three important researches (ACCORD trial [1] , ADVANCE trial[2] and the VADT trial [3]) despite that showed a significant reduction in the levels of HbA1c in patients with type 2 diabetes treated with an intensified strategy in relation to a “standard” care (with differences in the levels of HbA1c between the two branches of 1,1 %, 0,8% and 1,5% respectively), however ,they didn't obtain a similar reduction in the mayor adverse cardiovascular events (MACE: myocardial infarction, stroke and cardiovascular mortality).

Moreover, the ACCORD trial had to be interrupted due to an increase in the total mortality in the arm of patients with type 2 diabetes treated with intensified strategy.

Due to the uncertainty of the cardiovascular safety of some antihyperglycemic drugs, in 2008 the United States Food and Drug Administration issued a guideline addressing the pharmaceutical industry in which they established that the main requirement for the approval of a new drug was that it does not demonstrate an unacceptable rise in the cardiovascular risk[4]

After that the European Medical Agency and other regulatory entities published similar guidelines[5] that focused on the next points:

- In researches in phase 2-3 ,the adjudication of adverse cardiovascular events must be prospective and independent in order to facilitate a metaanalysis of these events in all the controlled studies of drugs versus placebo, drug plus standard therapy (add on) or with active comparators.

-The mayor cardiovascular end points defined as cardiovascular mortality, acute myocardial infarction and stroke(MACE), can be also evaluated with other criteria such as hospitalization due to unstable angina, revascularization procedures or heart failure (extended MACE of 4 points)

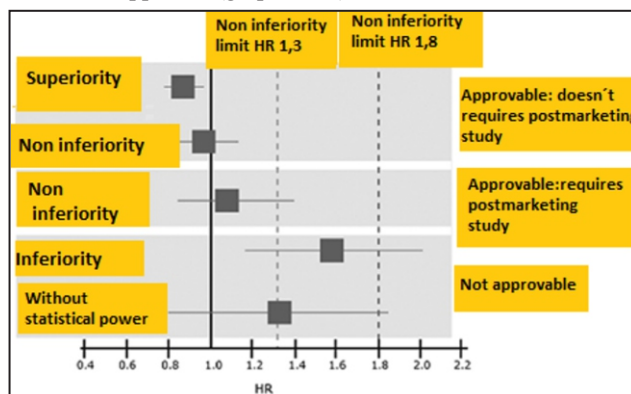
- A population of high risk of cardiovascular events (patients with type 2 diabetes of long duration, old patients or those with kidney failure) must be included and the drug safety must be assessed for at least for two years.

Using these criteria three possibles scenarios were described:

1) If the risk evaluation results in a Hazard ratio without differences between the groups but the superior arm of the confidence Interval 95% is below < 1,3 or if there is a HR of superiority (< 1) the drug should be approved and it doesn't requires further analysis post marketing

2) If the risk evaluation results in a Hazard ratio without differences between the groups ,but the superior arm of the confidence Interval 95% is between >1,3 and less than 1,8 the drug can be approved, but a safety cardiovascular study is required post marketing

3) If the risk evaluation concludes that there is a Hazard ratio with no statistical power but the superior arm of the confidence Interval 95% is superior to 1,8 ,or results inferior than the comparator (more risk) the drug should not be approved (graphic N 1)



GRAPHIC N 1

In the **table 1** are there the main results of those studies.

	LEADER	SUSTAIN-6	HARMONY	EMPAREG	CANVAS	DECLARE TIMI
NUMBER	9340	3927	10793	7020	10142	17160
DURATION	3,8 YEARS	2,1 YEARS	2,3 YEARS	3,1 YEARS	3,9 YEARS	4,2 years
ΔHbA1c	-0,40%	-0,7/-1,4%	-0,6%	-0,54/-0,60%	-0,58	-0,42%
ΔSYSTOLIC BP	-1,2 mmHg	-1,27/-2,6 mmHg	-0,67 mmHg	-4 mmHg	-3,9 mmHg	2,7 mmHg
Δ WEIGHT	-2,3 KG	-3,6/-4,9 kg	-0,8 kg	-1,8/-2,2 kg	-1,6 kg	- 1,8 kg
MACE REDUCTION	13% RRR (HR:0,87) (p:0,005)	26% RRR (HR:0,76) (p:0,001)	22%RRR (HR:0,78) (p:0,005)	14% RRR (HR:0,86) (p:0,04)	14%RRR (HR:0,86) (p:0,04)	7% (n.s)
CV MORTALITY	22% RRR (HR:0,78) (p:0,007)	-2% (n.s)	-7% n.s	38%RRR (HR:0,62) (p:0,001)	-13% (n.s)	0,2% (n.s)
NON FATAL MI	14% (n.s)	26% (n.s)	25%RRR (HR:0,75) (p:0,003)	13% (n.s)	15% (n.s)	-0,1% (n.s)
STROKE	14% (n.s)	39%RRR (HR:0,71) (p:0,04)	-14% (n.s)	+18% (n.s)	10% (n.s)	+ 0,2% (n.s)
HEART FAILURE	13% (n.s))	+11% (n.s)	-5% n.s	35%RRR (HR:0,65) (p:0,002)	22% (n.s)	17%RRR (HR:0,83) (p:0,005)
DEATH FOR ALL CAUSES	15%RRR (HR:0,85) (p:0,02)	+0,05 (n.s)		32%RRR (HR:0,68) (p:0,001)	13% (n.s)	

Table 1 The bolt letters imply statistical significance.RRR:reduction in relative risk

In the next decade, a new generation of researches were carried out using another class of drugs. Despite not obtaining such a great decrease in the HbA1c as the previous studies, a significant reduction in the events of cardiovascular mortality and major cardiovascular events (myocardial infarction and stroke) was demonstrated.

Two classes of drugs were used in these studies: the SGLT2 drugs and the analogs like glucagon peptide (GLP1) drugs (Table N 1). Using the first class of drugs, three important studies: the EMPAREG [6], the CANVAS [7] study and recently the DECLARE [8] study obtained a reduction in some of the three major adverse events (MACE) established or a significant reduction in events regarding heart failure.

When the second class of drugs were used: three major studies, the LEADER [9], the SUSTAIN 6 [10] and the HARMONY [11] study demonstrated a significant reduction in the number of events of cardiovascular mortality, or a decrease in the frequency of adverse events of myocardial infarction, and stroke.

So, taking into consideration the results described above, what are the main known effects of the glucagon like peptide agonist over the cardiovascular system?

At least, we can consider the following ones:

- 1) Expression of the GLP1 receptor in the left atrium
- 2) Direct action over cardiomyocytes
- 3) Antiinflammatory effects of the GLP1
- 4) Action of the GLP1 agonist on the endothelium cells
- 5) Action of the GLP1 agonist on the renin, angiotensin, aldosterone system
- 6) Natriuretic effect of the GLP1 agonist
- 7) Action on the atrial natriuretic peptide
- 8) Stabilization of the atherosclerotic plaque
- 9) Reduction in systolic and diastolic blood pressure.
- 10) Effects on lipids
- 11) Reduction of the hypoglycemic risk

1) EXPRESION OF THE GLP1 RECEPTOR IN THE LEFT ATRIUM

Both in the hearts of non human primates and in human heart, the receptors of GLP1 agonist have only been identified in the auricular area close to the sinoauricular node [12]. Nevertheless, some studies have detected partial GLP1 R mRNA transcripts by reverse transcriptase polymerase chain reaction techniques in the left ventricle. [13]

The clinical use of GLP1 agonist has been related to the rise in the heart rate in several studies. Researchers that have using 24hs monitoring shows that the variation in the heart rate depends on the class of GLP1 agonist that is used: long acting GLP1 agonist elevate the heart rate for 24 hs, whereas short acting drugs only lead to a transient rise in the heart rate. The relative contribution of the autonomic nervous system versus the direct effect of the GLP1 agonist over the GLP1R located in the proximity of the sinoauricular node is difficult to discern [14]

2) DIRECT ACTION OVER CARDIOMYOCITES

Adult rats cardiomyocytes treated with native GLP1 increases cyclic AMP levels, so is clear that their action is mediated by coupling with G Protein in a path similar to the Beta cell in pancreas [15]. Exposition of neonatal cardiomyocytes to exendin -4 increases Akt and extracellular regulated kinase (ERK) phosphorylation [16], this action may reduce cellular apoptosis after episodes of hypoxia/reoxygenation.

