GLP-1 receptor agonists show neuroprotective effects in animal models of diabetes

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Abstract

Enzyme-resistant receptor agonists of the incretin hormone glucagon-like peptide-1 (GLP-1) have shown positive therapeutic effects in people with type 2 diabetes mellitus (T2DM). T2DM has detrimental effects on brain function and impairment of cognition and memory formation has been described. One of the underlying mechanisms is most likely insulin de-sensitization in the brain, as insulin improves cognitive impairments and enhances learning. Treatment with GLP-1 receptor agonists improves memory formation and impairment of synaptic plasticity observed in animal models of diabetes-obesity. Furthermore, it has been shown that diabetes impairs growth factor signalling in the brain and reduces energy utilization in the cortex. Inflammation and apoptotic signalling was also increased. Treatment with GLP-1 receptor agonists improved neuronal growth and repair and reduced inflammation and apoptosis as well as oxidative stress. In comparison with the diabetes drug metformin, GLP-1 receptor agonists were able to improve glycemic control and reverse brain impairments, whereas metformin only normalized blood glucose levels. Clinical studies in non-diabetic patients with neurodegenerative disorders showed neuroprotective effects following administration with GLP-1 receptor agonists, demonstrating that neuroprotective effects are independent of blood glucose levels.

Highlights

- enzyme-resistant receptor agonists of GLP-1 are effective in treating diabetes
- GLP-1 plays important additional roles as a growth factor
- GLP-1 receptor agonists have protective effects in the brain
- impaired insulin signaling is restored by GLP-1 drugs
- other, glucose independent neuroprotective effects are found

Keywords: GLP-1; GIP; brain; neurons; growth factors, neurodegeneration, inflammation

1. Introduction

Glucagon-like peptide 1 (GLP-1) is a gut hormone secreted from enteroendocrine L-cells and is best known for its ability to enhance glucose-induced beta cell insulin secretion. In addition, GLP-1 also possesses a range of extrapancreatic actions which helps control blood glucose concentrations. Based on these observations, several GLP-1 receptor agonists that are resistant to enzyme degradation and have enhanced biological half-life in the blood stream have been developed as treatments for type 2 diabetes mellitus (T2DM) (Madsbad et al., 2008; Christensen et al., 2011; Campbell and Drucker, 2013). GLP-1 receptor agonists are effective, well received with few side effects, and widely used throughout the world to treat people with T2DM.

2. Diabetes and cognitive impairment

Studies have found evidence of cross-sectional and prospective associations between T2DM and cognitive impairment, memory and executive function (Stewart and Liolitsa, 1999; Yaffe et al., 2004). As vascular health is impaired by T2DM (Brands et al., 2004), both vascular and non-vascular factors are likely to play a role in causing this effect. Furthermore, T2DM has been identified as a risk factor for developing dementia (Luchsinger et al., 2004; Li et al., 2017). Epidemiological studies have shown a correlation between T2DM and the development of dementia in later life (Leibson et al., 1997; Luchsinger et al., 2004; Ristow, 2004; Strachan, 2005; Biessels et al., 2006; Haan, 2006). In one study, T2DM had been identified as a risk factor that doubled the likelihood of developing dementia (Janson et al., 2004). In a longitudinal study monitoring the health status of people over time, there was an increased risk of developing dementia in people with significantly elevated blood glucose levels (Schrijvers et al., 2010; Ohara et al., 2011; Li et al., 2017).

2.1 Insulin desensitization in the brain

A key parameter in developing T2DM is the desensitization of insulin signaling. Insulin signaling in T2DM is not only affected in the periphery, but in the brain as well (Gispen and Biessels, 2000; Biessels et al., 2002; Baker et al., 2011). In cognitive tests and in labelled deoxyglucose ^{18}FDG -PET brain imaging scans, it was found that people with T2DM exhibited poor performance and much reduced glucose uptake in cortical areas during cognitive testing (Baker et al., 2011). Reduced uptake of ^{18}FDG demonstrated reduced energy turnover and neuronal function. Moreover, brain tissue analysis of people with diabetes showed clear signs of neuropathology (Beeri et al., 2008). In animal models of T2DM, insulin signaling in the

brain was found to be markedly impaired (Yang et al., 2013; Agrawal et al., 2014).

2.2 Insulin boosts brain function

When insulin was administered via a nasal spray, people showed improved performance in several tests of attention, cognition and memory formation (Biessels et al., 2004; Freiherr et al., 2013). In clinical trials, verbal and spatial memory was improved after administration of a single dose of insulin (Benedict et al., 2008; Krug et al., 2010). Another study investigated the effects of 8 weeks of intranasal administration of insulin on memory and attention in healthy subjects in a double blind, placebo controlled trial. Blood glucose and plasma insulin levels were measured and did not differ between placebo and insulin treatment groups. After treatment, the delayed recall of words improved significantly (Benedict et al., 2004). Improvements in memory was even greater using insulin aspart, a fast-acting and long-lasting insulin analogue (Benedict et al., 2007).

2.3 Insulin plays important roles in brain function and neuronal growth

Insulin not only controls glucose utilization in the periphery (Hallschmid et al., 2004; Benedict et al., 2011; Hallschmid et al., 2012; Ott et al., 2012), but has additional roles in the brain. Insulin receptors have been identified in a variety of brain areas; the highest densities can be found in the cortex, olfactory bulb, hippocampus, and hypothalamus (Havrankova et al., 1978a; Havrankova et al., 1978b). Neurons express insulin receptors and their stimulation activates growth factor second messenger cascades that are vital for cell growth, repair and synaptic functions (de la Monte and Wands, 2005; Holscher, 2014). The observed cognitive impairments observed in people with T2DM and in animal models of diabetes can be explained by the loss of growth factor signaling. This in turn reduces the ability to withstand stressors and to repair damage that accumulates over time (Neth and Craft, 2017). In brain tissue of dementia patients, insulin signaling has also been shown to be severely impaired (Moloney et al., 2010; de la Monte, 2011; Talbot et al., 2012).

