BMJ open

Association of digital vascular function with cardiovascular risk factors: a population study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004399
Article Type:	Research
Date Submitted by the Author:	04-Nov-2013
Complete List of Authors:	Kuznetsova, Tatiana; University of Leuven, Cardiovascular Sciences Van Vlierberghe, Eline; University of Leuven, Knez, Judita; University of Leuven, Szczesny, Gregory; FLOMEDI Company, Thijs, Lutgarde; University of Leuven, Jozeau, Dominique; FLOMEDI Company, Balestra, Costantino; Haute Ecole Paul Henri Spaak, D'hooge, Jan; University of Leuven, Staessen, Jan; KU Leuven,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology
Keywords:	population, endothelial function, vasodilation, photoplethysmography

DIII 11/4/2013 11:03 AM
BMJ Open

Association of digital vascular function with cardiovascular risk factors: a population study

Short title: Correlates of endothelial function

Tatiana Kuznetsova[1], Eline Van Vlierberghe[1], Judita Knez[1], Gregory Szczesny[2], Lutgarde Thijs[1], Dominique Jozeau[2], Costantino Balestra[3], Jan D'hooge[4], Jan A. Staessen[1,5]

Word Counts: Manuscript 4636; Abstract 300;

Number: Tables 2, Figures 4

[1] Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium; [2]FLOMEDI Company, Brussels, Belgium; [3] Environmental, Occupational & Aging Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium; [4]Division of Cardiovascular Imaging and Dynamics, Department of Cardiovascular Sciences, University of Leuven, Belgium; and [5]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands.

Correspondence to Tatiana Kuznetsova, M.D., Ph.D., Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, Campus Sint Rafaël, Kapucijnenvoer 35, Box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kuznetsova@med.kuleuven.be

ABSTRACT

Objectives: Vasodilation of the peripheral arteries after reactive hyperaemia depends in part on release of nitric oxide from endothelial cells. Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and PWA hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). We sought to evaluate the correlates of digital PPG PWA hyperemic responses as a measure of peripheral vascular function.

Design: The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) is a population-based cohort study.

Setting: Respondents were examined at one centre in northern Belgium.

Participants: For this analysis, our sample consisted of 311 former participants (53.5% women; mean age 52.6 years; 43.1% hypertensive), who were examined from January 2010 until March 2012 (response rate 85.1%).

Primary outcome measures: Using a fingertip PPG device, we measured digital PWA at baseline and at 30-second intervals for 4 minutes during reactive hyperaemia induced by a 5-minute forearm cuff occlusion. We performed stepwise regression to identify correlates of the hyperaemic response ratio for each 30-second interval after cuff deflation.

Results The maximal hyperaemic response was detected in the 30- to 60-second interval. The explained variance for the PPG PWA ratio totaled from 9.2% at 0-30 second-interval and 22.5% at 60-90-second time interval. The hyperaemic response at each 30-second interval was significantly higher in women compared to men ($P \leq 0.001$). The PPG PWA changes at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$) and at 0-240-second intervals decreased with higher body mass index ($P \leq 0.035$). These associations with sex, current smoking and body mass index were mutually independent.

Conclusions Our study is the first to implement the new PPG technique to measure digital PWA hyperaemic changes in a general population. Hyperaemic response, as measured by PPG, inversely associated with traditional cardiovascular risk factors such as male sex, smoking and obesity.

ARTICLE SUMMARY

Article focus

- Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease. Vasodilation of the peripheral arteries after reactive hyperaemia depends in part on release of nitric oxide from endothelial cells.
- Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and its hyperaemic changes. Alternative approach is based on photoplethysmography (PPG).). This optical technique enables detecting blood volume changes in microvascular beds in response to hyperaemia.
- In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors.

Key messages

- We demonstrated that measurement of the hyperaemic response by the new PPG technique might be a useful tool in the detection of endothelial dysfunction associated with cardiovascular risk factors.
- We found that PPG pulse amplitude hyperaemic response was lower in men than in women and in smokers than nonsmokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.
- The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.

Strengths and limitations of this study

- Our study is the first to implement the new PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. A finger PPG is a low-cost and operator-independent technique compared to ultrasound in the assessment of peripheral vascular function.
- Under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques.
- Our sample size was smaller compared to other studies. On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function.
- Further prospective studies are required to validate the PPG technique for the non-invasive assessment of endothelial function.

Keywords Population ■ Vasodilation ■ Photoplethysmography ■ Endothelial function

INTRODUCTION

Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease.[1] In humans, endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with cardiovascular risk factors.[2] Vasodilation of the peripheral arteries during reactive hyperaemia after ischaemia depends in part on the release of nitric oxide from endothelial cells in response to increased shear stress.[3] This physiological response allows the non-invasive assessment of endothelial vasomotor function which can be measured based on the flow-mediated dilation (FMD) of the brachial artery[4] or on the fingertip pulse amplitude hyperaemic response.[3, 5, 6] Previous studies mainly applied fingertip peripheral arterial tonometry (PAT) to derive pulse wave amplitude and, therefore, the pulse amplitude changes during hyperaemia.[3, 5, 6] Another approach to derive information about the arterial pulse wave is based on photoplethysmography (PPG).[7] This optical technique enables detecting blood volume changes in microvascular beds during hyperaemia.[7] We sought to evaluate the correlates of digital PPG pulse amplitude hyperaemic responses as a measure of peripheral arterial function in a sample of a general population.

MATERIALS AND METHODS

Design and sample

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).[8] From August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address,

were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS random function. Households with a number matching the list were invited; household members older than 18 years were eligible. For the current analysis, our sample consisted of 378 former participants, who were examined from January 2010 until March 2012 (response rate 85.1%), including measurement of endothelial function with the PPG technique. We excluded 43 subjects with cardiac dysrhythmias, such as atrial fibrillation, pacemaker and frequent extrasystole. Because the PPG pulse amplitude was of insufficient quality to assess vascular function (n=14) or because the hyperaemic test was discontinued (n=10) we discarded a further 24 subjects. Thus, the number of participants statistically analysed totaled 311.

Determination of PPG pulse amplitude

We studied endothelial function in an air-conditioned room at constant temperature around 22°C after the subjects had rested for at least 20 minutes in the supine position. The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 hours before assessment of endothelial function. The blood pressure was the average of 5 auscultatory readings, obtained with a standard sphygmomanometer.

Digital pulse amplitude was measured with a PPG device (FLOMEDI Company, Brussels) transmitting infrared light at a wavelength of 940 nm and positioned on the tip of each index finger. Digital output from the PPG device was recorded through an analogue-to-digital converter (10 bit, sampling frequency 250 Hz). To determine the amplitude changes of the digital pulse curve in response to hyperaemia, we used a protocol as described by Hamburg *et al*.[6] Baseline PPG pulse amplitude was measured at each of the two index fingertips for 2 min 20 seconds. Next, arterial flow was interrupted for 5 minutes by an

inflatable cuff placed on the proximal forearm with an occlusion pressure of 200-220 mmHg (around 50 mmHg above the participant's systolic pressure). After cuff deflation, we analysed the PPG pulse amplitude at both fingers using a computerised, automated algorithm (FLOMEDI Company, Brussels) that provided the averaged pulse amplitude for each 30-second interval up to 4 minutes (see the PPG pulse tracking in Figure 1).

For each 30-second interval, the response of the PPG pulse wave amplitude to hyperaemia was calculated from the hyperaemic fingertip as the ratio of the post-deflation PPG pulse amplitude to the baseline amplitude (PA_{ht}/PA_{h0}, where PA is the pulse amplitude, h is the hyperaemic finger, t is time interval, and 0 is baseline). To obtain the PPG pulse amplitude ratio we divided PA_{ht}/PA_{h0} ratio by the corresponding ratio at the control hand (PA_{ct}/PA_{c0}, where c is the control finger).

To determine the inter-session reproducibility of the hyperaemic response, we analysed PPG ratios measured on two different occasions in 5 subjects. We determined the absolute and relative biases of the averaged and peak PPG pulse amplitude ratios between the two sessions as well as 95% limits of agreement between sessions. Absolute and relative biases between the two sessions were calculated according to Bland and Altman's method as (x1 – x2) vs averaged and (100*(x1 – x2)/averaged) vs averaged, respectively. The absolute biases of the averaged and peak PPG pulse amplitude ratios between the two sessions were 0.062 (95% confidence interval [CI]: –0.10 to 0.23) and 0.072 (95% CI: –0.049 to 0.19), respectively. The relative inter-session biases of the averaged and peak PPG pulse amplitude ratios were 3.29% (95% CI: –8.8% to 15.4%) and 4.87% (95% CI: –3.5% to 13.2%), respectively.

Other measurements

At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each subject's medical history, smoking and drinking habits, and intake of medications. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic (average of 5 consecutive auscultatory readings at the examination centre) or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Overweight was a body mass index between 25 and 30 kg/m². Obesity was a body mass index of 30 kg/m² or higher.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, NC). The central tendency and the spread of the data are reported as mean ± SD. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by computation of the coefficient of skewness, *i.e.*, the third moment about the mean divided by the cube of the standard deviation. We compared means and proportions by means of a sample t-test and by the χ^2-test, respectively. Significance was $P < 0.05$ on two-sided test.

We performed single and stepwise multiple regression to assess the independent correlations of the PPG pulse amplitude ratio during each 30-second interval with sex, age, body mass index, heart rate, systolic and diastolic blood pressures, current smoking, alcohol consumption, total cholesterol, treatment with antihypertensive or lipid-lowering drugs**,** and previous history of ischaemic heart disease. We set the *P*-values for variables to enter and to stay in the regression models at 0.10.

RESULTS

Characteristics of participants and PPG pulse amplitude

The participants included 154 (53.5%) women, and 134 (43.1%) hypertensive patients of whom 78 (25.1 %) were on antihypertensive drug treatment. Table 1 shows the clinical characteristics and PPG pulse amplitude measures of the study participants by sex. In this cohort, women had lower systolic and diastolic blood pressure and higher heart rate than men, less often reported alcohol consumption and had no history of ischaemic heart disease.

As shown in Figure 2, after forearm cuff deflation, the ratio of the PPG pulse amplitude to baseline rose rapidly in the hyperaemic fingertip, with maximal response occurring in the 30- to 60-second interval, whereas the changes of PPG amplitude in the control finger were minimal. Table 1 lists the mean values of the post-deflation PPG pulse amplitude ratio at each 30-second interval by sex. The hyperaemic response at each 30-second interval was significantly higher in women compared to men (Table 1). In both women and men, the maximal hyperaemic response was detected in the 30- to 60-second interval.

Determinants of PPG pulse amplitude ratio

We performed stepwise regression to assess the independent correlations of the hyperaemic response for each 30-second interval after cuff deflation with sex, age, body mass index, heart rate, systolic and diastolic blood pressures, total cholesterol, blood glucose, current smoking and alcohol intake, lipid-lowering treatment and previous history of ischaemic heart disease and diabetes. With age forced in the models, the explained variance for the PPG pulse amplitude ratio totaled from 9.2% at 0-30 second-interval and 22.5% at 60-90-second time interval (Table 2). The PPG PWA changes throughout 0-240-second intervals significantly decreased with male sex ($P \leq 0.0014$) and with body mass index ($P \leq 0.035$).

The hyperaemic response at 0- to 90-second intervals decreased with current smoking (*P*<0.0007). These associations with sex, body mass index and current smoking were mutually independent. In addition, the PPG pulse amplitude ratio at 30- to 60-second interval decreased with total cholesterol, but this association only reached borderline significance (*P*<0.06;Table 2).

Figure 3 illustrates the hyperaemic responses by the smoking status while adjusted for important covariables. The maximal hyperemic response in the 30-to 60-second interval was significantly lower in current smokers compared to non-smokers (1.37 vs 1.76; *P*<0.0001). Figure 4 shows the adjusted PPG pulse amplitude hyperaemic responses in subjects, divided into 3 categories according to their body mass index. In overweight (n=134; 1.51±0.060) and obese (n=64; 1.44±0.082) subjects the maximal hyperemic response was significantly lower compare to lean participants (n=113; 1.71±0.064).

DISCUSSION

In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors. We observed a time-dependent increase in digital PPG pulse amplitude that peaked in the 30- to 60-second interval after induction of reactive hyperaemia. In keeping with the literature,[6, 9-11] we found that PPG pulse amplitude hyperaemic response was higher in women than in men and in nonsmokers than smokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.

Endothelial function is often assessed non-invasively by vascular reactivity tests. Several methods are available to study endothelial function in the peripheral macrocirculation

(conduit arteries) and microcirculation (resistance arteries and arterioles).[2, 12] Measurement of the brachial artery diameter before and after several minutes of occlusion of the arterial flow to the forearm is the most widely used test to assess endothelium-dependent vasodilation.[4, 13, 14] The change in arterial diameter gives a measure of flow-mediated vasodilatation (FMD). This technique, however, is operator dependent, is costly and requires a long post-processing time. Measurement of microcirculatory reactive hyperaemia can be assessed by digital pulse amplitude measured by applanation tonometry[5, 6] or photoplethysmography.[15, 16] Lund[17] described the potential of the PPG technique for the assessment of vasodilation by using this technique to measure haemodynamic response to nitroglycerin.

We observed similar digital PPG pulse amplitude changes during the hyperaemic response compared with the method based on finger applanation tonometry.[6, 10, 11] In the Framingham study,[6, 10] similar to our study, the ratio of the digital pulse amplitude to baseline rose rapidly in the hyperaemic fingertip after forearm cuff deflation, and then slowly decreased towards baseline. However, we detected the maximal hyperaemic response in the 30- to 60-second interval, whereas in the Framingham study the pressure amplitude ratio was highest in the 60- to 90-second interval. The difference in the time of maximal hyperaemic response between the Framingham study and our report might be related to the fact that finger PAT measures pressure changes while photoplethysmography measures changes of the relative amount of blood volume.

We observed the relations between the hyperaemic PPG pulse amplitude response and cardiovascular risk factors. In our current study and in other community-based studies,[6, 10, 11] men had a less pronounced hyperaemic response than women, which is probably attributable to physiological differences in vessel diameter and wall thickness between the sexes. In line with other studies,[6, 10, 11] which used the PAT technique to evaluate endothelial

function, we demonstrated a significant inverse associations between PPG amplitude changes and smoking, obesity and total cholesterol. The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.[18] We also tested the influences of antihypertensive and antihyperlipidemic treatment on the hyperaemic PPG pulse amplitude response, which were not significant (results not shown).

Similar to other studies, in which finger applanation tonometry was used to assess the endothelial function,[10] we did not observe a significant relation between hyperaemic PPG pulse amplitude changes and age. On the other hand, previous studies reported lower endothelial function as assessed by FMD with advancing age.[13, 14] Differences in the age-related hyperaemic responses between microcirculatory and macrocirculatory reactivity might explain these divergent findings.[9] Moreover, recent studies demonstrated that brachial and digital measures of vascular function were uncorrelated with each other.[10, 19] It was suggested that FMD and PAT provide distinct information regarding vascular function in conduit versus smaller digital vessels.

The present study must be interpreted within the context of its potential limitations and strengths. First, PPG pulse amplitude registration is prone to measurement error due to higher variability in comparisons with the FMD technique.[20] On the other hand, assessment of the hyperaemic PPG pulse wave amplitude changes requires little training and is operator independent. Moreover, under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques. Second, our sample size was smaller compared to other studies.[10, 11] On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function.

In conclusion, our study is the first to implement the PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. We demonstrated that measurement of the hyperaemic response by the PPG technique might be a useful tool in the detection of endothelial dysfunction associated with smoking and obesity, while accounting for the differential hyperaemic response between men and women. Further prospective studies are required to validate the PPG technique for the non-invasive assessment of endothelial function.

Acknowledgments

The authors gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Dais Thijs and Hanne Truyens (Leuven, Belgium).

Contributors

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation of the data. TK, EVV, JK drafted the manuscript. All authors gave final approval of the final version.

Funding

The European Union (grants HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and ERC Advanced Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13 and G. 0881.13) gave support to the Research Unit Hypertension and Cardiovascular Epidemiology.

Disclosures

GC and DJ work at FLOMEDI, a spin-off company (Spin Off In Brussels - Innoviris) of the Technical Department of the Haute Ecole Paul Henri Spaak. The company designs and develops software and electronic medical devices in order to facilitate, simplify, and increase accuracy of non-invasive assessment of vascular stiffness.

None of the other authors declares a conflict of interest.

REFERENCES

1. Vita JA. Endothelial function. *Circulation* 2011;124:e906-e912.
2. Flammer AJ, Anderson T, Celermajer DS, *et al.* The assessment of endothelial function: from research into clinical practice. *Circulation* 2012;126:753-767.
3. Nohria A, Gerhard-Herman M, Creager MA, *et al.* Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006;101:545-548.
4. Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J* 2003;145:943-951.
5. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2013;146:168-174.
6. Hamburg NM, Keyes MJ, Larson MG, *et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467-2474.
7. Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiol Meas* 2007;28:R1-R39.
8. Kuznetsova T, Herbots L, López B, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009;2:105-112.
9. Brunner H, Cockcroft JR, Deanfield J, *et al.* Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:233-246.
10. Hamburg NM, Palmisano J, Larson MG, *et al.* Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011;57:390-396.

11. Schnabel RB, Schulz A, Wild PS, *et al.* Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. *Circ Cardiovasc Imaging* 2011;4:371-380.
12. Lekakis J, Abraham P, Abraham P, *et al.* Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18:775-789.
13. Benjamin EJ, Larson MG, Keyes MJ, *et al.* Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.
14. Nawrot TS, Staessen JA, Fagard RH, *et al.* Endothelial function and outdoor temperature. *Eur J Epidemiol* 2005;20:407-410.
15. Zahedi E, Jaafar R, Ali MA, *et al.* Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiol Meas* 2008;29:625-637.
16. Millasseau SC, Ritter JM, Takazawa K, *et al.* Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* 2006;24:1449-1456.
17. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates--a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79-86.
18. Steffen Y, Vuillaume G, Stolle K, *et al.* Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide* 2012;27:176-184.
19. Martin BJ, Gurtu V, Chan S, *et al.* The relationship between peripheral arterial tonometry and classic measures of endothelial function. *Vasc Med* 2013;18:13-18.
20. Donald AE, Charakida M, Cole TJ, *et al.* Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* 2006;48:1846-1850.

Legend to figures

Figure 1. Panel A shows a system incorporating two PPG devices transmitting infrared light, analogue-to-digital converter and forearm pressure cuff. Panel B shows the position of cuff and two PPG devices during the test. Panel C and D show recorded pulse amplitude tracing. In the arm undergoing hyperemia (panel C, top tracing, and panel D), baseline amplitude is recorded. During cuff inflation, flow is occluded and restores after cuff release (hyperemic period). In the contralateral control finger (panel C, bottom tracing), flow continues throughout, and pulse amplitude undergoes minimal changes.

Figure 2. PPG pulse amplitude response for the hyperemic (closed symbols) and control (open symbols) finger in women (circles) and men (squares). Women had more pronounced responses than men. Symbols are means, dashed line – 95% confidence interval.

Figure 3. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in smokers and nonsmokers subjects. Smokers had significantly lower response throughout the 0- to 120-second postdeflation intervals. Symbols are means and SE. Models are adjusted for sex, age, body mass index, and total cholesterol. *P<0.05, **P<0.01, ***P<0.001 vs nonsmokers.

Figure 4. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in subjects with normal body mass index (BMI), overweight (25 kg/m²≤BMI<30 kg/m²) and obesity (BMI≥30 kg/m²). Symbols are means and SE. Models are adjusted for sex, age, smoking, and total cholesterol. *P<0.05, **P<0.01, ***P<0.001 vs lean participants. $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001 vs lean participants.

