

Inflammatory Blockade Improves Human Pancreatic Islet Function and Viability

Zandong Yang^{a,*}, Meng Chen^a, Justin D. Ellett^a,
Jeffrey D. Carter^a, Kenneth L. Brayman^b
and Jerry L. Nadler^a

^aDepartment of Internal Medicine, Division of Endocrinology, Metabolism and

^bDepartment of Surgery, University of Virginia, Charlottesville, VA 22908, USA

*Corresponding author: Dr. Zandong Yang, zy4q@virginia.edu

The pathogenesis of pancreatic β -cell death in diabetes mellitus is still under investigation. Inflammation is likely to be one of the factors responsible for β -cell death during disease development. In this study, we have used a novel antiinflammatory compound, Lisofylline (LSF), to investigate the role of inflammatory blockade in protecting human pancreatic islets. LSF is a small synthetic molecule that reduces inflammatory cytokine production and action, improves β -cell mitochondrial metabolism, and regulates immune activities. The present study has demonstrated that the treatment of human islets with LSF not only allows the retention of glucose responsiveness and insulin secretion in the presence of multiple proinflammatory cytokines, but also enhances basal insulin secretion of β cells *in vitro*. LSF also significantly reduces islet apoptosis, protects β cells from proinflammatory cytokine damage, and maintains cellular viability. In a mouse transplantation model, insulin independence could be reached in diabetic recipient mice by implantation of 30% fewer islets when LSF was used in islet culture compared to the control group. These results demonstrate that LSF profoundly enhances β -cell function, and suggest the potential of using inflammatory blockade, such as LSF, to improve β -cell function for islet transplantation.

Key words: Inflammation, lisofylline, pancreatic islet, transplantation, Type 1 diabetes

Abbreviations: ELISA, enzyme-linked immunosorbent assay; LSF, Lisofylline; scid, severe combined immune deficient; STAT4, signal transducers and activators of transcription 4

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Introduction

Type 1 and late stages of Type 2 diabetes are characterized by pancreatic β -cell dysfunction and death (1,2). Mononuclear cell infiltration in pancreatic islets of Langerhans, termed insulinitis, is a pathological hallmark in the diagnosis of Type 1 diabetes (3). Several potential triggers of insulinitis have been suggested, but the pathogenesis of β -cell death is still under investigation. Inflammation is one contributor that may cause β -cell dysfunction and death (4,5). Therefore, it is possible that inflammatory blockade could be effective in the prevention and treatment of diabetes.

Previously, we demonstrated the ability to prevent autoimmune diabetes in experimental animal models by inflammatory blockade using LSF (6,7). LSF also prevented grafted β -cell destruction and diabetes recurrence by autoimmunity in an islet transplantation model (8). In the study of the mechanism of LSF's effect, we found that LSF promoted mitochondrial metabolism in murine β cells (9). In order to explore the effect of LSF's in human β cells, in this study, we cultured isolated human islets with LSF followed by testing β -cell response and function. We found that treatment of human islets with LSF enhanced basal insulin secretory function, and preserved glucose responsiveness and insulin secretion in the presence of multiple proinflammatory cytokines. LSF treatment significantly promoted islet metabolism and reduced apoptosis. Compared to the control group, the minimum number of islets required to reach insulin independence in diabetic recipient mice was reduced by 30% when LSF was used in islet culture. This study reveals the possibility that the use of LSF as a supplement in islet culture may enhance and protect β -cell function for transplantation.

Materials and Methods

Human islets

Human pancreatic islets were isolated from pancreata of 10 cadavers (8 men and 2 women) by our facility and by the facilities at the Washington University (St. Louis, MO), the University of Minnesota (Minneapolis, MN), and the University of Pennsylvania (Philadelphia, PA). The cold ischemia time in these isolations ranged from 8 to 14 h (average 11 h). The age of the donors ranged from 22 to 59 years old (average 46 years). The purity of the islet preparation ranged from 50% to 95% (average 85%). A research protocol to use human islets for investigation was approved by the Institutional Human Investigation Committee at the University of Virginia. Upon arrival, the medium in islet shipment was immediately replaced with

CMRL-1066 medium supplemented with 10% heat-inactivated FCS, 100 units/mL penicillin, 0.1 mg/mL streptomycin and 2 mmol/L L-glutamine (Invitrogen, Carlsbad, CA).

Reagents

LSF (1-(5-R-hydroxyhexyl)-3,7-dimethylxanthine, or CT-1501R) was provided from Cell Therapeutics, Inc. (Seattle, WA).

