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**Berggren**(54) **APOCIII AND THE TREATMENT AND  
DIAGNOSIS OF DIABETES**(75) Inventor: **Per-Olof Berggren, Solna (SE)**

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G01N 33/567(52) **U.S. Cl.** ..... **435/4; 435/7.2**(57) **ABSTRACT**

The present invention provides methods of identifying candidate compounds for the treatment of type I diabetes by identifying those test compounds that inhibit an apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells. The present invention further provides methods for treating patients with type I diabetes by administering to the patient an amount effective of an inhibitor of apoCIII to reduce apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells.

The present invention provides methods for diagnosing Type I diabetes or a propensity to develop type I diabetes by comparing an amount of sialylated apoCIII in a blood serum sample from a subject with an amount of sialylated apoCIII in a control blood serum sample and diagnosing those subjects with an elevated amount of sialylated apoCIII in the blood serum sample relative to the control as having type I diabetes, or having a propensity to develop type I diabetes.

The present invention provides methods for identifying diabetic patients to be treated with anti-apoCIII therapy, by determining an amount of apoCIII in a blood serum sample from a diabetic subject and comparing it to a control blood serum sample; and identifying those subjects with an elevated amount of apoCIII in the blood serum sample relative to the control blood serum sample as being subjects to be treated with anti-apoCIII therapy.

