

RESEARCH PAPER



Prolactin and oleic acid synergistically stimulate β -cell proliferation and growth in rat islets

Todd Clark Brelje, Nicholas V. Bhagroo , Laurence E. Stout, and Robert L. Sorenson

Department of Genetics, Cell Biology, and Development, University of Minnesota Medical School, Minneapolis, MN, USA

ABSTRACT

Islet adaptation to pregnancy is largely influenced by prolactin and placental lactogens. In addition serum lipids are significantly increased. Here, we report the novel observation that prolactin and oleic acid synergistically stimulate islet cell proliferation and islet growth. In neonatal rat islets, prolactin increased proliferation 6-fold, oleic acid 3.5-fold, and their combination 15-fold. The expression of insulin in these dividing cells establishes them as β -cells. Similar changes were seen in islet growth. This synergy is restricted to monounsaturated fatty acids and does not occur with other islet growth factors. Oleic acid increases prolactin-induced STAT5 phosphorylation, even though by itself it is unable to induce STAT5 phosphorylation. Their effects on Erk1/2 phosphorylation are additive. Some of the synergy requires the formation of oleoyl CoA and/or its metabolites. Unexpectedly, methyl oleic acid, a non-metabolizable analog of oleic acid, also shows synergy with prolactin. In summary, prolactin and oleic acid synergistically stimulate islet cell proliferation and islet growth in rat islets, oleic acid increases prolactin-induced STAT5 activation, and requires both the metabolism of oleic acid and non-metabolized oleic acid. Since oleic acid is the most abundant monounsaturated fatty acid in serum that is elevated during pregnancy, it may contribute to increased β -cell proliferation seen during pregnancy.

ARTICLE HISTORY

Received 4 November 2016
Revised 8 May 2017
Accepted 9 May 2017

KEYWORDS

β cell; cell division; islet; oleic acid; prolactin; proliferation

Introduction


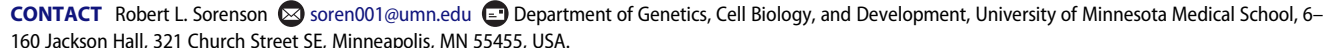
During pregnancy, there is an increased need for insulin to accommodate the developing insulin resistance and growing fetal compartment. Pancreatic islets meet this demand by increasing islet mass, insulin production and secretion.^{1,2} We have shown activation of prolactin receptors on β -cells induce these changes in islet function.^{3–5} It mediates the actions of both pituitary-derived prolactin and placental-derived lactogens. The key role of prolactin receptors in this adaptation is shown in heterozygous prolactin receptor-null (Prlr^{+/-}) mice. They have impaired insulin secretion and fail to increase islet mass during pregnancy.⁶ In addition, mRNA expression profiling of islets from pregnant mice have a pattern identical to islets cultured with placental lactogen.⁷


Serum lipids, triglycerides, and free fatty acids are also significantly increased during pregnancy.^{8–10} These elevations occur in rats between days 11–14 and peak

on the last 2 days of pregnancy. This increase in serum lipids is interesting because of the complex interaction between glucose and fatty acids in the regulation of β -cell function.^{11–13} Thus, lipids may be involved in the adaptation of β -cells in the latter half of pregnancy.

Because palmitic and oleic acids are the most abundant fatty acids,⁸ we examined their influence on islets cultured with prolactin.¹⁴ This simulates what islets experience during late pregnancy. This study showed elevated lipids may lead to enhanced insulin secretion in late pregnancy that is increasingly dependent on lipids and less sensitive to glucose. In addition, both fatty acids increased β -cell proliferation. While the effects of prolactin and palmitic acid were only additive, oleic acid was synergistic with prolactin.¹⁴

In this work, our goal was to investigate the synergy between prolactin and oleic acid. To our knowledge little information is currently available about oleic acid, either alone or in combination with prolactin,

CONTACT Robert L. Sorenson  soren001@umn.edu 

 Supplemental data for this article can be accessed on the [publisher's website](#).

© 2017 Todd Clark Brelje, Nicholas V. Bhagroo, Laurence E. Stout, and Robert L. Sorenson. Published with license by Taylor & Francis.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

aside from our previous experiment.¹⁴ In this study, we characterized their effects on β -cell proliferation and islet growth using rat islets.

Materials and methods

Hormones

Rat prolactin and growth hormone were provided by the National Hormone and Pituitary Program (AF Parlow, National Hormone and Pituitary Program, Harbor-UCLA Medical Center, Torrance, CA). Human IGF-1 and GLP-1 (Sigma-Aldrich, St. Louis, MO) were purchased commercially.

Materials

Oleic, palmitic, palmitoleic, linoleic, and methyl oleic acids, 5-bromo-2'-deoxyuridine (BrdU), etomoxir (Sigma), CAY10587, bisindolylmaleimide-1 (Cayman Chemical, Ann Arbor, MI), U0126 (Cell Signaling Technology, Danvers, MA), PKC ζ myristoylated pseudo substrate (Enzo Life Science, Plymouth Meeting, PA), triacsin C (Santa Cruz Biotechnology, Dallas, Santa Cruz, CA), GO-6976 (Tocris Bioscience, Bristol, UK) were purchased commercially.

Antibodies

Mouse anti-BrdU (Caltag Laboratories, San Francisco, CA), mouse anti-Ki67 (Sigma), mouse anti-PCNA (Abcam, Cambridge, MA), rabbit anti-phospho-STAT5 (Tyr694), rabbit anti-phospho-Erk1/2 (Thr202/Tyr204; Cell Signaling Technology) antibodies were purchased commercially. Anti- α -tubulin (12G10) was obtained from the Developmental Studies Hybridoma Bank (NICHD and The University of Iowa, Department of Biology Iowa City, IA).

Islets of Langerhans

Neonatal rat islets were isolated from 3–5-day-old Sprague–Dawley rats (Sasco, Omaha, NE) by a non-enzymatic culture method.¹⁵ After this initial culture, groups of 30 islets were cultured free floating in 24-well plates (Costar, Cambridge, MA) in 2 ml RPMI-1640 with 10 mM glucose supplemented with 10% horse serum (Gibco), 10 mM HEPES, 2 mM Gluta-Max (Gibco), and 1% penicillin–streptomycin–fungizone (PSF) antibiotic antimycotic (Sigma) at 37C with 5% CO₂ in air. Most experiments were done with 10%

horse serum and less frequently in serum-free media. Duplicate experiments show that relative differences were similar in both media.

Adult rat islets were isolated from Sprague–Dawley, 200–250 g, rats (Sasco, Omaha, NE)¹⁶ and cultured as described previously. All procedures using animals were approved by the Animal Care and Use Committee of the University of Minnesota.

Fatty acid solutions

Fatty acids were prepared as follows.¹⁷ 150 mM stock solutions were prepared by dissolving sodium salts of palmitic and oleic acids in 25% ethanol at 50 C. Aliquots of stock solutions were complexed with 10% fatty acid free albumin (in culture media) by stirring for 1 hour at 37 C and then diluted with additional culture media. The final albumin concentration was 1% with a molar ratio of fatty acid:albumin of 5:1 or less. The final ethanol concentration was \leq 0.06% (v:v).

The concentration of free fatty acids can be calculated using the method of Richieri et al.¹⁸ 0.6 mM oleic acid bound to 1% fatty acid free albumin in 10% horse serum (assuming 4.5% albumin in horse serum) results in 26.6 nM free fatty acids. Horse serum contributes a negligible amount of fatty acids (0.05 nM, assuming 0.5 mM free fatty acids in whole serum¹⁹). For experiments using serum-free media, oleic acid was reduced to give a similar concentration of free fatty acids. In the absence of horse serum, 0.4 mM oleic acid bound to 1% fatty free albumin results in 24.9 nM of free fatty acids.

Islet cell proliferation

Islet cell proliferation was assessed by examining BrdU incorporation as described previously.^{4,14,20,21} 10 μ M BrdU was added during the last 12 hr of culture. The islets were fixed for 30 min in 4% paraformaldehyde and DNA denatured for 30 min in 2 M HCl. Islets were double labeled by incubation with a 1:600 dilution of a mouse anti-BrdU antibody and a 1:1000 dilution of a guinea pig anti-insulin antibody. For secondary antibodies, a 1:200 dilution of FITC-conjugated donkey anti-guinea pig IgG and 1:600 CY3.18-conjugated donkey anti-rabbit IgG were used. Glass beads were included in the mounting media (Prolong Gold anti-fade reagent; Life Technologies, Grand Island, NY) to support the coverslips and prevent excessive deformation of the islets. As described previously,^{4,14,20,21} the number of BrdU-labeled nuclei/islet was determined by direct observation using a

conventional fluorescence microscope. The scattered BrdU-labeled nuclei (typically, 0 to 50) are counted while focusing through intact islets. A minimum of 75–100 islets were examined per treatment. Slides were coded so that the evaluator was unaware of the treatment groups.

