Trends in Endocrinology & Metabolism

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REVIEWS

Opinion

Heterogeneity and Similarities in GLP-1 Receptor Agonist Cardiovascular Outcomes Trials

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The latest recommendations from the American Diabetes Association and the European Association for the Study of Diabetes prioritize the use of drugs with proven cardiovascular (CV) benefit in patients with established CV disease. Especially among the glucagon-like peptide (GLP)-1 receptor agonists (GLP-1RA) class, results of cardiovascular outcomes trials (CVOT) have been heterogeneous. Baseline characteristics of the population, study design, drugs in the control arm, modifications of CV risk factors, including glycemic control, reduction of hypoglycemia, and the GLP-1RA direct effects on CV cells and tissues, were considered. Ultimately, the time of exposure to the GLP-1RA appears to be the factor most prominently explaining trial heterogeneity. Thus, the CV benefit should be regarded as a class effect of GLP-1RA, as largely similar results are seen for drugs sharing a common mechanism of action.

Heterogeneity in GLP-1RA CVOT: The Need for Interpretation

Since the 2008 FDA guidance (https://www.fda.gov), each new antidiabetes drug has to prove non-inferiority to placebo in terms of cardiovascular (CV) safety before approval for use in clinical care. This has led to a plethora of **cardiovascular outcomes trials** (**CVOT**; see Glossary) with dipeptidyl peptidase-4 (DPP-4) inhibitors [1–3], glucagon-like peptide (GLP)-1 receptor agonists (GLP-1RA) [4–10], and sodium-glucose cotransporter 2 (SGLT-2) inhibitors [11–13], which have shed light and generated questions on the actual CV benefits hypothesized for these molecules in preclinical and Phase II/III registration studies. Most GLP-1RA [4–10] and SGLT-2 inhibitors [11–13] decreased the incidence of **major adverse cardiovascular events (MACEs)** and all-cause mortality to a various extent. Full results of the Researching Cardiovascular Events with a Weekly Incretin in Diabetes (REWIND) [9] and Peptide Innovation for Early Diabetes Treatment 6 (PIONEER 6) [10] trials have just been disclosed, substantially aligning to the previous CVOT. Importantly, CVOT had an impact on the latest clinical recommendations from the American Diabetes Association and the European Association for the Study of Diabetes, which prioritize the use of GLP-1RA and SGLT-2 inhibitors in patients with established CV disease who are not meeting their individualized glycated hemoglobin A1c (HbA1c) targets with metformin [14,15]. Given the recommendation to use a medication associated with a 'proven CV benefit' and the heterogeneity of the CVOT results, particularly with GLP-1RA, it is unclear whether clinicians should prefer one drug over the others [16] or consider their beneficial impact on CV events and mortality as a class effect [17,18].

All CVOT with GLP-1RA had a 3-point MACE [i.e., CV mortality, non-fatal myocardial infarction (MI), and non-fatal stroke] as a primary outcome, except for the Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA) trial, which had a 4-point MACE including time to first occurrence of hospitalization for unstable angina [4]. ELIXA was the only trial in which GLP-1RA failed to show any CV benefit over standard of care, even though lixisenatide showed noninferiority on MACE

Highlights

ELIXA was the only GLP-1RA CVOT that failed to show any CV benefit over usual care. In LEADER, SUSTAIN-6, HARMONY Outcomes, and REWIND, GLP-1RA showed statistical superiority on MACEs compared to usual care, while EXSCEL (P=0.06) just grazed it and PIONEER 6 showed a nonsignificant 21% reduction (P=0.17). However, SUSTAIN-6 lacked a prespecified analysis for superiority.

Dulaglutide significantly decreased the incidence of MACEs by 12% in a population at a lower CV risk compared to previous CVOT.

Although GLP-1RA CVOT were not powered to detect such differences, GLP-1RA seemed to affect the individual MACE components according to specific patterns: liraglutide and oral semaglutide produced significant reductions of CV death; subcutaneous semaglutide and dulaglutide promoted a significant decrease in non-fatal stroke; albiglutide largely reduced non-fatal MI; and exenatide LAR did not show a significant effect in any component.

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[**hazard ratio (HR)**=1.02, 95% CI 0.89–1.17] and all-cause mortality (HR=0.94, 95% CI 0.78–1.13) [4]. Conversely, the effects of the other GLP-1RA on the composite primary endpoint were almost evenly favorable, as liraglutide (HR=0.87, 95% CI 0.78–0.97, *P*=0.01 for superiority) [5], subcutaneous semaglutide (HR=0.74, 95% CI 0.58–0.95, *P*=0.02 for superiority) [6], albiglutide (HR=0.78, 95% CI 0.68–0.90, *P*=0.0006 for superiority) [8], and dulaglutide (HR=0.88, 95% CI 0.79–0.99, *P*=0.026 for superiority) [9] demonstrated not only CV noninferiority but also statistical superiority to standard of care, while exenatide long-acting release (LAR; HR=0.91, 95% CI 0.83–1.00, *P*=0.06 for superiority) [7] just grazed it and oral semaglutide showed a nonsignificant reduction (HR=0.79, 95% CI 0.57–1.11, *P*=0.17 for superiority) [10]. However, not all GLP-1RA CVOT had prespecified statistical testing or were adequately powered for superiority, as in, the ELIXA [4], Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6) [6] and PIONEER 6 [10].

All GLP-1RA CVOT showed an individual pattern of change in MACE components: liraglutide exhibited a significant reduction in CV death (HR=0.78, 95% CI 0.66–0.93) with only a numerical reduction of non-fatal MI and stroke [5], subcutaneous semaglutide promoted a significant decrease in non-fatal stroke (HR=0.61, 95% CI 0.38–0.99) and a trend towards non-fatal MI reduction [6], albiglutide induced a statistically significant decrease in MI (HR=0.75, 95% CI 0.61–0.90) with little benefits on CV death and stroke [8], and dulaglutide primarily reduced non-fatal stroke (HR=0.76, 95% CI 0.61–0.95) [9]. In the Exenatide Study of Cardiovascular Event Lowering (EXSCEL) trial, there were no statistically significant differences between the two study arms for rates of CV death, MI, and stroke [7]. Oral semaglutide results on MACE were driven by a significant 51% reduction in CV death (HR=0.49, 95% CI 0.27–0.92) [10].