3. Protective effects of GLP-1 signaling in the brain

3.1 GLP-1 receptor agonists can reverse insulin de-sensitization in the brain

The incretin hormone GLP-1 is a growth factor and has similar properties to insulin. The GLP-1 receptor is a classic 7 membrane spanning G-protein coupled receptor of the glucagon class (Perry and Greig, 2002; Baggio and Drucker, 2007; Doyle and Egan, 2007; Holscher, 2014). GLP-1 and several of its analogues can cross the blood-brain-barrier (BBB) and exert

neuroprotective effects (Kastin et al., 2002; Kastin and Akerstrom, 2003; McClean et al., 2011; Hunter and Holscher, 2012; Christensen et al., 2015; Athauda et al., 2017). GLP-1 receptors are expressed in the brains of rodents, primates and humans (Merchenthaler et al., 1999; Cork et al., 2015; Heppner et al., 2015; Farr et al., 2016). GLP-1 receptor agonists such as exendin-4 or liraglutide can reverse insulin de-sensitization in the brain (Bomfim et al., 2012; Long-Smith et al., 2013). The localization and distribution of the insulin receptor and increased levels of insulin receptor substrate (IRS)-1 phosphorylated at serine 616 (IRS-1 pS(616)), a key marker of insulin resistance, was normalized in the brains of mice by treatment with liraglutide (Long-Smith et al., 2013). Liraglutide also improved key biomarkers in the brains of diabetic rats. The levels of insulin found in the brain were reduced, and there was a decrease in the phosphorylation of protein kinase B (AKT) and glycogen synthase kinase-3beta (GSK-3beta), which indicated decreased insulin signaling in rats with T2DM. Liraglutide treatment not only ameliorated hyperglycemia and peripheral insulin resistance, but also reversed brain insulin de-sensitization in a time-dependent manner (Yang et al., 2013). In the STZ model, insulin signaling was re-sensitized following activation of GLP-1 receptors, as illustrated by reduction of phospho-IRS1^{Ser1101} levels and by pAkt^{Ser473} upregulation and reactivation (Shi et al., 2017). Through GLP-1 receptor activation, cAMP/PKA/CREB growth factor signaling cascade is activated thus increasing gene expression of the insulin receptor, insulin, IRS-1, Akt and other growth factor-related proteins (Perfetti et al., 2000; Doyle and Egan, 2007; Park et al., 2010; Holscher, 2014; Talbot, 2014).

3.2 GLP-1 receptor agonists normalize cognitive impairments in T2DM

Several studies examining learning and memory in animal models show clear cognitive impairments induced by diabetes-obesity. In the high fat diet mouse model, memory formation was impaired and treatment with exendin-4 reversed this (Gault et al., 2010). Exendin-4 also protected streptozotocin (STZ)-induced diabetic rats from learning impairments as demonstrated using an elevated plus maze task and passive avoidance task (Gumuslu et al., 2016). Treatment with native GLP-1 also protected memory formation in STZ-treated rats (Iwai et al., 2009). Liraglutide protected STZ treated rats from impairments of learning a water maze task and a passive avoidance task, and improved motor impairments observed in the forced swimming test, open field, elevated plus maze, and rotarod motor coordination tests (Palleria et al., 2017). Liraglutide also normalized object recognition memory impairments in mice were maintained on a high fat diet (Porter et al., 2010). Furthermore, the DPP-4 inhibitor Sitagliptin, which elevates GLP-1 concentrations by reducing GLP-1 degradation, protected

memory formation in a high fat diet mouse model (Gault et al., 2015). Importantly, this effect is not entirely due to the normalization of blood glucose levels. When comparing effects of the enzyme-resistant GLP-1 analogue (Val8)GLP-1(GluPAL) with the diabetes drug metformin, it was found that both drugs effectively controlled blood glucose levels in high fat fed mice. However, the memory impairment observed in diabetic mice was not reversed in the metformin drug group alone, but only in the (Val8)GLP-1(GluPAL) treated group, see fig. 1 (Lennox et al., 2014). This clearly indicates that the neuroprotective effect of GLP-1 signaling goes beyond the regulation of glucose levels. GLP-1 receptor agonists have also shown neuroprotective effects in non-diabetic patients of Alzheimer's or Parkinson's disease, underscoring the protective effects that are independent of blood glucose regulation (Gejl et al., 2016; Athauda et al., 2017). In contrast, metformin enhances the risk of developing Alzheimer's or Parkinson's disease in people with T2DM, demonstrating that control of blood glucose is not sufficient to protect the brain in the same way that GLP-1 receptor agonists do (Hsu et al., 2011; Kuan et al., 2017).

3.3 GLP-1 receptor agonists normalize synaptic plasticity in the brain

Neurons communicate via synaptic activity, and long-term potentiation of synaptic activity (LTP) is considered to be the cellular correlate of memory (Bliss and Collingridge, 1993; Hölscher, 1999). When stimulating pyramidal neurons in area CA3 of hippocampal formation, the synapses projecting to CA1 neurons are upregulated. In diabetic animals, LTP has been found to be impaired. When treating high fat fed mice with liraglutide, the diabetes-induced block of LTP in the hippocampus was found to be reversed (Gault et al., 2010). Liraglutide also protected LTP formation in mice were maintained on a high fat diet (Porter et al., 2010). Native GLP-1 was also able to rescue impairments in synaptic transmission in STZ-treated rats (Iwai et al., 2009). In the *ob/ob* mouse model of diabetes, liraglutide rescued LTP in the hippocampus (Porter et al., 2013). GLP-1 has direct modulatory effects on synaptic activity, independent of the growth factor related effects, as shown in acute drug treatment in electrophysiological recording experiments (Gault and Holscher, 2008; Wang et al., 2013; Korol et al., 2014).

Similar to the effects on memory formation, when comparing the effects of the enzyme-resistant GLP-1 analogue (Val8)GLP-1(GluPAL) with the diabetes drug metformin, it was found that both drugs effectively controlled blood glucose levels in high fat fed mice, but the block of LTP observed in diabetic mice was not reversed in the metformin drug group, only in

the (Val8)GLP-1(GluPAL) treated group (Lennox et al., 2014) (see fig. 2).

