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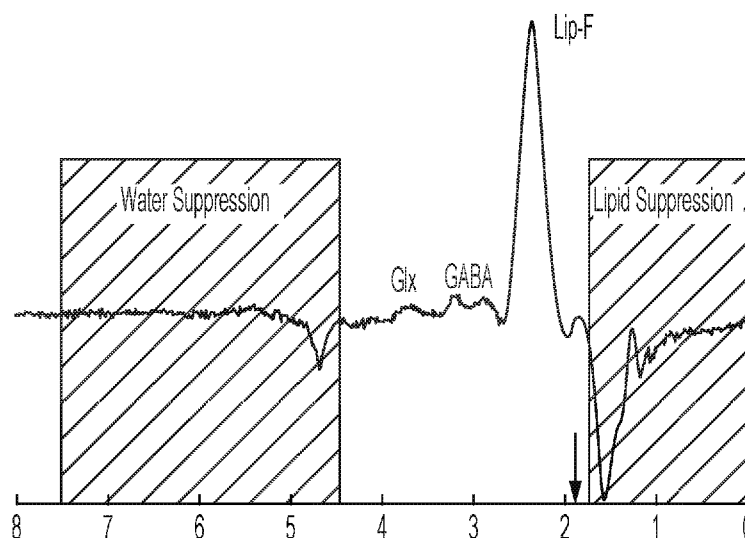


Figure 2

(57) Abstract: Aspects of the disclosure relate to systems and methods for obtaining and interpreting magnetic resonance spectroscopy (MRS) data obtained from the pancreas of a subject. In some embodiments, systems and methods of the disclosure relate to analyzing MRS spectra of metabolites, for example γ -Aminobutyric acid (GABA), to assess pancreatic islet density and function in a subject. In some embodiments, systems and methods described by the disclosure are useful for the diagnosis and/or treatment of diseases associated with impaired pancreatic function, for example diabetes.



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NON-INVASIVE DIAGNOSTIC BIOMARKER FOR PANCREATIC ISLET POPULATIONS

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S. provisional application serial number 62/573,281, filed October 17, 2017, entitled “NON-INVASIVE DIAGNOSTIC BIOMARKER FOR PANCREATIC ISLET POPULATIONS”, the entire contents of which are incorporated herein by reference.

GOVERNMENT SUPPORTED RESEARCH

This invention was made with government support under EB015909, EB016089, AA025306, TR001429, TR001427, TR001773, DK101120, DK104155 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Proton Magnetic resonance spectroscopy (1H-MRS) is an analytical technique that is often used to detect and quantify metabolic changes *in vivo* in a subject, for example in the brain of a subject. One advantage of 1H-MRS is that it is a non-invasive technique and does not require the use of ionizing radiation. However, the use of 1H-MRS to study metabolites in organs other than the brain faces many challenges, such as adequate signal acquisition, localization of 1H-MR spectra to the desired organ, and complications related to interpreting spectra obtained from different internal structures within a target organ.

SUMMARY

Aspects of the disclosure relate to systems and methods for obtaining and interpreting 1H-MRS data obtained from the pancreas of a subject. The disclosure is based, in part, on the surprising discovery that pancreatic islet function can be characterized using 1H-MRS spectra of certain metabolites (*e.g.*, γ -Aminobutyric acid, GABA and/or glutamate, Glx) that have been acquired from a subject and processed using methods described herein. In some embodiments, systems and methods described by the disclosure are useful for diagnosing and/or treating a subject having or suspected of having a disease associated with impaired pancreatic function, for example diabetes (*e.g.*, type 1 diabetes).

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In some aspects, the disclosure provides a method for detecting and/or quantifying GABA in a non-CNS tissue of a subject (*e.g.*, the pancreas of a subject), the method comprising acquiring a plurality of proton magnetic resonance (1H-MR) spectra using a high-field (*e.g.*, greater than or equal to 3 Tesla (T)) magnetic resonance imaging apparatus and, optionally, quantifying GABA concentration in the subject based upon the peak amplitude of a selected spectrum (or spectra) relative to at least one internal reference signal (*e.g.*, spectrum) obtained from the subject.

In some aspects, the disclosure provides a method for detecting and/or quantifying GABA in the pancreas of a subject, the method comprising acquiring a plurality of proton magnetic resonance (1H-MR) spectra from a multi-channel receiver array placed around the torso of a subject; performing subtractive J-difference editing on the 1H-MR spectrum obtained from each channel of the receiver array to produce edited difference spectra by selecting from one or more channels of the receiver array; and, optionally, quantifying GABA concentration in the subject based upon the 3.0 ppm peak amplitude of the selected difference spectra relative to at least one internal reference signal (*e.g.*, spectrum) obtained from the receiver array.

In some embodiments, the plurality of 1H-MR spectra are acquired using a magnetic field strength between 0.2 and 12 Tesla (T). In some embodiments, the plurality of 1H-MR spectra are acquired using a magnetic field strength between 1 and 3 T.

In some embodiments, acquisition of the plurality of 1H-MR spectra comprises a single voxel spectroscopy (SVS) pulse acquisition sequence. In some embodiments, the SVS pulse acquisition sequence comprises a point-resolved spectroscopy (PRESS) acquisition sequence.

In some embodiments, the echo time (TE) of the PRESS acquisition sequence is between 50 ms and 100 ms. In some embodiments, the TE is between 67 ms and 80 ms, optionally wherein the TE is 68 ms.

In some embodiments, acquisition of the plurality of 1H-MR spectra comprises administering one or more chemical-shift-selective editing pulses (*e.g.*, J-difference editing pulses). In some embodiments, the chemical-shift-selective editing pulses are applied at a chemical shift between -2.7 ppm and -2.8 ppm relative to water resonance of the subject. In some embodiments, chemical-shift-selective editing pulses comprise a Mescher-Garwood (MEGA) refocusing pulse sequence. In some embodiments, a MEGA refocusing pulse sequence is between 10 milliseconds (ms) and about 25 milliseconds (ms). In some embodiments, a MEGA refocusing pulse is 14 milliseconds (ms).