Table 1. Characteristics of participants

Clinical measurements				PPG pulse amplitude measures			
Characteristic	Women (*n* =154)	Men (*n* =157)	P-value	Characteristic	Women (*n* =154)	Men (*n* =156)	*P-value*
Anthropometrics				PPG ratio			
Age, y	53.51±12.2	51.8±14.5	0.26	Time interval (sec)			
Body mass index, kg/m^2	26.3±4.0	27.3±3.7	0.03	0-30	1.43 (0.87 to 2.02)	1.27 (0.83 to 1.84)	0.002
Systolic pressure, mm Hg	125.5±15.4	131.4±14.3	0.0006	30-60	1.93 (1.08 to 2.86)	1.46 (1.00 to 2.13)	<0.0001
Diastolic pressure, mm Hg	80.4±8.0	84.5±9.5	<0.0001	60-90	1.84 (1.10 to 2.50)	1.37 (0.97 to 1.93)	<0.0001
Heart rate, beats/minute	66.5±10.2	62.4±9.9	0.0003	90-120	1.64 (1.09 to 2.16)	1.27 (0.93 to 1.79)	<0.0001
Questionnaire data				120-150	1.49 (1.06 to 2.01)	1.20 (0.92 to 1.59)	<0.0001
Current smoking, n (%)	28 (18.2)	18 (11.5)	0.10	150-180	1.38 (1.00 to 1.84)	1.16 (0.89 to 1.46)	<0.0001
Alcohol, n (%)	39 (25.3)	94 (59.9)	<0.0001	180-210	1.30 (0.98 to 1.65)	1.14 (0.87 to 1.43)	<0.0001
Diabetes, n (%)	5 (3.3)	4 (2.6)	0.72	210-240	1.24 (0.95 to 1.65)	1.11 (0.87 to 1.33)	<0.0001
Hypertensive, n (%)	54 (35.1)	80 (51.0)	0.004				
Treated for hypertension, n (%)	38 (24.7)	40 (25.5)	0.87				
Beta-blockers, n (%)	18 (11.7)	23 (14.7)	0.44				
ACE or ARB, n (%)	12 (7.8)	15 (9.6)	0.58				
Diuretics or CCB, n (%)	22 (14.3)	19 (12.1)	0.57				
Previous history of IHD, n (%)	0 (0)	7 (4.5)	0.008				
Total cholesterol, mmol/l	5.2±1.00	5.0±0.96	0.037				
Lipid lowering agents, n (%)	10 (6.5)	8 (5.1)	0.60				

Values are mean (±SD), mean (10%-90%), or number of subjects (%). PPG indicates photoplethysmography, ACE indicates angiotensin-converting enzyme; ARB indicates angiotensin receptor blockers, CCB indicates calcium channel blockers, IHD indicates ischemic heart disease.

Table 2. Correlates of PPG ratios selected by stepwise regression

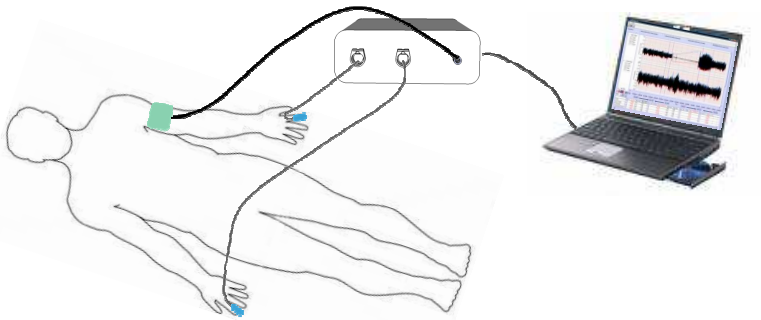
Parameter	PPG ratio							
	Time Intervals (sec)							
	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
Regression statistic								
Model R^2 (%)	9.2	21.4	22.5	19.6	18.1	16.3	12	9.6
Age (+10 years)*								
ß±SE	0.007 ± 0.020	-0.0007±0.028	0.005± 0.025	0.009± 0.021	0.009 ± 0.017	-0.0008 ± 0.014	0.006 ± 0.012	0.003 ± 0.011
	P = 0.73	*P* = 0.98	*P* = 0.85	*P* = 0.65	*P* = 0.60	*P* = 0.95	*P* = 0.65	*P* = 0.82
Partial r^2 (%)	0.04	0	0.01	0.07	0.09	0	0.07	0.02
Female (0,1)								
ß±SE	0.16 ± 0.05	0.49 ± 0.68	0.46 ± 0.061	0.35 ± 0.050	0.26 ± 0.041	0.20 ± 0.035	0.14 ± 0.031	0.11 ± 0.03
	P = 0.0014	*P* <0.0001	*P* <0.0001	*P* <0.0001	*P* <0.0001	*P* <0.0001	*P* <0.0001	*P* = 0.0001
Partial r^2 (%)	3.8	13.6	16.0	15.1	13.5	12	7.7	6.1
Current smoking (0,1)								
ß±SE	-0.30 ± 0.07	-0.39 ± 0.09	-0.29 ± 0.085	-0.14 ± 0.070	-	-	-	-
	P <.0001	*P* = <.0001	*P* = 0.0007	*P* = 0.047				
Partial r^2 (%)	4.0	3.9	3.1	1.1	-	-	-	-
Body mass index (kg/m²)								
ß±SE	-0.014 ± 0.007	-0.027 ±0.009	-0.032± 0.008	-0.025±0.007	-0.022 ± 0.005	-0.018 ± 0.005	-0.015 ± 0.004	-0.013 ± 0.004
	P = 0.035	*P* = 0.003	*P* <0.0001	*P* = 0.0002	*P* <0.0001	*P* <0.0001	*P* = 0.0003	*P* = 0.0008
Partial r^2 (%)	1.3	2.9	3.4	3.4	4.5	4.5	4.0	3.5
Total Cholesterol								
ß±SE	-	-0.066 ±0.035	-	-	-	-	-	-
		P = 0.06						
Partial r^2 (%)	-	0.9	-	-	-	-	-	-

Values are mutually adjusted partial regression coefficients ±SE. Age was forced into all models. The covariables considered in stepwise models included sex, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.

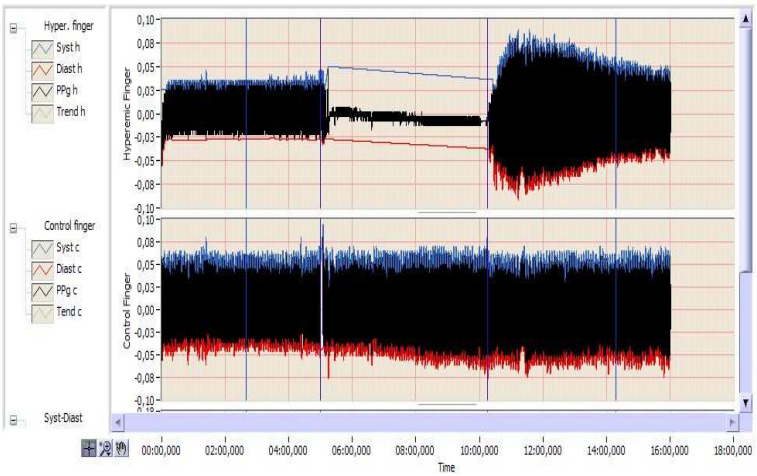
Figure 1

A

B



C



D

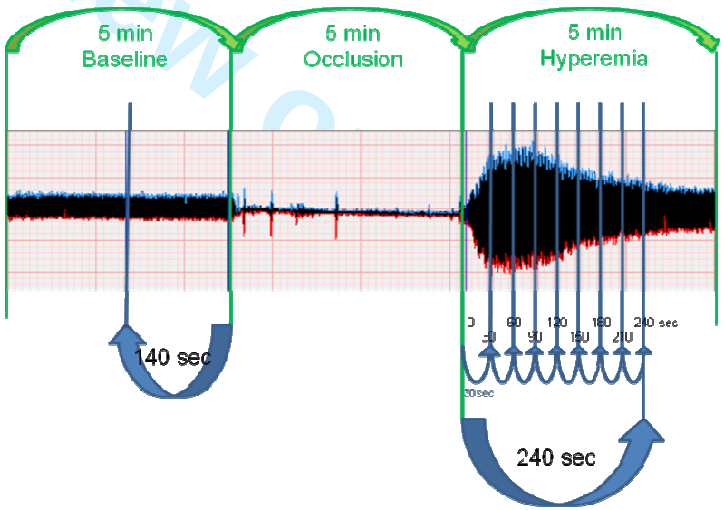


Figure 2

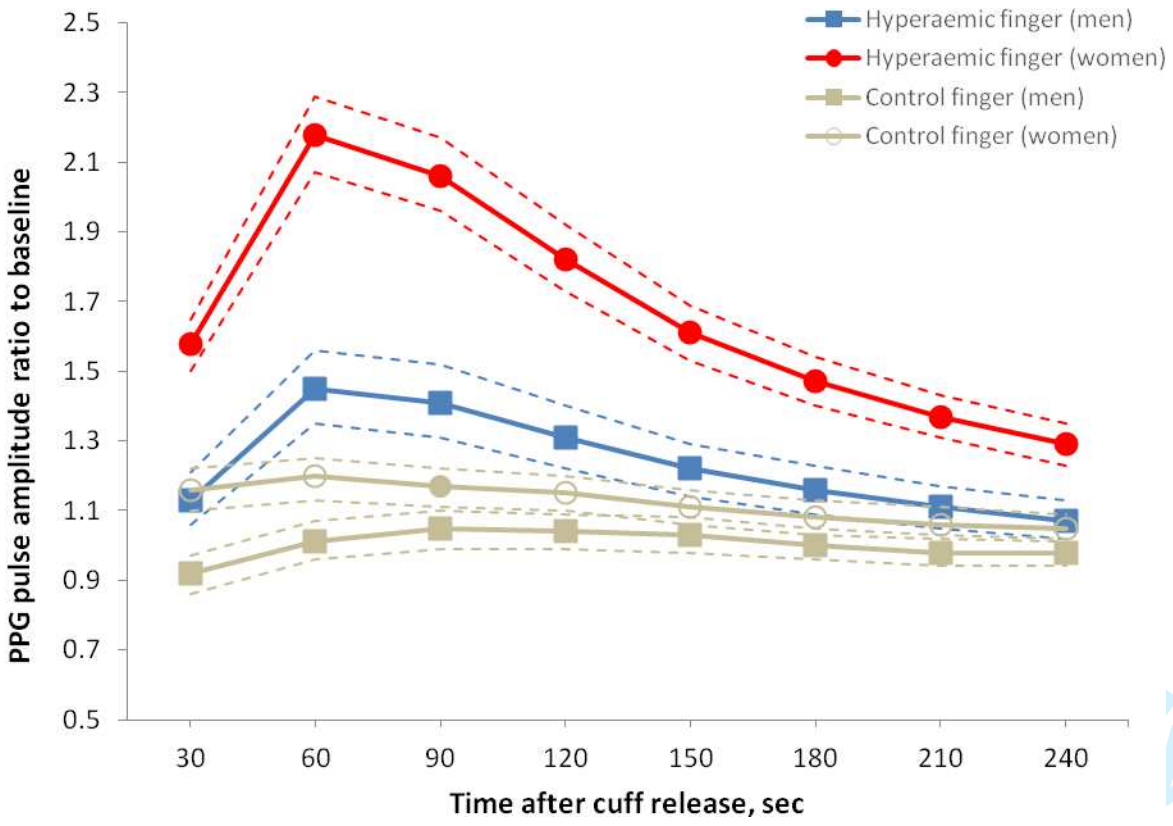

Hyperaemic finger (men)
Hyperaemic finger (women)
Control finger (men)
Control finger (women)
PPG pulse amplitude ratio to baseline
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 3

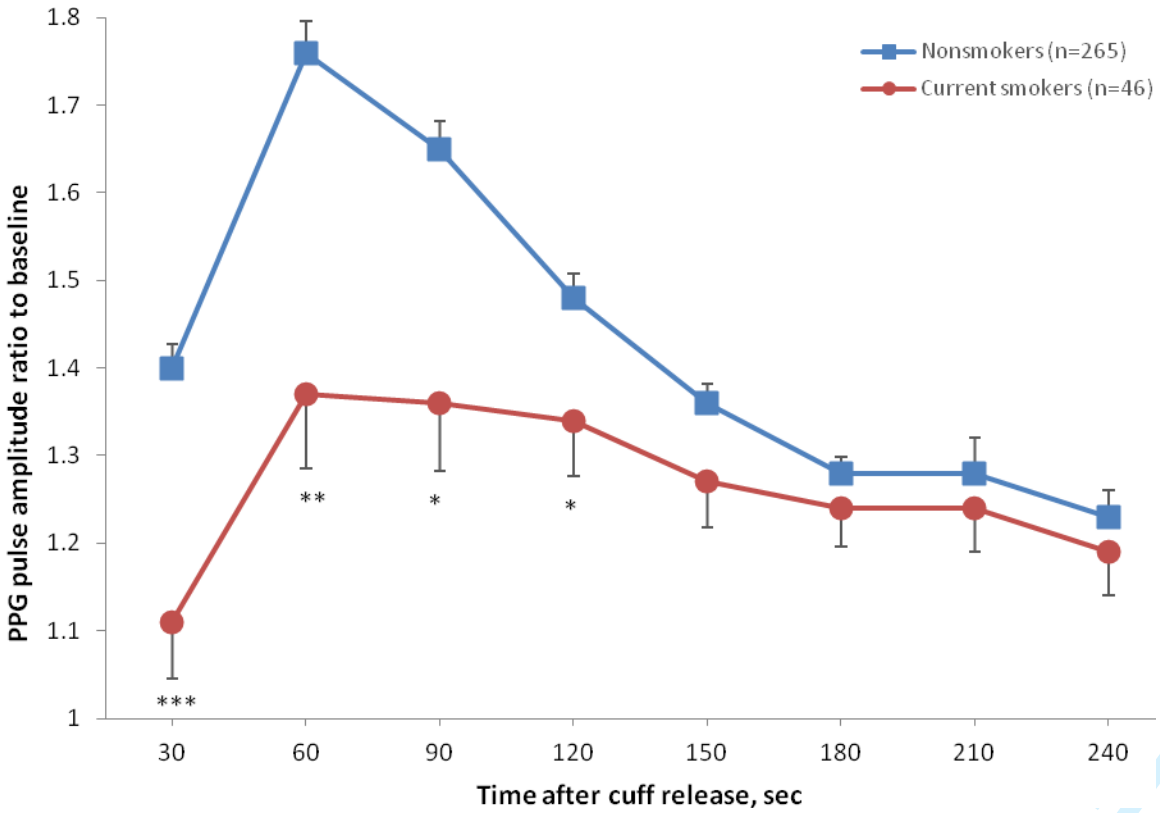
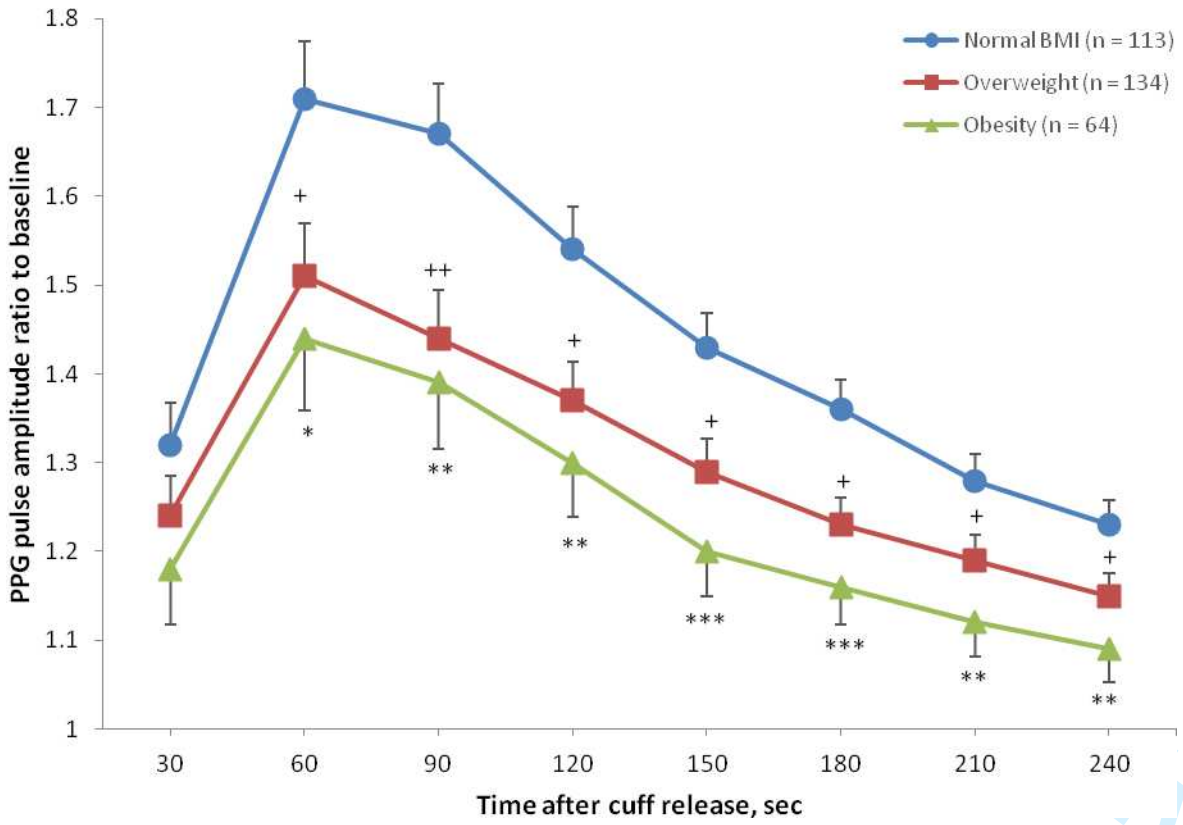

Nonsmokers (n=265)
Current smokers (n=46)
PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
***
**
*
*
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 4


Normal BMI (n = 113)
Overweight (n = 134)
Obesity (n = 64)
PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
30
60
90
120
150
180
210
240
Time after cuff release, sec

BMJ open

Association of digital vascular function with cardiovascular risk factors: a population study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004399.R1
Article Type:	Research
Date Submitted by the Author:	31-Jan-2014
Complete List of Authors:	Kuznetsova, Tatiana; University of Leuven, Cardiovascular Sciences Van Vlierberghe, Eline; University of Leuven, Knez, Judita; University of Leuven, Szczesny, Gregory; FLOMEDI Company, Thijs, Lutgarde; University of Leuven, Jozeau, Dominique; FLOMEDI Company, Balestra, Costantino; Haute Ecole Paul Henri Spaak, D'hooge, Jan; University of Leuven, Staessen, Jan; KU Leuven,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology
Keywords:	population, endothelial function, vasodilation, photoplethysmography

DIII 1/31/2014 5:04 AM
BMJ Open

Association of digital vascular function with cardiovascular risk factors: a population study

Short title: Correlates of endothelial function

Tatiana Kuznetsova[1], Eline Van Vlierberghe[1], Judita Knez[1], Gregory Szczesny[2], Lutgarde Thijs[1], Dominique Jozeau[2], Costantino Balestra[3], Jan D'hooge[4], Jan A. Staessen[1,5]

Word Counts: Manuscript 5137; Abstract 300;

Number: Tables 2, Figures 4

[1] Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium; [2]FLOMEDI Company, Brussels, Belgium; [3] Environmental, Occupational & Aging Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium; [4]Division of Cardiovascular Imaging and Dynamics, Department of Cardiovascular Sciences, University of Leuven, Belgium; and [5]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands.

Correspondence to Tatiana Kuznetsova, M.D., Ph.D., Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, Campus Sint Rafaël, Kapucijnenvoer 35, Box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kuznetsova@med.kuleuven.be

ABSTRACT

Objectives: Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells. Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and PWA hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). We sought to evaluate the correlates of digital PPG PWA hyperaemic responses as a measure of peripheral vascular function.

Design: The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) is a population-based cohort study.

Setting: Respondents were examined at one centre in northern Belgium.

Participants: For this analysis, our sample consisted of 311 former participants (53.5% women; mean age 52.6 years; 43.1% hypertensive), who were examined from January 2010 until March 2012 (response rate 85.1%).