3.4 Other neuroprotective effects of GLP-1 receptor agonists

3.4.1 Growth factor expression

GLP-1 receptor activation not only normalizes insulin signaling, but the impaired signaling of several other key growth factors, such as insulin-like growth factor 1 (IGF-1) (Moloney et al., 2010; Torres-Aleman, 2010), brain-derived neurotrophic factor (BDNF) (Park et al., 2010; Gumuslu et al., 2016), glia-derived neurotrophic factor (GDNF) (Allen et al., 2013; Yuan et al., 2017), and others. Exendin-4 normalized BDNF expression in the STZ rat model of diabetes (Gumuslu et al., 2016). Treatment of high fat fed mice with (Val8)GLP-1(GluPAL) normalized the expression of vascular endothelial growth factor (VEGF) (Lennox et al., 2014). Sitagliptin also enhanced VEGF expression (Gault et al., 2015). Other studies found normalization in expression and function of other growth factors after treatment with GLP-1 receptor agonists (Holscher, 2014; Yuan et al., 2017). These growth factors have neuroprotective effects and protect synapses and keep them functional under conditions of cellular stress (Cheng and Mattson, 1994; Yamada et al., 2001; Allen et al., 2013; Holscher, 2014).

3.4.2 Neurogenesis

While neurons do not divide and regenerate in most parts of the brain, there are specific brain regions such as the hippocampus/dentate gyrus where neurogenesis is still observed even in the adult brain. Neurogenesis is impaired in diabetic animals (Lang et al., 2009; Guo et al., 2010; Park et al., 2010). Exendin-4 normalized neurogenesis in STZ treated rats (Solmaz et al., 2015), while liraglutide normalized neurogenesis in *ob/ob* mice (Porter et al., 2013). Liraglutide or lixisenatide can enhance neurogenesis in wild type mice (Hunter and Holscher, 2012). Treatment with Sitagliptin also rescued neurogenesis in T2DM mice (Gault et al., 2015). Continuous neurogenesis is considered to be an important factor in long-term memory formation (Winocur et al., 2006).

3.4.3 Second messenger signalling for cell growth, repair, energy utilization and autophagy

GLP-1 signaling can compensate for the loss of other growth factors and insulin signaling in the brain. The main second messenger signaling pathway is the cAMP-PKA-CREB expression pathway (Doyle and Egan, 2007). However, other pathways such as Akt/PKB, AMPk and ERK kinase activity are also enhanced by GLP-1 receptor activation (Sharma et al., 2013; Jalewa et

al., 2016; Palleria et al., 2017). Genes that are activated include those relevant to energy utilization, for example, glucose uptake, mitochondrial function and replacement of damaged mitochondria (Lennox et al., 2014; Jalewa et al., 2016; Palleria et al., 2017); cell signaling that is linked to blocking apoptosis, for example, Bcl2 and Bax/BAD signaling and caspase activation (Baggio and Drucker, 2007; Kimura et al., 2009; Lupi et al., 2010); genes that control DNA repair (Yang et al., 2017), as well as control of chronic inflammation response in the brain that is observed in diabetics and that enhances oxidative stress (Parthsarathy and Holscher, 2013; Gault et al., 2015; Qin et al., 2016). In addition, autophagy, an important protective process that helps to eliminate cell debris that can become toxic if left to accumulate, is also enhanced and controlled by GLP-1 signaling (Jalewa et al., 2016; Panagaki et al., 2017).

4. Is GLP-1 unique?

Neuroprotective hormones that are released to signal energy availability and have cytoprotective properties form a large family. They include glucagon (Lund et al., 2011), insulin (Dailey, 2007), IGF-1 (Levine et al., 2012), leptin (Harvey, 2013), ghrelin (Gomez et al., 2009), oxyntomodulin (Pocai, 2014), adinopectin (Katsiki et al., 2011), GLP-1 (Baggio and Drucker, 2007), GLP-2 (Lund et al., 2011), GIP (Finan et al., 2016) and others. One might speculate if GLP-1 has a unique role to play in physiology or if its success is just a random finding, simply dictated by the fact that it was one of the first incretin hormones to be identified. However, it appears that there are differences between these hormones. The main reason why GIP had not been chosen to act as a novel treatment for type II diabetes even though it had been discovered first is because it was found to desensitize in diabetic patients (Vilsboll et al., 2002; Mohammad et al., 2014). Insulin obviously desensitizes, as does IGF-1 (Cohen et al., 2009), ghrelin (Theodoropoulou et al., 2012), leptin (Clemmensen et al., 2013), adinopectin (Satoh et al., 2005) and others. It appears that GLP-1 does not desensitize. What could be the reason for this? Perhaps analysing the mechanisms that cause desensitization will cast some light on this issue. In an acute inflammation response, the role of pro-inflammatory cytokines that are released by immune cells is to close down growth factor signalling (Musolino et al., 2017). Inflammation is observed in obesity, diabetes, Alzheimer's and Parkinson's disease (Craft, 2005; Holmes et al., 2009; Tansey and Goldberg, 2010; Ferrari and Tarelli, 2011; Stafeev et al., 2017). Pro-inflammatory cytokines such as TNF-α and growth factors/ anti-inflammatory cytokines counteract each other (Rossert et al., 2000; Calixto et al., 2004; Cotman et al., 2007; Bomfim et al., 2012; Musolino et al., 2017). The purpose appears to be to preserve energy and

to protect cells that are exposed to free radicals released during the inflammation response. When the acute inflammation response is coming to an end, anti-inflammatory cytokines are released in order to re-activate cell growth and energy utilization (Herder et al., 2013; Musolino et al., 2017). GLP-1 is one of such anti-inflammatory cytokines (Dozier et al., 2009; Shiraki et al., 2012; Parthsarathy and Holscher, 2013). Therefore, the reason why GLP-1 analogues are so successful in re-sensitizing insulin, IGF-1, and other growth factor signalling pathways is because GLP-1 signalling does not desensitize. If all growth factor signalling desensitized in the affected tissue, it would not be possible to reverse that situation. Some signalling pathways have to remain open to be accessible and to signal the end of the inflammatory response. GLP-1 appears to be one of those privileged signalling pathways. There are many more, and perhaps there is a field of treasures right there, waiting to be discovered.

5. Conclusion

GLP-1 receptor agonists are effective treatments for T2DM and are widely used throughout the world. The evidence presented here documents that the beneficial effects exceed those of simply enhancing insulin release during hyperglycemic episodes and helping to normalize blood glucose levels. Additional beneficial effects are observed that are directly induced by GLP-1 receptor activation in the brain and that are visible even in non-diabetic people, and not visible in diabetic people that show good control of T2DM by non-GLP-1 diabetes drugs. Further research is required to investigate the underlying mechanisms of these additional neuroprotective processes.

