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

Rehana Lovely, PhD[1], Jobayer Hossain, PhD[2], Jonathen P. Ramsey, BS[3], Venugopal Komakula, MD, MPH[3], Donald George, MD[4], David H. Farrell, PhD, FAHA[5], and Prabhakaran (Babu) Balagopal, PhD, FAHA[4]

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

From the [1]Department of Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX; [2]Department of Biomedical Research, Alfred I. DuPont Hospital for Children, Wilmington, DE; [3]Department of Biomedical Sciences, Missouri State University, Springfield, MO; [4]Nemours Children's Clinic, Jacksonville, FL; and [5]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR

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).

Table. Physical characteristics of the subjects

Characteristic	Lean group	Obese group		*P* value*
		Control	Intervention	
Patients, n	6 (3 M, 3 F)	7 (4 M, 3 F)	8 (4 M, 4 F)	
Age, years, mean ± SEM	16.0 ± 0.4	15.9 ± 0.5	15.6 ± 0.3	NS
BMI percentile, mean ± SEM	56.2 ± 1.1	99.1 ± 0.16	98.5 ± 0.9	<.01
Body fat, %, mean ± SEM	22.5 ± 2.1	43.6 ± 2.0	45.5 ± 2.3	<.01
Insulin, pmol/L, mean ± SEM	26.5 ± 12.6	129.3 ± 15.2	138.2 ± 9.0	<.01
Total fibrinogen, mg/dL, mean ± SEM	164 ± 7	353 ± 25	339 ± 25	<.01
CRP, mg/L, mean ± SEM	0.43 ± 0.13	3.95 ± 1.16	3.11 ± 0.72	<.01
IL-6, pg/mL, mean ± SEM	1.43 ± 0.14	4.42 ± 0.42	3.96 ± 0.31	<.01

F, female; *M*, male; *NS*, not significant.
*Unpaired 2-way *t* test, lean group versus the 2 obese groups (obese control and obese intervention) together. Although some of the data presented here were published previously,[11,32] data pertinent to the present study are included for clarity of discussion.

Baseline characteristics are summarized by lean, obese control, and obese intervention groups. Quantitative variables are presented as mean ± SEM. In addition to the mean, the median is also presented for variables that are nearly or marginally significantly deviated from the normal distribution. The 2-sample *t* test or Mann-Whitney *U* test, whichever was appropriate, was used to compare mean baseline characteristics of the control and intervention group. The Pearson or Spearman rank correlation coefficient, whichever was appropriate, was calculated between γ′ fibrinogen and risk factors for CVD, such as total fibrinogen, IL-6, BMI, insulin, homeostasis model of assessment–insulin resistance (HOMA-IR), and natural log-transformed high-sensitivity CRP. Only baseline data were used for this analysis. A backward stepwise variable selection method of multiple regression analysis of CVD risk factors was performed to identify the relative contribution of statistically significant predictors of γ′ fibrinogen. The 2-sample *t* test was used to examine the efficacy of intervention and compare the mean change in γ′ fibrinogen from baseline in the intervention and control groups of obese children. An ANCOVA model was used for the same comparison after adjusting for baseline γ′ fibrinogen. Change in γ′ fibrinogen concentration from baseline was used as the response variable, and the group (intervention and control) and baseline γ′ fibrinogen values were used as independent variables in the model. The Mann-Whitney *U* test was used to compare median percent change in γ′ fibrinogen from baseline. All tests were 2-tailed at a 5% level of significance. All statistical analyses were conducted using SPSS version 17.0 (SPSS Inc, Chicago, Illinois).

Results

The Table shows the baseline characteristics of the study subjects in each of the 3 groups: lean, obese intervention, and obese control groups. All subjects were age and maturity stage (Tanner stage >IV) matched. Because the 2 obese groups were similar in all variables, they were considered a single obese group for baseline comparisons with the lean group. Participants' physical characteristics and some of the biological factors have been reported previously,[8,28] but we have included some of these data pertinent to the present study in the Table for clarity of discussion. The concentration of γ′ fibrinogen was almost 2-fold higher ($P = .008$) in the obese participants compared with that in the lean control group (Figure 1, A). The ratio of γ′ fibrinogen to total fibrinogen did not differ significantly between the 2 groups (0.049 ± 0.009 in lean and 0.056 ± 0.005 in the obese groups; $P = .51$).

γ′ fibrinogen showed significant univariate correlations with various known risk factors for CVD. The correlations with BMI, insulin, IL-6, and log CRP ($P < .05$) are shown in Figure 2. To determine the significant influence of these variables on γ′ fibrinogen, we performed backward variable selection methods of multiple regression analysis, using 5 variables of CVD risk (BMI, HOMA-IR, IL-6, log CRP, and insulin) in the model. This analysis showed that at a significance level of $P < .05$, the best-fitting model involved only insulin (adjusted $R^2 = 0.594$; regression β-coefficient = 0.784; $P < .001$). Insulin alone explained approximately 60% of the total variance in γ′ fibrinogen in lean and obese participants.