Figure 1

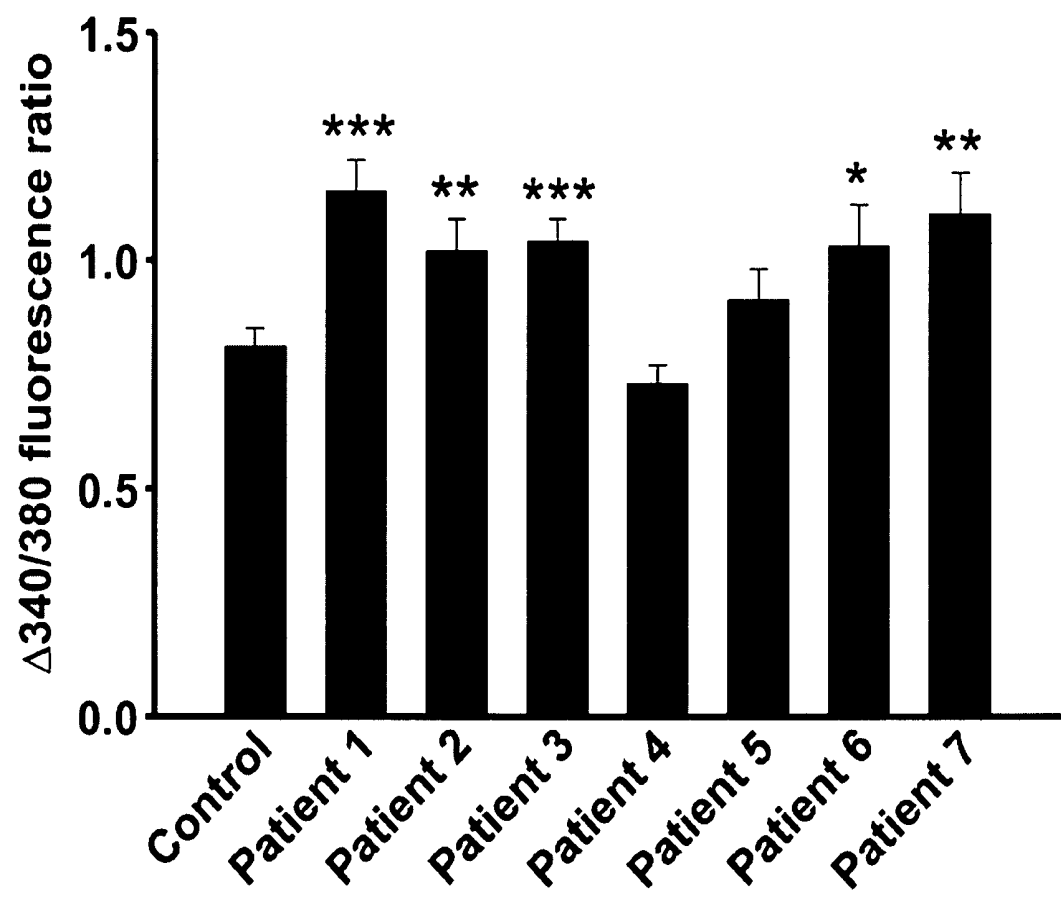


Figure 2

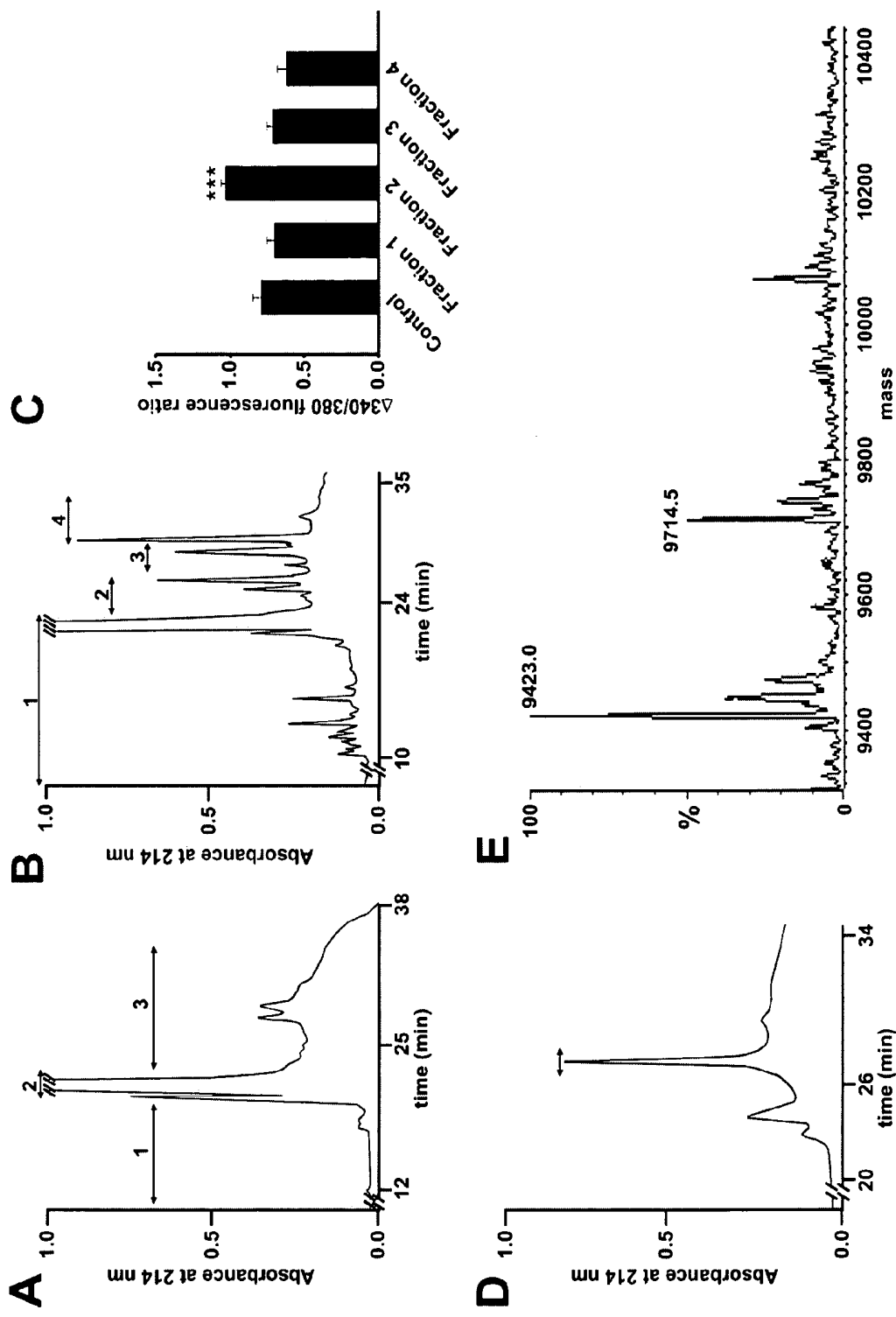


Figure 3

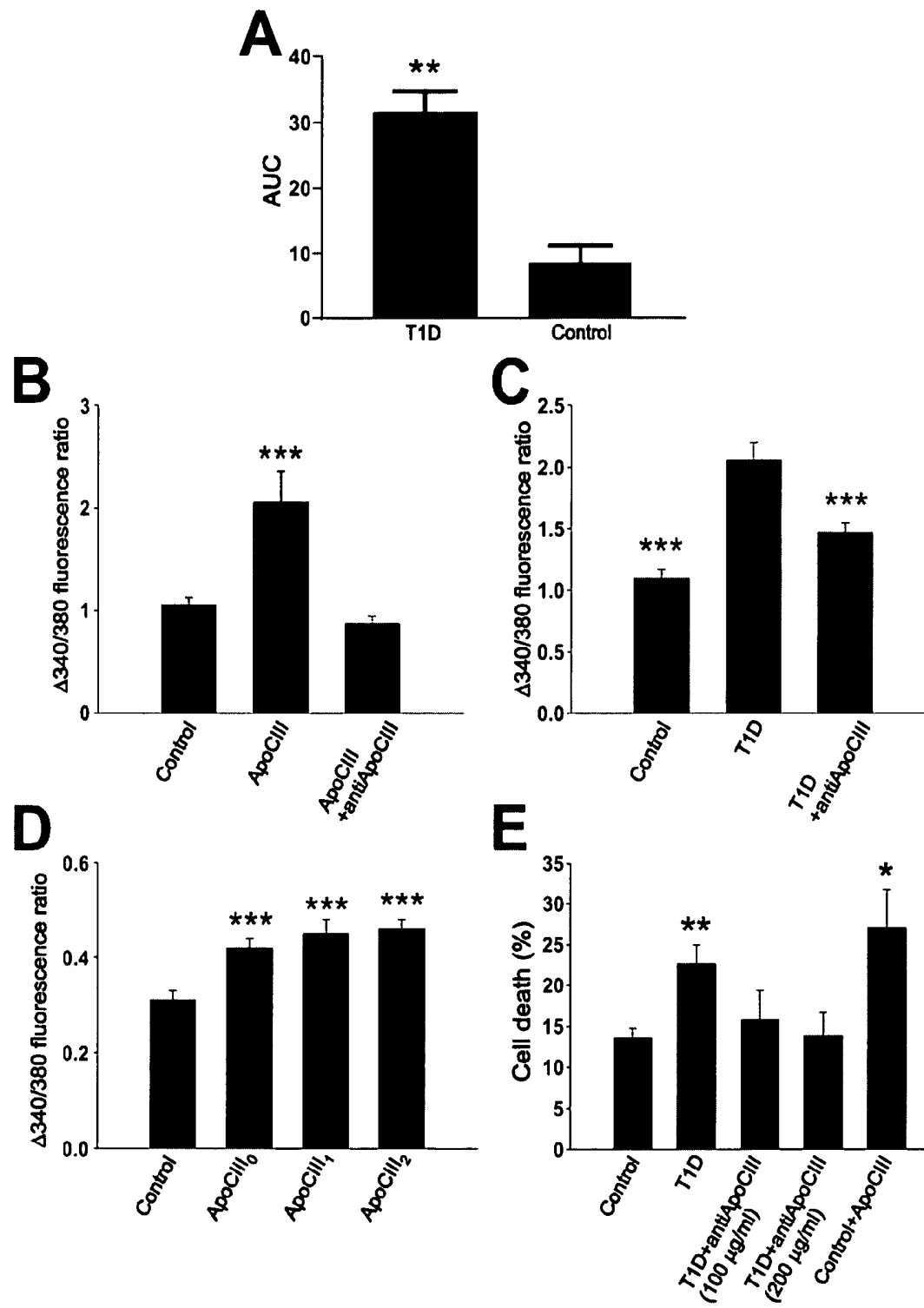
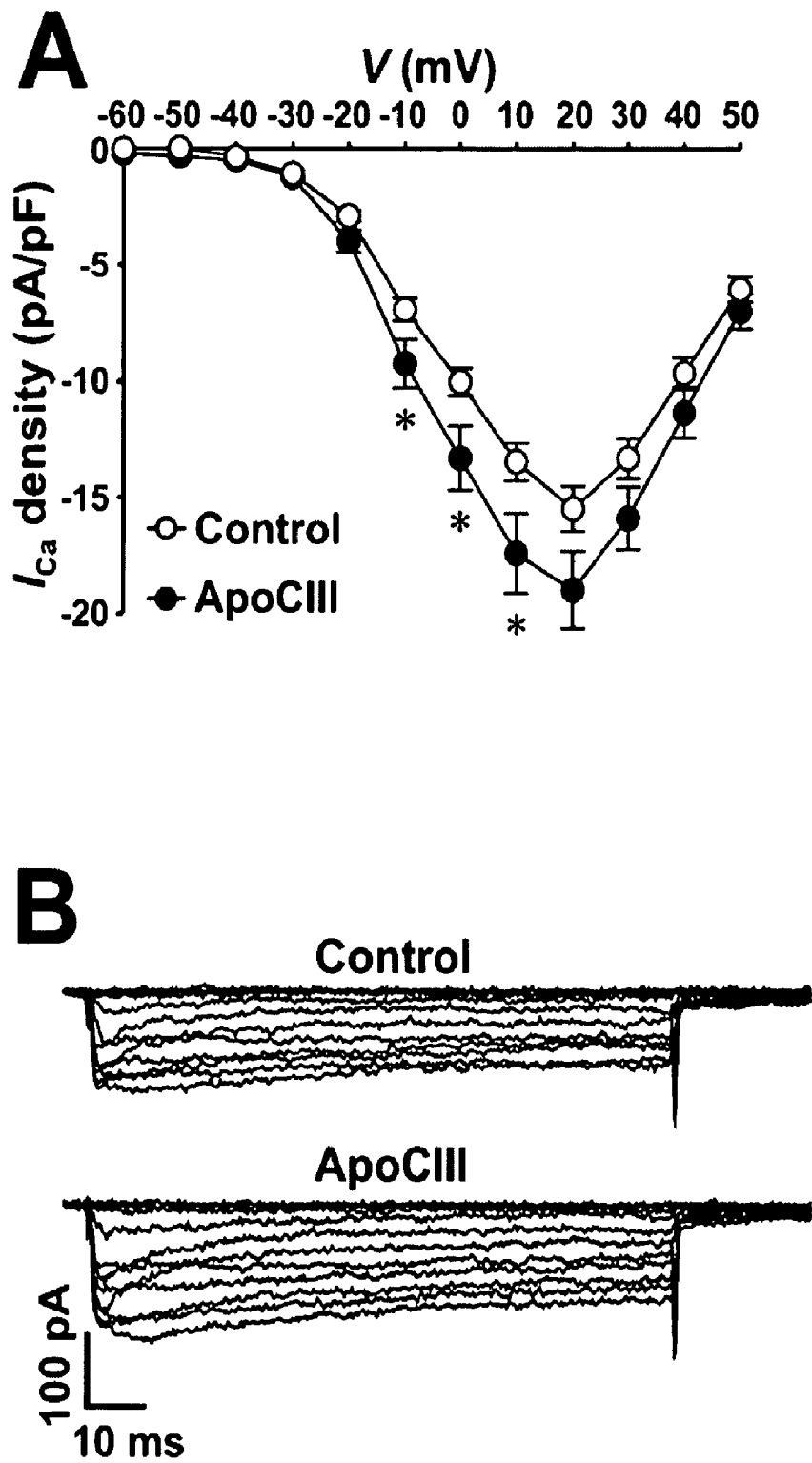


Figure 4



## APOCIII AND THE TREATMENT AND DIAGNOSIS OF DIABETES

### CROSS REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application 60/466,517 filed Apr. 29, 2003.

### BACKGROUND OF THE INVENTION

[0002] Voltage-gated L-type  $\text{Ca}^{2+}$ -channels have an important physiological role in pancreatic  $\beta$ -cell (" $\beta$ -cell") signal-transduction (1). These channels constitute an essential link between transient changes in membrane potential and insulin release from  $\beta$ -cells. Changes in cytoplasmic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in the  $\beta$ -cell are associated with the activation of a spectrum of intracellular signals and are strictly regulated, as prolonged high  $[\text{Ca}^{2+}]_i$  is harmful to the cells.

[0003] In type 1 diabetes (T1D), there is a specific destruction of the insulin secreting pancreatic  $\beta$ -cell. Sera from newly diagnosed type 1 diabetic (T1D) patients have been shown to increase the activity of voltage-gated L-type  $\text{Ca}^{2+}$ -channels in  $\beta$ -cells resulting in increased  $[\text{Ca}^{2+}]_i$  upon depolarization and  $\beta$ -cell apoptosis, effects that can be prevented by  $\text{Ca}^{2+}$ -channel blockers (2). However, it has not been determined what factor in T1D serum is responsible for the changes in  $[\text{Ca}^{2+}]_i$ .

