Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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Abstract

Background: Feline diabetes mellitus shares many similarities with human type 2 diabetes mellitus, including clinical, physiological and pathological features of the disease. The domestic cat spontaneously develops diabetes associated with insulin resistance in their middle age or later, with residual but declining insulin secretion. Humans and cats share largely the same environment and risk factors for diabetes, such as obesity and physical inactivity. Moreover, amyloid formation and loss of beta cells are found in the islets of the diabetic cat, as in humans. Altogether, the diabetic cat is a good model for type 2 diabetes in humans. The aims of the present study were to isolate feline islets using laser microdissection and to develop a quantitative method for detection of mRNA levels in islets of healthy and diabetic cats.

Results: By using the laser microdissection technique, we were able to meticulously sample islets from both healthy and diabetic cats. Insulin staining of separate sections showed many beta cells in islets from healthy cats, whereas few insulin positive cells were found in islets from diabetic cats. By quantitative real-time PCR, mRNA levels of the islet-specific genes *INS*, *PDX1*, *IAPP*, *CHGA* and *IA-2* could be detected in both healthy and diabetic cats.

Conclusions: Laser microdissection allows distinct studies of islets without contamination of acinar cells. Previous attempts in isolating feline islets with different collagenase-based protocols have led to damaged islets or islets coated with exocrine acinar cells, which either way compromise the results obtained from gene expression studies. The use of the laser microdissection technique eliminates these problems as shown in this study. Differences in gene expression between healthy and diabetic cats can reveal underlying mechanisms for beta cell dysfunction and decreased beta cell mass in human type 2 diabetes.

Keywords: Beta cell; Type 2 Diabetes mellitus; *Felis catus*; Gene expression; Islet isolation; Laser microdissection; Pancreatic islets

Abbreviations

LMD: Laser Microdissection; qPCR: quantitative real-time Polymerase Chain Reaction; T2DM: Type 2 Diabetes Mellitus; BP: Base Pair; *INS:* Insulin; *PDX1*: Pancreatic and Duodenal Homeobox 1; *CHGA*: Chromogranin A; *IA-2*: Islet Cell Antigen 2; *IAPP:* Islet Amyloid Polypeptide; *PNLIP*: Pancreatic Lipase; *ACTB*: Beta-Actin; *GAPDH*: Glyceraldehyde 3-Phosphate Dehydrogenase; *RPS7*: Ribosomal Protein S7; IHC: Immunohistochemistry; HRP: Horseradish Peroxidase; DAB: Diaminobenzidine Tetrahydrochloride

Introduction

Feline diabetes mellitus shares many similarities with Type 2 Diabetes Mellitus (T2DM) in humans, including clinical, physiological and pathological features of the disease. The domestic cat has thus been proposed a valuable animal model for T2DM [1,2]. Cats spontaneously develop diabetes associated with insulin resistance in their middle age or later, with residual but declining insulin secretion. Humans and cats largely share the same environment and also many of the risk factors for diabetes, such as obesity and physical inactivity. The diabetic cat may also develop late complications such as peripheral polyneuropathy, retinopathy, and nephropathy [1-5]. Amyloid deposition in islets, associated with an approximately 50% loss of beta-cell mass, is described in over 80% of cats with diabetes, as in human T2DM [6-9]. Amyloid formation is not seen in any of the rodent models of T2DM [1] and is a clear advantage for the feline model. Characterizing molecular mechanisms for beta cell dysfunction and decreased beta cell mass in feline diabetes may elucidate factors and mechanisms responsible for the development of type 2 diabetes in both cats and humans.

It is difficult to isolate feline islets without contamination with exocrine tissue, and it has been suggested that feline islets are particularly difficult to isolate with collagenase digestion because they are delimited with very little peri-islet matrix [10,11]. This leads to either damaged islets, or islets coated with acinar cells (referred to in the literature as islet-like cell clusters) [11,12], which either way compromise the results obtained from gene expression studies. The use of the Laser Microdissection (LMD) technique can eliminate some of the problems inherent in the use of collagenase-isolated islets [13]. The technique has been used successfully in human and rodent islet isolations [13-

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17]. LMD can be used selectively to cut out islets without visible contamination of acinar cells, and the technique also avoids some of the problems associated with trauma and stress to the cells by the enzymatic isolation that will cause changes in gene expression [13]. LMD of human beta-cells, recognized by their intrinsic autofluorescence, is performed on pancreatic tissue obtained from heart-beating cadaveric donors or from surgical samples from human pancreases [18]. The drawback with this approach is the long cold ischemia time or the small study material from surgical samples, since the islets only constitute 1-2% of the pancreas. In the cat, the whole pancreas can be removed from the euthanized animal and immediately snap frozen in liquid nitrogen, which enables us to study islets as close to reality as possible.

The aim of the present study was to develop a technique to isolate feline islets in order to perform gene expression studies in healthy and diabetic cats. This article reports a reliable method to obtain feline islets from both healthy and diabetic cats, with preserved RNA integrity for subsequent gene expression studies.