Animal and diabetic model

Male NOD.CB17-Prkdc^{scid}/J (NOD.scid) mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in a pathogen-free facility in the Center for Comparative Medicine at the University of Virginia. An experimental protocol to use animals was approved by the Institutional Animal Care and Use Committee. To induce diabetes, serial injections of streptozotocin (STZ, 40mg/kg/mouse/day, Sigma-Aldrich, St. Louis, MO) were given intraperitoneally (IP) to NOD.scid mice at 10–12 weeks of age as described (7). Blood glucose levels were measured in whole blood samples collected from tail veins by Accu-Chek Advantage Glucose Monitors (Roche, Indianapolis, IN). Diabetes was diagnosed when the blood glucose level was greater than 300 mg/dL on three consecutive measurements in a week. Diabetic mice were used as recipients for islet transplantation.

Islet treatment and insulin secretion study

Isolated human islets were cultured with recombinant human IL-1 β (5 ng/mL), IFN- γ (100 ng/mL) and TNF- α (10 ng/mL) (R&D Systems, Inc., Minneapolis, MN) at 37°C and 5% CO₂ for 18 h. At the same time when cytokines were added, LSF in the amount from 20 to 100 μ mol/L was supplemented in the cultures. Insulin secretion was tested as described previously (8,9). After 1-h incubation with a glucose-supplemented medium, insulin concentration was measured in supernatant samples by a human insulin enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostic, Windham, NH) with an established standard.

Apoptosis detection

Apoptosis in isolated islets was quantitatively detected using an ApoPercentage Apoptosis Assay Kit (Biocolor Ltd., Belfast, Northern Ireland) as described previously (9). Each set of 100 islets was seeded to a well in a gelatin-coated 96-well plate for 48 h. Red pink color (from an apoptosis-specific dye) was visualized in apoptotic islets under a light microscope. Quantitative measurement of islet apoptosis was obtained by spectrometry reading in the supernatant samples after release of apoptosis-specific dye from islets. Annexin V and propidium iodine (PI) were used as an additional assessment for apoptosis detection. After treatment with LSF and cytokines, islets were washed with PBS, and incubated with fluorescence-labeled annexin V and PI (Santa Cruz Biotechnology, Santa Cruz, CA) for 15 min at room temperature in the dark. Apoptotic cells labeled with fluorescent annexin showed up red in color, and could be visualized under a fluorescence microscope (Zeiss-Axioplan) with excitation filters. Phase contrast and fluorescence digital images of islets in each experimental condition were captured and analyzed by the SPOT Imaging System with a SPOT RT v3.4.5 program (SPOT Diagnostic Instruments, Inc., Sterling Height SA, MI).

Islet cell metabolism test

Mitochondrial metabolism in islets was tested by a reaction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) as described previously (9,10). After *in vitro* treatment with LSF and cytokines, islets were washed and incubated in Krebs-Ringer-bicarbonate-HEPES buffer (KRB) supplemented with 0.1 mg/mL MTT and with or without 28 mmol/L D-glucose (Sigma-Aldrich) for 1 h. After washing, insoluble MTT metabolites within islet cells were extracted by isopropanol (Sigma-Aldrich). The absorbance of an extract sample was measured as arbitrary

OD units at 590 nm by a spectrophotometer, SpectraMAXplus (Molecular Devices, Sunnyvale, CA).

Islet transplantation and nephrectomy

Islets were implanted in the left kidney renal capsules in diabetic NOD.scid recipients. Blood glucose levels of the recipient mice were monitored after transplantation. Nonfasting blood glucose levels higher than 250 mg/dL for three consecutive readings in a week were considered as evidence of loss of graft function. Human insulin was measured in serum samples of the recipients by human insulin ELISA kit (ALPCO Diagnostic). Nephrectomy was performed in several diabetes-controlled recipients in order to confirm the graft function (8).

Glucose-tolerance test

Glucose tolerance was tested in the recipient mice 3 weeks after transplantation. After fasting overnight, mice were injected with 200 μ L of normal saline containing 400 mg/mL D-glucose IP. Glucose levels were checked in whole blood samples collected from tail veins every 30 min after glucose injection.

Detection of total STAT4, phosphorylated STAT4, Caspase 3 and Caspase 8 proteins

After treatment with or without LSF overnight, islets were washed with PBS twice, and lysed for protein purification. Equal amount (50 μ g) of protein was used for detecting total STAT4, phosphorylated STAT4, Caspase 3 and Caspase 8 (all antibodies were purchased from Santa Cruz Biotechnology) in Western blots (11). The same blot was stripped to remove detecting antibodies used in previous experiments, and incubated with an anti-actin antibody (Santa Cruz Biotechnology) to ensure equal amount of protein loading

Detection of cytokine mRNA expression

RNA was extracted from the kidneys containing grafted islets and kidneys of normal NOD.scid mice that did not have surgery by a Total RNA Isolation System (Promega, Madison, WI). cDNA was synthesized from equal amount (2 μ g each) of RNA, and applied to semiquantitative RT-PCR reactions for cytokine gene expression (BD Bioscience, San Diego, CA). The mRNA of IFN- γ and TNF- α was detected, and compared quantitatively based on densitometry analysis of the RT-PCR results by a Typhoon 9400 Imager (Molecular Devices, Sunnyvale, CA).

