Novo Nordisk Inc.
Investigator-Initiated Research Trial Agreement

This Agreement is made and entered into as of the date signed by the last party to sign below (the "Effective Date") by and between:

Novo Nordisk Inc. (an affiliate of Novo Nordisk A/S of Denmark), having a business address at **100 College Road West, Princeton, New Jersey 08540** ("NOVO NORDISK"),

Board of Regents of the University of Oklahoma, 1000 Stanton L. Young Blvd., LIB-121, Oklahoma City, OK 73117 ("Institution") on behalf of **Dr. David Fields, Ph.D University of Oklahoma Health Science Center OUCP Diabetes & Endocrinology, 940 NE 13th St., CH 2B2426 Oklahoma City, OK 73104** ("Sponsor-Investigator")

RECITALS

WHEREAS, Sponsor-Investigator has designed a research study and wishes to receive certain support from NOVO NORDISK through Institution in order to carry out the research study (the "Study") described in the protocol entitled: **Impact of type 1 diabetes and being overweight cardiovascular risk factors and markers in children** (the "Protocol") at the Institution;

WHEREAS, NOVO NORDISK is willing to provide such support to the Sponsor-Investigator through Institution on the terms and conditions set forth in this Agreement with the understanding and agreement that NOVO NORDISK is not the sponsor of the Study;

WHEREAS, the research program contemplated by this agreement is of mutual interest and benefit to Institution and to NOVO NORDISK, will further the instructional and research objectives of Institution in a manner consistent with its status as a non-profit, state, educational institution, and may derive benefits for both NOVO NORDISK and Institution through the advancement of knowledge and through discovery and creation of new technologies.

NOW, THEREFORE, subject to the terms, conditions, and covenants hereinafter set forth, the parties agree as follows:

1. THE PARTIES' ROLES

1.1 Sponsor-Investigator. In connection with the conduct of the Study and this Agreement, Sponsor-Investigator shall at all times be considered a "Sponsor-Investigator" as that term is defined in 21 C.F.R § 312.3(b). Sponsor-Investigator has conceived and shall have the full responsibility for all aspects of the Study including without limitation its conduct, protocol design and all reporting related thereto, including without limitation, reporting of all information regarding adverse experiences and safety with respect to the Study, as such obligations are set forth in 21 C.F.R. §§ 312.31, 312.32, 312.33, 312.50, and 312.56. Sponsor-Investigator retains the right to use the data resulting from the Study for its own teaching and research purposes and programs subject to Section 7, 8, and 9 of this Agreement, provided, however, that in no event may such data be used for the commercial benefit of a third party.

1.2 NOVO NORDISK. NOVO NORDISK is not the "Sponsor" of the Study as such term is used in 21 C.F.R. Part 312, and Sponsor-Investigator shall not represent that NOVO

NORDISK is the sponsor of the Study at any time in any document, communication, or otherwise.

1.3 Institution. Obligations of the Sponsor-Investigator under this Agreement shall also apply to the Institution and Institution shall cause Sponsor-Investigator to comply with the terms hereof.

2. THE STUDY

2.1 Performance; Protocol Change. Sponsor-Investigator shall (a) perform the Study in accordance with the Protocol and (b) immediately inform NOVO NORDISK in writing of any changes in the Protocol that significantly affect the Study objectives or the research subject safety. Sponsor-Investigator shall have full and final discretion over changes to the Protocol, and shall notify NOVO NORDISK when such changes have been finalized. However, NOVO NORDISK's continued support for the Study shall be subject to NOVO NORDISK's acceptance of any such changes to the Protocol.

2.2 IRB Approval. Sponsor-Investigator shall submit the Protocol for review and approval to the appropriate review authorities, including, among others, the Institutional Review Board ("IRB") having jurisdiction over the facilities in which the Study will be conducted. Institution and Sponsor-Investigator shall provide NOVO NORDISK with written evidence of review and approval of the Study by the IRB prior to the initiation of the Study, and if applicable, the IRB's continuing review and approval of the Study.

2.3 Study Registration. Sponsor-Investigator is responsible for registering the Study with the Clinical Trials Data Bank pursuant to 42 U.S.C. § 282(j)(3) on clinicaltrials.gov no later than 21 days after the Study is opened for enrollment.

2.4 Adverse Events. Without limiting the generality of Section 1.1 above, Sponsor-Investigator shall assume and be solely responsible for reporting to the FDA any and all serious and unexpected adverse drug experiences associated with the use of any drug related to the Study as required under 21 C.F.R. § 312.32 and all other safety information associated with the use of such drug required under C.F.R. § 312.33. Sponsor-Investigator shall make such reports to the FDA, preferably on FDA form 3500, within fifteen (15) days of receiving information of any such serious and unexpected adverse drug experience whether or not the Study is being performed under an IND. Sponsor-Investigator will provide NOVO NORDISK with copies of any such reports at the same time they are submitted to the FDA. At NOVO NORDISK's request, Sponsor-Investigator and Institution will cooperate with NOVO NORDISK in investigating any such events.

