Obesity-Related Increased γ′ Fibrinogen Concentration in Children and Its Reduction by a Physical Activity-Based Lifestyle Intervention: A Randomized Controlled Study

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Objective To determine if elevated plasma γ′-fibrinogen, typically involved in the formation of fibrinolysis-resistant clots, confers an increased risk for cardiovascular disease (CVD) and thrombosis in children as it does in adults. Although obesity-related hyperfibrinogenemia is frequently reported in children, the role of γ′ fibrinogen and its response to physical activity-based lifestyle are less clear in this population.

Study design In a randomized controlled 3-month physical activity–based lifestyle intervention, γ′ fibrinogen concentration was measured in 21 children (aged 14-18 years; Tanner stage > IV), including 15 in the obese group and 6 in the normal weight group, with body mass index percentiles for age and sex of >95 and <85, respectively.

Results The relationships between γ′ fibrinogen and other risk factors for CVD, such as markers of insulin resistance and subclinical inflammation, along with body composition (as measured by dual-energy X-ray absortiometry), were assessed before and after the intervention. γ′ fibrinogen concentration was higher in the obese group compared with the normal weight group ($P < .05$) and was correlated with other risk factors for CVD (adjusted $R^2 = 0.9$; $P < .05$), and insulin emerged as the major predictor of γ′ fibrinogen. The intervention reduced γ′-fibrinogen concentration ($P < .05$).

Conclusion Our data reveal: (1) elevated γ′ fibrinogen concentrations in obese insulin-resistant children compared with normal lean controls; (2) a relationship between γ′ fibrinogen and other CVD risk factors; and (3) physical activity–induced reduction in γ′ fibrinogen in obese children. *(J Pediatr 2013;163:333-8)*.

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Hyperfibrinogenemia along with hypofibrinolysis constitute a hypercoagulable state resulting in the accumulation of fibrin, with an increased risk for thrombotic events and cardiovascular disease (CVD).[1-6] In both adults and children, obesity is characterized by a range of derangements in the key components of the hemostatic system, including the presence of hyperfibrinogenemia.[7-12] Fibrinogen exists as a hexamer containing 2 sets of 3 different chains (Aα, Bβ, and γ) linked to one another by disulfide bonds.[13] In light of this high degree of heterogeneity of fibrinogen in humans, the link between fibrinogen and increased thrombotic risk and its potential role as a risk factor in obesity-related CVD also may be closely related to its composition, given that the architecture of the fibrin clot formed depends on the composition of these individual chains.

In this context, γ′ fibrinogen, which constitutes approximately 7% of total fibrinogen,[14] has recently emerged as an important biomarker in CVD.[15-20] Functional studies have suggested that the clots formed from γ′ fibrinogen have increased resistance to fibrinolysis.[15,19,21-26] In 2 studies, only fibrin stiffness was independently correlated with premature coronary artery disease among a set of variables that included measures of fibrin morphology and mechanics, along with an array of established hemostatic, inflammatory, and metabolic risk indicators.[25,26]

Although recent evidence in animals and adult humans points to an important role for γ′ fibrinogen in the development of CVD and thrombosis, its role in children, especially in obesity-related CVD, remains less clear. The data on γ′ fibrinogen from studies in adults cannot be directly extended to children, given the different hormonal and metabolic milieu in these 2 populations. In addition,

BMI	Body mass index
BSA	Bovine serum albumin
CRP	C-reactive protein
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
HOMA-IR	Homeostasis model of assessment–insulin resistance
IL	Interleukin
PBS	Phosphate-buffered saline

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P. B. is supported by Nemours Research Programs and the American Heart Association Scientist Development Grant Award. Oregon Health & Science University (OHSU) and D.F. have significant interest in Gamma Therapeutics, a company that may have commercial interest in this research and technology; this potential conflict of interest has been reviewed and managed by OHSU. The other authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2013.01.004

a potential difference in the regulation of fibrinogen in children compared with adults and the elderly has been suggested recently.[8,27]

The aims of the present study were to determine γ′ fibrinogen concentrations in obese insulin-resistant and lean normal children, and to evaluate the impact of a physical activity–based lifestyle intervention on γ′ fibrinogen concentration in relation to adiposity and other risk factors for CVD in children.