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References

Agrawal R, Zhuang Y, Cummings BP, Stanhope KL, Graham JL, Havel PJ, Gomez-Pinilla F (2014) Deterioration of plasticity and metabolic homeostasis in the brain of the UCD-T2DM rat model of naturally occurring type-2 diabetes. Biochimica et biophysica acta 1842:1313-1323.

Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK (2013) GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacology & therapeutics 138:155-175.

Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, Hibbert S, Budnik N, Zampedri L, Dickson J, Li Y, Aviles-Olmos I, Warner TT, Limousin P, Lees AJ,

Greig NH, Tebbs S, Foltynie T (2017) Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. The Lancet 6736:31585-31584.
Baggio LL, Drucker DJ (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157.
Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S (2011) Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Archives of neurology 68:51-57.
Beeri MS, Schmeidler J, Silverman JM, Gandy S, Wysocki M, Hannigan CM, Purohit DP, Lesser G, Grossman HT, Haroutunian V (2008) Insulin in combination with other diabetes medication is associated with less Alzheimer neuropathology. Neurology 71:750-757.
Benedict C, Kern W, Schultes B, Born J, Hallschmid M (2008) Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. The Journal of clinical endocrinology and metabolism 93:1339-1344.
Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W (2004) Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29:1326-1334.
Benedict C, Brede S, Schioth HB, Lehnert H, Schultes B, Born J, Hallschmid M (2011) Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 60:114-118.
Benedict C, Hallschmid M, Schmitz K, Schultes B, Ratter F, Fehm HL, Born J, Kern W (2007) Intranasal insulin improves memory in humans: superiority of insulin aspart. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:239-243.
Biessels GJ, Bravenboer B, Gispen WH (2004) Glucose, insulin and the brain: modulation of cognition and synaptic plasticity in health and disease: a preface. European journal of pharmacology 490:1-4.
Biessels GJ, van der Heide LP, Kamal A, Bleys RL, Gispen WH (2002) Ageing and diabetes: implications for brain function. European journal of pharmacology 441:1-14.
Biessels GJ, De Leeuw FE, Lindeboom J, Barkhof F, Scheltens P (2006) Increased cortical atrophy in patients with Alzheimer's disease and type 2 diabetes mellitus. Journal of neurology, neurosurgery, and psychiatry 77:304-307.
Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.
Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, Silverman MA, Kazi H, Melo HM, McClean PL, Holscher C, Arnold SE, Talbot K, Klein WL, Munoz DP, Ferreira ST, De Felice FG (2012) An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. The Journal of clinical investigation 122:1339-1353.
Brands AM, Kessels RP, de Haan EH, Kappelle LJ, Biessels GJ (2004) Cerebral dysfunction in type 1 diabetes: effects of insulin, vascular risk factors and blood-glucose levels. European journal of pharmacology 490:159-168.
Calixto JB, Campos MM, Otuki MF, Santos AR (2004) Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med 70:93-103.

Campbell JE, Drucker DJ (2013) Pharmacology, physiology, and mechanisms of incretin hormone action. Cell metabolism 17:819-837.
Cheng B, Mattson MP (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. Brain Res 640:56-67.
Christensen M, Knop FK, Vilsboll T, Holst JJ (2011) Lixisenatide for type 2 diabetis mellitus. Expert opinion on investigational drugs 20:549-557.
Christensen M, Sparre-Ulrich AH, Hartmann B, Grevstad U, Rosenkilde MM, Holst JJ, Vilsboll T, Knop FK (2015) Transfer of liraglutide from blood to cerebrospinal fluid is minimal in patients with type 2 diabetes. Int J Obes (Lond) 39:1651-1654.
Clemmensen C, Chabenne J, Finan B, Sullivan L, Fischer K, Kuchler D, Sehrer L, Ograjsek T, Hofmann S, Schriever SS, Pfluger PT, Pinkstaff J, Tschop MH, Dimarchi R, Muller TD (2013) GLP-1/glucagon co-agonism restores leptin responsiveness in obese mice chronically maintained on an obesogenic diet. Diabetes 63:1422-1427.
Cohen E, Paulsson JF, Blinder P, Burstyn-Cohen T, Du D, Estepa G, Adame A, Pham HM, Holzenberger M, Kelly JW, Masliah E, Dillin A (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. Cell 139:1157-1169.
Cork SC, Richards JE, Holt MK, Gribble FM, Reimann F, Trapp S (2015) Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. Mol Metab 4:718-731.
Cotman CW, Berchtold NC, Christie LA (2007) Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci 30:464-472.
Craft S (2005) Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. Neurobiology of aging 26 Suppl 1:65-69.
Dailey GE (2007) Using prandial insulin to achieve glycemic control in type 2 diabetes. The Journal of family practice 56:735-742.
de la Monte SM (2011) Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease. Drugs 72:49-66.
de la Monte SM, Wands JR (2005) Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. Journal of Alzheimer's disease : JAD 7:45-61.
Doyle ME, Egan JM (2007) Mechanisms of action of glucagon-like peptide 1 in the pancreas. Pharmacology & therapeutics 113:546-593.
Dozier KC, Cureton EL, Kwan RO, Curran B, Sadjadi J, Victorino GP (2009) Glucagon-like peptide-1 protects mesenteric endothelium from injury during inflammation. Peptides 30:1735-1741.
Farr OM, Sofopoulos M, Tsoukas MA, Dincer F, Thakkar B, Sahin-Efe A, Filippaios A, Bowers J, Srnka A, Gavrieli A, Ko BJ, Liakou C, Kanyuch N, Tseleni-Balafouta S, Mantzoros CS (2016) GLP-1 receptors exist in the parietal cortex, hypothalamus and medulla of human brains and the GLP-1 analogue liraglutide alters brain activity related to highly desirable food cues in individuals with diabetes: a crossover, randomised, placebo-controlled trial. Diabetologia 59:954-965.
Ferrari CC, Tarelli R (2011) Parkinson's disease and systemic inflammation. Parkinson's disease 2011:436813.
Finan B, Muller TD, Clemmensen C, Perez-Tilve D, DiMarchi RD, Tschop MH (2016) Reappraisal of GIP Pharmacology for Metabolic Diseases. Trends Mol Med 22:359-376.