We also examined the impact of physical activity on γ′ fibrinogen concentration in relation to other risk factors for CVD. Figure 3 shows the decrease in the elevated levels of γ′ fibrinogen after the 3-month physical activity–based intervention in the obese group ($P = .03$). In the

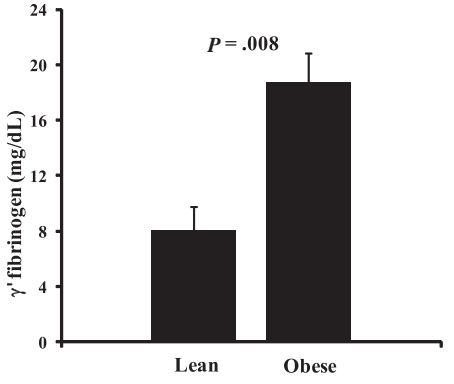

Figure 1. Baseline γ′ fibrinogen concentration (mg/dL) in lean and obese children. Data are mean ± SEM.

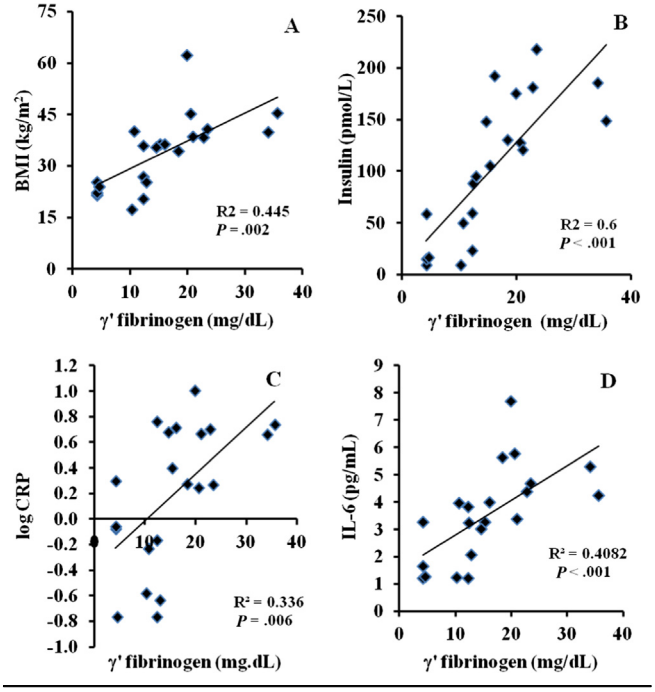

Figure 2. Relationships between γ′ fibrinogen concentration (mg/dL) and **A,** BMI (kg/m^2), **B,** insulin, (pmol/L), **C,** log CRP (arbitrary numbers), and **D,** IL-6 (pg/mL) in children.

corresponding control group, γ′ fibrinogen concentration tended to increase (P = .06) after 3 months. There was a significant (P = .002) difference in the mean change in γ′ fibrinogen concentration from baseline (preintervention) to after 3 months (postintervention) between control and intervention groups. The ratio of γ′ fibrinogen to total fibrinogen showed no significant changes after 3 months in both the intervention and control obese groups (P = .91 and .14, respectively). The 3-month intervention data on anthropometry, body composition, total fibrinogen, IL-6, CRP, and HOMA-IR have been reported previously.[8] There was also no direct relationship between the magnitude of reduction in the concentration of γ′ fibrinogen, total fibrinogen, and markers of inflammation, despite significant reductions in total fibrinogen, IL-6, CRP, and HOMA-IR after the intervention. In the obese control group, these factors did not show decreases similar to those in the obese intervention group during the 3-month period, and even tended to increase.

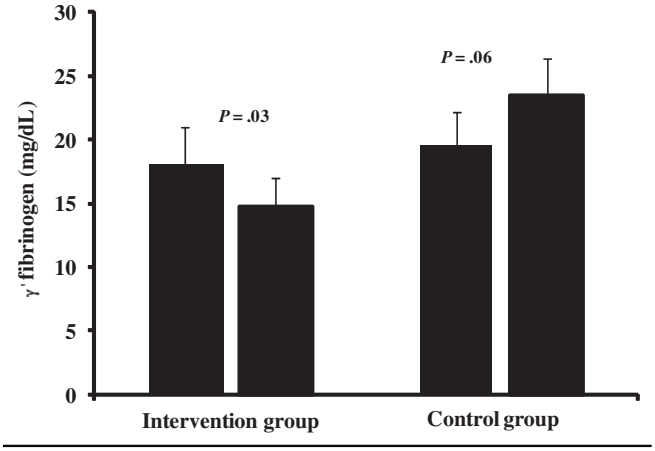

Figure 3. Effect of the physical activity–based lifestyle intervention on the concentration of γ′ fibrinogen (mg/dL) in the obese intervention group (n = 8) and obese control group (n = 7). Data are mean ± SEM.