Material and Methods

Animals

Two healthy and two diabetic cats were included in the study. Ethical approval for this study was obtained from the Uppsala Ethical Committee on Animal Experiments (Dnr C 262/12). All cats were euthanized on the owner's request for reasons unrelated to this study. Written consent was obtained from all owners before inclusion in the study. Diabetic cats were diagnosed based on clinical and laboratory findings consistent with persistent hyperglycemia. The concentration of fructosamine in serum is a reflection of mean blood glucose level over the preceding 1 to 3 weeks [19]. One of the diabetic cats (nr. 1) showed typical clinical signs of diabetes for several months prior to diagnosis and had lost more than 20% of its body weight. This cat also had markedly elevated serum fructosamine levels, 752 µmol/L (ref 190-350) and was euthanized. The second diabetic cat (nr. 2) had a short disease history and presented with only slightly elevated serum fructosamine levels, 461 µmol/l, and was euthanized due to reasons other than the diabetes. None of the cats had

received insulin treatment. Cats were sedated with different combinations of acepromazine (Plegicil® vet., Pharmaxim), medetomidine (Sedator®, Dechra Veterinary Products) and butorphanol (Dolorex vet., Intervet) given subcutaneously, prior to euthanasia with an intravenous overdose of sodium pentobarbital (Allfatal vet., Omnidea). Diagnosis of diabetes was confirmed by postmortem examination and histopathology of the pancreas. Pancreatic sections were also stained with Congo red as previously described [20] to confirm presence of amyloid in diabetic islets (Figure 1). Histopathological examination indicated no infiltration of immune cells in diabetic islets.

Preparation of tissue for laser microdissection

Immediately after euthanasia the pancreata were surgically excised from the cats under sterile conditions. The islet-dense splenic portion of the pancreas [21] was sectioned and embedded in frozen section medium (Richard-Allan Scientific NEG 50, Thermo Scientific, Kalamazoo, MI, USA) in cryomolds (Tissue-Tek® Cryomold®, Sakura Finetek Inc, Torrance, CA, USA) and snap frozen in liquid nitrogen. Mean time from point of death to snap freezing of pancreatic specimens was 13 min (range 10-17 min). Tissue samples were stored in -80°C until cryosectioning.

Frame slides (POL-Membrane 0.9 µm, Leica Microsystems, Wetzlar, Germany) were exposed to UV-light overnight for cross-linking of the membrane in order to improve cutting of the membrane. The cryostat, including accessories *i.e.* the sample holder, were cleaned with 70% ethanol. Brushes for cryosectioning and a glass cuvette with ice cold acetone were put in the cryostat and thereafter the UV-light in the cryostat was switched on for 30 min. 10 µm thick sections were mounted on frame slides and fixed in ice cold acetone for 2 min. The frame slide was then dried with cold air before storage in an RNase free 50 ml tube (Ambion, LifeTechnologies Europe BV, Stockholm, Sweden) in -80°C.

The frame slide with pancreas sections was thawed for 30 s before hydration in nuclease free water (Ambion) for 30 s. RNase free hematoxylin (Arcturus® HistoGene® Staining Solution, Applied Biosystems, Foster City, CA, USA) was applied to the sections for 90 s and washed away with nuclease free water for 30 s. The sections
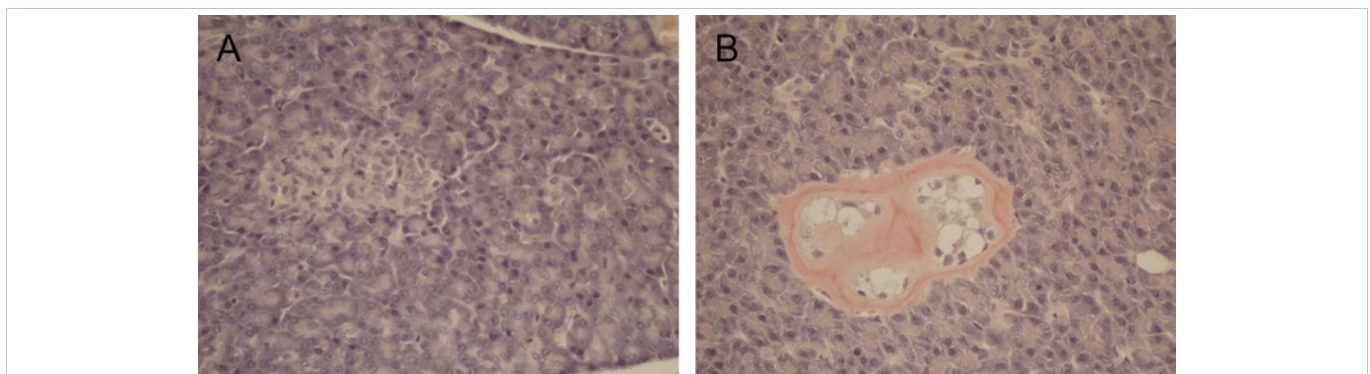
Figure 1: Representative images of Congo red staining on pancreatic sections from healthy cat (A) and diabetic cat (B). Islets in the diabetic cats stained positively with Congo red.

