Does Glucagon-like Peptide-1 Ameliorate Oxidative Stress in Diabetes? Evidence Based on Experimental and Clinical Studies

Karen Ekkelund Petersen\textsuperscript{a,b}, Günaj Rakipovski\textsuperscript{c}, Kirsten Raun\textsuperscript{b} and Jens Lykkesfeldt\textsuperscript{a,*}

\textsuperscript{a}Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; \textsuperscript{b}Department of Incretin & Obesity Pharmacology and \textsuperscript{c}Department of Diabetes Pharmacology, Novo Nordisk A/S, Maaloev, Denmark

Abstract: Glucagon-like peptide-1 (GLP-1) has shown to influence the oxidative stress status in a number of \textit{in vitro}, \textit{in vivo} and clinical studies. Well-known effects of GLP-1 including better glycemic control, decreased food intake, increased insulin release and increased insulin sensitivity may indirectly contribute to this phenomenon, but glucose-independent effects on ROS level, production and antioxidant capacity have been suggested to also play a role. The potential ‘antioxidant’ activity of GLP-1 along with other proposed glucose-independent modes of action related to ameliorating redox imbalance remains a controversial topic but could hold a therapeutic potential against micro- and macrovascular diabetic complications. This review discusses the presently available knowledge from experimental and clinical studies on the effects of GLP-1 on oxidative stress in diabetes and diabetes-related complications.

Keywords: Glucagon-like peptide-1, oxidative stress, diabetes, diabetic complications.

1. INTRODUCTION

Diabetes can lead to the development of late complications described as microvascular complications including retinopathy, nephropathy, neuropathy and macrovascular complications comprising cardiovascular diseases (CVD) [1], the latter being the leading cause of death among diabetic patients [2, 3]. The mechanisms underlying the development of complications in diabetes are still not completely understood. However, a hypothesis involving oxidative stress (OS) and inflammation as potent causative initiators of diabetic complications in general has been put forward [4, 5].

Glucagon-like peptide-1 (GLP-1) constitutes a relatively recent therapeutic option for type 2 diabetic patients. GLP-1 receptor agonists, as the approved pharmaceuticals liraglutide, exenatide, lixinatide, albiglutide and dulaglutide are designed to be more resistant to degradation than endogenous GLP-1 that with a half-life of less than two minutes has indirect effects making it important to investigate if these effects are dependent or independent of GLP-1’s effects on glucose homeostasis (insulinotropic and glucose-lowering effects). The present review discusses the available literature on the role of GLP-1 as a redox modulator.

2. DIABETES AND OXIDATIVE STRESS

The “unifying theory” proposed by Brownlee [4] in 2001 suggests that hyperglycemia - a hallmark of both type 1 and type 2 diabetes (T1D and T2D) - can lead to increased OS if the antioxidant capacity is exceeded. OS is characterized as an imbalance between produced reactive oxygen species (ROS)/reactive nitrogen species (RNS) and the antioxidant capacity. According to the hypothesis, OS plays a pivotal role in the development of diabetes related late complications in both T1D and T2D [4, 25, 26]. It should be
mentioned that OS may also play an important role in the development of both types of diabetes (reviewed in [27]). In short, β-cells are vulnerable to injury from high levels of ROS due to a comparatively low expression of antioxidant enzymes [28] and consequently, elevated OS conditions are likely to cause β-cell loss [28]. Importantly, loss of these cells cannot readily be restored because of their long lifespan and low proliferation rate [29, 30]. The loss of β-cells in turn results in further increasing blood glucose, OS and insulin resistance, thus starting a vicious circle (discussed in 2.1.).

2.1. Sources of Oxidative Stress in Diabetes

OS as measured both directly as ROS and by a large variety of biomarkers (Fig. 1) has consistently been shown to be elevated in diabetes [4, 14]. Mitochondrial production of the ROS, superoxide and increased activation of the protein kinase C (PKC) pathway in diabetic vascular tissue have been hypothesized to be major contributors to excess ROS production together with enzymatic systems such as nicotinamide adenine dinucleotide phosphate-oxidase (NOX), which is a membrane-bound superoxide producing enzyme [4]. Elevated plasma concentrations of glucose and free fatty acids (FFA), which is observed in T2D and poorly controlled T1D subjects seems to be the main drivers of ROS production [5, 31-34]. This is also the case in other disorders with elevated FFA’s as dyslipidemia and obesity (reviewed in [35]). Proinflammatory cytokines and excessive levels of nutrients can increase expression of phagocytic-like NOX in rat β-cells [36]. Thus, OS is an important factor in glucotoxicity and lipotoxicity as well as the combined glucolipotoxicity [37]. Superoxide produced under these conditions has shown to damage and impair mitochondrial integrity and function in vivo and in vitro [38, 39]. Mitochondrial dysfunction also leads to increased ROS leakage from the electron transport chain, and has been associated with induction of insulin resistance resulting in higher blood glucose levels [40]. Fig. (2) summarizes the putative mechanism leading to OS in diabetes.

2.2. Oxidative Stress and Diabetic Late Complications

Diabetes induced OS has been suggested to lead to both micro- and macrovascular complications. ROS may induce wide spread damage to cells including e.g. endothelial, retinal, mesangial and neural cells leading to the development of diabetic complications [5].

Damage is collectively caused by both direct effects on cellular components and damaging cells [41, 42] and indirectly through activation of the above mentioned pathways subsequently affecting cellular function (Fig. 2) and through induction of inflammation [4, 43]. Circulating ROS affect endothelial function and initiates inflammatory processes, both of which are thought to play an essential role in early atherogenesis (with atherosclerosis being the very core of diabetic macrovascular complications) as well as in hypertension and heart failures [4, 44-46].

Endothelial dysfunction, characterized by an imbalance between relaxing and contracting factors released by the endothelium, is seen in the early stages of atherosclerosis [56-58]. The vasodilator nitric oxide (NO) is produced by endothelial NO synthase (eNOS) [59]. OS contributes to a decreased NO bioavailability in several ways. OS lowers the production of NO through uncoupling of eNOS, turning production towards superoxide (Fig. 2). Degradation of NO is enhanced, as NO reacts efficiently with superoxide forming the deleterious peroxynitrite and cellular damage is induced [60, 61]. ROS can inactivate eNOS and other anti-atherosclerotic enzymes such as prostacyclin synthase and increase proatherosclerotic events by affecting vascular tone (through activation of PKC, increased flux in the polyol pathway and increased levels of AGE) [44, 62]. Monocytes infiltrate the vascular wall and differentiate into macrophages, which can accumulate oxidized low density lipoproteins (oxLDL) leading to the formation of foam cells [63]. These events in turn stimulate macrophage proliferation, T-lymphocyte attraction, smooth muscle cell proliferation and collagen accumulation, collectively resulting in plaque formation. OxLDL is able to activate NOX and appear to contribute to the development of atherosclerosis through this mechanism as well [55].

Diabetes has been diagnosed in approximately 400 million people worldwide [64] and diabetic late complications is likely to affect a large proportion of these. Trials investigating the relationship between blood glucose status and devel-
Fig. (2). Putative mechanisms leading to OS in diabetes. Five pathways are potentially activated, 1) Enlarged flux of monosaccharides through the polyol pathway [5]. 2) Increased formation of advanced glycation end-product (AGE) and 3) expression of advanced glycation end-product receptors (RAGE) [47]. 4) Activation of PKC [5] and 5) increased flux in the hexosamine pathway [5]. These are all downstream pathways from one common event, the production of ROS [5]. Damages through activation of the polyol pathway are conducted as NADPH is degraded, a process leading to a lower level of glutathione (GSH), thus decreasing antioxidant capacity. In addition, the pathway increases the level of the NOX substrate, NADH, thus favoring ROS production [48]. AGEs are formed when proteins or lipids are glycated in contact with for example glucose or other aldose sugars [49], and when these bind to RAGE the result can be production of ROS through activation of NOX [50, 51], xanthine oxidase (XO) and the mitochondrial electron transport chain [51]. The protein kinase C (PKC) pathway is stimulated when diacylglycerol (DAG) levels are elevated during hyperglycemia. The increased activity of PKC has many different effects on various genes, leading to changes in blood flow, increased inflammation and ROS production (by NOX) [52, 53]. A consequence can be an increase in NF-κB and release of inflammatory cytokines and growth factors in macrophages or mesangial cells [5]. Elevated glucose and FFA concentrations increase flow through the hexosamine pathway as well as induce OS [54]. The activation of these pathways initiates further ROS production. ROS can damage macromolecules including LDL. The resulting oxidized LDL (oxLDL) can lead to further NOX activation and thus further superoxide production [55]. The increase in ROS can uncouple eNOS switching eNOS from NO to superoxide production. This can lead to endothelial dysfunction (further described in section 2.2). The association between hyperglycemia, elevated FFA levels, mitochondrial dysfunction and ROS constitutes a vicious circle, in which the production of ROS during hyperglycemia worsens insulin resistance and thus further induce redox imbalance. These mechanisms are in particularly activated in cell types that are involved in the development of diabetic complications, as they are susceptible to hyperglycemic damage. These cells have a low capacity to reduce intracellular hyperglycemia, and include e.g. endothelial cells, mesangial cells, neurons and Schwann cells in peripheral nerves.

2.3. GLP-1 and Oxidative Stress

The anti-inflammatory action of GLP-1 has recently gained considerable interest as endogenous GLP-1 has been found to be increased in patients with chronic inflammatory diseases, where OS is implicated, as systolic heart failure (bearing in mind that atherosclerosis can cause heart failure) and to be associated with coronary plaque burden in patients [18, 19, 70]. Moreover, GLP-1 intervention has been shown to decrease inflammation in both preclinical in vivo [21, 71-74] and clinical studies [75-77]. While some studies have incorporated various measures of OS, this area deserves more focused attention. The present review discusses the current literature on GLP-1 with particular focus on the putative effects on OS status beyond anti-hyperglycemia and appetite regulation. The G-protein coupled GLP-1 receptor (GLP-1R) is found in a wide variety of cells. In pancreatic islets, activation of this receptor increases intracellular Ca²⁺, stimulates adenylate cyclase (increasing production of cAMP) and phospholipase C, and activates a number of pathways including protein kinase A (PKA), PKC, cAMP response element-binding protein (CREB), phosphatidylinositol-3 kinase, exchange protein activated by cAMP 2 (Epac2), and mitogen-activated protein kinase pathways ([78] and reviewed in [79]). Both cAMP and PKA are inhibitors of...
Current Diabetes Reviews, 2016, Vol. 12, No. 4 Petersen et al.

NOX [80-83]. However, some of these pathways vary from cell type to cell type, e.g. isoforms of PKC activates NOX in vascular tissues, where GLP-1 has here been shown to inactivate PKC and thus ROS production by NOX [84]. The role of GLP-1 in different cell types is under investigation and depends on various factors, such as stimuli (e.g. nutrient affecting the cells), species and the expression of the GLP-1R which has to be investigated with sensitive and specific antisera and detected in full length by PCR analysis [85]. Moreover, GLP-1 has been suggested to increase antioxidant capacity. Fig. (3) summarizes potential pathways through which GLP-1 may affect OS status and the following chapters discuss the in vitro, in vivo and clinical literature on GLP-1 and OS.

3. GLP-1 AND OXIDATIVE STRESS IN VITRO

Numerous in vitro studies have shown that GLP-1 and GLP-1 agonists reduces ROS and protects against apoptosis in several cell types when exposed to different stress factors e.g. high glucose levels or hydrogen peroxide [20, 21, 86-90] (Table 1). However, the observed effects on apoptosis in a number of these studies may not strictly be attributed to effects via OS, as GLP-1 per se activates antiapoptotic genes and thus independently decreases apoptosis [91]. But in general, in vitro studies have reported decreased levels of ROS [20, 21, 76, 84, 88-90, 92-99], NOX subunits or activity [20, 21, 84], malondialdehyde (MDA) [90, 95, 100] and increased levels of antioxidants and/or genes involved in antioxidant activity [21, 86, 87, 90, 92, 95, 100-103] following GLP-1 exposure. Whether these observed effects are GLP-1R dependent or independent can be investigated by using cleavage products of GLP-1 known not to interact with the receptor. The GLP-1 cleavage product, GLP-1(9-36) is not involved in glucose homeostasis [104], but has been shown to have cytoprotective effects on mouse cardiomyocytes exposed to hydrogen peroxide [105] and to decrease super...

\[ \text{Fig. (3). Putative mechanisms by which GLP-1 affects OS status by glucose independent mechanisms in cells expressing the GLP-1R and glucose dependent mechanisms in cells ± GLP-1R expression. Black arrows indicate glucose dependent mechanisms, white arrows glucose independent and grey indicates pathways that can be affected by both glucose dependent and glucose independent GLP-1 effects. Black/white arrow indicates that mechanism has not been clarified. GLP-1 lowers OS status through different mechanisms with the glucose dependent effects being the most apparent through reduced food intake, blood glucose and FFA. This reduces the ROS formed by the mitochondria which can decrease the activation of the five pathways outlined in Fig. (2). Glucose independent effects are mediated through GLP-1R stimulation, which can affect pathways involved in ROS formation. Stimulation of GLP-1R leads to increased levels of cAMP in the cell, which results in lower levels of ROS being produced by NOX and XO. The decrease in NOX activity can also be obtained by GLP-1 inactivation of PKC. Increased cAMP level by GLP-1R activation can increase PKA activity and by this route decrease the Rho/ROCK pathway leading to a decreased ROS production. The latter mentioned pathway can be activated by hyperglycemia e.g. though AGE formation (review in [69]) and can thus both be affected by glucose dependent and independent pathways. Decreases in AGE/RAGE interaction can lower XO, NOX and mitochondrial ROS production. Increased cAMP can lower the activity of Src kinase, which is an activator of NOX. This results in decreased NOX activity and ultimately lower superoxide production. Increased antioxidant capacity favors redox homeostasis and has been shown to be induced by GLP-1R activation and/or increased Nrf2 concentration or expression by GLP-1. A decreased ROS production can affect various pathways as PI3K/Akt and p38 MAPK and JNK activities resulting in a decrease in inflammatory (by decreasing NF-κB activity) and apoptotic pathways as discussed in the text.} \]
Table 1. *In vitro* studies of GLP-1 and oxidative stress.