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In some embodiments, the acquiring step is repeated between 100 and 1000 times. In some embodiments, the repetition time (TR) between acquisitions is between 500 milliseconds and 10 seconds.

In some embodiments, the subject is holding their breath during each acquisition of 1H-MR spectrum. In some embodiments the subject has exhaled before holding their breath.

In some embodiments, the multi-channel receiver array comprises at least four channels, at least eight channels, or at least 16 channels. In some embodiments, the multi-channel receiver array is a phased multi-channel receiver array.

In some embodiments, at least four channels of the multi-channel receiver array are positioned to acquire spectra in a position anterior to the subject. In some embodiments, at least four channels of the multi-channel receiver array are positioned to acquire spectra in a position posterior to the subject.

In some embodiments, eight channels of the multi-channel receiver array are positioned to acquire spectra in a position anterior to the subject and eight channels of the multi-channel receiver array are positioned to acquire spectra in a position posterior to the subject.

In some embodiments, at least one channel of the multi-channel receiver array acquires 1H-MR spectra from a voxel within the pancreas of the subject. In some embodiments, a voxel is of sufficient size to encompass the entire pancreas of a subject. In some embodiments, the voxel ranges from about 1 cm³ to about 250 cm³.

In some embodiments, a subject has a mechanically-restrained pancreas.

In some embodiments, concentration of GABA (*c*GABA) is quantified using the following equation:

$$c_{GABA} = c_{ref} \frac{S_{GABA}}{S_{ref}} R \kappa$$

where *C*_{ref} is the concentration of the reference signal, *S*_{GABA} and *S*_{ref} are the signal integrals of the GABA and reference signals respectively, *R* is a term to correct for differential relaxation of GABA and reference signals and *κ* is a constant to adjust for editing efficiency.

In some aspects, the disclosure provides a method for identifying compromised pancreatic islet function in a subject, the method comprising: quantifying a GABA concentration of the subject using a method as described by the disclosure; comparing the GABA concentration in the subject to a reference standard; and, determining that pancreatic islet function of the

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subject is compromised if the concentration of GABA in the subject is lower than the reference standard.

In some embodiments, a reference standard comprises a GABA concentration obtained from a healthy subject, for example a subject that does not have compromised pancreatic islet function (*e.g.*, a subject that does not have type 1 diabetes).

In some embodiments, methods described by the disclosure further comprise administering a therapeutic agent to a subject if the subject has been determined to have compromised pancreatic islet function.

In some aspects, the disclosure provides a method for treating diabetes in a subject, the method comprising administering a therapeutic agent to the subject, wherein the subject has been determined to have compromised pancreatic islet function by a method as described by the disclosure.

In some embodiments, compromised pancreatic islet function is a result of a subject having diabetes. In some embodiments, diabetes is type 1 diabetes. In some embodiments, the diabetes is type 2 diabetes.

In some embodiments, a type 1 diabetes therapeutic agent is insulin, insulin degludec, insulin isophane, insulin glulisine, insulin lispro, insulin aspart, glargine, detemir, or any combination thereof. In some embodiments, a type 2 diabetes therapeutic agent is metformin, a sulfonylurea, a DPP-4 inhibitor, a glucagon-like peptide (*e.g.*, an incretin mimetic), a thiazolidinedione, an alpha-glucosidase inhibitor (*e.g.*, acarbose, miglitol), a sodium glucose transporter (SGLT) 2 inhibitor, or any combination thereof.

BRIEF DESCRIPTION OF DRAWINGS

FIGs. 1A-1B shows schematic spectra of a J-difference editing (MEGA-PRESS) approach to MRS measurement of GABA. FIG. 1A shows editing pulses applied at 1.9 ppm suppress (“ON”) the GABA contribution to the 3 ppm glycine/GABA (GLX/GABA) peaks, which are revealed through the difference (“DIFF”) between the “ON” and “OFF” spectra. FIG. 1B shows the impact of the 1.9 ppm editing pulse on the 3 ppm peaks.

FIG. 2 shows a representative spectrum recorded from healthy human pancreas collected using methods described by the disclosure. Glx: glutamate/glutamine peak; GABA: γ -aminobutyric acid peak; Lip-lipid peak.

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DETAILED DESCRIPTION

Aspects of the disclosure relate to methods and compositions for the detection of certain metabolites (*e.g.*, glutamate, GABA) in non-CNS tissue, for example pancreatic tissue. The disclosure is based, in part, on the discovery that pancreatic islet function can be characterized using ¹H-MRS spectra of certain metabolites (*e.g.*, γ -Aminobutyric acid, GABA) that have been acquired from a subject and processed using methods described herein. Accordingly, systems and methods described by the disclosure are useful, in some embodiments, for diagnosing and/or treating a subject having or suspected of having a disease associated with impaired pancreatic function, for example diabetes (*e.g.*, type 1 diabetes).

In some aspects, the disclosure provides a method for detecting and/or quantifying γ -Aminobutyric acid (GABA) in the pancreas of a subject, the method comprising acquiring a plurality of proton magnetic resonance (¹H-MR) spectra from a multi-channel receiver array placed in proximity to (*e.g.*, around) the torso of a subject; performing subtractive J-difference editing on the ¹H-MR spectrum obtained from each channel of the receiver array to produce edited difference spectra by selecting from one or more channels of the receiver array; and, optionally, quantifying GABA concentration in the subject based upon the 3.0 ppm peak amplitude of the selected difference spectra relative to at least one internal reference signal (*e.g.*, spectrum) obtained from the receiver array.