[0004] We now demonstrate that apolipoprotein CIII (apoCIII) is increased in serum from T1D patients and that this serum factor both induces increased cytoplasmic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) and  $\beta$ -cell death.

### SUMMARY OF THE INVENTION

[0005] In one aspect, the present invention provides methods of identifying candidate compounds for the treatment of type I diabetes comprising contacting pancreatic  $\beta$  cells with an amount of apolipoprotein CIII ("apoCIII") effective to increase intracellular calcium concentration, in the presence of one or more test compounds, and identifying those test compounds that inhibit an apoCIII-induced increase in intracellular calcium concentration in the pancreatic  $\beta$  cells. In another aspect, the present invention provides methods for treating patients with type I diabetes comprising administering to the patient an amount effective of an inhibitor of apoCIII to reduce apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells.

[0006] In a further aspect, the present invention provides methods for diagnosing Type 1 diabetes or a propensity to develop type 1 diabetes comprising determining an amount of sialylated apoCIII in a blood serum sample from a subject; comparing the amount with an amount of sialylated apoCIII in a control blood serum sample; and diagnosing those subjects with an elevated amount of sialylated apoCIII in the blood serum sample relative to the control as having type I diabetes, or having a propensity to develop type I diabetes.

[0007] In a still further aspect, the present invention provides methods for identifying diabetic patients to be treated with anti-apoCIII therapy, comprising determining an amount of apoCIII in a blood serum sample from a diabetic subject and comparing it to a control blood serum sample; and identifying those subjects with an elevated amount of

apoCIII in the blood serum sample relative to the control blood serum sample as being subjects to be treated with anti-apoCIII therapy.

### BRIEF DESCRIPTION OF THE FIGURES

[0008] **FIG. 1** Changes in  $[\text{Ca}^{2+}]_i$  in pancreatic  $\beta$ -cells from mice exposed to T1D or control sera. Five out of seven T1D sera induced an enhanced increase in  $[\text{Ca}^{2+}]_i$ , when the cells were depolarized with high concentrations of  $\text{K}^+$  to open the voltage-gated  $\text{Ca}^{2+}$ -channel, compared to cells that had been exposed to normal serum (n=29, 28, 32, 47, 21, 27, 31 and 18, respectively), \*\*\*  $P<0.001$ , \*\*  $P<0.01$  and \*  $P<0.05$  versus control.

[0009] **FIG. 2** Stepwise separation and identification of the active fraction in T1D serum. A, After the first RP-HPLC separation the fraction marked 3 was found to give a higher increase in  $[\text{Ca}^{2+}]_i$ . B, Fraction 3 (**FIG. 2A**) was rerun on RP-HPLC under identical conditions. The fractions were again tested for  $[\text{Ca}^{2+}]_i$  stimulating activity (**FIG. 2C**), and one positive fraction (No. 2) was identified. D, The active fraction (**FIG. 2B**) was re-chromatographed. The fraction, inducing a higher increase in  $[\text{Ca}^{2+}]_i$  when  $\beta$ -cells were depolarized with high concentrations of  $\text{K}^+$ , is marked with a bar. C, Pancreatic  $\alpha$ -cells incubated with four fractions from RP-HPLC of diabetic sera from **FIG. 2B** (n=6, 11, 12, 11 and 10, respectively), \*\*\*  $P<0.001$  versus control. E, The active fraction from **FIG. 2C** was analyzed by electrospray mass spectrometry.

[0010] **FIG. 3** Amounts of apoCIII in T1D serum and effects on  $[\text{Ca}^{2+}]_i$  and cell death. A, Relative levels of apoCIII<sub>1</sub> in T1D and control sera, given as area under the curve (AUC), \*\* $P<0.01$  (n=5). B, Pancreatic  $\beta$ -cells were incubated with apoCIII or apoCIII+antibodies against human apoCIII (n=63, 35 and 33), \*\*\* $P<0.001$  versus control. C,  $\beta$ -cells were incubated with a control or a T1D serum and T1D serum+anti-apoCIII (n=18, 17 and 20), \*\*\* $P<0.001$  versus T1D serum. D, Mouse  $\beta$ -cells were incubated with apoCIII<sub>0</sub>, apoCIII<sub>1</sub>, apoCIII<sub>2</sub> or the vehicle (control) (n=54, 40, 48, 37), \*\*\*  $P<0.001$  versus control. E, The insulin secreting cell line RfNm5F was exposed to control and T1D sera and T1D serum with the addition of two concentrations of anti-apoCIII and finally control serum with apoCIII (n=5),  $P<0.05$  and \*\* $P<0.01$ , versus control.

[0011] **FIG. 4** Interaction of apoCIII with the voltage-gated L-type  $\text{Ca}^{2+}$  channel. A, Summary graph of current density-voltage relationships. ApoCIII-treated cells (filled circles, n=56) and control cells (open circles, n=55) were depolarized to potentials between -60 and 50 mV, in 10 mV increments, from a holding potential of -70 mV, \* $P<0.05$ . B, Sample whole-cell  $\text{Ca}^{2+}$  current traces from a control cell (cell capacitance: 4.3 pF) and a cell incubated with apoCIII (cell capacitance: 4.2 pF). Cells were depolarized by a set of voltage pulses (100 ms, 0.5 Hz) between -60 and 50 mV, in 10 mV increments, from a holding potential of -70 mV.

### DETAILED DESCRIPTION OF THE INVENTION

[0012] Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods

in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, Calif.), "Guide to Protein Purification" in *Methods in Enzymology* (M. P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, Calif.), *Culture of Animal Cells. A Manual of Basic Technique*, 2<sup>nd</sup> Ed. (R. I. Freshney. 1987. Liss, Inc. New York, N.Y.), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, Tex.).

[0013] In one aspect, the present invention provides methods of identifying candidate compounds for the treatment of type I diabetes comprising contacting pancreatic  $\beta$  cells with an amount effective of apolipoprotein CIII ("apoCIII") effective to increase intracellular calcium concentration in the presence of one or more test compounds, and identifying those test compounds that inhibit apoCIII-induced increase in intracellular calcium concentration in the pancreatic  $\beta$  cells.

[0014] As used herein, "apoCIII" refers to a protein comprising the amino acid sequence shown in SEQ ID NO:2 (Human) (NCBI accession number CAA25233), SEQ ID NO:4 (Rat) (NCBI accession number AAA40746), SEQ ID NO:6 (Macaque) (NCBI accession number CAA48419), (NCBI accession number NP\_075603), SEQ ID NO:8 (Mouse) or functional equivalents thereof.

[0015] The apoCIII may be substantially purified apoCIII, available, for example, from Sigma Chemical Company (St. Louis, Mo.), wherein "substantially purified" means that it is removed from its normal in vivo cellular environment. Alternatively, the apoCIII may be present in a mixture, such as blood serum from type I diabetic or partially or fully purified therefrom using standard techniques, such as those described below. In a preferred embodiment, substantially purified apoCIII is used.

[0016] As discussed below, there are three known isoforms of human apoCIII that have the same amino acid sequence, but which differ in their glycosylation pattern. Thus, in a preferred embodiment, glycosylated apoCIII is used, wherein the glycosylation is preferably sialylation. In an especially preferred embodiments, mono-sialylated or di-sialylated apoCIII is used. Such glycosylated forms may be purchased, for example, from Sigma Chemical Company, or may be partially or fully purified using standard techniques, such as those described below.

[0017] As used herein, "pancreatic  $\beta$  cells" are any population of cells that contains pancreatic  $\beta$  islet cells. The cells can be obtained from any mammalian species, or may be present within the mammalian species when the assays are conducted in vivo. Such pancreatic  $\beta$  islet cell populations include the pancreas, isolated pancreatic islets of Langerhans ("pancreatic islets"), isolated pancreatic  $\beta$  islet cells, and insulin secreting cell lines. Methods for pancreatic isolation are well known in the art, and methods for isolating pancreatic islets, can be found, for example, in Cejvan et al., *Diabetes* 52:1176-1181 (2003); Zambre et al., *Biochem. Pharmacol.* 57:1159-1164 (1999), and Fagan et al., *Surgery* 124:254-259 (1998), and references cited therein. Insulin secreting cell lines are available from the American Tissue Culture Collection ("ATCC") (Rockville, Md.). In a further embodiment where pancreatic  $\beta$  cells are used, they are

obtained from ob/ob mice, which contain more than 95%  $\beta$  cells in their islets, and are commercially available.

[0018] As used herein, "intracellular calcium concentration" refers to cytoplasmic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in the pancreatic  $\beta$ -cell. Such concentrations can be measured by any method known in the art, such as the use of fluorescent calcium indicators, as disclosed herein.