| Species | Cell Type | Study Design: Intervention (I), Duration (D) | Effect of GLP-1 Intervention on Oxidative Stress Markers | Survival/Apoptosis | Mechanisms | Comments |
|---------|-----------|---------------------------------------------|----------------------------------------------------------|-------------------|------------|---------|
| Mouse | Isolated mouse (C57BL/6 ± DIO) hepatocytes + H4IIE | I: GLP-1(28-36) amide (10 or 100 nmol/l) + tBHP (0.5 mmol/l). D: 24 hours prior to tBHP for 1 hour. | GLP-1(28-36)amide: ↓ ROS (CM-H2DCFDA by spectrofluorometer) (hepatocytes: p<0.028 for both ± DIO), H4IIE: p<0.006 (100 nmol/l) and p<0.002 (10 nmol/l). | | | [98] |
| Mouse | Atrial HL cardiomyocytes | I: GLP-1 (100 or 200 nmol/l), palmitate (750 μM). D: 30 minutes preincubation ± GLP-1 (7-36) then 6 hours + palmitate. | GLP-1 (7-36): ↓ XO activity (p<0.05). ↓ ROS and RNS (p<0.05) (dichlorodihydrofluorescin DioxyQ). ↑ GSH/GSSG ratio (200 nmol/l) (p<0.05). ↓ SOD activity (200 nmol/l) (p<0.05). | | | [116] |
| Mouse | Mouse pancreatic islet | I: GLP-1 (10 nmol/l) + glucose (20.8 mmol/l). D: 90 minutes. | GLP-1: ↓ Superoxide level (nitroblue tetrazolium assay) (p<0.05). | | | | |
| Hamster | Hamster pancreatic β-cell line (HIT-T15) | I: GLP-1 (10 nmol/l) in GS containing medium. D: 5 days. | GLP-1: ↓ ROS (p<0.01) (DCFH-DA probe). ↑ GR protein expression, thus only significant (p< 0.05) in medium control cells. | | | GLP-1: ↑ Nrf2 protein expression (p<0.01). ↑ MafA and PDX-1 protein expression (p<0.05). | [92] |
| Hamster | Hamster pancreatic β-cell line (HIT-T15) | I: GLP-1 (10 nmol/l) in GS containing medium. D: 5 days. | GLP-1: No effect on GSSG levels. ↑ GSH (p<0.001). GLP-1 increased proliferation (p<0.01, when compared with control medium, p<0.001 when compared to GS medium) rate and restored LDH release + caspase-3 activity to control cell levels. | | | | |
| | | | | | | | ROS may play an antagonistic role in the adenylate cyclase/cAMP/PKA pathway of insulin secretion [97]. |
| Species                          | Cell Type                        | Study Design: Intervention (I), Duration (D)                                                                 | Effect of GLP-1 Intervention on Oxidative Stress Markers                                                                 | Survival/Apoptosis                                                                 | Mechanisms                                                                                                                                                                                                 | Comments                                                                                                                                                                                                 |
|---------------------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cardiomyoblasts (H9c2)          |                                  | I: Exenatide treatment (1 nmol/l) prior to H<sub>2</sub>O<sub>2</sub> (200 µmol/l) exposure. D: 30 minutes     | Exenatide: ↓ ROS (DCFH-DA and flow cytometry) and ↓ MDA (p<0.05). ↑ T-SOD (p<0.05).                                           | Exenatide: ↑ viability (p<0.05) ↓ LDH and CK-MB release (p<0.05).                        |                                                                 | Protective effects of exenatide on glucose or H<sub>2</sub>O<sub>2</sub>-induced cell death might be downstream from OS. PKA dependent effect associated with activation of SERCA2a [109]. |
| Neonatal rat ventricular myocytes (NRVM) |                                  | I: Exendin-4 (30 mmol/l) ± D-glucose (5 or 33 mmol/l) or H<sub>2</sub>O<sub>2</sub> (250 µmol/l). D: 24-48 hours. | Exendin-4: No effect on ROS level (DHR123 fluorometry). Exendin-4: ↓ cell death (p<0.05) (trypan blue). No change in ROS level. | Exendin-4: ↓ Expression of markers of ER stress (CHOP, GRP78 and PDI).               |                                                                 |                                                                                                                                                                                                         |
| Rat Cardiac microvascular endothelial cells |                                 | I: GLP-1 (10<sup>-10</sup>, 10<sup>-7</sup>, 10<sup>-6</sup> or 10<sup>-5</sup> mol/l) ± glucose (25 mmol/l). D: 24 hours. | GLP-1 in high-glucose-medium: ↓ superoxide (lucigenin-enhanced chemiluminescence assay and DHE staining) (p<0.05 and 0.01 for 10<sup>-8</sup> mol/l). ↓ NOX activity (lucigenin-enhanced chemiluminescence assay) (p<0.05 and 0.01 for 10<sup>-8</sup> mol/l). ↓ p47<sub>phox</sub>, gp91<sub>phox</sub>, p22<sub>phox</sub> and p40<sub>phox</sub> protein level (p<0.05). | GLP-1 in high-glucose-medium: ↓ apoptosis (p<0.05) (terminal deoxynucleotidyl TUNEL assay). ↓ caspase-3 (p<0.05). | GLP-1 effects might be through cAMP/PKA/Rho-dependent mechanisms. + in vivo study. [20]                                                                 |                                                                                                                                                                                                         |
| Neonatal rat ventricular myocytes (NRVM) |                                 | I: Exendin-4 (0.1-10 nmol/l) + high glucose exposure (25 mmol/l). D: 12 hours.                               | Exendin-4: ↓ MDA concentration (p<0.05 for 1 and 10 nmol/l). ↓ SOD activity (p<0.05 for 1 and 10 nmol/l).                  | Exendin-4: ↓ LDH (p<0.05 for 1 and 10 nmol/l). ↓ CK (p<0.05 for 1 and 10 nmol/l).       | ↓ Rho (p<0.05). Fausudil and H89 reduced the effects of GLP-1.                                                                 | Hypothesis: (Hyperglycemia-induced) OS -> inflammatory cytokine secretion [100].                                                                                                                                |
| PC12                            |                                  | I: Methylglyoxal (1 mmol/l) ± GLP-1 (3.30 µg/ml). D: 30 min preincubation with GLP-1, then methylglyoxal for 24 hours. | GLP-1: ↑ GSH (p<0.05). ↑ GSH/GSSG (p<0.001).                                                                            | GLP-1: ↓ apoptosis (DAPI staining) (p<0.001).                                          | Rapamycin (mTOR inhibitor) reduced effects on GSH (p<0.05) and GSH/GSSG (p<0.001).                                                                 | P3K/Akt/mTOR/GCN pathway. [86]                                                                                                                                                                                                 |

Table 1. contd….
Table 1. contd….

| Species                | Cell Type                  | Study Design: Intervention (I), Duration (D)                                                                 | Effect of GLP-1 Intervention on Oxidative Stress Markers                                                                 | Survival/Apoptosis | Mechanisms                                                                                     | Comments                                                                 |
|------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Rat insulinoma cells   | (INS-1)                    | I: Exendin-4 (10 or 100 nmol/l) + IFN-γ (1 ng/ml) and IL-1 β (4 ng/ml) D: 5 hour preincubation with exendin-4 then 20 hours of IFN-γ and IL-1 β exposure. | Exendin-4: ↓ ROS (p<0.01 (10 nmol/l) and p<0.001 (100 nmol/l)) (DCFH-DA probe). Exendin-4: ↓ caspase-4 activity (p<0.001). |                    | Exendin-4: Actions on different proteins in the electron transport chain, e.g. NADH-ubiquinone oxidoreductase 75 kDa SU ↓ (p<0.05). ↑ cAMP dependent PKA (p<0.001). | [88]                                                                    |
| Cardio-myoblasts       | (H9c2)                     | I: Exenatide (50-600 nmol/l) + H/R (hypoxia chamber). D: 30 min prior to H/R.                                   | Exenatide (200 nmol/l): ↓ ROS (DCFA-DA fluorescence) (p<0.05). ↑ T-SOD (p<0.05). ↓ MDA (p<0.05). |                    | Exenatide: ↑ Viability (p<0.05 for 100, 200 and 400 nmol/l). ↓ Apoptosis (p<0.05 (200 nmol/l)). ↓ Caspase-3 (cleaved and activity) (p<0.05 (200 nmol/l)). | Improvement of mitochondrial function [95].                              |
| Rat insulinoma cells   |                             | I: Exendin-4 (20 nmol/l) + 0, 5, 20 and 40 nmol/l glucose. D: 4 hours.                                         | Exendin-4: ↓ TxNIP. Exendin-4: ↓ capase-3.                                                                     |                    | Forskolin and PDE → similar results as Exendin-4. Inhibitors: PKA or ePAC → attenuated effect of forskolin. | cAMP/PKA and/or ePAC and/or PDE pathway leading to ubiquitination and proteasomal degradation of TxNIP [103]. |
| Goto-Kakizaki islet cells |                            | I: Exendin-4 (100 μmol/l) + glucose (16.7 mmol/l after preincubation with 2.8 mmol/l for 20 minutes). D: 60 minutes. | Exendin-4: ↓ ROS (CM-H2DCFDA) (p<0.005). No effect on MnSOD activity.                                             |                    | Srk kinase inhibition by PP2 → ↓ ROS (p<0.05) (exendin-4 and PP2 did not further reduced ROS). Adenylate cyclase activator (forskolin) → ↓ ROS (p<0.01). Exendin-4 or forskolin + PKA inhibition (H-89) → no effect on the decreased ROS. General cAMP analog and cPAC specific cAMP analog → ↓ ROS (p<0.001),PK3 inhibitors (LY294002 and wortmannin) → ↓ ROS (p<0.01). MAPK/ERK kinase inhibitor (PD98059) → no ROS effect. EGFR kinase inhibitor (AG1478) → ↓ ROS (p<0.001). | The effects of exendin-4 and Src inhibitor (PP2) could not be produced in Wistar islet cells [112]. |
Table 1. contd....

| Species                        | Cell Type                               | Study Design: Intervention (I), Duration (D)                                                                 | Effect of GLP-1 Intervention on Oxidative Stress Markers                                                                 | Survival/Apoptosis                                          | Mechanisms                                                                 | Comments                                                                 |
|--------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Rat INS-1E and rat pancreatic islet cells. |                                         | I: Exendin-4 (10 nmol/l) pretreatment ± cytokines (10 ng/ml IL-1β, 50 ng/ml TNF-α, 50 ng/ml IFN-γ). D: Exendin-4 for 18 hours followed by cytokines for 18 hours. | Exendin-4: INS-1E: ↓ viability (p<0.01). INS-1E + islet cells: ↓ apoptosis and necrosis. | Exendin-4: ↓ phosphorylation of PKB (required for the protective effects shown). | Exendin-4 levels are mentioned to have been measured, but no data shown. Exendin-4 did not affect levels of either antioxidant. Conclusion: The reduction in ROS might be through lowering of ROS production rather that elimination. [94] |
| Human umbilical vein endothelial cells (HUVECs) |                                         | GLP-1 (10 nmol/l) or exendin-4 + (30 mol/l). D: GLP-1 for 30 minutes prior to H2O2 for one hour.             | GLP-1 or exendin-4: ↓ expression of NQO1 and HO-1 (p<0.05). GLP-1: ↑ expression of NQO1 (p<0.05). | GLP-1: ↑ CREB (p<0.05). Receptor mediated: exendin9-39 did not have any effect. Effects blocked by H89 or forskolin. | cAMP/PKA-dependent pathway involved. [102] |
| Human HUVECs                  |                                         | GLP-1 (0.03 or 0.3 nmol/l) in glycated bovine serum albumin (100 µg/ml). D: 4 hours.                          | GLP-1: ↓ superoxide (DHE staining) (p<0.01). Liraglutide or metformin: ↓ superoxide (DHE staining) (p<0.01). Liraglutide and metformin: ↓ superoxide (DHE staining) when compared to liraglutide or metformin alone (p<0.01). ↓ NOX activity (p<0.05). Prevention of p47 subtranslocation. | No effects on viability (> 94% in all samples). Liraglutide: ↓ DAG (p<0.01) ↑ AMPK phosphorylation (p<0.05) ↓ PKC (p<0.05) | Combination of liraglutide and metformin further reduced ROS production. Both by inhibition of the PKC-NOX pathway. [84] |
| Human aortic endothelial cells (HCAECs) |                                         | Liraglutide (30 nmol/l) and/or metformin (10 µM) treatment prior to high glucose exposure (25 mM). D: 48 hours. | Liraglutide or metformin: ↓ superoxide (DHE staining) (p<0.01). Liraglutide and metformin: ↓ superoxide (DHE staining) when compared to liraglutide or metformin alone (p<0.01). | No effects on viability (> 94% in all samples). Liraglutide: ↓ DAG (p<0.01) ↑ AMPK phosphorylation (p<0.05) ↓ PKC (p<0.05) | Combination of liraglutide and metformin further reduced ROS production. Both by inhibition of the PKC-NOX pathway. [84] |
| Species               | Cell Type               | Study Design: Intervention (I), Duration (D) | Effect of GLP-1 Intervention on Oxidative Stress Markers | Survival/Apoptosis | Mechanisms                                      | Comments |
|-----------------------|-------------------------|----------------------------------------------|--------------------------------------------------------|-------------------|------------------------------------------------|----------|
| HCAECs                | I: Exendin-4 (0.1-10 nmol/l) during palmitate exposure (125 μmol/l). D: 24 hours. | Exendin-4: ↓ ROS (DCFH-DA probe) (p<0.05). Exendin-4: ↓ DNA fragmentation (p<0.05 for 10 nmol/l). | GLP-1 dependent pathway (effects eliminated by exendin (9-39)) and better effects of GLP-1 than exendin-4 (not significant). No effect of GLP-1 (9-36). | Incubation with BH4 resulted in same reduction in ROS as exendin-4 (p<0.05). The effects on apoptosis might be through lower activation of PI3K/Akt pathway (which can be sensitive to ROS). | [89] |
| HUVECs                | I: Liraglutide (3, 30 or 300 nmol/l) + TNF-α (5 ng/ml). D: Liraglutide for 30 minutes prior to TNF-α for 90 minutes to 24 hours. | Liraglutide: ↓ ROS (CM-H2DCFDA by FACS calibur) (p<0.01 (30 nmol/l) and p<0.05 (300 nmol/l)). ↓ gp91phox (protein level and mRNA expression), p22phox (mRNA expression) (p<0.05). ↑ SOD-2 and CAT mRNA expression (p<0.05) (↑ SOD-1 and GPx (NS)). ↑ SOD-2, CAT (p<0.05) and GPx (p<0.01) protein level (↑ SOD-1 (NS)). | Liraglutide: ↓ Apoptosis (p<0.05 (3nmol/l) and p<0.01 (30 nmol/l)) (Annexin V-FITC apoptosis kit). Liraglutide (30 nmol/l): Eliminated PKC-α activation seen by TNF-α exposure. | Effects on ROS levels not blocked by exendin (9-36). NF-κB activity decreased by liraglutide. | [21] |
| Human proximal tubular cells | I: GLP-1 (0.2 nmol/l) + 100 μg/l AGE. D: 4 hours. | GLP-1: ↓ Superoxide (DHE staining) (p<0.01). ↓ ADMA levels (p<0.01). | GLP-1: ↓ RAGE mRNA level (p<0.01). This action was inhibited by siRNA’s against GLP-1R and RAGE. 8-Br-cAMP had same effects on RAGE expression as GLP-1. | RAGE gene suppression by GLP-1R-cAMP axis [93] |
| Human mesangial cells | I: GLP-1 (0.03 or 0.3 nmol/l) + AGE-BSA (100 μg/ml). D: 4 hours. | GLP-1: ↓ Superoxide (DHE staining) (p<0.01) (Br-cAMP had same effect). | GLP-1: ↓ RAGE mRNA level (p<0.01) (in bovine serum albumin alone). This action was inhibited by siRNA’s against GLP-1R and RAGE. 8-Br-cAMP had same effects on RAGE expression as GLP-1. | RAGE gene suppression by GLP-1R-cAMP axis. GLP-1 decreased MCP-1 (p<0.01) mRNA and protein. | [99] |
oxide levels in hippocampal slices [106]. This suggests that the effects may not only be mediated through the glucose-lowering effects of GLP-1 or by binding and signaling through the GLP-1R. The following sections examine the available data on GLP-1 mediated effects on antioxidant levels, ROS production, quenching of ROS, and protections against ROS induced damage.