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Page 2 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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were then dehydrated in 70% ethanol for 30 s, in 95% ethanol for 30 s, and finally in absolute ethanol for 30 s. The frame slide was air dried before laser microdissection performed with a Leica LMD6000 B microscope (Leica Microsystems). Islets were identified with both bright field and fluorescence (Figure 2 and 3). The cutting parameters with a 20x objective were set on: laser power 25, aperture 17, speed 20 and specimen balance 25. Approximately 2 million μm² of islet tissue was selected for each sample (two separate samples were collected from each cat) and approximately 2 million μm² of exocrine pancreatic tissue was collected from healthy cat nr 1. The laser microdissected samples were collected in 65 μl of lysis buffer (RNeasy Plus Micro Kit, Qiagen, Hilden, Germany) in the cap of a 0.5 ml RNase free microfuge tube (Ambion) during cutting. Thereafter, lysis buffer was added up to 350 μl and vortexed thoroughly before storage in -80°C.

RNA isolation

Total RNA was isolated from the laser microdissected feline islets according to the manufacturer's instructions (RNeasy Plus Micro Kit, Qiagen AB). Note that this kit contains a gDNA Eliminator spin column, which will remove the genomic DNA. The amount and purity (OD 260/280) of the total RNA was determined using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Yields of the LMD samples were in the range of 20-26 nanogram RNA. All RNA samples had OD 260/280 between 1.9 and 2.1, which is in the range for pure RNA. Due to the limited amount of isolated RNA, no further quality controls

could be included. The extracted total RNA was dissolved in nuclease free water and stored at -80°C until cDNA synthesis. Total RNA from two sets of laser microdissected islets from each cat was isolated.

cDNA synthesis

The RNA was transcribed to cDNA by Superscript First-Strand Synthesis Super Mix for qRT-PCR (Invitrogen, Life Technologies, Stockholm, Sweden) according to the manufacturer's instructions. Briefly, a mix of random hexamer primers and oligo (dT) primers were incubated with the Superscript III Reverse Transcriptase enzyme mix and RNA at 25°C for 10 min, followed by 50°C for 30 min and thereafter 85°C for 5 min to inactivate the enzyme. To remove the RNA template from the cDNA:RNA hybrid molecule after first-strand synthesis, the mixture was incubated with two units of RNaseH at 37°C for 20 min. The cDNA was stored at -20°C until use for qPCR. Two independent reverse transcriptase reactions were carried out for each RNA sample.

Primers

Primer pair selection criteria were set to generate short amplicons of 71-220 base pairs (bp) with an annealing temperature of 60°C and without predicted dimer formation using Primer BLAST (NCBI). For primer sequences used in this study see Table 1. The primers were purchased from Sigma-Aldrich and dissolved in nuclease free water (Ambion). A stock solution of each primer of 100 μM was prepared. From the stock solution, a working dilution of 10 μM was prepared.
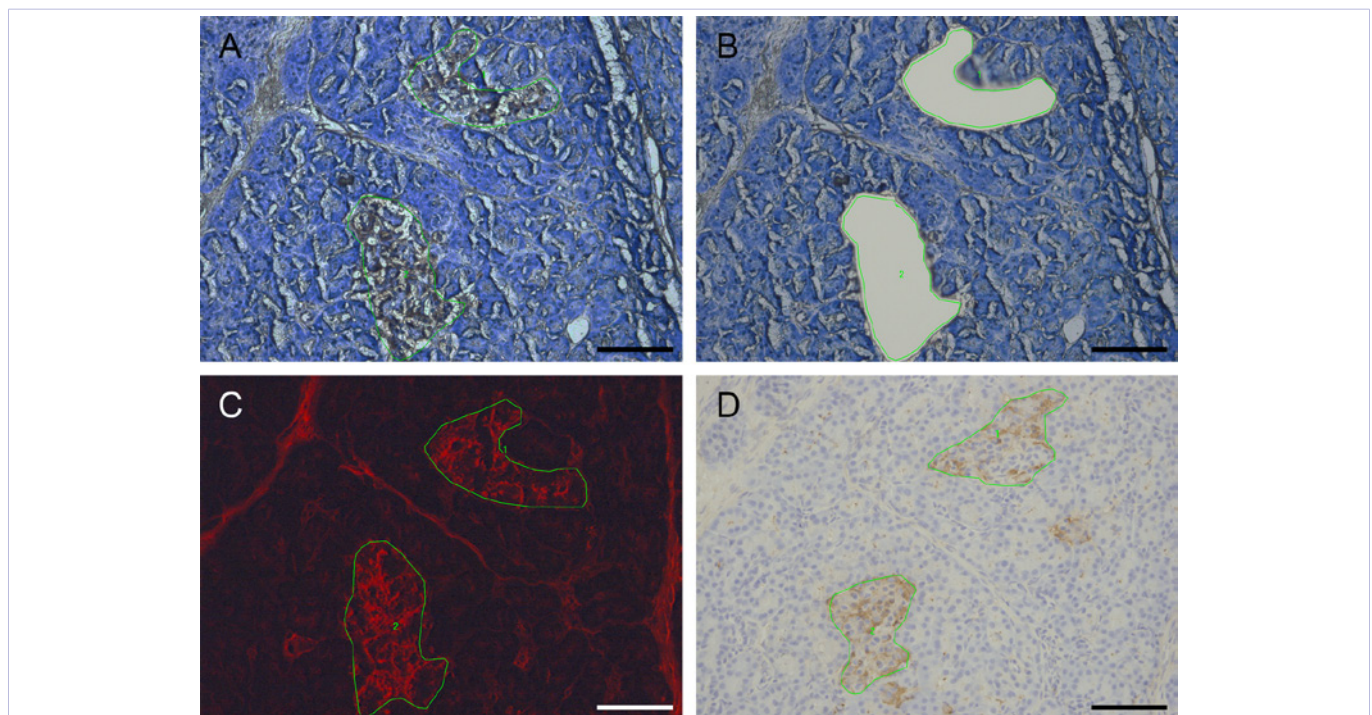
Figure 2: Micrographs of frozen pancreatic tissue from healthy cat
RNase free hematoxylin staining visualizing islets (marked with green line) in (A). Only selected area is laser microdissected (B). With fluorescence, the islets are clearly distinguished from connective tissue (C). Insulin (brown) is visualized with DAB (D). Scale bars represent 100 μm.

