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Objective—NADPH oxidase, one of the most important enzymes producing reactive oxidant species, is suggested to play a role in experimental atherosclerosis, but its role in human atherosclerosis is still unclear. We hypothesized that a reduced activity of NADPH oxidase might be linked to a reduced atherosclerotic burden.

Methods and Results—Thirty-one women carriers of hereditary deficiency of NOX2, the catalytic subunit of NADPH oxidase, were matched for sex and age with 31 controls and 31 obese women. Flow-mediated dilation and intima-media thickness, 2 surrogate markers of atherosclerosis, serum activity of NOX2, urinary isoprostanes, serum levels of nitrite/nitrate, and platelet production of isoprostanes and nitrite/nitrate were determined. Compared with controls (5.7±3.0% and 0.60±0.11 mm), carriers of NOX2 deficiency had higher flow-mediated dilation (9.2±5.0%; $P<0.001$) and lower intima-media thickness (0.50±0.11 mm; $P=0.002$), whereas obese women had lower flow-mediated dilation (3.2±2.1%; $P=0.007$) and higher intima-media thickness (0.71±0.15 mm; $P<0.001$). Compared with controls, carriers of NOX2 deficiency had lower urinary isoprostanes (132.6±87.3 versus 82.3±46.0 pg/mg creatinine; $P=0.007$) and serum NOX2 activity (24.9±19.3 versus 12.8±11.9 pg/mL; $P=0.004$) and higher serum nitrite/nitrate (23.8±7.6 versus 30.5±6.3 µmol/L; $P<0.001$), whereas obese women had higher urinary isoprostanes (132.6±87.3 versus 182.2±84.6 pg/mg creatinine; $P=0.008$) and serum NOX2 activity (24.9±19.3 versus 36.1±18.6 pg/mL; $P=0.008$) and lower serum nitrite/nitrate (23.8±7.6 versus 12.6±4.2 µmol/L; $P<0.001$). Flow-mediated dilation correlated with intima-media thickness ($r=–0.433$; $P<0.001$), serum NOX2 activity ($r=–325$; $P<0.001$), and urinary isoprostanes ($r=–0.314$; $P=0.002$). Ex vivo study showed that, compared with controls, platelets from carriers of NOX2 deficiency had lower isoprostanes ($P<0.001$) and higher nitrite/nitrate ($P<0.001$), whereas platelets from obese women had higher isoprostanes ($P<0.001$) and lower nitrite/nitrate ($P=0.013$).

Conclusion—The study shows reduced atherosclerotic burden in carriers of NOX2 deficiency, suggesting that oxidative stress generated by this enzymatic pathway is implicated in human atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2013;33:406-412.)**

Key Words: atherosclerosis ■ NADPH oxidase ■ oxidative stress

The oxidative stress theory of atherosclerosis is based on the concept that reactive oxidant species (ROS) generated by monocytes–macrophages and endothelial cells contribute to initiation of atherosclerotic process via oxidation of low-density lipoprotein.[1,2] Thus, ROS generated by enzymes, including myeloperoxidase, xanthine-oxidase, and NADPH oxidase, appear to be implicated in atherosclerosis.[3,4] Studies conducted in human atherosclerotic plaque demonstrated that NADPH oxidase is overexpressed and predominantly contributes to vascular oxidative stress.[5] Also, experimental studies demonstrated that the functional deficiency of NADPH oxidase is associated with reduced inflammation and atherosclerotic lesion.[6–8] However, it remains to be clarified whether ROS derived from NADPH oxidase have some role in the process of human atherosclerosis.

Chronic granulomatous disease (CGD) is a very rare genetic disorder[9,10] (1:250 000 individuals)[11] characterized by life-threatening infectious diseases.[12] It is characterized by defective activity of the innate immune system, caused by functional deficiency of NADPH oxidase subunits.[12] Among the NADPH oxidase subunits, the functional deficiency of gp91phox (NOX2), the catalytic subunit of NADPH oxidase, is the more frequent hereditary disorder.[12] In a previous study, we have shown that youth with hereditary deficiency of NOX2 (X-linked Chronic Granulomatous Disease, X-CGD) have reduced oxidative stress and enhanced flow-mediated