Histology and immunohistochemistry of the grafts

The kidneys containing implanted islets were fixed in 4% paraformaldehyde. Hematoxylin/eosin (H&E) stain was performed in 5 μ m paraffin sections. Adjacent sections were used for immunocytochemistry stain with an antiinsulin antibody (Santa Cruz Biotechnology) to identify insulin-positive cells in the grafts (11).

Statistics

Statistical analysis was performed using Prism version 3.0 software. A p-value < 0.05 (by ANOVA) was taken as an indication of a statistical significance.

Results

LSF preserves glucose responsiveness and insulin secretory function in human islets

Isolated human islets were incubated either with or without LSF at concentrations ranging from 20 to 100 μ mol/L in the presence or absence of a proinflammatory cytokine

cocktail (IL-1- β , 5 ng/mL; IFN- γ , 100 ng/mL and TNF- α , 10 ng/mL) for 18 h *in vitro*. A static insulin secretion assay was performed and indicated that LSF treatment preserved β -cell glucose responsiveness and insulin secretion in both basal (3 mmol/L or mM) and stimulatory (28 mM) glucose conditions in a dose-dependent manner. Without the presence of proinflammatory cytokines, the treatment of LSF at 50 μ mol/L and higher concentrations enhanced insulin secretion in the conditions of both basal and high-concentration glucose *in vitro* (Figure 1A). LSF was able

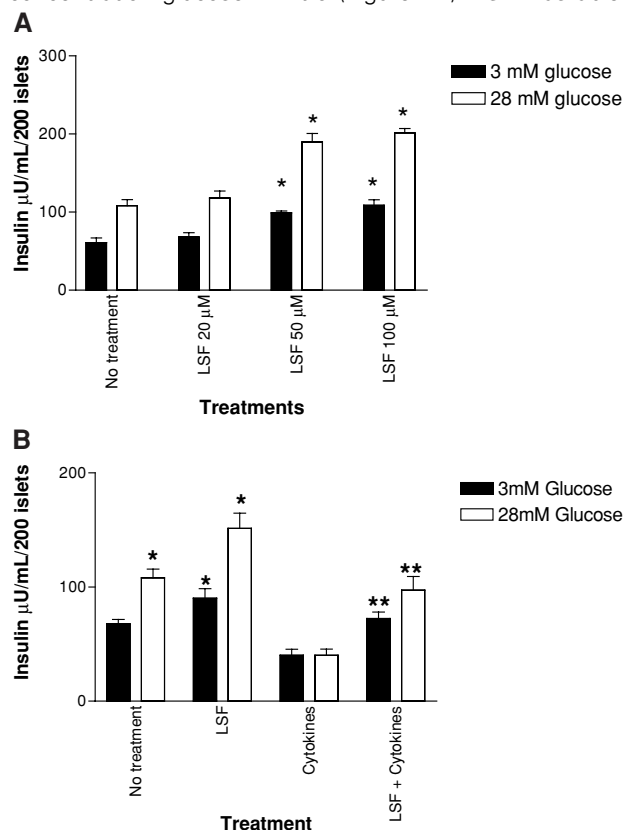


Figure 1: (A) Insulin secretion in the human islets treated with LSF. Isolated human islets (200 islets per group) were treated with different doses of LSF as indicated for 18 h. Insulin amounts reflect the insulin secretions that were under both 3 and 28 mmol/L (mM) glucose conditions for 1 h. The data present the results from three experiments. Each LSF-treated group was compared to no-treatment control, *indicates $p < 0.05$ in comparison to the corresponding data in the no-treatment groups, respectively. (B) Insulin secretion in human islets treated with LSF and cytokines. Isolated human islets (200 islets per group) were cultured for 18 h in the conditions indicated. For treatments, LSF 50 μ mol/L; the cytokine cocktail contained IL-1- β 5 ng/mL, IFN- γ 100 ng/mL, and TNF- α 10 ng/mL. Static insulin secretion in equal numbers of islets was tested in both 3 and 28 mmol/L glucose conditions. The data present the results from three experiments. In comparison between no-treatment and LSF-treated groups, *indicates $p < 0.01$ in either same concentration glucose or in same treatment. In comparison between cytokines and cytokines plus LSF groups, **indicates $p < 0.01$ in LSF-treated groups for both glucose conditions, respectively.

to restore insulin secretion and glucose responsiveness of islets in the presence of the proinflammatory cytokine cocktail (Figure 1B). These experiments suggested that LSF preserved β -cell function against the insult of proinflammatory cytokines, and directly enhanced β -cell insulin secretion.