Tissue sections

To identify the cells containing BrdU-labeled nuclei, tissue sections were prepared from groups of neonatal and adult islets. Sections were double labeled for BrdU and insulin as described previously and mounted in Prolong Gold anti-fade reagent. The proportion of cells with BrdU-labeled nuclei and express insulin was determined.

Islet volumes

The growth rate of individual islets was determined by culturing individual islets in 96 well plates. Each well contained 250 μ l of media of which half was changed every other day. Ten islets were used for each treatment. The attachment of islets is discouraged by the culture conditions (non-adherent multiwall plates) and agitation from media changes/handling. No indication of attachment or flattening was observed. Each islet was imaged on alternate days for 14 d. From these profiles, the volume of islets were calculated as described previously.¹⁵

Western blot analysis

Western blots were prepared from isolated islets as described previously.²² An equivalent amount of islet protein was loaded in each lane for electrophoresis. The probing antibodies were used at a dilution of 1:2000. Bound antibodies were detected using a 1:30,000 dilution of alkaline phosphatase-conjugated donkey secondary antibodies (Jackson ImmunoResearch Laboratories) and the chemiluminescent substrate CPD-Star (Applied Biosystems, Foster City, CA). A Bio-Rad GS-700 (Bio-Rad) was used for quantitative densitometry with the volume density of bands calculated as O.D. \times mm² after correction for tubulin as a loading control.

Expression of data and statistics

Data represent means \pm SEM of multiple independent experiments. Statistical analysis was performed using Student's paired t-test or analysis of variance with Newman-Keuls post-hoc test for multiple comparisons.

Results

Synergy between prolactin and oleic acid

To investigate the effects of prolactin and oleic acid on islet cell proliferation, islets were cultured with prolactin, oleic acid, or the combination of prolactin and oleic acid. Dividing cells were detected by adding BrdU during the last 12 hr (Fig. 1A). As expected, prolactin induced a 6.1-fold increase in BrdU-labeled nuclei/islet. Oleic acid had a smaller 3.5-fold increase. Remarkably, prolactin and oleic acid synergistically stimulated islet cell proliferation. A 15.6-fold increase was seen. This was significantly greater than the sum of their individual responses. Tissue sections were prepared from groups of islets for each treatment and double labeled for BrdU and insulin. More than 95% of the cells with BrdU-labeled nuclei were also stained for insulin. Thus, these dividing cells are β -cells.

To verify that BrdU incorporation reflects changes in cell division rather than other cellular processes (e.g., DNA damage and repair), islet cell proliferation was assessed using other cellular markers of proliferation. Similar increases in proliferation were also seen with Ki-67 and PCNA (Supplementary Fig. 1A and B).

A notable observation is that fatty acid free albumin, used to solubilize the oleic acid, affects islet cell proliferation. BrdU incorporation was significantly reduced when fatty acid free albumin was added by itself to culture media (Supplementary Fig. 1C). However, the relative increase with prolactin (\sim 6-fold) is the same in the absence or presence of fatty acid free albumin. This may reflect depletion of nutrients and/or growth factors from the serum by binding to the fatty acid free albumin. A similar phenomenon occurs with fatty acid-free albumin and insulin secretion.²³

Synergy between prolactin and oleic acid occurs across a broad range of glucose and oleic acid concentrations. Because saturated fatty acids reduce insulin production and β -cell viability, especially when high glucose is present,^{11–13} it was important to examine whether oleic acid exhibited similar toxicity. However, synergy is seen across all glucose concentrations (2.8 to 16.7 mM; Supplementary Figs. 2A and 2B). This implies it is not toxic at high glucose concentrations. BrdU incorporation was also increased with concentrations as low as 0.1 mM oleic acid (Supplementary Fig. 2C). Thus, circulating levels of oleic acid during pregnancy (0.4 to 0.6 mM)^{8–10} are sufficient to exhibit synergy with prolactin.

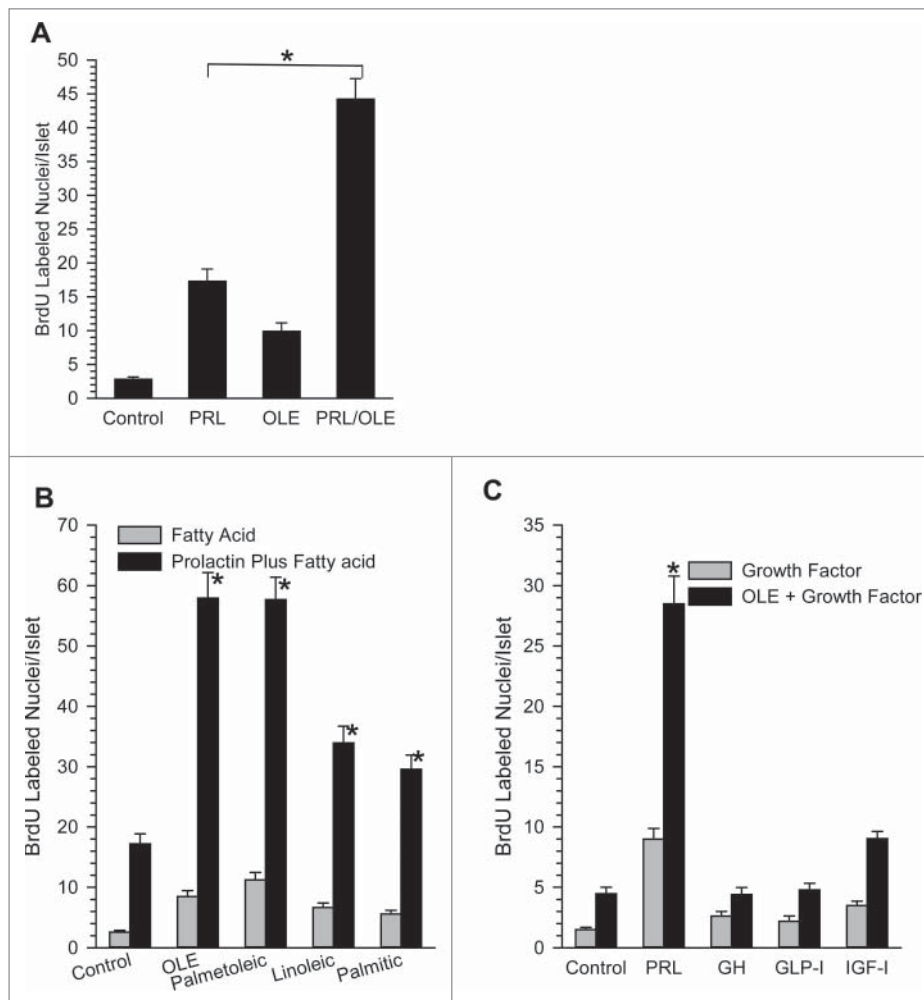


Figure 1. Synergy between prolactin (PRL) and oleic acid (OLE) on islet cell proliferation. Neonatal rat islets were cultured for 4 d with PRL (500 ng/ml), OLE (0.6 mM) or the combination of PRL and OLE in 10% horse serum. (A) PRL is synergistic with OLE on BrdU incorporation. All groups are different from the controls ($n = 24$, $p < 0.001$). $*p < 0.001$ for the difference between prolactin alone or in combination with OLE ($n = 24$). (B) Neonatal rat islets were cultured for 4 d with prolactin (PRL; 500 ng/ml), the indicated fatty acid (0.4 mM) or the combination of PRL and each fatty acid in 1% BSA. The largest synergy with PRL occurs with the monounsaturated fatty acids, OLE and palmitoleic acid. All fatty acids by themselves or with PRL increase BrdU incorporation. $*p < 0.05$ for differences between prolactin alone or in combination with each fatty acid ($n = 6$). (C) Neonatal rat islets were cultured for 4 d with the following growth factors: PRL (500 ng/ml), GH (500 ng/ml), GLP-1 (100 nM), and IGF-1 (15 nM) or the combination of each growth factor and OLE (0.4 mM) in 1% BSA. Only PRL shows synergy with OLE. The effects of GH, GLP-1 and IGF-1 were only additive with OLE. $*p < 0.05$ for the difference between prolactin alone or in combination with OLE ($n = 3$).

Synergy between prolactin and other fatty acids

To determine whether other fatty acids exhibit synergy with prolactin, islets were cultured with prolactin and other saturated and unsaturated fatty acids, individually or in combination (Fig. 1B). Prolactin induced a 6-fold increase in proliferation, while the fatty acids by themselves had less than 3-fold increases. Synergy was only seen with monounsaturated fatty acids, 20-fold for both oleic acid (C18) and palmitoleic acid (C16:1). In contrast, the saturated fatty acid, palmitic acid (C16), and polyunsaturated

fatty acid, linoleic acid (C18:2), produced smaller increase, less than 12-fold. These fatty acids are elevated during pregnancy.⁸⁻¹⁰ Thus multiple monounsaturated fatty acids may exhibit synergy with prolactin during pregnancy.