Freiherr J, Hallschmid M, Frey WH, 2nd, Brunner YF, Chapman CD, Holscher C, Craft S, De Felice FG, Benedict C (2013) Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. CNS drugs 27:505-514.

Gault VA, Holscher C (2008) GLP-1 agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. European journal of pharmacology 587:112-117.

Gault VA, Lennox R, Flatt PR (2015) Sitagliptin, a DPP-4 inhibitor, improves recognition memory, oxidative stress, hippocampal neurogenesis and up-regulates key genes involved in cognitive decline. Diabetes, obesity & metabolism 7:401-413.

Gault VA, Porter WD, Flatt PR, Holscher C (2010) Actions of exendin-4 therapy on cognitive function and hippocampal synaptic plasticity in mice fed a high-fat diet. Int J Obes (Lond) 34:1341-1344.

Gejl M, Gjedde A, Egefjord L, Møller A, Hansen SB, Vang K, Rodell AB, Braendgaard H, Gottrup H, Schacht A, Møller N, Brock B, Rungby J (2016) In Alzheimer's Disease, Six-Month Treatment with GLP-1 Analogue Prevents Decline of Brain Glucose Metabolism: Randomized, Placebo-Controlled, Double-Blind Clinical Trial. Frontiers in aging neuroscience 8:1-10.

Gispen WH, Biessels GJ (2000) Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci 23:542-549.

Gomez R, Lago F, Gomez-Reino JJ, Gualillo O (2009) Novel factors as therapeutic targets to treat diabetes. Focus on leptin and ghrelin. Expert opinion on therapeutic targets 13:583-591.

Gumuslu E, Mutlu O, Celikyurt IK, Ulak G, Akar F, Erden F, Ertan M (2016) Exenatide enhances cognitive performance and upregulates neurotrophic factor gene expression levels in diabetic mice. Fundam Clin Pharmacol.

Guo J, Yu C, Li H, Liu F, Feng R, Wang H, Meng Y, Li Z, Ju G, Wang J (2010) Impaired neural stem/progenitor cell proliferation in streptozotocin-induced and spontaneous diabetic mice. Neurosci Res.

Haan MN (2006) Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nat Clin Pract Neurol 2:159-166.

Hallschmid M, Higgs S, Thienel M, Ott V, Lehnert H (2012) Postprandial administration of intranasal insulin intensifies satiety and reduces intake of palatable snacks in women. Diabetes 61:782-789.

Hallschmid M, Benedict C, Schultes B, Fehm HL, Born J, Kern W (2004) Intranasal insulin reduces body fat in men but not in women. Diabetes 53:3024-3029.

Harvey J (2013) Leptin regulation of neuronal morphology and hippocampal synaptic function. Frontiers in synaptic neuroscience 5:3.

Havrankova J, Roth J, Brownstein M (1978a) Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272:827-829.

Havrankova J, Schmechel D, Roth J, Brownstein M (1978b) Identification of insulin in rat brain. Proc Natl Acad Sci U S A 75:5737-5741.

Heppner KM, Kirigiti M, Secher A, Paulsen SJ, Buckingham R, Pyke C, Knudsen LB, Vrang N, Grove KL (2015) Expression and distribution of glucagon-like peptide-1 receptor mRNA, protein and binding in the male nonhuman primate (Macaca mulatta) brain. Endocrinology 156:255-267.

Herder C, Carstensen M, Ouwens DM (2013) Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes, obesity & metabolism 15 Suppl 3:39-50.

Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, Culliford D, Perry VH (2009) Systemic inflammation and disease progression in Alzheimer disease. Neurology 73:768-774.

Holscher C (2014) Insulin, incretins and other growth factors as potential novel treatments for Alzheimer's and Parkinson's diseases. Biochemical Society transactions 42:593-599.

Hölscher C (1999) Synaptic plasticity and learning and memory: LTP and beyond. Journal of neuroscience research 58:62-75.

Hsu CC, Wahlqvist ML, Lee MS, Tsai HN (2011) Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. Journal of Alzheimer's disease : JAD 24:485-493.

Hunter K, Holscher C (2012) Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. BMC neuroscience 13:33-38.

Iwai T, Suzuki M, Kobayashi K, Mori K, Mogi Y, Oka JI (2009) The influences of juvenile diabetes on memory and hippocampal plasticity in rats: Improving effects of glucagon-like peptide-1. Neurosci Res.

Jalewa J, Sharma MK, Holscher C (2016) Novel incretin analogues improve autophagy and protect from mitochondrial stress induced by rotenone in SH-SY5Y cells. Journal of neurochemistry 139:55-67.

Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC (2004) Increased risk of type 2 diabetes in Alzheimer disease. Diabetes 53:474-481.

Kastin AJ, Akerstrom V (2003) Entry of exendin-4 into brain is rapid but may be limited at high doses. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 27:313-318.

Kastin AJ, Akerstrom V, Pan W (2002) Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. Journal of molecular neuroscience : MN 18:7-14.

Katsiki N, Mikhailidis DP, Gotzamani-Psarrakou A, Yovos JG, Karamitsos D (2011) Effect of various treatments on leptin, adiponectin, ghrelin and neuropeptide Y in patients with type 2 diabetes mellitus. Expert opinion on therapeutic targets 15:401-420.

Kimura R, Okouchi M, Fujioka H, Ichiyanagi A, Ryuge F, Mizuno T, Imaeda K, Okayama N, Kamiya Y, Asai K, Joh T (2009) Glucagon-like peptide-1 (GLP-1) protects against methylglyoxal-induced PC12 cell apoptosis through the PI3K/Akt/mTOR/GCLc/redox signaling pathway. Neuroscience 162:1212-1219.

Korol SV, Jin Z, Babateen O, Birnir B (2014) Glucagon-like peptide-1 (GLP-1) and exendin-4 transiently enhance GABAA receptor-mediated synaptic and tonic currents in rat hippocampal CA3 pyramidal neurons. Diabetes.

Krug R, Benedict C, Born J, Hallschmid M (2010) Comparable sensitivity of postmenopausal and young women to the effects of intranasal insulin on food intake and working memory. The Journal of clinical endocrinology and metabolism 95:E468-472.