[0019] As used herein, "increase intracellular calcium concentration" refers to increasing the concentration during the course of the assay above that seen in the absence of test compounds. The method does not require a specific amount of increase in intracellular calcium concentration over baseline, so long as the compound(s) promotes an increase in intracellular calcium concentration above that seen in the absence of test compounds. In a preferred embodiment, the increase is a statistically significant increase as measured by standard statistical measurements.

[0020] The contacting of the pancreatic  $\beta$  cells with the apoCIII may occur before, after, or simultaneously with contacting the cells with one or more test compounds. The contacting can be in vitro, in vivo, or ex vivo.

[0021] The present invention further provides compounds identified by the above screening methods, and their use for treating subjects with type I diabetes.

[0022] In another embodiment, the methods further comprise synthesizing the test compounds that inhibit apoCIII-induced increase in intracellular calcium concentration in the pancreatic  $\beta$  cells.

[0023] When the test compounds comprise polypeptide sequences, such polypeptides may be chemically synthesized or recombinantly expressed. Recombinant expression can be accomplished using standard methods in the art, as disclosed above. Such expression vectors can comprise bacterial or viral expression vectors, and such host cells can be prokaryotic or eukaryotic. Synthetic polypeptides, prepared using the well-known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc ( $\text{N}\alpha$ -amino protected  $\text{N}\alpha$ -t-butyloxycarbonyl) amino acid resin with standard deprotecting, neutralization, coupling and wash protocols, or standard base-labile  $\text{N}\alpha$ -amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids. Both Fmoc and Boc  $\text{N}\alpha$ -amino protected amino acids can be obtained from Sigma, Cambridge Research Biochemical, or other chemical companies familiar to those skilled in the art. In addition, the polypeptides can be synthesized with other  $\text{N}\alpha$ -t-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, such as by using automated synthesizers.

[0024] When the test compounds comprise antibodies, such antibodies can be polyclonal or monoclonal. The antibodies can be humanized, fully human, or murine forms of the antibodies. Such antibodies can be made by well-known methods, such as described in Harlow and Lane, *Antibodies; A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1988). In one example, preimmune serum is collected prior to the first immunization with, for example, apoCIII. A substantially

purified apoCIII, or antigenic fragments thereof, together with an appropriate adjuvant, are injected into an animal in an amount and at intervals sufficient to elicit an immune response. Animals are bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. At about 7 days after each booster immunization, or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about  $-20^{\circ}$  C. Polyclonal antibodies against apoCIII can then be purified directly by passing serum collected from the animal through a column to which non-antigen-related proteins prepared from the same expression system without apoCIII bound.

**[0025]** Monoclonal antibodies can be produced by obtaining spleen cells from the animal. (See Kohler and Milstein, *Nature* 256, 495-497 (1975)). In one example, monoclonal antibodies (mAb) of interest are prepared by immunizing inbred mice with apoCIII, or an antigenic fragment thereof. The mice are immunized by the IP or SC route in an amount and at intervals sufficient to elicit an immune response. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of by the intravenous (IV) route. Lymphocytes, from antibody positive mice are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner under conditions which will allow the formation of stable hybridomas. The antibody producing cells and fusion partner cells are fused in polyethylene glycol at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells and are screened for antibody production by an immunoassay such as solid phase immunoradioassay. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, *Soft Agar Techniques*, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press, 1973.

**[0026]** "Humanized antibody" refers to antibodies derived from a non-human antibody, such as a mouse monoclonal antibody. Alternatively, humanized antibodies can be derived from chimeric antibodies that retains or substantially retains the antigen-binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity as compared to the parental antibody when administered to humans. For example, chimeric antibodies can comprise human and murine antibody fragments, generally human constant and mouse variable regions. Since humanized antibodies are far less immunogenic in humans than the non-human monoclonal antibodies, they are preferred for subsequent therapeutic antibody use.

**[0027]** Humanized antibodies can be prepared using a variety of methods known in the art, including but not limited to (1) grafting complementarity determining regions from a non-human monoclonal antibody onto a human framework and constant region ("humanizing"), and (2) transplanting the non-human monoclonal antibody variable domains, but "cloaking" them with a human-like surface by replacement of surface residues ("veneering"). These meth-

ods are disclosed, for example, in, e.g., Jones et al., *Nature* 321:522-525 (1986); Morrison et al., *Proc. Natl. Acad. Sci., U.S.A.*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyer et al., *Science* 239:1534-1536 (1988); Padlan, *Molec. Immun.* 28:489-498 (1991); Padlan, *Molec. Immunol.* 31(3):169-217 (1994); and Kettleborough, C. A. et al., *Protein Eng.* 4(7):773-83 (1991).

**[0028]** The term antibody as used herein is intended to include antibody fragments thereof which are selectively reactive with apoCIII, or fragments thereof. Antibodies can be fragmented using conventional techniques, and the fragments screened for utility in the same manner as described herein for whole antibodies. For example,  $F(ab')_2$  fragments can be generated by treating antibody with pepsin. The resulting  $F(ab')_2$  fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

**[0029]** As used herein "selectively reactive" means that the antibodies recognize one or more epitope within apoCIII, but possess little or no detectable reactivity with control proteins, such as bovine serum albumin, under standard conditions such as those disclosed herein.

**[0030]** When the test compounds comprise nucleic acid sequences, such nucleic acids may be chemically synthesized or recombinantly expressed as well. Recombinant expression techniques are well known to those in the art (See, for example, Sambrook, et al., 1989, *supra*). The nucleic acids may be DNA or RNA, and may be single stranded or double. Similarly, such nucleic acids can be chemically or enzymatically synthesized by manual or automated reactions, using standard techniques in the art. If synthesized chemically or by in vitro enzymatic synthesis, the nucleic acid may be purified prior to introduction into the cell. For example, the nucleic acids can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the nucleic acids may be used with no or a minimum of purification to avoid losses due to sample processing.

**[0031]** When the test compounds comprise compounds other than polypeptides, antibodies, or nucleic acids, such compounds can be made by any of the variety of methods in the art for conducting organic chemical synthesis.

**[0032]** In another aspect, the present invention provides methods for treating patients with type I diabetes comprising administering to the patient an amount effective of an inhibitor of apoCIII to reduce apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells. As used herein, an "inhibitor" of apoCIII includes compounds that reduce the transcription of apoCIII DNA into RNA, compounds that reduce translation of the apoCIII RNA into protein, and compounds that reduce the function of apoCIII protein. Such inhibiting can be complete inhibition or partial inhibition, such that the expression and/or activity of the apoCIII is reduced, resulting in a reduced ability to increase intracellular calcium concentration. Such inhibitors are selected from the group consisting of antibodies that bind to apoCIII; antisense oligonucleotides directed against the apoCIII protein, DNA, or mRNA; small interfering RNAs directed against the apoCIII protein, DNA, or mRNA, and any other chemical or biological compound that can interfere with apoCIII activity. In one embodiment, the inhibitor is identified using the compounds of the present invention.



In another embodiment, the inhibitor is selected from the group consisting of (a) apoCIII-selective antibodies, (b) antisense nucleic acid constructs derived from the apoCIII mRNA sequence (SEQ ID NOS:1, 3, and 5) (NCBI accession numbers X00567 (Human); J02596 (Rat); and X68359 (Macaque), respectively), and (c) small interfering RNA sequences derived from the apoCIII mRNA sequence (SEQ ID NOS:1, 3, 5, and 7) (NCBI accession numbers X00567 (Human); J02596 (Rat); X68359 (Macaque), and NM\_023114 (Mouse) respectively).

**[0033]** Methods for making antibodies against apoCIII or fragments thereof are disclosed above. Antibodies against apoCIII are commercially available (for example, from Academy BioMedical Company (Texas, USA) Chemicon International (California, USA); United States Biological (Massachusetts, USA), Novus Biologicals (Colorado, USA) Rockland Immunochemicals (Pennsylvania, USA).

**[0034]** Methods for making antisense oligonucleotides and small interfering RNA sequences against the apoCIII mRNA sequence are well known to those of skill in the art, based on the apoCIII sequences disclosed herein. Antisense oligonucleotides will be complementary to the mRNA expressed from the apoCIII gene, in order to bind to the mRNA to inhibit translation.

**[0035]** In a preferred embodiment for using small interfering RNAs, the RNAs are double stranded RNAs. Methods for using such double stranded RNAs are as described, for example in U.S. Pat. No. 6,506,559. For example, RNA may be synthesized in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). If synthesized chemically or by in vitro enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

**[0036]** In another aspect, the present invention also provides pharmaceutical compositions, comprising an inhibitor of apoCIII activity and a pharmaceutically acceptable carrier. In a preferred embodiment, the apoCIII inhibitor is selected from the group consisting of an antibody reactive with apoCIII or a fragment thereof, an antisense oligonucleotide against the apoCIII mRNA sequence, and a small interfering RNA sequence directed against the apoCIII mRNA sequence.