In models of infarction in mouse C57BL/6 in which the main left coronary artery was ligated, the administration of a dose of 75-200 µg of liraglutide reduced its size in comparison to controls suggesting a direct effect over the affected cardiomyocytes. Liraglutide also modulated the expression and the activity of cardioprotective genes and proteins in the mouse heart, including Akt, GSK3, PPAR β , NrF2 and HO [17]

Additional studies with albiglutide, lixisenatide and exenatide demonstrated similar effects, and the cardioprotective action can be inhibited with the use of a specific inhibitor such as GLP1-RA exendin(9-39). This suggested a direct action over the canonical GLP1 receptor [18]

At pharmacological dose, intravenous GLP1 dose improved left ventricular function, maximum oxygen uptake and physical performance in subjects with heart failure. Similarly, intravenous exenatide reduce wedge pressure, and improved parameters of cardiac index, with better contractility however these facts were founded in vitro only in atrial cardiomyocytes but not in ventricular myocardium [19]

In the case of human diabetic subjects with acute myocardial infarction with ST-T elevation who undertook primary percutaneous coronary intervention, the treatment with liraglutide improved left ventricular ejection fraction at 3 months compared to placebo, reducing the hs-CRP, and increased the activity of endothelial Nitric oxide synthetase [20]

Nevertheless, while in the LEADER study, there was a non significant reduction of 13% in the frequency of heart failure, in a study that lasted 12 weeks with less number of patients, Lepore et al did not find an improvement in left ventricular ejection fraction, 6 minutes work testing, left ventricular structure and function, and parameters of glucose and oxygen utilization in obese and overweight patients with class II-III NYHA heart failure and ejection fraction less than 40% with the use of albiglutide versus placebo [21]. With similar findings, the FIGHT study [22] carried out in patients with severe heart failure (FE <26%) or history of previous hospitalization for heart failure during the former 180 days, did not find any significant improvement with the use of liraglutide compared to placebo regarding hospitalizations for new events of heart failure, mortality and levels of NTproBNP. More patients in the group liraglutide were rehospitalized due to heart failure.

Likewise, in the LIVE STUDY, in diabetic and non diabetic patients with stable heart failure (EF less than 45%) no improvements were founded with the use of liraglutide vs placebo over the ventricular systolic function and serious events cardiac events were superior in the first arm. [23]

Therefore, while there is not a clear evidence in favor of the use of GLP1 agonist in the setting of severe heart failure, a study carried out with a large number of high risk patients as the LEADER study have not demonstrated an increase in the risk of it. Conversely an inverse tendency was found in most of them.

3) **ANTIINFLAMMATORY ACTION OF GLP1 ANALOGS**

The enteroendocrine L cells can secrete GLP1 in response to proinflammatory stimuli.

This can in turn act promoting the expression of GLP1 receptors over the intraepithelial lymphocytes (iLES) with less locally intestinal inflammation.

GLP-1 may also indirectly reduce inflammation in different peripheral organs through weight loss or improved glucose control, or by targeting GLP-1Rs expressed on populations of circulating immune cells. Alternatively, GLP-1R activation may directly reduce inflammation in organs and cell types expressing the GLP-1R.

The gut has been found to be the biggest center of interrelation between the intestinal microflora and the immune system and an important target for the control of inflammation impacting not only in the local gut mucosa, but also in distant organs including the liver, adipose tissue, muscle, blood vessels, heart and pancreatic islets. Similarly, the enteroendocrine cells represent the largest specialized endocrine networks in human physiology [24]

In the case of rodents exposed to intestinal inflammation through the administration of lipopolysaccharides, the levels of derivatives of proglucagon derived peptides are increased. Interleukin 6 and LPS directly influence the increase in the secretion of GLP1 from a line of murine gut L cells [25]

The importance of basal intestinal GLP1 signaling for control of local inflammatory signals was demonstrated in GLP1^{-/-} knock out mice models in which exhibited gut microbial dysbiosis and markedly increased sensitivity to experimental induced colonic inflammation was observed [26]

The levels of GLP1 peptides are 6,9 fold elevated in critical care patients, and in those patients it can help predict the risk of mortality in sepsis and critical illness [27]

Anti-inflammatory effects of GLP-1R agonists were also demonstrated using human aortic endothelial cells (HAECs) pre-treated with liraglutide for 1 hr, and then exposed to TNF- α or LPS plus liraglutide for 3 hr. The induction of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin expression was attenuated, and adhesion of THP-1 monocytes to HAECs was reduced using liraglutide. These actions required increases in intracellular Ca²⁺, Ca²⁺/calmodulin-dependent protein kinase (CAMK)-, and AMPK-dependent signaling [28]

The GLP1 receptor agonist liraglutide and semaglutide significantly attenuated plaque lesion development in apolipoprotein E deficient mice (Apo E^{-/-}) and low density lipoprotein receptor (LDLR^{-/-}) mice, and this attenuation was independent of cholesterol and weight

lowering. This fact suggested a direct antiinflammatory action in the plaque tissue related to less leukocyte recruitment, reduction in leukocyte rolling, adhesion and extravasation, changes in the turnover of extracellular matrix proteins (metaloproteins), or reduction in the risk of plaque hemorrhage [29]

Nevertheless the expression of GLP1 R canonical receptor in mouse peritoneal or tissue macrophages are not fully described. So, the precise mechanism through the GLP1 modified immune systemic inflammation needs to be elucidated [30]

4) **ACTION OF GLP1 OVER ENDOTHELIUM CELLS**

Some pioneer studies have been suggesting the necessary presence of GLP1 receptors in human coronary endothelial cells. It was observed that the incubation of those cells with exendin-4 stimulates proliferation of human coronary artery endothelial cells and at the same time the activation of oxide nitric synthetase (eNOs), MAP kinase and PI3K/AKT dependent pathway. [31] Also, in human umbilical endothelial cells (HUVEC) some results were published which concluded that exenatide exerts direct protective effects on endothelial cells through the AMP/Akt/eNOs pathway in a GLP1 receptor dose dependent manner. [32]

However, the real presence of the GLP1 receptor in the coronary endothelial cells was questioned. The GLP1 receptor was cloned in 1992 and most of the researchers used immunochimistry methods. But when a careful Western Blotting characterization of multiple available polyclonal antibodies (Pba) against the GLP1 was done, the authors concluded that several of them did not detect authentic GLP1-R protein, even they used optimal enhanced methods. [33] After that a rigorous validation criteria for the use of any antiGLP1R antibody was defined [34]

The native GLP1, once modified by the DPP4 enzyme, present in the coronary endothelium, could have some vasodilatory action mediated by their metabolites GLP1 (9-36) and (7-36) which may increase blood flow with independence from the presence of GLP1 receptor. [35]

Despite these observations, in a randomized study on obese patients with prediabetes, the endothelial function assessed by digital reactive hyperemia, did not show any significant difference with the use of exenatide in comparison with metformin. [36] Likewise, there were not differences over the endothelial function measured by flow mediated dilation (FMD) in diabetic patients in a study comparing the action of liraglutide vs glargine insulin [37]

5) **ACTION OF GLP1 OVER THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM**

Using a new validated monoclonal antibody Pykes and cols. found the presence of GLP1R activity in smooth vascular muscle of the afferent arteriole closely to cells of the juxtaglomerular complex [38] with coexpressed simultaneous immunoreactivity for renin in normal primate and human kidney.

In a randomized, controlled, double-blinded, single day study

over twelve healthy patients, the 2 hs infusion of GLP1 had little effect over the glomerular filtration rate and the renal plasma flow compared to placebo. Nevertheless the renal clearance of sodium rose 40% and the concentration of angiotensin II decreased significantly (19%) whereas renin, aldosterone and the urinary excretion of angiotensinogen showed no significant changes. [39]

6) NATRIURETIC EFFECT OF GLP1

The GLP1 induced natriuretic effects in healthy subjects and in insulin resistant obese men. Intravenous infusion of GLP1 enhance sodium excretion, reduce H⁺ secretion and reduce glomerular hyperfiltration in obese men. These findings suggest an action at the proximal renal tubule and a potential renoprotective effect. [40]

7) ACTION ON THE ATRIAL NATRIURETIC FACTOR

In mice, after the infusion of angiotensin II to raise their blood pressure, the administration of GLP1 like peptide analogs augmented the secretion of atrial natriuretic peptide (ANP) through the activation of GLP1 receptor. [41] determining a decrease in it. But this effect is only observed in wild and not in KO GLP1 receptor mice.