Absence of synergy between other growth factors and oleic acid

We examined whether oleic acid exhibits synergy with other islet growth factors. Unlike the synergy with prolactin, oleic acid was only additive with growth

hormone, GLP-1 and IGF-1 (Fig. 1C) making the synergy between prolactin and oleic acid rather unique.

Prolactin receptor induced JAK2/STAT5 activation

Prolactin binding to the prolactin receptor activates JAK2 which then activates downstream signaling pathways including STAT5 phosphorylation, the Ras-MEK-Erk pathway, and the PI3K-Akt pathway, among others. To examine the role of JAK2 activation, it was inhibited using a high concentration of GO-6976 (Fig. 2A). BrdU incorporation with prolactin was dramatically reduced, while it was not affected by oleic acid. The combination of prolactin and oleic acid was similarly reduced. Thus, the actions of prolactin are JAK2 dependent, while those of oleic acid are JAK2 independent.

The transcription factor STAT5 is the critical effector of prolactin receptor activation. The effect of prolactin and oleic acid, individually or in combination, on the tyrosine phosphorylation of STAT5 was examined (Fig. 2C). As expected, prolactin caused robust phosphorylation of STAT5. None was seen with oleic acid. Unexpectedly, oleic acid increased the amount of prolactin-induced STAT5 phosphorylation by an additional 50%. This increased JAK2-STAT5 signaling likely contributes to their synergy.

Prolactin receptor induced Erk1/2 activation

Many growth factors require the activation of the Ras-MEK-ERK pathway to induce β -cell proliferation. The prolactin receptor and free fatty acid receptor 1 (FFA1) activate this pathway in β -cells.^{24,25} A MEK1/2 inhibitor (U0126) reduces BrdU incorporation by 35% in all groups (Fig. 2B). Their effects on Erk1/2 phosphorylation was also examined (Fig. 2D). Prolactin and oleic acid by themselves increased Erk1/2 phosphorylation. In combination, they did not further augment Erk1/2 phosphorylation beyond that seen when the individual responses were summed.

Role of fatty acid metabolism

We examined whether metabolism of oleic acid is required for synergy with prolactin. The first step in fatty acid metabolism is the conversion of long-chain fatty acids into the metabolically active CoA esters. Inhibition of long-chain fatty acyl CoA synthetase reduces the effects of prolactin and oleic acid on BrdU incorporation (Fig. 3A). A 15% inhibition was seen in the

controls and 40–50% inhibition with prolactin, oleic acid, and their combination. This shows that the conversion of oleic acid to oleoyl CoA and/or its metabolites contributes to synergy with prolactin. However, it does not require mitochondrial oxidation of oleic acid (Fig. 3B).

The effect of a non-metabolizable analog of oleic acid was also examined. Methyl oleic acid contains an alkyl group that blocks the carboxylate group required for esterification to oleoyl CoA.^{26–28} This renders the molecule inert. BrdU incorporation was increased 8.5-fold by prolactin, while methyl oleic acid was only increased 2.2-fold (Fig. 3C). Surprisingly, methyl oleic acid was synergistic with prolactin. However, the 17-fold increase was less than the 27-fold seen with the combination of prolactin and oleic acid. Thus, the presence of oleic acid molecules themselves is responsible for up to half of the synergy with prolactin.

Other oleic acid induced signaling pathways

Several other pathways are known to be activated by oleic acid. A well-recognized pathway is the activation of protein kinase C (PKC) by long-chain acyl CoA.²⁹ However, inhibition of various PKC isoforms had no effect (Supplementary Fig. 3A). The atypical isoform PKC ζ is critical for growth factor induced cell division in β -cells.³⁰ PKC ζ inhibition caused a similar 40% reduction in all groups (Supplementary Fig. 3B). FFA1 is a G protein-coupled cell surface receptor for long-chain fatty acids that stimulates proliferation in some cell types.³¹ FFA1 also mediates its positive effects on insulin secretion.²⁵ Although a FFA1 agonist (CAY10587) modestly increases BrdU incorporation, it did not show synergy with prolactin (Supplementary Fig. 3C). Similarly, a FFA1 antagonist (GW1100) did not block the synergy (Supplementary Fig. 3D). Thus, oleic acid-induced-PKC activation or binding to FFA1 is unlikely to contribute to the synergy.

Prolactin and oleic acid stimulate growth of neonatal rat islets

To examine the effects of prolactin and oleic acid on islet mass, individual islets were cultured for 14 d with prolactin and oleic acid, individually and in combination. Each islet was imaged on alternate days and changes in the volume of individual islets determined. This removes the effects of variability in islet size and permits the detection of much

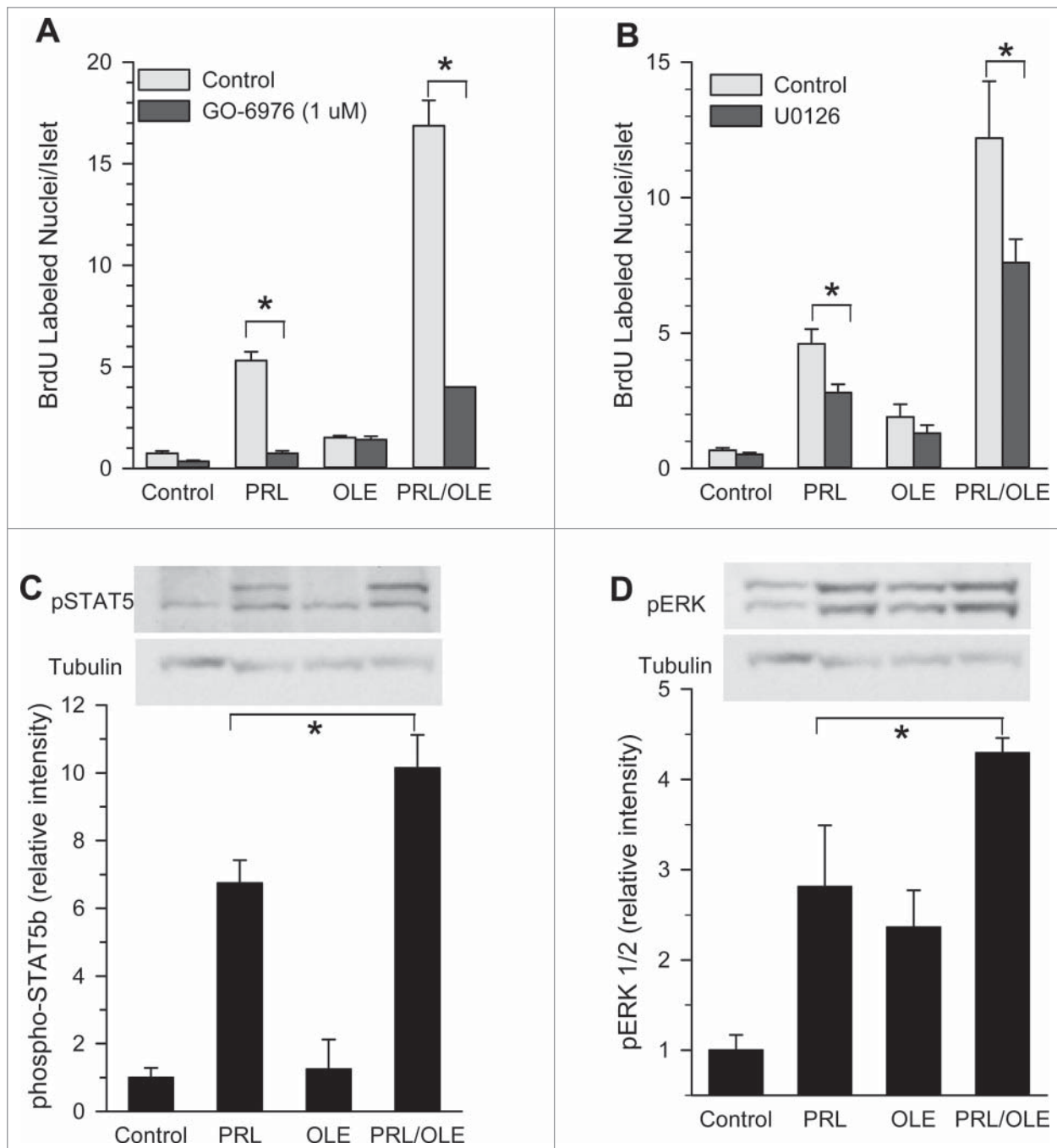


Figure 2. Synergy between prolactin (PRL) and oleic acid (OLE) on JAK2/STAT5B activation. Neonatal rat islets were cultured for 4 d with PRL (500 ng/ml), OLE (0.6 mM) or the combination of PRL and OLE in 10% horse serum. (A) JAK2 inhibition by Go-6976 (1 μ M) reduced BrdU incorporation by 90% with PRL and 70% with PRL/OLE. * $p < 0.001$ for differences between prolactin alone or in combination with OLE in the absence or presence of Go-6976 ($n = 3$). There was no effect on OLE-induced BrdU incorporation. (B) MEK inhibition by U0126 (10 μ M) reduced BrdU incorporation by 35% with PRL, OLE, and PRL/OLE. * $p < 0.05$ for the differences between prolactin alone or in combination with OLE in the absence or presence of U0126 ($n = 7$). (C) Densitometric analysis of western blots examining STAT5 phosphorylation. PRL increased phospho-STAT5. Although OLE had no effect on phospho-STAT5, it increased the amount of PRL-induced phospho-STAT5. * $p < 0.01$ for the difference between prolactin alone or in combination with OLE ($n = 7$). (D) Densitometric analysis of western blots show that both PRL and OLE induce phospho-Erk1/2 and that their effects are additive. * $p < 0.05$ for the difference between prolactin alone or in combination with OLE ($n = 3$).