The rates of death from any cause were lower in the intervention arm of the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) (HR=0.85, 95% CI 0.74–0.97) [5], PIONEER 6 (HR=0.51, 95% CI 0.31–0.84) [10], and EXSCEL trials, although in EXSCEL the difference was not considered statistically significant based on the hierarchical testing plan (HR=0.86, 95% CI 0.77–0.97) [7]. In SUSTAIN-6 [6], HARMONY Outcomes [8], and REWIND [9], the HR for all-cause death did not differ between the active treatment and control groups. Instead, no differences in hospitalization for heart failure were found in all published studies [4–10].

A recent meta-analysis of the results from ELIXA, LEADER, SUSTAIN-6, and EXSCEL showed a cumulative significant 10% **relative risk reduction** (**RRR**) in MACE (HR=0.90, 95% CI 0.82–0.99, *P*=0.033) and a 12% RRR in all-cause mortality (HR=0.88, 95% CI 0.81–0.95, *P*=0.002), with low-to-moderate heterogeneity among trials [19]. Results from HARMONY Outcomes [8], REWIND [9], and PIONEER 6 [10] do not diverge from this conclusion.

All GLP-1RA CVOT have been designed considering the effects of including the investigational drug in the intervention arm on top of standard drug therapy, compared with a similar drug regimen in which placebo was given, and the standard of care led to different treatments added during the trial [4–10]. While it was intended to achieve 'equipoise' for glycemia and similar control of other CV factors, actual changes in metabolic and CV parameters occurred differently in the two arms. This and other issues, including the characteristics of the type 2 diabetes (T2D) population enrolled, trial design, extent of exposure to the investigational drug and other medications, and specific signaling properties of individual GLP-1RA, could have potentially affected the individual CVOT results (Figure 1, Key Figure).

Is There a Target T2D Population in Which CV Benefit with GLP-1RA Can Be Seen?

The study populations from all GLP-1RA CVOT are averagely composed of men (65.7%) in their sixties [4–8,20,21]. Although REWIND involved a higher percentage of female participants

Glossary

Absolute risk reduction (ARR): arithmetic difference between the event rate in the control and experimental groups, respectively.
Apoptosis: a highly regulated programmed cell death; both defective and excessive apoptosis are linked to a wide variety of diseases.
cAMP: abbreviation for cyclic adenosine monophosphate; it is a second messenger involved in many relevant biological processes.
Cardiovascular outcomes trials (CVOT): trials designed to ensure that each new antihyperglycemic therapy did not induce an unacceptable increase in cardiovascular risk, mandatory after the FDA guidance issued in 2008.
Half-life: pharmacokinetic parameter that indicates the time it takes for the total amount of a drug in the body to be reduced by 50%.
Hazard ratio (HR): comparison between the probability of an event to happen in the experimental and control groups, respectively.
Major adverse cardiovascular events (MACEs): it constitutes the primary outcome of all GLP-1RA CVOT and comprises cardiovascular death, non-fatal stroke, and non-fatal myocardial infarction. ELIXA is the only GLP-1RA CVOT addressing a 4-point MACE, including hospitalization for unstable angina.
p-CREB: abbreviation for cAMP responding element–binding protein; it is a transcription factor that binds to certain DNA sequences to increase or decrease gene transcription. It has pleiotropic actions.
p-ERK1/2: abbreviation for extracellular signal-regulated protein kinases 1 and 2; they mediate cell proliferation and survival phosphorylating, thus activating multiple transcription factors.
p-JNK: abbreviation for c–Jun N–terminal kinases; they respond to stress stimuli (i.e., cytokines, UV shock) modifying by phosphorylation the activity of numerous proteins of the mitochondria or the nucleus. They are involved in apoptosis, inflammation, cell differentiation, and proliferation.
Relative risk reduction (RRR): difference between the event rate in the control and experimental groups, respectively, expressed as a proportion of the event rate in the control group.
Run-in phase: precedes the actual beginning of a clinical trial and can be