Therefore, this raised the possibility of an axis entero-atrial that explain the mayor cardiovascular effects of GLP1 analogs: the GLP1 secreted by L cells in the gut binds to GLP1 R in the atrial myocardium and induced ANP secretion via Epac 2. At the same time, ANP reduced blood pressure by increasing the permeability of blood vessels, inducing vasorelaxation and enhancing sodium secretion and inhibiting sodium reabsorption in the kidney. An additional mechanism of ANP mediated natriuresis includes increases in glomerular filtration rate, direct inhibition of renin, and suppression of aldosterone secretion from the adrenal zona glomerulosa cells. [42]

Nevertheless, in a study with a relative small number of hypertensive subjects with diabetes, liraglutide promoted natriuresis but did not increase circulating levels of ANP. [43] after 3 weeks of treatment. In another research a specific relationship between natriuresis and levels of pro atrial natriuretic peptide in healthy males was not found either. [44]

As a consequence, although it is clear that analogs glucagón like peptides promotes natriuresis their specific relationship with hemodynamic action peptides requires further researchs and explanations.

Even so, could there be another action attributable to the atrial natriuretic factor?

Some authors have demonstrated that the changes in the body composition and the magnitude of weight loss in obese diabetic patients induced by liraglutide are related to the levels of the ANP [45] Likewise, in an elegant ANP receptor knock out mouse model and a line of human adipocytes cells, Bordiccia and cols have demonstrated that in mouse and human adipocytes Atrial natriuretic peptide (ANP) and ventricular natriuretic peptide (BNP) activated PPAR α coactivator 1 α

(PGC-1 α) and mitochondrial uncoupling protein 1 (UCP1) expresión, induced mitochondriogenesis and increased uncoupled and total respiration. This effect results in increased lipolysis and energy expenditure, promoting "browning" of the white adipose tissue. This mechanism defines the heart as a central regulator of the adipose tissue biology. [46] In this sense, a potential action of the peptides ANP and BNP is that they could act as thermodynamic regulators dissipating much of the energy as heat and relieving cardiac work as a consequence.

8) STABILIZATION OF THE ATHEROSCLEROTIC PLAQUE PROGRESSION

In a apolipoprotein E deficient (apoE^{-/-}) mouse model the treatment with liraglutide inhibited the progression of early onset atherosclerotic plaques and enhance plaque stability core [47] It has also been confirmed that Lixisenatide confer protection against atherosclerosis: 2 months subcutaneous infusion of this compound decreased aortic arch lesion size in high cholesterol fed Apo E^{-/-} mice that were also heterozygous for insulin substrate 2 protein [48] Lixisenatide also improved plaque stability as determined by plaque collagen content and increased plaque fibrous cap thickness. Treatment of mouse aortic VSMC with exendin-4 reduced platelet derived growth factor that induced cell proliferation factor [49] In addition, liraglutide reduced oxidized low density lipoprotein-induced mitochondrial reactive oxygen species (ROS) generation in aortic human vascular smooth muscle cells. [50] In a study of patient who were submitted to carotid endarterectomy, atherosclerotic plaques were obtained from diabetics in comparison with no diabetics patients. Likewise, the plaques were compared between those diabetics patients treated with analogs glucagón like peptide or Iddp4 drugs versus non treated patients. Compared with plaques in non diabetic patients, diabetic plaques had more inflammation and oxidative stress along with less SIRT6 (sirtuins) expresión and collagen content. In those diabetics patient treated with GLP1 therapy, the plaques presented greater SIRT6 expression and less inflammation and oxidative stress, indicating more stable plaque phenotype in comparison with non treated diabetic patients. [51]

9) REDUCTION IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

In a metaanálisis Visboll et al. [52] have founded a reduction in the systolic and diastolic blood pressure in the diabetic patients treated with GLP1 analogs in comparison with other therapies. In other metaanálisis Wang and cols [53] founded that the treatment with liraglutide and exenatide reduced 1 to 5 mmHg the systolic and diastolic blood pressure compared with some others anti diabetic drugs including insulin, glimepiride and placebo. In a pooled analysis of six randomized clinical trials done over 2792 patients, the mean reduction of the systolic blood pressure and diastolic blood pressure with the use of liraglutide in comparison to placebo were evident after 2 weeks and maintain the effect a 26 weeks, the reduction was

independent of the magnitude of the weight loss and concomitant use of other antihypertensive medications [54] Also in the Duration 1 trial with exenatide once a week, an average number of nearly 50% of patients who have systolic blood pressure higher than 130 mmHg at the beginning of the study achieved a normal systolic blood pressure after 52 week. [55]

In the series of Lead 1 to 6 studies liraglutide consistently reduced systolic blood pressure in the monotherapy arm versus vs glimepiride[56], in combination with metformin [57] , in combination with sulfonilureas versus rosiglitazone [58] ,in combination with metformin and rosiglitazone[59] in comparison with exenatide [60] and sitagliptin[61]

10) **EFFECTS ON LIPIDS**

GLP1 R agonist reduce intestinal chylomicron production and secretion in normoglycemic and diabetic rodents and humans , and it has been shown that it also reduce postprandial triglycerides and apo B48. Some studies carried out over animals and humans have demonstrated reduction of liver fat and hepatic lipoprotein synthesis and secretion with these compounds. Nevertheless, neither enterocytes, the mayor cell type responsible for chylomicron synthesis and secretion and nor the hepatocytes express canonical GLP1 R [62] so it is possible that their action is produced by an indirect mecanism.

In type 2 diabetics ,liraglutide lowered the average postprandial triglycerides profile in comparison with placebo. [63] In the LEAD studies 1 to 6 the levels of total cholesterol, LDL cholesterol and triglycerides were significantly reduced in type 2 diabetics patients treated with liraglutide in dose of 1.8 mg compared with rosiglitazone, glimepiride, glargine insulina or exenatide.[64]

In an observational real life study that lasted 18th months in wich one hundred twenty one subjects with diabetes and metabolic syndrome were studied , the addition of liraglutide to metformin was associated with a reduccion in the levels of total and LDL cholesterol and triglycerides and in the carotid intima thickness assessed by B mode real time ultrasound .At the end of the observation period (18 months) 26% of the patients no longer fulfilled the criteria of metabolic syndrome[65]

In the SCALE ,a fifty six week, double blinded,placebo controled study,the levels of total cholesterol,VLDL,HDL and triglycerides were significantly reduced with the dose of 3 mg liraglutide but not with 1,8 mg.No effects on LDL cholesterol or free fatty acids were observed. [66]

Nevertheless, in the largest 3,8 years LEADER study not significant differences were observed between the liraglutide and placebo arm in terms of total colesterol,HDL cholesterol, triglycerides and LDL cholesterol. [9] Conversely in the EXSCCEL study, in wich exenatide was used once week a slight but significant difference was observed in the levels of low density cholesterol,(1,5 mg/dl or 0,04 mmol/l) and tryglicerides (1,8 mg or -0,02 mmol/l) between the treatment versus placebo arm. [67]

In the SUSTAIN 6 (10) study the difference of total cholesterol between the treatment with semaglutidie and placebo arm were

0,4 mg/dl in the dose of 0,5mg and 1 mg/dl in the dose of 1 mg. At the end of the study there was not a significant reduction in the levels of total cholesterol,LDL cholesterol tryglicerides and free fatty acids respect to the basal levels but the level of cholesterol HDL were superior and the tryglicerides inferior in the arm of 1 mg semaglutide in relation to placebo. Furthermore the level of LDL cholesterol was inferior in the arm of 0,5 mg of semaglutide regarding to placebo.