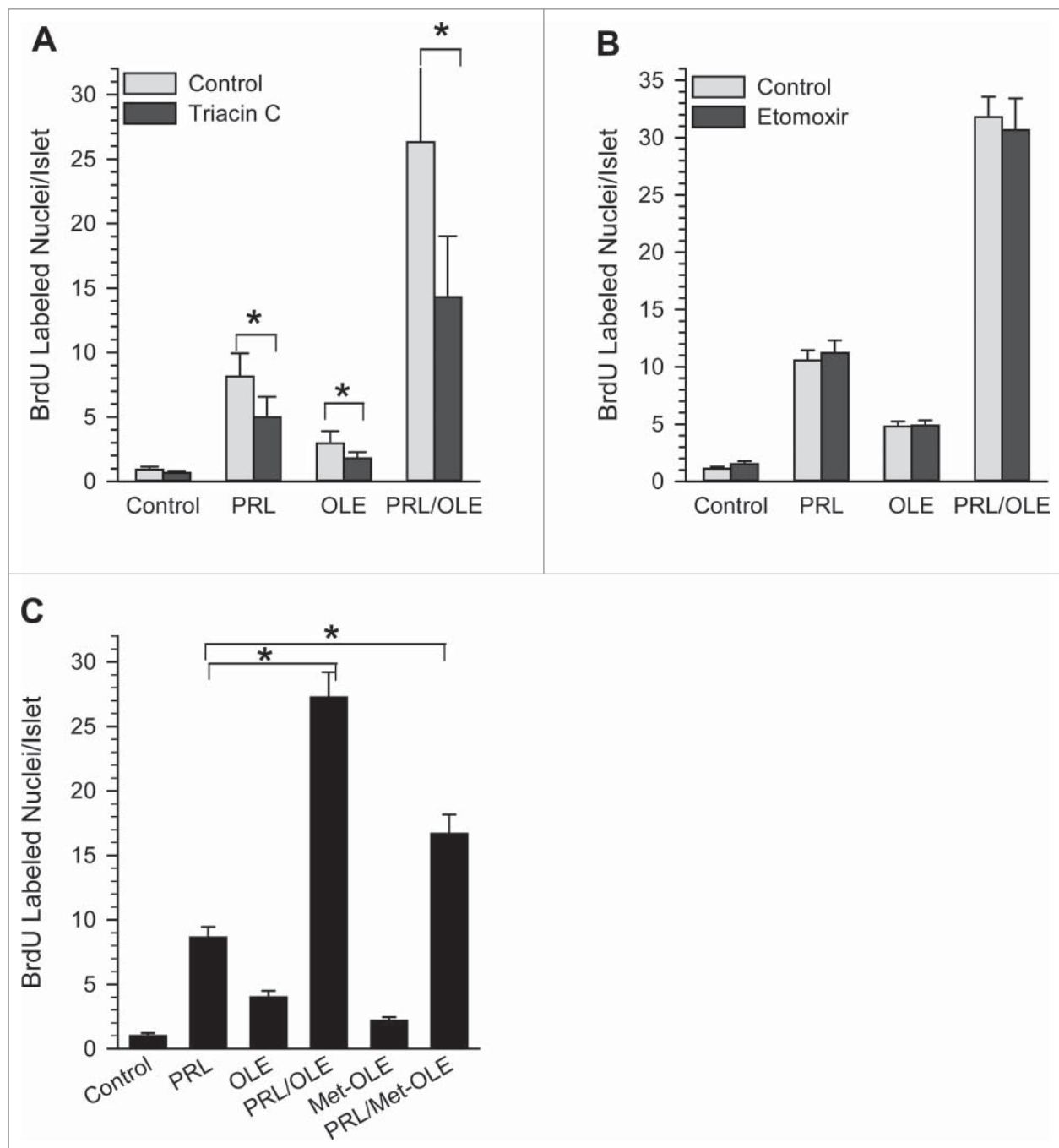


Figure 3. Fatty acid metabolism is involved in the synergy between prolactin (PRL) and oleic acid (OLE) on islet cell proliferation. Neonatal rat islets were cultured for 4 d with PRL (500 ng/ml), OLE (0.4 mM) or the combination of PRL and OLE in 1% BSA. (A) Triacin C (0.5 μ g/ml) inhibits long chain fatty acyl CoA synthetase and reduced BrdU incorporation by 40–50% with PRL, OLE, and PRL/OLE. * $p < 0.05$ for differences in the absence or presence of triacin C ($n = 6$). (B) Etomoxir (1 μ M) inhibits fatty acid oxidation and had no effect on BrdU incorporation in any group ($n = 3$, ns). (C) Methyl oleic acid (met-OLE) is a metabolically inert analog of OLE. Neonatal rat islets were cultured with PRL (500 ng/ml), met-OLE (0.4 mM) or the combination of PRL and met-OLE in 1% BSA for 4 d. Met-OLE by itself increased BrdU incorporation. Met-OLE/PRL also showed synergy with the increase being about half of that for PRL/OLE. * $p < 0.05$ for differences between prolactin alone or in combination with either OLE or Met-OLE ($n = 5$).

smaller changes in islet growth. Fig. 4A shows a typical experiment, while Fig. 4B is the average of multiple experiments. Prolactin increased islet volume by 60%, while oleic acid yielded a smaller 15%

increase. Similar to islet cell proliferation, prolactin and oleic acid showed synergy with a 210% increase in islet volume. This was considerably larger than the sum of their individual responses.

