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Fetal pancreatic islets express functional leptin receptors and leptin stimulates proliferation of fetal islet cells

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OBJECTIVE: Previous studies have demonstrated that leptin can stimulate proliferation of insulin-secreting tumor cell lines. The objective of this study was to characterize whether leptin could stimulate proliferation of primary β-cells too. Since adult β-cells have very limited capacity for replication, we examined the effect of leptin on islets of Langerhans obtained from fetal rats, in a tissue culture system.
METHODS: Leptin receptor mRNA and c-*fos* mRNA were measured by RT-PCR. Proliferation of fetal rat islet cells was measured by a WST-1 colorimetric assay and [^{3}H]-thymidine incorporation assay.
RESULTS: Leptin stimulated proliferation of serum-deprived fetal rat islet cells, as indicated by increased formation of formazan dye from a tetrazolium salt WST-1. Leptin stimulated DNA synthesis in islet cells, as indicated by increased [^{3}H]-thymidine incorporation into DNA. The effect of leptin on islet cell proliferation was on average 39–50% of the effect obtained with 10% fetal bovine serum. Leptin increased c-*fos* mRNA expression by 2.8-fold in isolated fetal islets after 30 min treatment. In fetal pancreatic islets, both the common extracellular portion (OB-R) and the intact long form (OB-Rb) of the leptin receptor were readily detected by reverse transcriptase polymerase chain reaction.
CONCLUSION: Functional leptin receptors are expressed in pancreatic islet cells, as early as during the fetal stage of development of these microorgans. Leptin stimulates proliferation of fetal islet cells and might play a role in determining islet cell mass at birth.
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Keywords: leptin; insulin-secreting cells; islets of Langerhans; fetal islets

Introduction

Leptin, the product of *ob* gene, is secreted primarily from fat cells and plays an important role in the regulation of food intake and metabolism.[1] These actions of leptin are mediated through the appetite regulatory centers in the hypothalamus, which express leptin receptors in abundance.[2,3] However, during recent years, leptin receptors have been identified in an increasing number of tissues outside the brain.[4,5] In these peripheral tissues, the level of expression of leptin receptors is generally low, which is not surprising, given that the concentration of leptin in the peripheral circulation is 100–200 times higher than that in the cerebrospinal fluid.[6,7] Leptin receptors exist in multiple isoforms, several of which (OB-Ra–OB-Re) are generated by alternative splicing.[8] OB-Rb, the intact form of leptin receptor, contains a long cytoplasmic domain of 302 amino acids that includes motifs for binding and activation of the Janus Kinase/Signal Transduction and Activator of Transcription (JAK/STAT). Consistent with this, OB-Rb activates the JAK/STAT signalling pathway and thereby mediates effect of leptin on food intake.[8–10] The roles of the shorter isoforms of leptin receptor are not fully understood.

Adult pancreatic islets express leptin receptors,[11–13] which are thought to mediate inhibition of insulin secretion and insulin biosynthesis.[14,15] It has been reported that leptin can stimulate proliferation of different β-cell-derived tumor cell lines.[16,17] Whether proliferation of primary β-cells is also stimulated by leptin is unknown. In adult islets only a minor proportion of β-cells take part in proliferation and the cells divide at a very slow rate. We therefore tested the effects of leptin on isolated fetal islets where the cells retain considerable replicative activity as opposed to their adult counterparts.[18] We demonstrate that fetal rat islets express full-length leptin receptors and that leptin stimulates proliferation of fetal islet cells.

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Materials and methods

Preparation and culture of fetal pancreatic islets

Pregnant Sprague–Dawley rats were killed by cervical dislocation on day 21 of gestation and their fetuses

rapidly removed. Islets were obtained from mildly digested fetal pancreas according to methods described by us before.[18,19] Briefly, pancreases were minced and digested for a short time with collagenase. The digest was carefully washed, plated in tissue culture dishes to allow attachment of islet-like clusters and cultured for one week at 37°C in a humidified atmosphere of 5% CO_2 in RPMI 1640 medium containing 11.1 mM glucose, 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml benzylpenicillin and 0.1 mg/ml streptomycin. Spherical islets, free of connective tissue, were then selected under stereomicroscope, transferred to 24-well plates and cultured under the conditions described below. Previous studies have shown that these islets contain 90–95% β-cells[18,19] and that they do not respond to classical fibroblast mitogens such as EGF and PDGF.[18]

Colorimetric assay for cell proliferation

We measured islet cell proliferation using two methods. We used an assay kit consisting of WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,-3-benzene disulfonate) and an electron coupling reagent. The assay is based on reduction of the tetrazolium salt to a water-soluble formazan compound by the respiratory chain enzymes of mitochondria. In this assay the amount of formazan dye formed correlates with the cell number.[20] Islets were handpicked and transferred to the wells of a 24-well plate (25 islets per well) containing serum-free RPMI-1640 medium. The islets were cultured overnight ($\sim$18 h) in this serum-free medium to achieve quiescence of the cells. In preliminary experiments we confirmed that fetal rat islets survive under serum-free culture conditions for prolonged periods, probably because insulin secreted by the islets acts as a growth factor.[21] After overnight incubation in serum-free medium we added leptin (100 nM, final concentration) or FBS (10%, final concentration). No addition was made to a third well which acted as basal control. After incubation for a further 24 h, we added 150 µl of WST-1 reagent to each well. Eight hours later, the plate was shaken for 1 min and absorbance measured at 450 nm against appropriate blanks using a Beckman DU 7500 spectrophotometer. Absorbance obtained at a reference wavelength (690 nm) was subtracted. Proliferation was expressed as the percentage of values observed in the absence of leptin and FBS, which was taken as 100%.