There were not significant differences in the levels of total cholesterol, LDL cholesterol, tryglicerides and HDL cholesterol between the treatment and placebo arm in the HARMONY study with the use of albiglutide versus placebo. [11]

11) **REDUCTION OF THE HYPOGLYCEMIC RISK**

In the LEADER study , the frequency of hypoglycemic events was 20% inferior in the group assigned to liraglutide versus the control group,and the frequency of severe hypoglycemia was 31% less,wich was highly significant. [9]] In the Exscel study using exenatide once a week there no were significant differences in the rate of severe hypogluceemic episodes between the treated and placebo arm. [67] In the ELIXA study with lixisenatide vs placebo in type 2 diabetics patients with and acute coronary syndrome, 16,6% of hypoglycemic episodes were reported in the lixisenatide group and 15,2% in the placebo group without statistical signficancy, but serious hypogluceemic episodes (that required assistance of a third person) were numerically less frequent with lixisenatide (14 patients reporting 16 events) than with placebo (24 patients reported 37 events)(68)

In the Harmony trial with a randomization of type 2 diabetes patients to albiglutide once a week versus placebo, the events of severe hypogluceemia were 44% inferior en the albiglutide arm (31 patients vs 55 patients) [11]

In a post hoc análisis made by Zinmann et al. over the LEADER study they found that 114 patients experienced severe hypoglycemia in the liraglutide arm vs 154 in the placebo arm (31% less) .In both arms, the patients who suffered severe hypoglycemia had longer diabetes duration, higher incidence of heart failure and use insulin more frequently at baseline.The most important fact about patients regardless they were treated or not was that those who were experiencing severe hypoglycemia were at greater risk of cardiovascular events and death, particularty shortly after of the hypoglycemic episode. A clear temporal association was observed between severe hypoglycemic episodes and subsequent risk of mayor adverse cardiovascular events (MACE),cardiovascular death ,non cardiovascular death or all cause death [69] These findings were similar to those described by Zoungas et al in the ADVANCE study [70] In a recent metaanálisis and systematic revisión,Fizpatrick et al. found a definitive association between hypogluceemia and cardiac arrhythmia. The hypoglycemia was associated to a less variation of the cardiac rhytm and mayor frequency of arrhythmia.Moreover ,the QTc interval was longer during the hypoglycaemic events than during the eugluceemic intervals. [71]

CAN WE SPEAK OF A CLASS EFFECT?

The GLP1 analogs differ in some pharmacokinetics and pharmacodynamics characteristics.

Exenatide and lixisenatide share a 53% and 50% aminoacid homology respectively with the native GLP1 [72], while liraglutide and semaglutide share an 97% and 94% of aminoacid homology respectively with the GLP1 native [73] At the same time, albiglutide and dulaglutide share an 94% and 91% of aminoacid homology respectively with the native GLP1.

Extensive research has been carried out in the last years in order to look for some molecular characteristics that determine that the compounds have a greater power as GLP1 receptor agonist such the synthesis of small molecules GLP1 agonist and some allosteric modulators, biased agonist and triagonist. [74]

Also, some pharmacodynamic characteristics may influence the differential action of GLP1 agonist :whereas exenatide and lixisenatide has a brief duration of action(2-5 hours) , liraglutide has an average half life of 13 hours and is suitable for once daily administration [75],and exenatide extended release and albiglutide can last up to a week [76]

Liraglutide has the addition of a C16 fatty acid that allows reversible binding to albumin wich slows degradation and reduces renal clearance. Additionally, it results in increased self association (wich slows absorption from the subcutaneous depot), reduced susceptibility to DPP-4(dipeptidyl peptidase -4) and prolongs its plasma half life ,protracting its action.Semaglutide has the addition of a C-18 di acid wich allows reversible binding to albumin,wich slows degradation and reduces renal clearance.The aminoacid substitution at position 8 protects against DPP4 degradation.Albiglutide has two GLP1 molecules attached to albumin to retain its therapeutic effect and a aminoacid substitution at position 8 that protects against DPP4 degradation while dulaglutide has two GLP1 DPP4 resistant moléculas covalently linked to a modified IgG4 Fragment that acts as carrier limiting the renal clearance of the molecule. [77]

If those characteristics has some effect on the cardiovascular outcomes is still a source of further debate.

CARDIOVASCULAR OUTCOMES

To date there are five major investigations that have explored the efficacy and safety of glucagón like peptide analogues in patients with high cardiovascular risk.

In the ELIXA trial 6068 patients were included ,with antecedents of myocardial infarction or hospitalization for inestable angina in the last 180 days.They were randomized to receive lixisenatide or placebo and the study lasted two years.During the observation period the HbA1c was 0,27% inferior on average in the lixisenatide arm vs the placebo arm, the differences in systolic blood pressure between the two groups was 0,8 mmHg and the group assigned to lixisenatide had a loss of 0,7 kg weight regarding the placebo arm.The end compositum objective was to evaluate the incidence of cardiovascular death,myocardial infarction,stroke or hospitalization for inestable angine (MACE) .13%(406 patients) of the patients in the lixisenatide arm versus 13,2% of those in the placebo groups (309 patients) suffered some of this

events wich confirm the non inferiority but not the superiority of the compound in relation to placebo.Also there were not differences in relation to heart failure or death for all causes. (68] The LEADER trial was a randomized, double blind study, the most extensive in terms of patients follow up:it lasted 3,8 years and it was also the one with the largest number of patients involved (9340 patients).

81,3% of the patients had high cardiovascular risk (previous non fatal myocardial infarction,stroke and/or renal failure with creatinine clearance less than 60 ml/min or heart failure in NYHA stage II-III). 18,7% of the patients were with high cardiovascular risk (micro and/or macroproteinuria,arterial hypertension or left ventricular hypertrophy,ventricular diastolic and/or systolic dysfunction and ankle/arm index less than 0,9)

The average difference in HbA1c between the group assigned to liraglutide versus placebo was 0,40% .The systolic blood pressure was 1,2 mmHg lowest in the liraglutide arm and, in average, the patients in this arm lost 2,3 kg more than patients in placebo .Also,there was a 13% reduction in the mayor cardiovascular combined events (MACE),wich was highly statistical significant.608 events in 4668 patients in the liraglutide arm vs 694 events in 4672 patients(14,9%) in the placebo arm .(Hazard ratio 0,87;95% confidence Interval (CI) 0,78 to 0,97, p<0,001 for non inferiority ,p:0,01 for superiority).The cardiovascular mortality was 22% inferior in the liraglutide arm than in the placebo,also highly significant.(Hazard ratio:0,78;95% CI 0,66 to 0,93 p:0,007). There was a non significant reduction in the number of non fatal myocardial infarctions (14%), stroke (14%) and hospitalization for heart failure in the liraglutide arm.

Regarding death by any causes, the patients assigned to the liraglutide group showed a significant reduction of 15% during the observational period (381 patients [8.2%]) compared to the placebo group (447 [9.6%]) (hazard ratio, 0.85; 95% CI, 0.74 to 0.97; P=0.02)

In the subgroup análisis, those patients with a glomerular filtration rate less than 60 ml/min benefitted more from the active treatment.(33% less MACE events vs placebo) than those with normal glomerular filtration rate. (hazard ratio:0,67 ;95% IC:0,54-0,83).The most common cause leading to discontinuation of the drug were gastrointestinal effects, and the incidence of pancreatitis was non significantly lower in the liraglutide group than in the placebo group .There were more episodes of gallstone disease with liraglutide, a finding that was previously reported.Also,there was a higher rate of retinopathy events with liraglutide than with placebo, although the difference was not significant.

The study demonstrate superiority of liraglutide compared to placebo in terms of reduction of mayor cardiovascular events,cardiovascular mortality and death of all causes The number of patients who would need to be treated to prevent one event in 3 years was 66 in the analysis of the primary outcome and 98 in the analysis of death from any cause (9]

In the EXSCEL TRIAL 14752 type 2 diabetic patiens of whom

the 73% had previous cardiovascular disease were followed for a median of 3,2 years. This was a randomized, double blind trial with the use of extended release exenatide at dose of 2 mg weekly versus a placebo controlled arm. Again, the primary outcome was the first occurrence of death from cardiovascular causes, non fatal myocardial infarction or non fatal stroke (MACE outcome). Secondary outcomes included death for any causes, death from cardiovascular causes, and the first occurrence of non fatal or fatal myocardial infarction, non fatal or fatal stroke, hospitalization for acute coronary syndrome and hospitalization for heart failure.