Nuclear magnetic resonance spectroscopy (MRS)

Generally, the disclosure relates to methods for analyzing an analyte (*e.g.*, GABA, creatine, *etc.*) in non-CNS tissue using nuclear magnetic resonance spectroscopy (NMRS or MRS). MRS is an analytical technique which uses strong magnetic fields and radiofrequency waves to determine the biophysical characteristics of molecules, such as small molecules, nucleic acids, peptides and proteins. Typically, MRS measurements are obtained by a magnetic resonance imaging system that generally comprises a strong magnet, a radiofrequency (RF) transmitting coil, a radiofrequency (RF) receiving coil, and one or more gradient coils that are connected to a control module (*e.g.*, comprising one or more computers). MR spectra may be obtained by measuring the resonance frequencies of one or more various isotopes within a subject, for example ¹H, ³¹P, ¹⁹F, ¹³C, or ²³Na. Clinical applications of ¹H-MRS are generally

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described, for example by Van der Graaf, *Eur. Biophys J.* 2010 Mar; 39(4): 527–540, the entire contents of which are incorporated herein by reference.

Acquisition of 1H-MRS Spectra

Acquisition of 1H-MR spectra relies on the generation of a magnetic field, which is usually measured in Tesla (T) units. Generally, clinical MRS is performed using magnetic fields with a strength greater than 1.5T. In some embodiments of methods described herein, a plurality of proton magnetic resonance (1H-MR) spectra are acquired from a subject at a magnetic field strength between 0.2T and 12T. In some embodiments, 1H-MR spectra are acquired at a magnetic field strength between 1T and 3T, between 1.5T and 5T, or between 3T and 9T. In some embodiments, 1H-MR spectra are acquired at a magnetic field strength of 3T.

In some embodiments, 1H-MR spectra are acquired using single voxel spectroscopy (SVS). As used herein, the term “single voxel spectroscopy” refers to a MRS acquisition technique that acquires a signal (*e.g.*, a radiofrequency signal or spectrum) from a three-dimensional volume of tissue (*e.g.*, a voxel). Generally, each plane (*e.g.*, X, Y, or Z) of a voxel can be between about 1 μm^3 and 500 cm^3 (*e.g.*, any volume between 1 cm^3 and 500 cm^3 , inclusive). In some embodiments, each plane (*e.g.*, X, Y, or Z) of a voxel is between about 10 cm^3 to about 250 cm^3 (*e.g.*, any volume between 10 and 250 cm^3). In some embodiments, each plane (*e.g.*, X, Y, or Z) of a voxel is between about 1.0 cm^3 to about 5 cm^3 . In some embodiments, a voxel is about 2.5 $\text{cm}^3 \times 2.5 \text{ cm}^3 \times 5.0 \text{ cm}^3$. In some embodiments, a voxel is of sufficient size to encompass the entire pancreas of a subject.

Aspects of the disclosure relate to measurement of GABA in the pancreas of a subject. Thus, in some embodiments, a voxel measured by SVS is located in a non-CNS tissue of a subject (*e.g.*, a mammal, such as a human, non-human primate, rodent, mouse, *etc.*). In some embodiments, a voxel is located in pancreatic tissue of the subject. In some embodiments, a voxel measured by SVS encompasses beta cells of the subject. In some embodiments, the beta cells comprise GABA. In some embodiments a voxel encompasses only pancreatic cells and structures of a subject (*e.g.*, does not encompass tissue other than pancreatic tissue).

Generally, 1H-MR signals are acquired by exciting the protons within a given sample (*e.g.*, a voxel) and measuring the amount of magnetization using a radiofrequency receiver. In some aspects, the disclosure relates to the discovery that certain combinations of pulse acquisition sequences result in acquisition of 1H-MR spectra (*e.g.*, a GABA peak) from

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pancreatic tissue of a subject. As used herein, the term “pulse acquisition sequence” refers to a series (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) of radiofrequency pulses that are passed through a subject to one or more radiofrequency receivers in order to generate an MRS spectrum.

As used herein, the term “chemical-shift-selective editing pulse” refers to a
5 radiofrequency pulse, or sequence of pulses (*e.g.*, 1, 2, 3, 4, 5, or more, radiofrequency pulses delivered to a sample with a fixed time period), that induces a change in resonance frequency of a particular target analyte (*e.g.*, glutamate, GABA, *etc.*) relative to other analytes in a sample, thereby increasing the signal-to-noise ratio of the target analyte relative to other analytes in a sample (*e.g.*, water, fat, creatine, *etc.*). Examples of chemical-shift-selective editing pulses
10 include but are not limited to water suppression editing pulses (*e.g.*, chemical-shift-selective, CHESS pulses), Stimulated Echo Acquisition Mode (STEAM pulses), Point-Resolved Spectroscopy (PRESS) editing pulses, J-difference editing pulses (*e.g.*, MEGA), *etc.*

In some embodiments, a chemical-shift-selective editing pulse comprises a point-resolved spectroscopy (PRESS) acquisition sequence. In some embodiments, a PRESS sequence
15 comprises, in the following order, a first 90° pulse, a first 180° pulse, and a second 90° pulse.

The echo time (TE) of a chemical-shift-selective editing pulse (*e.g.*, a PRESS pulse sequence) can vary. Without wishing to be bound by any particular theory, the long echo time (TE) used in PRESS sequences (*e.g.*, relative to the TE of other acquisition pulse sequences) increases signal-to-noise ratio for detection of certain analytes (*e.g.*, glutamate, GABA, *etc.*) by
20 reducing the number of detectable peaks in 1H-MR spectra acquired from a voxel of interest. As used herein, the term “echo time” or “TE” refers to the amount of time a magnetized proton (*e.g.*, a proton in an analyte) is in the transverse plane after an excitation before signal readout. In some embodiments, the echo time (TE) of a PRESS sequences ranges from about 10 milliseconds (ms) to about 500 ms. In some embodiments, the echo time (TE) of a PRESS
25 sequences ranges from about 10 milliseconds (ms) to about 100 ms (*e.g.*, about 10 ms, about 20 ms, about 30 ms, about 40 ms, about 50 ms, about 60 ms, about 70 ms, about 80 ms, about 90 ms, or about 100 ms). In some embodiments the TE of a PRESS sequence ranges from about 67 ms to about 80 ms (*e.g.*, 67 ms, 68 ms, 69 ms, 70 ms, 71 ms, 72 ms, 73 ms, 74 ms, 75 ms, 76 ms, 77 ms, 78 ms, 79 ms or 80 ms).