2.5 Change of Sponsor-Investigator. If for any reason Sponsor-Investigator becomes unavailable to conduct the Study, Institution and/or Sponsor-Investigator shall promptly so notify NOVO NORDISK. If the parties cannot agree upon a mutually acceptable successor to Sponsor-Investigator, all further enrollments of subjects into the Study shall immediately cease and this Agreement may be terminated by NOVO NORDISK.

2.6 Consents. Sponsor-Investigator and Institution shall obtain the informed consent of each Study subject (and/or their duly authorized representatives). In the event a Study subject is a minor or otherwise incompetent, the consent of the parent or legal guardian of such Study subject must be obtained prior to participation by such Study subject in the Study.

2.7 Compliance with Laws. Sponsor-Investigator and Institution shall comply and shall require all Sub-Investigators and other Study personnel to comply, with the Protocol and

with all applicable laws, rules, regulations and other governmental requirements relating to the performance of their respective responsibilities under this Agreement. Without in any way limiting the foregoing, these obligations shall include the following:

(a) Records. Records relating to the Study, including receipt and disposition of the Study Materials, will be retained for at least two (2) years after the completion or earlier termination of the Study. Source documents, such as patient charts, will be retained for not less than five (5) years.

(b) Reports. Sponsor-Investigator will provide NOVO NORDISK in confidence with copies of all reports related to the Study that are submitted to FDA, other governmental agencies, or an IRB, as well as any correspondence with entities related to the Study.

(c) Privacy. Sponsor-Investigator, Institution and NOVO NORDISK agree to comply with the applicable laws and regulations relating to the privacy and confidentiality of patient health information, including without limitation, the federal Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and the Standards for Individually Identifiable Health Information, 45 C.F.R. parts 160 and 164 ("the HIPAA Privacy Regulation"). Sponsor-Investigator and Institution shall take all actions necessary to comply with such laws and regulations, including agreeing to amend this Agreement as necessary for compliance. Sponsor-Investigator and Institution also shall require that all Study subjects execute appropriate written authorizations to permit the use and disclosure of protected health information in accordance with the HIPAA Privacy Regulation.

3. FUNDING

3.1 Support. Subject to the terms and conditions of this Agreement, NOVO NORDISK agrees to provide support to the Sponsor-Investigator for the Study in the form of funding in the amount of sixty two thousand dollars, seven hundred sixty dollars ($ 62,760) (the "Funding") to be provided to Institution in amounts and at times as provided in Appendix I attached hereto.

3.2 Use of Funding. The Funding provided by NOVO NORDISK in support of the Study shall be subject to Sponsor-Investigator's and Institution's compliance with the terms of this Agreement. The Funding shall be used solely for purposes related to the Study. If the Study is halted or abandoned, then a pro-rata portion of such funding representing that portion of the Study not performed less non-cancelable obligations shall be refunded to NOVO NORDISK. If any funds are unused at the conclusion of the Study, Sponsor-Investigator shall promptly return such funds to NOVO NORDISK.

4. SPONSOR-INVESTIGATOR REPORTS

4.1 Quarterly Reports. During the term of this Agreement, Sponsor-Investigator shall update NOVO NORDISK quarterly with a confidential written report of the progress and findings of the Study for tracking purposes.

4.2 Final Report. Sponsor-Investigator shall provide NOVO NORDISK with a confidential final written report of Study results within thirty (30) days of the conclusion of the Study.

5. TERM AND TERMINATION OF THE AGREEMENT

5.1 Term. This Agreement shall become effective on the Effective Date and shall continue in force until the completion of the Study unless earlier terminated as provided herein.

5.2 Termination. This Agreement may be terminated:

(a) immediately by any party upon notice to all other parties, if such party reasonably believes that termination is necessary to protect the safety or welfare of the Study subjects;

(b) by any party upon notice to all other parties for a breach of a material provision hereof by any other party; provided the aggrieved party shall have first provided the defaulting party with a notice of such material breach and the defaulting party shall have failed to cure such breach within thirty (30) days following receipt of written notice thereof from the aggrieved party;

(c) by NOVO NORDISK, upon thirty (30) days prior written notice: (i) if NOVO NORDISK believes there are scientific reasons for such termination; (ii) if the purpose of the Study has for any reason become obsolete or no longer has any validity or purpose; or (iii) for any other reason which NOVO NORDISK deems appropriate; or

(d) by written mutual agreement.