-At baseline, the median length of time that patients had had diabetes was 12 years, and the basal HbA1c was 8%. 16,2% of the patients had a history of heart failure. During the study the difference of HbA1c between the exenatide arm vs the placebo arm was 0,53%. The exenatide group had in average less -1,5 mmHg systolic blood pressure and the patients assigned to this arm lost .1,27 kg weight regarding to placebo. 11,4% of the patients in the exenatide arm (3,7 events persons /year) and 12,7% in the placebo arm (4 events persons/year) (Hazard ratio : 0,91,95% CI 0,83 to 1) suffered a primary composite event indicating that exenatide, administered once a week was no inferior to placebo with respect to safety (p < 0,001 for non inferiority) and was not superior to placebo with respect to efficacy (p:0,06 for superiority)

The risk of death from any cause was 6,9% in the exenatide group and 7,9% in the placebo group but this difference was not considered to be significantly on the basis of the hierarchical testing plan.

Patients randomly assigned to exenatide, administered once week had a lower risk of receiving an additional cointervention glucose lowering drug than patients in the placebo group and a lower risk of initiating long term insulin therapy. The rate of severe hypoglycemia didn't differ significantly between the two groups. The incidence of acute pancreatitis, pancreatic cancer, medullary thyroid carcinoma and serious adverse events did not differ significantly between the two groups. One of the limitations of this trial was the lost of follow up, the type 2 diabetic patients involved in the study had a mean exposure to the trial regimen of only 2,4 years with 25% of the study subjects being not fully maintained on the assigned trial regimen and more than 40% of the participants had prematurely discontinued their assigned study medication(67)

The SUSTAIN 6 study utilized semaglutide, a prolonged action GLP1 agonist, that allows its administration once a week in dose of 0,5 and 1 mg in comparison with placebo, in a double blind fashion. The research lasted 104 weeks (2,1 years) and included 3297 patients with high cardiovascular risk (83% with previous cardiovascular events, chronic kidney disease with the exception of patients with glomerular filtration estimated in less of 30 ml/min.) The rest of the patients had risk factors such as microalbuminuria or proteinuria, arterial hypertension or left ventricular hypertrophy, left diastolic or systolic dysfunction or ankle/ arm index less than 0,9. The average age of the patients was 65 years old, the overall duration of type 2 diabetes was

13,9 years and the mean glyated hemoglobin levels was 8,7% at the beginning.

The average difference in HbA1c between the 0,5 mg and 1 mg semaglutide arm in relation to placebo was -1,1% and 1,4% respectively. The systolic blood pressure was -1,27 mmHg and -2,6 mmHg inferior in the 0,5 and 1 mg arm. The 0,5 mg semaglutide arm lost 3,6 kg and the arm 1 mg -4,9 kg weight in comparison to placebo.

The primary composite outcome was the first occurrence of cardiovascular death, non fatal myocardial infarction or non fatal stroke .(MACE) The primary outcome occurred 26% less in the semaglutide treated patients : 108 of 1648 patients (6,6%) in the semaglutide group and 146 of 1649 patients (8,9%) in the placebo group, suffered one event. (Hazard ratio 0,74,95% CI 0,85-0,95 p< 0,001 for non inferiority and p<0,02 for superiority). It was necessary to treat 41 patients to reduce one primary composite outcome. The reduction of the primary objective was led by a significant reduction in the frequency of non fatal stroke in the patients assigned to the semaglutide arm: non fatal stroke occurred in 27 patients (1,6%) in the semaglutide group and 44(2,7%) in the placebo group (Hazard ratio 0,61;95% CI 0,38 to 0,99 p:0,04)

Non fatal myocardial infarction occurred in 47 patients (2,9%) in the semaglutide group and 64 (3,9%) in the placebo group, a difference that was not statistically significant. The risk of cardiovascular death was similar in both groups, 44 patients in the semaglutide group (2,7%) and 46 patients (2,8%) in the placebo group, also non significant.

The new or worsening nephropathy was reduced in 36% in the patients treated with semaglutide but there were an 76% increment in the diabetic retinopathy: 50 patients in the semaglutide group (3%) vs 29 patients in the placebo group (1,8%) suffered some diabetic retinopathy complications (need of retinal photocoagulation, use of intravitreal agents, vitreous hemorrhage or onset of diabetes related blindness) [69] There were a non significant raise in heart failure in both semaglutide arms (14%) and there was not differences in the mortality by all causes between the semaglutide and placebo treated. There were more pancreatitis episodes and pancreatic cancer in the placebo arm than in both semaglutide dose arms. The gallbladder disorder was similar in all groups.(10)

The last study published was the HARMONY TRIAL in which were involved 610 sites in 28 countries. Type 2 diabetes patients aged 40 years old and older with cardiovascular disease (myocardial infarction or at least 50% stenosis in one coronary artery or more, or previous coronary revascularization or cerebrovascular stroke or carotid stenosis or with intermittent claudication or ankle /arm index less than 0,9 or those whose received an peripheral vascular procedure) were submitted to a treatment with subcutaneous once week albiglutide or a placebo in addition to their standard care. The primary outcome were similar to the previous trial (MACE reduction). The mean age of the patients was 64,1 year, the average duration of diabetes was 14,1 years and the mean HbA1c was 8,7%. 71% of the patients had a history of coronary artery disease, 25% had

peripheral artery disease, 25% had previous cerebrovascular disease and 20% had a history of heart failure. The median duration of follow up was 1,6 years (maximum 2,6 years) Study treatment drug was taken for 87% of the total follow up for cardiovascular outcome in the albiglutide group and 85% of that time in the placebo group.

The mean difference of the HbA1c was 0,6% less in the albiglutide group in relation to placebo, the systolic blood pressure was 0,6 mmHg inferior in the first arm and the patients assigned to albiglutide lost in average 0,8 kg of body weight. The primary composite end point occurred in 338 (7%) of the 4731 patients assigned to albiglutide (4,57 events per 100 patients year) and in 428 (9%) of 4372 patients in the placebo group (5,87 events per 100 patients/year) which means a 22% reduction in the risk (HR 0,78 95% CI 0,68-0,90). Albiglutide was not inferior to placebo for cardiovascular safety ($p < 0,0001$ for non inferiority) and superior to placebo for efficacy ($p: 0,0006$). This means that we need to treat 50 patients during 1,6 years for reducing one event. There was a significant reduction in the incidence of myocardial infarction of 25% (HR: 0,61-0,90, 95% CI $p: 0,003$). Also there was a non significant reduction in the incidence of stroke events (HR: 0,86 CI 95% 0,66 to 1,14) and death for cardiovascular causes. Neither was a difference in relation to a hospitalization for heart failure. The frequency of severe hypoglycemia was low in the albiglutide and placebo arm and the number of pancreatitis and pancreatic cancer was less than 1% in both arms. There not where registry of medullary thyroid cancer.

There was not reporting of new or worsening retinopathy in the albiglutide group (11).

In the table 2 it is described a resume of this trials.

In a recent metaanalysis and systematic revision of twelve research identified in which only fourth of the trial described here were selected that involved 33.457 participants (ELIXA with lixisenatide, SUSTAIN 6 with semaglutide, LEADER with liraglutide and EXSCEL with exenatide extended released, HARMONY with albiglutide was not included) Bethel and cols. has concluded that compared with placebo, GLP1 receptor agonist showed a significant 10% relative risk reduction in the three point mayor adverse primary outcomes (MACE: cardiovascular mortality, non fatal myocardial infarction, and non fatal stroke) (HR 90 CI 95% 0,82-0,90; 0,03), a 13% reduction in relative risk of cardiovascular mortality (HR: 0,87, CI 95% 0,79-0,96 $p: 0,07$) and a 12% relative reduction in all causes mortality (HR: 0,88- CI 95%: 0,81-0,95 $p: 0,002$) with low to moderate between trial statistical heterogeneity (78).