[^{3}H]-thymidine incorporation assay

[^{3}H]-thymidine incorporation assay was performed as described by us earlier with small modifications.[18] In preliminary studies we optimized the assay to enable detection of modest stimulation of cell proliferation on prolonged (24 h) exposure to cytokines.[18] Fifty fetal islets per well were pre-incubated for 24 h in 24-well plates in 1 ml of RPMI 1640 medium containing 0.5% FBS and 2.8 mM glucose. Such pretreatment was regarded as essential since it stopped all DNA replication initiated earlier. We then added leptin (5 or 100 nM) or FBS (10%). A third well containing only 0.5% FBS and 2.8 mM glucose acted as control. Immediately after this, [^{3}H]-thymidine (0.5 µCi/ml) was added to the wells. After labeling with [^{3}H]-thymidine for exactly 24 h, the islets were transferred by capillary pipettes to Eppendorf tubes and washed with a balanced salt solution. Islets were then disrupted with 1% sodium lauryl sulfate, precipitated with ice-cold 10% trichloroacetic acid and pelleted by centrifugation. The pellet was dissolved in Soluene (Packard) and radioactivity incorporated in the pellet was measured by liquid scintillation counting. Radioactivity is expressed as dpm per 50 islets. Results are expressed as percentages of values obtained in the controls.

Leptin receptor mRNA level

Total RNA was isolated from 50 fetal islets by using TRI REAGENT™ (Sigma, Biosciences, USA) and isopropanol/ethanol precipitation. Integrity and loading of the RNA were studied by detection scanning of ribosomal rRNA bands (28S and 18S) in agarose gels. RNA samples containing RNase inhibitor (Amersham, UK) were treated immediately with RNase-free DNase I (Gibco/BRL, UK), and single-stranded cDNA synthesized using first-strand cDNA beads (Pharmacia Biotech, Sweden) and oligo $(dT)_{12-18}$ (Invitrogen). Different amounts of cDNA corresponding to that obtained from between one and 15 islets were used for reverse transcriptase polymerase chain reaction (RT-PCR). For amplifying the common extracellular part of the leptin receptor (OB-R), we used the following primer sequences: 5′-GCTGGATGAAAGGGGACTTG-3′ (sense) and 5′-GTGACTTCCATACGCAAACC-3′ (antisense) to amplify a 348 bp fragment of OB-R (GenBank U53144). To identify the full-length OB-Rb isoform we amplified two different fragments using the primer sequences 5′-AACTGATGAAGAGCAAGGGG-3′ (sense) and 5′-AGACAGTGAGCTGGGAATGG-3′ (antisense) to amplify a 332 bp fragment of OB-Rb (fragment-1) (GenBank D84551), or 5′-AGTGTCGATACAGCTTG-3′ (sense) and 5′-CTGAGAATGAAAGTTGTGG-3′ (antisense) to amplify a 399 bp fragment of OB-Rb (fragment-2) (GenBank D84551). PCR amplifications were performed with the following parameters: 95°C for 45 s (denaturing), 55°C for 45 s (annealing) and 72°C for 45 s (extension), in a thermocycler (Techne/Cambridge) for 37 cycles.

To study the regulation of leptin receptor level by glucose or serum we cultured islets in the presence of glucose (2.8 or 11 mM) or FBS (0.5% of 10%) for 48 h. Multiplex PCR assay was performed as described by Dukas *et al*.[22] The optimized linear range of co-amplification of two DNA fragments was obtained between 35 and 42 cycles and uniform amplification was maintained using a 5:1 ratio of OB-Rb (fragment-1):OB-R-common and a 4:1 ratio of OB-R-common:β-actin primer concentration. The

following β-actin primers were used: 5′-CTCTTT-AATGTCACGCACGAT-3′ (sense) and 5′-AGTGC-TGTGGGTGTAGGTACT-3′ (antisense) to amplify a 534 bp fragment of β-actin (GenBank J00691). We find that β-actin is more suitable than α-tubulin (see below) for use as an internal control for multiplex PCR assays of rare transcripts, since it is less abundant than tubulin. PCR fragments were cloned directly into a PCR-TRAP system for identification of sequences (GeneHunter Corp., USA) and the identity of fragments confirmed by sequencing using a Thermo-Sequenase terminator cycle sequencing kit (Amersham Life Sciences, UK). PCR products were either fractionated in a 1.5% standard agarose gel or 4% NuSieve 3:1 agarose (FMC BioProducts, USA) and bands were quantitated by scanning laser densitometry. Potential contamination from adipose tissues was investigated using the fatty acid binding protein (aP2)-specific primers by an RT-PCR amplification.