Primary outcome measures: Using a fingertip PPG device, we measured digital PWA at baseline and at 30-second intervals for 4 minutes during reactive hyperaemia induced by a 5-minute forearm cuff occlusion. We performed stepwise regression to identify correlates of the hyperaemic response ratio for each 30-second interval after cuff deflation.

Results The maximal hyperaemic response was detected in the 30- to 60-second interval. The explained variance for the PPG PWA ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval. The hyperaemic response at each 30-second interval was significantly higher in women compared to men ($P \leq 0.001$). The PPG PWA changes at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$) and at 0-240-second intervals decreased with higher body mass index ($P \leq 0.035$). These associations with sex, current smoking and body mass index were mutually independent.

Conclusions Our study is the first to implement the new PPG technique to measure digital PWA hyperaemic changes in a general population. Hyperaemic response, as measured by PPG, inversely associated with traditional cardiovascular risk factors such as male sex, smoking and obesity.

ARTICLE SUMMARY

Article focus

- Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease. Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells.
- Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and its hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). This optical technique enables detecting blood volume changes in microvascular beds in response to hyperaemia.
- In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors.

Key messages

- We demonstrated that measurement of the hyperaemic response by the new PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with cardiovascular risk factors.
- We found that PPG pulse amplitude hyperaemic response was lower in men than in women and in smokers than nonsmokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.
- The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.

Strengths and limitations of this study

- Our study is the first to implement the new PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. A finger PPG is a low-cost and operator-independent technique compared to ultrasound in the assessment of peripheral vascular function.
- Under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG technique.
- Our sample size was smaller compared to other studies. On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG technique in assessment of digital vascular function.
- Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Keywords Population ■ Vasodilation ■ Photoplethysmography ■ Endothelial function

INTRODUCTION

Endothelial dysfunction, a marker of reduced nitric oxide (NO) bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease.[1] In humans, endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with cardiovascular risk factors.[2] Vasodilation of the peripheral arteries during reactive hyperaemia after ischaemia depends in part on the release of nitric oxide from endothelial cells in response to increased shear stress.[3] This physiological response allows the non-invasive assessment of endothelial vasomotor function which can be measured based on the flow-mediated dilation (FMD) of the brachial artery[4] or on the fingertip pulse amplitude hyperaemic response.[3, 5, 6] Previous studies mainly applied fingertip peripheral arterial tonometry (PAT) to derive pulse wave amplitude and, therefore, the pulse amplitude changes during hyperaemia.[3, 5, 6] Another approach to derive information about the arterial pulse wave is based on photoplethysmography (PPG).[7] This optical technique enables detecting blood volume changes in microvascular beds during hyperaemia.[7] We sought to evaluate the correlates of digital PPG pulse amplitude hyperaemic responses as a measure of peripheral arterial function in a sample of a general population.

MATERIALS AND METHODS

Design and sample

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).[8] From August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address,

were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS random function. Households with a number matching the list were invited. The initial participation rate was 78.0%.

The FLEMENGHO study is on-going longitudinal population study and, therefore, the participants were repeatedly visited at home and examined at a local examination centre. From January 2010 until March 2012 a scheduled follow-up examination included also measurement of endothelial function with the PPG technique. From 444 invited participants for this examination, we obtained informed written consent from 378 subjects (response rate 85.1%). We excluded 43 subjects with cardiac dysrhythmias, such as atrial fibrillation, pacemaker and frequent extrasystole. Because the PPG pulse amplitude was of insufficient quality to assess vascular function (n=14) or because the hyperaemic test was discontinued (n=10) we discarded a further 24 subjects. Thus, the number of participants statistically analysed totaled 311.

Determination of PPG pulse amplitude

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 hours before the test. No medication was taken on the day of the examination. We studied endothelial function in an air-conditioned room at constant temperature around 22°C. To attain a cardiovascular steady-state before starting the test, the subjects had rested for at least 20 minutes in the supine position. Since peripheral vasoconstriction is correlated with the surrounding temperature, before the test, special care was taken to keep fingertips temperature around 35°C. The blood pressure was the average of 5 auscultatory readings, obtained with a standard sphygmomanometer. The blood pressure measurement was performed on the arm that served as control.

Digital pulse amplitude was measured with a PPG device (FLOMEDI Company, Brussels) transmitting infrared light at a wavelength of 940 nm and positioned on the tip of each index finger. Digital output from the PPG device was recorded through an analogue-to-digital converter (10 bit, sampling frequency 250 Hz). We expressed the amplitude of the PPG PWA signal in arbitrary units. To determine the amplitude changes of the digital pulse curve in response to hyperaemia, we used a protocol as described by Hamburg *et al*.[6] As shown in Figure 1, panel C and D, baseline PPG pulse amplitude was registered at each of the two index fingertips for at least 5 minutes to ensure a stable baseline PPG signal. For the analysis, we used PPG pulse amplitude that was measured for last 2 min 20 sec. Next, arterial flow was interrupted for 5 minutes by an inflatable cuff placed on the proximal forearm with an occlusion pressure of 200-220 mmHg (around 50 mmHg above the participant's systolic pressure). Complete cessation of blood flow to the hand is verified by the absence of a PPG signal from the occluded arm. After cuff deflation, we analysed the PPG pulse amplitude at both fingers using a computerised, automated algorithm (FLOMEDI Company, Brussels) that provided the averaged pulse amplitude for each 30-second interval up to 4 minutes (see the PPG pulse tracking in Figure 1).

For each 30-second interval, the response of the PPG pulse wave amplitude to hyperaemia was calculated from the hyperaemic fingertip as the ratio of the post-deflation PPG pulse amplitude to the baseline amplitude (PA_{ht}/PA_{h0}, where PA is the pulse amplitude, h is the hyperaemic finger, t is time interval, and 0 is baseline). To obtain the PPG pulse amplitude ratio we divided PA_{ht}/PA_{h0} ratio by the corresponding ratio at the control hand (PA_{ct}/PA_{c0}, where c is the control finger).

To determine the inter-session reproducibility of the hyperaemic response, we analysed PPG ratios measured on two different occasions in 5 subjects. We determined the

absolute and relative biases of the averaged PPG pulse amplitude ratios per each 30-second time interval between the two sessions as well as 95% limits of agreement between sessions. Absolute and relative biases between the two sessions were calculated according to Bland and Altman's method as (x1 – x2) vs averaged and (100*(x1 – x2)/averaged) vs averaged, respectively. The absolute and relative biases of the averaged PPG pulse amplitude ratios at each time interval between the two sessions were 0.062 (95% confidence interval [CI]: –0.10 to 0.23) and 3.29% (95% CI: –8.8% to 15.4%), respectively.

Other measurements

At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each subject's medical history, smoking and drinking habits, and intake of medications. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic (average of 5 consecutive auscultatory readings at the examination centre) or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Overweight was a body mass index between 25 and 30 kg/m². Obesity was a body mass index of 30 kg/m² or higher.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, NC). The central tendency and the spread of the data are reported as mean ± SD. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by computation of the coefficient of skewness, *i.e.*, the third moment about the mean divided by the cube of the standard deviation. We compared means and proportions by means of a sample t-test and by the χ^2-test, respectively. Significance was $P < 0.05$ on two-sided test.

We performed single and stepwise multiple regression to assess the independent correlations of the PPG pulse amplitude ratio during each 30-second interval with sex, age,

systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. We set the *P*-values for variables to enter and to stay in the regression models at 0.10.

RESULTS

Characteristics of participants and PPG pulse amplitude

The participants included 154 (53.5%) women, and 134 (43.1%) hypertensive patients of whom 78 (25.1 %) were on antihypertensive drug treatment. Table 1 shows the clinical characteristics and PPG pulse amplitude measures of the study participants by sex. In this cohort, women had lower systolic and diastolic blood pressure and higher heart rate than men, less often reported alcohol consumption and had no history of ischaemic heart disease.

The geometric means of the baseline PPG amplitude were 7.3 (5%-95% percentiles: 2.7 to 25.9) and 9.3 (5%-95% percentiles: 3.9 to 25.3) at the hyperaemic and control finger, respectively. We observed a high correlation between values of the baseline PPG amplitude recorded at both fingers (r=0.89, *P*<0.0001). As shown in Figure 2, after forearm cuff deflation, the ratio of the PPG pulse amplitude to baseline rose rapidly in the hyperaemic fingertip, with maximal response occurring in the 30- to 60-second interval, whereas the changes of PPG amplitude in the control finger were minimal. Table 1 lists the mean values of the post-deflation PPG pulse amplitude ratio at each 30-second interval by sex. The hyperaemic response at each 30-second interval was significantly higher in women compared to men (Table 1). In both women and men, the maximal hyperaemic response was detected in the 30- to 60-second interval.

Determinants of PPG pulse amplitude ratio

We performed stepwise regression to assess the independent correlations of the hyperaemic response for each 30-second interval after cuff deflation with sex, age, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. With age forced in the models, the explained variance for the PPG pulse amplitude ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval (Table 2). The PPG PWA changes throughout 0-240-second intervals significantly decreased with male sex ($P \leq 0.0004$) and with body mass index ($P \leq 0.017$). The hyperaemic response at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$). These associations with sex, body mass index and current smoking were mutually independent. In addition, the PPG pulse amplitude ratio at 30- to 60-second interval decreased with total cholesterol, but this association only reached borderline significance ($P=0.045$;Table 2). Blood glucose was also selected as an independent determinant of the PPG ratio (Table 2), but overall impact of this covariable is relatively small (explained about 1,5% of total variability). Moreover, blood glucose was not a significant determinant of the maximal peak of hyperaemic response which occurs at 30- to 60 second and 60- to 90-second intervals.

Figure 3 illustrates the hyperaemic responses by the smoking status while adjusted for important covariables. The maximal hyperaemic response in the 30-to 60-second interval was significantly lower in current smokers compared to non-smokers (1.37 vs 1.76; $P<0.0001$). Figure 4 shows the adjusted PPG pulse amplitude hyperaemic responses in subjects, divided into 3 categories according to their body mass index. In overweight (n=134; 1.51±0.060) and obese (n=64; 1.44±0.082) subjects the maximal hyperaemic response was significantly lower compared to lean participants (n=113; 1.71±0.064).

DISCUSSION

In our cohort recruited from a population study, we evaluated the relationship between PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, and cardiovascular risk factors. We observed a time-dependent increase in digital PPG pulse amplitude that peaked in the 30- to 60-second interval after induction of reactive hyperaemia. In keeping with the literature,[6, 9-11] we found that PPG pulse amplitude hyperaemic response was higher in women than in men and in nonsmokers than smokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.

Endothelial function is often assessed non-invasively by vascular reactivity tests. Several methods are available to study endothelial function in the peripheral macrocirculation (conduit arteries) and microcirculation (resistance arteries and arterioles).[2, 12] Measurement of the brachial artery diameter before and after 5 minutes of occlusion of the arterial flow to the forearm is the most widely used test to assess endothelium-dependent vasodilation.[4, 13, 14] The change in arterial diameter gives a measure of flow-mediated vasodilatation (FMD). This technique, however, is operator dependent, is costly and requires a long post-processing time. Measurement of microcirculatory reactive hyperaemia can be assessed by digital pulse amplitude measured by applanation tonometry[5, 6] or photoplethysmography.[15, 16] Lund[17] described the potential of the PPG technique for the assessment of vasodilation by using this technique to measure haemodynamic response to nitroglycerin. Moreover, Theunissen *et al*[18] observed in divers an increase in circulating NO after successive breath-hold dives. This increase in circulating NO level was associated with higher hyperaemic response measured using the same PPG device as in our study.

Both techniques for assessment of digital vascular function are non-operator-dependent, and the equipment is an order of magnitude less expensive than for ultrasonography. However, the tonometry method might be more expensive as compare to the PPG technique because of additional costs associated with changeable plethysmographic probes. Furthermore, the digital tonometry procedure is more complicated and less comfortable for patients because it requires attachment of a pneumo-electrical tube to an additional pneumatic digital cuff which should be constantly inflated during the test.

We observed similar digital PPG pulse amplitude changes during the hyperaemic response compared with results from studies using the finger applanation tonomtery based method.[6, 10, 11] In the Framingham study,[6, 10] similar to our study, the ratio of the digital pulse amplitude to baseline rose rapidly in the hyperaemic fingertip after forearm cuff deflation, and then slowly decreased towards baseline. However, we detected the maximal hyperaemic response in the 30- to 60-second interval, whereas in the Framingham study the pressure amplitude ratio was highest in the 60- to 90-second interval. The difference in the time of maximal hyperaemic response between the Framingham study and our report might be related to the fact that finger PAT measures pressure changes while photoplethysmography measures changes of the relative amount of blood volume.

We observed the relations between the hyperaemic PPG pulse amplitude response and cardiovascular risk factors. In our current study and in other community-based studies,[6, 10, 11] men had a less pronounced hyperaemic response than women, which is probably in part attributable to physiological differences in vessel diameter and wall thickness between the sexes. In line with other studies,[6, 10, 11] which used the PAT technique to evaluate endothelial function, we demonstrated a significant inverse associations between PPG amplitude changes and smoking, obesity and total cholesterol. The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and

metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.[19] We also tested the influences of antihypertensive and antihyperlipidemic treatment on the hyperaemic PPG pulse amplitude response, which were not significant (results not shown). The observed association between the PPG PWA ratio and blood glucose during some time intervals might be related to the optic technique which we used in our study.

Similar to other studies, in which finger applanation tonometry was used to assess the endothelial function,[10] we did not observe a significant relation between hyperaemic PPG pulse amplitude changes and age. On the other hand, previous studies reported lower hyperaemic response as assessed by FMD with advancing age.[13, 14] Differences in the age-related hyperaemic responses between microcirculatory and macrocirculatory reactivity might explain these divergent findings.[9] Moreover, recent studies demonstrated that brachial and digital measures of vascular function were uncorrelated with each other.[10, 20] It was suggested that FMD and PAT provide distinct information regarding vascular function in conduit versus smaller digital vessels.

The present study must be interpreted within the context of its potential limitations and strengths. First, PPG pulse amplitude registration is prone to measurement error due to higher variability in comparisons with the FMD technique.[21] On the other hand, assessment of the hyperaemic PPG pulse wave amplitude changes requires little training and is operator independent. Moreover, under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques. Second, placing the occlusion cuff above the site of hyperaemic response measurement might evoke a dilatory response that is related in part to ischaemia and, therefore, is not entirely mediated by NO. Third, our sample size was smaller compared to

other studies.[10,11] On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function. Forth, as shown in Table 2, in our study, we could explain only around 20% of variability of the PPG PWA ratios by traditional CV risk factors. The remaining variability might be influenced by genetic factors, inflammatory processes or other confounders that we did not consider in our study. Moreover, in our opinion, it is important to demonstrate in prospective studies that the hyperaemic response as assessed by the PPG technique might be an independent predictor of CV events.

In conclusion, our study is the first to implement the PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. We demonstrated that measurement of the hyperaemic response by the PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with smoking and obesity, while accounting for the differential hyperaemic response between men and women. Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Acknowledgments

The authors gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Dais Thijs and Hanne Truyens (Leuven, Belgium).

Contributors

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation of the data. TK, EVV, JK drafted the manuscript. All authors gave final approval of the final version.

Funding

The European Union (grants HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and ERC Advanced Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13 and G. 0881.13) gave support to the Research Unit Hypertension and Cardiovascular Epidemiology.

Disclosures

GC and DJ work at FLOMEDI, a spin-off company (Spin Off In Brussels - Innoviris) of the Technical Department of the Haute Ecole Paul Henri Spaak. The company designs and develops software and electronic medical devices in order to facilitate, simplify, and increase accuracy of non-invasive assessment of vascular stiffness.

None of the other authors declares a conflict of interest.

REFERENCES

1. Vita JA. Endothelial function. *Circulation* 2011;124:e906-e912.
2. Flammer AJ, Anderson T, Celermajer DS, *et al.* The assessment of endothelial function: from research into clinical practice. *Circulation* 2012;126:753-767.
3. Nohria A, Gerhard-Herman M, Creager MA, *et al.* Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006;101:545-548.
4. Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J* 2003;145:943-951.
5. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2013;146:168-174.
6. Hamburg NM, Keyes MJ, Larson MG, *et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467-2474.
7. Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiol Meas* 2007;28:R1-R39.
8. Kuznetsova T, Herbots L, López B, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009;2:105-112.
9. Brunner H, Cockcroft JR, Deanfield J, *et al.* Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:233-246.
10. Hamburg NM, Palmisano J, Larson MG, *et al.* Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011;57:390-396.

11. Schnabel RB, Schulz A, Wild PS, *et al.* Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. *Circ Cardiovasc Imaging* 2011;4:371-380.
12. Lekakis J, Abraham P, Abraham P, *et al.* Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18:775-789.
13. Benjamin EJ, Larson MG, Keyes MJ, *et al.* Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.
14. Nawrot TS, Staessen JA, Fagard RH, *et al.* Endothelial function and outdoor temperature. *Eur J Epidemiol* 2005;20:407-410.
15. Zahedi E, Jaafar R, Ali MA, *et al.* Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiol Meas* 2008;29:625-637.
16. Millasseau SC, Ritter JM, Takazawa K, *et al.* Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* 2006;24:1449-1456.
17. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates--a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79-86.
18. Theunissen S, Guerrero F, Sponsiello N, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med* 2013;40:135-144.
19. Steffen Y, Vuillaume G, Stolle K, *et al.* Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide* 2012;27:176-184.
20. Martin BJ, Gurtu V, Chan S, *et al.* The relationship between peripheral arterial tonometry and classic measures of endothelial function. *Vasc Med* 2013;18:13-18.

21. Donald AE, Charakida M, Cole TJ, *et al.* Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* 2006;48:1846-1850.

Legend to figures

Figure 1. Panel A shows a system incorporating two PPG devices transmitting infrared light, analogue-to-digital converter and forearm pressure cuff. Panel B shows the position of cuff and two PPG devices during the test. Panel C and D show recorded pulse amplitude tracing. In the arm undergoing hyperemia (panel C, top tracing, and panel D), baseline amplitude is recorded. During cuff inflation, flow is occluded and restores after cuff release (hyperaemic period). In the contralateral control finger (panel C, bottom tracing), flow continues throughout, and pulse amplitude undergoes minimal changes.

Figure 2. PPG pulse amplitude response for the hyperaemic (closed symbols) and control (open symbols) finger in women (circles) and men (squares). Women had more pronounced responses than men. Symbols are means, dashed line – 95% confidence interval.

Figure 3. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in smokers and nonsmokers subjects. Smokers had significantly lower response throughout the 0- to 120-second postdeflation intervals. Symbols are means and SE. Models are adjusted for sex, age, body mass index, total cholesterol and blood glucose. ***P<0.001 vs nonsmokers.

Figure 4. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in subjects with normal body mass index (BMI), overweight (25 kg/m²≤BMI<30 kg/m²) and obesity (BMI≥30 kg/m²). Symbols are means and SE. Models are adjusted for sex, age, smoking, total cholesterol and blood glucose. *P<0.05, **P<0.01, ***P<0.001 vs lean participants. $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001 vs lean participants.