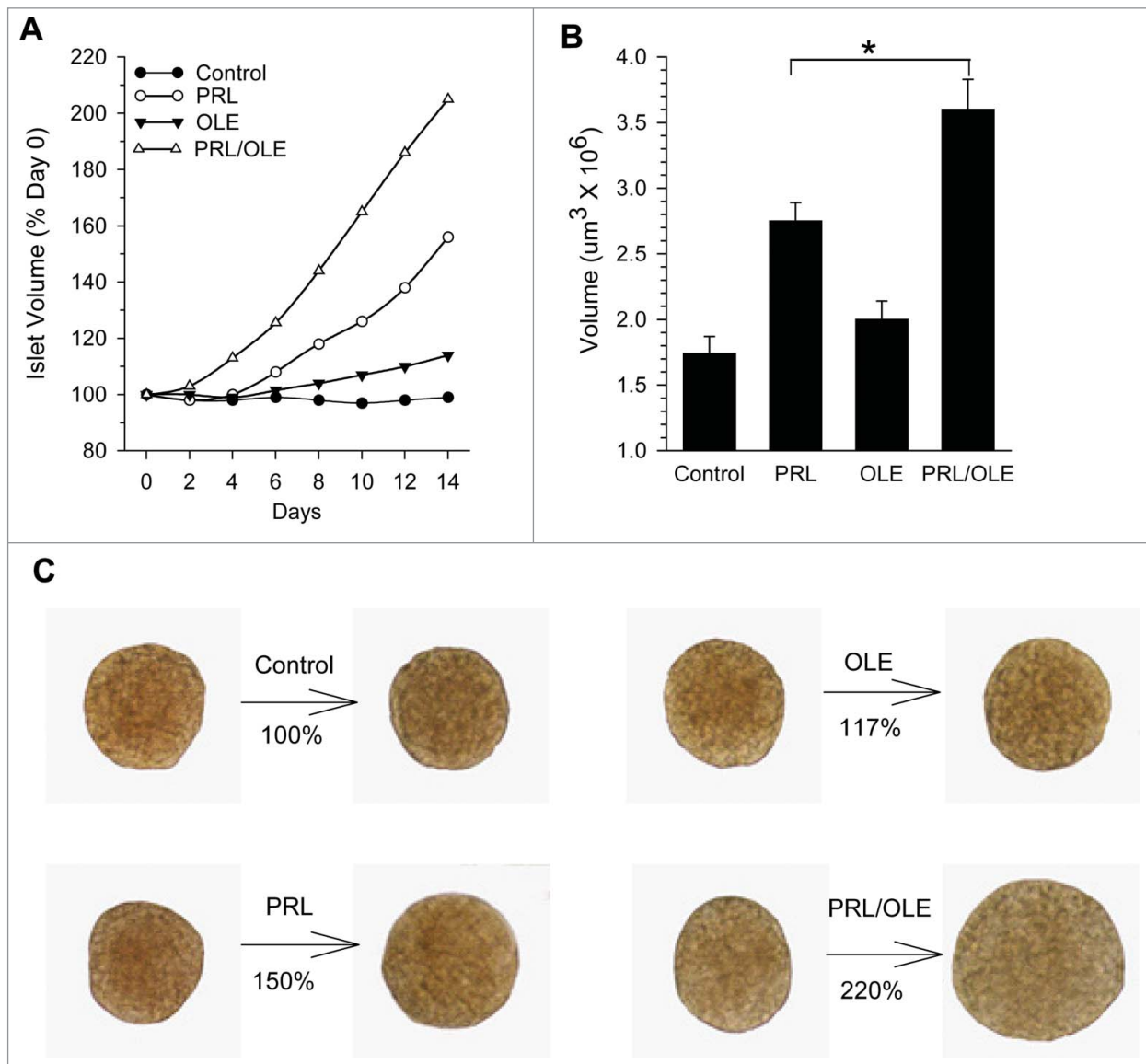


Figure 4. Prolactin (PRL) and oleic acid (OLE) stimulate the growth of neonatal rat islets. Individual neonatal rat islets were cultured for 14 d with PRL (500 ng/ml), OLE (0.6 mM) or the combination of PRL and OLE in 10% horse serum. Each group contained 10 islets cultured in individual wells. Each islet was photographed every 2 d and its volume calculated. (A) An example experiment showing the growth of individual islets. OLE enhances PRL-induced islet growth. (B) The results are shown from 7 experiments. PRL and OLE alone increased islet volumes, while the combination was synergistic. * $p < 0.01$ for the difference between prolactin alone or in combination with OLE ($n = 7$). (C) Images of the same islets at the start of the experiment and after culture with PRL, OLE, and PRL/OLE for 14 d. The percent indicates the calculated increase in islet volume at the end of the experiment for each of the islets.

Example images of an individual islet before and after 14 d are shown (Fig. 4C).

Prolactin and oleic acid stimulate growth of adult rat islets

Since most of our experiments used neonatal rat islets, it was important to confirm our observations with adult rat islets. Adult rat islets were cultured with prolactin and oleic acid, individually or in

combination (Fig. 5A). Prolactin induced a 3.3-fold increase in BrdU incorporation, while oleic acid had a lower 2.6-fold increase. Similar to neonatal rat islets, islet cell proliferation shows synergy with prolactin and oleic acid. The 12.9-fold increase was more than twice the sum of their individual responses.

To determine whether similar changes in islet mass occur in adults, isolated adult islets were followed in growth experiments as described previously (Fig. 5B).

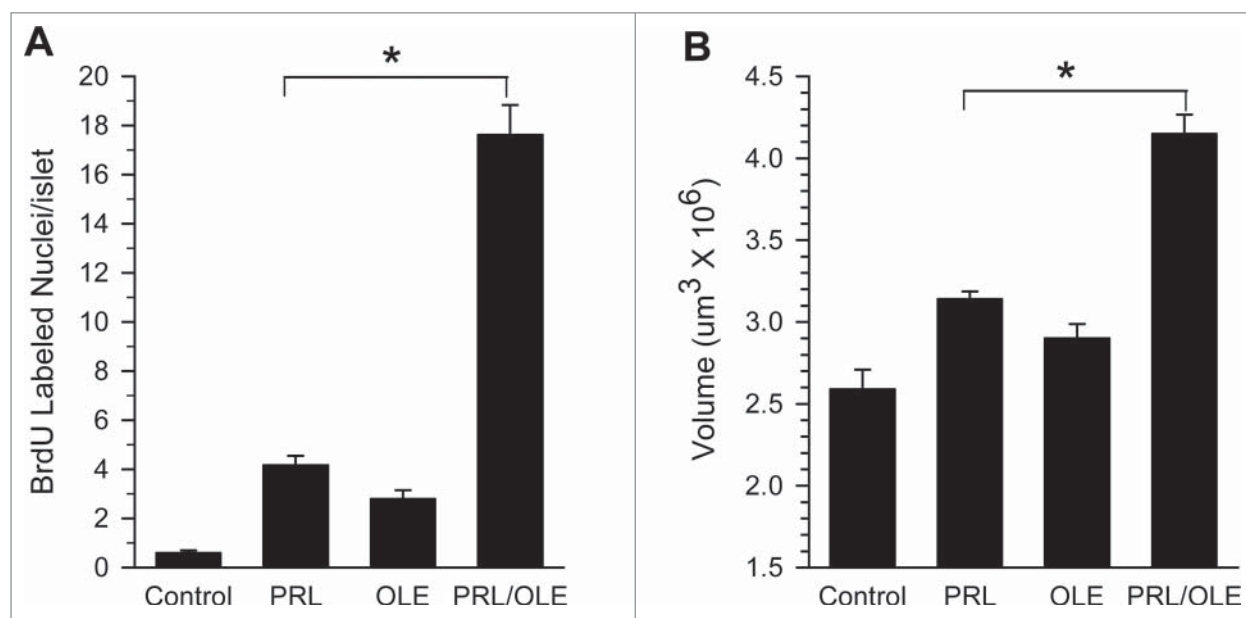


Figure 5. Prolactin (PRL) and oleic acid (OLE) stimulate the growth of adult rat islets. (A) Adult rat islets were cultured for 6 d with PRL (500 ng/ml), OLE (0.6 mM) or the combination of PRL and OLE in 10% horse serum. PRL, OLE, and PRL/OLE increased BrdU incorporation ($n = 9$, $p < 0.05$). PRL and OLE synergistically increased cell division. * $P < 0.05$ for the difference between prolactin alone or in combination with OLE ($n = 7$). (B) Individual adult rat islets were cultured for 14 d with PRL, OLE, or the combination of PRL and OLE in 10% horse serum. Each group contained 10 Islets cultured in individual wells. Each islet was photographed every 2 d and its volume calculated. The results are shown from 7 experiments. PRL, OLE, and PRL/OLE increased islet volumes ($n = 7$, $p < 0.05$). PRL and OLE synergistically increase islet growth. * $p < 0.05$ for the difference between prolactin alone or in combination with OLE ($n = 7$).

Prolactin increased islet volume by 20%, while oleic acid had a smaller 12% increase. Similar to neonatal rat islets, islet mass showed synergy with prolactin and oleic acid with the 60% increase about twice the sum of their individual responses.

Discussion

Prolactin/oleic acid synergy

This is the first study to investigate and characterize the effect of oleic acid, individually or in combination with prolactin, on islet cell proliferation and islet growth. The expression of insulin in more than 95% of the cells containing BrdU-labeled nuclei clearly established them as β -cells. Synergy was seen between prolactin and oleic acid. Previously, prolactin by itself has always shown the greatest effect on β -cell growth and in combination with other growth factors has been, at best, additive.³²

Prolactin/JAK2/STAT5 activation

Oleic and linoleic acids have been reported to induce STAT5 activation in breast cancer cells.³³ The

canonical JAK2/STAT5 signaling pathway is not involved. STAT5 activation requires phospholipase C, cyclooxygenase-2, lipoxygenases, and Src activities. The tyrosine kinase responsible for STAT5 phosphorylation was not identified. The authors suggest FFA1 may initiate this alternate pathway because it is required for oleic acid-induced proliferation of breast cells.³¹ However, oleic acid did not induce STAT5 phosphorylation in β -cells.

We found that oleic acid enhances prolactin-induced STAT5 phosphorylation. Previously, mono-unsaturated fatty acids have not been reported to influence JAK2/STAT5 signaling pathway. Among its target genes, STAT5 activation increases cyclin D2 transcription, inducing higher β -cell proliferation.^{34,35} This higher STAT5 activation should contribute to the synergy between prolactin and oleic acid. Similarly, synergy in STAT5 activation is seen between growth hormone (GH) and IGF-1 in β -cells.³⁶ IGF-1 enhances GH-induced STAT5 phosphorylation, but by itself has no effect on STAT5 activation.³⁶ Unlike prolactin and oleic acid, β -cell proliferation with GH and IGF-1 is additive.³⁶ However, a constitutively active STAT5b induces only a 6-fold increase in β -cell proliferation.³⁵

The much larger increase with prolactin and oleic acid suggests oleic acid has additional effects that contribute to its synergy with prolactin.