Kuan YC, Huang KW, Lin CL, Hu CJ, Kao CH (2017) Effects of metformin exposure on neurodegenerative diseases in elderly patients with type 2 diabetes mellitus. Prog Neuropsychopharmacol Biol Psychiatry 79:77-83.

Lang BT, Yan Y, Dempsey RJ, Vemuganti R (2009) Impaired neurogenesis in adult type-2 diabetic rats. Brain research 1258:25-33.

Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ (1997) Risk of dementia among persons with diabetes mellitus: a population-based cohort study. American journal of epidemiology 145:301-308.

Lennox R, Porter DW, Flatt PR, Holscher C, Irwin N, Gault VA (2014) Comparison of the independent and combined effects of sub-chronic therapy with metformin and a stable GLP-1 receptor agonist on cognitive function, hippocampal synaptic plasticity and metabolic control in high-fat fed mice. Neuropharmacology 86C:22-30.

Levine AJ, Harris CR, Puzio-Kuter AM (2012) The interfaces between signal transduction pathways: IGF-1/mTor, p53 and the Parkinson Disease pathway. Oncotarget 3:1301-1307.

Li TC, Yang CP, Tseng ST, Li CI, Liu CS, Lin WY, Hwang KL, Yang SY, Chiang JH, Lin CC (2017) Visit-to-Visit Variations in Fasting Plasma Glucose and HbA1c Associated With an Increased Risk of Alzheimer Disease: Taiwan Diabetes Study. Diabetes care 40:1210-1217.

Long-Smith CM, Manning S, McClean PL, Coakley MF, O'Halloran DJ, Holscher C, O'Neill C (2013) The diabetes drug liraglutide ameliorates aberrant insulin receptor localisation and signalling in parallel with decreasing both amyloid-beta plaque and glial pathology in a mouse model of Alzheimer's disease. Neuromolecular medicine 15:102-114.

Luchsinger JA, Tang MX, Shea S, Mayeux R (2004) Hyperinsulinemia and risk of Alzheimer disease. Neurology 63:1187-1192.

Lund A, Vilsboll T, Bagger JI, Holst JJ, Knop FK (2011) The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. American journal of physiology Endocrinology and metabolism 300:E1038-1046.

Lupi R, Del Guerra S, D'Aleo V, Boggi U, Filipponi F, Marchetti P (2010) The direct effects of GLP-1 and GIP, alone or in combination, on human pancreatic islets. Regulatory peptides 165:129-132.

Madsbad S, Krarup T, Deacon CF, Holst JJ (2008) Glucagon-like peptide receptor agonists and dipeptidyl peptidase-4 inhibitors in the treatment of diabetes: a review of clinical trials. Curr Opin Clin Nutr Metab Care 11:491-499.

McClean P, Parthsarathy V, Faivre E, Hölscher C (2011) The diabetes drug Liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:6587-6594.

Merchenthaler I, Lane M, Shughrue P (1999) Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. The Journal of comparative neurology 403:261-280.

Mohammad S, Patel RT, Bruno J, Panhwar MS, Wen J, McGraw TE (2014) A naturally occurring GIP receptor variant undergoes enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. Molecular and cellular biology 34:3618-3629.

Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C (2010) Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. Neurobiology of aging 31:224-243.

Musolino C, Allegra A, Innao V, Allegra AG, Pioggia G, Gangemi S (2017) Inflammatory and Anti-Inflammatory Equilibrium, Proliferative and Antiproliferative Balance: The Role of Cytokines in Multiple Myeloma. Mediators of inflammation 2017:1852517.

Neth BJ, Craft S (2017) Insulin Resistance and Alzheimer's Disease: Bioenergetic Linkages. Frontiers in aging neuroscience 9:345.

Ohara T, Doi Y, Ninomiya T, Hirakawa Y, Hata J, Iwaki T, Kanba S, Kiyohara Y (2011) Glucose tolerance status and risk of dementia in the community: The Hisayama Study. Neurology 77:1126-1134.

Ott V, Benedict C, Schultes B, Born J, Hallschmid M (2012) Intranasal administration of insulin to the brain impacts cognitive function and peripheral metabolism. Diabetes Obes Metab 14:214-221.

Palleria C, Leo A, Andreozzi F, Citraro R, Iannone M, Spiga R, Sesti G, Constanti A, De Sarro G, Arturi F, Russo E (2017) Liraglutide prevents cognitive decline in a rat model of streptozotocin-induced diabetes independently from its peripheral metabolic effects. Behavioural brain research 321:157-169.

Panagaki T, Michael M, Hölscher C (2017) Liraglutide restores chronic ER stress, autophagy impairments and apoptotic signalling in SH-SY5Y cells. Scientific Reports DOI:10.1038/s41598-41017-16488-x.

Park HR, Park M, Choi J, Park KY, Chung HY, Lee J (2010) A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. Neuroscience letters 482:235-239.

Parthsarathy V, Holscher C (2013) The type 2 diabetes drug liraglutide reduces chronic inflammation induced by irradiation in the mouse brain. European journal of pharmacology 700:42-50.

Perfetti R, Zhou J, Doyle ME, Egan JM (2000) Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology 141:4600-4605.

Perry T, Greig NH (2002) The glucagon-like peptides: a new genre in therapeutic targets for intervention in Alzheimer's disease. Journal of Alzheimer's disease : JAD 4:487-496.

Pocai A (2014) Action and therapeutic potential of oxyntomodulin. Mol Metab 3:241-251.

Porter DW, Kerr BD, Flatt PR, Holscher C, Gault VA (2010) Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance. Diabetes, obesity & metabolism 12:891-899.

Porter WD, Flatt PR, Holscher C, Gault VA (2013) Liraglutide improves hippocampal synaptic plasticity associated with increased expression of Mash1 in ob/ob mice. Int J Obes (Lond) 37:678-684.

Qin L, Chong T, Rodriguez R, Pugazhenthi S (2016) Glucagon-like peptide-1-mediated modulation of inflammatory pathways in the diabetic brain: Relevance to Alzheimer's disease. Current Alzheimer research 13:1346-1355.

Ristow M (2004) Neurodegenerative disorders associated with diabetes mellitus. J Mol Med 82:510-529.