**[0037]** The inhibitors may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids,

stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the inhibitors may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

**[0038]** The inhibitor may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (e.g., solutions, suspensions, or emulsions). Inhibitor may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the antibody, and are not harmful for the proposed application. The inhibitor may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

**[0039]** Thus, in a further aspect, the present invention provides a method for diagnosing Type I diabetes or a propensity to develop type I diabetes comprising

**[0040]** (a) determining an amount of sialylated apoCIII in a blood serum sample from a subject;

**[0041]** (b) comparing the amount with an amount of sialylated apoCIII in a control blood serum sample; and

**[0042]** (c) diagnosing those subjects with an elevated amount of sialylated apoCIII in the blood serum sample relative to the control as having type I diabetes, or having a propensity to develop type I diabetes.

**[0043]** As used herein, the "control blood serum sample" can be any relevant control that provides a suitable standard against which to compare the amount of sialylated apoCIII in the subject's serum sample, including but not limited to blood serum samples from a non-diabetic subject; previous blood serum samples from the subject being tested, and known reference standards.

**[0044]** As disclosed herein, the inventor has discovered that the sialylated forms of apoCIII predominate in type I diabetic patients, and that analyzing the levels of sialylated apoCIII in blood serum relative to control will provide a better read out for diagnosis of patients with type I diabetes or a propensity to develop type I diabetes than analyzing apoCIII levels as a whole.

**[0045]** In a further embodiment, the level of mono-sialylated apoCIII in the subject relative to control is analyzed; in another embodiment, the level of di-sialylated apoCIII in the subject relative to control is analyzed; and in a further embodiment, both measurements are made.

**[0046]** The method does not require a specific amount of increase in sialylated apoCIII in the blood serum sample over control, although it is preferred that the increase is a statistically significant increase as measured by standard statistical measurements.

[0047] The diagnostic methods of the invention can be used in combination with any other diagnostic methods known in the art, in order to increase the accuracy of the assays.

[0048] In a further aspect, the present invention provides methods for identifying diabetic patients to be treated with anti-apoCIII therapy, comprising

[0049] (a) determining an amount of apoCIII in a blood serum sample from a diabetic subject and comparing it to a control blood serum sample; and

[0050] (b) identifying those subjects with an elevated amount of apoCIII in the blood serum sample relative to the control blood serum sample as being subjects to be treated with anti-apoCIII therapy.

[0051] As used herein, the "control blood serum sample" can be any relevant control that provides a suitable standard against which to compare the amount of apoCIII in the subject's serum sample, including but not limited to blood serum samples from diabetic subjects that do not benefit or have not benefited from anti-apoCIII therapy, previous blood serum samples from the subject being tested, and known reference standards.

[0052] In an embodiment of this aspect of the invention, the method further comprises treating those subjects identified as subjects to be treated with anti-apoCIII therapy with anti-apoCIII therapeutic compounds. Such compounds include, but are not limited to, test compounds identified by the methods of the invention as inhibiting the apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells; apoCIII antibodies; and antisense nucleic acids and small interfering RNAs that selectively bind to apoCIII mRNA as disclosed herein.

[0053] In this aspect, the methods of the invention provide a means to identify the patient population that will most benefit from anti-apoCIII therapy, and thus to minimize use of the therapeutic methods on those patients that will not benefit. Such patients can be any patient population that will benefit from anti-apoCIII therapy, preferably diabetic patients (type I or II), and most preferably type I diabetic patients.

[0054] According to this aspect of the invention, the amount of apoCIII can be of total apoCIII, or, preferably, of sialylated apoCIII. In further embodiments, the level of mono-sialylated apoCIII in the subject relative to control is analyzed; in another embodiment, the level of di-sialylated apoCIII in the subject relative to control is analyzed; and in a further embodiment, both measurements are made.

[0055] The method does not require a specific amount of increase in apoCIII in the blood serum sample over control, although it is preferred that the increase is a statistically significant increase as measured by standard statistical measurements.

#### EXAMPLES

[0056] Materials and Methods

[0057] Media The basal medium used both for isolation of cells and for experiments was a HEPES buffer (pH 7.4), containing (in mM): 125 NaCl, 5.9 KCl, 1.3  $\text{CaCl}_2$ , 1.2  $\text{MgCl}_2$ , 25 HEPES. Bovine serum albumin was added to the

medium at a concentration of 1 mg/ml. For cell culture, RPMI 1640 medium was supplemented with 100  $\mu\text{g/ml}$  streptomycin, 100 IU penicillin and 10% fetal calf-, normal human- or diabetic serum.

[0058] Preparation of cells Adult mice from a local colony (3) were starved overnight. Pancreatic islets were isolated by a collagenase technique and cell suspensions were prepared as previously described (4, 5). Cells were seeded onto glass coverslips and cultured at 37° C. in a humidified atmosphere of 5%  $\text{CO}_2$  in air.

[0059] Preparation and purification of sera: Sera from T1D patients and control subjects were collected, identically sterile-processed and stored frozen at -20° C. until used. The sera were heat-inactivated by incubation at 56° C. for 30 min. Thereafter  $\beta$ -cells were incubated overnight in RPMI 1640 culture medium with 10% of the sera and changes in  $[\text{Ca}^{2+}]_i$  were recorded, subsequent to depolarization with 25 mM KCl. The five T1D sera that induced an enhanced  $[\text{Ca}^{2+}]_i$  response were centrifuged and the supernatant was passed through a 0.45 mm sterile filter. Samples were loaded on Sep-Pak  $\text{C}_{18}$  (Waters, Ma) preconditioned with 0.1% TFA. After a wash with 0.1% TFA, proteins were eluted with 60% acetonitrile in 0.1% TFA and thereafter lyophilized. Batches of one milligram of the lyophilized sample were dissolved in 500  $\mu\text{l}$  0.1% TFA, centrifuged and injected into a RP-HPLC with a Vydac  $\text{C}_{18}$  (0.46x25 cm) column (Grace Vydac, Hesperia, Calif.). The separation was made using a linear gradient of 20-60% acetonitrile in 0.1% TFA for 40 min at 1 ml/min. Fractions of 1 ml were collected and lyophilized.

[0060] Purification of isoforms of apolipoprotein CIII (apoCIII) ApoCIII was purified from human serum by adsorption to a lipid emulsion and delipidation, followed by chromatography of the lipid-associated proteins under denaturing conditions in guanidinium chloride and urea, respectively, as previously described (6). The apoCIII isoforms were dialyzed against ammonium bicarbonate and lyophilized before use.

[0061] Measurements of  $[\text{Ca}^{2+}]_i$  Cells, attached to coverslips, were pretreated with the different compounds as described in the results and thereafter incubated in basal medium with 2  $\mu\text{M}$  fura-2AM (Molecular Probes, Eugene, Oreg.) for 30 min. The coverslips were mounted as the bottom of an open chamber and cells were perfused with medium. Fluorescence signals were recorded with a SPEX Fluorolog-2 system connected to an inverted Zeiss Axiovert epifluorescence microscope. The excitation and emission wavelengths were 340/380 and 510 nm, respectively. The results are presented as 340/380 excitation ratios, directly representative of  $[\text{Ca}^{2+}]_i$  (7).

[0062] Patch clamp Whole-cell  $\text{Ca}^{2+}$  currents were recorded by using the perforated-patch variant of the whole-cell patch-clamp recording technique to eliminate the loss of soluble cytoplasmic components. Electrodes were filled with (in mM): 76  $\text{Cs}_2\text{SO}_4$ , 1  $\text{MgCl}_2$ , 10 KCl, 10 NaCl, and 5 HEPES (pH 7.35), as well as amphotericin B (0.24 mg/ml) to permeabilize the cell membrane and allow low-resistance electrical access without breaking the patch. Pancreatic  $\beta$ -cells were incubated in RPMI 1640 medium with apoCIII (10  $\mu\text{g/ml}$ ) or vehicle overnight. The cells were bathed in a solution containing (in mM): 138 NaCl, 10 tetraethylammonium chloride, 10  $\text{CaCl}_2$ , 5.6 KCl, 1.2  $\text{MgCl}_2$ , 5 HEPES and

3 D-glucose (pH 7.4). Whole-cell currents induced by voltage pulses (from a holding potential of  $-70$  mV to several clamping potentials from  $-60$  to  $50$  mV in  $10$  mV increments,  $100$  ms,  $0.5$  Hz) were filtered at  $1$  kHz and recorded. All recordings were made with an Axopatch 200 amplifier (Axon Instruments, Foster City, Calif.) at room temperature (about  $22^\circ$  C.). Acquisition and analysis of data were done using the software program pCLAMP6 (Axon Instruments, Foster City, Calif.).