Until date, no head to head cardiovascular outcomes trials have been done for this drug class, limiting detection of outcome differences between GLP1 receptor agonist of different structures and potency. Thus their differences could be assigned to a many factors related to the drugs (solubility, release, grade of drug absorption, peak drug levels, pharmacokinetic profile, degradation, metabolism, renal clearance, immunogenicity or potency on receptor signaling) either to the design of the different trials (population assigned, disease duration of type 2 diabetes, background

therapy, length of the follow up study, size of the population studied, event adjudication, time of exposition to drugs, number of patients needed to treat to obtain a benefit, number of patients needed to treat to obtain a harm and the statistical analysis).

Table 2: the bold letters imply statistically significance

Study	LEADER	SUSTAIN 6	ELIXA	EXSCEL	HARMONY
Patients involved	9340	3297	6068	14752	10793
Duration of the study	3,8 years	2,1 years	2 years	3,2 years	2,3 years
Δ HbA1c	-0,40%	-1,1/-1,4%	-0,27%	-0,53%	-0,6%
Δ Systolic blood pressure	-1,2 mmHg	-1,27/2,6 mmHg	-0,8 mmHg	-1,57 mmHg	-0,67 mmHg
Δ Weight	-2,3 kg	-3,6/-4,9 kg	-0,7 kg	-1,27 kg	-0,8 kg
Δ LDL cholesterol	n.s	-0,4/0,1 mg/dl	n.s	-1,5 mg/dl	n.s
M.A.C.E reduction	13% (HR: 0,87) (p: 0,005)	26% (HR: 0,76) (p: 0,001)	+0,2 (n.s)	-0,8 (n.s)	22% (HR: 0,78) (p: 0,005)
Cardiovascular mortality	22% (HR: 0,78) (p: 0,007)	-2% (n.s)	+2% (n.s)	-12% (n.s)	-7% (n.s)
Non fatal MI	14% (n.s)	26% (n.s)	+0,3% (n.s)	-3% (n.s)	25% (HR: 0,75) (p: 0,003)
Stroke	14% (n.s)	39% (HR: 0,71) (p: 0,04)	+12% (n.s)	-15% (n.s)	-14% (n.s)
Heart failure	13% (n.s)	+11% (n.s)	-0,3% (n.s)	-6% (n.s)	-5% (n.s)
Death for any cause	15% (HR: 0,85) (p: 0,02)	+0,05% (n.s)	-0,4% (n.s)	-14% (n.s)	

BIBLIOGRAFÍA

1. **The Action to Control Cardiovascular Risk in Diabetes Study Group.** Effects of Intensive Glucose Lowering in Type 2 Diabetes. N Engl J Med 2008; 358:2545-2559.
2. **The ADVANCE Collaborative Group.** Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 2008; 358:2560-2572
3. **Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven P, Zieve F, Marks J, Davis S, Hayward R, Warren SR, Pharm.D., Goldman S., for the VADT Investigators.** Glucose Control and Vascular Complications in Veterans with Type 2 Diabetes. N Engl J Med 2009; 360:129-139
4. **U.S. Department of Health and Human Services.** Guidance for Industry. Diabetes mellitus – Evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. Diciembre de 2008. En: <https://www.fda.gov/downloads/Drugs/Guidances/ucm071627.pdf>; consultado agosto 2017.
5. **Hirshberg B, Katz A.** Insights from cardiovascular outcome trials with novel antidiabetes agents: what have we learn? An industry perspective. Curr Diab Rep 2015; 15: 87.
6. **Zinman B, Wanner Ch., Lachin J., Fitchett D, Bluhmki E., Hantel S., Mattheus M, Devins T, Johansen O.D., Woerle H, Broedl U, Inzucchi S, for the EMPA-REG OUTCOME Investigators.:** Empagliflozin, Cardiovascular Outcomes, and

- Mortality in Type 2 Diabetes
N Engl J Med 2015; 373:2117-2128
7. **Neal N., Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondou N, Shaw W., Law G, Desai M, and Matthews DR, Phil. D**, for the CANVAS Program Collaborative Group Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes.
N Engl J Med 2017; 377:644-657
 8. **Wiviott SD., Raz I., Bonaca M, Mosenzon O, Kato E, Cahn A, Silverman M, Zelniker T A, Kuder JF., Murphy S.A., Bhatt DL, Leiter L.A., Mc Guire DK, Wilding JPH, Ruff CT, Guase-Nilson I.A.M, Fredriksson M, Johansson P.A., Lankilde M, Sabatini MS** for the DECLARE-TIMI: Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes
N. Engl. J. Med. 2019; 380:347-57
 9. **Marso SP, Daniels GH, Brown-Frandsen CH, Kristensen P, Mann J, Nauck MA, Nissen S.E, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockne Mr**, for the LEADER Steering Committee on behalf of the LEADER Trial Investigators Liraglutide and Cardiovascular outcomes in type 2 diabetes. N.Engl.J.Med. 2016; 375:311-322
 10. **Marso SP, Bain SC, Consoli A., Eliaschewitz FG, Jodar E., Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Hols AG, Pettersson J., Vilsboll T,** for the SUSTAIN-6 Invest. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes.
N Engl J Med. 2016; 375;1834-44.
 11. **Hernandez AF, Green JB, Janmohamed S, D'Agostino RB, Granger CHB, Jones NP, Leiter LA, Rosenberg AE, Sigmon KN, Somerville MC, Thorpe KM, McMurray JJ, o Del Prato S**, for the Harmony Outcomes committees and investigators. Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease
The Lancet 2018;392:10157: 1519-1529
 12. **Pyke C, Heller RS, Kirk RK, Orskov C, Reedtz-Runge S, Kastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB.** GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. 2014; 155:1280-1290.
 13. **Wallner M, Kolesnik E, Ablasser K, Khafaga M, Wakula P, Ljubojevic S, Thon-Gutsch EM, Sourij H, Kapl M, Edmunds NJ, Kuzmiski JB, Griffith DA, Knez I, Pieske B, von Lewinski D.** Exenatide exerts a PKA-dependent positive inotropic effect in human atrial myocardium: GLP-1R mediated effects in human myocardium. *J Mol Cell Cardiol*. 2015; 89(Pt B):365-375.
 14. **Smits MM, Muskiet MH, Tonneijck L, Hoekstra T, Kramer MH, Diamant M, van Raalte DH.** Exenatide acutely increases heart rate in parallel with augmented sympathetic nervous system activation in healthy overweightmales.
Br J Clin Pharmacol. 2016; 81:613-620.
 15. **Vila Petroff MG, Egan JM, Wang X, Sollott SJ.** Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes.
Circ Res. 2001; 89:445-452.
 16. **Ban K, Kim H, Cho J, Diamandis E, Backx PH, Drucker DJ, Husain M.** Glp-1(9–36) protects cardiomyocytes and endothelial cells from ischemiareperfusion injury via cytoprotective pathway.s independent of the glp-1receptor.
Endocrinology. 2010;151:1520-1531
 17. **Noyan-Ashraf M, Momen A, Ban K, Sadi A, Zhou YQ, Riazi AM, Baggio LL, R. Henkelman M, Husain M DruckerDJ**
Diabetes 2009; 58:975-983
 18. **Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM.** Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury.
Diabetes. 2005; 54:146-151.
 19. **Wallner M, Kolesnik E, Ablasser K, Khafaga M, Wakula P, Ljubojevic S, Thon-Gutsch EM, Sourij H, Kapl M, Edmunds NJ, Kuzmiski JB, Griffith DA, Knez I, Pieske B, von Lewinski D.** Exenatide exerts a PKA-dependent positive inotropic effect in human atrial myocardium: GLP-1R mediated effects in human myocardium.
J Mol Cell Cardiol. 2015; 89(Pt B):365-375.
 20. **Chen WR, Hu SY, Chen YD, Zhang Y, Qian G, M, Wang J, Yang JJ, Wang ZF, Tian F, Ning QX,** Effects of liraglutide on left ventricular function in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention
Am Heart Journal 2015; 170:845-854
 21. **Lepore JJ, Eric E, Demopoulos L, Haws T, Fang Z, Barbour AM, Fossler M, Davila-Roman VG., Russel SD., Gropler RJ** Effects of the Novel Long-Acting GLP-1 Agonist, Albiglutide, on Cardiac Function, Cardiac Metabolism, and Exercise Capacity in Patients with Chronic Heart Failure and Reduced Ejection Fraction. *JACC Heart failure* 2016 4;559-566
 22. **Margulies KB, Hernandez AF, Redfield MM., Givertz MM, Oliveira GH, Cole R., Mann DL., Whellan DJ, Kiernan MS, MS. Felker M, McNulty SE, Anstrom KJ., Shah MR, Braunwald E, Cappola TP,** for the NHLBI Heart Failure Clinical Research Network. Effects of Liraglutide on Clinical Stability Among Patients With Advanced Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial.
JAMA. 2016; 316:500-508.
 23. **Jorsa Al, Kistorp C, Holmager P, Stilling Tougaard R, Nielsen R, Hänselmann A, Nilsson B, Møller JE, Hjort J, Rasmussen J, Boesgaard TW,** Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE)—a multicentre, double-blind, randomised, placebo-controlled trial
Morten Schou, Lars Videbæk, Ida Gustafsson, Allan Flyvbjerg, Henrik Wiggers, and Lise Tarnow Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE)—a multicentre, double-blind, randomised, placebo-controlled trial
European Journal of Heart Failure 2017; 19, 69-77
 24. **Drucker D** Evolving concepts and translational relevance of enteroendocrine cell biology
J.Clin.Endocrinol.Metab.2016:101:-778-86