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dilation (FMD),[13] a surrogate marker of atherosclerosis that is predictive of cardiovascular events[14] in patients at risk or with established atherosclerosis. The fact that NADPH oxidase has vasoconstriction properties was confirmed in young adults with CGD, who were protected from ischemia-reperfusion injury.[15] Both studies,[13,15] however, could not exclude that concomitant therapy, including antibiotics and antifungal prophylaxis, influenced the results. To further explore this issue, we decided to study women relatives of X-CGD subjects, who were carriers of hereditary deficiency of NOX2 and were not under antibiotic or antifungal treatment. Therefore, we performed a cross-sectional study in which FMD and intima-media thickness (IMT), another surrogate marker of atherosclerosis,[16] have been measured in women carriers of NOX2 hereditary deficiency, in controls and in obese women, who were associated with NOX2 upregulation.[13] Herewith, we report for the first time that atherosclerosis burden, as assessed by FMD and IMT, is reduced in carriers of NOX2 hereditary deficiency.

Materials and Methods

Study Population

We performed a multicenter study in collaboration with the Italian Primary Immunodeficiencies Network. Among the women relatives of the 60 CGD patients registered in the National database,[12] we studied 31 women carriers of X-CGD who were willing to participate in the study. The group of carriers was composed of 23 mothers, 3 grandmothers, and 5 sisters of X-CGD patients.

Granulocyte function tests were performed to identify X-CGD carriers. Carrier detection of X-CGD was performed by searching for a mosaic pattern of oxidase-positive and oxidase-negative neutrophils in the nitroblue tetrazolium test[17] or dihydrorhodamine 123 flow cytometric analysis.[18] Genetic analysis of mutations was performed in 18 X-CGD carriers, as previously described.[19] Carriers of NOX2 hereditary deficiency were excluded if they were on antibiotic or antifungal treatment or assumed antibiotics or antifungal drugs in the previous month.

Thirty-one women, matched for age and atherosclerotic risk factors, were screened from routine visits and used as controls. Furthermore we included 31 age-matched obese women; BMI≥30 was used as cut-off to define obesity. In 2 women aged 9 and 10 years, obesity was defined as a BMI≥95th percentile.[20] Controls and obese patients were recruited from the outpatient clinic of our division at the I Clinica Medica of the Sapienza University of Rome.

Subjects were excluded from the study if they had liver disease, serious renal disorders (serum creatinine >2.8 mg/dL), cancer, myocardial infarction, unstable angina, acute cerebrovascular disease, deep venous thrombosis, or were on treatment with antioxidant vitamins.

The study was approved by the Ethical Committee. Each subject enrolled gave informed consent to participate in the study.

All participants received a questionnaire to quantitatively estimate the level of adherence to Mediterranean diet.[21]

Type 2 diabetes mellitus was diagnosed according to the American Diabetes Association definition.[22]

Hypercholesterolemia was classified as an low-density lipoprotein cholesterol level ≥160 mg/dL.[23]

Blood Sampling

After overnight fasting (12 hours) and supine rest for at least 10 minutes, blood samples were collected in vacutainers between 8 and 9 am (Vacutainer Systems, Belliver Industrial Estate) and centrifuged at 300*g* for 10 minutes to obtain supernatant, which was stored at −80°C until use.

Total cholesterol was measured by routine methods using an enzymatic colorimetric method on a Dimension RXL apparatus (Dade Behring AG, Switzerland).

Platelet Preparation

To obtain platelet-rich plasma (PRP), blood samples mixed with 3.8% sodium citrate (ratio 9:1) were centrifuged for 15 minutes at 180*g*. To avoid leukocyte contamination, only the top 75% of the PRP was collected, according to Pignatelli et al.[24]

Platelet pellets were obtained by double centrifugation (5 minutes, 300*g*) of PRP. Acid/citrate/dextrose (1:7 vol/vol) was added to avoid platelet activation during processing; samples were suspended in HEPES buffer in presence of 0.1% albumin, pH 7.35 (2×108/mL) per mL, and stimulated with or without 0.5 mmol/L arachidonic acid or with collagen (7 μg/mL; 10 minutes under stirring conditions). Supernatant was separated from cells by centrifugation (5 minutes, 300*g*) and stored until analysis.