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Page 3 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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Quantitative real-time PCR

The qPCR assay was performed using a Light Cycler 480t (Roche Diagnostic, Mannheim, Germany) and Light Cycler FastStart DNA Master PLUS SYBR Green I kit (Roche Diagnostic) for detection. The final reaction volume of 10 µl contained 0.5 µM of each primer, 2 µl 5x Light Cycler FastStart DNA Master PLUS SYBR Green mix, 5 µl water and 0.5 ng of cDNA. In the Non-Template Control (NTC), cDNA was substituted with nuclease free water. Samples without reverse transcriptase were also included. The qPCR reactions were carried out using an initial step of 10 min at 95°C to activate the *Taq* polymerase, followed by 45 cycles consisting of 10 s at 95°C, 5 s at 55°C, and elongation at 72°C, 10 s. The fluorescence was measured at the end of each cycle. A melting curve analysis was performed directly following PCR by continuously reading the fluorescence while slowly heating the reactions from 65°C to 95°C. All qPCR samples were run in duplicates. Moreover, cDNA was prepared twice from each RNA sample to ensure inter run specificity. In summary, all samples were run in quadruplicates per gene and RNA preparation, and thus eight samples per cat. To determine the PCR efficiency, the primer pairs were analyzed using a dilution curve with ten-fold cDNA template dilutions between 2 ng/µl and 0.02 ng/µl. The efficiency was calculated using the formula: Efficiency = $-1 + 10^{(-1/\text{slope})}$. As markers for islets, we selected the genes insulin (*INS*), Pancreatic and Duodenal Homeobox 1 (*PDX1*), Chromogranin A (*CHGA*), Islet Cell Antigen 2 (*IA-2*) and Islet Amyloid Polypeptide (*IAPP*) (Table 2). Pancreatic Lipase (*PNLIP*) was selected as a marker for exocrine pancreatic tissue. The expression stability of reference transcripts Beta-Actin (*ACTB*), Glyceraldehyde-3-phosphatase Dehydrogenase (*GAPDH)* and Ribosomal Protein 7 (*RPS7)* was evaluated using the Normfinder software [22]. The results are presented as Threshold Cycle values (Ct-values). The Ct-values were used to calculate the amount of PCR product compared with reference genes by subtracting the Ct-value for reference genes from the Ct-value for the gene studied (ΔCt). Relative mRNA expression was calculated as $2^{-\Delta Ct}$. Data are expressed as means of two islet LMD preparations from each cat ± SEM.

Agarose gel-electrophoresis

To confirm amplicon size, qPCR products were analyzed by electrophoresis using a 3% agarose gel (PCR-grade, Bio-Rad, Hercules, CA, USA). The PCR products were mixed with 5x loading buffer (Bio-Rad) before loading. A 50 bp ladder (Invitrogen) was used to determine the size of the PCR products. Electrophoresis was conducted using an electrical field of 5 V/cm for 80 min and the bands were visualized using GelRed (Biotium, Hayward, CA, USA) and detected using the Chemi Doc MP Imaging System (Bio-Rad).

Immunohistochemistry

Separate frozen pancreas sections were mounted on polysine coated glass slides (Thermo Scientific) and stored in the -80°C freezer. Sections were fixed in zinc fixative (IHC Zinc fixative (formalin free), BD Pharmingen, San Diego, CA, USA) for 10 min in room temperature before peroxidase blocking (Dako REAL Peroxidase-Blocking Solution, Glostrup, Denmark) for 5 min and thereafter protein block (Background Sniper, Biocare Medical, Concord, CA, USA) for 10 min. Sections were incubated with primary antibody (Guinea pig polyclonal insulin antibody, dilution 1:400, Fitzgerald, Concord, MA, USA) overnight in the refrigerator. A Horseradish Peroxidase (HRP) polymer system was used according to the manufacturer´s instructions (MACH 3 Rabbit HRP Polymer detection, Biocare Medical). Sections were developed in Diaminobenzidine (DAB) and counterstained with hematoxylin. After dehydration, sections were mounted with pertex (Histolab, Göteborg, Sweden). Dako wash buffer was used in all wash steps.

Table 1: Feline primer data.