Key Figure

Potential Factors in Cardiovascular Outcome Trials with GLP-1 Receptor Agonists

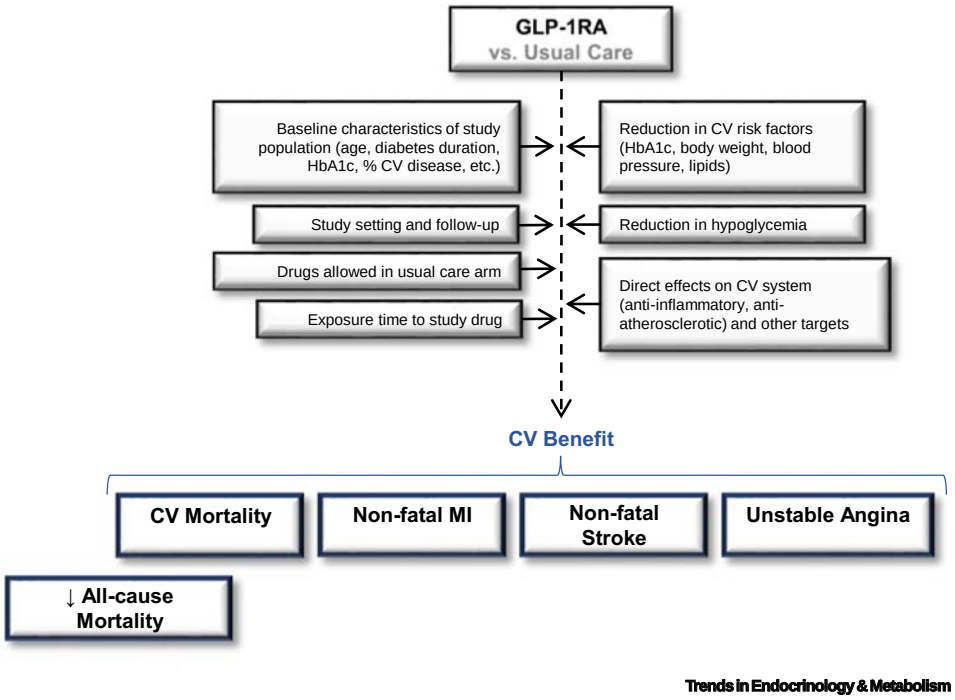


Figure 1. Glucagon-like peptide-1 receptor agonists (GLP-1RA) cardiovascular outcomes trials (CVOT) have been designed considering the effects of including the investigational drug in the intervention arm, in addition to other medications for controlling hyperglycemia and other cardiovascular (CV) risk factors, compared with a similar drug regimen in which placebo was given, and standard of care lead to different treatments added during the trial. Heterogeneity of GLP-1RA CVOT outcomes [CV mortality, non-fatal myocardial infarction (MI), non-fatal stroke, unstable angina, and all-cause mortality] might be potentially due to multiple factors, including differences in the characteristics of the type 2 diabetes population enrolled, trial design, extent of exposure to the investigational drug and other medications, actual differences in the control of hyperglycemia and other CV risk factors, variable rates of hypoglycemic episodes, and distinct signaling and biological properties of individual GLP–1RA. Abbreviation: HbA1c, glycated hemoglobin A1c.

passive (patients receive no treatment or placebo) or active (patients receive treatment). It is useful to assess patient compliance, washout previous medications, and standardize background therapy.

(46.3%), men were still more represented than women [20]. Mean diabetes duration, body mass index (BMI), low-density lipoprotein (LDL) cholesterol levels, and proportion of patients with arterial hypertension did not differ among the studies (Table 1). The definition of secondary prevention cohorts according to previous CV disease of the patients enrolled varied among trials, and so did the proportion of these patients. Specifically, ELIXA and HARMONY Outcomes involved only patients at the highest CV risk, as they all experienced prior CV diseases (i.e., an acute coronary syndrome within 180 days [4] or any CV disease at least 30 days prior to screening [8], respectively). By contrast, REWIND was unique in assessing a population mainly in primary prevention, with 70% of individuals without prior CV disease and only 8% affected by congestive heart failure [20]. Subgroup analyses from LEADER [5,22] and a recent systematic review and meta-analysis [23] suggest a CV benefit from GLP-1RA in individuals with established CV disease and not in those with only multiple CV risk factors. Accordingly, 5-year data from national Scandinavian registers showed that treatment with liraglutide was associated with significant MACE reduction compared to DPP-4 inhibitors, with the largest benefit in patients older than 65 years

Table 1. Baseline Characteristics of Study Population, Main Outcomes, and Use of New Antihyperglycemic and Cardiovascular Medications in Cardiovascular Outcomes Trials with glucagon-like peptide-1 Receptor Agonists

	ELIXA	LEADER	SUSTAIN-6	EXSCEL	HARMONY Outcomes	REWIND	PIONEER 6	
Mean diabetes duration (years)	9.3	12.8	13.9	12	14.1	10	14.9	Baseline risk level
Mean baseline HbA1c (%)	7.6	8.7	8.7	8.0	8.7	7.3	8.2	
Mean BMI (kg/m^2)	30.1	32.5	32.8	31.7	32.3	32.3	32.3	
History of CV disease (%)[a]	100	81.3	83	73.1	100	31.4	84.6	
Hypertension (%)	76.3	90	92.8	90.3[b]	86.4	93.2	95.3[b]	
eGFR <60 (%)	23.2	21.7	28.5	21.7	22.6	22.2	27	
Mortality rate (events/100 patient-year)[c]	3.1; 3.3	2.1; 2.5	1.8; 1.7	2.0; 2.3	2.4; 2.5	2.1; 2.3	1.1; 2.2	
Event rate of primary composite outcome (events/100 patient-year)[c]	6.4; 6.3	3.4; 3.9	3.2; 4.4	3.7; 4.0	4.6; 5.9	2.4; 2.7	2.9; 3.7	
MACE HR	1.02 (95% CI 0.89–1.17)	0.87 (95% CI 0.78–0.97) *P*=0.01[d]	0.74 (95% CI 0.58–0.95) *P*=0.02[d]	0.91 (95% CI 0.83–1.00) *P*=0.06[d]	0.78 (95% CI 0.68–0.90) *P*=0.0006[d]	0.88 (95% CI 0.79–0.99) *P*=0.026[d]	0.79 (95% CI 0.57–1.11) *P*=0.17[d]	Main outcomes
All-cause mortality HR	0.94 (95% CI 0.78–1.13)	0.85 (95% CI 0.74–0.97)	1.05 (95% CI 0.74–1.50)	0.86 (95% CI 0.77–0.97)	0.95 (95% CI 0.79–1.16)	0.90 (95% CI 0.80–1.01)	0.51 (95% CI 0.31–0.84)	
Introduction of SGLT-2 inhibitors during trial (%)[c]	–	2.1; 2.8	2.65; 5.65[e]	6.5; 9.4	3.1; 5.2	–	3.1; 7.0	New antihyperglycemic and CV medication usage during follow-up
Introduction of SU/insulin during trial (%)[c]	–	36.3; 54	13.1; 31.7[e]	16.3; 22.6	1.1; 4.3[f]	–	14.7; 31.4	
Introduction of statins during trial (%)[c]	–	9.4; 11.1	6.6; 8.8[e]	10.4; 11.1	–	–	9.1; 9.0[g]	
Introduction of calcium channel blockers during trial (%)[c]	–	10; 11.9	5.5; 9.3[e]	11.9; 12.6	–	–	–	
Refs	[4]	[5]	[6]	[7]	[8]	[9]	[10]	

