**ARIC Manuscript Proposal # 1001**

| | | |
|---|---|---|
| **PC Reviewed: 03/09/04** | **Status: A** | **Priority: 2** |
| **SC Reviewed: 03/10/04** | **Status: A** | **Priority: 2** |

**1.a. Full Title**: Oxidative Stress and Risk of Diabetes: The ARIC Study

**b. Abbreviated Title (Length 26 characters)**: Ox-LDL and Nitrotyrosine and diabetes risk

**2. Writing Group (list individual with lead responsibility first)**:

**Lead**: **Ron C. Hoogeveen, Ph.D.**
Address: Baylor College of Medicine
6565 Fannin Street, M.S. A-601
Houston, TX 77030
Phone: (713) 798-3407 Fax: (713) 798-7400
E-mail: ronh@bcm.tmc.edu

Writing group members: Christie M. Ballantyne
Heejung Bang
Bruce B. Duncan
Aaron Folsom
Gerardo Heiss
James Pankow
Other writing group members are invited to join.
(This proposal is based on the ancillary study Inflammatory Precursors of Type 2 Diabetes)

**3. Timeline**: 11/03 – 07/04

**4. Rationale**:

Type 2 diabetes is associated with increased cardiovascular morbidity and mortality.[1] In diabetic patients, postprandial hyperglycemia and hyperlipidemia drive the non-enzymatic oxidation and glycation of proteins and lipids, which can lead to a state of increased oxidative stress.[2] Experimental studies in cultured cells and animals indicate that oxidative modification of LDL enhances its atherogenicity.[3] Plasma levels of oxidized LDL have been shown to be significantly higher in patients with coronary artery disease compared to normal controls.[4] Furthermore, increased circulating levels of oxidized LDL have been found in diabetic patients and in subjects with impaired glucose tolerance.[5]

Hyperglycemia causes an increased production of nitric oxide (NO) and superoxide anion in human aortic endothelial cells,[6] which can lead to the formation of peroxynitrite, a powerful oxidant capable of nitrating tyrosine residues in endogenous proteins.[7] Therefore, the presence of nitrotyrosine in plasma proteins is considered an indirect marker of oxidative stress.[8] Reactive nitrogen species have been shown to be capable of nitrating the tyrosine residues of apo B and oxidized LDL recovered from human atherosclerotic aortas contain significantly higher levels of nitrotyrosine compared to LDL isolated from plasma of healthy donors.[9,10] Furthermore, a recent study showed that nitrotyrosine could be detected in the plasma of all 40 participating Type 2

diabetic patients, but not in 35 healthy control subjects.[11] Although data from numerous studies indicate that oxidized LDL is proinflammatory in nature, there is very limited data available on the relationship of oxidized LDL with either the degree of glucose intolerance or other oxidative stress markers. Therefore, we propose to investigate the association of plasma levels of oxidized LDL and nitrotyrosine with risk for type 2 diabetes.

References:


1. Isooma B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24:683-689.
2. Ceriello A, Taboga C, Tonutti L, et al. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: Effects of short- and long-term simvastatin treatment. Circulation 2002; 106:1211-1218.
3. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J. Biol. Chem. 1997; 272:20963-20966.
4. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler. Thromb. Vasc. Biol. 2001; 21:844-848.
5. Kopprasch S, et al. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes 2002; 51:3102-3105.
6. Consentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997; 96:25-28.
7. Ischiropolous H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. Arch. Biochem. Biophys. 1998; 356:1-11.
8. Ceriello A. Nitrotyrosine: new findings as a marker of postprandial oxidative stress. Int J Clin Pract Suppl 2002; 51-58.
9. Podrez EA, Schmitt D, Hoff HF, Hazen SL. Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro. J. Clin. Invest. 1999;103:1547-1560.
10. Leeuwenburgh, C. et al. reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. J. Biol. Chem. 1997; 272:1433-1436.
11. Ceriello A, Mercuri F, Quagliaro L, Assaloni R, Motz E, Tonutti L, Taboga C. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. Diabetologia 2001; 44:834-838.


**5. Main Hypothesis/Study Questions**:

Plasma levels of ox-LDL and nitrotyrosine are positively and independently associated with incident type 2 diabetes. These associations are stronger in dyslipidemic obese participants compared to normolipidemic lean individuals. Addition of an inflammation score will weaken the associations.

**6. Data (variables, time window, source, inclusions/exclusions)**:

Design: case-cohort study including a random sample of the visit 1 cohort free of diabetes and a random sample of incident diabetes cases ascertained at visits 2-4.
Primary Exposure Data: Oxidized LDL and nitrotyrosine measured on plasma samples from visit 1.
Covariates (from visit 1): age, gender, race, center, fasting glucose, fasting insulin, 2h glucose (visit 4), GAD-antibody, family history of diabetes, physical activity, BMI, WHR, hypertension, cigarette smoking, HDL-C, LDL-C, total cholesterol, triglycerides, NEFA, WBC, fibrinogen, vWF, IL-6, CRP, orosomucoid, sialic acid, adiponectin, leptin, complement C3, ICAM-1, ferritine, hemoglobin, ALT, GGT, medication use (incl. Statins, anti-hypertension and anti-diabetes medication).
Data analysis will include survival analysis with appropriate weighting for the case-cohort design and sampling scheme.

**7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes _X_ No**

**b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No**
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

**8.a. Will the DNA data be used in this manuscript? ____ Yes _X_ No**

**8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No**

**9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.**
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://bios.unc.edu/units/cscc/ARIC/stdy/studymem.html

_X_ Yes _____ No

**10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?**

**11. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.**