How oleic acid enhances prolactin-induced STAT5 activation, while alone has no effect on STAT5 activation, is unclear. However, some recent studies do provide insight. The protein tyrosine phosphatase-1B (PTP1B) inactivates many tyrosine-kinase receptors and soluble tyrosine kinases, including the insulin, IGF-1 receptors and JAK2 among others.³⁷⁻³⁹ PTP1B has a 70-fold preference for tandem phosphotyrosines in JAK2 over mono-phosphotyrosines such as in STAT5.³⁸ PTP1B is also a negative regulator of prolactin-induced STAT5 activation in breast cells.⁴⁰ Knock-down of PTP1B prolongs JAK2 phosphorylation allowing the production of more phosphorylated STAT5. Conversely, overexpression of PTP1B diminishes JAK2 phosphorylation and reduces STAT5 phosphorylation. In addition oleic acid enhances insulin receptor signaling in adipocytes.⁴¹ PTP1B inhibits insulin signaling by dephosphorylating the insulin receptor.³⁸ More interesting is the observation that unsaturated fatty acids (especially oleic acid) have been identified as potent inhibitors of PTP1B.^{39,41} PTP1B inhibition by oleic acid prevents the inactivation of the insulin receptor, thereby increasing insulin receptor signaling. Thus, oleic acid may inhibit PTP1B activation, slowing JAK2 inactivation, allowing more STAT5 phosphorylation.

Erk1/2

Activation of the Ras-MEK-Erk1/2 pathway is required for enhanced β -cell proliferation during pregnancy.⁴² Among the hormones/nutrients elevated during pregnancy, prolactin²⁴ and FFA1²⁵ receptors can activate this pathway in β -cells. MEK inhibition reduces BrdU incorporation to the same extent with prolactin alone and in combination with oleic acid. The absence of greater inhibition with the combination implies this pathway may not contribute to the synergy between prolactin and oleic acid. However, this is difficult to reconcile with their additive effects on Erk1/2 phosphorylation. The absence of synergy is surprising considering synergy is seen with other combinations of growth factors. For example, prolactin and PDGF synergize Erk1/2 activation (but not STAT5 or Akt) in human breast cancer cells.⁴³ Similarly growth hormone and EGF synergize ERK1/2

activation (but not STAT5, Akt, and PLC- γ) in preadipocytes.⁴³ Curiously, these results are the opposite of our observations. Prolactin and oleic acid synergize STAT5 activation, but not Erk1/2 activation. This implies synergy between prolactin and oleic acid is specific to certain signaling pathways. It is unclear to what extent the increased ERK1/2 activation contributes to the synergy between prolactin and oleic acid.

Fatty acid metabolism

The requirement for the metabolism of oleic acid was shown for synergy with prolactin. Inhibition of the conversion of long-chain fatty acids to their metabolically active CoA esters reduced the increase in islet cell proliferation. Notably, BrdU incorporation was reduced with prolactin, oleic acid, and when in combination. This shows that oleoyl CoA and/or its metabolites are required for some of the synergy with prolactin. The target(s) of oleoyl CoA is unclear. Similarly, palmitic acid-induced toxicity requires the formation of oleoyl CoA and is unaffected by inhibition of mitochondrial oxidation.⁴⁴

Methyl oleic acid is an analog of oleic acid that cannot be esterified to an active CoA ester. Because it is metabolically inert, all the actions of methyl oleic acid must occur through its interaction with critical binding site(s). Methyl palmitoleic acid also stimulates β -proliferation.²⁷ Methyl unsaturated fatty acids also do not bind to FFA1.²⁵ Yet, methyl oleic acid was synergistic with prolactin on BrdU incorporation. This suggests the presence of molecules of oleic acid themselves (i.e., non-metabolized oleic acid) are responsible for some of the synergy with prolactin.

Methyl/ethyl esters are reported to be capable of binding to the peroxisome-proliferator-activated receptor δ (PPRA δ).⁴⁵ In β -cells, it is the most abundant PPAR, the main transcriptional effector of unsaturated fatty acids, and a key regulator of fatty acid metabolism.⁴⁶ The combination of oleic acid and a retinoid-X-receptor (RXR) agonist also results in the synergistic activation of target genes and mitochondrial oxidation of fatty acids. However, β -cell proliferation was not examined here. PPAR δ activation by both oleic acid and alkylated monounsaturated fatty acids protect β -cells against saturated fatty acid-induced dysfunction.⁴⁶⁻⁴⁸ PPAR δ activation also increases expression of the important β -cell transcription factor MafA.^{47,48} MafA increases the transcription of the

prolactin receptor and cyclin D2.⁴⁹ STAT5 activation is necessary for prolactin-induced β -cell proliferation and cyclin D2 expression.³⁵ A siRNA specific for cyclin D2 markedly attenuates the effects of prolactin on β -cell proliferation.³⁴ This suggests prolactin-induced STAT5 activation and oleic acid-induced activation of PPAR δ may increase cyclin D2 greater than either alone. Similarly, MafA acts synergistically with Pdx1 and Beta2 to activate the insulin gene promoter.⁵⁰

Islet growth

Besides the effect of prolactin and oleic acid on islet cell proliferation, we examined their effects on islet growth. The increase in islet mass during pregnancy in rodents involves both hyperplasia and hypertrophy of β -cells.² Islet growth was determined here using a sensitive method whereby each islet was serially photographed at timed intervals. These results of the timed sequences confirmed that synergy between prolactin and oleic acid occurs in both β -cell proliferation and islet mass. However, the synergy with islet cell proliferation (15.6-fold) is much larger than with islet mass (2.1-fold). This is not surprising since the proportion of dividing β -cells within islets is quite low. In neonates, the percentage of dividing β -cells in rat islets is approximately $1.0 \pm 0.1\%$.⁵¹ The extent whereby an increase in cell size contributes to these changes in islet volume is unclear. Like many other growth factors, prolactin increases β -cell size by 30% in neonatal rat islets (unpublished).

Because pregnancy occurs in adults, it was important to examine the response of adult rat islets to prolactin and oleic acid. The percentage of dividing β -cells is only $0.12 \pm 0.10\%$ in adult rat islets.⁵¹ As expected, the changes in islet cell proliferation and islet mass were lower than in neonates. However, prolactin was still synergistic with oleic acid by both measures. The combination of prolactin and oleic acid increased islet mass by 60%. Although quite large, it is still less than the 200% increase observed during pregnancy in rodents.⁵² This suggests that the culture conditions are suboptimal or other factors may contribute to the increase in islet mass during pregnancy.

Human islets

It remains unclear whether a similar interaction between prolactin and oleic acid occurs during human pregnancy. The serum levels of prolactin and placental lactogens

increase in both rodents¹ and humans.⁸⁻¹⁰ Oleic acid may stimulate β -cell proliferation in adult human islets.⁵³ An increase in islet mass is seen in both species. In rodents, increased β -cell proliferation accounts for most of the increase in islet mass (2-fold).⁵² In humans, the absence of Ki67 expression in β -cells during pregnancy has been interpreted that dividing β -cells do not contribute to this increase in islet mass (1.4-fold).⁵⁴ Instead, the neogenesis of small islets was proposed. However, reports that adult human β -cells do not replicate may be erroneous due to a postmortem decline in replication markers such as Ki67.⁵⁵

The expression of the prolactin receptor has been suggested to be lower in human islets compared with rodent islets.^{56,57} In the transcriptomes of human and mouse β -cells, it was found that the prolactin and growth hormone receptors are expressed at much lower levels in non-pregnant humans compared with mice.⁵⁶ Similarly, it has been reported that prolactin receptors are not expressed in adult human islets and cannot induce STAT5 activation.⁵⁷ However, both studies used isolated, non-pregnant human islets. Expression of the prolactin receptor in β -cells may be downregulated in non-pregnant humans. In addition, the isolation protocol and/or culture conditions may reduce expression of the prolactin receptor. For example, an absence of the sex hormones, particularly estrogen and progesterone, which are elevated during pregnancy may be involved.⁵⁸ Estrogen is an important regulator of prolactin receptor expression in β -cells.⁵⁸ Prolactin and progesterone also synergistically increase prolactin receptor expression.⁵⁹ A reduction in β -cell differentiation during culture may also reduce MafA expression which is a transcriptional regulator of the prolactin receptor.⁴⁹ Thus, it is unclear whether β -cells express prolactin receptors during pregnancy in humans.

Summary

In summary, we report the novel observation that prolactin and oleic acid synergistically stimulate β -cell proliferation and islet growth in rat islets. Oleic acid increases prolactin-induced STAT5 phosphorylation. This synergy also requires non-metabolized oleic acid and formation of oleoyl CoA and/or its metabolites. Since oleic acid is the most abundant monounsaturated fatty acid in serum that is elevated during pregnancy, it may contribute to the increased β -cell proliferation observed during pregnancy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This research was partially supported by a grant funded by the National Institute of Health (DK33655).

Author contributions

T.C.B. and R.L.S. designed the study, analyzed, and interpreted the data and wrote the manuscript. N.V.B and L.E.S performed the laboratory experiments and collected the data.