30 The disclosure is based, in part, on the use of certain chemical-shift-selective editing pulses (*e.g.*, J-difference editing pulses) that separate or prevent formation of signal multiplets (*e.g.*, doublets, triplets, *etc.*) in spectra acquired from a voxel. In some embodiments of methods

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described herein, the chemical-shift-selective editing pulse is a J-difference editing pulse. Examples of J-difference editing pulses (*e.g.*, J-difference editing pulse sequences) include but are not limited to Mescher-Garwood PRESS (MEGA-PRESS), band-selective inversion with gradient dephasing (BASING), *etc.* In some embodiments, a chemical-shift-selective editing pulse is a MEGA-PRESS pulse sequence.

As used herein “MEGA-PRESS” refers to a J-difference editing pulse sequence described, for example by Mescher et al. (1998) *NMR Biomed.* 11(6):266-72, the entire contents of which are incorporated herein by reference. In some embodiments, a MEGA-PRESS editing pulse sequence comprises one or more (*e.g.*, 1, 2, 3, *etc.*) editing radiofrequency pulses applied at a chemical-shift that is between about -2.9 ppm and about -2.8 ppm relative to an internal reference signal (*e.g.*, a water signal). In some embodiments, a MEGA-PRESS editing pulse sequence is applied to the 1.9 ppm GABA peak of a ¹H-MR spectrum.

As used herein an “internal reference signal” refers to a known chemical peak present in a ¹H-MR spectrum, from which chemical shift of other analytes is measured, typically in parts per million (ppm) or in Hertz (Hz). Generally, MRS internal reference standards are known in the art, and include water, creatine, choline, *etc.* In some embodiments of methods described herein, the internal reference standard is a water signal (*e.g.*, the resonance of water in a particular voxel).

Generally, a plurality of ¹H-MR spectra are obtained from a voxel of interest (*e.g.*, a particular voxel selected within a subject) by repeating the acquiring step of methods described herein. In some embodiments, the acquisition step is repeated between 100 and 1000 times (*e.g.*, any integer between 100 and 1000, inclusive). In some embodiments, the acquisition step is repeated about 100, about 125, about 150, about 175, about 200, about 250, about 300, about 450, about 500, about 750, or 1000 times. In some embodiments, each repetition of the acquisition step comprises a MEGA-PRESS pulse sequence.

The repetition time between acquisition steps can vary. As used herein, the term “repetition time” or “TR” refers to the amount of time between each acquisition set of a method as described herein. In some embodiments, the repetition time (TR) between each acquisition step of a method described herein ranges from about 500 milliseconds (ms) to about 10 seconds. In some embodiments, the repetition time (TR) between each acquisition step of a method described herein ranges from about 10 seconds to about 5 seconds, about 8 seconds to about 2 seconds, about 3 seconds to about 750 ms, or about 1 second to about 500 ms.

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In some aspects, the disclosure relates to the discovery that obtaining ¹H-MR spectra from a multi-channel receiver array around the torso of a subject allows for the detection of GABA in the pancreas of the subject. As used herein, a “multi-channel receiver array” refers to a radiofrequency (RF) receiver array that comprises two or more receivers configured to detect a ¹H-MR signal (*e.g.*, radiofrequency signal) from one or more voxels of a subject. The disclosure is based, in part, on the discovery that MRS methods utilizing certain arrangements of receiver arrays (*e.g.*, positioning of receivers around the torso of a subject), in combination with particular acquisition and editing pulse sequences (*e.g.*, MEGA-PRESS), result in detection of ¹H-MR spectra comprising quantifiable GABA peaks from non-CNS tissue (*e.g.*, from one or more voxels comprising or consisting of pancreatic tissue or pancreatic cells).

In some embodiments, a multi-channel receiver array comprises two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) channels. In some embodiments, a multi-channel receiver array comprises 16 channels. The arrangement of channels of a multi-channel receiver array can vary (*e.g.*, positioning of the channels of an array can vary with respect to a subject). In some embodiments, at least eight channels (*e.g.*, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more channels) of an array are positioned to acquire spectra from a position anterior to a subject. For example, in some embodiments, at least eight channels of a multi-channel receiver array are placed in front (*e.g.*, anterior) of a subject’s torso. In some embodiments, at least eight channels of a multi-channel receiver array are placed in behind (*e.g.*, posterior) to a subject’s torso. In some embodiments, the receiver is placed 0.25 inches, 0.5 inches, 0.75 inches, 1 inch, 1.5 inches, 5 inches, 12 inches, 1.5 feet or 2 feet in front of a subject. In some embodiments, the receiver is placed 0.25 inches, 0.5 inches, 0.75 inches, 1 inch, 1.5 inches, 5 inches, 12 inches, 1.5 feet or 2 feet in front of a subject.

GABA and Pancreatic Function

In some aspects, the disclosure relates to the use of magnetic resonance spectroscopy methods described herein for detecting the presence (or absence) of GABA and/or the concentration of GABA in a non-CNS tissue (*e.g.*, pancreas) of a subject. As used herein, “γ-Aminobutyric acid (GABA)” refers to an amino acid having the following structure,



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that binds to its cognate receptors (*e.g.*, GABA_A and GABA_B) and typically functions, in vertebrates, as an inhibitory neurotransmitter in the central nervous system (CNS). However, GABA is also present in several vertebrate (*e.g.*, mammalian) peripheral tissues, for example, the intestines, stomach, kidneys, pancreas, fallopian tubes, uterus, ovaries, testes, kidneys, bladder, lungs, and liver.

Previously described techniques for quantifying GABA in a subject using MRS are focused on the central nervous system (CNS) of the subject. A “subject” generally refers to any mammalian organism that can be subjected to magnetic resonance imaging, including but not limited to humans, non-human primates (*e.g.*, monkeys, chimpanzees, *etc.*) rodents (*e.g.*, mice, rats, guinea pigs, hamsters *etc.*), dogs, cats, cows, *etc.* In some embodiments, a subject is a human subject. In some embodiments, a subject has or is suspected of having a disease associated with compromised pancreatic function, for example diabetes (*e.g.*, type 1 or type 2 diabetes).

