Effects of glucose-dependent insulinotropic polypeptide receptor knockout and a high-fat diet on cognitive function and hippocampal gene expression in mice

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Abstract. It has been previously demonstrated that compromise of glucose-dependent insulinotropic polypeptide receptor (GIPR) action and chronic consumption of a high-fat diet can independently impair memory and learning ability, however, the underlying pathology remain to be elucidated. The present study investigated the effects of GIPR knockout (KO), alone and in combination with a high-fat diet, on aspects of cognitive function and hippocampal gene expression in mice. In object recognition tests, normal mice exhibited effective memory, preferring to investigate the novel over the familiar object. However, wild-type (WT) mice fed a high-fat diet and GIPR KO mice fed a standard or high-fat diet demonstrated no such discrimination, suggesting the impairment of memory function. This decline in cognitive function was associated with marked changes in the expression levels of hippocampal genes involved in memory and learning. The chronic consumption of a high-fat diet decreased the hippocampal gene expression levels of mammalian target of rapamycin (mTOR), neurotrophic tyrosine kinase receptor type 2 (NTRK2) and synaptophysin. Notably, the GIPR KO mice fed a high-fat diet exhibited no reduction in the hippocampal expression of synaptophysin expression, however, the GIPR KO mice fed a standard rodent maintenance diet exhibited reduced hippocampal expression of mTOR compared with the WT controls. These data highlighted the importance of intact GIPR signalling and dietary composition in modulating memory and learning, and hippocampal pathways involved in the maintenance of synaptic plasticity, including mTOR and NTRK2, appear to be key in this regard.

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Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone, secreted by enteroendocrine K-cells, with established effects on insulin secretion and pancreatic β-cell function (1). Furthermore, GIP and its receptor (GIPR) have been identified in several areas of the brain, including the cortex, cerebellum and hippocampus (2-4). However, the role of GIP within the central nervous system remains to be elucidated. To date, GIP has been observed to exhibit a number of neuroprotective effects, inducing progenitor cell proliferation, improving learning and memory and enhancing synaptic plasticity (4,5). Indeed, the genetically-induced overexpression of GIP in mice improves their cognitive function (6). In addition, mice with a targeted deletion of the GIPR have been reported to have less than half number of newly proliferating cells in the hippocampus (4) and exhibit impaired learning and memory, compared with the wild-type (WT) controls (7). Overall, these preliminary results suggested that the GIPR is important in cognitive function in the central nervous system, however, the underlying mechanisms remain to be elucidated.

Evidence from previous studies has highlighted the association between obesity, type 2 diabetes and cognitive dysfunction (8-10). Previous studies in genetically obese ob/ob mice, Zucker fa/fa rats and high-fat diet fed obese-diabetic mice, have consistently reported impaired performance in learning and memory tests (11-14). Furthermore, a clear association has been demonstrated between chronic high-fat feeding, the increased secretion of GIP and the development of insulin resistance and obesity-associated diabetes (15). With the incidence of human obesity-associated diabetes expected to rise significantly, the occurrence of cognitive deficit disorders, including Alzheimer's disease, is also likely to increase (16). Therefore, it is necessary to elucidate the underlying pathological mechanisms and putative associations between a high-fat diet, GIPR signalling and cognitive decline.

The present study evaluated the role of GIPR signalling and a chronic high-fat diet, alone and in combination, on several aspects of cognitive function in mice. This included investigating learning and memory through object recognition tests (ORTs) and assessing the mechanisms involved by

analysing the expression levels of key hippocampal genes, which are involved in learning and memory formation. These investigations aimed to determine the importance of GIPR signalling and dietary composition in controlling the fundamental aspects of memory and learning.