LSF reduces apoptosis and promotes mitochondrial metabolism in islets

Using an *in situ* stain, apoptotic islet cells showed a red pink color intracellularly that represents condensation of the cytoplasm. Apoptotic islets can be morphologically visualized under a light microscope (Figure 2A). Apoptosis was quantified by measurement of the absorbance in the supernatant samples after releasing the intracellular stain from the islets. As compared to the control groups, LSF treatment reduced apoptosis in human islets *in vitro* (Figure 2B). In addition to the *in situ* stain of apoptotic cells, annexin V was used to detect phospholipid phosphatidylserine (PS). When a cell undergoes apoptosis, PS accumulated on the extracellular surface, and can be detected by binding annexin V. Therefore, we used a fluorescence-conjugated annexin V to detect apoptosis in human islets, and found that LSF treatment reduced apoptosis in human islet cells (Figure 2C). In order to determine apoptosis pathways, we examined several Caspase proteins. In LSF-treated islets, the levels of Caspase 8, but not Caspase 3, were decreased, suggesting that inhibition of Caspase 8 might be one of the mechanisms for reduction of apoptosis (Figure 2D). We used the MTT assay to analyze islet metabolic activity (9). LSF treatment promoted islet metabolism as shown by profoundly increased MTT metabolites (Figure 3). These data suggest that LSF was able to protect islet cells from apoptosis caused by proinflammatory cytokines. Furthermore, LSF directly increased islet cell metabolism.

Treatment of human islets with LSF improves grafted β -cell function in transplantation

In order to test the function of LSF-treated islets *in vivo*, we transplanted islets that were precultured with LSF to diabetic NOD.scid mice. In this study, correction of hyperglycemia in the recipients by grafting islets reflected the function of the transplanted β cells since immune rejection was eliminated because of severe combined immunodeficiency in NOD.scid recipients (12). Isolated islets were treated with 50 μ mol/L LSF for overnight before transplantation. After washing to eliminate free LSF from the culture medium, various numbers of islets were implanted under the renal capsules of the recipients. The mice that received islets without LSF pretreatment were used as controls. We found that insulin independence could be reached using reduced number of islets when LSF was added to the culture prior to transplantation. A 30% reduction in islet number was sufficient to control diabetes in the recipient mice when LSF was used in islet culture (Table 1). Human insulin was detectable in euglycemic recipients 1 and 3