[a]Definition of patients in secondary prevention varied among trials (ELIXA: acute coronary event within 180 days before trial enrollment. LEADER, SUSTAIN-6: aged 50 years or more with at least one CV condition [history of coronary heart disease, cerebrovascular disease, peripheral vascular disease, chronic kidney disease of stage 3 or greater, or chronic heart failure of New York Heart Association class II or III)]. EXSCEL: history of major clinical manifestation of coronary artery disease, ischemic cerebrovascular disease, or atherosclerotic peripheral arterial disease. HARMONY Outcomes: established disease of the coronary (MI, at least 50% stenosis in one coronary artery or more, or previous coronary revascularization), cerebrovascular (ischemic stroke, at least 50% carotid artery stenosis, or a previous carotid vascular procedure), or peripheral arterial circulation (intermittent claudication and an ankle to brachial index <0.9, non-traumatic amputation, or a previous peripheral vascular procedure). REWIND: history of MI, ischemic stroke, revascularization, hospitalization for unstable angina with concordant new ischemic ECG changes, or a positive stress test with concordant imaging. PIONEER 6: prior MI; prior stroke or transient ischemic attack; prior coronary, carotid or peripheral arterial revascularization; >50% stenosis on angiography or imaging of coronary, carotid or lower extremity arteries; documented history of symptomatic coronary heart disease; documented asymptomatic cardiac ischemia or chronic heart failure (New York Heart Association class II–III); or moderate renal impairment (eGFR 30–59 mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration equation).
[b]Percentage of hypertensive patients was indicated as percentage of patients on antihypertensive medications.
[c]Indicated for the GLP-1RA arm and placebo (on a background of standard of care) arm, respectively).
[d]*P* for superiority.
[e]Mean of % from 0.5 mg and 1 mg arms.
[f]Insulin alone or in combination with other antihyperglycemic medications.
[g]Indicated as new lipid-lowering drugs initiated during the trial. Abbreviations: HbA1c, glycated hemoglobin A1c; SU, sulfonylureas. (See [4–10].)

and with CV disease history [24]. The proportion of patients with baseline chronic kidney disease [estimated glomerular filtration rate (eGFR) <60 ml/min/m^2] or congestive heart failure was lower in EXSCEL [7] compared to most other published GLP-1RA CVOT, and one wonders whether a lower baseline risk profile of the population may have contributed to the lack of manifest superiority of exenatide LAR in this particular trial. However, challenging the concept that GLP-1RA

may exert CV benefit only in secondary prevention, REWIND recently showed that dulaglutide consistently reduced MACE in both patients with and without CV disease [9]. Moreover, mortality rates of the exposed populations similarly do not appear to have an impact on results: rates were similar in EXSCEL and PIONEER 6 [10] and lower in SUSTAIN-6 [6], yet while oral semaglutide failed to show superiority, subcutaneous semaglutide CV benefit was manifest.

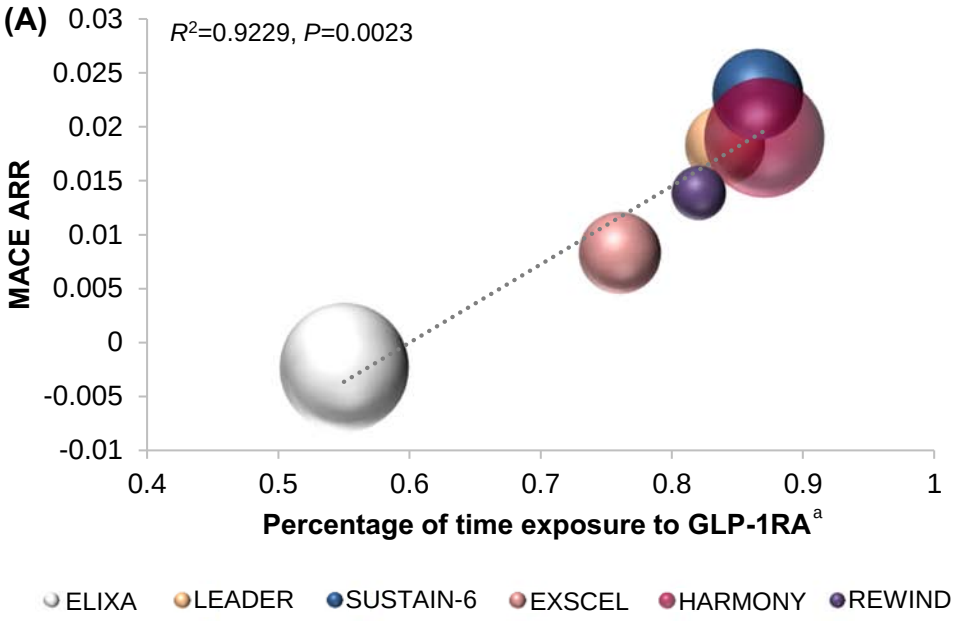
Does It Matter How the Study Is Designed and Executed to See Any CV Benefit?

EXSCEL [7] and HARMONY Outcomes [8] have the most pragmatic design, as they lack a **run-in phase**; have a visit interval of six and four months, respectively, resembling routine clinical practice; and allowed almost all diabetes medications in the usual care arm except for GLP-1RA. Nonetheless, the latter showed a clear reduction of non-fatal MI in the albiglutide arm [8], whereas this was not seen with exenatide LAR [7]. SUSTAIN-6 and PIONEER 6 also lacked a run-in phase, but follow-up visits were planned every three months or every seven weeks, respectively; the latter, despite more frequent contacts with patients, concluded for CV safety of oral semaglutide as noninferior to placebo [6,10]. Thus, it seems unlikely that the presence of a run-in phase might have directly influenced trial results.

Another potential interfering factor could be the greater drop-in of diabetes medications with proven CV benefit, such as SGLT-2 inhibitors in the control arm (9.4% vs. 6.5% in control and intervention arms, respectively, of EXSCEL; 7.0% vs. 3.1% in control and intervention arms, respectively, of PIONEER 6) [7,10] (Table 1). A recent analysis of EXSCEL intended to ascertain whether this could have blunted detection of exenatide LAR CV efficacy, as lower *P*-values were noted for 3-point MACE after censoring for sulfonylureas, SGLT-2 inhibitors, or any medications (Buse J.B. *et al.*, unpublished). However, the point estimates for MACE and all-cause death reductions were remarkably unchanged, while the number of events in each category became more exiguous. Moreover, in SUSTAIN-6 [6] and REWIND [9] there was greater use of SGLT-2 inhibitors in the control arm (4.9%/6.4% vs. 2.5%/2.8%, in 0.5 mg/1.0 mg control vs. intervention arms, respectively, of SUSTAIN-6; 7.3% vs. 5.3% in control and intervention arms, respectively, of REWIND), as in EXSCEL [7], yet this did not prevent semaglutide and dulaglutide from showing a statistically significant reduction of the primary outcome [6,9].

The prescription of antihypertensive drugs, especially calcium-channel blockers and angiotensin II receptor antagonists, diuretics, and statins, was significantly lower in the intervention arms of LEADER [5] and SUSTAIN-6 [6], respectively, compared to usual care, but similar in both arms of EXSCEL [7], REWIND [9], and PIONEER 6 [10] (Table 1). Thus, different drop-in of CV medications is also unlikely to have influenced results.