Urinary 8-Iso-Prostaglandin $F_{2\alpha}$ Assays

Morning urine samples were collected from all participants between 7:00 and 9:00 am and stored in 10-mL aliquots at –80°C until analysis. Concentration of urinary isoprostane (8-iso-PGF2α) was measured by a previously described and validated enzyme immuno-assay method.[25] Ten microliters of urine was extracted on a C-18 solid phase extraction column. The purification was tested for recovery by adding a radioactive tracer (tritiated 8-iso-PGF2α; Cayman chemical). The eluates were dried under nitrogen, recovered with 1 mL of buffer, and assayed in 8-iso-PGF2α–specific enzyme immuno-assay kit (Cayman chemical). Urinary 8-iso-PGF2α concentration was corrected for recovery and creatinine excretion. Values are expressed as pg/mg creatinine. Intraassay and interassay coefficients of variation were 2.1% and 4.5%, respectively.

Platelet 8-Iso-Prostaglandin $F_{2\alpha}$ Assays

Concentration of 8-iso-$PGF_{2\alpha}$ in supernatant of arachidonic acid (0.5 mmol/L)-stimulated PRP was measured by a previously described and validated enzyme immunoassay method (Cayman chemical, MI)[25,26] and expressed as pmol/L. Intraassay and interassay coefficients of variation were 4.4% and 8.8%, respectively.

Nitrite/Nitrate Serum and Platelet Level Measurement

A colorimetric assay kit (Tema Ricerca, Italy) was used to determine nitric oxide (NO) metabolites, nitrite/nitrate, in the serum and supernatant of PRP (platelets=3×10^8/mL) activated with collagen (7 μg/mL) at 37°C for 15 minutes, as previously described.[27] All samples were filtered through a 10 000 molecular weight cut-off spin filter to remove, in particular, hemoglobin. Intraassay and interassay coefficients of variation were 2.9% and 1.7%, respectively.

Serum and Platelet Soluble NOX2-Derived Peptide

Soluble NOX2-derived peptide (sNOX2-dp), a marker of NADPH oxidase activation, was detected in serum and platelets supernatant by ELISA method, as previously described by Pignatelli et al.[28] The peptide was recognized by the specific monoclonal antibody against the amino acidic sequence (224–268) of the extra membrane portion of NOX2. Values were expressed as pg/mL, intraassay and interassay coefficients of variation were 5.2% and 6%, respectively, for serum and platelets.

C-Reactive Protein

C-reactive protein was measured by commercially available immunoassays (Tema Ricerca, Italy). Intraassay and interassay coefficients of variation were 9.5% and 9.0%, respectively.

FMD and IMT

FMD and IMT were performed with a 7.5-MHz linear-array transducer ultrasound system (SonoScape, China). Ultrasound assessment of FMD was investigated according to the recently reported guidelines,[29] as previously described.[30] Briefly, the study was performed in a temperature-controlled room (22°C) with the subjects in a resting supine state between 8 and 10 am. Brachial artery diameter was imaged using a 7.5-MHz linear-array transducer ultrasound equipped with electronic callipers, vascular software for 2-dimensional imaging, color and spectral Doppler, and internal electrocardiogram; the brachial artery was imaged at a location 2 to 5 cm above the antecubital crease; to create a flow stimulus in the brachial artery, a sphygmomanometric cuff was placed on the forearm; the cuff was inflated at least 50 mm Hg above systolic pressure to occlude artery inflow for 5 minutes; all vasodilatation measurements were made at the end of diastole. FMD was expressed as a change in poststimulus diameter, evaluated as a percentage of the baseline diameter.

The coefficient of variation for FMD measurements, obtained in 3 separate occasions, was 12.5%.

Longitudinal ultrasonographic scans of the carotid artery were obtained on the same day as the studies of the brachial artery reactivity and included the evaluation of the right and left common carotid arteries, 1 cm proximal to the carotid bulb. Three measurements of IMT were obtained from the right and left carotid arteries, respectively, and were averaged to determine the mean IMT for both sides combined. The coefficient of variation for IMT measurements, obtained on 3 separate occasions, was 4.90%.

Statistical Analysis

We used linear mixed-effects models to compare means across groups because the subjects in the study were matched by age and sex. We used subject-specific random intercepts with clusters of random effects identified by the matched triplets (X-CGD carriers, controls, and obese). The group indicators were included as fixed effects. Results were further confirmed by nonparametric tests with the rank transformation.