Rossert J, Terraz-Durasnel C, Brideau G (2000) Growth factors, cytokines, and renal fibrosis during the course of diabetic nephropathy. Diabetes & metabolism 26 Suppl 4:16-24.

Satoh H, Nguyen MT, Trujillo M, Imamura T, Usui I, Scherer PE, Olefsky JM (2005) Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar rats. Diabetes 54:1304-1313.

Schrijvers EM, Witteman JC, Sijbrands EJ, Hofman A, Koudstaal PJ, Breteler MM (2010) Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. Neurology 75:1982-1987.

Sharma M, Jalewa J, Holscher C (2013) Neuroprotective and anti-apoptotic effects of Liraglutide on SH-SY5Y cells exposed to Methylglyoxal stress. Journal of neurochemistry 128:459-471.

Shi LJ, Zhang Z, Li L, Hölscher C (2017) A novel dual GLP-1 / GIP receptor agonist alleviates cognitive decline by re-sensitizing insulin signaling in the Alzheimer icv. STZ rat model. Behavioural brain research 237:65-74.

Shiraki A, Oyama JI, Komoda H, Asaka M, Komatsu A, Sakuma M, Kodama K, Sakamoto Y, Kotooka N, Hirase T, Node K (2012) The glucagon-like peptide 1 analog liraglutide reduces TNF-alpha-induced oxidative stress and inflammation in endothelial cells. Atherosclerosis 221:375-382.

Solmaz V, Cinar BP, Yigitturk G, Cavusoglu T, Taskiran D, Erbas O (2015) Exenatide reduces TNF-alpha expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats. European journal of pharmacology 765:482-487.

Stafeev IS, Vorotnikov AV, Ratner EI, Menshikov MY, Parfyonova YV (2017) Latent Inflammation and Insulin Resistance in Adipose Tissue. International journal of endocrinology 2017:5076732.

Stewart R, Liolitsa D (1999) Type 2 diabetes mellitus, cognitive impairment and dementia. Diabetic medicine : a journal of the British Diabetic Association 16:93-112.

Strachan MW (2005) Insulin and cognitive function in humans: experimental data and therapeutic considerations. Biochemical Society transactions 33:1037-1040.

Talbot K (2014) Brain insulin resistance in Alzheimer's disease and its potential treatment with GLP-1 analogs. Neurodegenerative disease management 4:31-40.

Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. The Journal of clinical investigation 122:1316-1338.

Tansey MG, Goldberg MS (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiology of disease 37:510-518.

Theodoropoulou A, Metallinos IC, Psyrogiannis A, Vagenakis GA, Kyriazopoulou V (2012) Ghrelin and leptin secretion in patients with moderate Alzheimer's disease. The journal of nutrition, health & aging 16:472-477.

Torres-Aleman I (2010) Toward a comprehensive neurobiology of IGF-I. Developmental neurobiology 70:384-396.

Vilsboll T, Krarup T, Madsbad S, Holst JJ (2002) Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. Diabetologia 45:1111-1119.

Wang XH, Yang W, Holscher C, Wang ZJ, Cai HY, Li QS, Qi JS (2013) Val(8)-GLP-1 remodels synaptic activity and intracellular calcium homeostasis impaired by amyloid beta peptide in rats. Journal of neuroscience research 91:568-577.

Winocur G, Wojtowicz JM, Sekeres M, Snyder JS, Wang S (2006) Inhibition of neurogenesis interferes with hippocampus-dependent memory function. Hippocampus 16:296-304.

Yaffe K, Blackwell T, Kanaya AM, Davidowitz N, Barrett-Connor E, Krueger K (2004) Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. Neurology 63:658-663.

Yamada M, Tanabe K, Wada K, Shimoke K, Ishikawa Y, Ikeuchi T, Koizumi S, Hatanaka H (2001) Differences in survival-promoting effects and intracellular signaling properties of BDNF and IGF-1 in cultured cerebral cortical neurons. Journal of neurochemistry 78:940-951.

Yang JL, Chen WY, Chen SD (2017) The Emerging Role of GLP-1 Receptors in DNA Repair: Implications in Neurological Disorders. Int J Mol Sci 18.

Yang Y, Zhang J, Ma D, Zhang M, Hu S, Shao S, Gong CX (2013) Subcutaneous administration of liraglutide ameliorates Alzheimer-associated tau hyperphosphorylation in rats with type 2 diabetes. Journal of Alzheimer's disease : JAD 37:637-648.

Yuan Z, Li L, Feng P, Xue G, Ji C, Li G, Hölscher C (2017) A novel GLP-1/GIP dual agonist is more effective than liraglutide in reducing inflammation and enhancing GDNF release in the MPTP mouse model of Parkinson’s disease. European journal of pharmacology 812:82-90.