Methods

The study protocol was approved by the Nemours Children's Clinic Research Review Committee and Baptist Medical Center/Wolfson Children's Hospital Institutional Review Committee. The design, methodology, and certain results of this study have been described in detail previously.[8,28] A total of 21 children, matched by age and pubertal status (aged 14-18 years; Tanner stage > IV) were included in this randomized controlled intervention study. Of these, 15 were obese and 6 were lean, with body mass index (BMI) percentiles for age and sex of >95 and <85, respectively. Exclusion criteria included the use of β-adrenergic blockers or steroids, active participation in any structured exercise activity for ≥20 minutes twice a week or more, on a diet program, tobacco use, alcohol abuse, heart disease, diabetes, and liver or kidney disease. Adolescent girls in their follicular cycle were not studied unless they completed their period at least 2 weeks earlier and could not have been pregnant. Only postpubertal (Tanner stage >IV) children were included in the study. Tanner staging was assigned based on physical examination by a pediatrician and/or nurse practitioner according to the criteria of Tanner for breast development and pubic hair in females and genital development and pubic hair in males.

After fulfillment of these inclusion and exclusion criteria, all control subjects were asked to maintain their lifestyle, and all participants instructed to maintain a physical activity and diet history for at least 3 days before the baseline study. All studies were completed in a supervised and metabolically controlled environment, to minimize the effect of confounders on the study outcome variables. The study subjects were admitted to the Clinical Research Center at Wolfson Children's Hospital on the evening of the study day. Body weight and height were measured and body composition was assessed by dual-energy X-ray absorptiometry (DEXA) with a Hologic QDR 4500-A machine (Hologic, Waltham, Massachusetts). All blood samples were collected in duplicate the next day (study day) after a supervised overnight fast at the Clinical Research Center. These samples were collected between 2000 and 2002 and small aliquots of samples were safely stored at −80°C, thus minimizing multiple freeze thawing cycles. All biochemical measurements were performed in duplicate.

The physical activity-based lifestyle intervention was adapted from the popular weight management program Shapedown, as described previously.[8,28] The 15 obese subjects were randomized and assigned to either the obese intervention group (n = 8; 4 males and 4 females) or the obese control group (n = 7; 4 females and 3 males). Participants in the obese intervention group met with a nutritionist once a week for 3 months following the baseline study. They were also advised to perform aerobic physical activity, mainly brisk walking, for at least 45 minutes at least 3 times a week for 3 months. One weekly session was monitored at the Nemours Children's Clinic by the investigators, and at least 1 parent also participated in these monitored sessions. The other 2 sessions were monitored by the parents. The physical activity was supplemented by dietary changes as advised by the nutritionist. However, dietary changes were not quantified during the entire period of the study. Although the participants in the obese control group received usual care and were not included in any specific lifestyle program during the 3-month study period, they received general advice on increased physical activity and diet. They were not actively monitored during the study period, unlike the participants in the intervention group. For all obese subjects, anthropometry, DEXA, and blood sampling were repeated at the end of the 3-month intervention period. The lean control subjects were studied only at baseline.

Plasma samples were assayed for γ′ fibrinogen, total fibrinogen, and other risk factors for CVD, including glucose, insulin, interleukin (IL)-6, and C-reactive protein (CRP) before and after the 3-month randomized controlled intervention along with measurement of body composition by DEXA. All measurements except γ′ fibrinogen were performed as reported previously.[8,28]

γ′ fibrinogen was assayed using an enzyme-linked immunosorbent assay method developed in our laboratory and described previously.[16] For this, 96-well Maxisorp plates were coated with 50 μL of 1.5 μg/mL monoclonal antibody 2.G2.H9 (Upstate, Charlottesville, Virginia) in phosphate-buffered saline (PBS). The plates were blocked for 1 hour at 37°C with bovine serum albumin (BSA) in 250 μL of PBS/1% BSA/0.1% Triton X-100. Plasma samples were diluted 1:1000 in PBS/5 mM EDTA/0.1% BSA/0.1% Triton X-100, and 50 μL was added in triplicate wells for 1 hour at 37°C. Wells were washed 3 times with 250 μL of PBS/0.1% Triton X-100. Then 50 μL of horseradish peroxidase–conjugated sheep anti-human fibrinogen (Innovative Research, Southfield, Michigan) was diluted 1:2500 in PBS/0.1% BSA/0.1% Triton X-100, then incubated in each well for 1 hour at 37°C. Wells were washed 3 times with 250 μL of PBS/0.1% Triton X-100, after which 50 μL of 3,3′,5,5′-tetramethylbenzidine Super-Sensitive One-Component HRP Microwell Substrate (BioFX Laboratories, Owings Mills, Maryland) was added to each well, and the resulting mixture was incubated for 30 minutes at 22°C. Then 50 μL of 450-nm liquid stop solution for 3,3′,5,5′-tetramethylbenzidine microwells (BioFX Laboratories) was added per well, and absorbance was read at 450 nm with a PowerWave XS microplate reader (BioTek, Winooski, Vermont). Absorbance values of the standards were fit to a nonlinear equation for a second-degree polynomial with the least squares error method using Kaleidagraph software (Synergy Software, Reading, Pennsylvania).