(e) by Institution upon 30 days written notice to NOVO NORDISK

5.3 Effects of Termination. Upon termination of this Agreement, for whatever reason, the Sponsor-Investigator shall immediately deliver and return to NOVO NORDISK any and all information, documentation and materials supplied by NOVO NORDISK under this Agreement. For archival, compliance, and legal defense or claim purposes, Institution may retain a copy of all data, information, and materials provided to Institution hereunder.

5.4 Further Assurances. Notwithstanding Section 5.2 above, the parties acknowledge that certain legal obligations relating to the administration of the Study may continue to exist after the expiration of this Agreement. The parties shall cooperate and shall take such further action as may be reasonably required in order to carry out the provisions and purposes of this Agreement and any legal and regulatory obligations associated herewith.

5.5 Survival. The parties agree that the obligations contained in Sections 6 ("Publication"), 7 ("Option and Right of First Refusal"), 8 ("Confidentiality"), 9 ("Indemnification"), and 10 ("Publicity") shall survive the termination of this Agreement, as well as any other terms which by their intent or meaning are intended to so survive. No termination hereunder shall constitute a waiver of any rights or causes of action that either party may have based upon events occurring prior to the termination date.

6. PUBLICATION

Sponsor-Investigator may publish or disclose publicly data or results of the evaluation under this Agreement, but such publication or disclosure must comply with Sponsor-Investigator's confidentiality obligations under this Agreement. If Sponsor-Investigator desires to publish or disclose publicly data or results of the evaluation under this Agreement, Sponsor-Investigator must provide NOVO NORDISK with a copy of any proposed publication or disclosure at least sixty (60) days prior to the earlier of (i) making any submission for publication or public

disclosure or (ii) making such publication or disclosure. During such sixty (60) days, NOVO NORDISK may provide comments or suggest revisions.

7. OPTION AND RIGHT OF FIRST REFUSAL

7.1
Rights to inventions, improvements and/or discoveries, whether or not patentable or copyrightable, relating to Study, made solely by employees of Institution shall belong to Institution ("Institution Intellectual Property"); however, NOVO NORDISK is granted a royalty-free, non-exclusive right to use such inventions/improvements, and/or discoveries for its internal non-commercial research purposes. Rights to inventions, improvements and/or discoveries, whether or not patentable or copyrightable, relating to Study, made solely by employees of NOVO NORDISK, shall belong to NOVO NORDISK ("NOVO NORDISK Intellectual Property"); however, Institution is granted a royalty-free, non-exclusive right to use such inventions/improvements, and/or discoveries for its internal research, teaching and service purposes. Rights to inventions, improvements and/or discoveries, whether or not patentable or copyrightable, which are made as a result of Study and are made jointly by one or more employees of Institution and by one or more employees of NOVO NORDISK in performance of Study shall belong to both the Institution and to NOVO NORDISK ("Joint Intellectual Property"). Ownership will be determined by mutual agreement based on the contributions of each party. Institution will provide NOVO NORDISK with a complete written disclosure of any Institution Intellectual Property or Joint Intellectual Property made under this Agreement. Institution grants to NOVO NORDISK the first option to negotiate an exclusive license to Institution or Joint Intellectual Property on commercially reasonable terms.

8. CONFIDENTIALITY

8.1 Confidential Information. Sponsor-Investigator and Institution recognize, acknowledge and agree that all information, whether written, oral or in any other form, relating to NOVO NORDISK and/or its affiliated companies' business, products and/or services, and learned or acquired in connection with activities contemplated by this Agreement, is highly confidential and proprietary in nature, and is a valuable and unique asset of NOVO NORDISK and shall be considered "Confidential Information" all of which would be understood by a reasonable person to be confidential. Sponsor-Investigator and Institution understand and agree that all Confidential Information (i) is to be preserved and protected with reasonable care, (ii) is to be disclosed only to Study personnel who require access to the Confidential Information for the activities contemplated by this Agreement, and (iii) is not to be used, directly or indirectly, for purposes unrelated to the activities contemplated by this Agreement without prior written authorization from an authorized officer of NOVO NORDISK.

NOVO NORDISK recognizes, acknowledges and agrees that all information, whether written, oral or in any other form, relating to INSTITUTION and/or its affiliated companies' business, products and/or services, and learned or acquired in connection with activities contemplated by this Agreement, is highly confidential and proprietary in nature, and is a valuable and unique asset of INSTITUTION and shall be considered "Confidential Information." NOVO NORDISK understand and agree that all Confidential Information (i) is to be preserved and protected with reasonable care, (ii) is to be disclosed only to Study personnel who require access to the Confidential Information for the activities contemplated by this Agreement, and (iii) is not to be used, directly or indirectly, for purposes unrelated to the activities contemplated by this Agreement without prior written authorization from an authorized officer of INSTITUTION.