Table 1. Characteristics of participants

Clinical measurements				PPG pulse amplitude measures			
Characteristic	Women (*n* =154)	Men (*n* =157)	P-value	Characteristic	Women (*n* =154)	Men (*n* =156)	*P-value*
Anthropometrics				PPG ratio			
Age, y	53.51±12.2	51.8±14.5	0.26	Time interval (sec)			
Body mass index, kg/m^2	26.3±4.0	27.3±3.7	0.03	0-30	1.43 (0.87 to 2.02)	1.27 (0.83 to 1.84)	0.002
Systolic pressure, mm Hg	125.5±15.4	131.4±14.3	0.0006	30-60	1.93 (1.08 to 2.86)	1.46 (1.00 to 2.13)	<0.0001
Diastolic pressure, mm Hg	80.4±8.0	84.5±9.5	<0.0001	60-90	1.84 (1.10 to 2.50)	1.37 (0.97 to 1.93)	<0.0001
Heart rate, beats/minute	66.5±10.2	62.4±9.9	0.0003	90-120	1.64 (1.09 to 2.16)	1.27 (0.93 to 1.79)	<0.0001
Questionnaire data				120-150	1.49 (1.06 to 2.01)	1.20 (0.92 to 1.59)	<0.0001
Current smoking, n (%)	28 (18.2)	18 (11.5)	0.10	150-180	1.38 (1.00 to 1.84)	1.16 (0.89 to 1.46)	<0.0001
Alcohol, n (%)	39 (25.3)	94 (59.9)	<0.0001	180-210	1.30 (0.98 to 1.65)	1.14 (0.87 to 1.43)	<0.0001
Diabetes, n (%)	5 (3.3)	4 (2.6)	0.72	210-240	1.24 (0.95 to 1.65)	1.11 (0.87 to 1.33)	<0.0001
Treated for hypertension, n (%)	38 (24.7)	40 (25.5)	0.87				
Beta-blockers, n (%)	18 (11.7)	23 (14.7)	0.44				
ACE or ARB, n (%)	12 (7.8)	15 (9.6)	0.58				
Diuretics or CCB, n (%)	22 (14.3)	19 (12.1)	0.57				
Previous history of IHD, n (%)	0 (0)	7 (4.5)	0.008				
Total cholesterol, mmol/l	5.2±1.00	5.0±0.96	0.037				
Lipid lowering agents, n (%)	10 (6.5)	8 (5.1)	0.60				

Values are mean (±SD), mean (10%-90%), or number of subjects (%). PPG indicates photoplethysmography, ACE indicates angiotensin-converting enzyme; ARB indicates angiotensin receptor blockers, CCB indicates calcium channel blockers, IHD indicates ischemic heart disease.

Table 2. Correlates of PPG ratios selected by stepwise regression

Parameter	PPG ratio							
	Time Intervals (sec)							
	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
Regression statistic								
Model R^2 (%)	9.7	21.4	22.5	19.8	19.2	16.3	13.2	12.2
Age (+10 years)*								
ß±SE	0.014±0.020	-0.0007±0.028	0.005±0.025	0.004±0.019	0.007±0.017	-0.0008±0.014	0.004±0.012	0.002±0.011
	P=0.45	*P*=0.98	*P*=0.85	*P*=0.85	*P*=0.68	*P*=0.95	*P*=0.72	*P*=0.91
Partial r^2 (%)	0.02	0	0.01	0.04	0.06	0	0.04	0.01
Female (0,1)								
ß±SE	0.16 ± 0.05	0.49 ± 0.68	0.46 ± 0.061	0.35 ± 0.050	0.26 ± 0.041	0.20 ± 0.035	0.14 ± 0.031	0.12 ± 0.03
	P=0.0004	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Partial r^2 (%)	3.9	13.6	16.0	15.1	13.5	12	7.7	6.1
Current smoking (0,1)								
ß±SE	-0.30 ± 0.07	-0.39 ± 0.09	-0.29 ± 0.085	-	-	-	-	-
	P=0.0004	*P*<0.0001	*P*=0.0007					
Partial r^2 (%)	4.0	3.7	3.1	-	-	-	-	-
Body mass index (kg/m²)								
ß±SE	-0.014±0.007	-0.027±0.009	-0.032±0.008	-0.025±0.007	-0.022±0.005	-0.018±0.005	-0.015±0.004	-0.013±0.004
	P=0.017	*P*=0.003	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*=0.0002	*P*=0.0008
Partial r^2 (%)	1.7	3.0	3.4	3.4	4.5	4.5	4.1	3.5
Total Cholesterol (+1mmol/l)								
ß±SE	-	-0.068 ±0.034	-	-	-	-	-	-
		P = 0.045						
Partial r^2 (%)	-	1.1	-	-	-	-	-	-
Blood Glucose (+1mmol/l)								
ß±SE	0.10 ± 0.04	-	-	0.07 ± 0.04	0.06 ± 0.03	-	0.05 ± 0.02	0.06 ± 0.02
	P=0.013			*P*=0.026	*P*=0.034		*P*=0.027	*P*=0.006
Partial r^2 (%)	1.9	-	-	1.3	1.2	-	1.4	2.2

Values are mutually adjusted partial regression coefficients ±SE. Age was forced into all models. The covariables considered in stepwise models included sex, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.

Figure 1

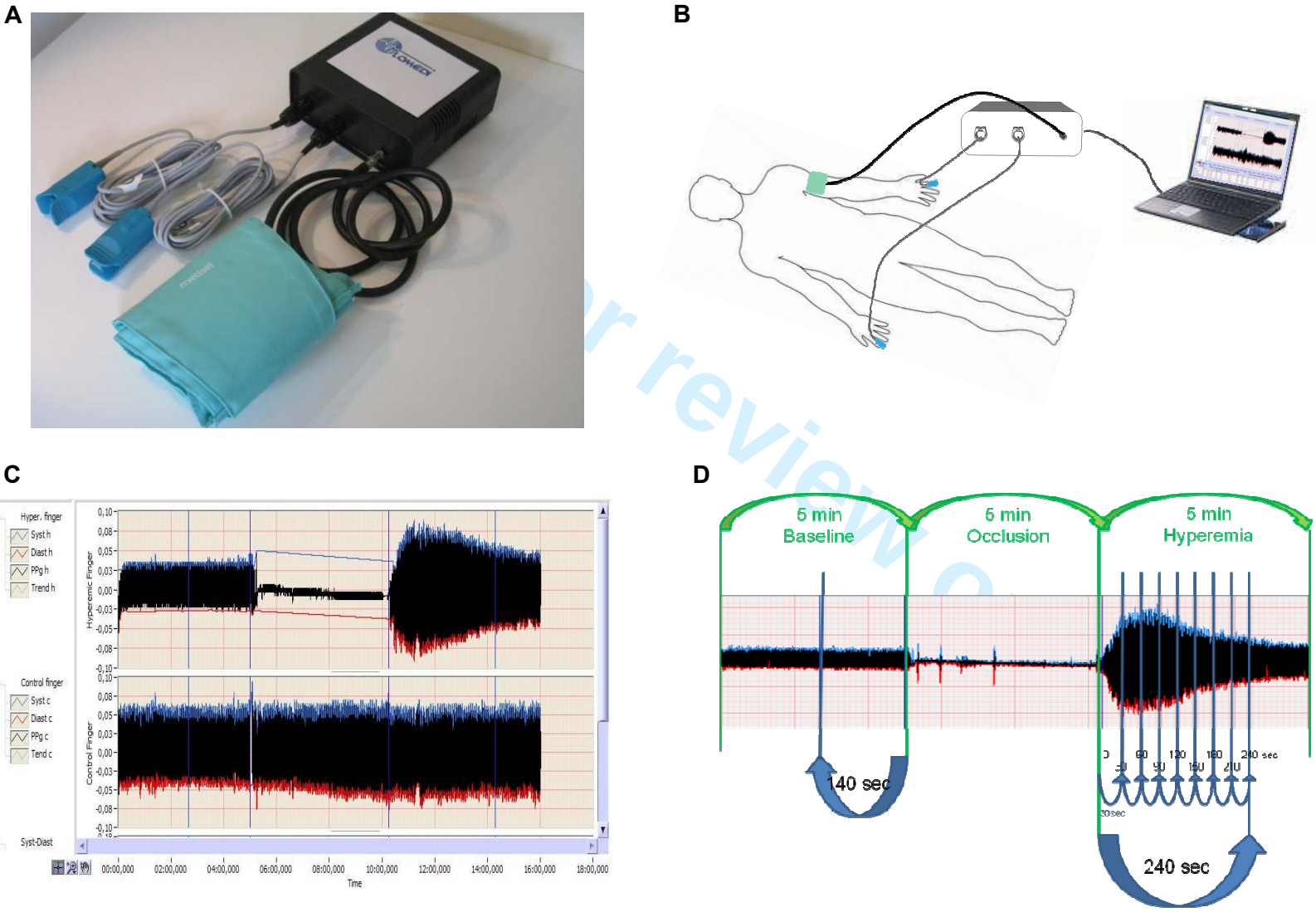

A
B
C
D
Hyper. finger
Syst h
Diast h
PPg h
Trend h
Control finger
Syst c
Diast c
PPg c
Tend c
Syst-Diast
Hyperemic Finger
Control Finger
Time
5 min
Baseline
5 min
Occlusion
5 mln
Hyperemia
140 sec
240 sec

Figure 2

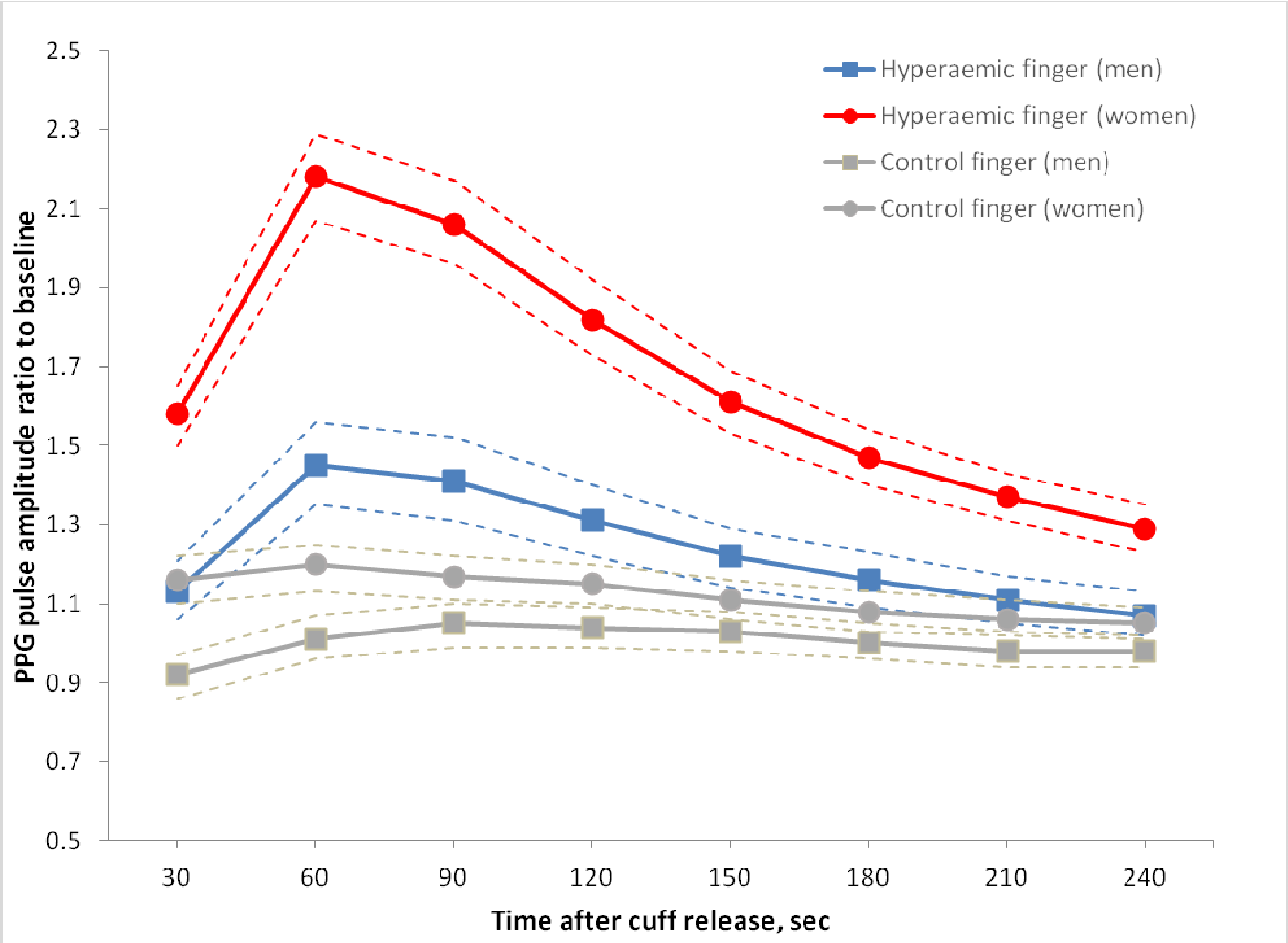

Hyperaemic finger (men)
Hyperaemic finger (women)
Control finger (men)
Control finger (women)
PPG pulse amplitude ratio to baseline
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 3

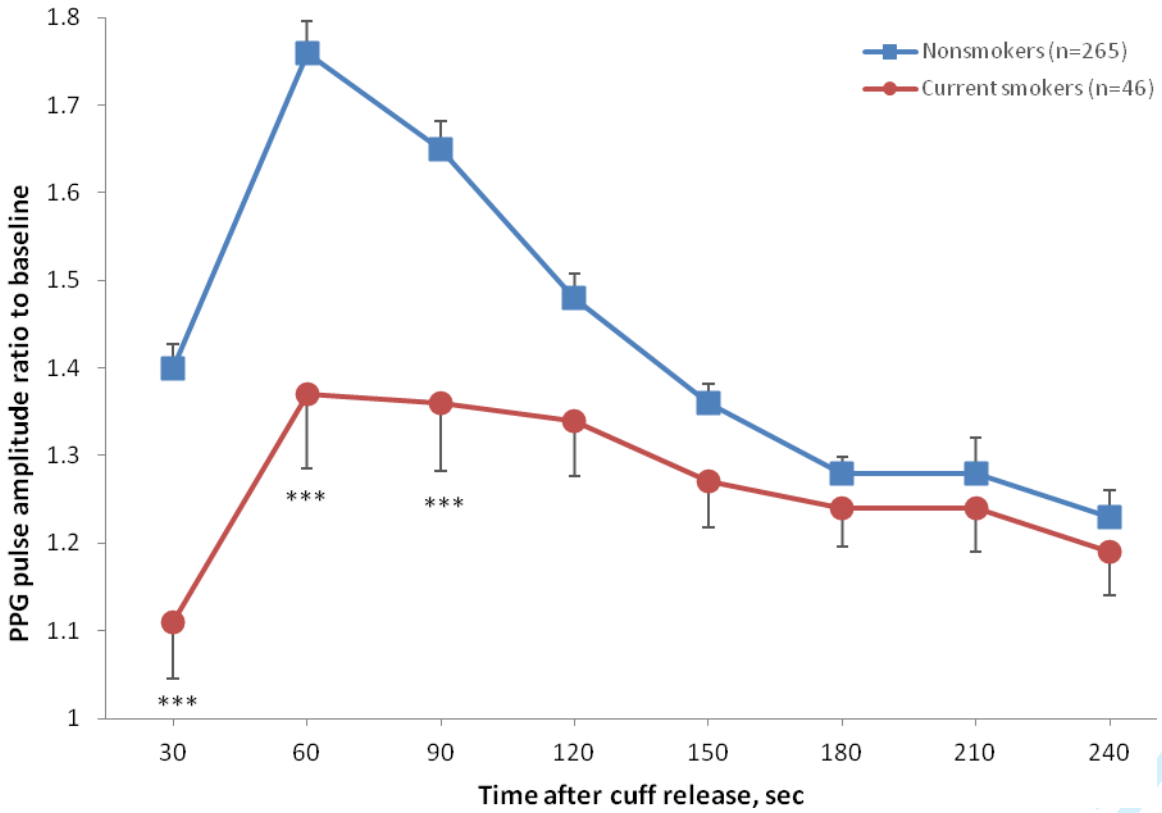
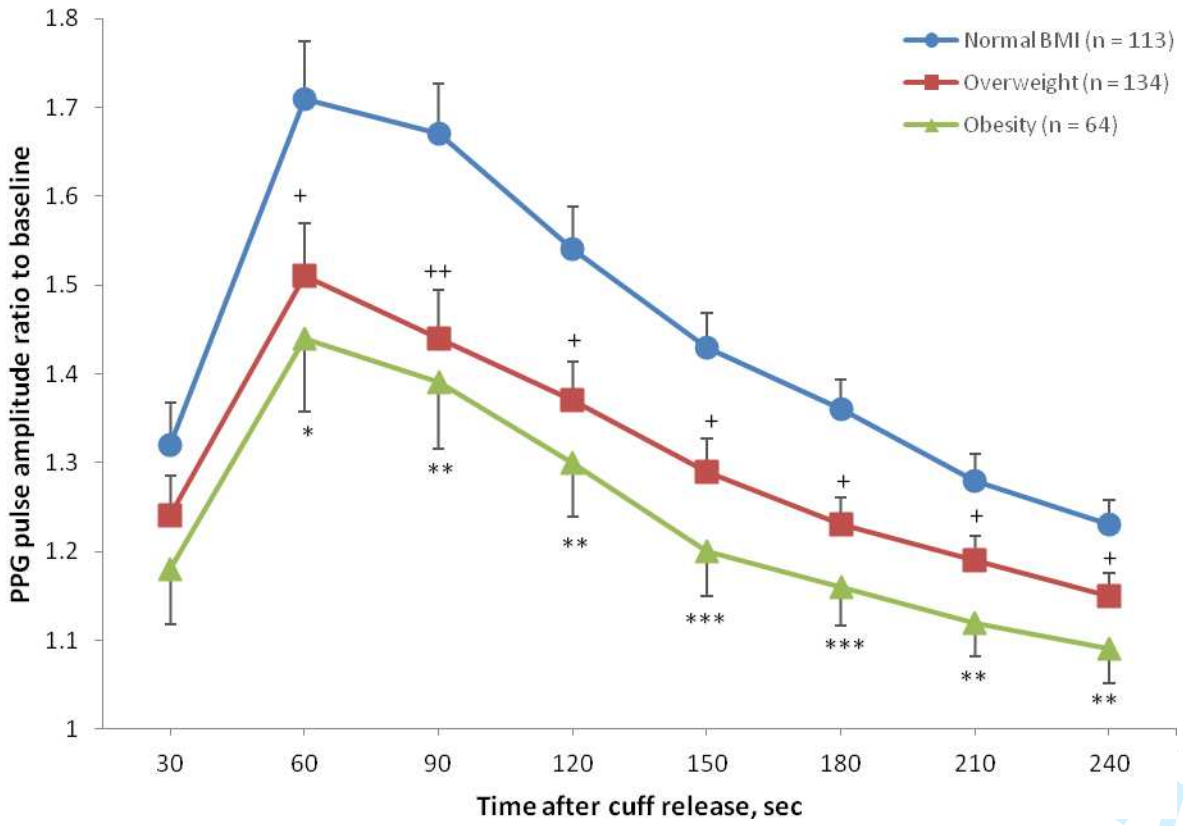
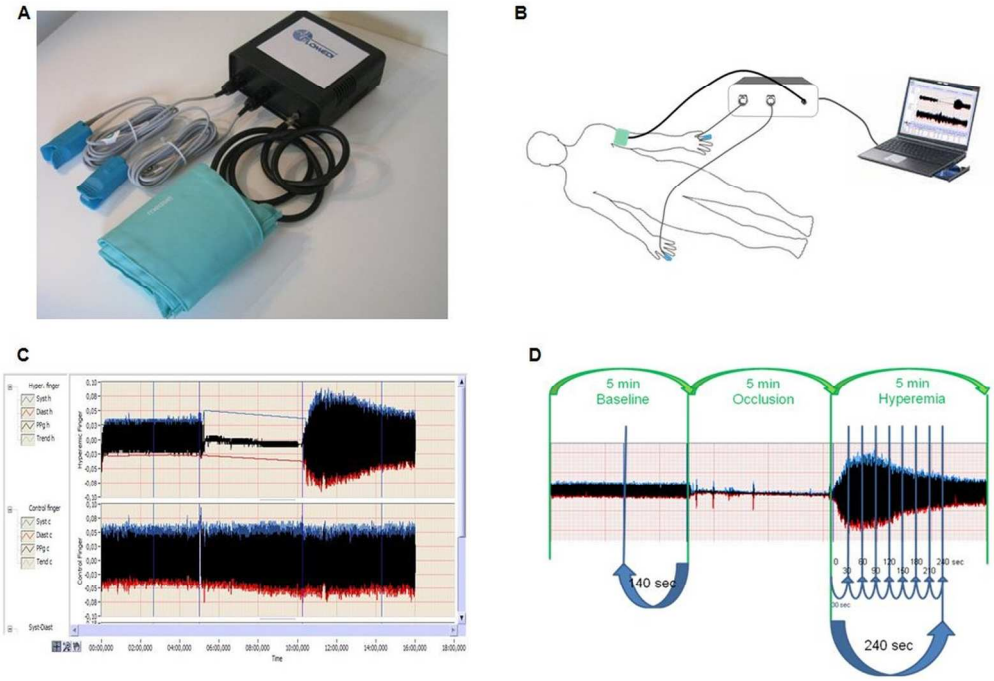
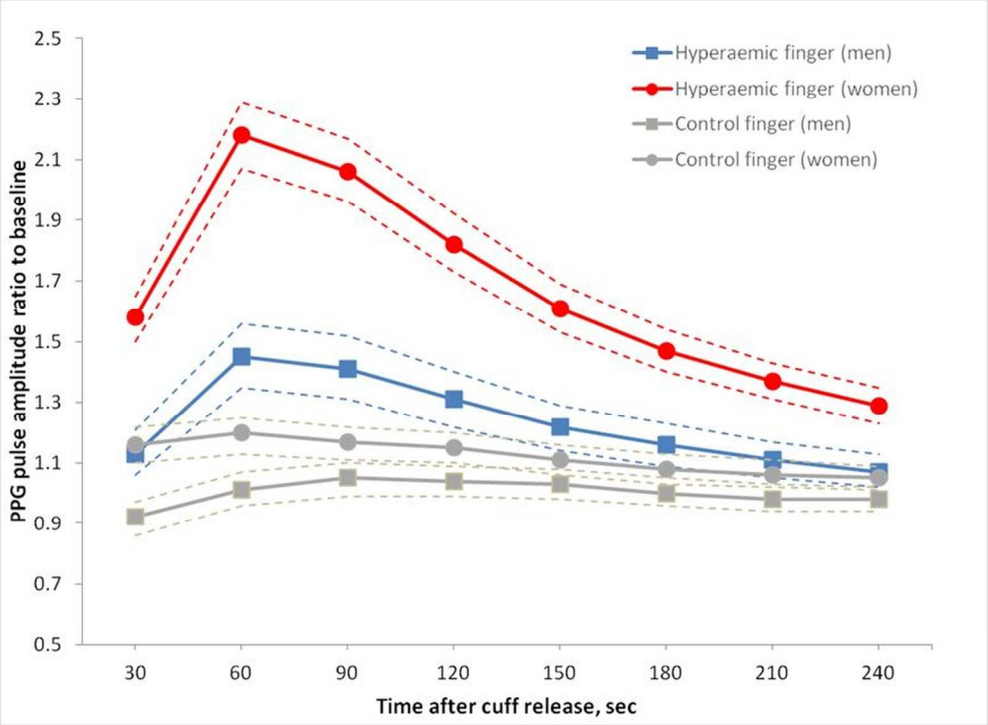

PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
Nonsmokers (n=265)
Current smokers (n=46)
***
***
***
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 4

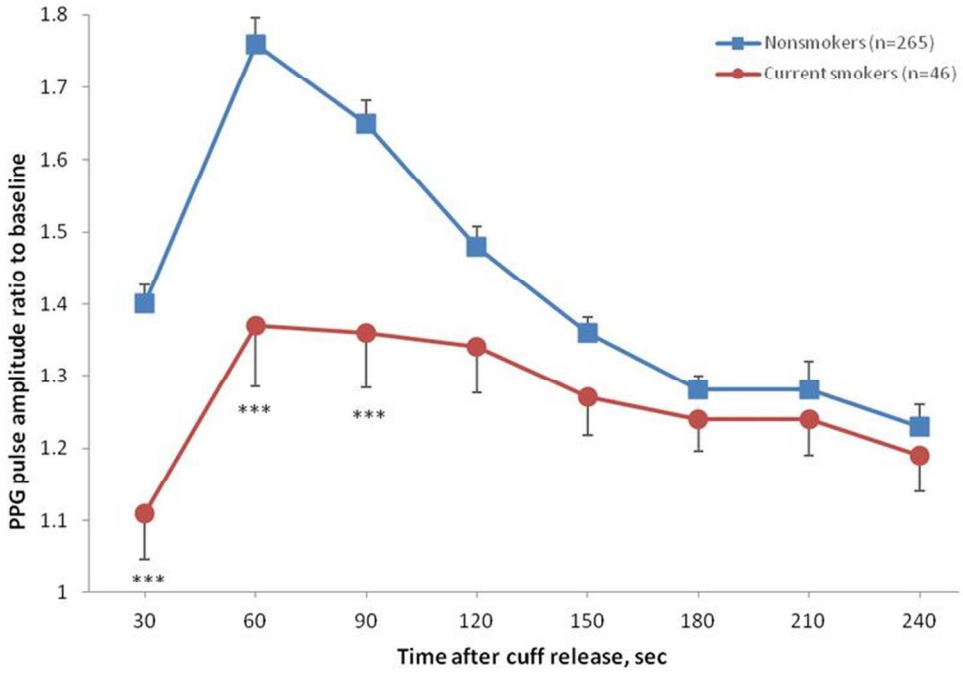

Normal BMI (n = 113)
Overweight (n = 134)
Obesity (n = 64)
PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
30
60
90
120
150
180
210
240
Time after cuff release, sec



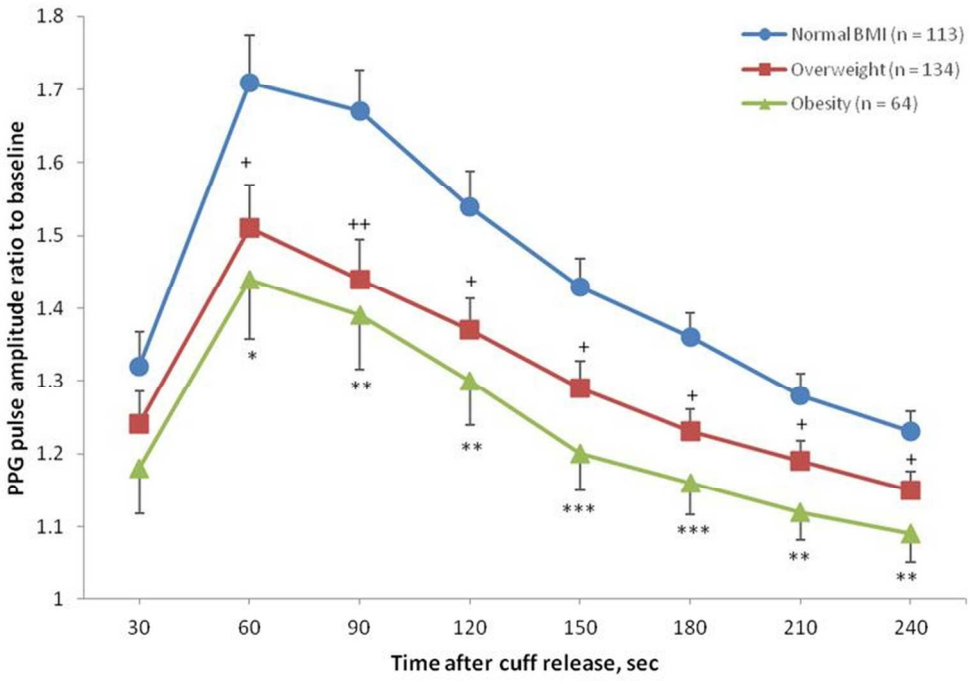
128x90mm (300 x 300 DPI)



121x90mm (300 x 300 DPI)



127x90mm (300 x 300 DPI)



127x90mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**Done**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**Done**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**Done**
Objectives	3	State specific objectives, including any prespecified hypotheses	**Done**
Methods			
Study design	4	Present key elements of study design early in the paper	**Done**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**Done**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	**Done**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**Done**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**Done**
Bias	9	Describe any efforts to address potential sources of bias	**Done**
Study size	10	Explain how the study size was arrived at	**Done**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**Done**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**Done**
		(*b*) Describe any methods used to examine subgroups and interactions	**Done**
		(*c*) Explain how missing data were addressed	NA

	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
	(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**Done**
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Done**
		(b) Indicate number of participants with missing data for each variable of interest	**Done**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*NA*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	**Done**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Done**
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Done**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Done**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Done**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Done**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Done**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**Done**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

DIII 1/31/2014 5:06 AM
BMJ Open

Association of digital vascular function with cardiovascular risk factors: a population study

Short title: Correlates of endothelial function

Tatiana Kuznetsova[1], Eline Van Vlierberghe[1], Judita Knez[1], Gregory Szczesny[2], Lutgarde Thijs[1], Dominique Jozeau[2], Costantino Balestra[3], Jan D'hooge[4], Jan A. Staessen[1,5]

Word Counts: Manuscript 5137; Abstract 300;

Number: Tables 2, Figures 4

[1] Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium; [2]FLOMEDI Company, Brussels, Belgium; [3] Environmental, Occupational & Aging Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium; [4]Division of Cardiovascular Imaging and Dynamics, Department of Cardiovascular Sciences, University of Leuven, Belgium; and [5]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands.

Correspondence to Tatiana Kuznetsova, M.D., Ph.D., Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, Campus Sint Rafaël, Kapucijnenvoer 35, Box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kuznetsova@med.kuleuven.be

ABSTRACT

Objectives: Vasodilation of the peripheral arteries **during** reactive hyperaemia depends in part on release of nitric oxide from endothelial cells. Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and PWA hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). We sought to evaluate the correlates of digital PPG PWA hyperaemic responses as a measure of peripheral vascular function.

Design: The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) is a population-based cohort study.

Setting: Respondents were examined at one centre in northern Belgium.

Participants: For this analysis, our sample consisted of 311 former participants (53.5% women; mean age 52.6 years; 43.1% hypertensive), who were examined from January 2010 until March 2012 (response rate 85.1%).

Primary outcome measures: Using a fingertip PPG device, we measured digital PWA at baseline and at 30-second intervals for 4 minutes during reactive hyperaemia induced by a 5-minute forearm cuff occlusion. We performed stepwise regression to identify correlates of the hyperaemic response ratio for each 30-second interval after cuff deflation.

Results The maximal hyperaemic response was detected in the 30- to 60-second interval. The explained variance for the PPG PWA ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval. The hyperaemic response at each 30-second interval was significantly higher in women compared to men ($P \leq 0.001$). The PPG PWA changes at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$) and at 0-240-second intervals decreased with higher body mass index ($P \leq 0.035$). These associations with sex, current smoking and body mass index were mutually independent.

Conclusions Our study is the first to implement the new PPG technique to measure digital PWA hyperaemic changes in a general population. Hyperaemic response, as measured by PPG, inversely associated with traditional cardiovascular risk factors such as male sex, smoking and obesity.

ARTICLE SUMMARY

Article focus

- Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease. Vasodilation of the peripheral arteries **during** reactive hyperaemia depends in part on release of nitric oxide from endothelial cells.
- Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and its hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). This optical technique enables detecting blood volume changes in microvascular beds in response to hyperaemia.
- In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors.

Key messages

- We demonstrated that measurement of the hyperaemic response by the new PPG technique might be a useful tool in the detection of **peripheral microvascular dysfunction** associated with cardiovascular risk factors.
- We found that PPG pulse amplitude hyperaemic response was lower in men than in women and in smokers than nonsmokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.
- The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.

Strengths and limitations of this study

- Our study is the first to implement the new PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. A finger PPG is a low-cost and operator-independent technique compared to ultrasound in the assessment of peripheral vascular function.
- Under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG technique.
- Our sample size was smaller compared to other studies. On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG technique in assessment of digital vascular function.
- **Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively**.

Keywords Population ■ Vasodilation ■ Photoplethysmography ■ Endothelial function

INTRODUCTION

Endothelial dysfunction, a marker of reduced nitric oxide (NO) bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease.[1] In humans, endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with cardiovascular risk factors.[2] Vasodilation of the peripheral arteries during reactive hyperaemia after ischaemia depends in part on the release of nitric oxide from endothelial cells in response to increased shear stress.[3] This physiological response allows the non-invasive assessment of endothelial vasomotor function which can be measured based on the flow-mediated dilation (FMD) of the brachial artery[4] or on the fingertip pulse amplitude hyperaemic response.[3, 5, 6] Previous studies mainly applied fingertip peripheral arterial tonometry (PAT) to derive pulse wave amplitude and, therefore, the pulse amplitude changes during hyperaemia.[3, 5, 6] Another approach to derive information about the arterial pulse wave is based on photoplethysmography (PPG).[7] This optical technique enables detecting blood volume changes in microvascular beds during hyperaemia.[7] We sought to evaluate the correlates of digital PPG pulse amplitude hyperaemic responses as a measure of peripheral arterial function in a sample of a general population.

MATERIALS AND METHODS

Design and sample

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).[8] From August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address,

were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS random function. Households with a number matching the list were invited. **The initial participation rate was 78.0%.**

The FLEMENGHO study is on-going longitudinal population study and, therefore, the participants were repeatedly visited at home and examined at a local examination centre. From January 2010 until March 2012 a scheduled follow-up examination included also measurement of endothelial function with the PPG technique. From 444 invited participants for this examination, we obtained informed written consent from 378 subjects (response rate 85.1%). We excluded 43 subjects with cardiac dysrhythmias, such as atrial fibrillation, pacemaker and frequent extrasystole. Because the PPG pulse amplitude was of insufficient quality to assess vascular function (n=14) or because the hyperaemic test was discontinued (n=10) we discarded a further 24 subjects. Thus, the number of participants statistically analysed totaled 311.

Determination of PPG pulse amplitude

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 hours before the test. No medication was taken on the day of the examination. We studied endothelial function in an air-conditioned room at constant temperature around 22°C. To attain a cardiovascular steady-state before starting the test, the subjects had rested for at least 20 minutes in the supine position. Since peripheral vasoconstriction is correlated with the surrounding temperature, before the test, special care was taken to keep fingertips temperature around 35°C. The blood pressure was the average of 5 auscultatory readings, obtained with a standard sphygmomanometer. The blood pressure measurement was performed on the arm that served as control.

Digital pulse amplitude was measured with a PPG device (FLOMEDI Company, Brussels) transmitting infrared light at a wavelength of 940 nm and positioned on the tip of each index finger. Digital output from the PPG device was recorded through an analogue-to-digital converter (10 bit, sampling frequency 250 Hz). **We expressed the amplitude of the PPG PWA signal in arbitrary units. To determine the amplitude changes of the digital pulse curve in response to hyperaemia, we used a protocol as described by Hamburg *et al*.[6] As shown in Figure 1, panel C and D, baseline PPG pulse amplitude was registered at each of the two index fingertips for at least 5 minutes to ensure a stable baseline PPG signal. For the analysis, we used PPG pulse amplitude that was measured for last 2 min 20 sec.** Next, arterial flow was interrupted for 5 minutes by an inflatable cuff placed on the proximal forearm with an occlusion pressure of 200-220 mmHg (around 50 mmHg above the participant's systolic pressure). **Complete cessation of blood flow to the hand is verified by the absence of a PPG signal from the occluded arm.** After cuff deflation, we analysed the PPG pulse amplitude at both fingers using a computerised, automated algorithm (FLOMEDI Company, Brussels) that provided the averaged pulse amplitude for each 30-second interval up to 4 minutes (see the PPG pulse tracking in Figure 1).

For each 30-second interval, the response of the PPG pulse wave amplitude to hyperaemia was calculated from the hyperaemic fingertip as the ratio of the post-deflation PPG pulse amplitude to the baseline amplitude (PA_{ht}/PA_{h0}, where PA is the pulse amplitude, h is the hyperaemic finger, t is time interval, and 0 is baseline). To obtain the PPG pulse amplitude ratio we divided PA_{ht}/PA_{h0} ratio by the corresponding ratio at the control hand (PA_{ct}/PA_{c0}, where c is the control finger).

To determine the inter-session reproducibility of the hyperaemic response, we analysed PPG ratios measured on two different occasions in 5 subjects. We determined the absolute and relative biases of the averaged PPG pulse amplitude ratios **per each 30-second time interval** between the two sessions as well as 95% limits of agreement between sessions. Absolute and relative biases between the two sessions were calculated according to Bland and Altman's method as (x1 – x2) vs averaged and (100*(x1 – x2)/averaged) vs averaged, respectively. **The absolute and relative biases of the averaged PPG pulse amplitude ratios at each time interval between the two sessions were 0.062 (95% confidence interval [CI]: –0.10 to 0.23) and 3.29% (95% CI: –8.8% to 15.4%), respectively.**

Other measurements

At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each subject's medical history, smoking and drinking habits, and intake of medications. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic (average of 5 consecutive auscultatory readings at the examination centre) or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Overweight was a body mass index between 25 and 30 kg/m². Obesity was a body mass index of 30 kg/m² or higher.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, NC). The central tendency and the spread of the data are reported as mean ± SD. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by computation of the coefficient of skewness, *i.e.*, the third moment about the mean divided by

the cube of the standard deviation. We compared means and proportions by means of a sample t-test and by the χ^2-test, respectively. Significance was $P < 0.05$ on two-sided test.

We performed single and stepwise multiple regression to assess the independent correlations of the PPG pulse amplitude ratio during each 30-second interval **with sex, age, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.** We set the *P*-values for variables to enter and to stay in the regression models at 0.10.

RESULTS

Characteristics of participants and PPG pulse amplitude

The participants included 154 (53.5%) women, and 134 (43.1%) hypertensive patients of whom 78 (25.1 %) were on antihypertensive drug treatment. Table 1 shows the clinical characteristics and PPG pulse amplitude measures of the study participants by sex. In this cohort, women had lower systolic and diastolic blood pressure and higher heart rate than men, less often reported alcohol consumption and had no history of ischaemic heart disease.

The geometric means of the baseline PPG amplitude were 7.3 (5%-95% percentiles: 2.7 to 25.9) and 9.3 (5%-95% percentiles: 3.9 to 25.3) at the hyperaemic and control finger, respectively. We observed a high correlation between values of the baseline PPG amplitude recorded at both fingers (r=0.89, *P*<0.0001). As shown in Figure 2, after forearm cuff deflation, the ratio of the PPG pulse amplitude to baseline rose rapidly in the hyperaemic fingertip, with maximal response occurring in the 30- to 60-second interval, whereas the changes of PPG amplitude in the control finger were minimal. Table 1 lists the mean values of the post-deflation PPG pulse amplitude ratio at each 30-second interval by

sex. The hyperaemic response at each 30-second interval was significantly higher in women compared to men (Table 1). In both women and men, the maximal hyperaemic response was detected in the 30- to 60-second interval.

Determinants of PPG pulse amplitude ratio

We performed stepwise regression to assess the independent correlations of the hyperaemic response for each 30-second interval after cuff deflation with sex, age, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. With age forced in the models, the explained variance for the PPG pulse amplitude ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval (Table 2). The PPG PWA changes throughout 0-240-second intervals significantly decreased with male sex ($P \leq 0.0004$) and with body mass index ($P \leq 0.017$). The hyperaemic response at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$). These associations with sex, body mass index and current smoking were mutually independent. In addition, the PPG pulse amplitude ratio at 30- to 60-second interval decreased with total cholesterol, but this association only reached borderline significance ($P=0.045$;Table 2). **Blood glucose was also selected as an independent determinant of the PPG ratio (Table 2), but overall impact of this covariable is relatively small (explained about 1,5% of total variability). Moreover, blood glucose was not a significant determinant of the maximal peak of hyperaemic response which occurs at 30- to 60 second and 60- to 90-second intervals.**

Figure 3 illustrates the hyperaemic responses by the smoking status while adjusted for important covariables. The maximal hyperaemic response in the 30-to 60-second interval was significantly lower in current smokers compared to non-smokers (1.37 vs 1.76;

P<0.0001). Figure 4 shows the adjusted PPG pulse amplitude hyperaemic responses in subjects, divided into 3 categories according to their body mass index. In overweight (n=134; 1.51±0.060) and obese (n=64; 1.44±0.082) subjects the maximal hyperaemic response was significantly lower compared to lean participants (n=113; 1.71±0.064).