Data are presented as mean±SD, unless indicated otherwise. Categorical variables were reported as counts (percentage); independence of categorical variables was tested by χ^2 test. The correlation analysis was carried out by Pearson correlation test. Statistical significance was defined at $P<0.05$. Statistical analysis was performed with SPSS 18.0 for Windows (SPSS Inc, Chicago, IL).

Sample Size Determination

On the basis of the data emerged by a pilot study, we computed the minimum sample size with respect to a 2-sample Student *t* test, considering as (1) a relevant difference for FMD values to be detected between the X-CGD carriers and controls $|\delta|\geq 3\%$, (2) standard deviations homogeneous between groups SDs=5, and (3) type I error probability $\alpha=0.05$ and power $1-\beta=0.90$. This resulted in a minimum sample size of 26 subjects for each group. Sample size calculations were performed using the software nQuery Advisor, version 5.0, (Statistical Solutions, Saugus, MA).

Results

Clinical characteristics of the 3 groups, including X-CGD carriers, obese subjects, and controls, are reported in the Table. Molecular characterization of 18 X-CGD carriers is reported in the Table in the online-only Data Supplement.

As expected, BMI was significantly higher in obese subjects compared with the other 2 groups. We did not observe differences in fruit and vegetable dietary intake among the 3 groups (data not shown). Four X-CGD carriers and 1 control were affected by autoimmune diseases. No significant difference of drug therapy, including statins, angiotensin-converting-enzyme-inhibitors, corticosteroids, and methotrexate was detected among the 3 groups. Furthermore, no difference of C-reactive protein serum levels was found in the groups.

At baseline brachial artery diameter did not differ within the 3 groups (Table). Compared with controls, X-CGD carriers had significantly higher FMD and lower IMT (Table and Figure 1A and 1B). Conversely, obese subjects had lower FMD and higher IMT compared with controls (Table and Figure 1A and 1B).

Oxidative stress, as assessed by blood sNOX2-dp and urinary isoprostanes, was different among the 3 groups. Thus, compared with controls, X-CGD carriers had lower (–50%) sNOX2-dp (Table and Figure 2A) and lower (–40%) urinary isoprostanes (Table and Figure 2B). Compared with controls, obese women had higher sNOX2-dp and urinary isoprostanes (Table and Figure 2A and 2B).

NO generation, as assessed by serum nitrite/nitrate, differed in the 3 groups. Thus, compared with controls, X-CGD carriers and obese patients had significantly higher and lower serum nitrite/nitrate, respectively (Figure 2C).

A correlation analysis in the overall population showed that FMD correlated inversely with sNOX2-dp ($R=-325$; $P=0.001$ and urinary isoprostanes ($R=-0.314$; $P=0.002$) and positively with IMT ($R=-0.433$; $P<0.001$).

Ex vivo study showed that, compared with controls, platelet NOX2 and isoprostanes were lower in X-CGD carriers and increased in obese women (Figure 3A and 3B); also, compared with controls, platelet nitrite/nitrate was higher in X-CGD carriers and reduced in obese women (Figure 3C).

Discussion

This study provides the first evidence that in carriers of hereditary deficiency of NOX2, the burden of atherosclerosis, as assessed by FMD and IMT, is reduced, suggesting a role for ROS generated by this enzymatic pathway in human atherosclerosis.

Our study hypothesis was that relatives of patients with X-CGD could represent an interesting clinical model to explore the oxidative stress theory of atherosclerosis because they should have less oxidative stress as a consequence of incomplete activity of NOX2 and potentially less atherosclerotic burden. Laboratory analyses confirmed ≈50% lowered activity of NOX2 in the systemic circulation in X-CGD carriers concidentally with reduced oxidative stress as documented by impaired formation of isoprostanes, a reliable marker of oxidative stress.[31] These findings, which further support the key role played by NOX2 in the formation of isoprostanes,[13] were corroborated by an in vitro study showing lower isoprostane formation by platelets from X-CDG carriers.