Materials and methods

Animals. Male C57BL/6 mice with genetic deletion of the GIPR were used in the present study, in addition to WT controls. These mice (32 mice in total, 10 GIPR KO and 10 WT mice on a high fat diet, 6 GIPR KO and WT mice on a normal diet; 6-8 weeks old; n=6-10) were derived from an in-house breeding colony, as described previously (17,18) and were age-matched and housed in an air-conditioned room at 22±2˚C with a 12 h light/dark cycle (08:00-20:00 h). The experimental animals had free access to drinking water, a standard rodent diet (10% fat, 30% protein and 60% carbohydrate; percentage of total energy of 12.99 kj/g; Trouw Nutrition, Cheshire, UK) or a high-fat diet (45% fat, 20% protein and 35% carbohydrate; percent of total energy of 26.15 kj/g; Special Diets Service, Essex, UK), as appropriate. Prior to commencement of experiments, all the mice were weighed and maintained on their respective diets for 120 days. The WT animals, which were fed a high-fat diet exhibited significantly increased body weight compared with the controls. All experiments were performed according to UK Home Office Regulations (UK Animals Scientific Procedures Act 1986), the 'Principles of Laboratory Animal Care' (NIH publication No. 86-23, revised 1985) and were approved by the University of Ulster Animal Ethics Review Committee.

Object recognition. At the end of the dietary intervention, the groups of mice were subjected to ORTs, as described previously (14). Briefly, the mice were initially habituated to the exploratory arena (58 cm diameter, 38 cm high) for 5 mins. Two identical random objects (2 marbles, 2.5 cm diameter or 2 die, 1.2 cm side length) were subsequently placed in the centre of the arena and, following 4 h exposure (the acquisition phase), one of the two objects was replaced by a novel object (a marble or dice) and the duration spent exploring each object during a 5 min trial phase was determined using a computerised tracking system (Tracker, Biosignal Group, New York, USA). The recognition index (RI), which was designated as the period of time spend exploring the novel object as opposed to the familiar object, was calculated, as described previously (19).

Hippocampal gene expression. Animals were sacrificed by lethal inhalation of CO_2 followed by cervical dislocation. Hippocampal tissue was excised at the end of the dietary intervention period by careful surgical excision, snap frozen and processed for gene expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) following total RNA extraction. The total RNA was extracted and purified using Tripure Isolation reagent (Roche Diagnostics, West Sussex, UK). The concentration and purity of the extracted RNA was determined using a nanophotometer (NanoPhotometer™ Pearl; Implen, Munich, Germany) at an absorbance of 260 nm. The cDNA was synthesised (final concentration, 1.5 μg) using a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics) according to the manufacturer's instructions. Gene expression analysis was performed using a Roche RealTime ready qPCR assay, LightCycler 480 Probes Master and a hot start reaction mix (Roche Diagnostics). The following RT-qPCR target specific primers were used: Glucagon-like peptide-1 receptor (GLP-1R); mammalian target of rapamycin (mTOR); neurotrophic tyrosine kinase receptor type 2 (NTRK2); sirtuin 1 (SIRT1); synaptophysin (SYP) and vascular endothelial growth factor (VEGF). The gene expression was normalised to the expression of hypoxanthine guanine phosphoribosyl transferase. Briefly, the specific primer (10 pmol in 20 μl reaction volume, 0.5 pmol/μl) and cDNA (40 ng/μl.) were added to each well (containing fluorescein-labelled short hydrolysis probes and PCR-grade water) to a final reaction volume of 10 μl. The PCR conditions were 95˚C for 10 min, followed by cDNA amplification for 45 cycles with 95˚C denaturation for 10 sec, 60˚C annealing for 30 sec and 72˚C elongation for 1 sec, followed by 30 sec cooling at 40˚C. The relative quantification was calculated using the $2^{-\Delta\Delta CT}$ method, to determine the differences in gene expression levels between the samples (20).