3.1. GLP-1 Effect on Antioxidant Level

Intervention with GLP-1 has been shown to upregulate superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities and increase glutathione (GSH) concentrations in media containing stressors such as glucose or hydrogen peroxide [21, 86, 87, 90, 95, 100], PKA activation following GLP-1R stimulation has been shown to protect endothelial cells against ROS through increased mRNA levels of NADPH dehydrogenase quinone 1 (NQO1) and heme-oxygenase 1 (HO-1) [102]. NQO1 and HO-1 both have some antioxidant activity [107, 108] and NQO1 also functions as a superoxide scavenger [107]. While these studies indicate an effect of GLP-1 on antioxidant capacity, increased levels of antioxidants could also result from adaptation to higher ROS levels, or decreased consumption through a lower ROS production and consequently, ROS levels and production should also be assessed [108].

3.2. GLP-1 Effect on ROS Level

GLP-1 is generally effective in lowering ROS levels in vitro [20, 21, 36, 76, 84, 88-90, 92-99]. However in one study, neonatal rat cardiomyocytes exposed to either high glucose concentrations or hydrogen peroxide showed that exendin-4 attenuated apoptosis without lowering ROS levels [109]. This result could suggest that effects of exendin-4 on apoptosis are downstream to that of OS, but differences in the study setup (cell type, glucose concentration and exposure time) may indicate otherwise [20, 84, 100]. A different technique was used for detection of ROS (dihydrorhodamine 123 (DHR123)), which primarily detects peroxynitrite, whereas neither hydrogen peroxide nor superoxide alone oxidizes DHR123 significantly [110]. If exendin-4 is able to reduce other reactive species than peroxynitrite, it would not be detected in this study. Thus, the technique used for measuring ROS levels is of major importance and overall conclusion on the levels of ROS cannot be done by only using techniques specific for only one type of ROS. Another important factor is the cell type. Potential glucose independent effects of GLP-1 is influenced by expression of the GLP-1R (as described in 2.4) and details in the expression of this in different cell types of the heart is still debated (reviewed in [111]). A study in Goto-Kakizaki rat islet cells exposed to high levels of glucose (16.7 mmol/l) showed a ROS concentration increase. Exendin-4 reduced this elevation in ROS without affecting MnSOD activity. Here different signaling
pathways of GLP-1 were investigated by adding a Src kinase inhibitor, an adenylate cyclase activator, cAMP and an ePAC-specific cAMP analog, a phosphatidylinositol-3 kinase inhibitor and an epidermal growth factor receptor kinase inhibitor, all of which led to decreased ROS concentrations, while PKA or mitogen-activated protein kinase extracellular signal-regulated (MAPK/ERK) kinase did not affect ROS concentrations. This study indicates that GLP-1 acts through suppression of Src kinase activity by decreasing cAMP levels and PKA and MAPK/ERK kinase independently but that ePAC, phosphatidylinositol-3 kinase and epidermal growth factor receptor kinase are involved [112]. Src kinase is an activator of NOX, and the decrease in ROS concentration by Src kinase activity is thus a potential route of mechanism [113].

In human aortic endothelial cells (HAECs) exposed to palmitate, exendin-4 has been shown to lower the level of ROS and at the same time reduce the level of DNA fragmentation used as a measure of apoptosis [89]. ROS can stimulate p38 mitogen-activated protein kinase and Jun N-terminal kinase, which can activate NOX by translocation of p47phox and p67phox subunits leading to the production of superoxide [114, 115]. Adding palmitate to the medium markedly increased the phosphorylation (and subsequently activity) of the kinases, a process that was significantly decreased by co-cultivating the cells with exendin-4 [89]. However, addition of trolox (a vitamin E analog) was not effective in decreasing palmitate-induced apoptosis even though it reduced ROS production, suggesting that the antiapoptotic effect of exendin-4 is mediated through lowering p38 MAPK and JNK activities. Another study of palmitate induced lipotoxicity in a cardiomyocyte cell line has shown a decrease in xanthine oxidase (XO) activity and ROS/RNS concentration and an increase in GSH/GSSG ratio. In this study, SOD activity was decreased [116]. The latter could be contributed to a decreased need of SOD as ROS production and concentrations were decreased.

3.3. GLP-1 Effect on ROS Production

GLP-1 and liraglutide has been shown to decrease NOX activity as well as protein and mRNA levels of NOX subunits [20, 21, 84]. By inhibition of Rho through the cAMP/PKA pathway, GLP-1 suppresses ROS production and expression of NOX subunits in cardiac microvascular endothelial cells [20]. High glucose-induced ROS production has been shown to be attenuated by liraglutide in endothelial cells [21, 84]. In a study by Batchuluun, B. et al. [84], lower levels of superoxide (detected by DHE) was observed along with a decrease in the PKC phosphorylation, translocation of PKCβ2, translocation of p47phox and decreased activity of NOX [84]. As total diacylglycerol (DAG) was decreased and AMP-activated protein kinase (AMPK) phosphorylation increased by liraglutide stimulation, this study suggests that DAG and AMPK may be involved in the GLP-1 lowering effects on ROS production.

In diabetes, inflammatory conditions in the pancreatic β-cells play a central role, making in vitro studies with different inflammatory cytokines of interest. Preincubation with exendin-4 was found to decrease ROS levels and caspase-4 activity in rat islet cells exposed to IFN-γ and IL-1β compared to controls [88]. Similar results were obtained when exposing HUVECs to TNF-α after liraglutide preincubation. Here, liraglutide resulted in decreased apoptosis, ROS production and expression NOX subunits (gp91phox and p22phox). These observations support findings that NOX activity is reduced by liraglutide and other GLP-1 analogues. A simultaneous increase in mRNA levels of SOD-2 and CAT as well as protein levels of SOD-2, CAT and GPx was found [21]. These data support the hypothesis that GLP-1 analogues improve antioxidant status in pancreatic β-cells and endothelial cells in spite of lower levels of ROS and suggest a mechanism by which OS may potentiate cytokine-induced β-cell apoptosis.

In vitro studies have also shown that GLP-1 can decrease the expression of RAGE [87, 93, 96, 99], which can potentially lead to decreased ROS production from NOX [50, 51], XO and the mitochondrial electron transport chain [51]. The observed effect was accompanied by lower levels of superoxide and higher levels of GSH, indicating an advantageous redox status in the cells.

From these data it can be concluded that GLP-1 shows very positive effects in vitro both by decreasing the production of ROS but also by increasing the antioxidant capacity. These outcomes are seen together with an antiapoptotic effect thus to some extend indicating an effect of these effects on OS status.

4. GLP-1 ANDOXIDATIVE STRESS IN VIVO

Most studies of GLP-1 in OS have been conducted in various disease models as outlined below. Thus, limited information is available on the effects of GLP-1 on OS status in healthy animals. However, one study examined the effect of exenatide on thioredoxin-interacting protein (TXNIP) [101]. In β-cells, TXNIP is a proapoptotic gene and expression of this gene is increased upon elevated levels of glucose, as seen in diabetes [117]. Expression of the gene inhibits the ROS scavenger, thioredoxin (Trx) and leads to increased ROS levels [118]. Exenatide injections for seven days in nondiabetic mice reduced the expression of TXNIP in pancreatic islets and suggested that this GLP-1 analogue is able to reduce apoptosis of mouse β-cells in vivo [101]. The available in vivo data are summarized in Table 2.

4.1. Ischemia/reperfusion (I/R) Models

In mice subjected to acute left middle cerebral artery occlusion (MCAO), single injections of exendin-4 at different time points after reperfusion reduced 8-hydroxy-2′-deoxyguanosine (8-OHdG) and anti-4-hydroxy 2-hexenal (HHE) positive cells in the brain at both 24 hours, 72 hours and seven days after I/R [119]. This effect of GLP-1 on OS has been supported by a study showing that four days of liraglutide treatment, in a model of traumatic brain injury induced by cryolesion (probe cooled in liquid nitrogen), can decrease in vivo visualization of ROS/RNS in female C57BL/6 mice. The effect was accompanied by a reduction in α-spectrin, a marker of necrosis and apoptosis, indicating a reduction in these cellular events [120]. In CL57BL/6 mice, seven days of liraglutide treatment resulted in higher expression and protein concentration of Nrf2 and HO-1 in...
the heart suggesting that liraglutide can increase antioxidant capacity [121].

In rats, the preventive effect of exendin-4 has also been examined in a MCAO model. Administration seven days prior to I/R resulted in significantly lower levels of MDA and higher levels of SOD and GSH [122]. This suggests that exendin-4 exerted its protective effect through increased antioxidant status capable of protecting against macromolecular damage induced by ROS. Similar results using exenatide were obtained in a model where the left coronary artery was ligated for 30 minutes followed by two hours of reperfusion. Here, exenatide administration for two weeks prior to I/R resulted in a significant decrease in MDA and increases in total SOD, CAT, and GPx [90]. Similarly, in a renal model of I/R, exenatide treatment for two weeks prior to I/R showed significant reductions in XO, MDA, combined with an increase in antioxidant capacity (GSH, GPx, SOD and CAT) [123]. In this study, a reduction in ROS producing enzymes was also observed, indicating that GLP-1 can prevent the production of ROS in vivo. Comparable effects of exenatide has been found after induction of diabetes with streptozotocin (STZ); thus, in kidney I/R induced after 10 days of exenatide, decreased kidney content of MDA and XO activity was observed and accompanied by increases in GSH concentration, GPx protein concentration, and SOD and CAT activities [124]. Collectively, these results suggest that prophylactic administration of GLP-1 analogues in I/R models may partially protect against OS both by induction of antioxidant capacity and by reduction of ROS producing enzymes. Therapeutic administration of GLP-1 in rats following I/R has been shown effective as well. A single administration of liraglutide acutely after reperfusion has been shown to reduce infarct volumes in a transient MCAO model [125]. Derivatives of reactive oxygen metabolites (d-ROMs) - a crude measure of peroxides [126] were significantly reduced in plasma in the liraglutide treated rats. Likewise, intrauterine growth retardation (IUGR) followed by six days of exendin-4 administration resulted in decreased thiobarbituric acid reactive substances (TBARS; a crude measure of lipid hydroperoxides with questionable specificity and quite inconsistent findings [127]) in the livers of the offspring, thus indicating a reduction in lipid peroxidation in newborn rats treated with exendin-4 [128]. Furthermore, antioxidant status was improved as shown by increased liver MnSOD and GSH/GSSG ratio.

The potential long term effect of a 6-day exendin-4 administration to newborn rats on cardiac OS status was examined by evaluating the cardiac response to ex vivo I/R after 4-6 weeks and 8-9 months [129]. Surprisingly, the study showed improved recovery in hearts from the exendin-4 treated groups, which was attributed to reductions in oxidative phosphorylation possibly through epigenetic alterations, as no differences between groups in TBARS and MnSOD activity were observed [129].