FMD is recognized as a hallmark of systemic atherosclerosis and a useful marker to stratify the risk of cardiovascular disease in patients at risk,[29] or with established clinically manifested atherosclerosis.[14,32–35] Several experimental studies have shown a pivotal role of NADPH oxidase in modulating arterial tone.[8] This was particularly evident in animal knockout models of NADPH oxidase in which an increased arterial dilation was detected compared with wild-type.[36] A role for NADPH oxidase in inhibiting arterial dilation has been suggested also in humans in whom impaired artery dilation was

Table. Clinical Characteristics of X-Chronic Granulomatous Disease (X-CGD) Carriers, Controls, and Obese Patients

	X-CGD Carriers (n=31)	*P* Value*	Controls (n=31)	*P* Value*	Obese Patients (n=31)
Age, y	41.6±14.3	…	41.2±14.3	…	42.6±13.7
Sex	31 women	…	31 women	…	31 women
Systolic blood pressure, mm Hg	116±6	0.518	117±7	0.013	120±5
Diastolic blood pressure, mm Hg	72±8	0.672	72±10	0.078	75±7
BMI	24.2±4.3	0.692	24.8±5.7	0.002	32.5±4.8
Total cholesterol, mg/dL	199.8±79.7	0.882	196.7±88.7	0.02	244.9±81.3
Triglycerides, mg/dL	83.30±22.56	0.38	88.31±19.34	0.21	93.84±12.65
LDL, mg/dL	153.5±68.5	0.60	145.9±70.7	0.06	187.1±68.1
HDL, mg/dL	52.87±13.27	0.54	50.62±16.0	0.18	45.67±13.87
C-reactive protein, mg/L	2.24±1.0	0.09	1.78±1.1	0.566	1.94±1.1
Current smokers and	11/31	1.0	11/31	1.0	10/31
Cigarettes/d	12.4±4.9	0.812	12.0±3.7	0.921	11.8±5.2
Hypertension	4/31	1.0	4/31	0.919	5/31
Hypercholesterolemia	0/31	0.472	2/31	0.256	6/31
Type 2 diabetes mellitus	0/31	1.0	1/31	0.351	4/31
sNOX2-dp, pg/mL	12.8±11.9	0.004	24.9±19.3	0.008	36.1±18.6
Isoprostanes, pg/mg creatinine	82.3±46.0	0.007	132.6±87.3	0.008	182.2±84.6
NOx, μmol/L	30.5±6.3	<0.001	23.8±7.6	<0.001	12.6±4.2
IMT, mm	0.50±0.11	0.002	0.60±0.11	<0.001	0.71±0.15
FMD, %	9.2±5.0	<0.001	5.7±3.0	0.007	3.2±2.1
Brachial artery diameter (mm) at rest	3.20±0.45	0.263	3.07±0.35	0.08	3.27±0.57
Brachial artery diameter (mm) after 5 min of forearm occlusion	3.49±0.48	0.04	3.24±0.33	0.248	3.38±0.59
Drugs					
Statin	0/31	0.472	2/31	0.256	6/31
Angiotensin-converting enzyme inhibitors	4/31	1.0	4/31	1.0	5/31
Corticosteroid therapy	2/31	1.0	1/31	1.0	0/31
Methotrexate	1/31	1.0	1/31	1.0	0/31
Hydroxychloroquine	2/31	0.472	0/31	1.0	0/31
Lupus like-illness					
Photosensitive skin rashes	7/31	0.058	1/31	1.0	0/31
Mouth ulcers	3/31	0.605	1/31	1.0	0/31
Joint pains	3/31	0.605	1/31	1.0	0/31
Rectocolitis	1/31	1.0	0/31	1.0	0/31
Hypothyroidism	5/31	0.062	0/31	1.0	0/31

BMI indicates body mass index; FMD, flow-mediated dilation; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; and sNOX2-dp, soluble NOX2-derived peptide.

*Compared with controls.

associated with endothelial overexpression of the NADPH oxidase subunit p47phox.[37]

The present study supports and extends previous findings in youth,[13,15] showing that NOX2 possesses vasoconstriction property also in carriers of NOX2 deficiency, who disclosed, in fact, enhanced FMD compared with controls.