C-*fos* mRNA expression

Fetal isets were cultured for ~18 h in serum-free RPMI 1640 medium. Islets (50 per tube) were transferred to Eppendorf tubes containing pre-warmed serum-free RPMI and the tubes left in the incubator. After 1 h, leptin (100 nM) or control buffer was added to the tubes. Thirty minutes later the tubes were put on ice, centrifuged in refrigerated centrifuge at 5000 rpm, and the supernatant carefully removed. Fifty microliters of RNase-free water containing RNase inhibitor (330 U/ml, Amersham-Pharmacia, Sweden) were added to each tube. The level of expresion of mRNA of c-*fos* relative to that of endogenously expressed α-tubulin mRNA in the islets was measured by quantitative PCR assay.[23] Optimal linear regions of PCR amplification of c-*fos* and α-tubulin mRNA were obtained over 32−40 cycles. Serial dilutions (1:1, 1:3, 1:9) of cDNA from control and leptin-treated islets were used at fixed number of PCR cycles (36 cycles) and the differences in mRNA levels then calculated as described previously.[23] The following primer sequences were used: 5′-TGCAGCTCCCAC-CAGTGTCTAC-3′ (sense) and 5′-GGAGAATCC-GAAGGGAATGGAA-3′ (antisense) to amplify a 499 bp fragment of c-*fos* mRNA (GenBank X06769). A set of commercially available primers (Clontech) was used to amplify a 540 bp fragment of α-tubulin mRNA.

Materials

Recombinant human leptin was from R&D Systems Europe (UK). [Methyl-^{3}H]-thymidine was from Amersham (UK). The cell proliferation reagent kit WST-1 was from Boehringer Mannheim (Mannheim, Germany). FBS was from GIBCO BRL. Sources of other reagents are indicated in the text.

Statistical analysis

Results of [^{3}H]-thymidine incorporation, WST-1 assay and c-*fos* mRNA expression were tested for statistical significance by Student's unpaired *t*-test.

Results

Leptin stimulates fetal islet cell proliferation

The dose-response of leptin in stimulating cell proliferation was established in β-cell-derived tumor cells in our previous study and has not been repeated here because of limited availability of fetal rat islets.[16] We used leptin at a concentration of 100 nM, which was reported to be the maximally effective concentration of leptin for stimulating cell proliferation.[16] We first used a non-radioactive method for rapid measurement of islet-cell proliferation. This assay utilizes tetrazolium salt WST-1, which on cleavage by mitochondrial dehydrogenases yields a water-soluble reaction product. In this assay, an increase in the amount of

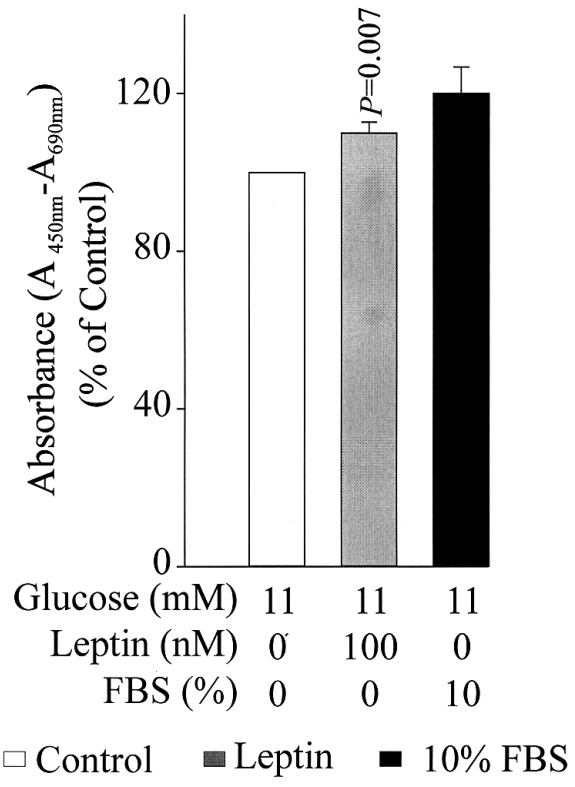

Figure 1 Fetal islet cell proliferation measured by WST-1 assay. Islets (25 per well) obtained from rat fetuses were incubated overnight in 24-well plates in the absence of FBS in RPMI-1640 medium conatining 11 mM glucose. Islets were then exposed to leptin (100 nM) or FBS (10%) for 24 h. After a further 8 h incubation with WST-1, absorbance was measured at 450 and 690 nm (reference). Values are expressed as percentages of that obtained from control islets cultured in the absence of leptin and FBS. Leptin increased islet cell proliferation by 10% (±2.8%; $n=5$ experiments with islets from fetuses of five rats; $P=0.007$). Under similar conditions 10% FBS increased islet cell proliferation by 20% (±6.7%; $n=5$; $P=0.015$).