A relevant issue is the percentage of time that participants were exposed to the specific trial treatment regimen. EXSCEL was characterized by a lower exposure time to exenatide LAR [7], which was instead between 80% and 90% with lixisenatide [4], liraglutide [5], semaglutide [6], albiglutide [8], and dulaglutide [9]. Indeed, exposure to both exenatide LAR and placebo was low (i.e., 76% and 75%, respectively) in EXSCEL, possibly due to the unhandy injection device [7]. However, the reported exposure time to lixisenatide (88%) [4] may not correspond to the real time of drug action since the lixisenatide **half-life** ($T_{1/2}$) is markedly shorter (~3 h) than that of other GLP-1RA [25], as also highlighted by studies assessing heart rate changes in response to lixisenatide or liraglutide, respectively, showing increases in heart rate with lixisenatide mainly during daytime [26]. We have correlated the percentage of time patients were exposed to individual GLP-1RA during the CVOT to the MACE **absolute risk reduction** (ARR) and HR in the individual trial. Lixisenatide has been also included, assuming it took 5× its $T_{1/2}$ to be fully eliminated, leading to an approximate 60% 24-h exposure time [27], compared to ~100% for the other GLP-1RA; however, this approach



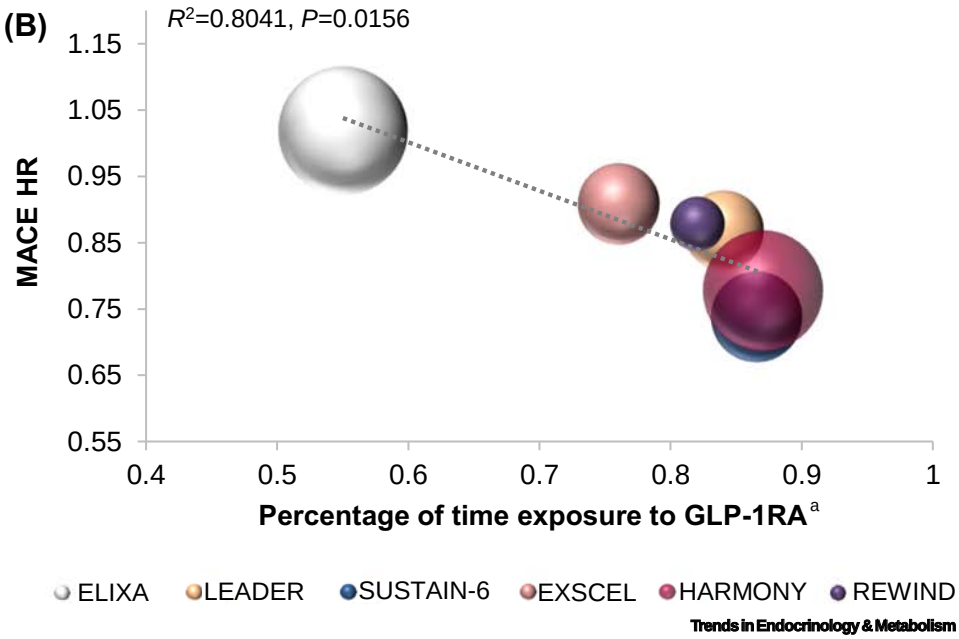


Figure 2. Correlation between Percentage of Time Exposure to Study Drug and Major Adverse Cardiovascular Effect Absolute Risk Reduction (A) and Hazard Ratio (B). Major adverse cardiovascular events (MACE) absolute risk reduction (ARR) (A) and hazard ratio (HR) (B) are plotted on the vertical axis as a function of percentage of time exposure to study regimen. Sphere size represents the baseline cardiovascular (CV) risk of the study population, expressed as MACE incidence rate in the control arm (number of events per 100 patient–year). Despite a reported median exposure to study regimen of 88% in Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA), actual exposure to lixisenatide could be regarded as much lower than this, acknowledging that its short half-life of approximately 3 h prevents the establishment of a steady–state. Considering lixisenatide to be completely eliminated after 15 h, this leads to an approximate 60% 24-h exposure time compared to nearly 100% for the other glucagon-like peptide–1 receptor agonists (GLP-1RA). For this

(Figure legend continued at the bottom of the next page.)

may have some limitations due to uncertainties on the exact duration of action of lixisenatide. Results from PIONEER 6 were not included in this analysis, as mean percentage of time exposure to study drug across the trial has not been disclosed [10]. Interestingly, the time of exposure to the investigational GLP-1RA was positively correlated to the MACE ARR (R^2=0. 9229, P=0. 0023) and negatively correlated to MACE HR (R^2=0.8041, P=0.0156; Figure 2). In contrast, the baseline CV risk of the study population, expressed as MACE incidence rate in the control arm (number of events per 100 patient-year), showed no meaningful association with MACE ARR (R^2=0.0912, P=0.51).

What Is the Contribution of Correction of Hyperglycemia and Control of Other CV Risk Factors to the CV Benefit?

In all CVOT, a reduction of HbA1c was detected in the intervention compared to usual care arm (Figure 3) [4–10], and differences in glucose control between the two arms may potentially have an impact on CV risk. The historical trials of intensive vs. less intensive glucose control {including the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Study [28], Action in Diabetes and Vascular Disease (ADVANCE) study [29], Veterans Affairs Diabetes Trial (VADT) [30], and the UK Prospective Diabetes Study (UKPDS) [31]}, exhibiting a difference in HbA1c levels between the intensive and control arms between 0.8–1.5% [32] and a relatively long follow-up (min. 3.5 years in ACCORD [28], max. 10 years in UKPDS [31]), all failed to show a significant reduction in MACE and mortality [32]. Only after an additional several-year follow-up extension, the effect of a previous intensive glucose control on macrovascular complications eventually emerged in UKPDS, VADT, and ACCORD (not in ADVANCE), mainly driven by MI reduction [32]. This suggests that glucose control plays a limited role particularly in patients with established CV disease, and that the CV benefit seen in the GLP-1RA CVOT, which had shorter duration and achieved smaller between-arm differences in HbA1c compared to the early trials, is mainly due to their extra-glycemic effects.

