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MOLECULAR METABOLISM

Limited impact on glucose homeostasis of leptin receptor deletion from insulin- or proglucagon-expressing cells

Helen Soedling [1], David J. Hodson [1], Alice E. Adrianssens [2], Fiona M. Gribble [2], Frank Reimann [2], Stefan Trapp [3], Guy A. Rutter [1,*]

ABSTRACT

Aims/hypothesis: The adipose tissue-derived hormone leptin plays an important role in the maintenance of body weight and glucose homeostasis. Leptin mediates its effects by interaction with leptin receptors (LepRb), which are highly expressed in the hypothalamus and other brain centres, and at lower levels in the periphery. Previous studies have used relatively promiscuous or inefficient *Cre* deleter strains, respectively, to explore the roles of LepR in pancreatic β and α cells. Here, we use two newly-developed *Cre* lines to explore the role of leptin signalling in insulin and proglucagon-expressing cells.

Methods: Leptin receptor expression was measured in isolated mouse islets and highly-purified islet cells by RNASeq and quantitative RT-PCR. Mice lacking leptin signalling in pancreatic β, or in α and other proglucagon-expressing cells, were generated using Ins1*Cre*- or iGlu*Cre*-mediated recombination respectively of *flox*'d leptin receptor alleles. *In vivo* glucose homeostasis, changes in body weight, pancreatic histology and hormone secretion from isolated islets were assessed using standard techniques.

Results: Leptin receptor mRNA levels were at or below the level of detection in wild-type adult mouse isolated islets and purified cells, and leptin signalling to Stat3 phosphorylation was undetectable. Whereas male mice further deleted for leptin receptors in β cells exhibited no abnormalities in glucose tolerance up to 16 weeks of age, females transiently displayed improved glucose tolerance at 8 weeks (11.2 ± 3.2% decrease in area under curve; $p < 0.05$), and improved (39.0 ± 13.0%, $P < 0.05$) glucose-stimulated insulin secretion *in vitro.* No differences were seen between genotypes in body weight, fasting glucose or β/α cell ratio. Deletion of LepR from α-cells, a minority of β cells, and a subset of proglucagon-expressing cells in the brain, exerted no effects on body weight, glucose or insulin tolerance, nor on pancreatic hormone secretion assessed *in vivo* and *in vitro.*

Conclusions/interpretation: The use here of a highly selective *Cre* recombinase indicates that leptin signalling plays a relatively minor, age- and sex-dependent role in the control of β cell function in the mouse. No *in vivo* role for leptin receptors on α cells, nor in other proglucagon-expressing cells, was detected in this study.

Keywords Leptin; Insulin; Glucagon; GLP-1; Diabetes; β cell; α cell; L-cell

1. INTRODUCTION

Type 2 Diabetes mellitus (T2D) currently affects approximately 380 million individuals worldwide [1] and is characterized by elevated blood glucose levels. The treatment of T2D complications, which include cardiovascular disease, retinopathy and peripheral nerve damage, typically consumes 10–20% of the health care budgets of westernized societies [2]. Whilst insulin, secreted from pancreatic islet β cells, acts to lower blood glucose levels, glucagon, secreted by pancreatic α cells, increases glycaemia. Defects in the release or actions of either hormone can thus contribute to the disease [3].

Obesity, which affects ~1 in 4 adults in the UK (www.hscic.gov.uk/catalogue/PUB10364), is an important risk factor for T2D and promotes both insulin resistance and β cell failure [4]. The adipose tissue-derived hormone leptin is an important satiety factor which acts on the feeding centres in the brain to suppress appetite [5]. Human mutations in either the leptin (obese, or *ob*) [6,7] or the leptin receptor (*LepR*) [8] gene lead to severe obesity from an early age. Demonstrating a conserved role for the hormone across mammalian species, mice carrying mutations in the homologous genes (*ob/ob* [9] or *db/db*) [10] display severe hyperphagia, obesity and disturbed glucose homeostasis.