formazan dye formation indicates an increase in the number of cells. In these experiments islets were pre-incubated for ~18 h in serum-free RPMI-1640 medium containing 11 mM glucose prior to addition of leptin (100 nM) or FBS (10%). Twenty-four hours after addition of leptin or FBS, islet cell number was estimated from the amount of formazan dye formed. Both leptin and FBS increased formazan dye formation as compared to the untreated controls (Figure 1). The effects of leptin and FBS on proliferation of fetal islet cells obtained from different pregnant rats and measured by WST-1 assay varied considerably. Leptin increased fetal cells proliferation by 5–20% as compared to the untreated controls (mean 10 ± s.e.m. 2.8%, $n=5$, $P=0.007$). Under similar conditions FBS increased the same by 5–46% of the controls (mean 20 ± s.e.m. 6.7%, $n=5$, $P=0.015$). The effect of leptin on islet cell proliferation was on the average 50% of that achieved with 10% FBS.

In another set of experiments, we tested the effect of leptin on islet cell proliferation by measuring [^{3}H]-thymidine incorporation, which is widely accepted as an index of DNA synthesis. In these experiments we used a protocol slightly different from that used in the WST-1 assay. Islets were incubated in RPMI 1640

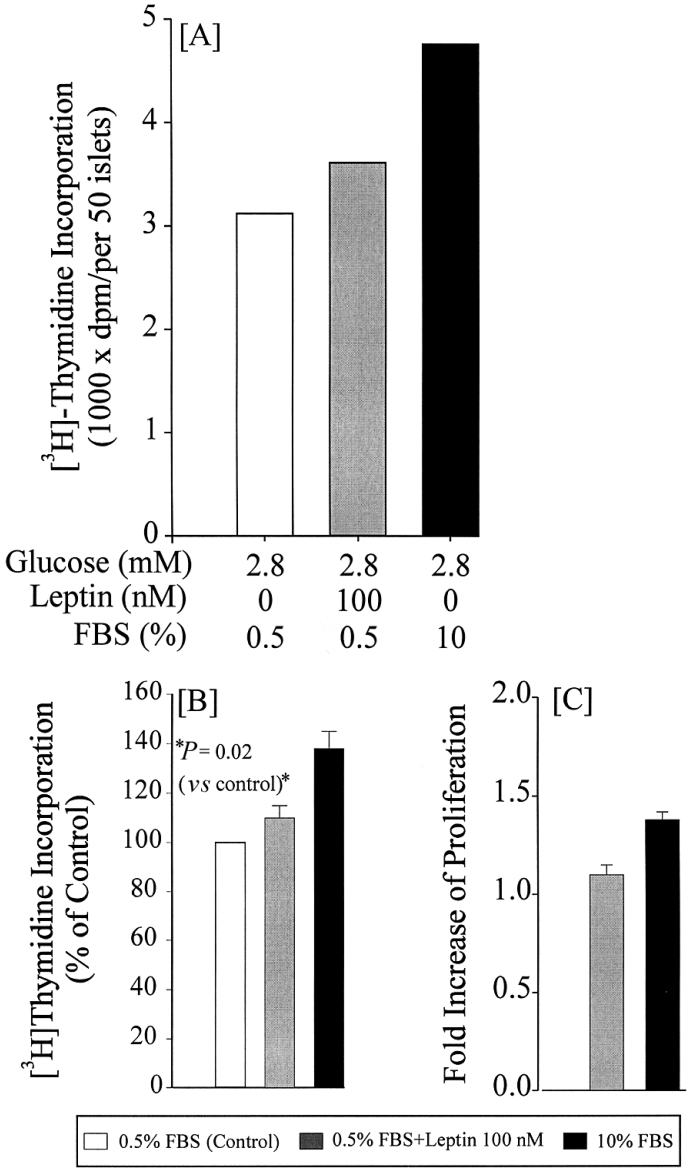

Figure 2 Stimulation of DNA synthesis in fetal islet cells by leptin. Islets (50 per well) were pre-incubated for 24 h in RPMI-1640 medium containing 0.5% FBS and 2.8 mM glucose. [^{3}H]-thymidine incorporation into DNA was measured as described in the text. (A) Thymidine incorporation stimulated by 100 nM leptin or 10% FBS. The figure is representative of three different experiments done with fetal islets obtained from three pregnant rats. (B) Data are expressed as percentages of proliferation in the basal controls, ie in the presence of 0.5% only. Values indicate [^{3}H]-thymidine incorporation in the presence of 100 nM leptin and that in the presence of 10% FBS. Results are mean ± s.e.m. values of determinations from three independent experiments. Leptin increased islet cell proliferation by 18% (± 5%; $P=0.02$ *vs* control, $n=3$). FBS increased islet cell proliferation by 46% (± 7%, $P<0.001$, $n=3$). (C) Results are expressed as -fold increase of proliferation where proliferation obtained in the presence of 0.5% FBS is normalized to 1. Data are mean ± s.e.m. ($n=3$ experiments with islets obtained from fetuses of three pregnant rats).