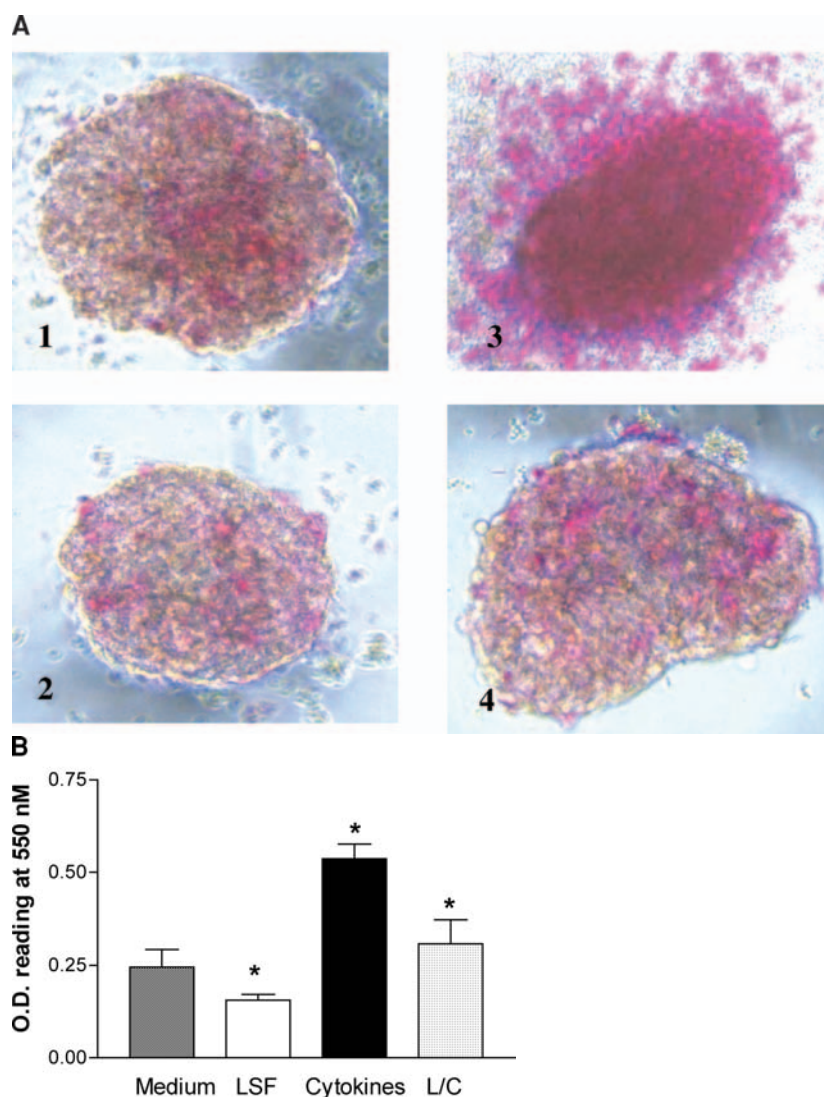


Figure 2: (A) Morphological detection of apoptosis in human islets. Isolated human islets were treated for 18 h in the conditions indicated. For treatments, LSF 50 $\mu\text{mol/L}$; the cytokine cocktail contained IL-1- β 5 ng/mL, IFN- γ 100 ng/mL and TNF- α 10 ng/mL. Apoptotic cells, (in red color) were detected after treatment of reagents provided in ApoPercentage Apoptosis Assay Kit. (1) no treatment; (2) LSF 50 $\mu\text{mol/L}$ treated; (3) the cytokine cocktail (IL-1- β 5 ng/mL, IFN- γ 100 ng/mL and TNF- α 10 ng/mL) treated; and (4) LSF and the cytokine cocktail treated. Amplification: $\times 200$. **(B)** Quantitative detection of apoptosis in human islets. Isolated human islets (100 islets per group) were treated for 18 h in the conditions indicated. For treatments, LSF 50 $\mu\text{mol/L}$; the cytokine cocktail contained IL-1- β 5 ng/mL, IFN- γ 100 ng/mL and TNF- α 10 ng/mL. Quantitative detection of apoptotic islets was measured after treatment of reagents provided in ApoPercentage Apoptosis Assay Kit. The data present the results from three experiments. *Indicates $p < 0.05$ when comparing with non-treated islet samples. L/C: LSF plus cytokines. (C) Apoptosis was also detected by fluorescence-conjugated annexin V and PI. Human islets were incubated with fluorescence-conjugated annexin V and PI, and were examined through a fluorescence filter (upper panels), and in a bright field for phase contrast (lower panels). A representative image from each study conditions was shown. Red stain indicates fluorescence-conjugated annexin positive cells (apoptotic cells), green stain indicates negative for both annexin V and PI. Amplification of the imaging is $\times 200$. (D) Detection of Caspase 3 and 8 in islets. After incubation with or without 50 $\mu\text{mol/L}$ LSF, islets were washed and lysed. Equal amount of protein samples (50 μg each) were used to detect Caspase 3 and 8 proteins in Western blot. Same blots were used to detect Caspase 3 after removal of anti-Caspase 8 antibodies. The upper panel shows detection of Caspase 8, the lower one demonstrates the results of Caspase 3 detection. The samples of lines are (1) the islets cultured without LSF, (2) the islets treated with LSF. This result was reproducible in three experiments, and the representative data are shown.

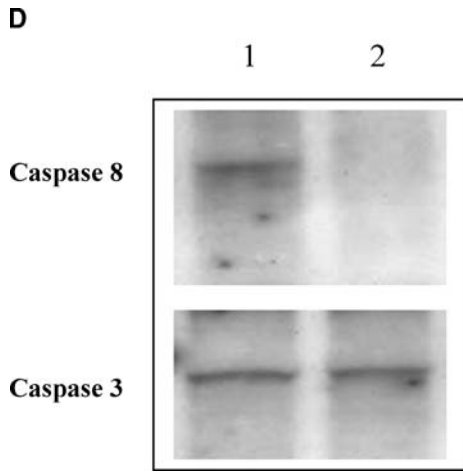
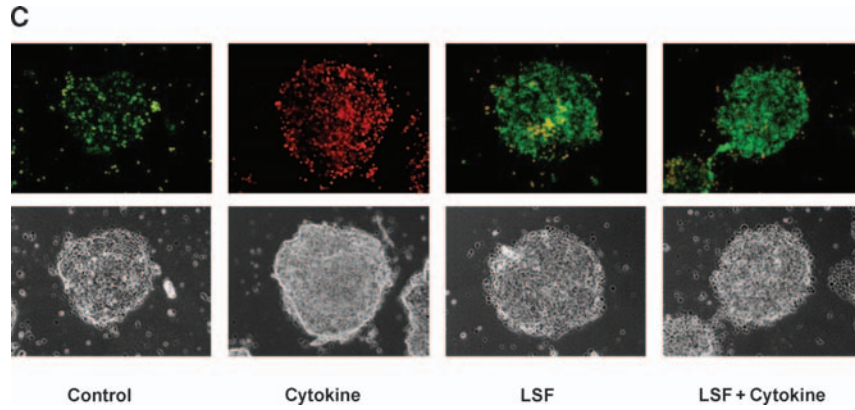


Table 1: Human islet survival in diabetic NOD.scid recipients

Islet treatment	Numbers of islet implanted/mouse	Individual survival (days × mice)
None	700	100*, >150 × 3
None	500	4 × 3, 5 × 2, 6
LSF	500	77*, 87*, >150 × 4
None	400	2, 3 × 4, 4
LSF	400	3, 4 × 4, 7*

The days of survival reflected the recipient mice maintained euglycemic condition without insulin. Isolated islets used in the paired groups were obtained from the same donor.

*Indicates the date when nephrectomy was performed.

Figure 2: Continued.