While the observed HbA1c changes evidently negate the possibility to achieve glycemic equipoise with such trial design, they generally reflect the relative potency of individual GLP-1RA in glucose-lowering and promotion of weight loss. For example, the larger HbA1c and body weight reductions observed in SUSTAIN-6 [6] than in LEADER [5] and EXSCEL [7] are in line with the greater efficacy of subcutaneous semaglutide (1 mg) compared with liraglutide and exenatide LAR in head-to-head Phase III studies [33–35]. Accordingly, the glucose and weight lowering effects observed in REWIND and PIONEER 6 are respectively similar and higher compared to those in LEADER, resembling dulaglutide [35] and oral semaglutide [36,37] behavior compared to liraglutide in head-to-head studies. Recently, Taylor (2018) has suggested that the magnitude of cardioprotection might be related to the normalized HbA1c lowering capacity of individual GLP-1RA, as derived from comparisons in head-to-head trials [38]. However, the results from HARMONY Outcomes blunt this correlation, since albiglutide, with a relatively low HbA1c lowering

reason, for lixisenatide, exposure (reduced from 88% to 55%) was determined not only by discontinuations of treatment, but also by the fact that effective drug levels are maintained for a shorter time after each injection even in patients taking the drug. Time of exposure to the investigational GLP–1RA is positively correlated with the MACE ARR (R^2=0.9229, P=0.0023) and, accordingly, negatively correlated with the MACE HR (R^2=0.8041, P=0.0156). As it can be inferred from the opposite position of the spheres representing ELIXA and cardiovascular safety and efficacy of albiglutide (HARMONY Outcomes) trials (both enrolling patients at the highest CV risk) on the regression line, baseline CV risk level does not seem to be related to changes in CV outcomes. [a]The percentage of time exposure to study drug is expressed as a median in ELIXA, HARMONY Outcomes, and Researching Cardiovascular Events with a Weekly Incretin in Diabetes (REWIND), and as a mean in Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER), Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6), and Exenatide Study of Cardiovascular Event Lowering (EXSCEL). (See [4–9].).