Two studies have been performed using porcine models of I/R. Exenatide intervention (started just prior to reperfusion and continued for two days after) in a cardiac I/R model resulted in higher activity of SOD and CAT as well as decreased 8-OHdG in the heart tissue [22]. In the same study, the exenatide group showed a 40 % reduction in infarction size. In the second experiment, treatment with human recombinant GLP-1 for four hours from one minute after reperfusion in a model of cardiac arrest resulted in lower levels of 8-iso-Prostaglandin F2\(_{	ext{a}}\) (8-iso-PGF\(_{2\alpha}\)) in plasma [130]. In contrast to the first study, the GLP-1 analogue did not have any effect on the SOD activity. The authors concluded that the decrease in the level of 8-iso-PGF\(_{2\alpha}\) might be through non-enzymatic pathways, i.e. through a reduction in ROS [130]. Firstly, the decrease in 8-iso-PGF\(_{2\alpha}\) could be due to changes in many other pathways as discussed through this review. Secondly, the decrease in 8-iso-PGF\(_{2\alpha}\) could still be explained by SOD activity as the duration of these two experiments are different, maybe SOD is utilized to decrease superoxide levels and the amount of 8-iso-PGF\(_{2\alpha}\), and the difference in the time allowed to increase SOD in these experiments are different. Catalase and SOD was only elevated at measurements after 72 hours. Unfortunately, the studies did not measure other antioxidants such as GSH or CAT. If the overall antioxidant capacity was indeed unchanged, the decrease in 8-iso-PGF\(_{2\alpha}\) may be attributed to a decreased production of ROS by GLP-1, as GLP-1 has shown glucose independent effects on ROS production by down regulating NOX in both heart and kidney in in vivo studies [71, 131]. Importantly, the observation in Dokken et al. [130] shows that GLP-1 protects against I/R in isolated hearts, suggesting that the effects are independent of circulating insulin and blood glucose levels [130].

Collectively, these results support the hypothesis that GLP-1 can reduce tissue damage in I/R models (decrease infarct volume size, improve recovery, decrease DNA fragmentation etc.) [22, 119, 122, 124, 125, 129, 130] by lowering ROS production, oxidative damaged macromolecules, and/or increase antioxidant status. This indication of lower nuclear DNA damage and lipid peroxidation accompanied by increased intracellular cAMP supports the hypothesis that induction of cAMP signaling takes part in the protective effects of GLP-1. Further mechanistic details are needed to determine if the effects of GLP-1 are mediated through lowering the production of ROS or scavenging of ROS. It has already been summarized by Birnbaump et al. (2012) that the protective effects observed by GLP-1 on injury after I/R are primarily due to GLP-1R activation, cAMP concentration increase intracellular and PKA activation, which leads to activation of several pathways as mentioned previously [132]. E.g. CREB has shown to be involved in reducing OS status both in vitro and in vivo potentially by activation of OS defense mechanisms [102, 119].

### 4.2 Obese and/or Spontaneously Diabetic Animal Models

As mentioned in the introduction, hyperglycemia may affect OS status in several ways. This issue becomes particularly important when studying effects of GLP-1 on OS in obese and diabetic animal models, as GLP-1’s effects on body weight and blood glucose are easily mistaken for glucose independent effects, i.e. direct effects of GLP-1 on OS. Thus, body weight and blood glucose must be closely monitored, properly controlled for and taken into account when concluding on data from such experiments.

A 60-day exendin-4 treatment of ob/ob mice showed a reduction in TBARS in liver contents compared to untreated
Does Glucagon-like Peptide-1 Ameliorate Oxidative Stress in Diabetes?

Animals [133]. The treated animals lost 14% of their body weight compared to vehicle controls, and as the experiment did not include a weight-matched control group, it cannot be ruled out that the effect of exendin-4 on TBARS in the liver may to some extent result from the reduction in body weight. Additionally normalization to liver lipids could help exclude the possibility that the observed effects on TBARS were due to the reduction in the liver lipid content. Measurement of antioxidant levels and ROS producing enzymes could be helpful on evaluation on potential glucose independent effects as well. Forty days of exendin-4 infusion has been shown to reduce the degree of cardiac remodeling in two different obese pre diabetic animal models, the hyperphagic, insulin resistance and dyslipidemic, KKAY mouse and the diet induced obese (DIO) mouse [131]. Exendin-4 decreased the levels of cardiac superoxide and NOX4 in both models. In the DIO model - but not the KKAY mouse - cardiac SOD-1 and GPx increased concurrently, showing that the choice of model often affects the readouts obtained in the evaluation of OS. Here, factors such as difference in obesity status and GLP-1R expression could play a role. In the same study, exendin-4 decreased both total cholesterol and triglycerides, systemically and in the heart but the potential indirect effect on ROS status caused by the improvement of dyslipidemia was not independently controlled for. Moreover, as NOX is down regulated by exendin-4, the GLP-1R activator might have a glucose independent effect on the ROS producing enzyme. Exendin-4 did not affect food intake (given as an individual average of the entire experimental course), body weight or blood glucose, but only the blood glucose during a challenge (oral glucose tolerance test) [131]. Even though the KKAY model is generally considered insulin resistant [134], the effect of exendin-4 was significant in the oral glucose tolerance test, indicating that the treatment ameliorates glucose intolerance in the model, which was also the case in the DIO model. Similar difficulties in determining the independent effects of weight loss, food intake and blood glucose lowering were apparent in a study in db/db mice, in which two weeks of liraglutide intervention, in a relevant dosing regime in rodent models (200 µg/kg twice daily), resulted in increased GPx activity and a no significant increase in CAT in pancreatic tissue, while no effect on antioxidant gene expression was observed [135].

The first study on the effect of GLP-1 in the kidney was performed in db/db mice. Here, exendin-4 treatment for 8 weeks decreased urinary 8-OHdG and decreased immunohistochemical expression of 8-OHdG in the kidney. Exendin-4 did not decrease blood glucose, which could be due to a low number of β-cells in db/db mice [136], but body weight was decreased by exendin-4 treatment making it difficult to rule out an effect of body weight on the OS status. However, the observed effects could be induced by increased GLP-1R and peroxisome proliferator-activator receptor-α expression [137]. In a mouse model of diabetic nephropathy, the KK/Ta-Akita mouse, activation of the GLP-1R by liraglutide for four weeks has been shown to decrease NOX4 expression and its activity, a decrease in glomerular superoxide and an increase in glomerular NO, via an elevation of cAMP and PKA levels [138]. This resulted in lower levels of TBARS and a reduction in kidney damage as measured by decreased albuminuria, glomerular filtration rate, kidney hypertrophy, mesangial expansion score, fibronectin staining intensity, glomerular basement membrane and podocyte number. SOD-1 down-regulation in this mouse model plays an important role in the development of nephropathy [139] and thus the reduced superoxide production in the kidneys in the presence of liraglutide is likely to cause the attenuated kidney damage observed in this study. Importantly, no changes were observed in blood glucose or plasma insulin in the diabetic animals, as this mouse model has dysfunctional β-cells [140]. The body weight was not affected either, suggesting that the observed protective mechanisms did not occur through the known metabolic effects of liraglutide.

4.3. Animal Models of Induced Diabetes

The acute effect of exendin-4 under hyperglycemic conditions (blood glucose = 20 mmol/l) has been investigated in one study using C57BL/7 mice undergoing hyperglycemic clamp studies, where blood glucose was fixed at a predetermined concentration. Intracerebral infusion of exendin-4 lowered ROS level in the hypothalamus and decreased the GSSG/GSH ratio. This study showed increased whole body glucose utilization in hyperglycemic mice [141]. The study indicates an improved OS status but glucose dependent effects cannot be ruled out as the intracerebral injection of exendin-4 could improve glucose homeostasis locally even though the systemic blood glucose is kept constant.

Prophylactic intervention with human recombinant GLP-1 seven days prior to STZ administration and 23 days after diabetes induction has been shown to decrease plasma and pancreatic levels of MDA, increased SOD and GPx (ns in pancreas) in Chinese Kun Ming mice [142]. Treatment with exendin-4 for 30 days to BALB/c mice in a post STZ administration regimen, resulted in a protection of β-cells from apoptosis probably through increased antioxidant capacity [143]. Exendin-4 increased pancreatic CAT, GPx and SOD in diabetic but not in non-diabetic exendin-4 treated animals. In addition, exendin-4 reduced blood glucose and increased body weight, an effect that may be attributed to increased β-cell mass and improved blood glucose regulation. Food intake was not recorded but effects of altered food intake would be expected to be most pronounced in the beginning of the study. Therefore, decreased blood glucose may at least in part explain the higher levels of antioxidants observed. Increased expression of Nr2, a nuclear factor involved in expression of antioxidant genes and genes involved in the antioxidant defense [144], has been shown in a STZ induced T1D mouse model treated with exendin-4 after STZ injections [145]. MDA levels were decreased in the liver indicating a protective effect of exendin-4 through regulation of antioxidant genes.

In diabetic Wistar rats, ten weeks of exenatide treatment initiated five days after STZ exposure led to decreased blood glucose levels after four and eight weeks and improved glucose tolerance as measured by intraperitoneal glucose tolerance test (IPGTT) at the end of the ten weeks [146]. Immunofluorescence staining of the pancreas for CAT and glutathione reductase (GR) revealed an increase in treated animals (co-localized with insulin in the islets of Langerhans) together with an elevation in GPx gene expression. The results may suggest that exenatide increases antioxidant level.
and gene expression, but an indirect effect through blood glucose lowering cannot be excluded. A similar study with GLP-1 yielded comparable results as ten weeks of GLP-1 treatment increased the percentage of GR containing cells in the pancreas and increased GR gene expression in STZ induced diabetic Wistar rats. The GLP-1 treated diabetic rats had a higher body weight and a lower blood glucose (both fasting and during an IPGTT), while GLP-1 did not affect body weight in nondiabetic rats. Moreover, the latter only showed a reduction in blood glucose at two time points during the IPGTT (no effect on fasting blood glucose) but still displayed increased GR gene expression [147]. This could indicate a glucose independent effect of GLP-1 on GR gene expression. Exendin-4 treatment of Sprague Dawley rats for eight weeks starting one week after STZ administration decreased urinary 8-OHdG, glomerular 8-OHdG content, expression of Nox-4 in cortex and Nox-4 content in kidney endothelial cells (detected by immunofluorescence staining) without any effect on blood glucose level, blood pressure, food intake or body weight [71]. These results indicate a glucose independent effect of exendin-4 on ROS induced DNA damage, a decrease in ROS production and a decrease in NF-kB, implicating a favorable effect on inflammation [71]. However, these measurements were made at the end of the experiment (week 8) and the initial glucose dependent effects were not accounted for. Similar results were obtained in a four week liraglutide treatment study in Wistar rats, resulting in decreased mRNA expression of Nox-4, gp91phox, p22phox and p47phox and protein levels of NOX4, gp91phox and p47phox. Kidney superoxide levels were lower in the liraglutide treated group compared to controls and both urinary 8-OHdG and MDA were decreased. The observed effects were not accompanied by a change in food intake, body weight, blood glucose or blood pressure at day 28 [148]. In another study in Sprague Dawley rats, antioxidant enzyme activity (GR, SOD and CAT) was increased by four weeks of exenatide treatment together with a decrease in MDA, a result shown in a low dose and high dose regime (1.0 μg/kg and 5.0 μg/kg once daily) without returning blood glucose and insulin levels to normal in the low dose regime [149]. However, a significant decrease in blood glucose and an increase in serum insulin were observed. Even though their levels did not return to normal, the data do not seem to support the authors’ conclusions of a glucose independent effect of exenatide on lipid peroxidation resulting from an improved oxidative status seemingly by increased antioxidant activity. In the same study a short term intervention with exenatide had the same effects on OS measurements, but as neither food intake or body weight was measured in these experiments, it is likely that the effect of exenatide on food intake (especially in the beginning of the treatment period) and on body weight could cause these effects. Shorter treatment regimens (two weeks) has also proven effective as e.g. a two-week exendin-4 administration by intraperitoneal infusion pump led to a reduction in urinary 8-OHdG content and increased body weight in male STZ treated diabetic Wistar rats [93]. In this study, RAGE expression was decreased and as depicted in Fig. (3), this could provide a mechanism explaining the effects of exendin-4 on ROS production and damage. Again, food intake was unfortunately not measured, which could have been interesting in this short treatment regimen.

Effects of GLP-1 treatment on body weight in STZ-induced diabetic animals are interesting as the animals both are hyperphagic and diabetic and when treatment increases body weight in some of these studies, it could indicate a remaining β-cell mass with the potential for GLP-1 to exert insulinotropic effects. C-peptide measures could be used to reveal the degree of β-cell function in such studies.

4.4. Animal Models of Cardiovascular Function and Disease

GLP-1 has shown protective effects on cardiovascular function in both apolipoprotein E (APO E) knockout mice and humans making the underlying mechanisms of these effects relevant to investigate [72, 150-152]. In APO E knockout mice, prophylactic intervention with human GLP-1 for four weeks (initiated simultaneously with a change to atherogenic diet) suppressed the formation of atherosclerotic lesions (surface area of atherosclerotic lesions, plaque size and macrophage infiltration) [150]. A reduction in oxLDL accumulation in isolated macrophages was also observed but without concurrent effects on plasma glucose or insulin when compared to control groups. However, as atherosclerotic development and OS status is highly dependent on food intake in this model [153], the duration of study becomes important as effects of lowered food intake (which are most pronounced in the beginning of GLP-1 intervention) may be misinterpreted as potential glucose independent effects of GLP-1 on atherosclerosis and OS. Administration of GLP-1 prior to change in diet can circumvent the most pronounced effect of GLP-1 on food intake and thereby on the intake of an atherogenic diet. Longer studies are needed both in prevention and treatment regimens to evaluate further on the latter effects of GLP-1.

In Wistar rats, seven days of exendin-4 injections prevented the induction of vascular endothelial dysfunction following streptozotocin treatment as evaluated by Ach-induced relaxation and integrity of endothelial lining [154]. The data suggest that the effect result from an increased production of eNOS by activation of Akt, as the effects of exendin-4 were attenuated by the eNOS inhibitor L-NAME. A significantly reduction in TBARS was observed in the exendin-4 treated when compared with control animals [154] implying effects on OS status. However, as a report showed that contractile force in aortic rings can be preserved by GLP-1 in STZ induced diabetic rats but not with exendin-4 while both treatments lead to reduced plasma MDA [155], the two effects may not be related. On the other hand, the lack of contractile effects of exendin-4 may be due to the low doses used, although they were clearly high enough to reduce lipid oxidation.