Statistical analysis. One-way analysis of variance, followed by Newman-Keuls post-hoc test were used for statistical analysis using Prism 5 software (Graph-Pad Prism®, San Diego, CA, USA). The data are expressed as the mean ± standard error of the mean. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of GIPR KO and high-fat diet alone, and in combination, on object recognition. In the ORT, no difference or bias was observed in the RI between any of the groups of mice during the acquisition period. During the trial phase, the WT mice fed a normal diet had significantly ($P<0.05$) increased RI compared with the acquisition phase (Fig. 1A), indicating a preference to examine the novel object. By contrast, the WT mice fed a high-fat diet and the GIPR KO mice fed either the standard maintenance or the high-fat diet failed to discriminate between the novel and the familiar object during the trial phase (Fig. 1B-D).

Effects of GIPR KO and high-fat feeding alone, and in combination, on hippocampal gene expression levels. The assessment of the hippocampal gene expression levels revealed that a high-fat diet significantly decreased the expression levels of mTOR, NTRK2 and SYP ($P<0.001$, $P<0.05$ and $P<0.05$; respectively) in the WT mice (Fig. 2A-C). Notably, the GIPR KO mice fed a standard rodent diet also exhibited significantly ($P<0.05$) decreased hippocampal expression of mTOR compared with the WT mice (Fig. 2A). Similarly, the GIPR KO mice fed a high-fat diet exhibited significantly ($P<0.05$) decreased expression of mTOR, however, this was higher ($P<0.001$) compared with the WT mice fed a high-fat diet (Fig. 2A). In addition, the GIPR KO mice fed a high-fat diet also exhibited decreased hippocampal gene expression of NTRK2 compared with the WT controls (Fig. 2B). The expression levels of hippocampal GLP-1R, SIRT1 and VEGF

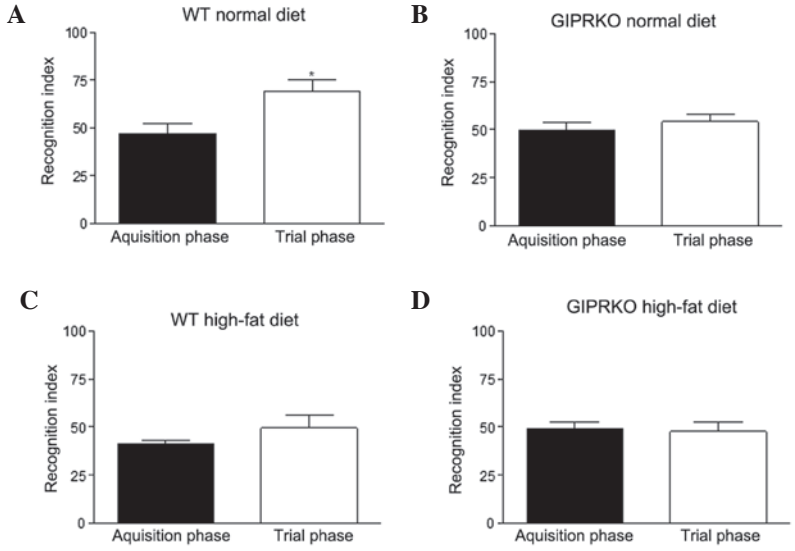

Figure 1. Effects of (A) normal diet, (B) GIPR KO, (C) high-fat feeding and (D) GIPR KO in combination with a high-fat feeding on recognition memory. Experiments were performed following 120 days of dietary intervention. An object recognition test (5 min) was performed using two familiar objects during an acquisition phase and following introduction of a novel object 4 h later for the trial phase. The recognition index was the percentage of time spent exploring the novel, vs. familiar object. Data are expressed as the mean ± standard error of the mean for 10 mice (*P<0.05, compared with the acquisition phase). WT, wild-type; GIPR, glucose-dependent insulinotropic polypeptide receptor; KO, knockout.