FMD is prevalently dependent on NO release from endothelium,[38] as also suggested by the significant correlation between FMD and serum plasma nitroso compounds.[39] Oxidative stress seems to play a pivotal role in modulating FMD via interfering with NO bioavailability and biosynthesis.[30] In accordance with this, we found an inverse correlation between FMD and urinary isoprostanes and an overexpression of NO metabolites in carriers of NOX2 deficiency, suggesting a link between enhanced artery dilation and impaired oxidative stress. We recognized that the analysis of serum nitrite/nitrate may be influenced by several confounding factors, including intraindividual variability, dietary nitrate uptake, inhalation of atmospheric gaseous nitrogen oxides, salivary formation, and renal function.[40] However, these findings were supported by ex vivo study demonstrating downexpression and overexpression of isoprostanes and nitrite/nitrate, respectively, in platelets from X-CGD carriers.

Other confounding factors could be reasonably excluded, as X-CGD carriers and controls were well matched for age, dietary habit, atherosclerotic risk factors, and concomitant therapy. A peculiar characteristic of X-CGD carriers was the coexistence of lupus-like illness, which is a feature found in

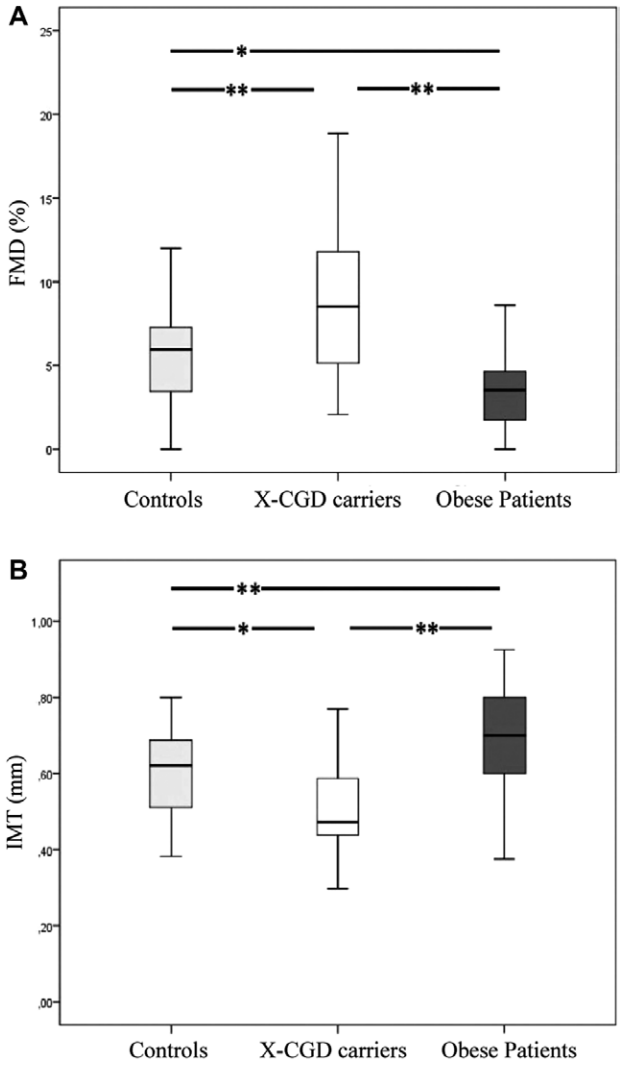

Figure 1. The box plots depict the values of flow-mediated dilation (FMD; **A**) and intima-media thickness (IMT; **B**), namely the minimum and maximum values, the upper and lower quartiles, and the median in X-linked Chronic Granulomatous Disease (X-CGD) carriers, controls, and obese patients. The median is identified by a line inside the box. The length of the box represents the interquartile range. The extreme lines of the box plot depict the maximum and the minimum of the nonoutlier range. **P*<0.05, ***P*<0.001.