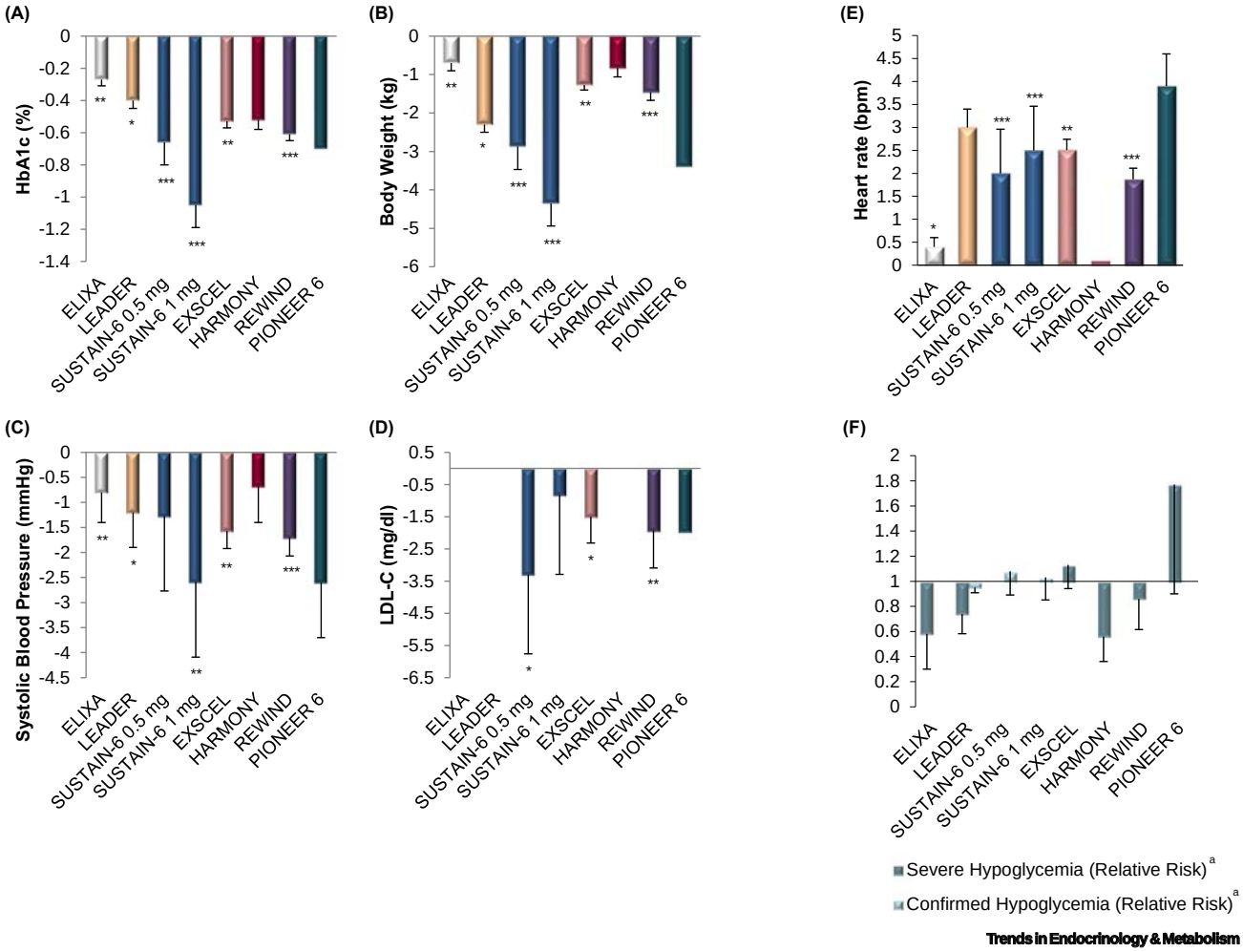


Figure 3. Mean Changes in Cardiovascular Risk Factors, Heart Rate, and Hypoglycemic Events across glucagon-like peptide-1 Receptor Agonists Cardiovascular Outcomes Trials (Intervention vs. Placebo). In all glucagon-like peptide-1 receptor agonists (GLP-1RA) cardiovascular outcome trials, a reduction of HbA1c (A), body weight (B), systolic blood pressure (C), and low-density lipoprotein cholesterol (D), as well as an increase in heart rate (E), were detected in the intervention arm compared to usual care. The magnitude of these changes echoed that observed in head-to-head studies. However, despite smaller effects on glycemic control, weight loss, and blood pressure, albiglutide showed a cardiovascular (CV) benefit similar to that of liraglutide and semaglutide. Similarly, only liraglutide showed a significant reduction in severe and confirmed hypoglycemic episodes (F), thus making this feature unlikely to be relevant in explaining the heterogeneity in GLP-1RA CV outcomes. Bars express between-arms differences. *P<0.05; **P<0.001; ***P<0.0001. [a]Confirmed hypoglycemia was defined as plasma glucose <56 mg/dl (3.1 mmol per liter). Severe hypoglycemia was defined as a hypoglycemic episode requiring assistance from a third party. In Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6), confirmed and severe hypoglycemia were reported without any distinctions and are herein indicated as confirmed hypoglycemia. (See [4–10].). Abbreviations: HbA1c, glycated hemoglobin A1c; ELIXA, Evaluation of Lixisenatide in Acute Coronary Syndrome; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, cardiovascular safety and efficacy of albiglutide; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; PIONEER 6, Peptide Innovation for Early Diabetes Treatment 6; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes.

capacity, caused HbA1c changes of lower magnitude than in LEADER [5] and EXSCEL [7] and yet reduced incidence of MACE by 22% [8].

In all CVOT, reductions of systolic blood pressure, body weight, and LDL-cholesterol, as well as an increase in heart rate, were also detected in the intervention compared to control arms (Figure 3) [4–10]. Verma *et al*. recently carried out a post-hoc analysis from LEADER showing

that the CV benefit of liraglutide was independent from baseline LDL-cholesterol and statin use, persisting even in the setting of an optimal lipid profile [39].

Relative increases in heart rate are related to the duration and entity of GLP-1 receptor (GLP-1R) engagement [26]. Yet, the role of changes in heart rate also seems negligible, as they were smallest in ELIXA [4], and this trial did not show any hint of CV benefit, whereas clear benefit occurred in the presence of mean 3 beats per minute (bpm) increases in heart rate, as in LEADER [5]. Notably, an even higher between-arms difference in heart rate (3.9 bpm) was noted in PIONEER 6, which failed to prove CV superiority regarding 3-point MACE yet exhibited a significant reduction of CV mortality (HR=0.49, 95% CI 0.27–0.92) [10].

Only LEADER [5] showed a significant reduction in severe (rate ratio 0.69, 95% CI 0.51–0.93) or confirmed (rate ratio 0.80, 95% CI 0.74–0.88) hypoglycemic events between GLP-1RA and control arms, whilst ELIXA [4], HARMONY Outcomes [8], and REWIND [9] detected only a numerical non-significant reduction, EXSCEL [7] and SUSTAIN-6 [6] found no difference, and PIONEER 6 found an increase in the intervention arm but only in patients already treated with insulin or sulfonylureas [10] (Figure 3). A post-hoc analysis of LEADER showed that there was an association between severe or confirmed hypoglycemia and CV events and mortality; however, the effect size was small and the protective effect of liraglutide on MACE was similar whether hypoglycemia occurred or not (*P* for interaction=0.90) [40]. Thus, sparing of hypoglycemic episodes does not seem to be a major mechanism for the GLP-1RA-mediated CV benefit.

Do Exendin-4- versus GLP-1-Based Agonists Differ in Signaling and Bioeffects?

Lixisenatide and exenatide are based on the backbone of exendin-4, a hormone identified in the Gila monster (*Heloderma suspectum*), whereas liraglutide, semaglutide, albiglutide, and dulaglutide are modified versions of active human GLP-1 (7–36); hence, the first group of agents has 53% amino acid sequence homology with human GLP-1 while the latter is almost identical to it (up to 97% linear homology) [41]. Accordingly, lixisenatide and exenatide induce more antibody formation than human GLP-1-based agonists, which could potentially cause decreased drug efficacy and increased risk of injection site reactions. Data from 17 clinical trials with exenatide twice daily and once weekly showed that the latter was the most immunogenic [42]. Immunogenicity appears to have little relevance on efficacy and safety of currently used GLP-1RA [35,41,43] but injection site reactions were a reason for premature permanent drug discontinuation during EXSCEL (3.4% vs. 1.8% in the exenatide and control arms, respectively), perhaps contributing to less CV benefit [7].

Fascinatingly, the GLP-1 receptor is capable of biased agonism, consisting in the ability of diverse ligands to bind to the same receptor with each promoting a different cellular response [44]. The interaction between different ligands and the extracellular loops (ECLs) of the GLP-1 receptor (GLP-1R) is crucial both to affinity and, through induction of conformational transitions, to engage intracellular effectors for signal propagation [44]. GLP-1 and exendin-4 appear to engage the same region (ECL2) of GLP-1R, albeit with different contribution of individual amino acid residues, exhibiting similar effects on **cAMP** generation. However, exendin-4 displays slightly less efficient Ca^{2+} mobilization and relies more on ECL3 for activation of receptor coupling to **p-ERK1/2** than GLP-1 [44]. Nonetheless, despite their potential to differently engage the GLP-1R, there is no evidence that exendin-4-like agonists possess a dampened ability to activate GLP-1R signaling when compared to human GLP-1-like agonists [45]. Indeed, cardioprotection against ischemia, angiogenesis, proliferation, and survival of endothelial cells and cardiac progenitor cells, endothelium-dependent vasodilation, and impaired release of inflammatory cytokines were similarly observed with GLP-1, liraglutide, exenatide, and

lixisenatide [33,45–47]. The GLP-1R is expressed in all four chambers of the human heart [45], and both exendin-4 and GLP-1 might exert protective effects in the setting of diabetic cardiomyopathy and myocardial ischemia, preserving the pool of cardiac progenitor cells through activation of **p-CREB** and reduction of **p-JNK**-mediated **apoptosis** [48,49]. GLP-1R agonism may reduce CV inflammation and atherosclerosis through multiple, yet not fully ascertained mechanisms [50,51]. Indeed, several additional observations on GLP-1 affecting multiple biological responses relevant to CV protection have been made. However, the salutary role of GLP-1RA on cardiac metabolism, decreasing lipolysis in favor of glucose oxidation, has to be proven in the setting of insulin resistance [52].