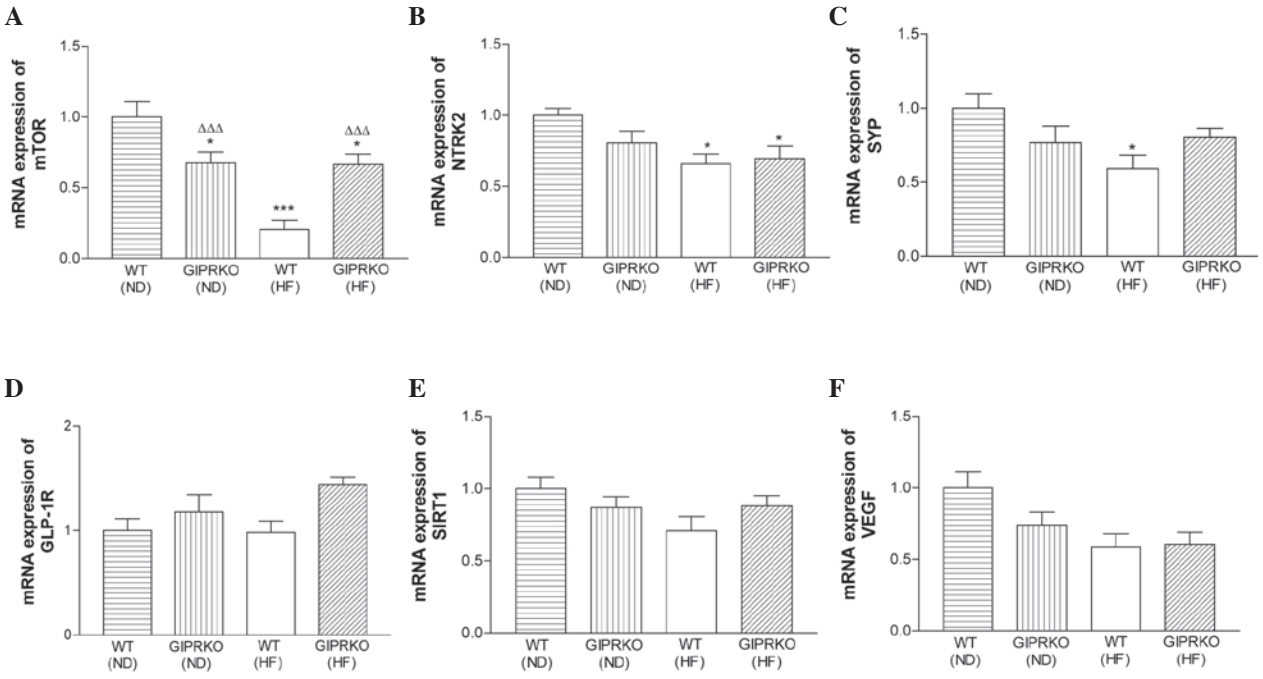

Figure 2. Effects of GIPR KO alone or in combination with a high-fat diet on hippocampal gene expression. The mRNA expression levels of (A) mTOR, (B) NTRK2, (C) SYP, (D) GLP-1R, (E) SITR1 and (F) VEGF were examined following 120 days of dietary intervention. The data are expressed as the mean ± standard error of the mean for six mice (*P<0.05 and ***P<0.001, compared with WT mice fed a normal diet; $^{\Delta\Delta\Delta}$P<0.001, compared with WT mice fed a high-fat diet). WT, wild-type; GIPR, glucose-dependent insulinotropic polypeptide receptor; KO, knockout; ND, normal diet; HD, high-fat diet; mTOR, mammalian target of rapamycin; NTRK2, neurotrophic tyrosine kinase receptor type 2; SYP, synaptophysin; GLP-1R, glucagon-like peptide 1 receptor; SITR1, sirtuin 1; VEGF, vascular endothelial growth factor.

were not significantly different in any of the groups of mice (Fig. 2D and E).

Discussion

Chemical and genetic reduction of GIPR signalling, or chronic consumption of a high-fat diet, have been demonstrated independently to impair learning and memory ability (4,7,12,13). However, the precise mechanisms and pathologies underlying these phenomena remain to be elucidated. The present study investigated the effects of genetic GIPR KO alone, and in combination with a chronic high-fat diet, on recognition memory and the expression levels of a panel of hippocampal genes, which are involved in maintaining normal cognitive function.