8.2 Exceptions. Notwithstanding the foregoing, Sponsor-Investigator's and Institution's obligations of confidentiality shall not apply to any information that (i) is, at the time of disclosure, properly and legally in the public domain, (ii) properly and legally comes into the public domain after disclosure hereunder through no breach of this Agreement, (iii) can be shown to have been in Sponsor-Investigator and/or Institution's possession at the time of disclosure hereunder, (iv) is acquired by Sponsor-Investigator and/or Institution from a third party who legally and properly received the Confidential Information, or (v) is required to be disclosed by law, regulation or court order; provided, or (vi) is disclosed to regulatory or oversight authorities, however, that the party intending to make such a disclosure of Confidential Information shall provide NOVO NORDISK with notice as soon as reasonably practicable so that NOVO NORDISK may contest such potential use or disclosure.

Notwithstanding the foregoing, NOVO NORDISK's obligations of confidentiality shall not apply to any information that (i) is, at the time of disclosure, properly and legally in the public domain, (ii) properly and legally comes into the public domain after disclosure hereunder through no breach of this Agreement, (iii) can be shown to have been in NOVO NORDISK's possession at the time of disclosure hereunder, (iv) is acquired by NOVO NORDISK from a third party who legally and properly received the Confidential Information, or (v) is required to be disclosed by law, regulation or court order; provided, however, that the party intending to make such a disclosure of Confidential Information shall provide INSTITUTION with notice as soon as reasonably practicable so that INSTITUTION may contest such potential use or disclosure.

9. INDEMNIFICATION

Each party will be responsible for its own negligent and intentional acts and omissions with the University's liability governed by the terms of the Oklahoma Governmental Tort Claims Act, 51 Okl. St. §§ 151 et seq.

10. PUBLICITY

Except as otherwise permitted under Section 6 ("Publication"), neither party shall originate any publicity, news release or other public announcement, written or verbal, whether to the public press or otherwise, relating to this Agreement, the Protocol, the Study conducted hereunder, or to any amendment(s) thereto without the other party's prior written consent, which consent shall not be unreasonably withheld.

11. NOTICES

Any and all notices and other communications required or permitted to be given hereunder except service of process, shall be made in writing and shall be personally delivered, sent by registered or certified mail, or sent by facsimile or electronic mail, addressed as follows, unless such address is changed by written notice hereunder:

If to Sponsor-Investigator: (Technical)	Dr. David Fields, Ph.D University of Oklahoma Health Science Center OUCP Diabetes & Endocrinology, 940 NE 13th St., CH 2B2426 Oklahoma City, OK 73104 Phone: 405 271 8000 ext 43083 Fax: 405 271 3093

If to Institution: (Business)	University of Oklahoma Health Sciences Center Office of Research Administration Attn: Kasie L. Nichols, M.S. 1000 Stanton L. Young Blvd., LIB-121 Oklahoma City, OK 73117
If to NOVO NORDISK:	Novo Nordisk Inc. Attn: Therese Leach 100 College Road West Princeton, New Jersey 08540 Fax: 609-580-2142
With a copy to:	Novo Nordisk Inc. Attn: Legal Department 100 College Road West Princeton, New Jersey 08540 Fax: (609) 919-7741

Any such notice shall be effective on the day it was delivered or transmitted or if mailed, on the date of its receipt.

12. MISCELLANEOUS PROVISIONS

12.1 Relationship of the Parties. It is expressly agreed that the relationship of NOVO NORDISK, Sponsor-Investigator, and Institution shall not constitute a partnership, joint venture, or agency. NOVO NORDISK, Sponsor-Investigator, and Institution, with respect to common activities of all, are independent contractors. Neither NOVO NORDISK, Sponsor-Investigator, nor Institution shall have the authority to make any statement, representations, or commitments of any kind, or to take any action, which shall be binding on the other, without the prior consent of the other parties to do so.

12.2 Entire Agreement. This Agreement contains the entire agreement between the parties hereto pertaining to the subject matter hereof and supersedes all prior and contemporaneous agreements, except those contemplated hereunder or not inconsistent herewith.

12.3 Force Majeure. No party shall be held liable or responsible to another party nor be deemed to have defaulted under or breached this Agreement for failure or delay in fulfilling or performing any term of this Agreement when such failure or delay is caused by or results from causes beyond the reasonable control of the affected party, including without limitation, fire, floods, embargoes, war, acts of war (whether war is declared or not), insurrections, riots, civil commotions, strikes, lockouts or other labor disturbances, acts of God or acts, omissions or delays in acting by any governmental authority or another party; provided, however, that the party so affected shall use reasonable efforts to avoid or remove such causes of non-performance, and shall continue performance hereunder with reasonable dispatch wherever or whenever such causes are removed. Each party shall provide the other parties with prompt written notice of any delay or failure to perform that occurs by reason of force majeure.