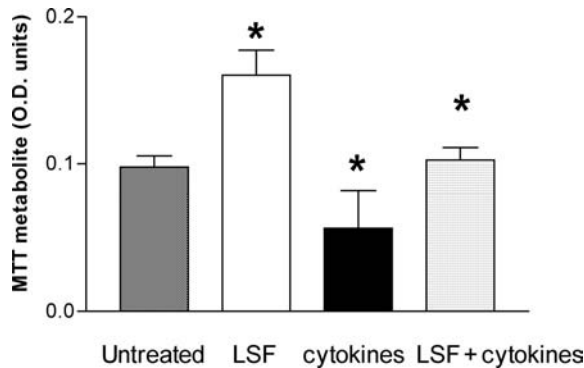


Figure 3: Cellular metabolism test in isolated islets by MTT assay. Isolated human islets were treated for 18 h in the conditions indicated. For treatments, LSF 50 μ mol/L; the cytokine cocktail contained IL-1- β 5 ng/mL, IFN- γ 100 ng/mL and TNF- α 10 ng/mL. Islet metabolism was detected by MTT assay. The data present the results from three experiments. *Indicates $p < 0.05$ when comparing with untreated islet samples.

A

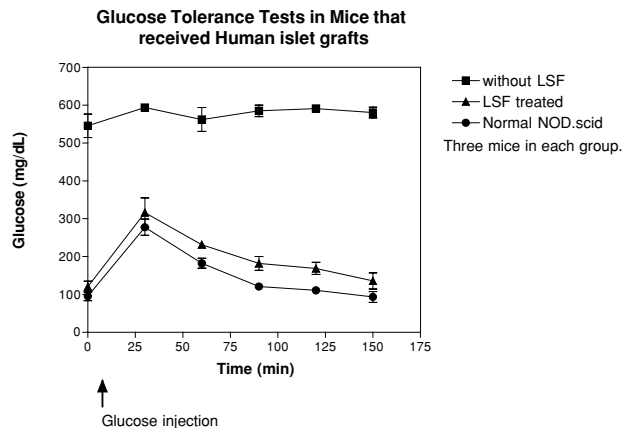
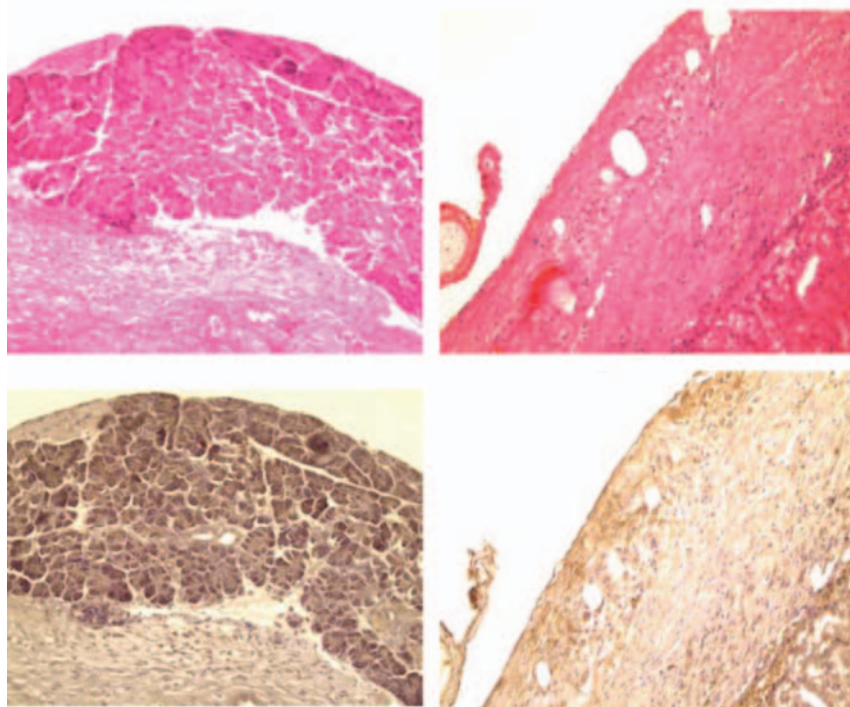


Figure 4: (A) Glucose-tolerance test. Three weeks after islet implantation, the recipient mice were IP injected with 200 μ L of normal saline supplemented with 400 mg/mL D-glucose once. Blood glucose levels were monitored before and after glucose injection, and shown. **(B)** Histology and immunocytochemistry of the islet grafts. Paraffin sections of the kidneys were obtained from the recipient mice 3 weeks after transplantation. In adjacent sections, the tissues in the upper panel were stained with hematoxylin and eosin (H&E), those in the lower panel were incubated with an antiinsulin antibody. Insulin positive cells show dark-brown stain. Similar observations were seen in the same study group ($n = 3$ for each group). Amplification of the imaging is $\times 200$.