Gene	Oligo	Sequence (5´-3´)	Amplicon size (bp)
ACTB	forward reverse	GTGGATCAGCAAGCAGGAGT CACTTGCCAGGGCAAACCTA	136
GAPDH	forward reverse	GCCATCAATGACCCCTTCAT GCCGTGGAATTTGCCGT	82
RPS7	forward reverse	AAAATCCAAGTCCGGCTCGT TCACACGGATTCTCTTGCCC	220
INS1	forward reverse	CATCGTGGAGCAATGCTGTG GCGCCCTCTAGTTGCAGTAA	71
IAPP	forward reverse	AGAAGCAATGTGCCTCCTGA AGAAATTTGCCAGGCGTTGG	152
IA-2	forward reverse	CCAAGGGCTCAGGACGAAAT GATCCAGAGATCAGCACCCG	190
CHGA	forward reverse	ACACACTATCCAAGCCCAGC GGCACCTTGGAGAGCAAGAT	137
PDX1	forward reverse	AAGTCTACCAAGGCTCACGC TGCGTGTCCGTTTGTTTTCC	85
PNLIP	forward reverse	CAAACAAGTGCTTCCCCTGC TTCCTTCCAGACAGCGTGAC	172

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Page 4 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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Table 2: Specifications of the tested feline genes including reference genes.

| Gene	Name	Function	GenBank Acc. Nr.
ACTB	beta-Actin	Major cytoskeletal protein	XM_003997266
CHGA	Chromogranin A	Secreted protein produced by endocrine cells	XM_003987967
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Glycolytic enzyme	NM_001009307
IA-2	Islet cell antigen 2, receptor-type tyrosine-protein phosphatase-like N isoform 2	Receptor-type tyrosine-protein phosphatase	XM_003991192
IAPP	Islet amyloid polypeptide	Secreted peptide produced by beta cells	NM_001043338
INS	Insulin	Peptide hormone, lowers blood glucose	NM_001009272
PDX1	Pancreatic and duodenal homeobox 1	Transcription factor, beta cell differentiation marker	XM_003980305
RPS7	Ribosomal protein S7	Ribosomal protein	NM_001009832
PNLIP	Pancreatic lipase	Enzyme produced in exocrine pancreas	XM_006938207
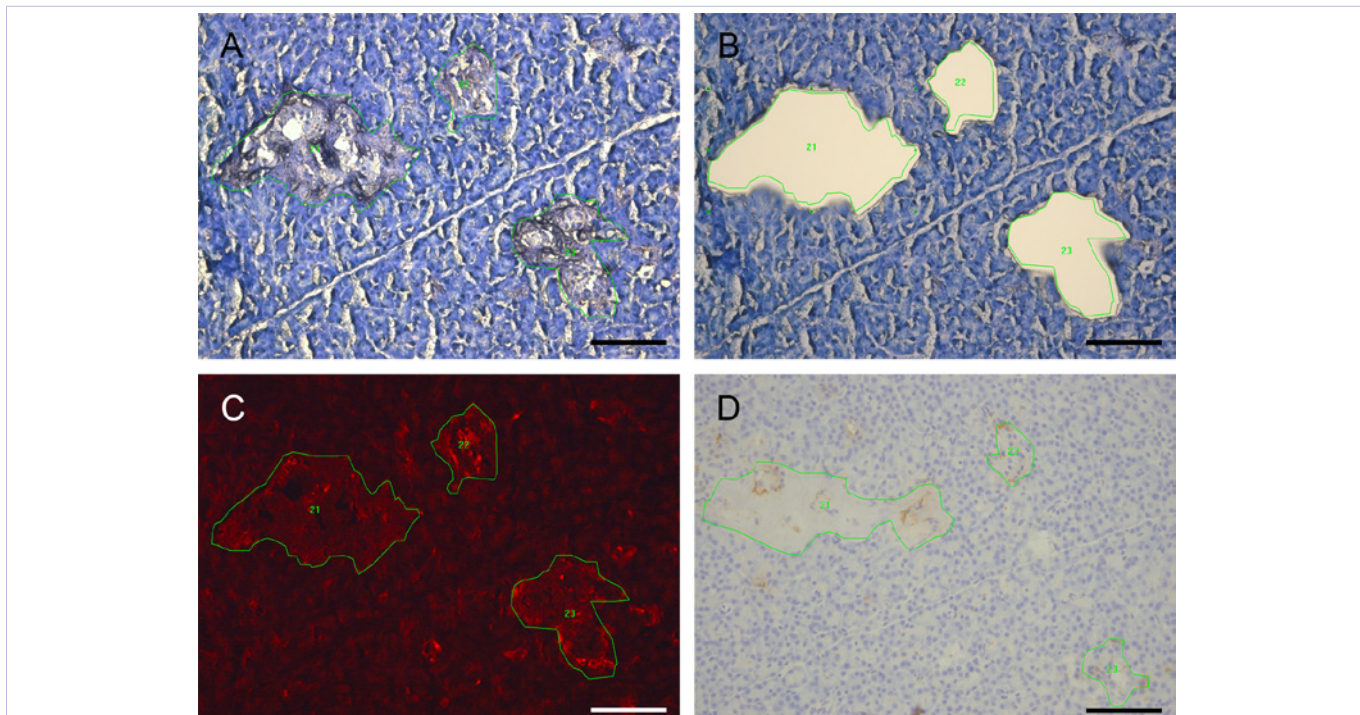
Figure 3: Micrographs of frozen pancreatic tissue from diabetic cat
RNase free hematoxylin staining visualizing islets (marked with green line) in (A). Only selected area is laser microdissected (B). Islets are visualized by fluorescence with a Texas Red filter (C). Insulin (brown) is visualized with DAB (D). Scale bars represent 100 μm.