ORCID

Nicholas V. Bhagroo  <http://orcid.org/0000-0003-3204-3905>

References

- [1] Parsons JA, Brelje TC, Sorenson RL. Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* 1992; 130:1459–66; PMID:1537300; <https://doi.org/10.1210/endo.130.3.1537300>
- [2] Sorenson RL, Brelje TC. Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm Metab Res* 1997; 29:301–7; PMID:9230352; <https://doi.org/10.1055/s-2007-979040>
- [3] Brelje TC, Allaire P, Hegre O, Sorenson RL. Effect of prolactin versus growth hormone on islet function and the importance of using homologous mammosomatotropic hormones. *Endocrinology* 1989; 125:2392–9; PMID:2676483; <https://doi.org/10.1210/endo-125-5-2392>
- [4] Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG, Sorenson RL. Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. *Endocrinology* 1993; 132:879–87; PMID:8425500; <https://doi.org/10.1210/endo.132.2.8425500>
- [5] Brelje TC, Sorenson RL. Nutrient and hormonal regulation of the threshold of glucose-stimulated insulin secretion in isolated rat pancreases. *Endocrinology* 1988; 123:1582–90; PMID:3042373; <https://doi.org/10.1210/endo-123-3-1582>
- [6] Huang C, Snider F, Cross JC. Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. *Endocrinology* 2009; 150:1618–26; PMID:19036882; <https://doi.org/10.1210/en.2008-1003>
- [7] Goyvaerts L, Lemaire K, Arijs I, Auffret J, Granvik M, Van Lommel L, Binart N, in't Veld P, Schuit F, Schraenen A. Prolactin receptors and placental lactogen drive male mouse pancreatic islets to pregnancy-related mRNA changes. *PLoS One* 2015; 10:e0121868; PMID:25816302; <https://doi.org/10.1371/journal.pone.0121868>
- [8] Chen ZY, Yang J, Cunnane SC. Gestational hyperlipidemia in the rat is characterized by accumulation of n-6 and n-3 fatty acids, especially docosahexaenoic acid. *Biochem Biophys Acta* 1992; 1127:263–9; [https://doi.org/10.1016/0005-2760\(92\)90230-S](https://doi.org/10.1016/0005-2760(92)90230-S)
- [9] Knopp RH, Saudek CD, Arky RA, O'Sullivan JB. Two Phases of tissue metabolism in pregnancy: Maternal Adaptations for fetal growth. *Endocrinology* 1973; 92:984–8; PMID:4686327; <https://doi.org/10.1210/endo-92-4-984>
- [10] Scow RO, Chernick SS, Brinley MS. Hyperlipemia and ketosis in the pregnant rat. *Am J Physiol* 1964; 206:796–804; PMID:14166175
- [11] Nolan CJ, Madiraju MS, Delghingaro-Augusto V, Peyot ML, Prentki M. Fatty Acid Signaling in the β -Cell and Insulin Secretion. *Diabetes* 2006; 55 Suppl 2:S16–23; PMID:17130640; <https://doi.org/10.2337/db06-S003>
- [12] Poitout V, Hagman D, Stein R, Artner I, Robertson RP, Harmon JS. Regulation of the insulin gene by glucose and fatty acids. *J Nutr* 2006; 136:873–6; PMID:16549443
- [13] Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest* 2006; 116:1802–12; PMID:16823478; <https://doi.org/10.1172/JCI29103>
- [14] Brelje TC, Bhagroo NV, Stout LE, Sorenson RL. Beneficial effects of lipids and prolactin on insulin secretion and beta-cell proliferation: a role for lipids in the adaptation of islets to pregnancy. *J Endocrinol* 2008; 197:265–76; PMID:18434356; <https://doi.org/10.1677/JOE-07-0657>
- [15] Hegre OD, Marshall S, Schulte BA, Hickey GE, Williams F, Sorenson RL, Serie JR. Nonenzymic in vitro isolation of perinatal islets of Langerhans. *In Vitro* 1983; 19:611–20; PMID:6136459; <https://doi.org/10.1007/BF02619574>
- [16] Gotoh M, Maki T, Satomi S, Porter J, Bonner-Weir S, O'Hara CJ, et al. Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. *Transplantation* 1997; 43:725–30; <https://doi.org/10.1097/00007890-198705000-00024>
- [17] Briaud I, Harmon JS, Kelpel CL, Segu VB, Poitout V. Lipotoxicity of the pancreatic beta-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. *Diabetes* 2001; 50:315–21; PMID:11272142; <https://doi.org/10.2337/diabetes.50.2.315>
- [18] Richieri GV, Anel A, Kleinfeld AM. Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* 1993; 32:7574–80; PMID:8338853; <https://doi.org/10.1021/bi00080a032>
- [19] Zimmerman NI, Wickler SJ, Rodiek AV, Hower MA. Free fatty acids in exercising Arabian horses fed two common diets. *J Nutr* 1992; 122:145–50; PMID:1729464

- [20] Brelje TC, Parsons JA, Sorenson RL. Regulation of islet beta-cell proliferation by prolactin in rat islets. *Diabetes* 1994; 43:263–73; PMID:7904577; <https://doi.org/10.2337/diab.43.2.263>
- [21] Brelje TC, Sorenson RL. Role of prolactin versus growth hormone on islet B-cell proliferation in vitro: implications for pregnancy. *Endocrinology* 1991; 128:45–57; PMID:1986937; <https://doi.org/10.1210/endo-128-1-45>
- [22] Brelje TC, Stout LE, Bhagroo NV, Sorenson RL. Distinctive roles for prolactin and growth hormone in the activation of signal transducer and activator of transcription 5 in pancreatic islets of langerhans. *Endocrinology* 2004; 145:4162–75; PMID:15142985; <https://doi.org/10.1210/en.2004-0201>
- [23] Parker SM, Moore PC, Johnson LM, Poitout V. Palmitate potentiation of glucose-induced insulin release: a study using 2-bromopalmitate. *Metabolism* 2003; 52:1367–71; PMID:14564691; [https://doi.org/10.1016/S0026-0495\(03\)00279-8](https://doi.org/10.1016/S0026-0495(03)00279-8)
- [24] Amaral ME, Cunha DA, Anhe GF, Ueno M, Carneiro EM, Velloso LA, Bordin S, Boschero AC. Participation of prolactin receptors and phosphatidylinositol 3-kinase and MAP kinase pathways in the increase in pancreatic islet mass and sensitivity to glucose during pregnancy. *J Endocrinol* 2004; 183:469–76; PMID:15590973; <https://doi.org/10.1677/joe.1.05547>
- [25] Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, et al. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 2003; 422:173–6; PMID:12629551; <https://doi.org/10.1038/nature01478>
- [26] Dhayal S, Welters HJ, Morgan NG. Structural requirements for the cytoprotective actions of mono-unsaturated fatty acids in the pancreatic beta-cell line, BRIN-BD11. *Br J Pharmacol* 2008; 153:1718–27; PMID:18297101; <https://doi.org/10.1038/bjp.2008.43>
- [27] Diakogiannaki E, Dhayal S, Childs CE, Calder PC, Welters HJ, Morgan NG. Mechanisms involved in the cytotoxic and cytoprotective actions of saturated versus monounsaturated long-chain fatty acids in pancreatic beta-cells. *J Endocrinol* 2007; 194:283–91; PMID:17641278; <https://doi.org/10.1677/JOE-07-0082>
- [28] Morgan NG, Dhayal S. Unsaturated fatty acids as cytoprotective agents in the pancreatic beta-cell. *Prostaglandins Leukot Essent Fatty Acids* 2010; 82:231–6; PMID:20206490; <https://doi.org/10.1016/j.plefa.2010.02.018>
- [29] Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ. Fatty acid and phorbol ester-mediated interference of mitogenic signaling via novel protein kinase C isoforms in pancreatic beta-cells (INS-1). *J Mol Endocrinol* 2003; 30:271–86; PMID:12790799; <https://doi.org/10.1677/jme.0.0300271>
- [30] Vasavada RC, Wang L, Fujinaka Y, Takane KK, Rosa TC, Mellado-Gil JM, Friedman PA, Garcia-Ocaña A. Protein kinase C-zeta activation markedly enhances beta-cell proliferation: an essential role in growth factor mediated beta-cell mitogenesis. *Diabetes* 2007; 56:2732–43; PMID:17686945; <https://doi.org/10.2337/db07-0461>
- [31] Hardy S, St-Onge GG, Joly E, Langelier Y, Prentki M. Oleate promotes the proliferation of breast cancer cells via the G protein-coupled receptor GPR40. *J Biol Chem* 2005; 280:13285–91; PMID:15695516; <https://doi.org/10.1074/jbc.M410922200>
- [32] Brelje TC, Sorenson RL. The physiological roles of prolactin, growth hormone and placental lactogen in the regulation of islet beta cell proliferation. In: Sarvetnik N, ed. *Pancreatic Growth and Regeneration*. New York: Karger Landes Systems, 1997:1–30.
- [33] Soto-Guzman A, Villegas-Comonfort S, Cortes-Reynosa P, Perez Salazar E. Role of arachidonic acid metabolism in Stat5 activation induced by oleic acid in MDA-MB-231 breast cancer cells. *Prostaglandins Leukot Essent Fatty Acids* 2013; 88:243–9; PMID:23332799; <https://doi.org/10.1016/j.plefa.2012.12.003>
- [34] Arumugam R, Fleenor D, Lu D, Freemark M. Differential and complementary effects of glucose and prolactin on islet DNA synthesis and gene expression. *Endocrinology* 2011; 152:856–68; PMID:21239441; <https://doi.org/10.1210/en.2010-1258>
- [35] Friedrichsen BN, Richter HE, Hansen JA, Rhodes CJ, Nielsen JH, Billestrup N, Møldrup A. Signal transducer and activator of transcription 5 activation is sufficient to drive transcriptional induction of cyclin D2 gene and proliferation of rat pancreatic beta-cells. *Mol Endocrinol* 2003; 17:945–58; PMID:12586844; <https://doi.org/10.1210/me.2002-0356>
- [36] Ma F, Wei Z, Shi C, Gan Y, Lu J, Frank SJ, Balducci J, Huang Y. Signaling cross talk between growth hormone (GH) and insulin-like growth factor-I (IGF-I) in pancreatic islet beta-cells. *Mol Endocrinol* 2011; 25:2119–33; PMID:22034225; <https://doi.org/10.1210/me.2011-1052>
- [37] Li J, Liu X, Chu H, Fu X, Li T, Hu L, Xing S, Li G, Gu J, Zhao ZJ. Specific dephosphorylation of Janus Kinase 2 by protein tyrosine phosphatases. *Proteomics* 2015; 15:68–76; PMID:25354842; <https://doi.org/10.1002/pmic.201400146>
- [38] Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol Cell* 2000; 6:1401–12; PMID:11163213; [https://doi.org/10.1016/S1097-2765\(00\)00137-4](https://doi.org/10.1016/S1097-2765(00)00137-4)
- [39] Shibata E, Kanno T, Tsuchiya A, Kuribayashi K, Tabata C, Nakano T, Nishizaki T. Free fatty acids inhibit protein tyrosine phosphatase 1B and activate Akt. *Cell Physiol Biochem* 2013; 32:871–9; PMID:24107614; <https://doi.org/10.1159/000354489>
- [40] Johnson KJ, Peck AR, Liu C, Tran TH, Utama FE, Sjolund AB, Schaber JD, Witkiewicz AK, Rui H. PTP1B suppresses prolactin activation of Stat5 in breast cancer cells. *Am J Pathol* 2010; 177:2971–83; PMID:20952588; <https://doi.org/10.2353/ajpath.2010.090399>
- [41] Tsuchiya A, Nagaya H, Kanno T, Nishizaki T. Oleic acid stimulates glucose uptake into adipocytes by enhancing insulin receptor signaling. *J Pharmacol Sci* 2014; 126:337–43; PMID:25391857; <https://doi.org/10.1254/jphs.14182FP>