Collectively, the above results suggest that GLP-1 can lower the OS status in various animal models, both preventive and treatment studies, by lowering ROS producing enzymes and increasing antioxidant capacity, thus lowering the damage induced by high levels of ROS in the animals. Many different organ systems are involved and thus more detailed studies are needed to elucidate the effects of GLP-1 on OS.
Table 2. *In vivo* studies of GLP-1 and oxidative stress.

| Species | Type | Study Design: Model (M), Intervention (I) and Treatment Duration (TD) | Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless Otherwise Stated) | Pathological Changes (Compared to Control Unless OTHERWISE Stated) | Comments |
|---------|------|--------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------|----------|
| I/R     | M: C57BL/6 mice.  
I: MCAO and exendin-4 0.1, 1, 10 or 50 μg/100μL/mouse (iv).  
TD: Single injection at different time points after I/R. | Brain:  
↓ 8-OHdG and HHE (p<0.001). | Exendin-4 > 10 μg reduced infarct area and volume (p<0.001), best effect when administrated at time 0 (p<0.001). | ↑ CREB, indicating actions through cAMP/CREB pathways [119]. |
|         | M: C57BL/6 mice (n=12).  
I: Liraglutide 200 μg/kg (ip twice daily.  
TD: 7 days prior to MI. | Heart:  
↑ Nrf2 expression and protein concentration (p<0.05).  
↑ HO-1 expression and protein concentration (p<0.05). | Liraglutide:  
Mice subjected to MI had improved recovery (left ventricular developed pressure) (p<0.01). | Nrf2 and HO-1 was measured prior to MI in other mice [121]. |
| Acute clamp | M: C57BL/6 + GLP-1R (-/-) mice (n=62).  
I: Catheters (lateral cerebral ventricle + left femoral vein) + ultrasonic flow probe. hyperinsulinemic-euglycemic (5.5 mmol/l) or hyperglycemic (20 mmol/l) clamps. Intracerebral infusion of exendin-4 or exendin-9 (0.5 pmol/kg/min). H2O2 (2 or 20 nmol/min) was infused for the last hour.  
TD: 3 hours. | Brain (hypothalamus):  
↓ ROS (CM-H2DCFDA fluorescence) compared to hyperglycemic and normoglycemic controls (p<0.05).  
↓ GSSG/GSH ratio (20 nmol/l) (p<0.05). | Glucose dependent exendin-4 induced ROS reduction.  
Decreased eNOS activity during hyperglycemia.  
Hypothalamic ROS decrease was associated with reduction of femoral blood flow [141]. |
| Mice   | M: C3H/HeJ mice.  
I: Exendin-4 24 nmol/kg (ip).  
TD: 7 days. | Langerhans islets:  
↓ caspase-3 (p<0.05) and bax (p<0.001) mRNA level. | Langerhans islets:  
↓ TxNIP expression (p<0.001). | [101] |
| Cryo-lesion | M: C3H/HeJ mice.  
I: Exendin-4 24 nmol/kg (ip).  
TD: 7 days. | Langerhans islets:  
↓ caspase-3 (p<0.05) and bax (p<0.001) mRNA level. | Langerhans islets:  
↓ TxNIP expression (p<0.001). | [101] |
| Prevention | M: APO E (-/-) mice.  
I: GLP-1(7-36) amide 2.2 nmol/kg/day (sc. pumps).  
TD: 30 days. | Isolated peritoneal macrophages:  
45% reduction in oxLDL induced cholesteryl ester accumulation (p<0.0001). | Oil red O staining and MOMA-2 staining of aorta.  
Significant reduction in:  
Surface area of atherosclerotic lesions (p<0.0001), plaque size (p<0.005) and macrophage infiltration (p<0.05). | oxLDL induced cholesteryl ester accumulation was assessed in cultured macrophages from treated mice. [150] |
| Species | Type | Study Design: Model (M), Intervention (I) and Treatment Duration (TD) | Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless Otherwise Stated) | Pathological Changes (Compared to Control Unless OTHERWISE Stated) | Comments |
|---------|------|---------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------|-----------|
|         |      |                                                                     | Kidney:                                                                         | Kidney:                                                            | + Adenylate cyclase inhibitor (SQ22536) or PKA inhibitor (H-89) → eliminated effects of liraglutide. |
| M:      | KK/Ta-Akita mice \( \delta \) (n= 5 pr. group). I: Liraglutide 200 µg/kg/day (sc). TD: 4 weeks. | M:                                                                 | TBARS level (p<0.05). NOX activity (p<0.001). NOX4 expression (p<0.001). Superoxide level (DHE staining) (p<0.001). ♦ NO fluorescence intensity (p<0.0001). | ▼ albuminuria (p<0.01). glomerular filtration rate (p<0.01). Kidney hypertrophy (p<0.05). Kidney histology: Mesangial expansion score, fibronectin staining intensity, glomerular basement membrane (p<0.001). Podocyte number (p<0.001). | No difference in blood glucose or plasma insulin. Experiments GLP-1R knockout mice supports that effects are mediated through the GLP-1R [138] |
|         |      |                                                                     | Plasma:                                                                         | Pancreas:                                                          | [142]. |
|         |      |                                                                     | MDA (p<0.05). SOD (p<0.05). GPx (p<0.05). Pancreas: MDA (p<0.001). SOD (p<0.05). | Islet number/area (p<0.05). Histological changes. | |
|         |      |                                                                     | Pancreas: db/db: GPx (p<0.01) after 2 weeks.                                   | Pancreas: db/db: Caspase-1, Caspase-3 and Cad (p<0.05) expression after 2 days and 2 weeks. Bcl2 (p<0.05) expression after 2 weeks (NS after 2 days). | ‡ ER stress (Xbp1 expression) (p<0.05) after 2 weeks. [135] |
|         |      |                                                                     | Heart:                                                                          | Cardiac lipid accumulation ♦ (p<0.01) and fibrosis. No weight loss observed. | No weight loss observed. [131] |
| M:      | db/db and m/m \( \delta \) mice. I: Liraglutide 200 µg/kg twice daily (sc). TD: 2 weeks. | M: db/db: Gp (p<0.01) after 2 weeks.                                           | Superoxide (DHE staining) (p<0.05). Mfn1/Mfn2 (p<0.05). Mitochondria specific OS (MTR) (p<0.01). PARKIN + PINK-1 (p<0.01 and 0.05). Protein levels of: Nox-4 (p<0.01). SOD-1 (DIO) (p<0.05). GPx (DIO) (p<0.01). Nox-2 and TRx no change. | | |
| M:      | KKAy and DIO mice. I: Exendin-4 24 nmol/kg/day (sc pumps). TD: 40 days. | M: db/db: Caspase-1, Caspase-3 and Cad (p<0.05) expression after 2 days and 2 weeks. Bcl2 (p<0.05) expression after 2 weeks (NS after 2 days). | | | |
|         |      |                                                                     | Heart:                                                                          | Cardiac lipid accumulation ♦ (p<0.01) and fibrosis. No weight loss observed. | No weight loss observed. [131] |
## Table 2. contd…

| Species | Type | Study Design: Model (M), Intervention (I) and Treatment Duration (TD) | Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless Otherwise Stated) | Pathological Changes (Compared to Control Unless OTHERWISE Stated) | Comments |
|---------|------|-------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------|
| M: db/db (db/m as controls) (n=27). I: Exendin-4 (0.5 or 1.0 nmol/kg) ip. TD: 8 weeks. | | Urinary (24 hours): 8-OHdG (p<0.05 for db/db vs. control db/db and p<0.01 for db/m vs. control db/db). | Kidney: ↓ glomeruli lipid accumulation (oil red O). ↓ collagen IV (p<0.05 and p<0.01). ↓ TGF-β (p<0.05 and p<0.01). ↓ mesangial matrix expansion. ↓ glomerular hypertrophy. ↓ macrophage infiltration (F4/80 staining) (p<0.05 and p<0.01). ↓ apoptotic cells (caspase-3 positive cells) (p<0.05 in 1.0 nmol/kg). | Induction of peroxisome proliferator-activator receptor-α and GLP-1R expression. Decrease in GLP-1R positive cells in the glomeruli of db/db mice. Increased by exendin-4 treatment. No effect on food intake and blood glucose but decreased body weight in both db/db dose groups [137]. |
| M: Ob/ob mice. I: Exendin-4 (10 μg/kg for 14 days → 10 μg/kg or 20 μg/kg). TD: 60 days. | | Liver: ↓ TBARS (p<0.05 for 20 μg/kg). | Significant reduction in liver lipid content (p<0.05 for 10 μg/kg and 0.01 for 20 μg/kg). | [133] |
| M: C57BL/6 (n=24). I: STZ + exendin-4 (8 μg/kg) sc ± omeprazole. TD: From 14 days after first STZ injection and for 28 days. | | Liver: ↓ MDA (p<0.01). (± omeprazole p<0.001). ↑ Nrf2 level (p<0.01). | Liver: ↓ triglycerides (p<0.01). (+ omeprazole p<0.001). Nrf2 controls antioxidant genes and is important in protecting the liver against OS [145]. |
| M: BALB/c mice. I: STZ + exendin-4 3 μg/kg (sc). TD: 30 days after STZ injection. | | Pancreas (activity of): ↑ CAT (p<0.05) ↑ GPx (p<0.01) ↑ SOD (p<0.05) | No observed effects on apoptosis (TUNEL assay and cleaved caspase-3). No effect on CAT, GPx and SOD in nondiabetic mice. [143] |
| M: SD rat pups. I: Exendin-4 (1.0 nmol/kg) sc. Isolation of heart at 4-6 weeks or 8-9 months and subjected to I/R ex vivo. TD: 6 days from the day of delivery. | | Heart homogenates: No effect on TBARS and MnSOD activity in either group. Nor on nonenzymatic antioxidant capacity (Biovision kit). | Heart: Improved recovery from I/R (percent recovery of left ventricular end diastolic pressure). | [129]. |
| M: Wistar rats. I: MCAO and liraglutide (1 ml, 700 μg/kg (IP) TD: Single dose one hour after reperfusion. | | Peripheral blood: ↓ d-ROMS (0.93) (p<0.05) | Significant reduction in infarct volume (p< 0.05). | [125] |
| Species | Type | Study Design: Model (M), Intervention (I) and Treatment Duration (TD) | Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless Otherwise Stated) | Pathological Changes (Compared to Control Unless OTHERWISE Stated) | Comments |
|---------|------|---------------------------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------|---------|
| M: SD rats | | Brain: ↓ MDA (0.75) (p<0.001) ↑ GSH (1.45) (p<0.05) ↑ SOD (1.67) (p<0.01). | Significant reduction in infarct volume (p<0.001). | [122] |
| I: MCAO and exendin-4 0.5 μg/kg twice daily (ip). TD: 7 days prior to I/R. | | | | |
| M: Sprague Dawley rats | (n=32). | Heart homogenates: ↓ MDA (p<0.05). ↑ T-SOD, CAT and GPx (p<0.05). | | [90] |
| I: Exenatide 10 μg/kg/day (ip). Ligation of left coronary artery for 30 min followed by 2 h reperfusion. TD: 2 weeks prior to MI/R. | | | | |
| M: Wistar rats | | Liver: ↓ MDA (p<0.001) ↓ XO (p<0.001) ↑ GSH (p<0.01) ↑ SOD (p<0.001) ↑ GPx (p<0.05) ↑ CAT (p<0.01) | Significant reduction in ALT, AST and ALP after I/R (p<0.01). | [123] |
| I: STZ induced diabetes + surgical induced renal I/R. Exenatide 10 μg twice daily (sc.). TD: 2 weeks prior to I/R. | | | | |
| M: Wistar rats | | Kidney: ↓ DNA fragmentation. Preservation of normal morphology. | After STZ but before I/R [124]. | |
| I: STZ + renal I/R (after 10 days of exenatide (10 μg twice daily (sc)) intervention) TD: 2 weeks from 2 weeks after STZ. | | | | |
| M: neonatal SD rats | | Liver: ↓ MnSOD (p<0.05). ↓ TBARS (p<0.05). ↑ GSH/GSSG (p<0.05). | | |
| I: IUGR + exendin-4 1nmol/kg (sc). TD: 6 days from day of life. | | | | |
| M: Wistar rats | | Significant reduction in serum TBARS (STZ or methionine +/- exendin-4) (p<0.05). Significant prevention of diabetes or hyperhomocysteinemia induced VED. | Inhibition of eNOS by L-NAME → no effect of exendin-4 [154] | |
| I: STZ or methionine + exendin-4 1 μg/kg (ip). TD: 7 days. | | | | |
| M: Wistar rats | | Kidney: ↓ glucosuric area, macrophage infiltration in glomeruli, glomerular and tubulointerstitial fibrosis (p<0.01). | Inhibition of RAGE gene expression in both dose groups (p<0.01) [93]. | |
| I: STZ + Exendin-4 0.3 or 1.5 μg/kg/h (ip pumps). TD: 2 weeks. | | | | |
| Species          | Type          | Study Design: Model (M), Intervention (I) and Treatment Duration (TD)                                                                 | Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless Otherwise Stated) | Pathological Changes (Compared to Control Unless OTHERWISE Stated) | Comments                                                                 |
|------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------|------------------------------------------------------------------------|
| **Heart:**       | **M:** SD rats ♂. I: STZ + exenatide (1.0 or 5.0 µg/kg once daily) sc. TD: 1 or 4 weeks from 4 days after STZ.                  |                                                                                       |                                                                         |                                                                         | Low dose treatment does not return blood glucose and serum insulin to normal, but still affects MDA, GR, SOD and CAT [149]. |
|                  | **I:**        |                                                                                                                                   |                                                                                       | **Heart:**                                                       |                                                                         |                                                                       |
|                  | **TD:**       |                                                                                                                                   |                                                                                       | **Plasma:**                                                     |                                                                         |                                                                       |
|                  | **Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless OTHERWISE Stated)**                                    |                                                                                       |                                                                                       | **Heart:**                                                       |                                                                         |                                                                       |
|                  | **Pathological Changes (Compared to Control Unless OTHERWISE Stated)**                                                      |                                                                                       |                                                                                       | **Heart:**                                                       |                                                                         |                                                                       |
|                  | **Comments**  |                                                                                                                                   |                                                                                       | **Heart:**                                                       |                                                                         |                                                                       |
| **Plasma:**      | **M:** Wistar rats ♂+♀. I: STZ + GLP-1 (1 µg/kg twice daily) or exendin-4 (0.1 µg/kg/day) ip. TD: 4 weeks.                   |                                                                                       |                                                                         | Aortic rings: GLP-1 reversed the decreased contractive force in diabetic rats. |                                                                         | [155].                                                                |
|                  | **I:**        |                                                                                                                                   | Kidney:                                                                                |                                                                         |                                                                         |                                                                       |
|                  | **TD:**       |                                                                                                                                   |                                                                                       |                                                                                       |                                                                         |                                                                       |
|                  | **Kidney:**   |                                                                                                                                   |                                                                                       | Kidney morphology:                                              |                                                                         |                                                                       |
|                  | **Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless OTHERWISE Stated)**                                    |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Pathological Changes (Compared to Control Unless OTHERWISE Stated)**                                                      |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Comments**  |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
| **Kidney:**      | **M:** Sprague Dawley rats ♂ (n=23) I: STZ + exendin-4 10 µg/kg/day (ip). TD: 8 weeks from one week after STZ.                |                                                                                       |                                                                         |                                                                                       | NF-κβ p65 binding activity decreased by exendin-4 in diabetic group [71] |
|                  | **I:**        |                                                                                                                                   |                                                                                       | Kidney microinflammation:                                     |                                                                         |                                                                       |
|                  | **TD:**       |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Kidney:**   |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Pathological Changes (Compared to Control Unless OTHERWISE Stated)**                                                      |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Comments**  |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
| **Kidney:**      | **M:** Wistar rats ♂. I: STZ + exenatide 1 µg/kg (ip). TD: 10 weeks from day five after STZ.                                |                                                                                       |                                                                         |                                                                                       | Focus on insulin release.                                             | [146]                                                                 |
|                  | **I:**        |                                                                                                                                   |                                                                                       |                                                                                       |                                                                         |                                                                       |
|                  | **Pancreas:** |                                                                                                                                   |                                                                                       |                                                                                       |                                                                         |                                                                       |
|                  | **Serum:**    |                                                                                                                                   |                                                                                       |                                                                                       |                                                                         |                                                                       |
|                  | **Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless OTHERWISE Stated)**                                    |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Pathological Changes (Compared to Control Unless OTHERWISE Stated)**                                                      |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Comments**  |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
| **Kidney:**      | **M:** Wistar rats ♂ (n=40). I: STZ + GLP-1 30 ng/kg (ip). TD: 10 weeks from day five after STZ.                            |                                                                                       |                                                                         |                                                                                       |                                                                         |                                                                       |
|                  | **I:**        |                                                                                                                                   |                                                                                       | Kidney microinflammation:                                     |                                                                         |                                                                       |
|                  | **TD:**       |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Effect of GLP-1 on Oxidative Stress Markers (Compared to Control Unless OTHERWISE Stated)**                                    |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Pathological Changes (Compared to Control Unless OTHERWISE Stated)**                                                      |                                                                                       |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
|                  | **Comments**  |                                                                                                                                   |                                                                                       | **Kidney morphology:**                                          |                                                                         |                                                                       |
and mechanisms involved. Particularly, by assessing the balance between ROS, ROS production and antioxidant concentration and activity in all studies and trying to eliminate the confounding of GLP-1 on blood glucose, body weight and food intake. Moreover, it is important to investigate if and to what extent the effects observed in animal models may translate to humans bearing in mind the comparatively high doses used in animals compared to clinically relevant dosing regimens.