25. **Kahles F, Meyer C, Mollmann J, Diebold S, Findeisen H.M., Leberherz C, Trautwein C, Koch A, Tacke F, Marx N, and Lehrke M** GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. *Diabetes* 2014; 63:3221-3229
26. **Yusta B, Baggio L.L., Koehler J, Holland D, Cao X, Pinnell L.J., Johnson-Henry K.C., Yeung W, Surette M.G., Bang K.W., et al.** GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. *Diabetes* 2015; 64:2537-2549.
27. **Leberherz C, Schlieper G, Mollmann J, Kahles F, Schwarz M, Brunsing J, Dimkovic N, Koch A, Trautwein C, Floge J.** GLP-1 levels predict mortality in patients with critical illness as well as end-stage renal disease. *Am. J. Med.* 2017; 130:833-841.e3.
28. **Krasner N.M., Ido Y, Ruderman N.B., and Cacicedo J.M.** Glucagon like peptide-1 (GLP-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and AMPK dependent mechanism. *PLoS ONE* 2014;9:e97554.
29. **Rakipovski G, Rolin B, Nøhr J, Klewe LB, Frederiksen KS, Augustin R, Hecksher-Sørensen J, Ingvorsen C, Polex-Wolf J, Knudsen LB** The GLP-1 Analogs Liraglutide and Semaglutide Reduce Atherosclerosis in ApoE^{-/-} and LDLr^{-/-} Mice by a Mechanism That Includes Inflammatory Pathways. *JACC: Basic Transl Sci Science* 2018;3:844-857
30. **Drucker D** The Cardiovascular biology of Glucagon like peptide-1 *Cell Metabolism* 2016; 24:15-30
31. **Erdogdu O, Nathanson D, Sjöholm A, Nyström T, Zhang Q.** Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. *Mol and Cell Endocrinology* 2010; 325:26-35
32. **Wei R, Ma S, Wang CH, Ke J, Yang J, Li W, Liu Y, Hou W, Feng X, Wang G, Hong T** Exenatide exerts direct protective effects on endothelial cells through the AMPK/Akt/eNOS pathway in a GLP-1 receptor-dependent manner. *Am J. Physiol Endocrinol Metab.* 2016; 310:E947-E957
33. **Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, Streutker C, Holland D, Cao X, Baggio LL, Drucker DJ** 2012 GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE^{-/-} mice. *Endocrinology* 2012; 154:127-139
34. **Pyke Ch, Bjerre Knudsen L** The Glucagon Like Peptide 1 receptor-or not? *Endocrinology* 2013;154:4-8
35. **Ban K,; M. Noyan-Ashraf H, Hoefler J; Bolz SS; Drucker D, Husain M,** Cardioprotective and Vasodilatory Actions of Glucagon-Like Peptide 1 Receptor Are Mediated Through Both Glucagon-Like Peptide 1 Receptor-Dependent and -Independent Pathways *Circulation* 2008; 117:2340-2350
36. **Kelly AS, Bergenstal RM, Gonzalez-Campoy JM, Katz H Bank AJ:** Effects of Exenatide vs. Metformin on endothelial function in obese patients with pre-diabetes: a randomized trial *Cardiovascular Diabetology* 2012; 11:64.2-7
37. **Nomoto H, i Miyoshi H, Furumoto T, Oba K, i Tsutsui H, Miyoshi A, Kondo T, Tsuchida T, Atsumi T, Manda N, Kurihara Y, Aoki S, SAIS Study Group** :A Comparison of the Effects of the GLP-1 Analogue Liraglutide and Insulin Glargine on Endothelial Function and Metabolic Parameters: A Randomized, Controlled Trial Sapporo Athero-Incretin Study 2 (SAIS2) *PLOS ONE* 2015; 10:e0135854.
38. **Pyke CH R. Heller S, Kirk RK, Ørskov C, Reedtz-Runge S, Kastrup P, Hvelplund A, Bardram L, Calatayud D, Bjerre Knudsen L.** GLP-1 Receptor Localization in Monkey and Human Tissue: Novel Distribution Revealed With Extensively Validated Monoclonal Antibody. *Endocrinology* 2014;155:1280-1290
39. **Skov J, Dejgaard A, Frøkiær J, Holst JJ, Jonassen T, Rittig S, Christiansen J.S.** Glucagon-Like Peptide-1 (GLP-1): Effect on Kidney Hemodynamics and Renin-Angiotensin-Aldosterone System in Healthy Men. *J.Clin.Endocrinol.Metab* 2013; 98: E664-E671
40. **Gutzwiller JP, Schopp S, Bock A, Zehnder C, Hube AR, Kreyenbuehl M, Heike H, Drewe J, Henzen CH, Goeke B, Beglinger CH** Glucagon-Like Peptide 1 Induces Natriuresis in Healthy Subjects and in Insulin-Resistant Obese Men. *J.Clin.Endocrinol.Metab.* 2004; 89:3055-3061
41. **Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ.** GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nature Medicine* 201; 19:567-575
42. **Buglioni A, Burnett J.** A gut heart connection in cardiometabolic regulation *Nat Med.* 2013; 19: 534-536.
43. **Lovshin JA, Barnie A, De Almeida A, Logan A, Zinman B, Drucker D** Liraglutide Promotes Natriuresis but Does Not Increase Circulating Levels of Atrial Natriuretic Peptide in Hypertensive Subjects With Type 2 Diabetes. *Diabetes Care* 2015; 38:132-139
44. **Skov J, Holst JJ, Gøtzte JP, Frøkiær J, Christiansen JS** Glucagon-like peptide-1: effect on pro-atrial natriuretic peptide in healthy males. *Endocrine Connections* 2014; 3:11-16
45. **Li Ch J, Yu Q, Yu P, Yu TL, Zhang QM, Lu S, Yu DM** Changes in liraglutide-induced body composition are related to modifications in plasma cardiac natriuretic peptides levels in obese type 2 diabetic patients. *Cardiovascular Diabetology* 2014;13:36:1-11
46. **Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang Ch, i Takahashi N, Sarzani R, Collins S.** Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes *J.Clin. Invest.* 2012;122:1022-1036
47. **Gaspari T, Welungoda I, Widdop RE, Simpson RW E Dear AE** The GLP-1 receptor agonist liraglutide inhibits progression of vascular disease via effects on atherogenesis, plaque stability and endothelial function in an ApoE^{-/-} mouse model. *Diabetes & Vascular Disease Research* 2013; 10:353-360
48. **Vinue A, Navarro J, Herrero-Cervera A, Garcia-Cubas M, Andres-Blasco I, Martinez-Hervas S:** The GLP-1 analogue lixisenatide decreases atherosclerosis in insulinresistant