- [42] Gupta RK, Gao N, Gorski RK, White P, Hardy OT, Rafiq K, Brestelli JE, Chen G, Stoeckert CJ Jr, Kaestner KH. Expansion of adult beta-cell mass in response to increased metabolic demand is dependent on HNF-4alpha. *Genes Dev* 2007; 21:756–69; PMID:17403778; <https://doi.org/10.1101/gad.1535507>
- [43] Li X, Huang Y, Jiang J, Frank SJ. Synergy in ERK activation by cytokine receptors and tyrosine kinase growth factor receptors. *Cell Signal* 2011; 23:417–24; PMID:20946955; <https://doi.org/10.1016/j.cellsig.2010.10.016>
- [44] El-Assaad W, Buteau J, Peyot ML, Nolan C, Roduit R, Hardy S, Joly E, Dbaibo G, Rosenberg L, Prentki M. Saturated fatty acids synergize with elevated glucose to cause pancreatic beta-cell death. *Endocrinology* 2003; 144:4154–63; PMID:12933690; <https://doi.org/10.1210/en.2003-0410>
- [45] Schmidt A, Vogel RL, Witherup KM, Rutledge SJ, Pitzemberger SM, Adam M, Rodan GA. Identification of fatty acid methyl ester as naturally occurring transcriptional regulators of the members of the peroxisome proliferator-activated receptor family. *Lipids* 1996; 31:1115–24; PMID:8934443; <https://doi.org/10.1007/BF02524285>
- [46] Ravnskjaer K, Frigerio F, Boergesen M, Nielsen T, Maechler P, Mandrup S. PPARdelta is a fatty acid sensor that enhances mitochondrial oxidation in insulin-secreting cells and protects against fatty acid-induced dysfunction. *J Lipid Res* 2010; 51:1370–9; PMID:19965574; <https://doi.org/10.1194/jlr.M001123>
- [47] Cao M, Long Y, Tong Y, Wan J, Tong N. Activation of PPARdelta up-regulates the expression of insulin gene transcription factor MafA and ameliorates glucose-induced insulin secretion impaired by palmitate. *Mol Cell Biochem* 2012; 366:183–9; PMID:22466807; <https://doi.org/10.1007/s11010-012-1296-9>
- [48] Cao M, Tong Y, Lv Q, Chen X, Long Y, Jiang L, Wan J, Zhang Y, Zhang F, Tong N. PPARdelta Activation Rescues Pancreatic beta-Cell Line INS-1E from Palmitate-Induced Endoplasmic Reticulum Stress through Enhanced Fatty Acid Oxidation. *PPAR Res* 2012; 2012:680684; PMID:22792088; <https://doi.org/10.1155/2012/680684>
- [49] Eto K, Nishimura W, Oishi H, Udagawa H, Kawaguchi M, Hiramoto M, Fujiwara T, Takahashi S, Yasuda K. MafA is required for postnatal proliferation of pancreatic beta-cells. *PLoS One* 2014; 9:e104184; PMID:25126749; <https://doi.org/10.1371/journal.pone.0104184>
- [50] Aramata S, Han SI, Yasuda K, Kataoka K. Synergistic activation of the insulin gene promoter by the beta-cell enriched transcription factors MafA, Beta2, and Pdx1. *Biochim Biophys Acta* 2005; 1730:41–6; PMID:15993959; <https://doi.org/10.1016/j.bbaexp.2005.05.009>
- [51] Montanya E, Nacher V, Biarnes M, Soler J. Linear correlation between beta-cell mass and body weight throughout the lifespan in Lewis rats: role of beta-cell hyperplasia and hypertrophy. *Diabetes* 2000; 49:1341–6; PMID:10923635; <https://doi.org/10.2337/diabetes.49.8.1341>
- [52] Parsons JA, Bartke A, Sorenson RL. Number and size of islets of Langerhans in pregnant, human growth hormone-expressing transgenic, and pituitary dwarf mice: effect of lactogenic hormones. *Endocrinology* 1995; 136:2013–21; PMID:7720649; <https://doi.org/10.1210/endo.136.5.7720649>
- [53] Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. *Diabetes* 2003; 52:726–33; PMID:12606514; <https://doi.org/10.2337/diabetes.52.3.726>
- [54] Butler AE, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, Butler PC. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* 2010; 53:2167–76; PMID:20523966; <https://doi.org/10.1007/s00125-010-1809-6>
- [55] Sullivan BA, Hollister-Lock J, Bonner-Weir S, Weir GC. Reduced Ki67 Staining in the Postmortem State Calls Into Question Past Conclusions About the Lack of Turnover of Adult Human beta-Cells. *Diabetes* 2015; 64:1698–702; PMID:25488899; <https://doi.org/10.2337/db14-1675>
- [56] Benner C, van der Meulen T, Caceres E, Tigyi K, Donaldson CJ, Huising MO. The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression. *BMC Genomics* 2014; 15:620; PMID:25051960; <https://doi.org/10.1186/1471-2164-15-620>
- [57] Chen H, Kleinberger JW, Takane KK, Salim F, Fiaschi-Taesch N, Pappas K, Parsons R, Jiang J, Zhang Y, Liu H, et al. Augmented Stat5 Signaling Bypasses Multiple Impediments to Lactogen-Mediated Proliferation in Human beta-Cells. *Diabetes* 2015; 64:3784–97; PMID:26159175; <https://doi.org/10.2337/db15-0083>
- [58] Moldrup A, Petersen ED, Nielsen JH. Effects of sex and pregnancy hormones on growth hormone and prolactin receptor gene expression in insulin-producing cells. *Endocrinology* 1993; 133:1165–72; PMID:8365359; <https://doi.org/10.1210/endo.133.3.8365359>
- [59] Dong J, Tsai-Morris CH, Dufau ML. A novel estradiol/estrogen receptor alpha-dependent transcriptional mechanism controls expression of the human prolactin receptor. *J Biol Chem* 2006; 281:18825–36; PMID:16651265; <https://doi.org/10.1074/jbc.M512826200>