A subject “having” a disease is a subject who exhibits signs and symptoms of a disease (*e.g.*, diabetes) and has been diagnosed by a medical doctor, for example after a confirmatory diagnostic test (*e.g.*, a blood glucose test in the case of diabetes). A subject “suspected of having a disease” is a subject who exhibits signs and symptoms of a disease (*e.g.*, diabetes) but has not been diagnosed by a medical doctor and/or has not been administered a diagnostic test. In some embodiments, a subject suspected of having diabetes is a pre-diabetic subject, for example as described in Gundy (2012) *J Am Coll Cardiol.* 59(7):635-43.

In some embodiments, methods described herein detect the presence (or absence) of GABA in non-CNS tissue of a subject. As used herein “non-CNS tissue” refers to tissue and cells of a subject that are not part of the central nervous system (*e.g.*, brain, spinal cord, cerebrospinal fluid, *etc.*) of a subject. In some embodiments, non-CNS tissue is pancreatic tissue or pancreatic cells.

In some embodiments, GABA is present in beta cells (β cells) of the pancreas. β cells, which form a large percentage of the cells in pancreatic islets, control the production and release of insulin, and therefore play an important role in diseases associated with regulation of blood glucose concentrations (*e.g.*, diabetes mellitus, such as type 1 diabetes, type 2 diabetes, gestational diabetes, *etc.*). Without wishing to be bound by any particular theory, the disclosure is based, in part, on the discovery that quantification of GABA concentrations in the pancreas (*e.g.*, β cells of the pancreas) of a subject using ¹H-MRS is indicative of pancreatic islet function

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of the subject, and thus is useful for determining whether a subject has or is at risk of having a disease characterized by compromised pancreatic islet function. Various methods of determining the concentration or amount of an analyte based upon a MRS spectrum are known in the art. In some embodiments, GABA concentration in a subject is determined using the following equation:

$$C_{GABA} = C_{ref} \frac{S_{GABA}}{S_{ref}} R \kappa$$

where C_{ref} is the concentration of the reference signal, S_{GABA} and S_{ref} are the signal integrals of the GABA and reference signals respectively, R is a term to correct for differential relaxation of GABA and reference signals and κ is a constant to adjust for editing efficiency.

In some aspects, the disclosure relates to methods of identifying and/or treating compromised pancreatic function (*e.g.*, a disease associated with compromised pancreatic function, such as diabetes) in a subject. In some embodiments, the disease is type 1 diabetes. In some embodiments, the disease is type 2 diabetes. In some embodiments, methods of identifying a subject having compromised pancreatic function comprise the steps of quantifying the amount (*e.g.*, concentration) of GABA in the pancreas of the subject (*e.g.*, using methods described herein), comparing the GABA amount (or concentration) to a reference standard, and determining that pancreatic islet function of the subject is compromised if the amount of GABA in the subject is lower than the reference standard.

As used herein, “reference standard” refers to an amount (*e.g.*, concentration) of GABA quantified from a healthy subject (*e.g.*, a subject not having compromised pancreatic function, or a disease associated with compromised pancreatic function, such as diabetes). The threshold amount (*e.g.*, the amount by which a GABA amount or concentration from a subject is lower than a reference standard) which results in a determination that a subject has compromised pancreatic islet function can vary. In some embodiments, the amount of GABA detected in the subject is between about 1% and about 99% lower than a reference standard. In some embodiments, the amount of GABA detected in the subject is between about 1% and about 10% lower, about 5% and about 20% lower, about 15% and about 25% lower, about 20% and about 40% lower, about 30% and about 75% lower, or about 50% and 100% lower than the reference standard. In some embodiments, the amount of GABA detected in the subject is between about 1-fold and 100-fold less than a reference standard (*e.g.*, about 1-fold, 2-fold, 5-fold, 10-

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fold, 25-fold, 50-fold, 75-fold, or 100-fold lower). In some embodiments, the amount of GABA detected in the subject is more than 100-fold lower than a reference standard.

In some aspects, the disclosure provides a method of treating diabetes comprising administering a therapeutic agent to a subject who has been determined to have compromised pancreatic function by a method described herein. Therapeutic agents for the treatment of diabetes are known in the art, for example as described by Kang (2012) *Korean J Anesthesiol.* 63(3):195–202. Examples of therapeutic agents for treatment of type 1 diabetes include but are not limited to insulin (*e.g.*, natural isolated insulin, recombinant insulin, *etc.*), insulin degludec, insulin isophane, insulin glulisine, insulin lispro, insulin aspart, glargine, detemir, or any combination thereof. Examples of therapeutic agents for treatment of type 2 diabetes include but are not limited to metformin, a sulfonylurea, a DPP-4 inhibitor, a glucagon-like peptide (*e.g.*, an incretin mimetic), a thiazolidinedione, an alpha-glucosidase inhibitor (*e.g.*, acarbose, miglitol), a sodium glucose transporter (SGLT) 2 inhibitor, or any combination thereof.

EXAMPLES

J-difference editing: MEGA-PRESS

In vivo detection of low-concentration metabolites such as GABA requires a targeted experiment because the GABA signals in the traditional MR spectrum are overlapped by signals from more concentrated metabolites. J-difference editing is a mechanism to remove the overlapping signals and retain only a signal of interest (*e.g.*, GABA). The removal of confounding signals is achieved by subtracting two slightly different experimental conditions (hence *difference editing*). MEGA-PRESS is one type of J-difference editing that enhances the GABA signal present in a ¹H-MR spectrum.

For example, when used for detection of GABA, the two experimental conditions of MEGA-PRESS differ in how they treat the coupling (typically represented by the symbol *J*) within the GABA molecule. The majority of signals in the MR spectra produced by the two conditions behave the same. However, the GABA signals (*e.g.*, peaks) of the two conditions behave differently, and thus subtracting the spectra of the two conditions removes confounding signals and retains only the GABA signals (*e.g.*, peaks).