- mice by modulating macrophage phenotype. *Diabetologia*. 2017; 60:1801-12.
49. **Goto H, Nomiya T, Mita T, Yasunari E, Azuma K, Komiya K.** Exendin-4, a glucagon-like peptide-1 receptor agonist, reduces intimal thickening after vascular injury. *Biochem. Biophys. Res. Commun.* 2011; 405:79-84.
 50. **Dai Y, Mercanti F, Dai D, Wang X, Ding Z, Pothineni NV.** LOX-1, a bridge between GLP-1R and mitochondrial ROS generation in human vascular smooth muscle cells. *Biochem Biophys Res Commun.* 2013; 437:62-6.
 51. **Balestrieri ML, Rizzo MR, Barbieri MB, Paolisso P, D'Onofrio N, Giovane A, Siniscalchi M, Minicucci F, Sardu C, D'Andrea D, Mauro C, Ferraraccio F, Servillo L, Chirico F, Caiazzo P, Paolisso G, Marfella R, Sirtuin** 6 Expression and Inflammatory Activity in Diabetic Atherosclerotic Plaques: Effects of Incretin Treatment. *Diabetes* 2015;64:1395-1406
 52. **Visboll T, Christensen M, Junker A, Knopp F.** Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *Brit Med J* 2012;344 :d7771
 53. **B. Wang J., Zhong, H. Lin, Z. Zhao, Z. Yan, H. He, Y. Ni, D. Liu Z. ,Zhu Z** Blood pressure-lowering effects of GLP-1 receptor agonists exenatide and liraglutide: a meta-analysis of clinical trials *Diabetes Obesity and Metabolism* 2013, 15:737-749.
 54. **Fonseca VA, De Vries JH, Henry RR, Donsmark M, Thomsen HF, Plutzky J** Reductions in systolic blood pressure with liraglutide in patients with type 2 diabetes: Insights from a patient-level pooled analysis of six randomized clinical trials. *J Diabetes Complications* 2014; 28:399-405
 55. **Buse JB, Drucker DJ, Taylor KL, Kim T, Walsh B, Hu H.** DURATION-1: exenatide once weekly produces sustained glycemic control and weight loss over 52 weeks. *Diabetes Care.* 2010; 33:1255-61.
 56. **Garber A, Henry R, Ratner R, Garcia Hernandez P, Rodriguez Pattzi H, Olvera Alvarez I.** Liraglutide vs glimepiride monotherapy for type 2 diabetes (LEAD 3) a randomized, 52 week phase III double blind parallel trial *The Lancet* 373; 9662:473-481
 57. **Nauck M, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, Düring M, Matthews DR.** Efficacy and Safety Comparison of Liraglutide, Glimepiride, and Placebo, All in Combination With Metformin, in Type 2 Diabetes. *Diabetes care* 2009; 32:84-90
 58. **Marre, M.; Shaw, J.; Brandle, M.; Bebakar, W.M.W.; Kamaruddin, N.A.; Strand, J.; Zdravkovic, M.; LeThi, T.D.; Colagiuri L.** Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). *Diabetic Medicine* 2009; 25:268-278
 59. **Zinman B, Gerich J, Buse JB, Lewin A, Schwartz S, Raskin P, Hale PM, Zdravkovic M, Blonde L, and the LEAD-4 Study Investigators** Efficacy and Safety of the Human Glucagon-Like Peptide-1 Analog Liraglutide in Combination With Metformin and Thiazolidinedione in Patients With Type 2 Diabetes (LEAD-4 Met+TZD) *Diabetes Care* 2009; 32:1224-30
 60. **Buse, J.B.; Rosenstock, J.; Sesti, G.; Schmidt, W.E.; Montanya, E.; Brett, J.H.; Zychma, M.; Blonde, L.** Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6) *The Lancet* 2009;374:9683:39-47
 61. **Pratley, R.E.; Nauck, M.; Bailey, T.; Montanya, E.; Cuddihy, R.; Filetti, S.; Thomsen, A.B.; Sondergaard, R.E.; Davies, M.** Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial *The Lancet* 2010;375:9724:1447-1456
 62. **Drucker D.J** The Cardiovascular Biology of Glucagon-like Peptide-1. *Cell Metabolism* 2016; 24:15-30
 63. **Hermansen K** Liraglutide improves the average postprandial triglycerides profile in subjects with type 2 diabetes *Diabetes* 2012,61 A272-1058 P
 64. **Fonseca VA.** Liraglutide effects on fasting lipids levels Lead 1-6: metaanalysis International Diabetes Federation 21st World Diabetes Congress Abstracts, December 2011, Dubai, UAE.
 65. **Rizzo M., Rizvi A, Patti AM, Nikolic D, Giglio RV, Castellino G, Volti GL, Caprio M, Montalto G, Provenzano V, Genovese S, Ceriello A** Liraglutide improves metabolic parameters and carotid intima-media thickness in diabetic patients with the metabolic syndrome: an 18-month prospective study. *Cardiovasc. Diabetol* 2016.15:162
 66. **Davies MJ; Bergenstal R; Bode B; Kushner RF; Lewin A. Vang Skjoth T; Haahr Andreasen A; Bjorn Jensen CH., De Fronzo, R;** Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes The SCALE Diabetes Randomized Clinical Trial. *JAMA.* 2015;314:687-699.
 67. **Holman RR, Bethel MA, Mentz RJ, Thompson VP, Lokhnygina Y, Buse JB, Chan JC, Choi J, Gustavson SM, Iqbal N, Maggioni AP, Marso, SP** for the EXSCEL Study Group. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. *N.Engl.J.Med.* 2017;377:1228-1239
 68. **Pfeffer MA Claggett B, Diaz R, Dickstein K., Gerstein HC, Køber LV., Lawson FC., Ping L, Wei X., Lewis EF, Maggioni AP, McMurray JJV,** for the ELIXA Investigators Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome *N Engl J Med* 2015; 373:2247-2257
 69. **Zinman B., Marso SP, Christiansen E, Calanna S, Rasmussen S., Buse JB,** and the LEADER Publication Committee on behalf of the LEADER Trial Investigators: Hypoglycemia, Cardiovascular Outcomes, and Death: The LEADER Experience *Diabetes Care* 2018; 41:1783-1791
 70. **Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Biostat. M, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP,** et al., for the

ADVANCE Collaborative Group Severe Hypoglycemia and Risks of Vascular Events and Death .

N Engl J Med 2010; 363:1410-1418

71. **Fitzpatrick C, Chatterjee S, Seidu S, Bodicoat D, Andre G, Davies M, Khunti K.** Association of hypoglycaemia and risk of Cardiac arrhythmia in patients with diabetes mellitus systematic review and meta-analysis *Diabetes Obesity and Metabolism* 2018;20:2169-2178
72. **Ratner, R.E.; Rosenstock, J.; Boka, G.** Dose-dependent effects of the once-daily GLP-1 receptor agonist lixisenatide in patients with Type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled trial. *Diabetic Medicine*: 2010;27:9:1024-1032
73. **Nauck M** *Diabetologia* 2012;55(Suppl 1):S7
74. **Drucker D.** Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1 *Cell Metabolism* 2018;27:741-756
75. **Meier JJ.** *Nat Rev Endocrinol* 2012;8:728-742
76. **Knop FK, Brønden A, Vilsbøll T.** Exenatide: pharmacokinetics, clinical use, and future directions. *Expert Opin Pharmacother* 2017;18:555-571
77. **Chris de Graaf, Dan Donnelly, Denise Wootten, Jesper Lau, Patrick M. Sexton, Laurence J. Miller, Jung-Mo Ahn, Jiayu Liao, Madeleine M. Fletcher, Dehua Yang, Alastair J. H. Brown, Caihong Zhou, Jiejie Deng, and Ming-Wei Wang** Glucagon-Like Peptide-1 and Its Class B G Protein-Coupled Receptors: A Long March to Therapeutic Successes *Pharmacol. Rev* 68:953-1013
78. **M Angelyn Bethel, Rishi A Patel, Peter Merrill, Yuliya Lokhnygina, John B Buse, Robert J Mentz, Neha J Pagidipati, Juliana C Chan, Stephanie M Gustavson, Nayyar Iqbal, Aldo P Maggioni, Peter Öhman, Neil R Poulter, Ambady Ramachandran, Bernard Zinman, Adrian F Hernandez, Rury R Holman, for the EXSCEL Study Group.** Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis *Lancet Diabetes Endocrinol* 2018; 6:105–13