12.4 Debarment Certification. Institution warrants that Institution is not and does not use in any capacity hereunder the services of any person debarred under the Generic Drug Enforcement Act of 1992 subsections 306(A) or 306(B); or any testing facility disqualified under CFR Part 58, Subpart K, or a clinical investigator disqualified under 21 CFR

312.70, in connection with any of the services performed by Institution pursuant to this Agreement. Institution will immediately disclose in writing to NOVO NORDISK if any person, testing facility or clinical investigator engaged in the performance of services under this agreement is disqualified or debarred, or if any action, suit, claim, investigation or legal or administrative proceeding is pending or threatened, relating to the debarment or disqualification of Institution or any person performing services hereunder. Institution will require subsites to covenant that they are in compliance with the requirements of this section.

12.5 No Obligation to Prescribe. The Funding provided hereunder imposes no obligation, express or implied, for Sponsor-Investigator to prescribe, provide favorable formulary status for, or otherwise support NOVO NORDISK products, except such NOVO NORDISK products as are required to be dispensed or administered to subjects under the Protocol.

12.6 Amendments. Any alteration, modification, or amendment to this Agreement must be in writing and signed by all parties.

12.7 Assignment. This Agreement, any portion hereof, or any obligation hereunder shall not be assigned by Sponsor-Investigator or Institution.

12.8 Governing Law. This Agreement This Agreement shall be governed by the laws of the state of Oklahoma, without giving force and effect to its choice of law provisions. Any legal action in connection with this Agreement shall be filed in a court of competent jurisdiction in the state of Oklahoma, to which jurisdiction and venue NOVO NORDISK expressly agrees.

12.9 Invalidity. In the event that any provision of this Agreement is deemed by a court of competent jurisdiction to be in violation of any federal, state or local statutes, laws, rules or regulations, or is otherwise declared invalid or unenforceable by such court, the parties agree that such provision shall be of no force or effect and the remaining provisions shall remain valid and in full force and effect as though such superseded provision was not contained in this Agreement.

12.10 Waiver. No waiver of breach, term, provision or condition of this Agreement shall be considered valid unless in writing and signed by the party giving such waiver and no such waiver shall be deemed a waiver of any subsequent breach, term provision or condition contained in this Agreement. No failure on the part of any party to exercise, and no delay in exercising any right, remedy, power or privilege shall operate as a waiver thereof.

Notwithstanding anything to the contrary herein, the parties agree that Institution may post the following information on its web site regarding the study solely for patient recruiting purposes, with prior approval of the Institution 's IRB if required: Institution reference number, Institution contact information, full study title, disease site, inclusion and exclusion criteria, age range to identify when pediatric patients are involved.

If a provision of the attachments incorporated herein by reference or of the Protocol conflicts with or contains obligations or provisions that are in addition to those contained in this Agreement with regard to the Institution's responsibility, this Agreement shall take precedence on all such matters.

As applicable to Institution, the provisions of Executive Order 11246, as amended by EO 11375 and EO 11141 and as supplemented in Department of Labor regulations (41 CFR Part 60 et. seq.) are incorporated into this Agreement and must be included in any subcontracts

awarded involving this Agreement. The Institution represents that all services are provided without discrimination on the basis of race, color, religion, national origin, disability, political beliefs, sex, or veteran's status; it does not maintain nor provide for their employees any segregated facilities, nor will the Institution permit its employees to perform their services at any location where segregated facilities are maintained. In addition, the Institution agrees to comply with the applicable provisions of Section 504 of the Rehabilitation Act and the Vietnam Era Veteran's Assistance Act of 1974, 38 U.S.C. §4212.

IN WITNESS WHEREOF the parties hereto have executed this Agreement by their duly authorized representatives.

NOVO NORDISK INC.

By: [signature] Name: ALAN C MOSES

Title: Chief Medical Officer Date: 2 July 2007

INSTITUTION

By: [signature] Name: KASIE L NICHOLS

Title: Kasie L. Nichols, MS
Associate Director Date: 25 July 2007

Read and Acknowledged
SPONSOR-INVESTIGATOR

By: David Fields Date: 7/10/2007

REVIEWED
NOVO NORDISK LEGAL
DATE: 6/28/07
INITIALS: MHI

RESEARCH PROTOCOL OUTLINE

Title: The impact of type 1 diabetes and being overweight on cardiovascular risk factors and markers in children.