DISCUSSION

In our cohort recruited from a population study, we evaluated the relationship between PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, and cardiovascular risk factors. We observed a time-dependent increase in digital PPG pulse amplitude that peaked in the 30- to 60-second interval after induction of reactive hyperaemia. In keeping with the literature,[6, 9-11] we found that PPG pulse amplitude hyperaemic response was higher in women than in men and in nonsmokers than smokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.

Endothelial function is often assessed non-invasively by vascular reactivity tests. Several methods are available to study endothelial function in the peripheral macrocirculation (conduit arteries) and microcirculation (resistance arteries and arterioles).[2, 12] Measurement of the brachial artery diameter before and after **5 minutes** of occlusion of the arterial flow to the forearm is the most widely used test to assess endothelium-dependent vasodilation.[4, 13, 14] The change in arterial diameter gives a measure of flow-mediated vasodilatation (FMD). This technique, however, is operator dependent, is costly and requires a long post-processing time. Measurement of microcirculatory reactive hyperaemia can be assessed by digital pulse amplitude measured by applanation tonometry[5, 6] or photoplethysmography.[15, 16] Lund[17] described the potential of the PPG technique for the assessment of vasodilation by

using this technique to measure haemodynamic response to nitroglycerin. **Moreover, Theunissen *et al*[18] observed in divers an increase in circulating NO after successive breath-hold dives. This increase in circulating NO level was associated with higher hyperaemic response measured using the same PPG device as in our study.**

Both techniques for assessment of digital vascular function are non-operator-dependent, and the equipment is an order of magnitude less expensive than for ultrasonography. However, the tonometry method might be more expensive as compare to the PPG technique because of additional costs associated with changeable plethysmographic probes. Furthermore, the digital tonometry procedure is more complicated and less comfortable for patients because it requires attachment of a pneumo-electrical tube to an additional pneumatic digital cuff which should be constantly inflated during the test.

We observed similar digital PPG pulse amplitude changes during the hyperaemic response **compared with results from studies using the finger applanation tonomtery based method**.[6, 10, 11] In the Framingham study,[6, 10] similar to our study, the ratio of the digital pulse amplitude to baseline rose rapidly in the hyperaemic fingertip after forearm cuff deflation, and then slowly decreased towards baseline. However, we detected the maximal hyperaemic response in the 30- to 60-second interval, whereas in the Framingham study the pressure amplitude ratio was highest in the 60- to 90-second interval. The difference in the time of maximal hyperaemic response between the Framingham study and our report might be related to the fact that finger PAT measures pressure changes while photoplethysmography measures changes of the relative amount of blood volume.

We observed the relations between the hyperaemic PPG pulse amplitude response and cardiovascular risk factors. In our current study and in other community-based studies,[6, 10, 11] men had a less pronounced hyperaemic response than women, which is probably in

part attributable to physiological differences in vessel diameter and wall thickness between the sexes. In line with other studies,[6, 10, 11] which used the PAT technique to evaluate endothelial function, we demonstrated a significant inverse associations between PPG amplitude changes and smoking, obesity and total cholesterol. The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.[19] We also tested the influences of antihypertensive and antihyperlipidemic treatment on the hyperaemic PPG pulse amplitude response, which were not significant (results not shown). **The observed association between the PPG PWA ratio and blood glucose during some time intervals might be related to the optic technique which we used in our study.**

Similar to other studies, in which finger applanation tonometry was used to assess the endothelial function,[10] we did not observe a significant relation between hyperaemic PPG pulse amplitude changes and age. On the other hand, previous studies reported lower hyperaemic response as assessed by FMD with advancing age.[13, 14] Differences in the age-related hyperaemic responses between microcirculatory and macrocirculatory reactivity might explain these divergent findings.[9] Moreover, recent studies demonstrated that brachial and digital measures of vascular function were uncorrelated with each other.[10, 20] It was suggested that FMD and PAT provide distinct information regarding vascular function in conduit versus smaller digital vessels.

The present study must be interpreted within the context of its potential limitations and strengths. First, PPG pulse amplitude registration is prone to measurement error due to higher variability in comparisons with the FMD technique.[21] On the other hand, assessment of the hyperaemic PPG pulse wave amplitude changes requires little training and is operator

independent. Moreover, under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques. **Second, placing the occlusion cuff above the site of hyperaemic response measurement might evoke a dilatory response that is related in part to ischaemia and, therefore, is not entirely mediated by NO.** Third, our sample size was smaller compared to other studies.[10,11] On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function. **Forth, as shown in Table 2, in our study, we could explain only around 20% of variability of the PPG PWA ratios by traditional CV risk factors. The remaining variability might be influenced by genetic factors, inflammatory processes or other confounders that we did not consider in our study. Moreover, in our opinion, it is important to demonstrate in prospective studies that the hyperaemic response as assessed by the PPG technique might be an independent predictor of CV events.**

In conclusion, our study is the first to implement the PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. We demonstrated that measurement of the hyperaemic response by the PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with smoking and obesity, while accounting for the differential hyperaemic response between men and women. **Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.**

Acknowledgments

The authors gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Dais Thijs and Hanne Truyens (Leuven, Belgium).

Contributors

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation of the data. TK, EVV, JK drafted the manuscript. All authors gave final approval of the final version.

Funding

The European Union (grants HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and ERC Advanced Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13 and G. 0881.13) gave support to the Research Unit Hypertension and Cardiovascular Epidemiology.

Disclosures

GC and DJ work at FLOMEDI, a spin-off company (Spin Off In Brussels - Innoviris) of the Technical Department of the Haute Ecole Paul Henri Spaak. The company designs and develops software and electronic medical devices in order to facilitate, simplify, and increase accuracy of non-invasive assessment of vascular stiffness.

None of the other authors declares a conflict of interest.

REFERENCES

1. Vita JA. Endothelial function. *Circulation* 2011;124:e906-e912.
2. Flammer AJ, Anderson T, Celermajer DS, *et al.* The assessment of endothelial function: from research into clinical practice. *Circulation* 2012;126:753-767.
3. Nohria A, Gerhard-Herman M, Creager MA, *et al.* Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006;101:545-548.
4. Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J* 2003;145:943-951.
5. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2013;146:168-174.
6. Hamburg NM, Keyes MJ, Larson MG, *et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467-2474.
7. Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiol Meas* 2007;28:R1-R39.
8. Kuznetsova T, Herbots L, López B, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009;2:105-112.
9. Brunner H, Cockcroft JR, Deanfield J, *et al.* Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:233-246.
10. Hamburg NM, Palmisano J, Larson MG, *et al.* Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011;57:390-396.

11. Schnabel RB, Schulz A, Wild PS, *et al.* Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. *Circ Cardiovasc Imaging* 2011;4:371-380.
12. Lekakis J, Abraham P, Abraham P, *et al.* Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18:775-789.
13. Benjamin EJ, Larson MG, Keyes MJ, *et al.* Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.
14. Nawrot TS, Staessen JA, Fagard RH, *et al.* Endothelial function and outdoor temperature. *Eur J Epidemiol* 2005;20:407-410.
15. Zahedi E, Jaafar R, Ali MA, *et al.* Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiol Meas* 2008;29:625-637.
16. Millasseau SC, Ritter JM, Takazawa K, *et al.* Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* 2006;24:1449-1456.
17. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates--a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79-86.
18. Theunissen S, Guerrero F, Sponsiello N, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med* 2013;40:135-144.
19. Steffen Y, Vuillaume G, Stolle K, *et al.* Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide* 2012;27:176-184.
20. Martin BJ, Gurtu V, Chan S, *et al.* The relationship between peripheral arterial tonometry and classic measures of endothelial function. *Vasc Med* 2013;18:13-18.

21. Donald AE, Charakida M, Cole TJ, *et al.* Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* 2006;48:1846-1850.

Legend to figures

Figure 1. Panel A shows a system incorporating two PPG devices transmitting infrared light, analogue-to-digital converter and forearm pressure cuff. Panel B shows the position of cuff and two PPG devices during the test. Panel C and D show recorded pulse amplitude tracing. In the arm undergoing hyperemia (panel C, top tracing, and panel D), baseline amplitude is recorded. During cuff inflation, flow is occluded and restores after cuff release (hyperaemic period). In the contralateral control finger (panel C, bottom tracing), flow continues throughout, and pulse amplitude undergoes minimal changes.

Figure 2. PPG pulse amplitude response for the hyperaemic (closed symbols) and control (open symbols) finger in women (circles) and men (squares). Women had more pronounced responses than men. Symbols are means, dashed line – 95% confidence interval.

Figure 3. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in smokers and nonsmokers subjects. Smokers had significantly lower response throughout the 0- to 120-second postdeflation intervals. Symbols are means and SE. Models are adjusted for sex, age, body mass index, total cholesterol and blood glucose. ***P<0.001 vs nonsmokers.

Figure 4. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in subjects with normal body mass index (BMI), overweight (25 kg/m²≤BMI<30 kg/m²) and obesity (BMI≥30 kg/m²). Symbols are means and SE. Models are adjusted for sex, age, smoking, total cholesterol and blood glucose. *P<0.05, **P<0.01, ***P<0.001 vs lean participants. $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001 vs lean participants.

Table 1. Characteristics of participants

Clinical measurements				PPG pulse amplitude measures			
Characteristic	Women (*n* =154)	Men (*n* =157)	P-value	Characteristic	Women (*n* =154)	Men (*n* =156)	*P-value*
Anthropometrics				PPG ratio			
Age, y	53.51±12.2	51.8±14.5	0.26	Time interval (sec)			
Body mass index, kg/m^2	26.3±4.0	27.3±3.7	0.03	0-30	1.43 (0.87 to 2.02)	1.27 (0.83 to 1.84)	0.002
Systolic pressure, mm Hg	125.5±15.4	131.4±14.3	0.0006	30-60	1.93 (1.08 to 2.86)	1.46 (1.00 to 2.13)	<0.0001
Diastolic pressure, mm Hg	80.4±8.0	84.5±9.5	<0.0001	60-90	1.84 (1.10 to 2.50)	1.37 (0.97 to 1.93)	<0.0001
Heart rate, beats/minute	66.5±10.2	62.4±9.9	0.0003	90-120	1.64 (1.09 to 2.16)	1.27 (0.93 to 1.79)	<0.0001
Questionnaire data				120-150	1.49 (1.06 to 2.01)	1.20 (0.92 to 1.59)	<0.0001
Current smoking, n (%)	28 (18.2)	18 (11.5)	0.10	150-180	1.38 (1.00 to 1.84)	1.16 (0.89 to 1.46)	<0.0001
Alcohol, n (%)	39 (25.3)	94 (59.9)	<0.0001	180-210	1.30 (0.98 to 1.65)	1.14 (0.87 to 1.43)	<0.0001
Diabetes, n (%)	5 (3.3)	4 (2.6)	0.72	210-240	1.24 (0.95 to 1.65)	1.11 (0.87 to 1.33)	<0.0001
Treated for hypertension, n (%)	38 (24.7)	40 (25.5)	0.87				
Beta-blockers, n (%)	18 (11.7)	23 (14.7)	0.44				
ACE or ARB, n (%)	12 (7.8)	15 (9.6)	0.58				
Diuretics or CCB, n (%)	22 (14.3)	19 (12.1)	0.57				
Previous history of IHD, n (%)	0 (0)	7 (4.5)	0.008				
Total cholesterol, mmol/l	5.2±1.00	5.0±0.96	0.037				
Lipid lowering agents, n (%)	10 (6.5)	8 (5.1)	0.60				

Values are mean (±SD), mean (10%-90%), or number of subjects (%). PPG indicates photoplethysmography, ACE indicates angiotensin-converting enzyme; ARB indicates angiotensin receptor blockers, CCB indicates calcium channel blockers, IHD indicates ischemic heart disease.

Table 2. Correlates of PPG ratios selected by stepwise regression

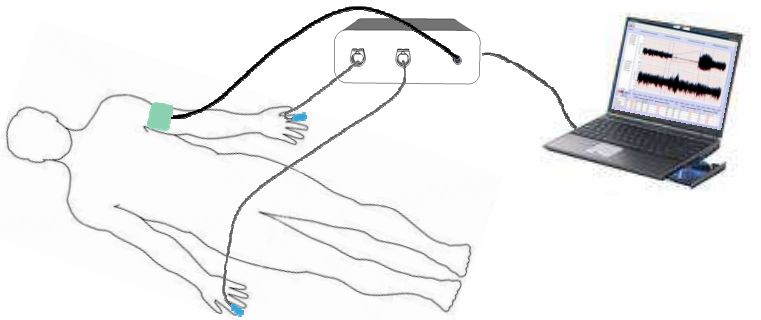
Parameter	PPG ratio							
	Time Intervals (sec)							
	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
Regression statistic								
Model R^2 (%)	9.7	21.4	22.5	19.8	19.2	16.3	13.2	12.2
Age (+10 years)*								
ß±SE	0.014±0.020	-0.0007±0.028	0.005±0.025	0.004±0.019	0.007±0.017	-0.0008±0.014	0.004±0.012	0.002±0.011
	P=0.45	*P*=0.98	*P*=0.85	*P*=0.85	*P*=0.68	*P*=0.95	*P*=0.72	*P*=0.91
Partial r^2 (%)	0.02	0	0.01	0.04	0.06	0	0.04	0.01
Female (0,1)								
ß±SE	0.16 ± 0.05	0.49 ± 0.68	0.46 ± 0.061	0.35 ± 0.050	0.26 ± 0.041	0.20 ± 0.035	0.14 ± 0.031	0.12 ± 0.03
	P=0.0004	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Partial r^2 (%)	3.9	13.6	16.0	15.1	13.5	12	7.7	6.1
Current smoking (0,1)								
ß±SE	-0.30 ± 0.07	-0.39 ± 0.09	-0.29 ± 0.085	-	-	-	-	-
	P=0.0004	*P*<0.0001	*P*=0.0007					
Partial r^2 (%)	4.0	3.7	3.1	-	-	-	-	-
Body mass index (kg/m²)								
ß±SE	-0.014±0.007	-0.027±0.009	-0.032±0.008	-0.025±0.007	-0.022±0.005	-0.018±0.005	-0.015±0.004	-0.013±0.004
	P=0.017	*P*=0.003	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*=0.0002	*P*=0.0008
Partial r^2 (%)	1.7	3.0	3.4	3.4	4.5	4.5	4.1	3.5
Total Cholesterol (+1mmol/l)								
ß±SE	-	-0.068 ±0.034	-	-	-	-	-	-
		P = 0.045						
Partial r^2 (%)	-	1.1	-	-	-	-	-	-
Blood Glucose (+1mmol/l)								
ß±SE	0.10 ± 0.04	-	-	0.07 ± 0.04	0.06 ± 0.03	-	0.05 ± 0.02	0.06 ± 0.02
	P=0.013			*P*=0.026	*P*=0.034		*P*=0.027	*P*=0.006
Partial r^2 (%)	1.9	-	-	1.3	1.2	-	1.4	2.2

Values are mutually adjusted partial regression coefficients ±SE. Age was forced into all models. The covariables considered in stepwise models included sex, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.

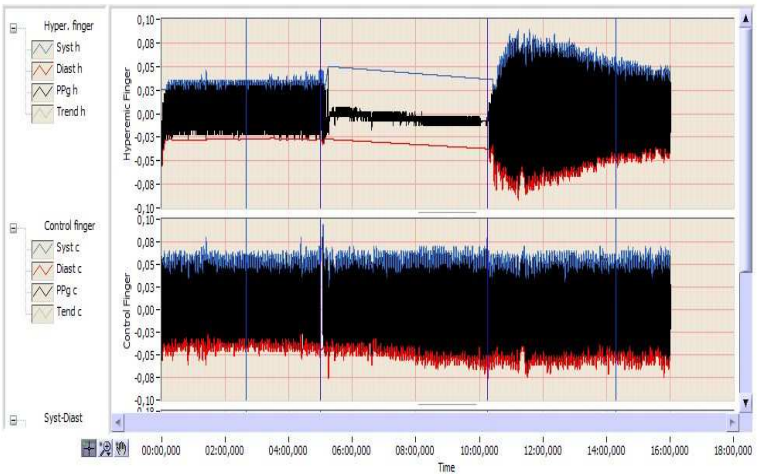
Figure 1

A

B



C



D

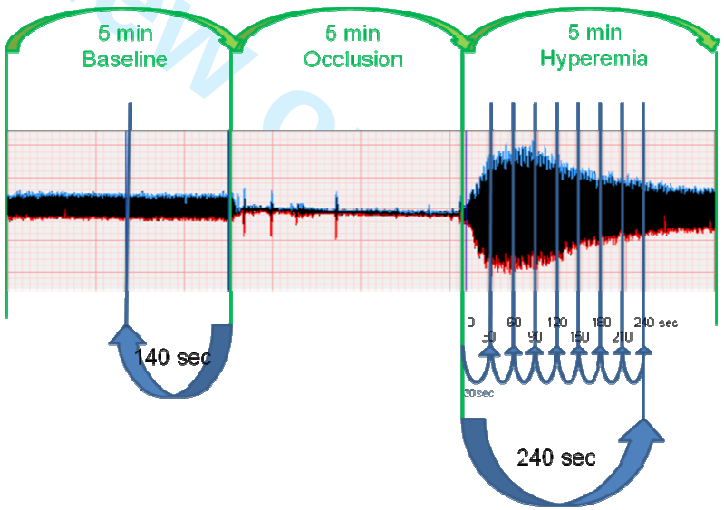


Figure 2

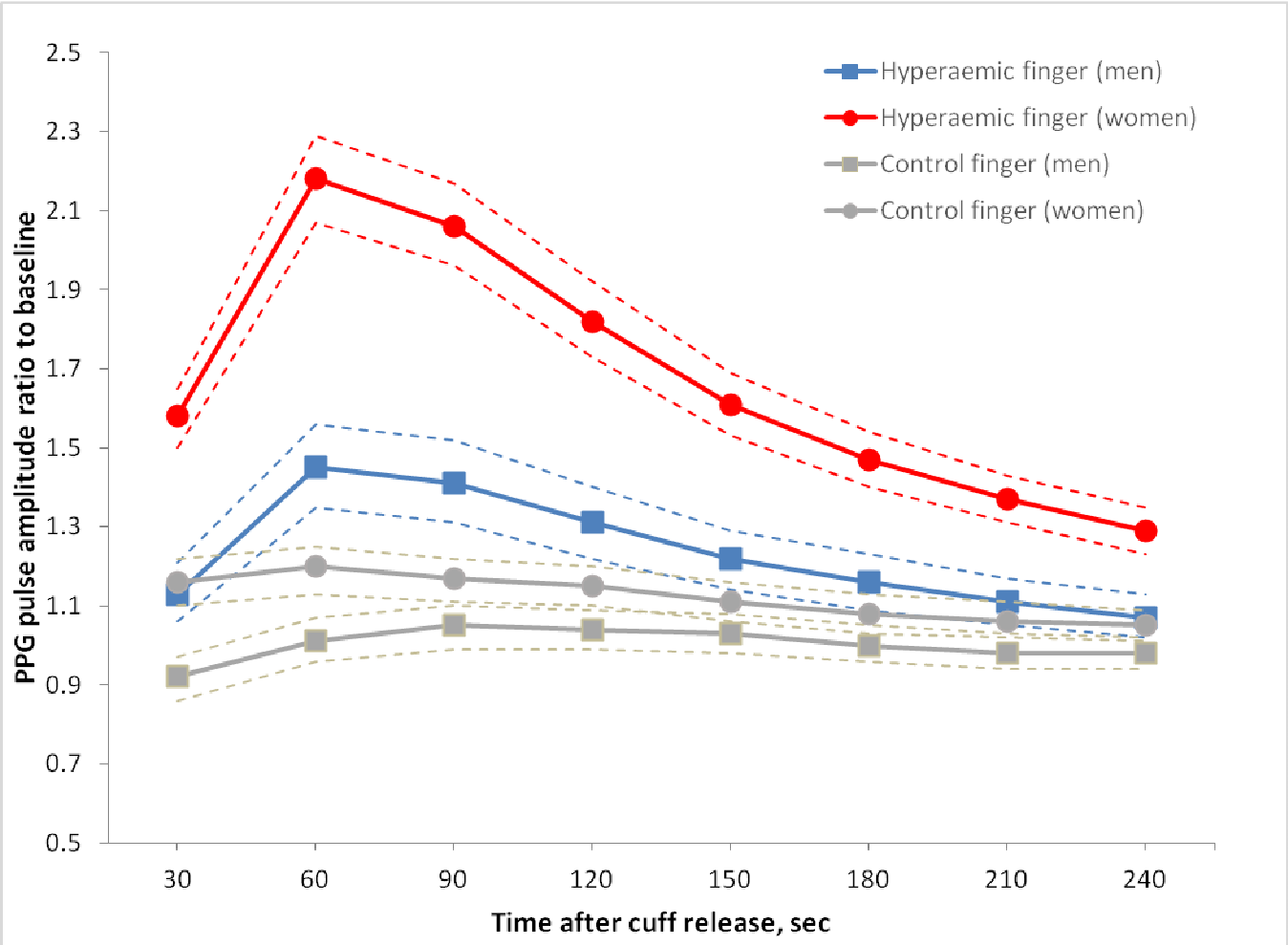

Hyperaemic finger (men)
Hyperaemic finger (women)
Control finger (men)
Control finger (women)
PPG pulse amplitude ratio to baseline
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 3