Results

Laser microdissection of islets and immunohisto-chemistry

By the use of RNase-free hematoxylin to stain frozen pancreatic cat tissue for 90 seconds, we were able to identify islets for laser microdissection (Figure 2A and 3A). We found that islets showed fluorescence visualized with a Texas Red filter (Figure 2C and 3C). Connective tissue, which also showed fluorescence, was distinguished from islets by its characteristic fibrous appearance. The selected islets could therefore be distinctly cut out without visible contamination with exocrine pancreatic tissue (Figure 2B and 3B). Separate pancreatic sections from adjacent areas used to stain for insulin with Immunohistochemistry (IHC) revealed high insulin content in islets of healthy cats (Figure 2D) whereas less insulin-positive cells were found in diabetic cats (Figure 3D).

Specificity of the islet-specific genes used in the qPCR assay

Primers for qPCR were designed using PrimerBlast (NCBI). The specificity of each primer pair (Tables 1 and 2) was verified by melting curve analysis followed by gel-electrophoresis. A

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Page 5 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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single specific peak in the melting curve from each primer pair indicates that the qPCR has only amplified one product (data not shown). The detected Melting Temperatures (Tm) had a PCR product-specific variation of less than 0.3°C. All qPCR reactions were then analyzed by agarose gel-electrophoresis (Figures 4A and 4B), which confirmed that each primer pair was specific for the cDNA. A single band at the expected size of the amplicon for each primer pair could be detected. The non-template control where the cDNA was substituted with water, confirms the absence of primer dimers (data not shown). In the samples where the reverse transcription step was omitted, no amplified product could be detected (data not shown). This confirms that genomic DNA did not interfere with the PCR results.

Efficiency of the PCR

To determine the PCR efficiency, the primer pairs were further analyzed using a dilution curve with ten-fold cDNA template dilutions between 2 ng/µl and 0.02 ng/µl. The PCR efficiency of the primer pairs was determined to be in the range of 90 to 100% (data not shown).

Transcript levels of islet-specific genes

The normalization analysis, using the Normfinder software, indicated that the best normalization was obtained by using the geometric mean of the expression of the reference genes *GAPDH* and *RPS7*. This normalization was used to compare the islet-specific transcripts in all samples. To characterize the islets collected by laser microdissection from two healthy and two diabetic cats, the normalized mRNA expression levels of *INS*, *PDX1*, *CHGA*, *IA-2* and *IAPP* were determined (Figure 5) and compared. Within and between run variations were found to be low/moderate and reproducible results were obtained.

Purity of islet preparation

In order to investigate the purity of the LMD islets we performed qPCR on healthy cat nr 1 using the primers for PNLIP. It is highly expressed in the exocrine tissue of the pancreas but is expressed at very low levels in islets [23]. The islet sample was compared to an LMD sample of exocrine pancreatic tissue.

The signal for the exocrine control gene *PNLIP* was 82 times higher in the exocrine tissue compared to islets. This indicates that the islet preparation contains less than 1.2% exocrine cells (Figure 6).
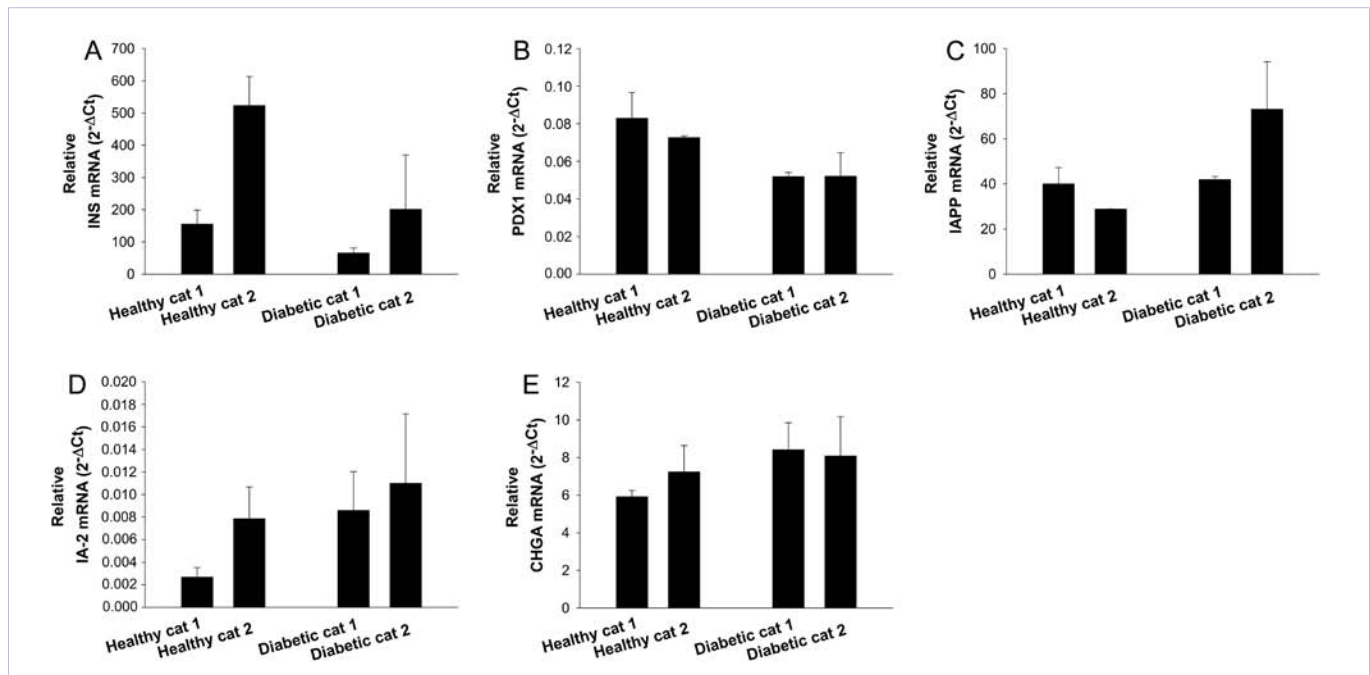
Discussion

