

## ARIC Manuscript Proposal # 1707

PC Reviewed: 10/12/10  
SC Reviewed: \_\_\_\_\_

Status: A  
Status: \_\_\_\_\_

Priority: 2  
Priority: \_\_\_\_\_

**1. a. Full Title:**

Relationship between circulating levels of IL-18 and the risk of type 2 diabetes in the Atherosclerosis Risk in Communities (ARIC) study

**b. Abbreviated Title (Length 26 characters):**

IL-18 and diabetes

**2. Writing Group:**

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(This proposal is based on the ancillary study Inflammatory Precursors of Type 2 Diabetes)

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. SN [please confirm with your initials electronically or in writing]

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### **3. Timeline:**

All laboratory analyses have been completed. Statistical analysis and manuscript preparation will start immediately upon approval of the manuscript proposal.

### **4. Rationale:**

#### *Magnitude of the Problem:*

Type 2 Diabetes is a leading cause of morbidity and mortality in most developed countries and there is substantial evidence that it is assuming epidemic proportions in many developing and newly industrialized nations. (1) The associated microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease) related with diabetes contribute to significant morbidity and mortality, reduced life expectancy, diminished quality of life and enormous health costs. (2) Diabetes is a considerable cause of premature mortality, a situation that is likely to worsen, particularly in low and middle income countries as diabetes prevalence increases. (3) Thus, there is a growing need for identification of the pathologic pathways of this disease with an ultimate aim of reducing the disease burden

#### *Role of IL-18:*

Increasing evidence suggests a role of pathophysiological factors involving proinflammatory cytokines, adipokines, and oxidative stress in insulin resistance and Type 2 Diabetes. (4) Several proteins have been identified in these inflammatory pathways, including the pro-inflammatory molecule Interleukin 18 (IL-18). IL-18 was first described as an interferon- $\gamma$  inducing factor. (5) It is now known to have other functions, including augmentation of cell adhesion molecules, synthesis of nitric oxide, chemokine production, induction of Fas ligand and stimulation of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin-6 (IL-6) production. (5) It is believed to be primarily produced by macrophages and dendritic cells throughout the body. (5) Though adipocytes have also been shown to produce IL-18, the non-adipocyte cells seem to be the major source of IL-18 in the adipose tissue. (5, 6) It is also believed to be produced by muscles in the insulin-resistant state. (7)

IL-18 has been associated with insulin resistance measured by HOMA (8, 9) IVGTT (10) or clamps (11). Cross-sectional studies have also shown that levels of IL-18 are higher in patients with type 2 diabetes when compared with the general population. (12, 13) In one prospective cohort, diabetes incidence was associated with higher levels

of IL-18 at baseline after adjustment for the other known risk factors for diabetes and insulin resistance. (14) However, the independent and direct contribution of IL-18 to the development of Type 2 Diabetes is still unknown.

The proposed study would be undertaken to determine whether higher plasma levels of IL-18 are associated with the development of Type 2 Diabetes in the middle-aged African Americans as well as white population. Additionally, we will investigate whether possible interactions between plasma levels of IL-18, free fatty acids, and markers of liver function modulate this association.

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

Circulating levels of IL-18 in the middle age are associated with the development of diabetes. Additionally, the interaction of IL-18 with free fatty acids, markers of liver function, and inflammatory markers [Interleukin -1(IL-1), Interleukin-6 (IL-6), Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and cell adhesion molecules (CAM)] will be examined in a secondary analyses.

### **6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).**

The case-cohort design, which was previously used to investigate the role of an inflammation score based on biomarkers, total adiponectin, leptin, and other biomarkers in the development of diabetes in ARIC (15, 16, 17) will be applied in this study. From eligible members of this baseline cohort, we selected and measured analytes on ethnicity-stratified (50% white, 50% African American) random samples of both cases of incident diabetes and eligible members of the full cohort (1,198 individuals in total). A few of the incident cases of diabetes overlapped with the cohort random sample, and a few were selected only via the cohort sample. Of those sampled, we excluded 45 for incomplete fasting (<8 h) or for not having values for all covariates.