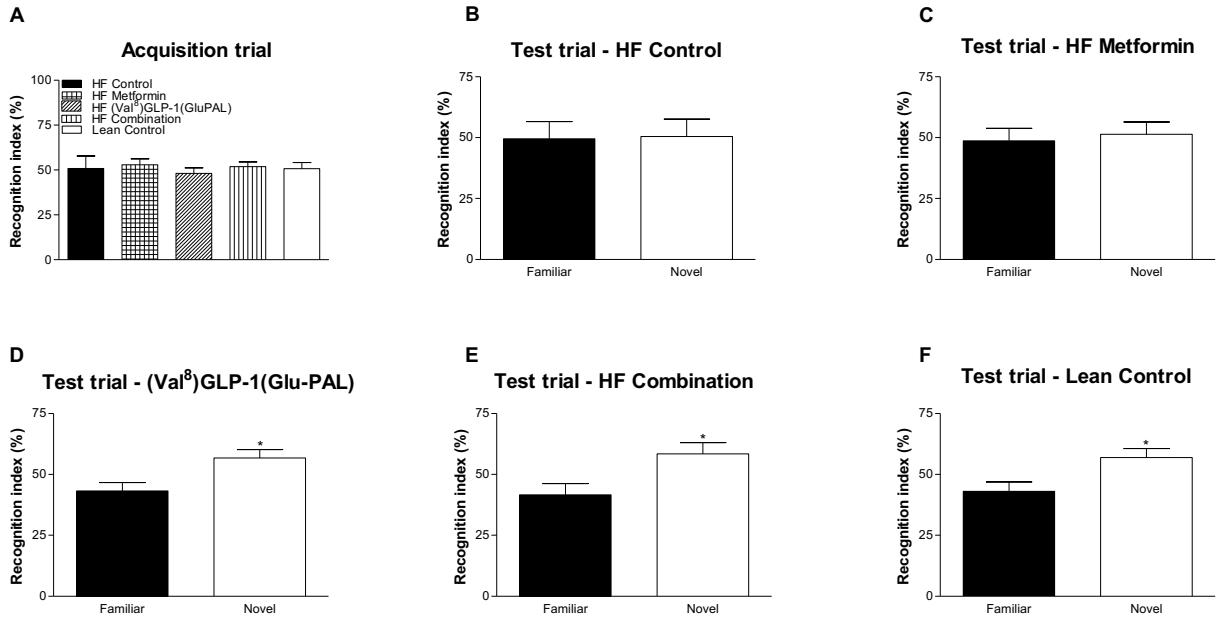

Figure 1: Effects of 20 days treatment with (Val^8)GLP-1(GluPAL), metformin or combined drug administration on recognition memory in high fat fed mice. Acquisition (A) and test (B-F) tasks in high fat fed mice. The recognition index (RI) was defined as the amount of time exploring the familiar (tA) or novel object (tB) over the total time spent exploring both objects x 100: (tA or tB/(tA+tB))∗100. Values are means ± SE for ten mice. *P < 0.05 compared with saline-treated HF control mice. For technical details, see (Lennox et al., 2014). This Figure has been reproduced with permission (Lenox et al., 2014).

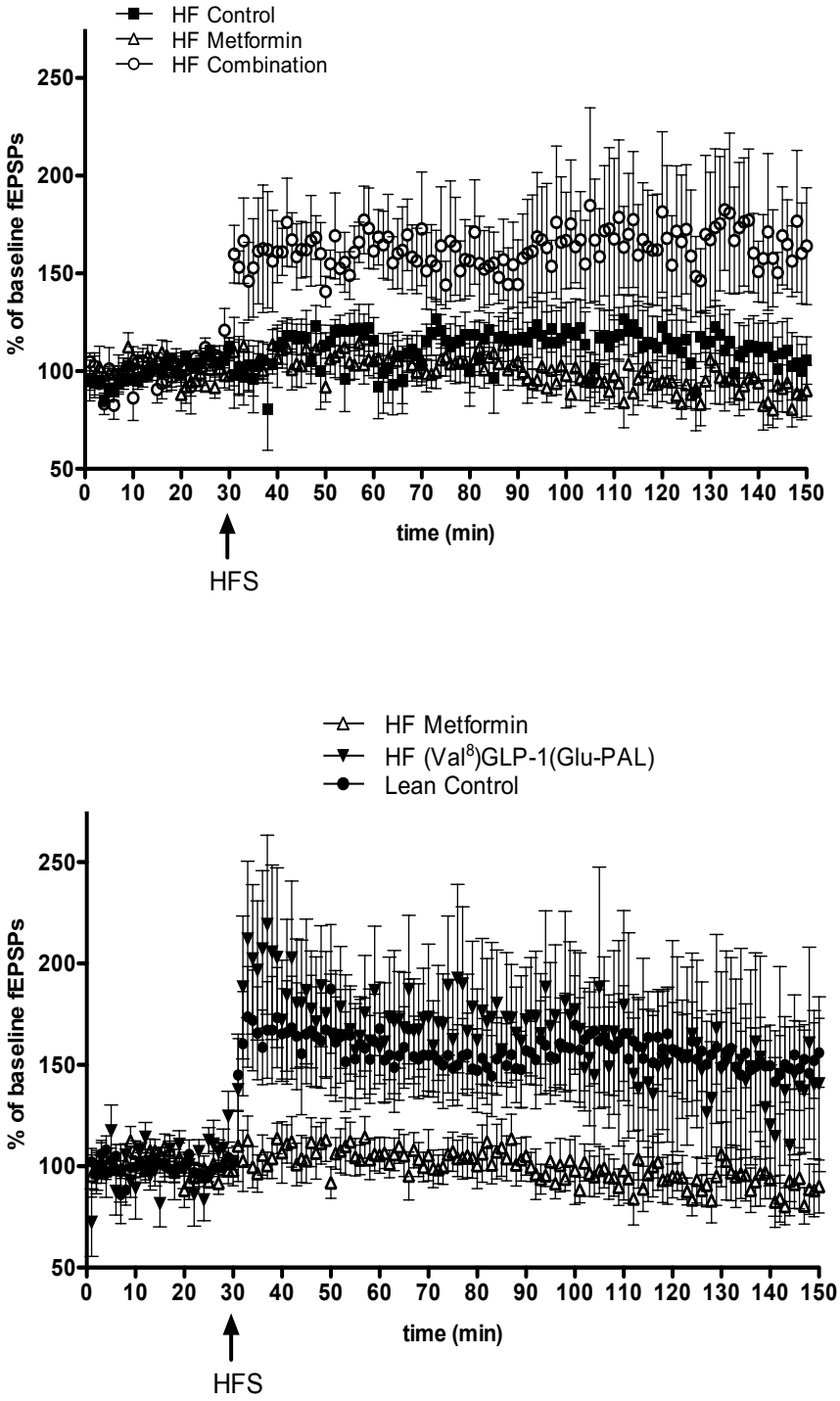

Figure 2: Effects of 20 days treatment with (Val^8)GLP-1(GluPAL), metformin or combined drug administration on measurements of LTP in the hippocampal CA1 region in high fat fed mice. Field excitatory postsynaptic potentials were recorded from stratum radiatum in the CA1 region of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral/commissural pathway. Values are means ± SEM for six mice. Treatment with the GLP-1 analogue ameliorated LTP as shown by a two-level two-way ANOVA indicating a significant difference between *HF* saline controls and GLP-1 analogue -treated mice ($P < 0.001$) and over time ($P <$

0.001). Similarly, a two-level two-way ANOVA showed a significant difference in LTP between *HF s*aline controls and the combination treatment metformin and GLP-1 analogue ($P < 0.001$) and over time ($P < 0.001$). However, no statistical difference was found between metformin treated HF mice and Saline treated HF mice. For technical details, see (Lennox et al., 2014). This Figure has been reproduced with permission (Lenox et al., 2014).