B



LSF-treated Islets

Untreated Islets

Figure 4: Continued.

weeks after transplantation (data not shown). Three weeks after transplantation, an intraperitoneal glucose-tolerance test was performed in the recipient mice that each received 500 islets. All three previously diabetic mice that received LSF-treated islets responded to glucose stimulation similarly to nondiabetic NOD.scid mice. However, all three mice that had islet engraftment without LSF culture remained hyperglycemic and showed no response to glucose challenge (Figure 4A). Removal of kidneys containing islet grafts caused recurrence of diabetes in the long-term cured recipients within 2–3 days ($n = 4$ as indicated in Table 1). Histology and insulin immunocytochemistry showed large islet clusters and many cells positively stained with insulin (Figure 4B). This result indicates that LSF-mediated enhancement of β -cell function can lead to efficient reversal of diabetes by replacing fewer islets after preculture with LSF.

LSF reduces the gene expression of proinflammatory cytokines and suppresses STAT4 phosphorylation in β cells

In contrast to our previous studies (6–8), LSF in this study was only given to isolated islets *in vitro* before transplantation. Although we know that systemic administration of LSF could reduce proinflammatory cytokines in mice (6,7), whether a similar effect could be reached locally or systemically after transplant of LSF-treated islets has not been tested. In this experiment, we used separate groups of recipient mice that each was implanted with 500 un-

treated or LSF-treated islets in the renal capsule, and detected cytokine gene expression by quantitative RT-PCR. Nondiabetic NOD.scid mice without surgery were used as controls. Since the NOD.scid recipient mice are severely dysfunctional in both T and B lymphocytes, we only tested IFN- γ and TNF- α , and found that the gene expression of both cytokines was reduced in the kidneys containing LSF-treated islets, compared to the kidneys implanted with islets cultured without LSF (Figure 5A). However, we did not observe significant differences in cytokine levels in serum samples collected from the same recipients (data not shown). In LSF-treated islets, STAT4 phosphorylation was suppressed (Figure 5B). These studies suggest that a local effect of cytokine gene suppression could be a consequence of inhibition of STAT4 signal transduction following the implantation of LSF-treated islets.

Discussion

β -cell damage in diabetes has been suggested as a result in part from an inflammatory process (4). Therefore, inflammatory blockade should be beneficial in disease attenuation or prevention (4–9,13,14). Both clinical and basic research studies demonstrate that immune cells and inflammatory mediators, including cytokines, play vital roles in the pathogenesis of diabetes and its complications. Thus, control of activated immune cells and suppression of inflammatory mediator production may prevent and control diabetes.

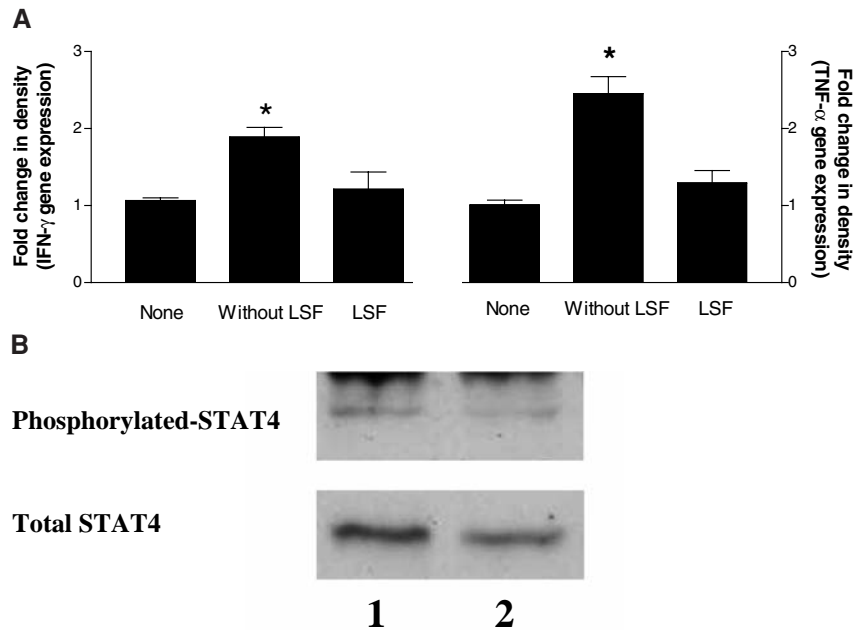


Figure 5: (A) Gene expression of IFN- γ and TNF- α in the grafts. Total RNA samples were extracted from the whole kidney containing islet grafts and the kidneys from normal NOD.scid mice. Equal amount of RNA (2 μ g each) was used for quantitative RT-PCR. The data show relative quantitation of each cytokine among sample groups (three mice from each group). *Indicates $p < 0.05$ when comparing with the samples with the kidneys of normal mice. (B) Detection of STAT4 and phosphorylated STAT4 in islets. After incubation with or without 50 μ mol/L LSF, islets were washed and lysed. Equal amount of protein samples (50 μ g each) were used in Western blot to detect STAT4 and phosphorylated STAT4 protein. The upper panel shows the detection of phosphorylated STAT4, the lower panel is for total STAT4. The samples in lines are (1) the islets cultured without LSF, (2) the islets treated with LSF. This result was reproducible in three experiments, and the representative data are shown.