The element that differs between the two experimental conditions of MEGA-PRESS described in this example is the editing pulses, which are selective for GABA. When applied at 1.9 ppm, the editing pulses refocus the evolution of a J-coupling within the GABA molecule,

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altering the appearance of the GABA signal in the spectrum, as shown in FIGs. 1A-1B. FIG. 1A shows editing pulses applied at 1.9 ppm suppress (“ON”) the GABA contribution to the 3 ppm glycine/GABA (GLX/GABA) peaks, which are revealed through the difference (“DIFF”) between the “ON” and “OFF” spectra. Note that the “OFF” spectrum is produced in the absence of an editing pulse. FIG. 1B shows that the 1.9 ppm editing pulse enhances the 3 ppm GABA signal (*e.g.*, peaks) present in the “DIFF” spectrum.

Cardiac triggering and breath hold

Acquisition of 1H-MR spectra is generally repeated a large number of times (typically >100) as the single-measurement signal-to-noise ratio (SNR) is high. With repeated acquisitions, the SNR of the acquired data is sufficient for quantification. For MRS of brain tissue, the repetition time (TR) between repeat acquisitions is usually fixed (to ~2 s). Applications of MRS in the body must account for organ movement within the body cavity as a function of the cardiac and respiratory cycle. For example, in pancreas, these motions have two main effects: the position of the pancreas within the body cavity will change with cardiac and/or respiratory motion, and the magnetic field within and around the pancreas will change with cardiac and/or respiratory motion.

To accommodate effects of the cardiac cycle, acquisitions are performed with cardiac and/or respiration triggering, and/or breath-hold, and/or mechanical stabilization (*e.g.*, a rigid band placed around the torso of a subject). The cardiac cycle is determined by fingertip pulse oximeter or ECG connected to the scanner physiological monitoring system. The respiration cycle is determined by respiration sensor connected to scanner. Each TR is triggered following a delay following the cardiac cycle. The breath hold procedure involves instruction to participant to hold breath at a paced interval during which one or more acquisitions may be made.

MRS of GABA in the pancreas

J-difference-edited MRS of GABA was performed in the pancreas using a modified MEGA-PRESS sequence with PRESS localization and MEGA J-difference editing. Data were acquired using a 16-channel phased-array body coil (*e.g.*, 8 channels placed on the abdomen of the subject and 8 channels placed on the back of the subject in windows of ~32 seconds during which the participant was instructed to breath-hold (exhaled). Editing was performed with 14-ms editing pulses applied at 1.9 ppm in editing-ON scans to refocus evolution of the GABA coupling. Other acquisition parameters included TE= 68 ms, a voxel size sufficient to

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encompass the portion of pancreas being sampled during physiological rhythm associated movement (*e.g.*, respiration, cardiac, peristalsis, *etc.*) while avoiding digestive tract, 2 kHz spectral width. More than 2,000 data points were acquired. Channels were weighted in a phase-sensitive manner to improve SNR of reconstructed spectra. Spectral registration was used to frequency-and-phase correct the individual transients. Data were Fourier transformed after windowing by a 5-Hz exponential and the difference spectrum was calculated.

In vivo MRS detection of GABA

FIG. 2 shows a representative spectrum recorded from healthy human pancreas collected using methods described as follows. One healthy male subject (aged 32 years, weight 85 kg) was recruited. The subject was positioned supine in a 3.0T MRI scanner and the subject's abdomen was bound with an elasticated band (30 cm width) to reduce the freedom to move of the abdominal organs, and a 16-channel phased array body coil was placed anterior and posterior to the subject, at the level of the pancreas. Based on T2-weighted anatomical imaging (cardiac triggered FSE with respiratory gating), a $2.5 \times 2.5 \times 5 \text{ cm}^3$ voxel was placed so as to include as much pancreas tissue as possible. Shimming of the voxel was performed using Philips 'pencil-beam' voxel-projection method. Chemical-shift-selective water and fat suppression was performed using dual band saturation (Zhu, Ouwerkerk, & Barker, 2010). Acquisition parameters include: MEGA-PRESS sequence; TE/TR 68/2000 ms; 320 averages of 2048 data points, sampled at 2 kHz; 14-ms editing pulses applied alternately at 1.9 ppm, to refocus evolution of the coupling to the GABA signal at 3 ppm, and 7.46, allowing the GABA coupling to evolve. Coil combination was performed based on the amplitude and phase of the unsuppressed water signal from each channel of the 16-channel torso coil. 3 Hz exponential line broadening was applied prior to Fourier transformation and display of the spectrum in FIG. 2. Data indicate selective positioning of receiver arrays combined with J-difference editing allows for detection of GABA at 3T in pancreas. Co-editing of additional predicted resonances from Glx (due to coupling to Glx signals around 2 ppm) and lipid signal Lip-F was also performed (FIG. 2).

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CLAIMS

What is claimed is:

1. A method for quantifying GABA in a subject, the method comprising:
 - (i) acquiring a plurality of proton magnetic resonance (1H-MR) spectra from a multi-channel receiver array placed around the torso of a subject;
 - (ii) performing subtractive J-difference editing on the 1H-MR spectrum obtained from each channel of the receiver array to produce edited difference spectra;
 - (iii) generating at least one edited difference spectra having a peak at 3.0 ppm by selecting from one or more channels of the receiver array; and,
 - (iv) quantifying GABA concentration in the subject based upon the 3.0 ppm peak amplitude of the difference spectra selected in (iii) relative to at least one internal reference signal (*e.g.*, spectrum) obtained from the receiver array.
2. The method of claim 1, wherein the plurality of 1H-MR spectra are acquired using a magnetic field strength between 0.2 and 12 Tesla (T).
3. The method of claim 1 or 2, wherein the plurality of 1H-MR spectra are acquired using a magnetic field strength between 1 and 3 T.
4. The method of any one of claims 1 to 3, wherein acquisition of the plurality of 1H-MR spectra comprises a single voxel spectroscopy (SVS) pulse acquisition sequence.
5. The method of any claim 4, wherein the SVS pulse acquisition sequence comprises a point-resolved spectroscopy (PRESS) acquisition sequence.
6. The method of claim 5, wherein the echo time (TE) of the PRESS acquisition sequence is between 50 ms and 100 ms.