medium containing 2.8 mM glucose and 0.5% FBS for 24 h, after which leptin (100 nM) or FBS (10%) was added for a further 24 h. Under these conditions, leptin increased the rate of DNA synthesis, as indicated by increased incorporation of [^{3}H]-thymidine into DNA (Figure 2A). When used at a concentration of 5 nM, leptin induced a small increase in [^{3}H]-thymidine incorporation in three out of five experiments, which did not reach the level of statistical significance (data not shown). Significant stimulation was observed with 100 nM leptin ($P=0.02$, $n=3$). The increase in thymidine incorporation stimulated by 100 nM leptin was 10–27% (mean $18\pm5\%$, $n=3$) over that obtained in controls, ie in the presence of 0.5% serum only. In comparison, the increase in thymidine incorporation stimulated by 10% FBS was 38–49% (mean $46\pm7\%$, $n=3$) over basal (Figure 2B). On the average, the effect of leptin on cell proliferation was $\sim$39% of the maximal effect obtained with 10% FBS. In these experiments, the increase in proliferation of fetal islet cells induced by 100 nM leptin varied from 1.1 to 1.27-fold (1.18 ± 0.05, mean $\pm$ s.e.m., $n=3$), as compared with proliferation in the absence of leptin and FBS. Under similar conditions, the increase in proliferation obtained by 10% FBS ranged from 1.38 to 1.49-fold (1.46 ± 0.04, $n=3$; Figure 2C).

Leptin increases c-*fos* mRNA levels in fetal pancreatic islets

We tested whether leptin could induce transcription of the immediate early gene c-*fos*, a functional nuclear response described before in adult islets and insulinoma cells.[12,16] Figure 3 shows ratios of c-*fos* and α-tubulin from optimized linear regions of PCR condition, generated from equal volumes of cDNA. Treatment by leptin for 30 min caused a 2.8 ± 0.35-fold ($P<0.0018$, $n=3$) increase in c-*fos* mRNA levels in fetal islet cells.

Leptin receptor mRNA in fetal islets

We used RT-PCR for detecting transcripts for the extracellular part of the leptin receptor that is common to all isoforms (OB-R), and the full-length OB-Rb in fetal pancreatic islets. For detection of OB-Rb transcripts, we amplified two different fragments, both of which were readily detectable in as few as five islets. The common OB-R transcripts were more abundant and were detectable in as little as a single islet (Figure 4). Since previous studies suggested that serum can confer leptin unresponsiveness in clonal β-cells,[16] we examined whether factors like FBS (0.5% *vs* 10%, for 48 h) or glucose (2.8 *vs* 11 mM, for 48 h) might alter OB-R and OB-Rb transcript levels. We used multiplex PCR assay to measure the levels of OB-R mRNA relative to β-actin mRNA and OB-Rb mRNA relative to OB-R mRNA. In this method, the number of primer–template matches formed can modify the efficiency between different PCR amplifications.[22] We found that the leptin receptor transcript levels

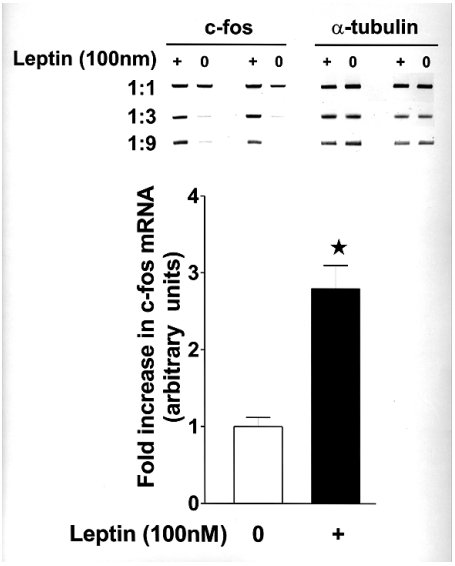

Figure 3 Leptin increases c-*fos* mRNA level in fetal islets. The figure shows c-*fos* and α-tubulin mRNA amplified by PCR from fetal islets treated with leptin. Serial dilutions of cDNA samples (1:1, 1:3, 1:9) were used at a fixed number of PCR cycles (36 cycles) to obtain linear regions of PCR amplification. Results from two groups of islets are shown in upper panel of the figure and quantitation of three independent experiments is shown in the bottom panel. Results are expressed as mean $\pm$ s.e.m. of -fold increase in c-*fos* mRNA. *$P<0.0018$ *vs* 0 leptin.