Principal Investigator: David Fields, Ph.D., Department of Pediatrics

Co-Investigators: Dr Sowmya Krishnan, M.D., Department of Pediatrics
Dr Piers R. Blackett, M.D., Department of Pediatrics
Dr Kenneth Copeland, M.D., Department of Pediatrics

ABSTRACT:

Type 1 diabetes mellitus is a chronic disease affecting many children all over the world. In the United States the prevalence rate is 1.7 per 1000 among individuals less than 19years of age (1). There have been significant improvements in its treatment since the discovery of insulin in 1922. The recent Diabetic Control and Complications Trial (DCCT) showed that better glycemic control leads to a significant decrease in complications due to diabetes (2); however an intensive insulin regimen is also associated with increased weight gain (3). It is yet to be determined if this is associated with the metabolic syndrome which is commonly seen in non diabetic obese children. To better elucidate this concern we propose to do a study to look at the presence of metabolic syndrome and a variety of cardiovascular risk factors in 4 study groups. Groups 1 and 2 will consist of 20 children each with type 1 diabetes who are normal weight and overweight respectively. Groups 3 and 4 will consist of 20 children each without diabetes who are of normal weight and overweight respectively.

Specific aims are:

(1) To define the body composition (specifically body fat and lean tissue) in children with type 1 diabetes who are normal weight and in children with type 1 diabetes who are overweight and compare those to similar children without diabetes;

(2) To define the degree of vascular elasticity in children with type 1 diabetes who are normal weight and in children with type 1 diabetes who are overweight and compare those to similar children without diabetes;

(3) To determine the lipid profile and serum apolipoprotein apoC-III in children with type 1 diabetes who are normal weight and children with type 1 diabetes who are overweight and compare those to similar children without diabetes.

Design and Methods: A matched pair-wise cross sectional study design consisting of 4 study groups will allow us to look at the impact of type 1 diabetes and being overweight independently on cardiovascular risk factors as measured by body composition, vascular elasticity and fasting lipid profile and to see if this effect is additive.

A. SPECIFIC AIMS

Obesity among children is becoming a growing concern because of the long term cardiovascular risk starting in childhood. This is especially important in childhood type 1 diabetes mellitus as with weight gain children may be getting insulin resistant and thus may be potentiating their cardiovascular risk factors. This aspect has not been well studied in children. Our study will be one of the first to look at all of the cardiovascular risk factors in children with type 1 diabetes and our study is well designed to look at the independent effect of being overweight on the cardiovascular risk factors. We hypothesize that children who are overweight with type 1 diabetes mellitus will have the worst cardiovascular risk factors and thus weight gain is one of the other modifiable risk factors that clinicians should pay close attention to when treating children with type 1 diabetes. *Our long-term goal* is to better understand the role of insulin resistance in the pathogenesis of the long term complications seen in children with type 1 diabetes.

Aim 1: Define the body composition in children with type 1 diabetes who are normal weight and in children with type 1 diabetes who are overweight and compare those to similar children without diabetes. We postulate that overweight children will have greater truncal mass than normal weight children and children with type 1 diabetes will have greater truncal fat mass compared to children who do not have type 1 diabetes.

Aim 2:Define the degree of vascular elasticity in children with type 1 diabetes who are normal weight and in children with type 1 diabetes who are overweight and compare those to similar children without diabetes. We postulate that overweight children will have reduced vascular elasticity compared to normal weight children and that children with type 1 diabetes will have reduced vascular elasticity compared to children who do not have type 1 diabetes.

Aim 3:Determine the lipid profile and serum apolipoprotein apoC-III in children with type 1 diabetes who are normal weight and children with type 1 diabetes who are overweight and compare it to similar children without diabetes. We postulate that overweight children will have higher serum cholesterol, triglyceride, apoC-III, LpB:C-III, LDL cholesterol and lower HDL cholesterol and apo C-III ratio(LpA:CIII to LpB:C-III) compared to normal weight children and that children with type 1 diabetes will have higher fasting triglyceride, cholesterol, apoC-III, LpB:C-III, LDL cholesterol and lower HDL cholesterol levels and apo C-III ratio compared to children without type 1 diabetes.

B. BACKGROUND AND SIGNIFICANCE

Background: Type 1 diabetes mellitus is an autoimmune disease caused by selective destruction of the insulin producing (beta) - cells in the islets of Langerhan in the pancreas. It is characterized by disturbed metabolism of carbohydrate, protein and fat resulting from a deficiency in insulin. Description of this disease dates back to the ancient Egyptian times. It is the second most common chronic disease in childhood after asthma and occurs in 1 of every 1500 children by the age 5 and in 1 of every 350 children by age 18. In the United States the prevalence among school age children is approximately 1.9 in 1000 (1). The annual incidence in United States remains 12 to 15 per 100,000 of the childhood population. Mortality and morbidity due to diabetes stem from metabolic derangements and from the long term complications that affect small and large vessels, resulting in nephropathy, neuropathy, ischemic heart disease, and arterial obstruction with gangrene of extremities. Cardiovascular disease (CVD) constitutes the major cause of morbidity and mortality in both type 1 and type 2 diabetes. Macrovascular CVD, its

treatment and link to diabetes have been investigated primarily in type 2 diabetes patients with relatively little known about risks for CVD specific to type 1 diabetes.