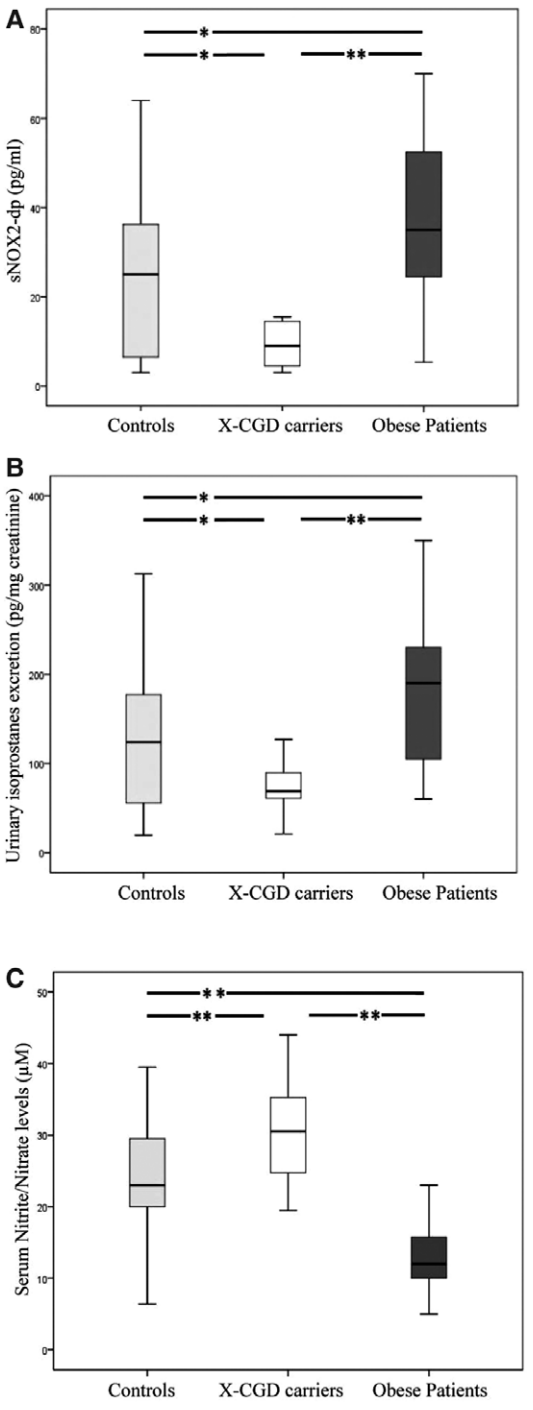

Figure 2. Box plots of serum levels of soluble NOX2-derived peptide (sNOX2-dp; **A**), urinary isoprostanes excretion (**B**), and serum nitrite/nitrate levels (**C**) in X-linked Chronic Granulomatous Disease (X-CGD) carriers, controls, and obese patients. **P*<0.05, ***P*<0.001.

≈30% of our population (Table). The prevalence was slightly lower than that previously observed,[41] but small sample size and different inclusion criteria may have accounted for that. However, this should not have biased our results, as lupus-like illness is associated with accelerated, but not with reduced, atherosclerosis.[42]

The lower IMT in carriers of NOX2 deficiency was another important evidence in favor of the reduced atherosclerotic burden in subjects with impaired ROS production. Thus, IMT is a noninvasive diagnostic measure of atherosclerosis that correlates with histology and predicts cardiovascular events, including myocardial infarction and stroke.[43] Of note, IMT significantly correlated with FMD, suggesting an interplay between the carotid atherosclerotic burden and artery dilatation.

The relationship between NOX2-derived oxidative stress and atherosclerotic burden was further corroborated by the behavior of oxidative stress and surrogate markers of atherosclerosis in obese women. Thus, they showed opposite features compared with X-CGD carriers, inasmuch as low FMD and high IMT were associated with upregulation of NOX2 and urinary isoprostane overexpression. These changes were supported by an ex vivo study, as platelets from obese women disclosed increase of isoprostanes and reduction of NO compared with controls.