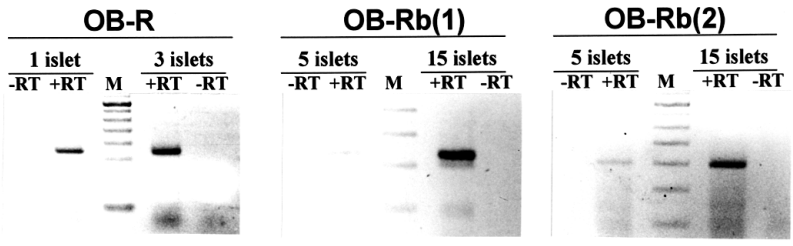

Figure 4 RT-PCR detection of the common extracellular part of leptin receptor OB-R (348 bp) and the full-length leptin receptor OB-Rb in fetal rat islets. Two different sets of primers were used to detect the OB-Rb isoform, OB-Rb (fragment-1, 332 bp) and OB-Rb (fragment-2; 399 bp) as described in the research design and methods. Different volumes of cDNA representing RNA obtained from between $\frac{1}{2}$ and 15 islets were used.

were not affected by varying the concentration of glucose or FBS (Figure 5).

Discussion

Islets obtained from rat fetuses are used as a convenient model for examining effects of cytokines and growth-factors on proliferation of pancreatic β-cells.[18,19] Leptin stimulated proliferation of isolated serum-deprived fetal rat islet cells. The magnitude of this stimulation was 39–50% of the maximal effect obtained with 10% FBS. Since assays for quantification of cell proliferation have potential pitfalls, we used two independent methods, ie [^{3}H]-thymidine incorporation and tetrazolium assays.[20,24] An increase in proliferation of fetal islet cells was observed in all experiments by these two independent methods. The difference in the magnitudes of stimulation observed in WST-1 assay and [^{3}H]-thymidine incorporation was probably due to slightly different conditions used in the two sets of experiments. Since β-cells constitute 90–95% of the cells in these islets,[19] it is likely that islet cell proliferation observed in our study reflects mainly proliferation of β-cells. Consistent with stimulation of islet cell proliferation, leptin also induced expression of the immediate-early gene c-*fos* in fetal rat islets.

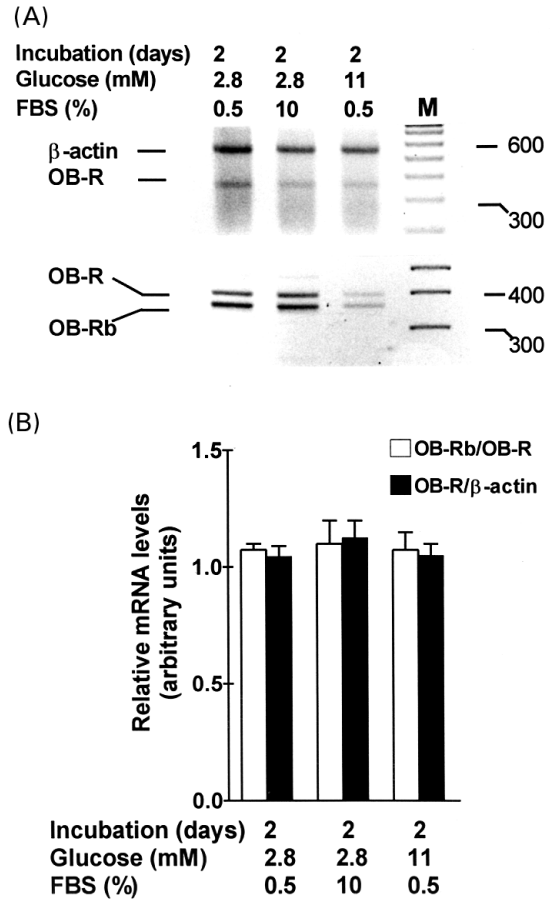

Figure 5 The effect of glucose and fetal bovine serum on leptin receptor mRNA level; multiple PCR analysis of the mRNA levels of OB-R common extracellular parts and the full-length OB-Rb isoform in rat fetal islets incubated for 2 days in medium containing different concentrations of glucose (2.8 or 11 mM) and FBS (0.5% or 10%). (A) Representative gels showing co-amplification of the β-actin (534 bp) and OB-R common (348 bp) in upper panel, and OB-R common and OB-Rb (fragment-1; 332 bp) in lower panel. (B) Results from the experiment in (A) were reproduced in three separate experiments, quantitated and expressed as mean ± s.e.m.

We found that fetal islet cells express high levels of mRNA for OB-R, which is common to all splice variants of leptin receptor. However, it is the full-length receptor, OB-Rb that can mediate leptin signaling. To detect whether these cells express mRNA for OB-Rb, we amplified two fragments of the receptor by RT-PCR. The amplified fragments were detected in cDNA obtained from as little as five islets suggesting that this form of leptin receptor is expressed in fetal islet cells. OB-Rb activates the JAK/STAT pathway in many cells including insulinoma cells and adult islet cells.[25] Activation of this pathway is an important mechanism of signalling for proliferation of β-cells and is utilized by ligands like growth hormone and prolactin.[26,27] OB-Rb also mediates expression of the immediate-early gene c-*fos* which is often used as a criterion for functional leptin signaling.[16,28,30] c-*fos* is required for cell-cycle progression and is targeted by activated STAT-3, which results in induction of the protein.[31,32] It is thus likely that leptin-induced expression of c-*fos* in fetal islet cells is mediated by OB-Rb through activation of STAT-3. Further clarification of the signaling mechanisms by which leptin stimulates fetal islet cell proliferation is currently underway in our laboratory. It could be argued that leptin stimulated fetal islet cell proliferation by stimulating some other cytokine receptors. However, the presence of signaling-competent isoform OB-Rb in fetal islets makes it likely that the leptin-effect was mediated by activation of its native receptor. Furthermore, in islets of adult *db*/*db* mice which do not express functional leptin receptors, we did not find any evidence that leptin can activate other cytokine receptors.[12] Previous studies demonstrated that the level of expression of leptin receptors can be altered by various factors including growth hormone and glucocorticoids.[28,29] We examined the effects of glucose and FBS and found that they did not cause detectable alteration in the level of expression of leptin receptor mRNA in fetal islet cells.