5. EFFECT OF GLP-1 ON OXIDATIVE STRESS IN CLINICAL STUDIES

The relationship between fasting GLP-1 concentration and OS has been investigated in a retrospective study where 72 T2D patients (65.3±8.6 yrs.) and 14 (63.4±8.7 yrs.) non-diabetic subjects were included. It revealed an inverse correlation between nitrotyrosine concentrations and GLP-1 independently of age, gender, BMI, HbA1c, anti-diabetic treatment, and antihyperglycemic treatment. In this study, 8-OHdG was increased in the diabetic patients as well [116]. The effect of GLP-1 on OS status in humans has been investigated in both acute studies and longer term clinical studies. The available clinical data are summarized in Table 3.

5.1. Acute Effects in Diabetic Patients

Ceriello and coworkers have performed several acute cross over experiments with hypo- or hyperglycemia in both T1D and T2D diabetic patients with focus on the effects of GLP-1 on OS [77, 156-159]. In one study, 30 male and female T1D patients, mean aged about 24 years and diagnosed for 7-8 years, were exposed to hypo- or hyperglycemic conditions [77]. Hypoglycemia has been shown to induce ROS production in an in vivo rat model in the CNS comparable to that of hyperglycemia [43, 160]. In the above study, two hours of hypo- or hyperglycemia and an simultaneous GLP-1 infusion to T1D patients decreased plasma 8-iso-PGF2α and nitrotyrosine levels, although they remained significantly elevated compared to baseline. In a similar study of hyperglycemia in T2D patients, GLP-1 also reduced plasma 8-iso-PGF2α and nitrotyrosine levels significantly [156]. The study was repeated after two months of optimized glycemic control of the diabetic patients. In these clamp experiments, which do not correspond to any physiological situation, two months of improved glycemic control per se decreased basal levels of 8-iso-PGF2α and nitrotyrosine while the following GLP-1 treatment no longer improved the measures as it had done at baseline. The study suggests that the effect of GLP-1 on OS status is more pronounced when subjects are exposed to hyperglycemic conditions for longer periods of time, which may indicate that GLP-1 exerts a protective effect only under hyperglycemic conditions. However, these patients might have lost weight during the two months, and to evaluate on the effect of better glycemic control, a group of body weight and/or HbA1c matched patients should have been included, depending on whether the glycemic control aimed at lowering the HbA1c or fluctuations in blood glucose.

Effects of the antioxidant vitamin C and/or GLP-1 on OS status were examined in 20 T1D patients during acute hypoglycemia [158]. GLP-1 alone reduced both 8-iso-PGF2α and nitrotyrosine significantly and vitamin C alone reduced the levels of these two biomarkers even more. Interestingly, simultaneous administration of both GLP-1 and vitamin C
resulted in levels of 8-iso-PGF$_{2a}$ and nitrotyrosine not being different from baseline measurements. This can be contributed to different actions of vitamin C and GLP-1, vitamin C being a scavenger of ROS and GLP-1 acting through induction of intracellular antioxidant defenses, respectively. Similar results were obtained in T1D patients in a study of effects of GLP-1 and/or vitamin C on the glycemic changes during I/R like conditions, where hypoglycemia conditions were followed by either normo- or hyperglycemia [157]. During hypoglycemia, levels of both 8-iso-PGF$_{2a}$ and nitrotyrosine increased, and these levels increased even more when hyperglycemia replaced hypoglycemia as compared to normoglycemia. GLP-1 lowered 8-iso-PGF$_{2a}$ and nitrotyrosine but again, vitamin C had a more pronounced effect. In this case, simultaneous infusions of GLP-1 and vitamin C lowered 8-iso-PGF$_{2a}$ and nitrotyrosine to baseline level. The finding that GLP-1 is less effective than vitamin C could be caused by a reduction of sensitivity to GLP-1 in hypoglycemia as hyperglycemic conditions can increase the effects of GLP-1 on OS status as discussed above.

In the above experiments, the clamp technique was used and insulin was administrated. As insulin has effects on OS level and vasodilatory effects as well, the administration of insulin should be considered when evaluating on the effects of GLP-1 on OS [161-165]. It has been shown that combined administration of insulin and GLP-1 in such clamp studies can lower 8-iso-PGF$_{2a}$ and nitrotyrosine levels more than GLP-1 by itself [159].

5.2. Short-Term Intervention Studies (< 6 Months)

A pilot study in 20 female and male T2D patients on metformin (57 ± 13 years) liraglutide treatment for two months lowered HbA1c significantly together with a decrease in blood concentrations of lipid peroxides (LOOH) and HO-1 and an increase in GSH and ghrelin. Body weight, fasting blood glucose and waist circumference were not affected in this study, and there was no correlation between LOOH and GSH concentrations, which implies a glucose independent effect of liraglutide on OS status [166]. Ghrelin has previously been associated with decrease in OS status in obese subjects [167]. Liraglutide induced ghrelin regulation could have an effect on OS status. The data on HO-1 in this study are not in line with the in vitro data of Oeseburg et al (2010), where HO-1 were increased by GLP-1, as it has antioxidant properties. T2D patients have increased levels of HO-1 [168] and the effect of liraglutide in this study could lower the need of HO-1 protection against OS and thus the concentration of HO-1. ROS was measured using DHE staining in this study, but no significant effect was found, which could support the hypothesis that antioxidant protection is present in diabetes. No metabolic studies were performed and only fasting blood glucose was measured, which together with the lowering of HbA1c makes it difficult to rule out that the effects on OS could be attributed in part to improved glycemic control.

In isolated mononuclear cells from obese T2D patients undergoing diabetes therapy, a 22 ± 9 % reduction in superoxide production was observed when the patients were treated with exenatide for 6 or 12 weeks prior to sampling. This was significantly different from baseline and from pla-cebo, an effect seemingly independent of weight loss as no change in body weight was observed over the course of the study. The lack of weight loss could be due to the short duration of the study and that the patients were on insulin therapy throughout the study. An acute study was also performed; here a single injection of exenatide (5 μg) reduced superoxide release from isolated mononuclear cells as well [24]. FFA was reduced in the single injection experiment but glucose and insulin were not measured. In the 12 week study fasting blood glucose, HbA1c and FFA were significantly lowered and insulin increased by exenatide treatment. Thus, regulatory effects on glucose, lowered FFA and increased insulin cannot be excluded to have an effect in this study. NF-κβ binding and mRNA levels of inflammatory markers (TNF-α, JNK-1, IL-1β, TLR-2, and TLR-4) were all decreased as well. The link between inflammation and OS has been suggested to be initiated by ROS activating NF-κβ subsequently inducing proinflammatory cytokines. However, increased NF-κβ may be induced by elevated blood glucose [169].

Reductions in inflammatory markers and 8-iso-PGF$_{2a}$ have been shown in T2D patients following five months treatment with exenatide. Consistent with the expected effects of GLP-1 in T2D patients, these biochemical changes were accompanied by reduced body weights and BMIs, improved glucose profiles, reduced HbA1c, mean glucose levels and limited blood glucose excursions. To investigate the potential glucose independent effects of exenatide on 8-iso-PGF$_{2a}$, an analysis of covariance analysis was performed, showing that the effect of exenatide was independent of glycemic regulation and body weight [75]. Thus, this study supports that glucose excursions per se increase OS [170], since the glucose excursions measured in this study correlated with 8-iso-PGF$_{2a}$. However, as 8-iso-PGF$_{2a}$ is decreased by exenatide, a glucose dependent effects of GLP-1 on OS status cannot be ruled out.

A comparison of the first line drug for prediabetic patients, metformin, and exenatide has been performed in male and female non-diabetic but obese and prediabetic patients with a mean age of 58.5±10.0 yrs. having either impaired fasting glucose, elevated HbA1c, or impaired glucose tolerance [171]. Three months of treatment resulted in no significant differences between exenatide or metformin groups on either oxLDL levels or endothelial function. The metformin group had a significantly lower oxLDL level after treatment than when compared to baseline. Unfortunately, the study had a small sample size and did not include a placebo group as baseline was used as control and albeit not significantly different, there was a difference in baseline data between the groups. Thus, the metformin treated group had a higher baseline concentration of oxLDL perhaps leaving a higher window of opportunity for effect of treatment. The effects of metformin could be due to altered antioxidant activity and antioxidant homeostasis [172, 173]. In another study, three months of exenatide treatment to obese children and adolescents did not reduce oxLDL levels even though BMI were significantly decreased [174]. These studies indicate that the effect of GLP-1 on OS may be restricted to or at least most pronounced when individuals are challenged with diabetes and e.g. postprandial glucose excursions. This is suggested
| Duration of treatment | Study Design: Subjects (S), Intervention (I) and Treatment Duration (TD) | End point: Effect of GLP-1 Intervention on Oxidative Stress Markers (Compared to control Unless Otherwise Stated) | Comments |
|-----------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------|----------|
| Hours                 | S: T1D (n=30). I: Hypo- (2.9 ± 0.1 mmol/l) or hyperglycemic (15 mmol/l) clamp ± GLP-1 infusion (0.4 pmol/kg/min). TD: 2 hours. | Plasma: ↓ 8-iso-PGF₂α + nitrotyrosine (hypo- and hyperglycemia) (p<0.01) but significantly higher than baseline (p<0.01). | [77] |
| S: T2D (n=16) + controls (n=12). I: Hyperglycemic (15 mmol/l) 0-1 hours and 12.8 mmol/l at 1-2 hours clamp. Repeated after two months of better glycemic control (insulin therapy). TD: 2 hours. | Plasma: ↓ 8-iso-PGF₂α + nitrotyrosine (p<0.05) (after one and two hours in T2D, after one in controls). More pronounced effect after two months of better glycemic control. | [156] |
| S: T1D (n=20). I: Hypoglycemic (2.9 ± 0.1 mmol/l) clamp. Vitamin C (30 mg/min) and/or GLP-1 (0.4 pmol/kg/min). TD: 2 hours. | Plasma: ↓ 8-iso-PGF₂α + nitrotyrosine (GLP-1 or vitamin C (more pronounced (p<0.05)). GLP-1 + vitamin C: 8-iso-PGF₂α and nitrotyrosine at one and two hours not significantly different from baseline measurements. | [158] |
| S: T1D (n=15). I: Hypoglycemia (2.9 ± 0.1 mmol/l) superseded with either normoglycemia (4.5 mmol/l) or hyperglycemia (15 mmol/l) Vitamin C (30 mg/min) and/or GLP-1 (0.4 pmol/kg/min). TD: 4 hours (2 hours of hypoglycemia and 2 hours of hyper- or normoglycemia). | Plasma: ↓ 8-iso-PGF₂α + nitrotyrosine (p<0.01) (GLP-1 or vitamin C). Not significantly different from baseline when administrated together. | [157] |
| S: T2D (n=24) receiving dietary or metformin intervention. I: Gr. 1: Normoglycaemic-normoinsulinaemic (blood glucose = 5 mmol/l) or normoglycaemic-hyperinsulinaemic (insulin infusion 0.1 mU/kg/min, blood glucose = 5 mmol/l) ± GLP-1 (0.4 pmol/kg/min). Gr. 2: Hyperglycaemic-normoinsulinaemic (blood glucose = 15 mmol/l) or hyperglycaemic-hyperinsulinaemic (insulin infusion 0.1 mU/kg/min, blood glucose = 15 mmol/l) ± GLP-1 (0.4 pmol/kg/min). TD: 2 hours. | Plasma: ↓ 8-iso-PGF₂α + nitrotyrosine (p<0.05), further decreased by hyperinsulinemia. | [159] |
| S: T2D (n=20). I: Liraglutide (0.6 mg daily for two weeks then 1.2 mg daily). TD: 2 months. | Blood; ↓ LOOH (p<0.05). ↑ GSH (p<0.01). ↓ HO-1 (p<0.05). Decreased HbA1c (p<0.0001) and increased ghrelin concentrations (p<0.01) [166]. And acute study was performed as well testing superoxide generation in MNCs after a single dose of exenatide (5 μg) → superoxide was reduced after 6 hours compared to baseline and placebo (p<0.05) [24]. | |
| S: T2D (n=12) on metformin. I: Exenatide (10 μg twice daily). Measuring superoxide release from isolated peripheral blood mononuclear cells. TD: 3 months. | Isolated peripheral blood MNCs: ↓ superoxide release (chemiluminescence) (p<0.05). | |
| S: Prediabetic patients (n=50) I: Exenatide (5 μg for 4 weeks then 10 μg twice daily) or metformin (500 mg for 4 weeks then 1000 mg twice daily). TD: 3 months. | Plasma: ↓ oxLDL in metformin group (p<0.05) but no significant difference between the metformin and exenatide group. | [171] |
| S: Obese but nondiabetic children and adolescents (age 9–16 years old) (n=12). I: Exenatide (5 μg for 4 weeks then 10 μg twice daily). TD: 3 months. | Plasma: No effect on oxLDL. Significant BMI decrease | [174] |
by Tesouro et al. (2013) as well, as healthy obese subjects do not respond to GLP-1 therapy when measuring endothelial vasodilation [175]. But aspects such as duration of the studies, obesity level of the patients, and its effect on blood glucose should be evaluated more thoroughly in future studies, which could also include more biomarkers of OS as a single marker does not give a complete picture.