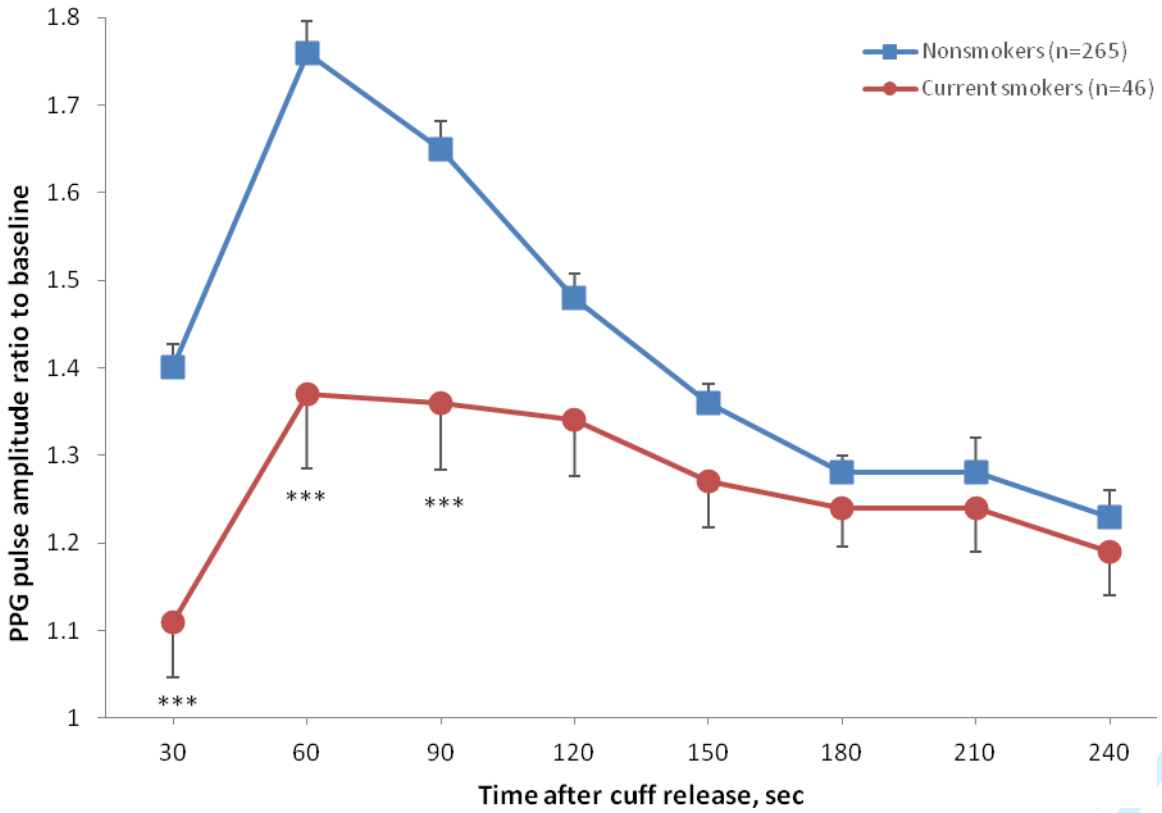
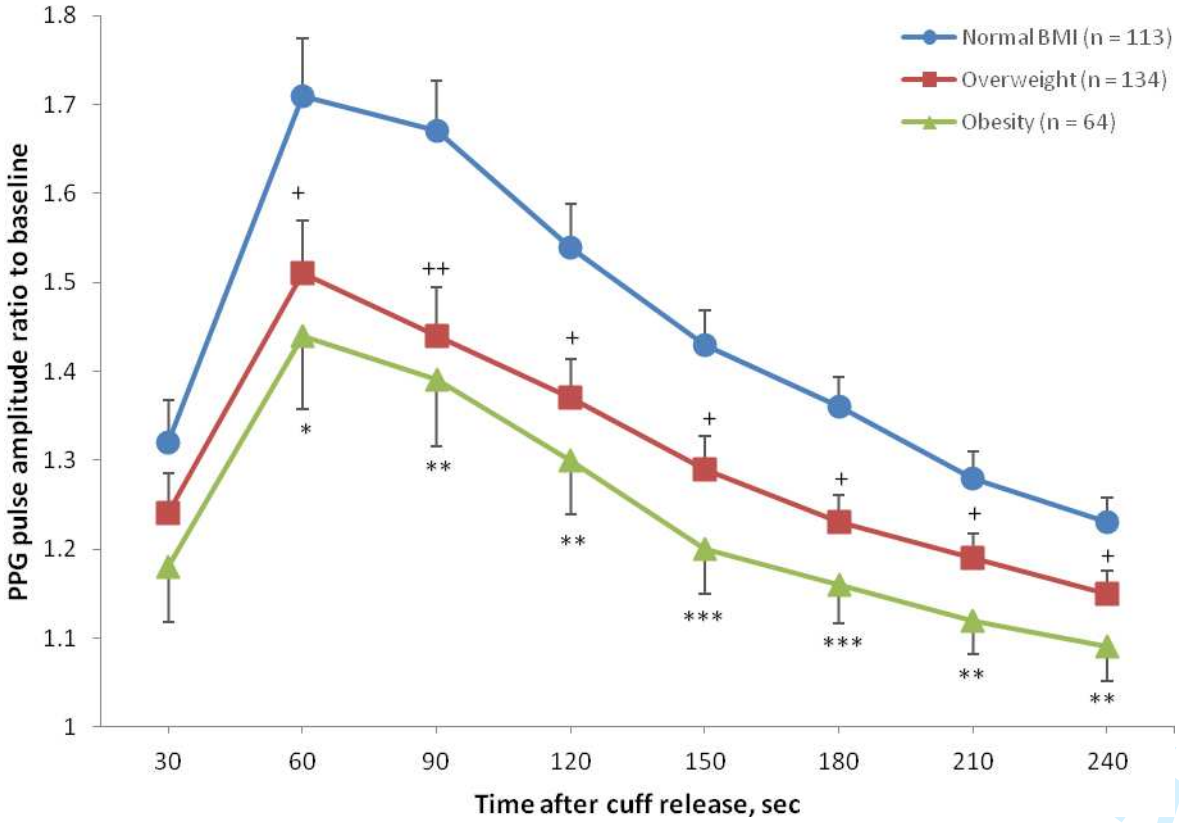

PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
Nonsmokers (n=265)
Current smokers (n=46)
***
***
***
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 4


Normal BMI (n = 113)
Overweight (n = 134)
Obesity (n = 64)
PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
30
60
90
120
150
180
210
240
Time after cuff release, sec

BMJ open

Association of digital vascular function with cardiovascular risk factors: a population study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004399.R2
Article Type:	Research
Date Submitted by the Author:	24-Feb-2014
Complete List of Authors:	Kuznetsova, Tatiana; University of Leuven, Cardiovascular Sciences Van Vlierberghe, Eline; University of Leuven, Knez, Judita; University of Leuven, Szczesny, Gregory; FLOMEDI Company, Thijs, Lutgarde; University of Leuven, Jozeau, Dominique; FLOMEDI Company, Balestra, Costantino; Haute Ecole Paul Henri Spaak, D'hooge, Jan; University of Leuven, Staessen, Jan; KU Leuven,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology
Keywords:	population, endothelial function, vasodilation, photoplethysmography

DIII 2/27/2014 9:44 AM
BMJ Open

Association of digital vascular function with cardiovascular risk factors: a population study

Short title: Correlates of digital vascular function

Tatiana Kuznetsova[1], Eline Van Vlierberghe[1], Judita Knez[1], Gregory Szczesny[2], Lutgarde Thijs[1], Dominique Jozeau[2], Costantino Balestra[3], Jan D'hooge[4], Jan A. Staessen[1,5]

Word Counts: Manuscript 5266; Abstract 300;

Number: Tables 2, Figures 4

[1] Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium; [2]FLOMEDI Company, Brussels, Belgium; [3] Environmental, Occupational & Aging Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium; [4]Division of Cardiovascular Imaging and Dynamics, Department of Cardiovascular Sciences, University of Leuven, Belgium; and [5]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands.

Correspondence to Tatiana Kuznetsova, M.D., Ph.D., Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, Campus Sint Rafaël, Kapucijnenvoer 35, Box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kuznetsova@med.kuleuven.be

ABSTRACT

Objectives: Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells. Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and PWA hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). We sought to evaluate the correlates of digital PPG PWA hyperaemic responses as a measure of peripheral vascular function.

Design: The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) is a population-based cohort study.

Setting: Respondents were examined at one centre in northern Belgium.

Participants: For this analysis, our sample consisted of 311 former participants (53.5% women; mean age 52.6 years; 43.1% hypertensive), who were examined from January 2010 until March 2012 (response rate 85.1%).

Primary outcome measures: Using a fingertip PPG device, we measured digital PWA at baseline and at 30-second intervals for 4 minutes during reactive hyperaemia induced by a 5-minute forearm cuff occlusion. We performed stepwise regression to identify correlates of the hyperaemic response ratio for each 30-second interval after cuff deflation.

Results The maximal hyperaemic response was detected in the 30- to 60-second interval. The explained variance for the PPG PWA ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval. The hyperaemic response at each 30-second interval was significantly higher in women compared to men ($P \leq 0.001$). The PPG PWA changes at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$) and at 0-240-second intervals decreased with higher body mass index ($P \leq 0.035$). These associations with sex, current smoking and body mass index were mutually independent.

Conclusions Our study is the first to implement the new PPG technique to measure digital PWA hyperaemic changes in a general population. Hyperaemic response, as measured by PPG, inversely associated with traditional cardiovascular risk factors such as male sex, smoking and obesity.

ARTICLE SUMMARY

Article focus

- Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease. Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells.
- Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and its hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). This optical technique enables detecting blood volume changes in microvascular beds in response to hyperaemia.
- In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors.

Key messages

- We demonstrated that measurement of the hyperaemic response by the new PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with cardiovascular risk factors.
- We found that PPG pulse amplitude hyperaemic response was lower in men than in women and in smokers than nonsmokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.
- The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.

Strengths and limitations of this study

- Our study is the first to implement the new PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. A finger PPG is a low-cost and operator-independent technique compared to ultrasound in the assessment of peripheral vascular function.
- Under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG technique.
- Our sample size was smaller compared to other studies. On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG technique in assessment of digital vascular function.
- Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Keywords Population ■ Vasodilation ■ Photoplethysmography ■ Endothelial function

INTRODUCTION

Endothelial dysfunction, a marker of reduced nitric oxide (NO) bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease.[1] In humans, endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with cardiovascular risk factors.[2] Vasodilation of the peripheral arteries during reactive hyperaemia after ischaemia depends in part on the release of nitric oxide from endothelial cells in response to increased shear stress.[3] This physiological response allows the non-invasive assessment of endothelial vasomotor function which can be measured based on the flow-mediated dilation (FMD) of the brachial artery[4] or on the fingertip pulse amplitude hyperaemic response.[3, 5, 6] Previous studies mainly applied fingertip peripheral arterial tonometry (PAT) to derive pulse wave amplitude and, therefore, the pulse amplitude changes during hyperaemia.[3, 5, 6] Another approach to derive information about the arterial pulse wave is based on photoplethysmography (PPG).[7] This optical technique enables detecting blood volume changes in microvascular beds during hyperaemia.[7] We sought to evaluate the correlates of digital PPG pulse amplitude hyperaemic responses as a measure of peripheral arterial function in a sample of a general population.

MATERIALS AND METHODS

Design and sample

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).[8] From August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address,

were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS random function. Households with a number matching the list were invited. The initial participation rate was 78.0%.

The FLEMENGHO study is on-going longitudinal population study and, therefore, the participants were repeatedly visited at home and examined at a local examination centre. From January 2010 until March 2012 a scheduled follow-up examination included also measurement of digital vascular function with the PPG technique. From 444 invited participants for this examination, we obtained informed written consent from 378 subjects (response rate 85.1%). We excluded 43 subjects with cardiac dysrhythmias, such as atrial fibrillation, pacemaker and frequent extrasystole. Because the PPG pulse amplitude was of insufficient quality to assess vascular function (n=14) or because the hyperaemic test was discontinued (n=10) we discarded a further 24 subjects. Thus, the number of participants statistically analysed totaled 311.

Determination of PPG pulse amplitude

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 hours before the test. No medication was taken on the day of the examination. We studied digital vascular function in an air-conditioned room at constant temperature around 22°C. To attain a cardiovascular steady-state before starting the test, the subjects had rested for at least 20 minutes in the supine position. Since peripheral vasoconstriction is correlated with the surrounding temperature, before the test, special care was taken to keep fingertips temperature around 35°C. The blood pressure was the average of 5 auscultatory readings, obtained with a standard sphygmomanometer. The blood pressure measurement was performed on the arm that served as control.

Digital pulse amplitude was measured with a PPG device (FLOMEDI Company, Brussels) transmitting infrared light at a wavelength of 940 nm and positioned on the tip of each index finger. Digital output from the PPG device was recorded through an analogue-to-digital converter (10 bit, sampling frequency 250 Hz). We expressed the amplitude of the PPG PWA signal in arbitrary units. To determine the amplitude changes of the digital pulse curve in response to hyperaemia, we used a protocol as described by Hamburg *et al*.[6] As shown in Figure 1, panel C and D, baseline PPG pulse amplitude was registered at each of the two index fingertips for at least 5 minutes to ensure a stable baseline PPG signal. For the analysis, we used PPG pulse amplitude that was measured for last 2 min 20 sec. Next, arterial flow was interrupted for 5 minutes by an inflatable cuff placed on the proximal forearm with an occlusion pressure of 200-220 mmHg (around 50 mmHg above the participant's systolic pressure). Complete cessation of blood flow to the hand is verified by the absence of a PPG signal from the occluded arm. After cuff deflation, we analysed the PPG pulse amplitude at both fingers using a computerised, automated algorithm (FLOMEDI Company, Brussels) that provided the averaged pulse amplitude for each 30-second interval up to 4 minutes (see the PPG pulse tracking in Figure 1).

For each 30-second interval, the response of the PPG pulse wave amplitude to hyperaemia was calculated from the hyperaemic fingertip as the ratio of the post-deflation PPG pulse amplitude to the baseline amplitude (PA_{ht}/PA_{h0}, where PA is the pulse amplitude, h is the hyperaemic finger, t is time interval, and 0 is baseline). To obtain the PPG pulse amplitude ratio we divided PA_{ht}/PA_{h0} ratio by the corresponding ratio at the control hand (PA_{ct}/PA_{c0}, where c is the control finger).

To determine the inter-session reproducibility of the hyperaemic response, we analysed PPG ratios measured on two different occasions in 5 subjects. We determined the

absolute and relative biases of the averaged PPG pulse amplitude ratios per each 30-second time interval between the two sessions as well as 95% limits of agreement between sessions. Absolute and relative biases between the two sessions were calculated according to Bland and Altman's method as (x1 – x2) vs averaged and (100*(x1 – x2)/averaged) vs averaged, respectively. The absolute and relative biases of the averaged PPG pulse amplitude ratios at each time interval between the two sessions were 0.062 (95% confidence interval [CI]: –0.10 to 0.23) and 3.29% (95% CI: –8.8% to 15.4%), respectively.

Other measurements

At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each subject's medical history, smoking and drinking habits, and intake of medications. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic (average of 5 consecutive auscultatory readings at the examination centre) or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Overweight was a body mass index between 25 and 30 kg/m². Obesity was a body mass index of 30 kg/m² or higher.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, NC). The central tendency and the spread of the data are reported as mean ± SD. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by computation of the coefficient of skewness, *i.e.*, the third moment about the mean divided by the cube of the standard deviation. We compared means and proportions by means of a sample t-test and by the χ^2-test, respectively. Significance was $P < 0.05$ on two-sided test.

We performed single and stepwise multiple regression to assess the independent correlations of the PPG pulse amplitude ratio during each 30-second interval with sex, age,

systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. We set the *P*-values for variables to enter and to stay in the regression models at 0.10.

RESULTS

Characteristics of participants and PPG pulse amplitude

The participants included 154 (53.5%) women, and 134 (43.1%) hypertensive patients of whom 78 (25.1 %) were on antihypertensive drug treatment. Table 1 shows the clinical characteristics and PPG pulse amplitude measures of the study participants by sex. In this cohort, women had lower systolic and diastolic blood pressure and higher heart rate than men, less often reported alcohol consumption and had no history of ischaemic heart disease.

The geometric means of the baseline PPG amplitude were 7.3 (5%-95% percentiles: 2.7 to 25.9) and 9.3 (5%-95% percentiles: 3.9 to 25.3) at the hyperaemic and control finger, respectively. We observed a high correlation between values of the baseline PPG amplitude recorded at both fingers (r=0.89, *P*<0.0001). As shown in Figure 2, after forearm cuff deflation, the ratio of the PPG pulse amplitude to baseline rose rapidly in the hyperaemic fingertip, with maximal response occurring in the 30- to 60-second interval, whereas the changes of PPG amplitude in the control finger were minimal. Table 1 lists the mean values of the post-deflation PPG pulse amplitude ratio at each 30-second interval by sex. The hyperaemic response at each 30-second interval was significantly higher in women compared to men (Table 1). In both women and men, the maximal hyperaemic response was detected in the 30- to 60-second interval.

Determinants of PPG pulse amplitude ratio

We performed stepwise regression to assess the independent correlations of the hyperaemic response for each 30-second interval after cuff deflation with sex, age, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. With age forced in the models, the explained variance for the PPG pulse amplitude ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval (Table 2). The PPG PWA changes throughout 0-240-second intervals significantly decreased with male sex ($P \leq 0.0004$) and with body mass index ($P \leq 0.017$). The hyperaemic response at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$). These associations with sex, body mass index and current smoking were mutually independent. In addition, the PPG pulse amplitude ratio at 30- to 60-second interval decreased with total cholesterol, but this association only reached borderline significance ($P=0.045$;Table 2). Blood glucose was also selected as an independent determinant of the PPG ratio (Table 2), but overall impact of this covariable is relatively small (explained about 1,5% of total variability). Moreover, blood glucose was not a significant determinant of the maximal peak of hyperaemic response which occurs at 30- to 60 second and 60- to 90-second intervals.