In summary, we have demonstrated that fetal islet cells express intact leptin receptors (OB-Rb) and that leptin stimulates proliferation of these cells. The cell types that express leptin receptors and proliferate in response to leptin are predominantly the β-cells, since they constitute over 90% of cells in these islets.[19] β-Cell proliferation stimulated by leptin in these *in vitro* experiments and under minimal conditions was 39–50% of maximal. *In vivo*, leptin may stimulate proliferation of β-cells by acting synergistically with other growth factors. Mice lacking leptin receptors (*db*/*db*) may have fewer β-cells and atrophic islets depending on the background on which *db* gene is expressed.[33–35] However, many factors determine islet cell proliferation in the complex *in vivo*

environment. Despite lacking leptin receptors, *db*/*db* islets can often be large due to factors like hyperglycemia and hyperinsulinemia, which stimulate β-cell proliferation. Islet cell mass increases mainly during embryonic and fetal life. Hormones secreted from placenta, for example growth hormone, insulin-like growth factor 1 and prolactin, exert trophic effects on fetal islet cells.[36] Leptin is present in the fetal circulation where it is partly derived from the placenta.[37–39] Our study suggests that leptin may play a role in determining islet cell mass at birth. Stimulation of β-cell proliferation by leptin may also have relevance in adults, since it is known that obese animals and human often have large islets.[40–42]

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References

1 Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763–770.
2 McKinley MJ, Oldfield BJ. The brain as an endocrine target for peptide hormones. *Trends Endocrinol Metab* 1998; **9**: 349–354.
3 Tartaglia LA. The leptin receptor. *J Biol Chem* 1997; **272**: 6093–6096.
4 Cawthorne MA, Morton NM, Pallett AL, Liu YL, Emilsson V. Peripheral metabolic actions of leptin. *Proc Nutr Soc* 1998; **57**: 449–453.
5 Lollmann B, Gruninger S, Stricker-Krongrad A, Chiesi M. Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and, e in different mouse tissues. *Biochem Biophys Res Commun* 1997; **238**: 648–652.
6 Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med* 1996; **2**: 589–593.
7 Dötsch J, Adelmann M, Englaro P, Dotsch A, Hanze J, Blum WF, Kiess W, Rascher, W. Relation of leptin and neuropeptide Y in human blood and cerebrospinal fluid. *J Neurol Sci* 1997; **151**: 185–188.
8 Lee G-H, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 1996; **379**: 632–635.
9 Chen H, Churfat O, Tartaglia LA, Woolf EA, Wang X, Ellis SJ, Lukey ND, Culpepper J, Moore KJ, Breitbart LF, Duyk GM, Tepper RL, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db*/*db* mice. *Cell* 1996; **84**: 491–495.
10 Ghilardi N, Skoda RC. The leptin receptor activates Janus Kinase 2 and signals for proliferation in a factor-dependent cell line. *Mol Endocrinol* 1997; **11**: 393–399.
11 Poitout V, Rouault C, Guerre-Millo M, Briaud I, Reach G. Inhibition of insulin secretion by leptin in normal rodent islets of Langerhans. *Endocrinology* 1998; **139**: 822–826.
12 Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 1997; **46**: 313–316.
13 Kieffer TJ, Heller RS, Habener JF. Leptin receptors expressed on pancreatic β-cells. *Biochem Biophys Res Commun* 1996; **224**: 522–527.
14 Pallett AL, Morton NM, Emilsson V, Cawthorne MA. Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochem Biophys Res Commun* 1997; **238**: 267–270.
15 Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR. Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, *in vivo*, in mice. *J Clin Invest* 1997; **100**: 2729–2736.
16 Islam MS, Morton NM, Hansson A, Emilsson V. Rat insulinoma-derived pancreatic β-cells express a functional leptin receptor that mediates a proliferative response. *Biochem Biophys Res Commun* 1997; **238**: 851–855.
17 Tanabe K, Okuya S, Tanizawa Y, Matsutani A, Oka Y. Leptin induces proliferation of pancreatic β-cell line MIN6 through activation of mitogen-activated protein kinase. *Biochem Biophys Res Commun* 1997; **241**: 765–768.
18 Sjöholm Å. Differential effects of cytokines on long-term mitogenic and secretory responses of fetal rat pancreatic β-cells. *Am J Physiol* 1992; **263**: C114–C120.
19 Hellerström CH, Lewis NJ, Borg H, Johnson R, Freinkel N. Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. *Diabetes* 1979; **28**: 769–776.
20 Marshall NJ, Goodwin CJ, Holt SJ. A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function. *Growth Regul* 1995; **5**: 69–84.
21 McEvoy RC, Leung PE. Tissue culture of fetal rat islets: comparison of serum-supplemented and serum-free, defined medium on the maintenance, growth, and differentiation of A, B, and D cells. *Endocrinology* 1982; **111**: 1568–1575.
22 Dukas K, Sarfati P, Vaysse N, Pradayrol L. Quantitation of changes in the expression of multiple genes by simultaneous polymerase chain reaction. *Anal Biochem* 1993; **215**: 66–72.
23 Liu YL, Emilsson V, Cawthorne MA. Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (*ob*/*ob*) mice. *FEBS Lett* 1997; **411**: 351–355.
24 Maurer HR. Potential pitfalls of [^{3}H]-thymidine techniques to measure cell proliferation. *Cell Tissue Kinet* 1981; **14**: 111–120.
25 Morton NM, Emilsson V, de Groot RP, Pallett AL, Cawthorne MA. Leptin signalling in pancreatic islets and clonal insulin secreting cells. *J Mol Endocrinol* 1999; **22**: 173–184.
26 Stout LE, Svensson AM, Sorenson RL. Prolactin regulation of islet-derived INS-1 cells: characteristics and immunocytochemical analysis of STAT5 translocation. *Endocrinology* 1997; **138**: 1592–1603.
27 Sekine N, Wollheim CB, Fujita T. GH signalling in pancreatic β-cells. *Endocr J* 1998; **45**: S33–S40.
28 Uotani S, Bjorbaek C, Tornoe J, Flier JS. Functional properties of leptin receptor isoform internalisation and degradation of leptin and ligand-induced receptor downregulation. *Diabetes* 1999; **48**: 279–286.
29 Cai A, Hyde JF. Upregulation of leptin receptor gene expression in the anterior pituitary of human growth hormone-releasing hormone transgenic mice. *Endocrinology* 1998; **139**: 420–423.
30 Bjoerbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem* 1997; **272**: 32686–32695.