### 5.3. Longer Term Intervention studies (> 6 Months)

Very limited data are available on long-term effects of GLP-1 administration on OS in humans as only two studies have been performed. Here, one year of exenatide treatment resulted in improved postprandial glucose homeostasis and dyslipidemia in 60 metformin treated T2D patients subjected to a standardized meal after an overnight fasting [23]. Exenatide treatment lowered plasma MDA significantly both compared to baseline and to a parallel insulin glargine treatment group. The postprandial oxLDL/LDL ratio was significantly reduced from baseline measures in the exenatide group, but no significant difference was observed between the two treatment groups. The glucose lowering effects of both treatments may explain these findings. The treatment period of one year was followed by a period of five weeks off drug, after which the subjects had the standardized mixed meal and the above measurements were repeated, displaying a return of all parameters to their baseline values [23]. The study shows that exenatide lowers excursions in glucose and lipids after a meal and improved biochemical markers of oxidative damage to lipids but does not provide a lasting effect following discontinuation of treatment. Again measuring other biomarkers of ROS level, antioxidant capacity and damaged end products could help elucidate the mechanisms behind the observed effects. In another long-term study, eight months of liraglutide intervention (mean dose 0.74 mg/day) in T2D patients did not result in a reduction of blood MDA even though it lowered d-ROMs when compared to baseline [176]. Blood glucose and plasma insulin was significantly lower, which may explain the effect on d-ROMs. The absence of effect on MDA could be due to the low doses used in this study as the normal human dose is 1.2 mg/day in T2D. In the former mentioned exenatide study, the normal dosing regimen of exenatide was used.

Part of the evaluation of the effects of GLP-1 on diabetic late complications and in particular CVD will be done in the LEADER trial, which was initiated in 2010 and will follow patients for up to five years. This trial evaluates the effect of liraglutide on CVD [177]. Such a trial makes studying the mechanisms behind a potential effect even more warranted, as the clinical studies available so far have not been suitable for separating glucose dependent effects from glucose independent effects.

### CONCLUSION

Besides the well-established metabolic effects of GLP-1 that in several ways may positively affect redox homeostasis,
glucose independent effect on OS may also play a role and can be investigated by choosing other compounds exerting distinct parts of the GLP-1 activity complex. Better glycemic control is known to lower the risk of developing diabetic complications such as CVD [2]. Decreased food intake caused by diet restriction has been shown repeatedly to affect OS levels in rodents, primarily lowering oxidative damage and increasing GSH, but the effect on antioxidant status varies from tissue to tissue [178]. Moreover, caloric restriction is an effective way to extend lifespan in rodents supporting the role of OS in late onset disease development [179]. However, from the clinical studies available so far, it seems that lowering blood glucose may be the main driver and not body weight reduction in humans.

This review summarizes the effects of GLP-1 on OS status in different treatment or intervention regimes, both in in vitro, in vivo and in the clinical setting. The less pronounced effects of GLP-1 in clinical studies may possibly be explained by something as simple as the lower doses used, as in vitro and in vivo studies in general have used doses well above those approved clinically while the differences between “mouse and man” are not to be neglected. There is no clear evidence supporting a single mechanism driving the effects of GLP-1 on redox homeostasis, but it seems that both ROS production and antioxidant capacity can be affected in a positive direction, favoring lower OS status and lower levels of damaged end products. The binding of GLP-1 to the GLP-1R increases cAMP, activates PKA and downstream pathways from this, which can explain the glucose independent pathways of GLP-1 induced improvement of OS status.

Collectively, the available data demonstrates that GLP-1 can decrease OS but also that this effect is dependent on dose, diabetic status, obesity etc. This effect of GLP-1 may have therapeutic potential if OS is in fact causally related to the development of late-onset diabetic complications.

CONFLICT OF INTEREST
KR and GR are employees at Novo Nordisk A/S. The authors declare no conflicts of interest regarding the present work.

ACKNOWLEDGEMENTS
KEP and JL are supported by the LifePharm Centre for in vivo pharmacology at University of Copenhagen. The present review was conceived by all authors. KEP wrote the draft manuscript, which was subsequently edited by all authors.

REFERENCES
[1] Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med. 1993; 328(23): 1676-85.
[2] Mazzone T. Intensive glucose lowering and cardiovascular disease prevention in diabetes: Reconciling the recent clinical trial data. Circulation. 2010; 122(21): 2201-11.
[3] Rydéén L, Standl E, Malgorzata B, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: Executive summary. The task force on diabetes and cardiovascular diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J. 2007; 28(1): 88-136.
[4] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414(6865): 813-20.
[5] Brownlee M. The pathobiology of diabetic complications: A unifying mechanism. Diabetes. 2005; 54(6): 1615-25.
[6] Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocr Metab. 1995; 80(3): 952-7.
[7] Holst JJ. Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. Expert Opin Emerg Dr. 2004; 9(1): 155-66.
[8] Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia. 1985; 28(9): 704-7.
[9] Keymam BF, Williams G, Ghatei MA, et al. Glucagon-like peptide-1 7-36: A physiological incretin in man. Lancet. 1987; 2(8571): 1300-4.
[10] Zander M, Madsbad S, Madsen JL, et al. Effect of 6-week course of glucagon-like peptide 1 on glycemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 2002; 359(9309): 824-30.
[11] Ursuova IA, Farilla L, Hui H, et al. GLP-1 inhibition of pancreatic islet cell apoptosis. Trends Endocrin Metab. 2008; 19(1): 27-33.
[12] Tourel C, Baille D, Meile MJ, et al. Glucagon-Like Peptide-1 and Exendin-4 Stimulate beta-Cell Neogenesis in Streptozotocin-Treated Newborn Rats Resulting in Persistently Improved Glucose Homeostasis at Adult Age. Diabetes. 2001; 50(7): 1562-70.
[13] Gutzwiller JP, Drewe J, Göke B, et al. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. Am J Physiol-Regul. 1999; 276(5 45-5): 1541-4.
[14] Ceriello A. Acute hyperglycaemia and oxidative stress generation. Diabetic Med. 1997; 14(SUPPL 3): 45-9.
[15] Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycaemia in patients with type 2 diabetes. J Am Med Assoc. 2006; 295(14): 1681-7.
[16] Ellingsgaard H, Hausenfeld L, Schulzer B, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med. 2011; 17(11): 1481-9.
[17] Kahles F, Meyer C, Möllmann J, et al. GLP-1 Secretion Is Increased by Inflammatory Stimuli in an IL-6-Dependent Manner, Leading to Hyperinsulinemia and Blood Glucose Lowering. Diabete. 2014; 63(11): 3221-9.
[18] Piotrowski K, Becker M, Zugwurst J, et al. Concentrating distributions of GLP-1 are associated with coronary atherosclerosis in humans. Cardiovasc Diabetol 2013; 12(1).
[19] Hattori A, Kawamura I, Yamada Y, et al. Elevated plasma GLP-1 levels and enhanced expression of cardiac GLP-1 receptors as markers of left ventricular systolic dysfunction: A cross-sectional study. BMJ Open; 2013; 3(9).
[20] Wang D, Luo P, Wang Y, et al. Glucagon-like peptide-1 protects against cardiac microvascular injury in diabetes via a cAMP/PKA/Rho-dependent mechanism. Diabetes. 2013; 62(5): 1697-708.
[21] Shiraki A, Oyama J, Komoda H, et al. The glucagon-like peptide 1 analog tiraglutide reduces TNF-alfa-induced oxidative stress and inflammation in endothelial cells. Atherosclerosis. 2012; 221(2): 375-82.
[22] Timmers L, Henriques JPS, de Klein DVP, et al. Exenatide Reduces Infarct Size and Improves Cardiac Function in a Porcine Model of Ischemia and Reperfusion Injury. J Am Coll Cardiol. 2009; 53(6): 501-10.
[23] Bunck MC, Cornéz A, Eliasson B, et al. One-year treatment with exenatide vs. Insulin Glargine: Effects on postprandial glycaemia, lipid profiles, and oxidative stress. Atherosclerosis. 2010; 212(1): 223-9.
[24] Chaudhuri A, Ghanim H, Vora M, et al. Exenatide exerts a potent antiinflammatory effect. J Clin Endocr Metab. 2012; 97(1): 198-207.
[25] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vase Biol. 2004; 24(5): 816-23.
Does Glucagon-like Peptide-1 Ameliorate Oxidative Stress in Diabetes?

[26] Maiese K, Chong ZZ, Shang YC. Mechanistic insights into diabetes mellitus and oxidative stress. Curr Med Chem. 2007; 14(16): 1729-38.

[27] Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol. 2003; 17(1): 24-38.

[28] Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med. 1996; 20(3): 463-6.

[29] Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: Defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Invest. 2002; 32(SUPPL 3): 14-23.

[30] Chop M, Hughes SJ, Igillo-Esteve M, et al. The long lifespan and low turnover of human islet beta cells estimated by mathematical modeling of lipofuscin accumulation. Diabetologia. 2010; 53(2): 321-30.

[31] Reaven GM, Hollembuck C, Jeng CY, et al. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes. 1988; 37(8): 1020-4.

[32] Zuniga-Gujardo S, Zinnman B. The metabolic response to the euglycemic insulin clamp in type 2 diabetes and normal humans. Metabolism. 1985; 34(10): 926-30.

[33] Fraze E, Donner CC, Swislocki e, et al. Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: Evidence for insulin resistance. J Clin Endocrin Metab. 1985; 61(5): 807-11.

[34] McGarry JD. What if Minkowski had been ageusic? An alternative pathway to diabetes-induced oxidative stress. J Am Soc Nephrol. 2012; 44(SUPPL 1): 2-16.

[35] Matsuda M, Shimomura I. Increased oxidative stress in obesity: Implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. Obes Res Clin Pract. 2013; 7(5): 330-41.

[36] Morgan D, Oliveira-Emilio HR, Keane D, et al. Oxidative stress, Noxs, and hypertension. Vasc Pharmacol. 2012; 57(5-6): 139-49.

[37] Poulson HE, Specht E, Broedbaek K, et al. RNA modifications by oxidative damage in mammalian cells. Trends Biochem Sci. 1997; 22(12): 477-81.

[38] Diouf A, Hassan M, Yousif H, et al. Pathogenesis and pathophysiology of accelerated atherosclerosis in the diabetic heart. Mol Cell Biochem. 2009; 331(1-2): 89-116.

[39] Mortensen A, Lykkesfeldt J. Does vitamin C enhance nitric oxide availability in a tetrahydrobiopterin-dependent manner? in vitro, in vivo and clinical studies. Nitric Oxide Bio Chem. 2014; 36: 51-7.

[40] Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet; 1998; 352(9131): 837-53.

[41] Dreuxse A, Hassan M, Yousif H, et al. Pathogenesis and pathophysiology of accelerated atherosclerosis in the diabetic heart. Mol Cell Biochem. 2009; 331(1-2): 89-116.

[42] Laschke M, Fanti P, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: Avenues for a mechanistic-based therapeutic approach. Curr Diabetes Rev. 2011; 7(5): 313-24.

[43] Pitocco D, Tesaro A, Alessandro R, et al. Oxidative stress in diabetes: Implications for vascular and other complications. Int J Mol Sci. 2013; 14(11): 21525-50.

[44] Turner R. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet; 1998; 352(9131): 837-53.

[45] Basta G, Lazzeroni G, Del Turco S, et al. At least 2 distinct pathways generating reactive oxygen species mediate vascular cell adhesion molecule-1 induction by advanced glycation end products. Arterioscler Thromb Vase Biol. 2005; 25(7): 1401-7.

[46] Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. Cerc Res; 2010; 106(8): 1319-31.