**[0063]** Protein characterization Primary sequence was obtained in ABI 494C and cLC sequencers. Protein molecular weights were determined by electrospray mass spectrometry (AutoSpec hybrid tandem mass spectrometer, Micro-mass). For recording of positive-ion conventional-ES spectra, samples ( $16$  pmol/ml) were introduced into the ES interface by infusion or loop injection at a flow rate of  $3$  ml/min. To determine the position of the glycosylation, the native protein was digested with trypsin  $1:10$  w/w (Promega, Madison, Wis.). The resulting fragments were separated by HPLC using a Vydac  $C_8$  ( $2.1 \times 150$  mm) and a gradient of  $0$ - $50\%$  B in  $50$  min (buffer A,  $5\%$  acetonitrile/ $0.1\%$  TFA; B,  $80\%$  acetonitrile/ $0.1\%$  TFA). The fragments separated were applied to mass analysis.

**[0064]** Quantification of apoCIII Sera were collected and prepared as described above. The relative amounts of apoCIII in T1D serum and control serum, respectively were evaluated by comparisons of the peak area corresponding to apoCIII in the second RP-HPLC.

**[0065]** Flow cytometric analysis of cell death RINm5F cells were cultured for  $36$  h in the presence of  $10\%$  control serum, control serum and  $40$   $\mu$ g/ml apoCIII or T1D serum with or without  $100$  or  $200$   $\mu$ g/ml anti-apoCIII. The whole cell population was collected and stained with EGFP-conjugated Annexin V and propidium iodide (PI) (BD Pharmingen) and analyzed on a FACScan using CELLQuest acquisition software (Becton Dickinson, Immunocytometry System). FACS gating, based on forward and side scatter, was used to exclude cellular debris and autofluorescence and typically  $10,000$  cells were selected for analysis.

**[0066]** Statistical analysis Statistical significance was evaluated by Student's *t*-test and *P* values  $<0.05$  were considered significant. Data are expressed as means  $\pm$  SEM.

**[0067]** Results and Discussion

**[0068]** ApoCIII plays a key role in the regulation of the metabolism of triglyceride-rich lipoprotein (TRL) (8). It controls the catabolism of TRL by inhibiting the activity of lipoprotein lipase (LPL) (9, 10), thereby inducing hypertriglyceridemia. ApoCIII also inhibits the binding of remnant lipoproteins to catabolic receptors like the LDL receptor related protein (LRP) (11). When the apoCIII gene was disrupted in knock-out mice, there was a  $70\%$  reduction in triglyceride levels (12). Overexpression of human apoCIII in transgenic mice results in hypertriglyceridemia (13). ApoCIII is a  $79$ -residue,  $8.8$  kDa polypeptide (14) with three known isoforms that differ in the extent of glycosylation, CIII<sub>0</sub> (no sialic acid), CIII<sub>1</sub> (one sialic acid residue), CIII<sub>2</sub> (two sialic acid residues), contributing approximately  $10\%$ ,  $55\%$  and  $35\%$ , respectively, of total plasma apoCIII (15). Mutagenesis of the glycosylation site and expression in stable cell lines suggest that intracellular glycosylation is not required for the transport and secretion functions (16). Lack

of glycosylation does not affect the affinity of apoCIII for VLDL and HDL (16). Insulin is involved in the regulation of the apoCIII gene and induces a dose-dependent down-regulation of the transcriptional activity. Overexpression of the apoCIII gene could contribute to the hypertriglyceridemia seen in T1D patients (17). However, mice transgenic for the human apoCIII gene are neither insulin-resistant nor hyperinsulinemic (18). The concentration of apoCIII has previously been found to be higher in diabetic patients than in normal subjects (19-27). In insulin deficient rats there was no significant change in apoCIII in one study (28), while others have reported an increase in the proportions of the sialylated apoCIII (29, 30).

**[0069]** We have tested sera from seven newly diagnosed T1D patients (Table 1). Mouse pancreatic  $\beta$ -cells were cultured overnight with  $10\%$  sera from patients or normal subjects. Sera from five of the patients induced a significantly higher increase in  $[Ca^{2+}]_i$ , when cells were depolarized with  $25$  mM KCl, leading to an opening of voltage-gated L-type  $Ca^{2+}$  -channels, than sera from healthy blood donors (FIG. 1). Positive sera were pooled, concentrated and fractionated by reversed phase (RP)—HPLC. When fractions were tested on isolated mouse pancreatic  $\alpha$ -cells, one fraction (No. 3, FIG. 2A) eluting between  $52$ - $60\%$  acetonitrile, induced a more pronounced increase in  $[Ca^{2+}]_i$ , when cells were depolarized with high concentrations of  $K^+$ . After further purification of the component(s) in this fraction by repeated RP-HPLC runs (FIG. 2B,D), all fractions obtained were tested for effects on  $[Ca^{2+}]_i$  by incubation with mouse  $\alpha$ -cells overnight. The results from this second purification (FIG. 2B) showed a higher activity in fraction 2 (FIG. 2C). The protein that induced an increase in  $[Ca^{2+}]_i$ , indicated by the bar in FIG. 2D was determined. Sequence information was obtained both by C-terminal and N-terminal degradations. The sequences were identical to those of human apoCIII for  $20$  N-terminal and  $5$  C-terminal residues.

TABLE 1

Characterization of the T1D patients.

Pa-tient	Sex	Age (years)	Duration of T1D (weeks)	Medication	ICA	GAD	IA-2
1	M	32	<1	No*	Pos	Pos	Pos
2	F	32	12	No*	Neg	Pos	Neg
3	F	31	<1	No*	Pos	Pos	Pos
4	F	23	<1	No*	Pos	Neg	ND
5	M	19	<1	No*	Neg	Neg	Pos
6	F	35	<1	No*	Pos	Pos	Pos
7	F	33	28	No*	Pos	Pos	ND

Gender of the patients is designated as F, female, and M, male. The presence (Pos), absence (Neg) or no data available (ND) of antibodies to islet cells (ICA), GAD and tyrosine phosphatase IA2 (IA-2) are marked in the table. Healthy blood donors, all negative for ICA, GAD and IA-2, served as sources of control sera.

\*Insulin was the only medication administered.

**[0070]** We analyzed the apoCIII purified from T1D sera by mass spectrometry for subcomponent identification. The major components had apparent masses of  $9423$  and  $9714$  Da (FIG. 2E), corresponding to the mono- and di-glycosylated forms of apoCIII (theoretical, calculated values are: CIII<sub>0</sub>  $8764$  Da, CIII<sub>1</sub>  $9420$  Da, CIII<sub>2</sub>  $9712$  Da). To determine the positions of glycosylation, the protein was digested with trypsin and the fragments were separated by RP-HPLC. When the separated fragments were analyzed by mass

spectrometry, seven of the eight fragments showed masses identical to the theoretical values. The mass difference was localized to the C-terminal fragment, previously shown to be glycosylated (31). The absence of a non-glycosylated C-terminal fragment indicated that the isolated apoCIII forms were glycosylated. The relative amounts of apoCIII in T1D and control sera were evaluated by comparisons of the peak area corresponding to apoCIII in the second RP-HPLC (FIG. 3A). In T1D sera the levels of the sialylated isoforms of apoCIII (apoCIII<sub>1</sub> and apoCIII<sub>2</sub>) were four-fold higher than in non-diabetic sera. The non-sialylated isoform (apoCIII<sub>0</sub>) could not be detected.

[0071] The concentration of apoCIII has been reported to be between 6-14 mg/dl in control subjects and 9-27 mg/dl in diabetics (19, 20, 24-27). These variations may to a certain extent reflect the fact that various methods have been used for the determinations. In our experiments we have used 10% T1D serum in the culture medium instead of 10% fetal calf serum normally used, and therefore we chose to use concentrations in the range 10-50  $\mu$ g/ml. We have tested concentrations from 1-50  $\mu$ g/ml and with 1, 3 and 6  $\mu$ g/ml we did not see any effects, but with the concentrations 10-50  $\mu$ g/ml we had responses.