The prevalence of cardiovascular risk factors in a large population of children, adolescents and young adults with type 1 diabetes in Germany was reviewed by Schwab *et al.* They looked at various risk factors including obesity, hypertension, dyslipidemia, poor glycemic control and smoking in 27,358 patients. They found that more than half of the patients in each group had at least one cardiovascular risk factor (2). The Epidemiology of Diabetes Interventions and Complications (EDIC) study a long term follow up of the Diabetes Control and Complications Trial (DCCT) showed that the group that received intensive therapy during the DCCT had slower progression of intimal medial thickness than the conventional therapy group as measured 6 years after the DCCT ended (3). However intensive diabetes therapy in the DCCT frequently caused excessive weight gain (25% of subjects) and those who gained weight excessively were much more likely to develop components of the metabolic syndrome including dyslipidemia and hypertension (4). Few studies have looked at the cardiovascular risk profile in obese adolescents with type 1 diabetes who have good glycemic control. With the increased incidence of obesity in children, its close correlation to insulin resistance and its strong negative impact on cardiovascular health it is necessary to understand obesity in children with other chronic conditions. It is all the more interesting in children with type 1 diabetes for when adolescent children gain weight they may develop insulin resistance and thus an additional component more typical of type 2 diabetes.

Significance: Mortality rates in patients with type 1 diabetes mellitus exceed those in the general population despite improvements in care. Although not associated with many of the CVD risk factors recognized in type 2 diabetes, the age adjusted relative risk for CVD in type 1 diabetes may exceed that in type 2 diabetes. Though the DCCT showed that intensive insulin regimen is better in preventing the microvascular complications of diabetes it is unclear what happens when these patients gain excessive weight and develop insulin resistance. This study will describe the cardiovascular risk profile in children with type 1 diabetes with good glycemic control compared to obese children without diabetes. If results show that children with type 1 diabetes with good glycemic control have similar or worse cardiovascular risk profile as obese adolescents because of weight gain we may be looking at the addition of other pharmacological agents in the future to ameliorate this effect. This may result in children with type 1 diabetes having a better life expectancy, as well as improved health throughout life.

Review of Literature pertinent to this study

Body Composition: It is well known that obesity is associated with hyperinsulinemia and insulin resistance. Hyperinsulinemia in itself is also responsible for central fat distribution by inhibiting lipolysis and facilitating lipogenesis according to Kabadi *et al* who studied the influence of chronic insulin therapy in central adiposity (5). Weight gain and heightened risk of obesity as well as increased risk of hypoglycemia represented the most conspicuous side effects of intensive treatment observed in the DCCT trial. Body composition in adults with type 1 diabetes since childhood was studied by C.M Inberg *et al.* They found significant correlations between insulin dosage and whole body fat mass in diabetic females and between serum cholesterol levels and abdominal fat mass in diabetic males (6). In another study on adolescent girls with type 1 diabetes the observed overweight in adolescent females with type 1 diabetes was explained by an increased fat mass. Abdominal fat accumulation was associated with poor glycemic control, increased need for insulin and elevated lipid levels (7). An increase in muscle mass was shown to

cause the weight gain in a study done by Bartz *et al*. Using bioimpedance measurements in 274 diabetic children they showed that the weight gain was not due to increase in fat mass but due to increase in muscle mass (8). The number of daily insulin injection influenced the accumulation of body fat in girls with type 1 diabetics in a study done by Stefan Sarnblad *et al* (9). Ferrante E *et al* studied nutritional status, obesity and metabolic control in children with type 1 Diabetes mellitus. The study showed a high frequency (34.5%) of obesity and overweight in children with type 1 diabetes. There was higher frequency in diabetic females (10). Greenfield *et al* studied insulin resistance, intraabdominal fat, cardiovascular risk factors, and androgens in healthy young women with type 1 diabetes mellitus. They showed that there was greater insulin resistance in young women with type 1 diabetics compared with nondiabetic controls, unrelated to abdominal adiposity, lipids or androgens (11). De Block *et al* studied the impact of overweight on chronic microvascular complications in adult type 1 diabetic patients. Retinopathy and nephropathy were more prevalent in overweight type 1 diabetic patients with BMI>=25kg/m^2. However logistic regression analysis showed that diabetes duration and A1C remained the main determinants for retinopathy and neuropathy (12). Few studies have compared children with type 1 diabetes with obese children.