Discussion

Although the exact role of the increased γ′ fibrinogen concentrations found in the present study and/or its threshold level that might impart detrimental effects in children remain unknown, the increased γ′ fibrinogen concentrations in obese children compared with lean children found in this study may have significant clinical relevance considering its potential for forming more fibrinolysis-resistant clots and its involvement in CVD and thrombosis in adults.[16,19-22,29] Clinically, hypofibrinolysis has been associated with an increased incidence of myocardial infarction and venous thrombosis.[30-33] Although our present data suggest an association between γ′ fibrinogen concentration and other risk factors for CVD, including total fibrinogen, future prospective studies should attempt to decipher the exact role of γ′ fibrinogen and its relationship to CVD and thrombosis. A recent study demonstrated a causative role for elevated total fibrinogen levels in thrombosis in vivo,[6] and another study suggested independence of γ′ fibrinogen and total fibrinogen levels under inflammatory settings.[18]

Because cardiovascular risk reduction is crucial early in the clinical course of obesity, the precipitous drop in the elevated γ′ fibrinogen concentrations in obese children after the 3-month intervention is notable, for 2 reasons. First, no studies in either children or adults have suggested that elevated levels of γ′ fibrinogen can be reduced by exercise or other modalities. Second, the reduction in γ′ fibrinogen concentration after our 3-month intervention supports the idea that even modest interventions are beneficial if initiated sufficiently early. It further suggests the plasticity of the tissues in children compared with adults and their susceptibility to even modest lifestyle changes. The reduction in γ′ fibrinogen concentration in the present study was accompanied by similar reductions in fat mass, insulinemia, HOMA-IR, total fibrinogen, and inflammatory factors, including CRP and IL-6. Whether elevated γ′ fibrinogen levels in adults show a similar response to a physical activity–based lifestyle intervention remains to be determined.

Although the present study was not designed to identify the underlying mechanisms of γ′ fibrinogen regulation in children, it is likely that the overall obesity-related inflammatory state present in these children may play a role in the elevation of γ′ fibrinogen and possibly its subsequent reduction in response to physical activity. The strong association between

γ′ fibrinogen and IL-6 and the synchronized reduction in both γ′ fibrinogen and IL-6 in response to the lifestyle intervention suggest this possibility. IL-6, a known stimulator of acute-phase proteins, plays an important role in regulating hepatic production of fibrinogen[34] and is also elevated in the obese children.[28] Interestingly, IL-6 appears to disproportionately up-regulate γ′ fibrinogen compared with total fibrinogen in HepG2 human liver cells.[35] Previous studies have suggested a possible role of insulin in fibrinogen synthesis.[36,37] In the present study, insulin emerged as the major predictor, explaining ~60% of the variance in γ′ fibrinogen. The postintervention decrease in insulin levels and the corresponding decrease in γ′ fibrinogen as seen in the present study also fit well with the potential role of insulin in γ′ fibrinogen regulation. Whether the reduction in γ′ fibrinogen is related to a decrease in its fractional synthesis rate, as reported for total fibrinogen in children,[8] or to an enhanced rate of degradation awaits further investigation. We did not specifically measure the turnover of γ′ fibrinogen in the present study.

This study has several limitations, including its relatively small sample size. In addition, measurements were performed retrospectively using frozen plasma samples. However, careful storage and avoidance of multiple freeze-thaw cycles likely rendered any impact on the samples negligible. Whether γ′ fibrinogen undergoes diurnal variation is not clear, but this possibility was minimized in the present study because the samples were obtained at similar times during the day and under steady-state conditions. For clarity of discussion of the novel data on γ′ fibrinogen, we have included previously reported data[8,28] also in the **Table**, and used them to help interpret our γ′ fibrinogen data.

Risk prediction for obesity-related comorbidities, such as CVD and type 2 diabetes, remains suboptimal even after the introduction of global risk assessment by various scores. Thus, the search for additional biomarkers is of critical importance. Based on our present data, the potential role of γ′ fibrinogen warrants further investigation. ■

We thank all of the volunteers for participating in this study. We also thank Linda Russell and Leslie Berry for their excellent support in conducting the study, the nursing staff of the Clinical Research Center at the Wolfson Children's Hospital for their superb assistance with clinical aspects of the study, and Melodie Gerry for her secretarial assistance. The skilled technical assistance of Brenda Sager, Lynda Everline, Astride Altomare, Karl Mann, and Shawn Sweeten is most appreciated. We also thank Drs Norman Patton and Edward Bayne for help with the study, and Dr Samuel Gidding for helpful suggestions.

Submitted for publication Aug 28, 2012; last revision received Nov 12, 2012; accepted Jan 2, 2013.

Reprint requests: Prabhakaran (Babu) Balagopal, PhD, Nemours Children's Clinic & Mayo Clinic College of Medicine, 807 Children's Way, Jacksonville, FL 32207. E-mail: bbalagop@nemours.org

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