The ORT is a popular and informative tool used for assessing recognition memory in rodents (21). In the present study, WT mice maintained on a standard rodent maintenance diet exhibited normal recognition memory, preferring to investigate the novel object during ORTs. In accordance with previous findings, the GIPR KO mice or mice fed a chronic high-fat diet demonstrated significantly impaired recognition memory (7,13). Therefore, it was not unexpected that the GIPR KO mice fed a high-fat diet also exhibited markedly reduced memory function. However, the absence of additive detrimental effects of GIPR annulment combined with a high-fat diet on recognition memory, suggested that similar pathways may be involved in mediating this action, although this requires further investigation.

Normal hippocampal function is particularly important for the preservation of recognition memory (22). Experimental evidence suggested that the deleterious effect of chronic high-fat feeding on learning and memory is associated with altered hippocampal gene expression levels (23). Furthermore, the GIPR KO mice exhibited significant reductions in progenitor cell proliferation in the dentate gyrus of the hippocampus (7). Notably, GIPR KO mice and mice fed a chronic high-fat diet have previously been demonstrated to inhibit long term potentiation (LTP) in the hippocampus, the major cellular mechanism underlying learning and memory (7,13). Therefore, to extend this previous investigation, the present study investigated the effects of GIPR KO alone, and in combination with a high-fat diet, on the expression levels of hippocampal genes, which are known to be involved in cognitive function.

In the present study, the hippocampal expression of mTOR, a protein kinase with an established role in the maintenance of LTP (24), was significantly reduced by GIPR KO and a high-fat diet. Therefore, the expression of mTOR and subsequent activity clearly have major implications on the cognitive ability of each of these groups of mice. Notably, GIPR KO partially reversed the severe detrimental effect of a high-fat diet on the expression of mTOR, although the expression levels remained reduced compared with the WT controls. This may appear marginally contradictory, however, it may be linked to the opportunity for lifelong adaptive mechanisms in genetic KO animals (17). GLP-1 action, also associated with positive effects on cognition (13), has previously been observed to be upregulated in GIPR KO mice (17). In the present study, increased hippocampal expression of GLP-1R was observed in each group of GIPR KO mice, however was not statistically significant. Similarly, the hippocampal expression of SYP was significantly reduced by a chronic high-fat diet, however, concurrent GIPR KO restored the expression of SYP almost to normal levels. SIRT1 has previously been revealed as an important factor for normal cognitive function and object recognition (23,25), however, the hippocampal expression of SIRT1 was unaffected by GIPR KO or a high-fat diet in the present study.

NTRK2, a gene encoding for the tyrosine kinase receptor, TrkB, on neuronal cells, is important in hippocampal synaptic plasticity and neurogenesis (26). The present study revealed that the chronic consumption of a high-fat diet by the WT or GIPR KO mice significantly reduced the hippocampal expression of NTRK2. Therefore, a potential mechanism for the reported impairment of neurogenesis by high-fat feeding (27) may be associated with a reduced expression of NTRK2, which may ultimately decrease the activity of its ligand, brain-derived neurotrophic factor (BDNF). BDNF has well recognised beneficial actions on the growth and differentiation of neurons and synapses (28). Notably, the expression of VEGF, which is important in hippocampal neurogenesis (29,30), was not significantly reduced in all GIPR KO and high-fat fed mice. Taken together, GIPR KO deletion and a chronic high-fat diet may impair hippocampal neurogenesis via complementary pathways.

In conclusion, GIPR KO and chronic consumption of a high-fat diet exhibited negative effects on hippocampal-dependent recognition memory. The pathways involved in the maintenance of hippocampal synaptic plasticity, including SYP, NTRK2 and particularly mTOR, appeared to be key in this respect. In addition, the results suggested that a deficit in hippocampal neurogenesis may also be important in the observed reduction in recognition memory. These findings highlight the importance of GIPR signalling and dietary content for the maintenance of normal cognitive function.

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