Cases were defined on the basis of 1) a reported physician diagnosis, 2) use of antidiabetic medications, 3) a fasting ( $\geq 8$  h) glucose value  $\geq 7.0$  mmol/l, or 4) a nonfasting glucose value of  $\geq 11.1$  mmol/l. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the previous one, as previously described (15, 16, 17).

Data used as covariates will include baseline measurements of age, gender, center, ethnicity, parental history of diabetes, smoking, body mass index (BMI), waist-hip ratio (WHR), hypertension, fasting glucose and insulin, plasma levels of free fatty acids, gamma glutaryl transferase (GGT), and aminoalanine transaminase (ALT) as well as other biomarkers associated with inflammation measured in the cohort and previously in the ancillary study in question. Stratification/adjustment using these covariates will be done to assess the presence of effect modification and/or confounding. The primary independent variable will be baseline IL-18.

Statistical analyses will be performed using the SAS (SAS Institute Inc., Cary, NC) and SUDAAN statistical software packages, based on the case-cohort sampling design. Weighted ANCOVA will be used to compute adjusted means and proportions of socio demographic variables and risk factors. Weighted Spearman correlations will be applied

MS # 853 Duncan et al Diabetes 2003  
MS # 862 Pankow et al Diabetes Care 2004  
MS # 1001 Hoogeveen et al Diabetologia 2007  
MS # 417 Wang et al Am J Clin Nutr 2003

X   Yes             No

  X      **A. primarily the result of an ancillary study (list number\* 1995.09)**  
          **B. primarily based on ARIC data with ancillary data playing a minor**  
**role (usually control variables; list number(s)\* \_\_\_\_\_**  
**\_\_\_\_\_)**

**12. 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.**

1. International Diabetes Federation: Diabetes Atlas. 3<sup>rd</sup> Edition. 2007.
2. World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: Report of a WHO/IDF consultation. Geneva, World Health Org., 2006
3. Roglic G, Unwin N. Mortality attributable to diabetes: Estimates for the year 2010. *Diab Res Clin Prac* 2010; 27:15–19
4. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obes Relat Metab Disord* 2003:S25–S28
5. Dinarello CA. Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. *Am J Clin Nutr* 2006; 83:447S–455S
6. Skurk T, Kolb H, Muller-Scholze S, Röhrig K, Hauner H, Herder C. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. *Eur J Endocrinol.* 2005 Jun;152(6):863-8.
7. Krogh-Madsen R, Plomgaard P, Møller K, Mittendorfer B, Pedersen BK. Influence of TNF- $\alpha$  and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. *Am J Physiol Endocrinol Metab* 2006; 291: 108–114
8. Bruun JM, Stallknecht B, Helge JW, Richelsen B. Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. *Eur J Endocrinol.* 2007 Oct; 157(4):465-71.
9. Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK. Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. *Clin Immunol* 2005; 117:152–160
10. Bosch M, Lopez-Bermejo A, Vendrell J, Musri M, Ricart W, Fernandez-Real JM. Circulating IL-18 concentration is associated with insulin sensitivity and glucose tolerance through increased fat-free mass. *Diabetologia* 2005; 48:1841–1843

11. Strackowski M, Kowalska I, Nikolajuk A, Otziomek E, Adamska A, Karolczuk Zarachowicz M, Gorska M. Increased serum interleukin-18 concentration is associated with hypoadiponectinemia in obesity, independently of insulin resistance. *Int J Obes* 2007; 31:221–225
12. Aso Y, Okumura K, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intima-media wall thickness in patients with type 2 diabetes. *Diabetes Care* 2003; 26:2622–2627
13. Esposito K, Nappo F, Giugliano F, Di Palo C, Ciotola M, Barbieri M, Paolisso G, Giugliano D. Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care* 2003; 26:1647
14. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984–2002. *Diabetes* 2005; 54:2932–2938
15. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003 Jul;52(7):1799-805.
16. Duncan BB, Schmidt MI, Pankow J, Bang H, Couper D, Ballantyne CM, et al. Adiponectin and the development of type 2 diabetes - the ARIC Study. *Diabetes* 2004;53(9).
17. Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, et al. Leptin and incident type 2 diabetes: risk or protection? *Diabetologia* 2006 Sep;49(9):2086-96.