Concluding Remarks and Future Perspectives

The results from CVOT with GLP-1RA are apparently puzzling with some studies showing CV protection [5,6,8,9], two studies missing the statistical significance for superiority [7,10], and one study not even suggesting a benefit [4]. Such a heterogeneity regarding the primary outcome is even higher when considering the individual MACE components [4–10].

The characteristics of the T2D population define the level of CV risk and number of events that can be observed during the study follow-up, thus potentially influencing the ability of the GLP-1RA being tested to affect the outcomes. If GLP-1RA can interfere with the atherosclerotic process [45,46,51–55], it is conceivable that this effect will be more evident in a population with established atherosclerotic disease. As seen with cholesterol-lowering drugs, which provide progressively greater risk reductions in MACE in patients with higher baseline LDL-cholesterol level [56], the likelihood to see CV protection from GLP-1RA could be greater the higher the CV risk of the exposed T2D subjects. However, this conclusion is apparently at odds with the lack of benefit in ELIXA [4], in which patients were at the highest CV risk, and the CV superiority of dulaglutide vs. placebo both in primary and secondary prevention cohorts in REWIND, the CVOT enrolling the lowest-risk patients [9]. Indeed, the CV benefit of GLP-1RA could be observed also in lower-risk T2D patients, provided that the observation is sufficiently extended to collect a statistically adequate number of MACE. Accordingly, in our analysis, we could not find any association between the MACE rates in the control arm, expressing the overall CV risk, and GLP-1RA-induced ARR of MACE.

Despite the potential limitations of our analysis, we believe that much attention should be payed to the percentage of time patients are exposed to the GLP-1RA, as this parameter appears to be correlated with the MACE ARR and HR (Figure 2). The extent of exposure to the investigational drug is likely to be influenced by type of trial (explanatory vs. pragmatic) and its design, pharmacokinetics, and adherence and persistence to treatment. The low extent of drug exposure to lixisenatide in ELIXA [4] and exenatide LAR in EXSCEL [7], due to different reasons, likely hid any statistically significant CV benefit of these two GLP-1RA. In PIONEER 6, 84.7% of patients assigned to the intervention arm were on treatment at their last visit, most of whom were taking the highest dose of the study drug (69.5%) [10]. Oral semaglutide was characterized by a favorable tolerability profile in Phase III studies [36,37,57–59], and a high level of therapeutic adherence is associated with low discontinuation rates and extended drug exposure [60,61], the grounds enabling the CV properties of GLP-1RA to become clinically evident. However, the percentage of time exposure to oral semaglutide across the trial has not been disclosed [10], and thus we did not include data from PIONEER 6 in our analysis. Moreover, in PIONEER 6 a relevant proportion of patients in the intervention arm was not on the highest dose of the study drug (i.e., 15.2%) [10].

When comparing GLP-1 RA with usual care, differences in HbA1c, blood pressure, body weight, and lipids do not seem to provide the clue for why some CVOT were successful and others

Outstanding Questions

All GLP-1RA showed a different impact on single components of the primary endpoint; is it due to the play of chance or to molecule-specific tropism towards different tissues (e.g., see the effects of subcutaneous semaglutide and dulaglutide on stroke prevention)?

EXSCEL failed to show CV benefit of exenatide LAR, perhaps because of relatively high discontinuation rate. Will results of FREEDOM-CVO with ITCA 650, evaluating continuous exenatide delivery via a subcutaneous implanted pump, rule out the issue of low patient exposure to this investigational drug? However, FREEDOM-CVO is not powered for superiority.

PIONEER 6 is the CVOT with the shortest follow-up, and both PIONEER 6 and SUSTAIN-6 involved fewer patients than other CVOT. Could the divergent findings on all-cause death between subcutaneous and oral semaglutide in SUSTAIN-6 (no change) and PIONEER 6 (reduction) be explained by a too small number of events collected in these two trials?

The proportion of time patients were exposed to oral semaglutide in PIONEER 6 is not known, yet supposed to be high due to its route of administration and overall tolerability. Will this fit the model 'the higher the exposure, the greater the benefit'?

Will results from genetic testing on participants to EXSCEL and HARMONY Outcomes be able to clarify if any gene variants are associated with GLP-1RA-mediated CV efficacy?

Higher resting heart rate is associated with poor outcomes; could the trend towards lesser CV efficacy hinted for patients with heart failure in a subgroup analysis of SUSTAIN-6 be due to the GLP-1RA-mediated heart rate increase in these high-risk patients?

Will head-to-head comparisons in preclinical and clinical models, if available, discover differences in the direct anti-atherosclerotic effects of distinct GLP-1RA?

were not. In general, differences in HbA1c in EXCEL [7] and HARMONY Outcomes [8] were smaller than in SUSTAIN-6 [6] and similar to those in LEADER [5]. Interestingly, the CV benefit of dulaglutide in REWIND occurred in patients with a baseline HbA1c level close to 7%, much lower than in all other trials [9]. Overall, there is no consistent correlation between changes in these CV risk factors and the observed results, highlighting that the CV benefit of GLP-1RA may rely in their ability to directly halt the atherosclerotic process [51–55]. Indeed, GLP-1RA may be complementary to traditional optimal control of CV risk factors, as recently suggested [39].

Preliminary evidence shows that dual agonists may have beneficial effects on heart metabolism [glucagon-GLP-1, glucose-dependent insulinotropic polypeptide (GIP)-GLP-1] and atherosclerotic plaques (GIP-GLP-1). Will these agents promote a similar or greater CV benefit compared to GLP-1 mono-agonists?

Exendin-4-based and GLP-1-based agonists have the potential to differ in signaling and biological effects, yet preclinical and clinical studies all agree on attributing to both exendin-4 and human GLP-1 based GLP-1RA the same beneficial effects on heart metabolism and viability, endothelial function and integrity, and systemic inflammation [33,34,45].

Thus, it seems reasonable to consider CV protection a 'class effect' of GLP-1RA tightly dependent upon the time of drug exposure. Future research is needed to fully disclose potential differences among specific molecules (see Outstanding Questions).

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