Vascular elasticity: Cardiovascular diseases account for the major morbidity and early mortality seen in type 1 diabetic patients. Evidence regarding the associations of adult coronary artery disease risk factors with atherosclerosis in young persons has been described (13). Analysis of the pulse wave form by tonometry of applanation provides a noninvasive means to record local arterial pressure and wave reflection (14). In 2002 Jarvisalo *et al* in their study of carotid artery intima media thickness in children with type 1 diabetes concluded that diabetes is an independent risk factor for increased carotid intima media thickness in children (15). Cheung *et al* studied the micro-vascular abnormalities in pediatric diabetic patients. They found that micro-vascular abnormalities commonly found in adult patients existed in the conjunctival circulation of all pediatric type 1 diabetes patients in varying degrees despite their young age (16). Stakos *et al* studied cardiovascular effects of type 1 diabetes mellitus in children. They found impaired carotid artery structure and function and decreased elastic properties of the aorta before demonstrable changes in left ventricular structure and function could be detected (17). Haller *et al* studied arterial stiffness in children with type 1 diabetes using radial artery tonometry. They found that children with type 1 diabetes have increased arterial stiffness compared with healthy control subjects (18). DCCT looked at intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. The mean progression of the intima-media thickness was significantly less in the group that had received intensive insulin therapy versus the one on conventional treatment. To date no study has compared children with type 1 diabetes and healthy obese children.

Lipid profile: Data on dyslipidemia in type 1 diabetic patients is scarce. Ladela *et al* evaluated the lipid profile in type 1 diabetic patients and its correlation to glycemic control. They found a high prevalence of hypercholesterolemia(54%) in diabetic patients (19). Duration of diabetes correlated with LDL cholesterol level while insulin dose correlated with triglyceride levels. Balkau *et al* in 1998 found a correlation between high daily insulin dose and triglyceride concentrations (20). Purnell *et al* looked at the influence of excessive weight gain with intensive therapy of type 1 diabetic patients on lipid level and blood pressure from the DCCT trial. They found that in the intensively treated group the patients who had the highest weight gain also had an increase in their blood pressure, triglycerides, cholesterol and LDL cholesterol levels. The mean values when compared between the group with the highest quartile weight

gain and the lowest was for triglycerides,-0.99 mmol/l vs 0.79mmol/l; LDL cholesterol, 122mg/dl vs 106 mg/dl and apolipoprotein B, 0.89g/l vs 0.78g/l; all P<0.001. They concluded that intensive insulin regimen resulting in excessive weight gain caused unfavorable changes in lipid profile and blood pressure similar to those seen in insulin resistance syndrome. Thus these patients may be at increased risk of coronary artery disease with time (21). The correlation between visceral obesity, hepatic lipase activity and dyslipidemia in subjects from the DCCT who gained excessive weight was studied by Shalamar *et al.* They concluded in their study that elevated IAF in subjects with type 1 diabetes was related to an atherosclerotic dyslipidemia similar to that seen in individuals without diabetes who have metabolic syndrome. DCCT related weight gain positively correlated with subsequent intraabdominal fat (22). Using a validated diabetes model projected life expectancy, quality adjusted life expectancy, and total lifetime costs of complications in type 1 diabetes cohorts Palmer *et al* studied if the increased body weight and associated worsening of lipid profiles and BP would negate the benefits of improved glycemic control seen with intensive insulin regimen. Their results showed that intensive control even with weight gain did better but intensive therapy with no weight gain had the higher life expectancy and quality adjusted life expectancy and lower cost of complications compared to patients on intensive insulin regimen who gained weight (23).

C. PRELIMINARY STUDIES

None

D. RESEARCH DESIGN AND METHODS

1. Research design: This will be a matched pair wise cross sectional study of type 1 diabetic and non diabetic children between the ages of 13- 18 years who are of either normal weight or are overweight. Children will be screened for medical eligibility to participate in the study at their initial visit. If found eligible they will be administered a standard questionnaire for pertinent history, 24 hour food recall and physical activity. A complete physical exam will be done. All females of child bearing potential (that is menstruating) will undergo a urine pregnancy test. Children will have their body composition measured by Dual energy X-ray absorptiometry (DXA) and vascular elasticity measured using the HDI/ pulsewave analyzer. Blood will be drawn to check the fasting lipid profile, insulin, glucose, HbA1C and apolipoprotein C-III levels. They will be given a physical activity monitor to wear on their ankle for a period of 5 days.

2. Subjects.

The study population will consist of 4 groups of subjects. Group 1 will consist of 20 type 1 diabetic children who are of normal weight and group 2 will be 20 type 1 diabetic children who are overweight. Group 3 will consist of 20 healthy non diabetic children who are of normal weight while group 4 will consist of 20 non diabetic healthy children who are overweight. At baseline all subjects will be Tanner stage 3 Breasts or Genetilia or higher. The study sample will be structured in 20 groups of 4 kids where each quartet is comprised of 1 child from each of the 4 groups (Type 1diabetic normal weight, type 1 diabetic overweight, non diabetic normal weight and non diabetic overweight). Children in each quartet will be matched for age (± 2 years) and sex.