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7. The method of claim 6, wherein the TE is between 67 ms and 80 ms, optionally wherein the TE is 68 ms.

8. The method of any one of claims 1 to 7, wherein acquisition of the plurality of
5 ¹H-MR spectra comprises chemical-shift-selective editing pulses (*e.g.*, J-difference editing pulses).

9. The method of claim 8, wherein the chemical-shift-selective editing pulses (*e.g.*, J-difference editing pulses) are applied at a chemical shift between -2.7 ppm and -2.8 ppm
10 relative to the internal reference signal (*e.g.*, water resonance).

10. The method of claim 9, wherein the chemical-shift-selective pulses comprise a Mescher-Garwood (MEGA) refocusing pulse sequence.

15 11. The method of claim 10, wherein each of the chemical-shift-selective pulses has a duration between 10 and 25 ms, optionally wherein the MEGA refocusing pulse sequence is 14 milliseconds (ms).

12. The method of any one of claims 1 to 11, wherein the acquiring step is repeated
20 between 100 and 1000 times.

13. The method of claim 12, wherein the repetition time (TR) between acquisitions is between 500 ms and 10 seconds.

25 14. The method of any one of claims 1 to 13, wherein the subject is holding their breath during each acquisition of ¹H-MR spectrum, optionally wherein the subject has exhaled before holding their breath.

15. The method of any one of claims 1 to 14, wherein the multi-channel receiver
30 array comprises at least four channels, at least eight channels, or at least 16 channels.

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16. The method of any one of claims 1 to 15, wherein the multi-channel receiver array is a phased multi-channel receiver array.

17. The method of any one of claims 1 to 16, wherein at least four channels of the multi-channel receiver array are positioned to acquire spectra from a position anterior to the subject.

18. The method of any one of claims 1 to 17, wherein at least four channels of the multi-channel receiver array are positioned to acquire spectra from a position posterior to the subject.

19. The method of any one of claims 1 to 8, wherein eight channels of the multi-channel receiver array are positioned to acquire spectra from a position posterior to the subject and eight channels of the multi-channel receiver array are positioned to acquire spectra from a position anterior to the subject.

20. The method of any one of claims 1 to 19, wherein at least one channel of the multi-channel receiver array acquires ¹H-MR spectra from a voxel within the pancreas of the subject.

21. The method of claim 20, wherein the voxel ranges from about 1 cm³ to about 250 cm³, optionally wherein the voxel is of a size sufficient to encompass the entire pancreas of the subject.

22. The method of any one of claims 1 to 21, wherein the subject has a mechanically-restrained pancreas.

23. The method of any one of claims 1 to 22, wherein the concentration of GABA (cGABA) is quantified using the following equation:

$$c_{GABA} = c_{ref} \frac{S_{GABA}}{S_{ref}} R_K$$

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where C_{ref} is the concentration of the reference signal, S_{GABA} and S_{ref} are the signal integrals of the GABA and reference signals respectively, R is a term to correct for differential relaxation of GABA and reference signals and κ is a constant to adjust for editing efficiency.

5 24. The method of any one of claims 1 to 23, wherein the internal reference signal (*e.g.*, spectrum) is derived from water, creatine, or glutamate.

 25. A method for identifying compromised pancreatic islet function in a subject, the method comprising:

10 quantifying a GABA concentration of the subject using the method of any one of claims 1 to 24;

 comparing the GABA concentration in the subject to a reference standard; and,
 determining that pancreatic islet function of the subject is compromised if the concentration of GABA in the subject is lower than the reference standard.

15 26. The method of claim 25, wherein the reference standard comprises a GABA concentration obtained from a healthy subject, for example a subject that does not have compromised pancreatic islet function.

20 27. The method of claim 25 or 26, wherein the method further comprises administering a therapeutic agent to the subject if the subject has been determined to have compromised pancreatic islet function.

25 28. A method for treating diabetes in a subject, the method comprising administering a therapeutic agent to the subject, wherein the subject has been determined to have compromised pancreatic islet function by the method of any one of claims 25-27.

 29. The method of any one of claims 25-28, wherein the compromised pancreatic islet function is reduced function or loss of function in pancreatic beta cells.

30 30. The method of claim 28 or 29, wherein the diabetes is type 1 diabetes.

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31. The method of any one of claims 28-30, wherein the therapeutic agent is insulin, insulin degludec, insulin isophane, insulin glulisine, insulin lispro, insulin aspart, glargine, detemir, or any combination thereof.

5 32. The method of claim 28 or 29, wherein the diabetes is type 2 diabetes, optionally wherein the therapeutic agent is metformin, a sulfonylurea, a DPP-4 inhibitor, a glucagon-like peptide (*e.g.*, an incretin mimetic), a thiazolidinedione, an alpha-glucosidase inhibitor (*e.g.*, acarbose, miglitol), a sodium glucose transporter (SGLT) 2 inhibitor, or any combination thereof.