[1]Section of Cell Biology and Functional Genomics, Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Imperial College London, du Cane Road, London W12 0NN, UK [2]University of Cambridge Metabolic Research Laboratories, Cambridge, UK [3]Centre for Cardiovascular and Metabolic Neuroscience, Department of Neuroscience, Physiology & Pharmacology, University College London, London, UK

*Corresponding author. E-mail: g.rutter@imperial.ac.uk (G.A. Rutter).

Abbreviations: AUC, area under the curve; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; K_{ATP}, ATP-sensitive K^+ channel; GTT, ITT, glucose and insulin tolerance test, respectively; IP, intraperitoneal; NTS, nucleus tractus solitarius

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In addition to the central actions of leptin, roles for the hormone have also been suggested in the periphery. Thus, LepR expression has been described in the endocrine pancreas [11,12], and several studies [12–18] have reported an action of leptin to inhibit insulin secretion. Furthermore, over-expression of insulin receptors in the islets of LepR-deficient Zucker diabetic fatty (ZDF) rats restores glucose-stimulated insulin secretion (GSIS) [19]. Whether physiological concentrations of leptin ($\leq$1 nM) also inhibit secretion, however, has been contested [20]. Recent work [21] suggests that the actions of leptin on insulin release involve the stimulation of AMPK-activation protein kinase (AMPK) and trafficking of ATP-sensitive K^+ (K_{ATP}) channels to the plasma membrane. These changes are thus expected to impede the glucose-dependent closure of K_{ATP} channels, voltage-gated Ca^{2+} channel opening and Ca^{2+} influx that trigger insulin secretion in response to glucose [22].

Using a *Cre* deleter strain driven by the rat insulin 2 promoter (RIP2) Covey et al. [23] deleted critical *flox*'d exons in the gene encoding the active, long form of the leptin receptor (LepRb) *in vivo* in murine pancreatic β cells and in a specific population of neurons in the hypothalamus (RIP2*Cre* neurons). These animals developed obesity, fasting hyperinsulinaemia, impaired glucose-stimulated insulin release and glucose intolerance. On the other hand, Morioka et al. [24] disrupted the leptin receptor in all pancreatic cells using a *Pdx1* promoter-driven *Cre* and these mice, in contrast, exhibited normal body weight, improved glucose tolerance and elevated plasma insulin. However, when challenged with high fat diet, Pdx1*Cre*:$LepR^{F/F}$ mice became glucose intolerant.

The difference between these studies is thus likely to be the result of using different *Cre* lines and hence deletion in partially overlapping, but different, cell types: crucially, in neither case was the leptin receptor deleted exclusively in pancreatic β cells. Thus, the RIP2*Cre* promoter used by Covey et al. is active in β cells and also in several areas of the brain, particularly in the hypothalamus [25]. By contrast, the Pdx1*Cre* used by Morioka et al. is expressed in adult β-cells and also deletes in glucagon-secreting pancreatic α cells as well intestinal glucagon-like peptide 1- (GLP-1) secreting L-cells and several other cell types with roles in glucose homeostasis [26]. The Pdx1*Cre* line also deletes in neuronal populations responsive to leptin [25].

Our first aim here, therefore, was to examine the effects of deleting the long form of the leptin receptor (LepRb) highly selectively in pancreatic β cells using the novel Ins1*Cre* deleter strain [27,28]. Selectivity with this strain is achieved firstly by the use of the Insulin *I* promoter, whose expression is essentially confined to pancreatic β cells [29]. By contrast, the Insulin *II* gene, which drives RIP2*Cre* promoter expression, is expressed in multiple brain regions [25,30]. Secondly, precise developmental and spatial control of *Cre* expression by Ins1*Cre* is enhanced by its targeted insertion (knock-in) at the *Ins1* locus. Finally, this line also avoids risks associated with the use of other insulin promoter-driven *Cre*s [31] of *Cre*-independent actions due to co-expression of growth hormone. The present strategy is expected, therefore, to distinguish between the actions of leptin on glucose homeostasis *via* the pancreatic β cell and those acting *via* the brain and elsewhere.