[47] Inoguchi T, Sonta T, Tsubouchi H, et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: Role of vascular NAD(P)H oxidase. J Am Soc Nephrol. 2003; 14(SUPPL 3): 227-32.

[48] Kaneto H, Xu G, Song KH, et al. Activation of the Hexosamine Pathway Leads to Deterioration of Pancreatic ß-Cell Function through the Induction of Oxidative Stress. J Biol Chem. 2001; 276833: 31099-104.

[49] O'Donnell RW, Johnson DK, Ziegler LM, et al. Endothelial NADPH oxidase: Mechanism of activation by low-density lipoprotein. Endothelium J Endothelial Cell Res. 2003; 10(6): 297-1.

[50] Lüscher TF, Barton M. Biology of the endothelium. Clin Cardiol. 1997; 20(SUPPL 12): 3-10.

[51] Miller JD, Chu Y, Castaneda LE, et al. Vascular function during prolonged progression and regression of atherosclerosis in mice. Arterioscl Throm Vasc Biol. 2013; 33(5): 459-65.

[52] Moyer B, Hemmings B. Bioactivation of nitric oxide by NADPH oxidase in mammalian cells. Trends Biochem Sci. 1997; 22(12): 477-81.

[53] Mathews C, Hulka B, et al. The role of oxidative stress in the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993; 329(14): 877-86.

[54] Fraccasso R, Bracciofiori G, et al. Oxidant stress and antioxidants: A review. J Biochem Mol Toxicol. 2003; 17(1): 24-38.

[55] Arakawa M, Mita T, Azuma K, et al. Inhibition of Monocyte Adhesion to Endothelial Cells and Attenuation of Atherosclerotic Lesion by a Glucagon-like Peptide-1 Receptor Agonist, Exendin-4. Diabetes. 2010; 59(4): 1030-7.

[56] Lee YS, Park MS, CHoung JS, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. Diabetes. 2012; 55(9): 2456-68.
Wang Y, Parleveit ET, Geerling JI, et al. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. Br J Pharmacol. 2014; 171(3): 723-34.

Wu JD, Xu XH, Zhu J, et al. Effect of exenatide on inflammatory and oxidative stress markers in patients with type 2 diabetes mellitus. Diabetes Technol Ther. 2011; 13(2): 143-8.

He L, Wong CK, Cheung KK, et al. Anti-inflammatory effects of exendin-4, a glucagon-like peptide-1 analog, on human peripheral lymphocytes in patients with type 2 diabetes. J Diabetes Invest. 2013; 4(4): 382-92.

Cerillo A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. Diabetes Care. 2013; 36(8): 2346-50.

Drucker DJ, Philippe J, Mojsov S, et al. Glucagon-like peptide 1 stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. P Natl Acad Sci USA. 1987; 84(10): 3434-8.

Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology. 2007; 132(6): 2131-57.

Savitha G, Salimath BP. Cross-talk between protein kinase C and protein kinase A down-regulates the respiratory burst in polymorphonuclear leukocytes. Cell Signal. 1993; 5(2): 107-17.

Kim JS, Diebold BA, Babiör BM. Reduced levels of cyclic AMP contribute to the enhanced oxidative stress in vascular smooth muscle cells from spontaneously hypertensive rats. Can J Physiol Pharmacol. 2008; 86(4): 190-8.

Kim JS, Diebold BA, Babiör BM, et al. Regulation of Noyx1 activity via protein kinase A-mediated phosphorylation of NoxA1 and 14-3-3 binding. J Biol Chem. 2007; 282(48): 34787-900.

Batchuluun B, Inoguchi T, Sonoda N, et al. Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway in human aortic endothelial cells. Atherosclerosis. 2014; 232(1): 156-64.

Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab. 2013; 17(6): 819-37.

Kimura R, Okouchi M, Fujioka H, et al. Exenatide inhibits advanced glycation end product receptor level. Metab Clin Exp. 2011; 60(9): 1271-7.

Cai Y, Hu X, Yi B, et al. Glucagon-like peptide-1 receptor agonist protects against hyperglycemia-induced cardiomyocyte injury by inhibiting high mobility group box 1 expression. Mol Biol rep. 2012; 39(12): 10705-11.

Chen J, Couto FM, Minn AH, et al. Exenatide inhibits beta-cell apoptosis by decreasing thioredoxin-interacting protein. Biochem Biophys Res Co. 2006; 346(3): 1067-74.

Oeseburg H, De Boer RA, Buikema H, et al. Glucagon-like peptide 1 prevents reactive oxygen species-induced endothelial cell senescence through the activation of protein kinase A. Arterioscl Thromb Vasc Biol. 2010; 30(7): 1407-14.

Shao W, Yu Z, Fantus IG, et al. Cyclic AMP signaling stimulates proteasomal degradation of thioredoxin interacting protein (TxNIP) in pancreatic beta-cells. Cell Signal. 2010; 22(8): 1240-6.

Rolin B, Deacon CF, Carr RD, et al. The major glucagon-like peptide-1 metabolite, GLP-1-(9-36)-amide, does not affect glucose or insulin levels in mice. Eur J Pharmacol. 2004; 494(2-3): 283-8.

Ban K, Kim KH, Cho CK, et al. Glucagon-like Peptide (GLP)-1(9-36)amide-mediated cytoprotection is blocked by exendin(9-39) yet does not require the known GLP-1 receptor. Endocrinology. 2010; 151(4): 1520-31.

Ma T, Du X, Pick JE, et al. Glucagon-like peptide-1 cleavage product GLP-1(29-36)amide rescues synaptic plasticity and memory deficits in Alzheimer's disease model mice. J Neurosci. 2012; 32(40): 13701-8.

Siegel D, Gustafson DL, Dehn DL, et al. NAD(P)H:quinone oxidoreductase 1; Role as a superoxide scavenger. Mol Pharmacol. 2004; 65(5): 1238-47.

Turkesven S, Kruger A, Mingone CJ, et al. Antioxidant mechanism of hemoxegenase-1 involves an increase in superoxide dismutase and catalase in experimental diabetes. Am J Physiol Heart Circ Physiol. 2005; 289(2 58-2): 701-7.

Younce CW, Burmeister MA, Ayala JE. Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endogenous MAPK and NADPH oxidase-dependent generation of reactive oxygen species. Apoptosis. 2011; 16(4): 347-58.
Does Glucagon-like Peptide-1 Ameliorate Oxidative Stress in Diabetes?

Minn AH, Hafele C, Shalev A. Thio-redox-in-interacting protein is stimulated by glucose through a carbohydrate response element and induces B-cell apoptosis. Endocrinology. 2005; 146(5): 2397-405.

Schulze PC, Yoshioka J, Takahashi T, et al. Hyperglycemia promotes oxidative stress through inhibition of thio-redox-in-interacting protein. J Biol Chem. 2004; 279(29): 30369-74.

Teramoto S, Miyamoto N, Yatomi K, et al. Exendin-4, a glucagon-like peptide-1 receptor agonist, provides neuroprotection in mice transient focal cerebral ischemia. J Cereb Blood F Met. 2011; 31(8): 1696-705.

DellaValle B, Hempel C, Johansen FF, et al. GLP-1 improves neuropathology after murine cold lesion brain damage. Ann Clin Transl Neurol. 2014; 1(9): 721-32.

Noyan-Ashraf MH, Abdul Momen M, Ban K, et al. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes. 2009; 58(4): 751-62.

Briyal S, Gulati K, Gulati A. Repeated administration of exendin-4 reduces focal cerebral ischemia-induced infarction in rats. Brain Res. 2012; 1427: 23-34.

Vaghasiya JD, Sheth NR, Bhalodia YS, et al. Exaggerated liver injury induced by renal ischemia reperfusion in diabetes: Effect of exenatide. Saudi J Gastroenterol. 2010; 16(3): 174-80.

Lotfy M, Singh J, Rashed H, et al. Mechanism of the beneficial and protective effects of exenatide in diabetic rats. J Endocrinol. 2014; 220(3): 291-304.

Sato K, Kameda M, Yasuhara T, et al. Neuroprotective effects of liraglutide for stroke model of rats. Int J Mol Sci. 2013; 14(11): 21513-24.

Cesaremo MR, Belcaro G, Carratelli M, et al. A simple test to monitor oxidative stress. 18(2). Int Angiol. 1999; 127: 30.

Esterbauer H, Gebicki J, Puhl H, et al. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces B-cell apoptosis. Endocrinology. 2005; 146(5): 2397-405.

Strehler BI, Libonati JR, Selak MA, et al. Neonatal Exendin-4 leads to protection from reperfusion injury and reduced rates of oxidative phosphorylation in the adult rat heart. Cardiovascular Drugs Ther. 2010; 24(3): 197-205.

Raab EL, Vuguin PM, Stoffers DA, et al. Neonatal exendin-4 treatment reduces oxidative stress and prevents hepatic insulin resistance in intruterine growth-retarded rats. Am J Physiol-Reg I. 2009; 297(6): 1785-94.

Lotfy M, Singh J, Rashed H, et al. The effect of glucagon-like peptide-1 receptor agonist liraglutide inhibits progression of vascular disease via effects on atherogenesis, plaque stability and endothelial function in an ApoE-/- mouse model. Diab Vasc Dis Res. 2013; 10(4): 353-60.

Esterbauer H, Gebicki J, Puhl H, et al. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med. 1992; 13(4): 341-90.

Minn AH, Hafele C, Shalev A. Thio-redox-in-interacting protein is stimulated by glucose through a carbohydrate response element and induces B-cell apoptosis. Endocrinology. 2005; 146(5): 2397-405.

Cesaremo MR, Belcaro G, Carratelli M, et al. A simple test to monitor oxidative stress. 18(2). Int Angiol. 1999; 127: 30.

Esterbauer H, Gebicki J, Puhl H, et al. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med. 1992; 13(4): 341-90.

Minn AH, Hafele C, Shalev A. Thio-redox-in-interacting protein is stimulated by glucose through a carbohydrate response element and induces B-cell apoptosis. Endocrinology. 2005; 146(5): 2397-405.

Cesaremo MR, Belcaro G, Carratelli M, et al. A simple test to monitor oxidative stress. 18(2). Int Angiol. 1999; 127: 30.
[161] Dandona P, Chaudhuri A, Ghanim H, et al. Insulin as an Anti-Inflammatory and Antiatherogenic Modulator. J Am Coll Cardiol. 2009; 53(SUPPL 5): 14-20.

[162] Monnier L, Colette C, Mas E, et al. Regulation of oxidative stress by glycaemic control: Evidence for an independent inhibitory effect of insulin therapy. Diabetologia. 2010; 53(3): 562-71.

[163] Dandona P, Aljada A, Mohanty P, et al. Insulin inhibits intranuclear nuclear factor kB and stimulates kB in mononuclear cells in obese subjects: Evidence for an anti-inflammatory effect? J Clin Endocrinol Metab. 2001; 86(7): 3257-65.

[164] Taddei S, Virdis A, Mattei P, et al. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. Circulation. 1995; 92(10): 2911-8.

[165] Rask-Madsen C, Dominguez H, Ihleman N, et al. Tumor Necrosis Factor-alfa Inhibits Insulin's Stimulating Effect on Glucose Uptake and Endothelium-Dependent Vasodilation in Humans. Circulation. 2003; 108(15): 1815-21.

[166] Rizzo M, Abate N, Chandalia M, et al. Liraglutide reduces oxidative stress and restores heme oxygenase-1 and ghrelin levels in patients with type 2 diabetes: A prospective pilot study. J Clin Endocrinol Metab. 2015; 100(2): 603-6.

[167] Suematsu M, Katsuki A, Sumida Y, et al. Decreased circulating levels of active ghrelin are associated with increased oxidative stress in obese subjects. Eur J Endocrinol. 2005; 153(3): 403-7.

[168] Bao W, Song F, Li X, et al. Plasma heme oxygenase-1 concentration is elevated in individuals with type 2 diabetes mellitus. PLoS ONE. 2010; 5(8).

[169] Dhindsa S, Tripathy D, Mohanty P, et al. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kB in mononuclear cells. Metabolism. 2004; 53(3): 330-4.

[170] Rakipovski G, Raun K, Lykkesfeldt J. Fluctuating hyperglycaemia increases oxidative stress response in lean rats compared to sustained hyperglycaemia despite lower glycaemic exposure. Diab Vasc Dis Res. 2011; 8(4): 295-8.

[171] Kelly AS, Bergenstal RM, Gonzalez-Campoy JM, et al. Effects of Exenatide vs. Metformin on endothelial function in obese patients with pre-diabetes: a randomized trial. Cardiovasc Diabetol. 2012; 11.

[172] Rojas LBA, Gomes MB. Metformin: An old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr. 2013; 5(1).

[173] Araki E, Nishikawa T. Oxidative stress: A cause and therapeutic target of diabetic complications. J Diabetes Invest. 2010; 1(3): 90-6.

[174] Kelly AS, Metzig AM, Rudser KD, et al. Exenatide as a weight-loss therapy in extreme pediatric obesity: A randomized, controlled pilot study. Obesity. 2012; 20(2): 364-70.

[175] Tesauro M, Schinzari F, Adamo A, et al. Effects of GLP-1 on forearm vasodilator function and glucose disposal during hyperinsulinemia in the metabolic syndrome. Diabetes Care. 2013; 36(3): 683-9.

[176] Okada K, Kotani K, Yagyu H, et al. Effects of treatment with liraglutide on oxidative stress and cardiac natriuretic peptide levels in patients with type 2 diabetes mellitus. Endocrine. 2014; 47(3): 962-4.

[177] Marso SP, Pouler NR, Nissen SE, et al. Design of the liraglutide effect and action in diabetes: Evaluation of cardiovascular outcome results (LEADER) trial. Am Heart J. 2013; 166(5): 823-30.

[178] Walsh ME, Shi Y, Van Remmen H. The effects of dietary restriction on oxidative stress in rodents. Free Radic Biol Med. 2014; 66: 88-99.

[179] Swindell WR. Dietary restriction in rats and mice: A meta-analysis and review of the evidence for genotype-dependent effects on lifespan. Ageing Res Rev. 2012; 11(2): 254-70.