In the present study, we demonstrate that laser microdissection allows distinct isolation of pancreatic islets from both healthy and diabetic cats, with preserved RNA integrity for further studies, i.e. gene expression profiling. Feline islets have been considered particularly difficult to isolate with standard collagenase protocols due to a lack of peri-islet matrix, and previous isolation attempts have resulted in islets surrounded by a rim of exocrine tissue [10,11,24]. It is advantageous to minimize the amount of exocrine tissue contamination when performing gene expression studies on pancreatic islets.

In order to prevent degradation of RNA we tried to keep the staining protocol as short as possible. We found that a staining time of 90 s was optimal for visualization of islets, as compared to the slightly shorter staining protocols used for isolation of rodent islets [17,25], or as in human islet laser microdissection, where the intrinsic autofluorescence of human β-cells allows isolation without prior staining [13-15]. The autofluorescence of rodent β-cells is not strong enough to be useful for LMD [17], and although we found fluorescence of stained feline islets using the Texas red filter, this fluorescence was not strong enough to be used without staining of slides to allow for identification and isolation of islets.

We found mRNA expression of all islet-specific genes, as well as the reference genes, in all our samples. As markers for islets, we selected the genes *INS*, *PDX1*, *CHGA*, *IA-2* and *IAPP*. Since islets in majority consist of beta cells [9], we wanted to investigate the mRNA levels of insulin. PDX1 is a transcription factor, which is important in the maturation and survival of beta cells [26], CHGA is
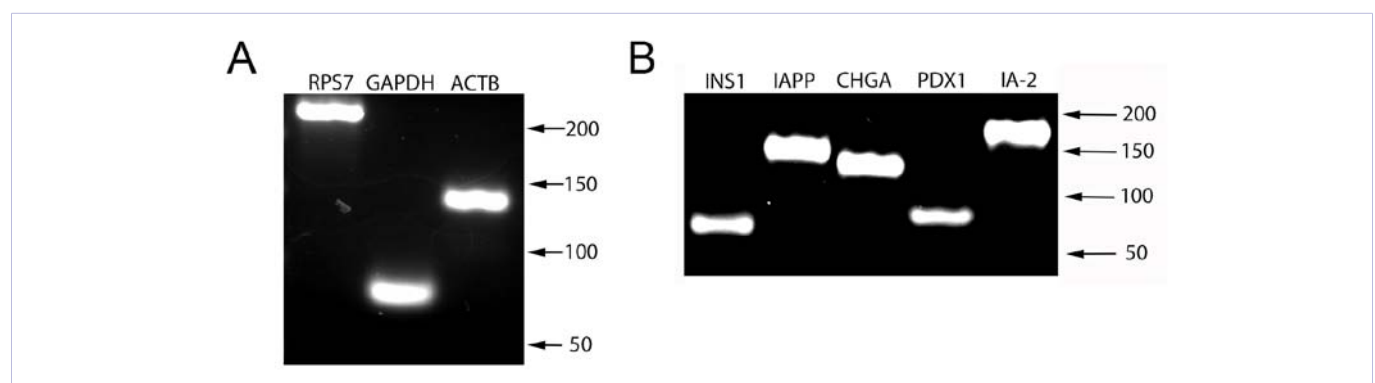
Figure 4: Specificity of the reference genes and islet-specific genes used in the qPCR assay
qPCR products from the reference genes visualized on a 3% agarose gel stained with GelRed. Each primer pair was subjected to PCR using reverse transcribed total RNA as template. All primer pairs generated a single product of the predicted size. Arrows indicate DNA molecular ladder (bp). (A) Lane 1: primer for *RPS7* (220 bp). Lane 2: primer for *GAPDH* (82 bp). Lane 3: primer for *ACTB* (136 bp). (B) Lane 1: primers for *INS* (71 bp). Lane 2: primers for *IAPP* (152 bp). Lane 3: primers for *CHGA* (137 bp). Lane 4: primers for *PDX1* (85 bp). Lane 5: primers for *IA-2* (190 bp).