The possibility has also recently been explored that leptin receptors play a role in pancreatic α cells to control glucagon secretion [32]. However, whilst the latter studies failed to identify any defects in glucose homeostasis after LepR deletion using a *Cre* expressed under the control of a short fragment of the glucagon promoter [33], recombination was only achieved in ~43% of α cells. Correspondingly, we [34] and others [35,36] have similarly found that the latter *Cre* deletes in only a minority (13–45%) of α cells. This leaves open the possibility that the effects of deletion described by Tuduri et al. [32] might be masked by changes in the remaining, non-recombined α cell population. To test this hypothesis, our second aim here was to use an alternative glucagon promoter-driven *Cre*, iGlu*Cre* [37], based on a much longer (~100 kB) region upstream of the proglucagon gene, which drives recombination in the vast majority of α cells. However, *Cre* is also expressed with this line at other sites of proglucagon expression including the nucleus tractus solitarius (NTS) of the hindbrain [38], and in intestinal L-cells, both of which express leptin receptors [37,39]. Both of these cell types secrete the incretin glucagon-like peptide-1 (GLP-1) and thus impaired leptin receptor signalling, particularly in the neuronal population [40], might affect body weight, glucose homeostasis, or both.

2. MATERIALS AND METHODS

2.1. Generation of mice

Mice bearing $LepR^F$ alleles on an FVB background were kindly provided by Dr Streamson Chua (Columbia University) and, after backcrossing twice to C57BL/6 mice, bred to Ins1*Cre* [28] or iGlu*Cre* [37] mice (C57BL6 background) as indicated. Further crosses to Rosa26tdRFP were performed with iGlu*Cre*$LepR^{F/F}$ mice to allow labelling of recombined cells. Animals (2–5) were maintained in separately ventilated cages in a specific pathogen-free environment. All procedures were approved by the U.K. Home Office and were compliant with the Animals (Scientific Procedures) Act 1986 of the United Kingdom (PPL 70/7349). Genotyping was performed as described ([41] and see Results). Primer sequences are given in Table 1.

2.2. Studies of glucose homeostasis and hormone release *in vivo*

Intraperitoneal glucose (1 g/kg) or insulin (0.75 U/mL) tolerance tests were performed on mice fasted for 16 h or 5 h, respectively, as described [42], using an automated glucometer (Accucheck, Roche, Burgess Hill, U.K.). Plasma insulin and glucagon levels were measured with ELISA kits from Cis Bio (Bagnols-sur-Cèze, France) and Mercodia (Uppsala, Sweden), respectively.

2.3. Assay of insulin and glucagon secretion from isolated islets

Islet isolation by collagenase injection and pancreatic distension was performed as described [43]. Islets were cultured for 24–36 h in RPMI medium supplemented with 10% foetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Hormone release was measured during 30 or 60 min (insulin or glucagon, respectively) static incubation of batches of six (insulin) or 20 (glucagon) islets in 0.5 mL modified Kreb's Ringer medium comprising (mM): 120 NaCl, 4.8 KCl, 24 $NaHCO_3$, 0.5 Na_2HPO_4, 5 HEPES, 2.5 $CaCl_2$, 1.2 $MgCl_2$ and 5 D-glucose, pH 7.4, at 37 °C. Secreted and total hormone levels were measured by homogeneous time-resolved fluorescence-based (HTRF) assay (Cisbio).

Table 1 — Primer sequences.

Target	Forward primer sequence 5′-3′	Reverse primer sequence 5′-3′
Tissue control	FER2-Q2: accttcagaccttggcgttggagg	FER2-R2: cctgaggttcctgttgctgtgactcc
Ins1Cre	Cre jva rv 2: gccagattacgtatatcctggcag	Cre jva fw 2: ttcactggttatgcggcgg
$LepR^F$	103: tgagttccctcatgattctgg	See below
$LepR^F$	104: cagccgaccaatgcttatt	105: acaggcttgagaacatgaacac
iCre	Cre002: gacaggcaggccttctctgaa	Cre003: cttctccacaccagctgtgga
β-catenin	RM41: aaggtagagtgatgaaagttgtt	RM42: caccatgtcctctgtctattc
tdRFP	Anti: ctacaggaacaggtggtgg	Sense: ctgttcctggggcatggc

