1.a. Full Title: Shedding of GP Ibα from platelets and risk for PAD

b. Abbreviated Title (Length 26 characters): GP Ibα and PAD

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

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3. Timeline: Glycocalicin measurements on Visit 3 (296) and 4 (489) EDTA plasma samples of PAD patients and controls were completed in May of 2007 and data submitted to the ARIC CC. Manuscript preparation will start upon approval by the ARIC publication committee. We anticipate that the manuscript will be completed in 2007.

4. Rationale:
Platelets play a critical role in hemostasis at the site of vessel injury. They also contribute to the initiation and propagation of atherothrombosis (1;2). Platelets function through their surface receptors that bind specific ligands or counter-receptors to trigger intracellular signals. Once activated by the ligand-receptor interactions, platelets aggregate to form thrombi at the site of plaque rupture as commonly observed in patients with myocardial infarction. Platelets will also aggregate with each other or with leukocytes in plasma. The aggregates travel down stream to
occlude smaller vessels, leading to thromboembolism, typically seen in stroke. In addition to thrombotic occlusion of the vessels, activated platelets release a large number of inflammatory cyto- and chemokines that stimulate and propagate injury to endothelial cells and promote proliferation of smooth muscle and fibroblast cells. The functional state of circulating platelets has, therefore, been widely used as a marker for atherothrombosis. However, platelets are anucleated cells that can not be cultured in vitro and stored at low temperature. These biological and technical limitations make studying platelet function in large populations, especially for longitudinal epidemiologic studies, extremely difficult. It is, therefore, highly desirable to identify plasma markers that could correlate with or be indicative of platelet functions. We hypothesize that glycocalcicin, the extracellular portion of the platelet receptor for von Willebrand factor (VWF), can serve an indicator for platelets activation and may correlate with the development and severity of peripheral artery disease (PAD).

Glycocalcicin is the extracellular domain of the ligand binding subunit of the platelet VWF receptor the GP Ib-IX-V complex (3). The receptor is critical for initiating platelet adhesion to VWF and collagen exposed at the site of injury. It is also involved in a process called shear-induced platelet aggregation (SIPA) at the area of high fluid shear stress (4). SIPA is encountered in the area of stenosis and responsible for platelet-derived thromboembolism. The GP Ib-IX-V complex is composed four subunits of GP Ibα, GP Ibβ, GP IX, and GP V with a stoichiometry of 2:2:2:1 (5). All four subunits belong to the family of leucine-rich proteins that are known to be involved in protein-protein interactions. The ligand binding subunit GP Ibα binds VWF, thrombin, P-selectin, and CD11b (MAC-1) (6-8), whereas the other subunits are required for efficient expression of the complex on the surface of platelets. GP Ibα is a type I transmembrane protein, with the ligand-binding region contained within the most N-terminal 300 amino acids (45 kDa fragment). The N-terminal binding domain is projected 80 nm above platelet surface by a rigid mucin stalk, primarily for sensing changes in fluid shear stress. GP Ibα is expressed on resting platelet in approximately 20,000/platelet, but its surface density reduces about 30% upon platelet activation. Traditionally, the reduction is attributed to the translocation of GP Ibα into the open canonical system (9), but a recent study strongly suggests that GP Ibα is proteolytically shedded by matrix metalloprotease (10), especially during systemic inflammation. The entire extracellular domain can thus be released into circulation as a soluble polypeptide called glycocalcicin. The level of plasma glycocalcicin is therefore correlated with platelet activation and shedding.

In addition to being a marker for platelet activation, as an adhesive ligand, glycocalcicin may also play a key role in mediating platelet and leukocyte adhesion to the vessel wall and platelet-leukocyte interaction in solution. Measurements of plasma glycocalcin could, therefore, provide new insights into the role of platelets in the initiation and propagation of atherothrombosis.

5. **Main Hypothesis/Study Questions:**
The working hypothesis is that plasma glycocalcicin is higher in patients with PAD as compared to controls. Glycocalcicin can, therefore, serve as marker for platelet activation and GP Ibα shedding.

Because plasma glycocalcicin has never been measured in large populations, the normal levels and their gender and ethnic differences are not known. The current study will provide this critical information, which can be valuable for studying a role of platelets and glycocalcicin in the development of PAD and other disease processes.
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).

We will test the hypothesis in age-eligible residents of the ARIC study areas, who were invited to take part in the ARIC study. This will be a case–control study design. For the study, PAD was defined by ankle–brachial index (ABI), which, together with ultrasonography, was measured on individuals at the third or fourth exams.

The inclusion criteria for the case–control study were defined as individuals who had valid ABI data at exam 3 or 4. Exclusion criteria included individuals in the exam 3 and 4, who were diagnosed as either coronary heart disease or stroke before exam 3. In addition, Bernard-Soulier syndrome and other types of thrombocytopenia (platelet count less than 100,000/µl) were exclusions applied to the selected cases and controls prior to analysis. The participants were divided into two groups. The Case group included individuals, whose ABI measurements were below the sex-specific cutpoints at exam 3 and 4. For each exam group, an equal number of noncase individuals were chosen as controls, stratified by field center. We proposed to analyze data from individuals consisted of a 1:1 case:control ratio. We had 148 PAD cases at exam 3 and 234 new PAD cases at exam 4. The sample size selected is 296 at visit 3 and 448 at visit 4 (total 744).

Glycocalycin was measured by an ELISA method using EDTA plasma obtained at the time of ABI measurement (exam 3 or exam 4).

Multivariable logistic regression models will be used to evaluate the relation between PAD status and plasma glycocalcin, modeled both as continuous variables and in tertiles. Consideration will be given to the effect of measurement error on the results of the analyses. Consideration will also be given to using SUDAAN software to adjust for sampling strategy in the logistic regression models, and SUDAAN will definitively be used for descriptive statistics. The established cardiovascular risk factors evaluated as potential confounders in the logistic regression models will include age, gender, race, center, BMI, years of cigarette smoking, incident CHD, incident diabetes, lipids (triglycerides, total cholesterol, LDL-C, HDL-C), and if possible platelet markers (surface expression of GP Iba).

Since glycocalcin has never been measured in large normal population, correlation of plasma levels to age, gender, and ethnicity will also be performed in control population alone. We will correlate glycocalyn with plasma cholesterol to measure whether the shedding is affected by membrane lipids, which correlate with plasma cholesterol content.

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://www.cscc.unc.edu/ARIC/search.php

__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. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ____ Yes  _X__ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _2004.09_)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________  __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

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.
Reference List