31 Cosenza SC, Yumet G, Soprano DR, Soprano KJ. Induction of c-*fos* and c-*jun* mRNA at the M/G1 border is required for cell cycle progression. *J Cell Biochem* 1994; **55**: 503–512.
32 Gronowski AM, Zhong Z, Wen Z, Thomas MJ, Darnell JE Jr, Rotwein P. *In vivo* growth hormone treatment rapidly stimulates the tyrosine phosphorylation and activation of STAT3. *Mol Endocrinol* 1995; **9**: 171–177.
33 Diani AR, Peterson T, Sawada GA, Wyse BM, Gilchrist BJ, Hearron AE, Chang AY. Ciglitazone, a new hypoglycaemic agent. 4. Effect on pancreatic islets of C57BL/6J-*ob*/*ob* and C57BL/KsJ-*db*/*db* mice. *Diabetologia* 1984; **27**: 225–234.
34 Coleman DL, Schwizer RW, Leiter EH. Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and in aged normal mice. *Diabetes* 1984; **33**: 26–32.
35 Baetens D, Stefan Y, Ravazzola M, Malaisse-Lagae F, Coleman DL, Orci L. Alteration of islet cell populations in spontaneously diabetic mice. *Diabetes* 1978; **27**: 1–7.
36 Scharfmann R, Czernichow P. Differentiation and growth of pancreatic β-cells. *Diabetes Metab* 1996; **22**: 223–228.
37 Yura S, Sagawa N, Mise H, Mori T, Masuzaki H, Ogawa Y, Nakao K. A positive umbilical venous-arterial difference of leptin level and its rapid decline after birth. *Am J Obstet Gynecol* 1998; **178**: 926–930.
38 Lepercq J, Cauzac M, Lahlou N, Timsit J, Girard J, Auwerx J, Hauguel-de Mouzon S. Overexpression of placental leptin in diabetic pregnancy: a critical role for insulin. *Diabetes* 1998; **47**: 847–850.
39 Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature Med* 1997; **3**: 1029–1033.
40 Ogilvie RF. The islands of Langerhans in 19 cases of obesity. *J Pathol* 1933; **37**: 473–481.
41 Lucocq JM, Findlay JA. Islet organ, blood glucose and glucose tolerance of lean and obese Mongolian gerbils. A quantitative study. *Cell Tissue Res* 1981; **220**: 623–636.
42 Brandhorst D, Brandhorst H, Hering BJ, Federlin K, Bretzel RG. Islet isolation from the pancreas of large mammals and humans: 10 y of experience. *Exp Clin Endocrinol Diabetes* 1995; **103**(Suppl 2): 3–14.