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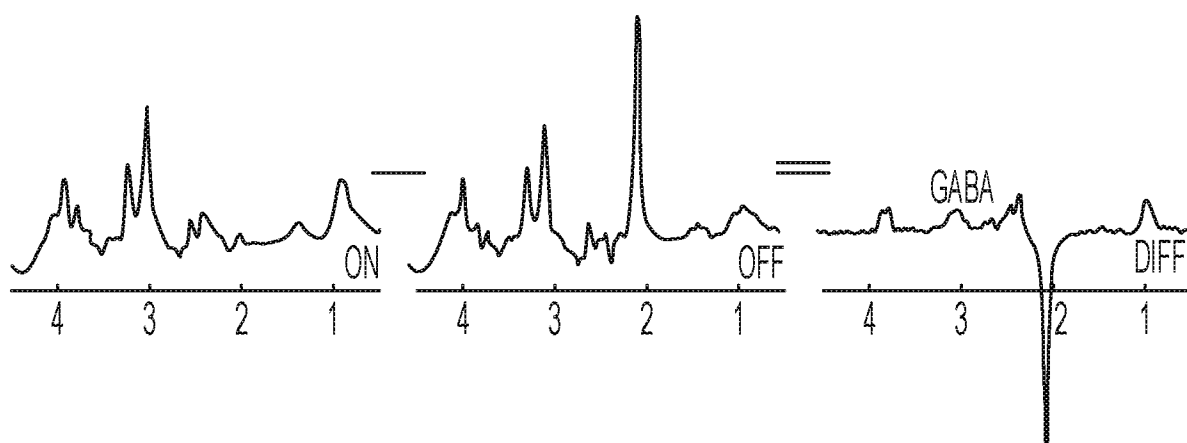


Figure 1A

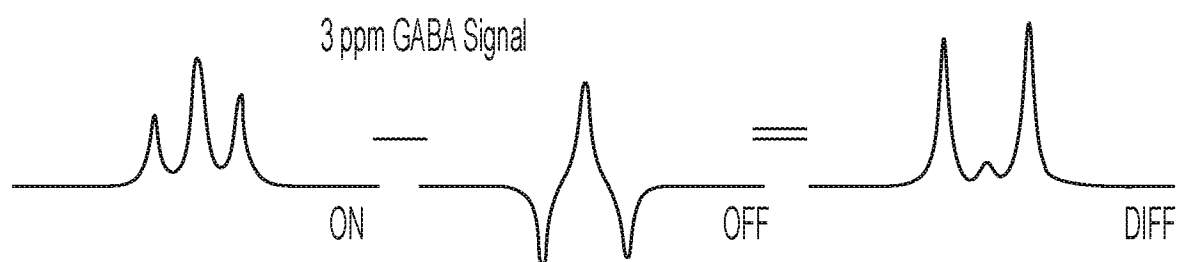


Figure 1B

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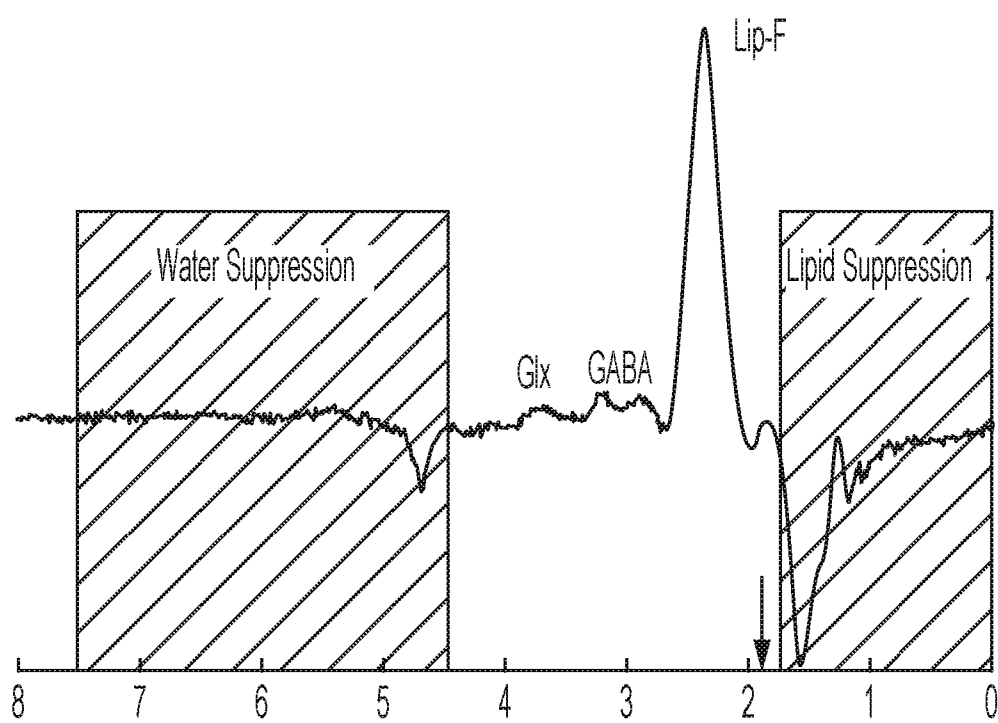


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/056287

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/197; G01N 24/08; G01R 33/465 (2018.01)

CPC - A61K 31/197; G01N 24/087; G01N 24/088; G01R 33/465 (2018.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 600/410; 702/19 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PORGES et al. "Frontal Gamma-Aminobutyric Acid Concentrations Are Associated With Cognitive Performance in Older Adults," Biological Psychiatry: Cognitive Neuroscience and Neuroimaging, 31 January 2017 (31.01.2017), Vol. 2, No. 1, Pgs. 38-44. entire document	1-3
Y	BEGOVAZ et al. "Pancreatic adipose tissue infiltration, parenchymal steatosis and beta cell function in humans," Diabetologia, 05 March 2015 (05.03.2015), Vol. 58, Iss. 7, Pgs. 1646-1655. entire document	1-3
A	EDDEN et al. "Measuring T2 in vivo with J-difference editing: Application to GABA at 3 tesla," Journal of Magnetic Resonance Imaging, 01 November 2011 (01.11.2011), Vol. 35, Iss. 1, Pgs. 229-234. entire document	1-3
A	US 2015/0153324 A1 (INJE UNIVERSITY INDUSTRY-ACADEMIC COOPERATION FOUNDATION) 04 June 2015 (04.06.2015) entire document	1-3
A	WO 2016/174425 A1 (OXFORD UNIVERSITY INNOVATION LIMITED) 03 November 2016 (03.11.2016) entire document	1-3
A	SOLTANI et al. "GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes," Proceedings of the National Academy of Sciences USA, 27 June 2011 (27.06.2011), Vol. 108, No. 28, Pgs. 11692-11697. entire document	1-3

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 November 2018

Date of mailing of the international search report

11 DEC 2018

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/056287

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.