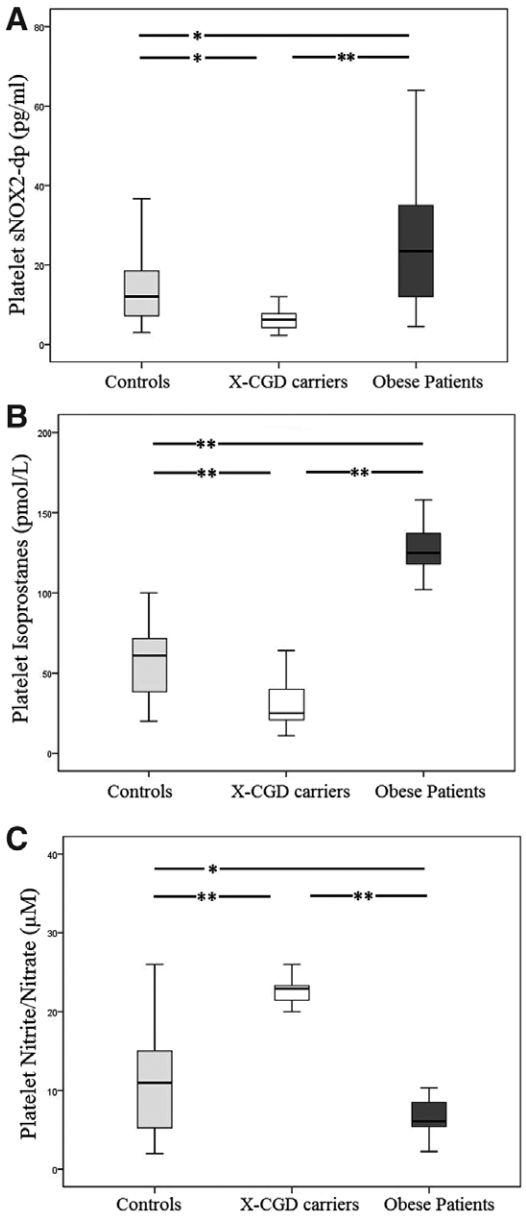

Figure 3. Ex vivo study: Box plots of platelet levels of soluble NOX2-derived peptide (sNOX2-dp; **A**), isoprostanes (**B**), and nitrite/nitrate (**C**) in X-linked Chronic Granulomatous Disease (X-CGD) carriers, controls, and obese patients. **P*<0.05, ***P*<0.001.

This study has implications and limitations. The interplay between NOX2 regulation and 2 surrogate markers of atherosclerosis suggests that this enzymatic pathway may be implicated in the process of atherosclerosis via the production of ROS. Our findings are apparently in contrast with a recent experimental study in mice in which NOX2 overexpression was associated with enhanced vascular oxidative stress and macrophage recruitment in the vessel wall, but had scarce impact in atherosclerotic progression.[44] This issue also needs to be investigated in humans by prospective analysis of the relationship between s-NOX2dp and surrogate markers of atherosclerosis in patients at risk of atherosclerotic disease.

A limitation of the study is the relatively small sample size. However, despite CGD being a very rare disease and the difficulty of finding carriers of NOX2 hereditary deficiency, our samples had adequate power to discover a difference between the X-CGD carriers and controls. Another limitation is that the study was done in women; therefore, the data cannot be extrapolated to men. Further study should be performed in non–X-linked CGD carriers to see whether NADPH oxidase is implicated in the atherosclerotic process also in men. To the best of our knowledge, the relationship between CGD and atherosclerosis is still unknown; prospective study should be done to see the progression of atherosclerosis in this population. This would be useful also to support our speculation that reduced IMT and enhanced FMD reflects NOX2-related atherosclerotic progression. Finally, we have only indirect evidence suggesting a role for NO in enhancing FMD of X-CGD carriers, and further study in human arteries should be done to explore such hypothesis.

In conclusion, this study provides evidence that, in humans, NOX2 activity is implicated in artery changes that are related to human atherosclerosis disease and could provide a new tool to follow-up atherosclerotic progression, and eventually its clinical complications.

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Disclosures

None.

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Table I

gp91phox mutations of 18 X-CGD carriers.

Carrier X-CGD	cDNA nucleotide change	Amino acid change
1	c.1+?_252+?del	p.M1_A84del
2	c.1+?_252+?del	p.M1_A84del
3	Del ≥550 kb+XK gene	Undetectable
4	c.252G>A	r.142_252del
5	c.388C>T	p.R130X
6	c.1287delT + c.1290delC	p.C428fs
7	c.1123G>T	p.E375X
8	c.1123G>T	p.E375X
9	c.469C>T	p.R157X
10	c.1357T>A	p.W453R
11	c.742dupA	p.ile248asnFsX36
12	p. Arg290Stop	p. Arg290Stop
13	c.937G>A	p.Glu309Lys
14	c.937G>A	p.Glu309Lys
15	c.937G>A	p.Glu309Lys
16	del 32,72 Kb (CYBB gene deleted)	Undetectable
17	del 32,72 Kb (CYBB gene deleted)	Undetectable
18	ca-cc change intron 4 in CYBB gene	Unknown