[0072] Commercially available apoCIII (Sigma), which constitutes a mixture of apoCIII<sub>1</sub> and apoCIII<sub>2</sub>, was tested at a concentration of 10  $\mu$ g/ml and was shown to stimulate  $\text{Ca}^{2+}$  influx as the product isolated from T1D sera (FIG. 3B). Co-incubation of  $\beta$ -cells with 100  $\mu$ g/ml of a polyclonal antibody against human apoCIII (Academy BioMedical Company, Houston, Tex.) blocked the activity of both the commercial apoCIII and the T1D serum (FIG. 3B,C). The polyclonal antibody had no activity by itself (data not shown). When testing the three isoforms of apoCIII by incubation of  $\beta$ -cells at a concentration of 10  $\mu$ g/ml, both the glycosylated (CIII<sub>1</sub> and CIII<sub>2</sub>) and the un-glycosylated isoform caused significantly higher increase in  $[\text{Ca}^{2+}]_i$  than cells that had been incubated with only the vehicle, 0.1% trifluoroacetic acid (TFA) (FIG. 3D). To study the effect of possible binding of apoCIII to serum lipoproteins in the culture medium, cells were incubated in basal buffer containing no serum and 10  $\mu$ g/ml apoCIII<sub>1</sub> for 2 and 6 h. There was a significantly elevated increase in  $[\text{Ca}^{2+}]_i$  upon depolarization in all the experiments where the cells had been exposed to apoCIII<sub>1</sub> for 6 h, but only in one out of three experiments where the incubation time was only 2 h (data not shown).

[0073] There was a higher percentage of dead cells in the cell population exposed to T1D serum. This effect was prevented by the addition of anti-apoCIII (FIG. 3E). Furthermore, the addition of pure apoCIII to culture medium with control serum resulted in an increased cell death.

[0074] To elucidate the molecular mechanism underlying the stimulatory effect of apoCIII on  $[\text{Ca}^{2+}]_i$ , the activity of voltage-gated  $\text{Ca}^{2+}$ -channels was analyzed in  $\alpha$ -cells incubated with 10  $\mu$ g/ml apoCIII. ApoCIII-treated cells displayed larger  $\text{Ca}^{2+}$ -channel currents than control cells during depolarizations in the range -10 to 10 mV, from a holding potential of -70 mV (FIG. 4A,B). These data demonstrate that apoCIII modulated the activity of the voltage-gated L-type  $\text{Ca}^{2+}$ -channel and that the effect occurred in the range of physiological depolarizations. So far immunoblot experiments have not revealed a direct interaction of apoCIII

with the  $\text{Ca}^{2+}$ -channel (data not shown). Future experiment will clarify to what extent this reflects imperfectness in the immunoprecipitation protocol or the actual true situation.

[0075] Our study shows that the sialylated forms of apoCIII were on average four-fold higher in sera from newly diagnosed T1D patients than in sera from healthy subjects. ApoCIII induced both an increase in  $[\text{Ca}^{2+}]_i$  and  $\alpha$ -cell death. The molecular mechanism underlying the stimulatory effect of apoCIII on  $[\text{Ca}^{2+}]_i$  reflected an activation of the voltage-gated L-type  $\text{Ca}^{2+}$ -channel. Addition of an antibody against apoCIII blocked the effects of both T1D serum and apoCIII on  $[\text{Ca}^{2+}]_i$  as well as on  $\beta$ -cell death. This suggests that the  $\text{Ca}^{2+}$  dependent cytotoxic effect of T1D serum on the pancreatic  $\beta$ -cell is mediated by apoCIII.

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ctcagagatt gccacaccg ttcacttcca cctccgcagc caagagatca gctactgacc 2460
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<210> SEQ ID NO 4  
<211> LENGTH: 101  
<212> TYPE: PRT  
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 4

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1           5           10           15
Ser Ala Arg Ala Asp Glu Gly Glu Gly Ser Leu Leu Leu Gly Ser Met
          20           25           30
Gln Gly Tyr Met Glu Gln Ala Ser Lys Thr Val Gln Asp Ala Leu Ser
          35           40           45
Ser Met Gln Glu Ser Asp Ile Ala Val Val Ala Ser Arg Gly Trp Met
          50           55           60
Asp Asn Arg Phe Lys Ser Leu Lys Gly Tyr Trp Ser Lys Phe Thr Asp
65           70           75           80
Lys Phe Thr Gly Leu Trp Glu Ser Gly Pro Glu Asp Gln Leu Thr Thr
          85           90           95
Pro Thr Leu Glu Pro
          100

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<210> SEQ ID NO 5

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<211> LENGTH: 3262
<212> TYPE: DNA
<213> ORGANISM: Macaca fascicularis
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (858)..(912)
<223> OTHER INFORMATION:
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<221> NAME/KEY: CDS
<222> LOCATION: (1048)..(1171)
<223> OTHER INFORMATION:
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2871)..(2988)
<223> OTHER INFORMATION:

<400> SEQUENCE: 5

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gccagcttgc tgcccttggc cgccctgccc caggccctgg tctctgatca gcaggtgacc      120
tttgcccagt gccctgggtc ctcatgtccc gctgccctgg agacaatata aaacaggctc      180
agaacctccc tgccctgccc ctctgttcat ccctagaggc agctgctcca ggtaatgccc      240
tctggggagg ggaagaggga ggggaggagg atgaagagga gcaagaggag ctccctgccc      300
agcccagcca gcaagcctgg agaaacactt gctagagcta aggaagcctc ggagctggac      360
gggtgcccc aacctctcat cataacctga agaaaatgga gggccgggag ggtgtgcaact      420
tgcccaaagc tacacagggg gtggggctgg aaatggttcc aagtgcaggc ttccccgtca      480
ttctgcaggc ttagggctgg aggaagcctt agacagccca gtcctacca gacagggaaa      540
ctgaggcctg gagagggcca gaaagcccca aagtcacaca gcattgtggc tgcaactggac      600
agagaccagt ccagaccgca ggtgccttga tgtccagtct ggtgggtttt ctgctccatc      660
ccacctacct ccttttgggc ccctcactag tccccttctg agagcccgta ttagcaggaa      720
gcaggccctt actccctctg gcagaccgag ctcatgtccc accttagggg ccatgccacc      780
tgtccaggga ggggtccaga ggcattgggg cctgggggtg ccctcacagg acaattcctt      840
gcaggaacag aggcgcc atg cag ccc cgg gta ctc ctt gtt gct gcc ctg      890
                Met Gln Pro Arg Val Leu Leu Val Ala Ala Leu
                1             5             10

ctg tca ctc ctg gcc tct gcc a gtaagcactt ggtgggactg ggctgggggc      942
Leu Ser Leu Leu Ala Ser Ala
                15

aggatggagg tggcttgggg atcccagtc taatgggtgg tcaagcagga gctcagggct      1002
cgcctagagg ccgateccacc actctcagcc ctgctcttcc ctgag ga gct tca gag      1058
                Arg Ala Ser Glu
                20

gcc gag gac acc tcc ctt ctt ggc ttc atg cag ggc tac atg cag cat      1106
Ala Glu Asp Thr Ser Leu Leu Gly Phe Met Gln Gly Tyr Met Gln His
                25             30             35

gcc acc aag acc gcc aag gat gca ctg acc agc gtc cag gag tcc cag      1154
Ala Thr Lys Thr Ala Lys Asp Ala Leu Thr Ser Val Gln Glu Ser Gln
                40             45             50

gtg gcc cag cag gcc ag gtacacccgc tggcctccct ccccatccct      1201
Val Ala Gln Gln Ala Arg
55

catgccagct ccctccattc ccacccgcc tgccctgggt agatcccagc aatggaatgg      1261
aggtgccagc ctcccctggt cctgtgcctc ttggcctcc tctttcctca cagggccttg      1321

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cctctgcctc ccaggttcac cccattctcc tgcctcagcc tccaagtag ctgggattac	1621
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tttccatggg tcagcgggga tggcttggg atgaaactgc tccacctcag atcaccaggc	1921
attggattct cctaagaag cctccacccc gacctctgag atgcgcagtt cacaacaggt	1981
ttcacactcc tgtgagaatc taatgccgcc taacctgaca gaaggcggg cttgggcggg	2041
attcctctgt caccatcac tcaactttgt ctgtgcagcc tggctcctaa ctggccatgg	2101
accagtaccc atctgtgact tgggggctgg ggacctctg gctaggggtt tgccttggga	2161
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tggggccagg gtgagtagaa gtgtgtcctt cctctcccat cctgccccg cccatcggtc	2281
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ctttgcagat gagaaaattg aggtctcagag cgattagggt acctgcccc gatcacacaa	2461
ctaataatc ctccaatgac tttccaaatg agaggctgcc tccctctgtc ctacctgct	2521
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aagttgagag ggtggcagg agagccagtg gggacatgg tgtgggtccc atagttgcct	2821
ccaaaggagt tctcatgccc tgetctgttg cttcccccta ctgatttag a ggc tgg	2877
	Gly Trp
gtg acc gat ggc ttc agt tcc ctg aaa gac tac tgg agc acc gtt aag	2925
Val Thr Asp Gly Phe Ser Ser Leu Lys Asp Tyr Trp Ser Thr Val Lys	
65 70 75	
gac aag tta tct ggg ttc tgg gat ttg aac cct gag gcc aaa ccc act	2973
Asp Lys Leu Ser Gly Phe Trp Asp Leu Asn Pro Glu Ala Lys Pro Thr	
80 85 90	
ctg gct gag gct gcc tgagacctca ataccceaag tccacctgcc tgtccatcct	3028
Leu Ala Glu Ala Ala	
95	
gccagctcct tgggtcctgc agcctccagg gctgccccg taggttgctt aaaagggaca	3088
gtattctcag tgcctccta ccgcacctca tgcctggccc cctccaggc aggggtgtcct	3148
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&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 99