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Page 6 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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Figure 5: Relative mRNA transcript levels in laser microdissected feline islets from healthy and diabetic cats
Relative gene expression for *INS* (A), *PDX1* (B), *IAPP* (C), *IA-2* (D) and *CHGA* (E) in two healthy and two diabetic cats. Values are normalized to the geometric mean of the reference genes *RPS7* and *GAPDH.* Data are expressed as means of two islet LMD preparations from each cat ± SEM.
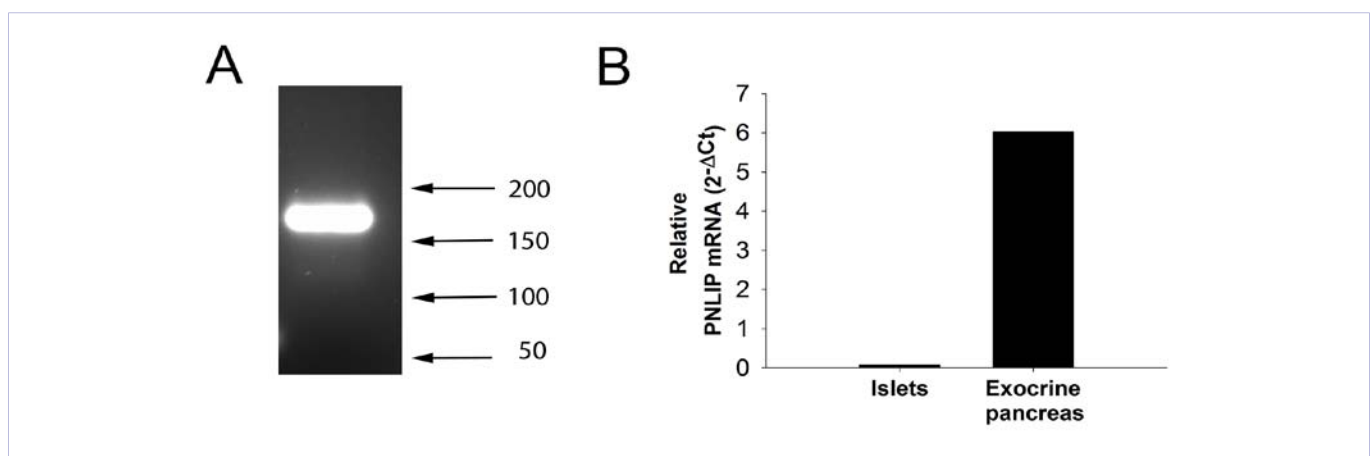
Figure 6: Purity of islet preparation
(A) Specificity of the exocrine pancreatic gene used in the qPCR assay and relative mRNA transcript levels in laser microdissected feline islets and exocrine pancreatic tissue. qPCR product from the exocrine gene visualized on a 3% agarose gel stained with GelRed. The primer pair was subjected to PCR using reverse transcribed total RNA as template. The primer pair generated a single product of the predicted size. Arrows indicate DNA molecular ladder (bp). Lane 1: primer for *PNLIP* (172 bp). (B) Relative gene expression for *PNLIP* in islets and exocrine tissue from healthy cat no 1. Values are normalized to the reference gene *RPS7.*

a general marker for endocrine cells, whereas IA-2, (also referred as Islet Cell Autoantigen (ICA) 512) is an islet-specific membrane protein found in all islet endocrine cells [27]. Moreover, IA-2 is also well known as a diabetes-specific autoantigen [28]. CHGA and IA-2 were included in our study in case we could not detect the mRNA levels of insulin in the diabetic cats. Aggregates of IAPP result in amyloid formation in islets, which is found in diabetic cats, as well as in humans with T2DM [8]. Congo red staining

on pancreatic sections from diabetic cats confirmed presence of amyloid in islets, whereas islets from healthy cats were not stained with Congo red.

Great care was taken to only include the visible islets themselves in the laser microdissection while carefully avoiding exocrine tissue, and by cutting out all islets under direct surveillance. Moreover, PNLIP was included in the study as an

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Page 7 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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exocrine control. Our islet preparation was found to be pure with as low as 1.2% of exocrine cells.

One of the diabetic cats (nr.1) showed, as expected, a lower expression of insulin compared to both healthy cats, whereas the other diabetic cat (nr. 2) showed expression of insulin in the same range as the healthy cats. This could be explained by the first cat being in a later stage of the disease, with a declining insulin secretion; whereas the second cat was more recently diagnosed and may still have had a normal insulin production. Measuring circulating serum insulin levels may help elucidate these observations, and no conclusions could be drawn from this finding due to the small number of cats included in the study.

Immunohistochemistry was used to confirm presence of insulin-positive cells in pancreatic specimens. We found, not surprisingly, that these cells were more abundant in healthy cats than in diabetic cats. The immunohistochemistry slides were prepared from sections in close proximity to the laser microdissected sections and thus help verifying the presence and location of islets.

Laser microdissection allows distinct studies of islets with minimal contamination of acinar cells. Our results demonstrate that laser microdissected islets both from healthy and diabetic cats can effectively be utilized for quantitative PCR studies. Future studies using LMD and q-PCR will help define expression profiles of healthy and diabetic cats. We are currently recruiting additional healthy and diabetic cats in order to expand the study and investigate a larger set of genes important in islet function. Differences in gene expression between healthy and diabetic cats may reveal underlying mechanisms for beta cell dysfunction and decreased beta cell mass in human T2DM.

Acknowledgement

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Page 8 of 9

Laser Microdissection of Pancreatic Islets Allows for Quantitative Real-Time PCR Detection of Islet-Specific Gene Expression in Healthy and Diabetic Cats

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Page 9 of 9