Islet transplantation is currently under active clinical evaluation as a new therapeutic modality for patients with Type 1 diabetes (15). However, short tissue supply and loss of functional β -cell mass during isolation and following implantation are two of major obstacles in islet transplantation. Apoptosis is a primary mechanism for loss of function in β cells, and can be initiated at the time of tissue procurement, islet isolation or transplantation (16). Apoptosis is also responsible in part for autoimmune destruction of β cells (17) and a consequence of proinflammatory cytokine effects (18). Therefore, blockade of apoptosis, either directly or through inhibition of inflammation, could be a novel approach to protect β cells from cellular- and noncellular-mediated destruction.

Our previous studies showed a beneficial effect of LSF in β -cell survival and in diabetes prevention (6–9). In this study, we demonstrated for the first time the effectiveness of LSF in prevention of human islet death. We showed that short-term treatment with LSF *in vitro* enhanced human islet metabolism and β -cell insulin secretion, and reduced proinflammatory cytokine-induced apoptosis. These effects were associated with promotion of mitochondrial metabolism. Enhancement of mitochondrial metabolism may also explain improvement in insulin secretory capacity

of β cells in both basal and high glucose conditions following LSF treatment (9). We emphasized the ability of LSF to promote β -cell mitochondrial metabolism because mitochondrial function regulates β -cell insulin secretion and controls the endpoint of apoptosis (19–22). In a preliminary study, we showed that LSF inhibited TNF- α -induced apoptosis in β cells through suppression of the Caspase 8 pathway (23), revealing a molecular pathway related to β -cell death. In this study, we further confirmed this mechanism and excluded the association of Caspase 3. Reduction of apoptosis by LSF in human islets was determined by two different assays that assess cellular membrane and intracellular changes, respectively. Our observations suggest that LSF could be useful to preserve and augment β -cell function. The use of LSF as a supplemental reagent in islet isolation to reduce β -cell death is attractive.

We also tested the effect of LSF in β -cell function *in vivo* using an islet transplantation model. In the NOD.scid mice, we focused on the functional evaluation of human islets. We compared β -cell function *in vivo* by using different numbers of islets in transplantation. Our hypothesis was that improved β -cell function should reverse diabetes effectively by using fewer numbers of islets that precultured with LSF. As expected, we found that LSF-pretreated islets

had better insulin secretory function since transplantation of 30% fewer islets was able to normalize hyperglycemia in the recipients compared to those using the vehicle-treated islets. The results from glucose-tolerance tests indicated that β cells in LSF-treated islets were functional and sufficient to control diabetes in the recipients. This impression was also supported by the observations from histology, immunocytochemistry, human insulin measurement and the nephrectomy studies. This reduction in islet requirement is significant since it may allow using fewer numbers of islets to achieve insulin independences. For a safety concern, LSF has been used in several clinical trials without major adverse effects (24,25). Therefore, LSF could be ready for new clinical testing including islet transplantation.

The antiinflammatory property of LSF is related to its ability to suppress proinflammatory cytokines (26–29). In this study, we observed a reduction of proinflammatory gene expression in the local tissue around implanted LSF-treated islets. A general effect of LSF to reduce inflammation depends on systemic administration, while local protection can be obtained by islets or β cells that were previously cultured with LSF. Although we used IFN- γ and TNF- α as two representatives for proinflammatory cytokines because of the use of NOD.scid mice, we expect that LSF could inhibit other proinflammatory cytokines as indicated by previous studies (6–8,27–29). In transplantation, local protection may be more desirable in order to avoid adverse effects from general immunosuppression.

One mechanism of LSF-mediated protection can likely be explained by the ability to inhibit STAT4-mediated IL-12 signaling (11,30). Interruption of STAT4-mediated IL-12 signaling clearly leads to suppression of immune Th1 cell activation and inflammatory cytokine production (31–33). Both suppression of Th1 cell function and reduction of proinflammatory cytokine production could be responsible for LSF's effect to prevent both autoimmune diabetes and islet destruction following transplantation (30,34). In this study, we tested this mechanism in human islets, and found that LSF was able to directly suppress STAT4 phosphorylation in the islets. In addition to the effect of interruption of STAT4-mediated IL-12 signaling and suppression of Th1 cell function, suppression of STAT4 activation in β cells may be subsequently responsible for increased insulin gene expression by LSF (Z. Yang et al., unpublished observation). Additional studies are undergoing to test this hypothesis.

In summary, we have demonstrated the potential for LSF in islet transplantation. Our studies provide the rationale supporting an investigation using LSF or its analogs as supplements in pancreas organ procurement, islet isolation and clinical transplantation.

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