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&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Macaca fascicularis

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 1 5 10 15

Ser Ala Arg Ala Ser Glu Ala Glu Asp Thr Ser Leu Leu Gly Phe Met  
 20 25 30

Gln Gly Tyr Met Gln His Ala Thr Lys Thr Ala Lys Asp Ala Leu Thr  
 35 40 45

Ser Val Gln Glu Ser Gln Val Ala Gln Gln Ala Arg Gly Trp Val Thr  
 50 55 60

Asp Gly Phe Ser Ser Leu Lys Asp Tyr Trp Ser Thr Val Lys Asp Lys  
 65 70 75 80

Leu Ser Gly Phe Trp Asp Leu Asn Pro Glu Ala Lys Pro Thr Leu Ala  
 85 90 95

Glu Ala Ala

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 2411

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Mus musculus

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: CDS

&lt;222&gt; LOCATION: (54)..(350)

&lt;223&gt; OTHER INFORMATION:

&lt;400&gt; SEQUENCE: 7

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agg cag tcg agg tta gtg agg act gcg agg cag aca ctt tgc tgt gtt 104  
 Arg Gln Ser Arg Leu Val Arg Thr Ala Arg Gln Thr Leu Cys Cys Val  
 5 10 15

caa atc caa gtc aag ggt aca aaa atg cag agc aat aaa gcc ttt aac 152  
 Gln Ile Gln Val Lys Gly Thr Lys Met Gln Ser Asn Lys Ala Phe Asn  
 20 25 30

ttg gag aag cag aat cat act cca agg aag cat cat cag cat cac cac 200  
 Leu Glu Lys Gln Asn His Thr Pro Arg Lys His His Gln His His His  
 35 40 45

cag cag cac cat cag cag caa cag cag cag cag cag caa cag cca ccc 248  
 Gln Gln His His Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro  
 50 55 60 65

cca cca ata cct gca aat ggc cag cag gcc agc agc cag aat gaa ggc 296  
 Pro Pro Ile Pro Ala Asn Gly Gln Gln Ala Ser Ser Gln Asn Glu Gly  
 70 75 80

ttg act att gac ctg aag aat ttt agg aaa cca gga gag aag acc ttt 344  
 Leu Thr Ile Asp Leu Lys Asn Phe Arg Lys Pro Gly Glu Lys Thr Phe  
 85 90 95

aca cag cgtagccgtc tctttgtggg caatcttccc cctgatatca ctgaggagga 400  
 Thr Gln

aatgaggaaa ctatttgaga aatatggaaa agcaggcgaa gttttcattc ataaggataa 460

aggctttggc ttatttcgct tggaacacag aaccctagcg gaaattgtca aagtggagct 520

ggacaacatg cccctccgtg ggaagcagct gcgagtgcgc ttcgctgtc acagtgcac 580

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&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 99

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 8

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 1 5 10 15

Val Gln Ile Gln Val Lys Gly Thr Lys Met Gln Ser Asn Lys Ala Phe  
 20 25 30

Asn Leu Glu Lys Gln Asn His Thr Pro Arg Lys His His Gln His His  
 35 40 45

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His	Gln	Gln	His	His	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Pro
50					55					60					
Pro	Pro	Pro	Ile	Pro	Ala	Asn	Gly	Gln	Gln	Ala	Ser	Ser	Gln	Asn	Glu
65					70					75					80
Gly	Leu	Thr	Ile	Asp	Leu	Lys	Asn	Phe	Arg	Lys	Pro	Gly	Glu	Lys	Thr
				85					90					95	
Phe	Thr	Gln													

---

I claim:

1. A method of identifying candidate compounds for the treatment of type I diabetes comprising contacting pancreatic  $\beta$  cells with an amount of apolipoprotein CIII ("apoCIII") effective to increase intracellular calcium concentration in the presence of one or more test compounds and identifying those test compounds that inhibit an apoCIII-induced increase in intracellular calcium concentration in the pancreatic  $\beta$  cells.

2. The method of claim 1 wherein the apoCIII comprises sialylated apoCIII.

3. The method of claim 1 wherein the apoCIII is substantially purified.

4. The method of claim 1 further comprising synthesizing the test compounds that inhibit an apoCIII-induced increase in intracellular calcium concentration in the pancreatic  $\beta$  cells.

5. A method for treating patients with type I diabetes comprising administering to a patient with type I diabetes an amount effective of an inhibitor of apoCIII to reduce apoCIII-induced increase in intracellular calcium concentration in pancreatic  $\beta$  cells, wherein the inhibitor is selected from the group consisting of an apoCIII-selective antibody, an antisense oligonucleotide directed against the apoCIII mRNA, and a small interfering RNA directed against the apoCIII mRNA.

7. A method for diagnosing Type I diabetes or a propensity to develop type I diabetes in a subject comprising

(a) determining an amount of sialylated apoCIII in a blood serum sample from a subject;

(b) comparing the amount with an amount of sialylated apoCIII in a control blood serum sample; and

(c) diagnosing those subjects with an elevated amount of sialylated apoCIII in the blood serum sample relative to the control as having type I diabetes, or having a propensity to develop type I diabetes.

8. A method for identifying diabetic patients to be treated with anti-apoCIII therapy, comprising

(a) determining an amount of apoCIII in a blood serum sample from a diabetic subject and comparing it to a control blood serum sample; and

(b) identifying those subjects with an elevated amount of apoCIII in the blood serum sample relative to the control blood serum sample as being subjects to be treated with anti-apoCIII therapy.

\* \* \* \* \*

专利名称(译)	ApoCIII与糖尿病的治疗和诊断		
公开(公告)号	<a href="#">US20040224304A1</a>	公开(公告)日	2004-11-11
申请号	US10/834525	申请日	2004-04-29
申请(专利权)人(译)	BIOSTRATUM INC.		
当前申请(专利权)人(译)	BIOSTRATUM INC.		
[标]发明人	BERGGREN PER OLOF		
发明人	BERGGREN, PER-OLOF		
IPC分类号	G01N33/564 C12Q1/00 G01N33/53 G01N33/567		
CPC分类号	G01N33/564 G01N2333/775 C12N15/113 G01N30/02 C07K16/18 G01N2800/042 A61P3/00 A61P3/10 C07K2317/76 C12N2310/11 C12N2310/14 G01N33/5041 G01N33/507 G01N33/92 G01N2440/38 G01N2500/10 G01N2800/50 G01N2800/52		
优先权	60/466517 2003-04-29 US		
其他公开文献	US9062306		
外部链接	<a href="#">Espacenet</a> <a href="#">USPTO</a>		

#### 摘要(译)

本发明提供了通过鉴定那些抑制apoCIII诱导的胰腺β细胞中细胞内钙浓度增加的试验化合物来鉴定用于治疗I型糖尿病的候选化合物的方法。本发明进一步提供了通过向患者施用有效量的apoCIII抑制剂以减少apoCIII诱导的胰腺β细胞中细胞内钙浓度增加来治疗患有I型糖尿病的患者。本发明提供了通过比较来自受试者的血清样品中唾液酸化的apoCIII的量与对照血清样品中唾液酸化的apoCIII的量并诊断那些受试者的方法来诊断I型糖尿病或发展I型糖尿病的倾向。相对于具有I型糖尿病或具有发展I型糖尿病倾向的对照，血清样品中唾液酸化的apoCIII的量升高。本发明提供了通过测定来自糖尿病受试者的血清样品中的apoCIII的量并将其与对照血清样品进行比较来鉴定用抗apoCIII疗法治疗的糖尿病患者的方法。并且鉴定血清样品中具有升高量的apoCIII的受试者相对于对照血清样品作为待用抗apoCIII疗法治疗的受试者。

Figure 1