Figure 3 illustrates the hyperaemic responses by the smoking status while adjusted for important covariables. The maximal hyperaemic response in the 30-to 60-second interval was significantly lower in current smokers compared to non-smokers (1.37 vs 1.76; $P<0.0001$). Figure 4 shows the adjusted PPG pulse amplitude hyperaemic responses in subjects, divided into 3 categories according to their body mass index. In overweight (n=134; 1.51±0.060) and obese (n=64; 1.44±0.082) subjects the maximal hyperaemic response was significantly lower compared to lean participants (n=113; 1.71±0.064).

DISCUSSION

In our cohort recruited from a population study, we evaluated the relationship between PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, and cardiovascular risk factors. We observed a time-dependent increase in digital PPG pulse amplitude that peaked in the 30- to 60-second interval after induction of reactive hyperaemia. In keeping with the literature,[6, 9-11] we found that PPG pulse amplitude hyperaemic response was higher in women than in men and in nonsmokers than smokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.

Endothelial function is often estimated non-invasively by vascular reactivity tests. Several methods are available to study endothelial function in the peripheral macrocirculation (conduit arteries) and microcirculation (resistance arteries and arterioles).[2, 12] Measurement of the brachial artery diameter before and after 5 minutes of occlusion of the arterial flow to the forearm is the most widely used test to assess endothelium-dependent vasodilation.[4, 13, 14] The change in arterial diameter gives a measure of flow-mediated vasodilatation (FMD). This technique, however, is operator dependent, is costly and requires a long post-processing time. Measurement of microcirculatory reactive hyperaemia can be assessed by digital pulse amplitude measured by applanation tonometry[5, 6] or photoplethysmography.[15, 16] Lund[17] described the potential of the PPG technique for the assessment of vasodilation by using this technique to measure haemodynamic response to nitroglycerin. Moreover, Theunissen *et al*[18] observed in divers an increase in circulating NO after successive breath-hold dives. This increase in circulating NO level was associated with higher hyperaemic response measured using the same PPG device as in our study.

Both techniques for assessment of digital vascular function are non-operator-dependent, and the equipment is an order of magnitude less expensive than for ultrasonography. However, the tonometry method might be more expensive as compare to the PPG technique because of additional costs associated with changeable plethysmographic probes. Furthermore, the digital tonometry procedure is more complicated and less comfortable for patients because it requires attachment of a pneumo-electrical tube to an additional pneumatic digital cuff which should be constantly inflated during the test.

We observed similar digital PPG pulse amplitude changes during the hyperaemic response compared with results from studies using the finger applanation tonomtery based method.[6, 10, 11] In the Framingham study,[6, 10] similar to our study, the ratio of the digital pulse amplitude to baseline rose rapidly in the hyperaemic fingertip after forearm cuff deflation, and then slowly decreased towards baseline. However, we detected the maximal hyperaemic response in the 30- to 60-second interval, whereas in the Framingham study the pressure amplitude ratio was highest in the 60- to 90-second interval. The difference in the time of maximal hyperaemic response between the Framingham study and our report might be related to the fact that finger PAT measures pressure changes while photoplethysmography measures changes of the relative amount of blood volume.

We observed the relations between the hyperaemic PPG pulse amplitude response and cardiovascular risk factors. In our current study and in other community-based studies,[6, 10, 11] men had a less pronounced hyperaemic response than women, which is probably in part attributable to physiological differences in vessel diameter and wall thickness between the sexes. In line with other studies,[6, 10, 11] which used the PAT technique to evaluate digital vascular function, we demonstrated a significant inverse associations between PPG amplitude changes and smoking, obesity and total cholesterol. The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and

metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.[19] We also tested the influences of antihypertensive and antihyperlipidemic treatment on the hyperaemic PPG pulse amplitude response, which were not significant (results not shown). The observed association between the PPG PWA ratio and blood glucose during some time intervals might be related to the optic technique which we used in our study.

Similar to other studies, in which finger applanation tonometry was used to assess the digital vascular function,[10] we did not observe a significant relation between hyperaemic PPG pulse amplitude changes and age. On the other hand, previous studies reported lower hyperaemic response as assessed by FMD with advancing age.[13, 14] Differences in the age-related hyperaemic responses between microcirculatory and macrocirculatory reactivity might explain these divergent findings.[9] Moreover, recent studies demonstrated that brachial and digital measures of vascular function were uncorrelated with each other.[10, 20] It was suggested that FMD and PAT provide distinct information regarding vascular function in conduit versus smaller digital vessels. In our study we also did not observe the difference in hyperaemic response between patients with hypertension and normotensive participants. Similar finding was observed in other epidemological study[10] that used applanation tonometry in assessing microvascular function. We could speculate that the PPG reactive hyperaemia index (microvasculature) is more sensitive to metabolic factors, especially body mass index, smoking and total cholesterol and less sensitive to systemic hemodynamic factors such as high blood pressure.

The present study must be interpreted within the context of its potential limitations and strengths. First, PPG pulse amplitude registration is prone to measurement error due to higher variability in comparisons with the FMD technique.[21] On the other hand, assessment

of the hyperaemic PPG pulse wave amplitude changes requires little training and is operator independent. Moreover, under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques. Second, placing the occlusion cuff above the site of hyperaemic response measurement might evoke a dilatory response that is related in part to ischaemia and, therefore, is not entirely mediated by NO. Moreover, the sex difference in the hyperaemic response observed in our study might be in part attributable to physiological differences in vessel diameter (allometric differences) and, therefore, could not also entirely explained by low NO realize in men. Further studies should account for the differential response to hyperaemia between men and women. Third, our sample size was smaller compared to other studies.[10,11] On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function. Forth, as shown in Table 2, in our study, we could explain only around 20% of variability of the PPG PWA ratios by traditional cardiovascular risk factors. The remaining variability might be influenced by genetic factors, inflammatory processes or other confounders that we did not consider in our study. Moreover, in our opinion, it is important to demonstrate in prospective studies that the hyperaemic response as assessed by the PPG technique might be an independent predictor of cardiovascular events.

In conclusion, our study is the first to implement the PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. We demonstrated that measurement of the hyperaemic response by the PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with smoking and obesity, while accounting for the differential hyperaemic response between men and women. Further research including clinical and prospective epidemiological studies are required to validate

the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Acknowledgments

The authors gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Dais Thijs and Hanne Truyens (Leuven, Belgium).

Contributors

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation of the data. TK, EVV, JK drafted the manuscript. All authors gave final approval of the final version.

Funding

The European Union (grants HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and ERC Advanced Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13 and G. 0881.13) gave support to the Research Unit Hypertension and Cardiovascular Epidemiology.

Disclosures Data Sharing Statement

GC and DJ work at FLOMEDI, a spin-off company (Spin Off In Brussels - Innoviris) of the Technical Department of the Haute Ecole Paul Henri Spaak. The company designs and develops software and electronic medical devices in order to facilitate, simplify, and increase accuracy of non-invasive assessment of vascular stiffness.

None of the other authors declares a conflict of interest.

Data Sharing Statement

Data and documentation for the FLEMENGHO study are available at the Study Coordination Office, Research Unit Hypertension and Cardiovascular Epidemiology, University of Leuven.

REFERENCES

1. Vita JA. Endothelial function. *Circulation* 2011;124:e906-e912.
2. Flammer AJ, Anderson T, Celermajer DS, *et al.* The assessment of endothelial function: from research into clinical practice. *Circulation* 2012;126:753-767.
3. Nohria A, Gerhard-Herman M, Creager MA, *et al.* Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006;101:545-548.
4. Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J* 2003;145:943-951.
5. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2013;146:168-174.
6. Hamburg NM, Keyes MJ, Larson MG, *et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467-2474.
7. Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiol Meas* 2007;28:R1-R39.
8. Kuznetsova T, Herbots L, López B, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009;2:105-112.
9. Brunner H, Cockcroft JR, Deanfield J, *et al.* Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:233-246.
10. Hamburg NM, Palmisano J, Larson MG, *et al.* Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011;57:390-396.

11. Schnabel RB, Schulz A, Wild PS, *et al.* Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. *Circ Cardiovasc Imaging* 2011;4:371-380.
12. Lekakis J, Abraham P, Abraham P, *et al.* Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18:775-789.
13. Benjamin EJ, Larson MG, Keyes MJ, *et al.* Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.
14. Nawrot TS, Staessen JA, Fagard RH, *et al.* Endothelial function and outdoor temperature. *Eur J Epidemiol* 2005;20:407-410.
15. Zahedi E, Jaafar R, Ali MA, *et al.* Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiol Meas* 2008;29:625-637.
16. Millasseau SC, Ritter JM, Takazawa K, *et al.* Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* 2006;24:1449-1456.
17. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates--a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79-86.
18. Theunissen S, Guerrero F, Sponsiello N, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med* 2013;40:135-144.
19. Steffen Y, Vuillaume G, Stolle K, *et al.* Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide* 2012;27:176-184.
20. Martin BJ, Gurtu V, Chan S, *et al.* The relationship between peripheral arterial tonometry and classic measures of endothelial function. *Vasc Med* 2013;18:13-18.

21. Donald AE, Charakida M, Cole TJ, *et al.* Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* 2006;48:1846-1850.

Legend to figures

Figure 1. Panel A shows a system incorporating two PPG devices transmitting infrared light, analogue-to-digital converter and forearm pressure cuff. Panel B shows the position of cuff and two PPG devices during the test. Panel C and D show recorded pulse amplitude tracing. In the arm undergoing hyperemia (panel C, top tracing, and panel D), baseline amplitude is recorded. During cuff inflation, flow is occluded and restores after cuff release (hyperaemic period). In the contralateral control finger (panel C, bottom tracing), flow continues throughout, and pulse amplitude undergoes minimal changes.

Figure 2. PPG pulse amplitude response for the hyperaemic (closed symbols) and control (open symbols) finger in women (circles) and men (squares). Women had more pronounced responses than men. Symbols are means, dashed line – 95% confidence interval.

Figure 3. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in smokers and nonsmokers subjects. Smokers had significantly lower response throughout the 0- to 120-second postdeflation intervals. Symbols are means and SE. Models are adjusted for sex, age, body mass index, total cholesterol and blood glucose. ***P<0.001 vs nonsmokers.

Figure 4. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in subjects with normal body mass index (BMI), overweight (25 kg/m²≤BMI<30 kg/m²) and obesity (BMI≥30 kg/m²). Symbols are means and SE. Models are adjusted for sex, age, smoking, total cholesterol and blood glucose. *P<0.05, **P<0.01, ***P<0.001 vs lean participants. $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001 vs lean participants.

Table 1. Characteristics of participants

Clinical measurements				PPG pulse amplitude measures			
Characteristic	Women (*n* =154)	Men (*n* =157)	P-value	Characteristic	Women (*n* =154)	Men (*n* =156)	*P-value*
Anthropometrics				PPG ratio			
Age, y	53.51±12.2	51.8±14.5	0.26	Time interval (sec)			
Body mass index, kg/m^2	26.3±4.0	27.3±3.7	0.03	0-30	1.43 (0.87 to 2.02)	1.27 (0.83 to 1.84)	0.002
Systolic pressure, mm Hg	125.5±15.4	131.4±14.3	0.0006	30-60	1.93 (1.08 to 2.86)	1.46 (1.00 to 2.13)	<0.0001
Diastolic pressure, mm Hg	80.4±8.0	84.5±9.5	<0.0001	60-90	1.84 (1.10 to 2.50)	1.37 (0.97 to 1.93)	<0.0001
Heart rate, beats/minute	66.5±10.2	62.4±9.9	0.0003	90-120	1.64 (1.09 to 2.16)	1.27 (0.93 to 1.79)	<0.0001
Questionnaire data				120-150	1.49 (1.06 to 2.01)	1.20 (0.92 to 1.59)	<0.0001
Current smoking, n (%)	28 (18.2)	18 (11.5)	0.10	150-180	1.38 (1.00 to 1.84)	1.16 (0.89 to 1.46)	<0.0001
Alcohol, n (%)	39 (25.3)	94 (59.9)	<0.0001	180-210	1.30 (0.98 to 1.65)	1.14 (0.87 to 1.43)	<0.0001
Diabetes, n (%)	5 (3.3)	4 (2.6)	0.72	210-240	1.24 (0.95 to 1.65)	1.11 (0.87 to 1.33)	<0.0001
Treated for hypertension, n (%)	38 (24.7)	40 (25.5)	0.87				
Beta-blockers, n (%)	18 (11.7)	23 (14.7)	0.44				
ACE or ARB, n (%)	12 (7.8)	15 (9.6)	0.58				
Diuretics or CCB, n (%)	22 (14.3)	19 (12.1)	0.57				
Previous history of IHD, n (%)	0 (0)	7 (4.5)	0.008				
Total cholesterol, mmol/l	5.2±1.00	5.0±0.96	0.037				
Lipid lowering agents, n (%)	10 (6.5)	8 (5.1)	0.60				

Values are mean (±SD), mean (10%-90%), or number of subjects (%). PPG indicates photoplethysmography, ACE indicates angiotensin-converting enzyme; ARB indicates angiotensin receptor blockers, CCB indicates calcium channel blockers, IHD indicates ischemic heart disease.

Table 2. Correlates of PPG ratios selected by stepwise regression

Parameter	PPG ratio							
	Time Intervals (sec)							
	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
Regression statistic								
Model R^2 (%)	9.7	21.4	22.5	19.8	19.2	16.3	13.2	12.2
Age (+10 years)*								
ß±SE	0.014±0.020	-0.0007±0.028	0.005±0.025	0.004±0.019	0.007±0.017	-0.0008±0.014	0.004±0.012	0.002±0.011
	P=0.45	*P*=0.98	*P*=0.85	*P*=0.85	*P*=0.68	*P*=0.95	*P*=0.72	*P*=0.91
Partial r^2 (%)	0.02	0	0.01	0.04	0.06	0	0.04	0.01
Female (0,1)								
ß±SE	0.16 ± 0.05	0.49 ± 0.68	0.46 ± 0.061	0.35 ± 0.050	0.26 ± 0.041	0.20 ± 0.035	0.14 ± 0.031	0.12 ± 0.03
	P=0.0004	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Partial r^2 (%)	3.9	13.6	16.0	15.1	13.5	12	7.7	6.1
Current smoking (0,1)								
ß±SE	-0.30 ± 0.07	-0.39 ± 0.09	-0.29 ± 0.085	-	-	-	-	-
	P=0.0004	*P*<0.0001	*P*=0.0007					
Partial r^2 (%)	4.0	3.7	3.1	-	-	-	-	-
Body mass index (kg/m²)								
ß±SE	-0.014±0.007	-0.027±0.009	-0.032±0.008	-0.025±0.007	-0.022±0.005	-0.018±0.005	-0.015±0.004	-0.013±0.004
	P=0.017	*P*=0.003	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*=0.0002	*P*=0.0008
Partial r^2 (%)	1.7	3.0	3.4	3.4	4.5	4.5	4.1	3.5
Total Cholesterol (+1mmol/l)								
ß±SE	-	-0.068 ±0.034	-	-	-	-	-	-
		P = 0.045						
Partial r^2 (%)	-	1.1	-	-	-	-	-	-
Blood Glucose (+1mmol/l)								
ß±SE	0.10 ± 0.04	-	-	0.07 ± 0.04	0.06 ± 0.03	-	0.05 ± 0.02	0.06 ± 0.02
	P=0.013			*P*=0.026	*P*=0.034		*P*=0.027	*P*=0.006
Partial r^2 (%)	1.9	-	-	1.3	1.2	-	1.4	2.2

Values are mutually adjusted partial regression coefficients ±SE. Age was forced into all models. The covariables considered in stepwise models included sex, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.

DIII 2/24/2014 5:54 PM
BMJ Open

Association of digital vascular function with cardiovascular risk factors: a population study

Short title: Correlates of **digital vascular** function

Tatiana Kuznetsova[1], Eline Van Vlierberghe[1], Judita Knez[1], Gregory Szczesny[2], Lutgarde Thijs[1], Dominique Jozeau[2], Costantino Balestra[3], Jan D'hooge[4], Jan A. Staessen[1,5]

Word Counts: Manuscript 5137; Abstract 300;

Number: Tables 2, Figures 4

[1] Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium; [2]FLOMEDI Company, Brussels, Belgium; [3] Environmental, Occupational & Aging Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium; [4]Division of Cardiovascular Imaging and Dynamics, Department of Cardiovascular Sciences, University of Leuven, Belgium; and [5]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands.

Correspondence to Tatiana Kuznetsova, M.D., Ph.D., Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, Campus Sint Rafaël, Kapucijnenvoer 35, Box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kuznetsova@med.kuleuven.be

ABSTRACT

Objectives: Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells. Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and PWA hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). We sought to evaluate the correlates of digital PPG PWA hyperaemic responses as a measure of peripheral vascular function.

Design: The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) is a population-based cohort study.

Setting: Respondents were examined at one centre in northern Belgium.

Participants: For this analysis, our sample consisted of 311 former participants (53.5% women; mean age 52.6 years; 43.1% hypertensive), who were examined from January 2010 until March 2012 (response rate 85.1%).

Primary outcome measures: Using a fingertip PPG device, we measured digital PWA at baseline and at 30-second intervals for 4 minutes during reactive hyperaemia induced by a 5-minute forearm cuff occlusion. We performed stepwise regression to identify correlates of the hyperaemic response ratio for each 30-second interval after cuff deflation.

Results The maximal hyperaemic response was detected in the 30- to 60-second interval. The explained variance for the PPG PWA ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval. The hyperaemic response at each 30-second interval was significantly higher in women compared to men ($P \leq 0.001$). The PPG PWA changes at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$) and at 0-240-second intervals decreased with higher body mass index ($P \leq 0.035$). These associations with sex, current smoking and body mass index were mutually independent.

Conclusions Our study is the first to implement the new PPG technique to measure digital PWA hyperaemic changes in a general population. Hyperaemic response, as measured by PPG, inversely associated with traditional cardiovascular risk factors such as male sex, smoking and obesity.

ARTICLE SUMMARY

Article focus

- Endothelial dysfunction, a marker of reduced nitric oxide bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease. Vasodilation of the peripheral arteries during reactive hyperaemia depends in part on release of nitric oxide from endothelial cells.
- Previous studies mainly employed a fingertip tonometric device to derive pulse wave amplitude (PWA) and its hyperaemic changes. Alternative approach is based on photoplethysmography (PPG). This optical technique enables detecting blood volume changes in microvascular beds in response to hyperaemia.
- In our cohort recruited from a population study, we evaluated the relation of PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, to cardiovascular risk factors.

Key messages

- We demonstrated that measurement of the hyperaemic response by the new PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with cardiovascular risk factors.
- We found that PPG pulse amplitude hyperaemic response was lower in men than in women and in smokers than nonsmokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.
- The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.

Strengths and limitations of this study

- Our study is the first to implement the new PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. A finger PPG is a low-cost and operator-independent technique compared to ultrasound in the assessment of peripheral vascular function.
- Under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG technique.
- Our sample size was smaller compared to other studies. On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG technique in assessment of digital vascular function.
- Further research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Keywords Population ■ Vasodilation ■ Photoplethysmography ■ Endothelial function

INTRODUCTION

Endothelial dysfunction, a marker of reduced nitric oxide (NO) bioavailability, contributes to atherosclerosis and the pathogenesis of cardiovascular disease.[1] In humans, endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with cardiovascular risk factors.[2] Vasodilation of the peripheral arteries during reactive hyperaemia after ischaemia depends in part on the release of nitric oxide from endothelial cells in response to increased shear stress.[3] This physiological response allows the non-invasive assessment of endothelial vasomotor function which can be measured based on the flow-mediated dilation (FMD) of the brachial artery[4] or on the fingertip pulse amplitude hyperaemic response.[3, 5, 6] Previous studies mainly applied fingertip peripheral arterial tonometry (PAT) to derive pulse wave amplitude and, therefore, the pulse amplitude changes during hyperaemia.[3, 5, 6] Another approach to derive information about the arterial pulse wave is based on photoplethysmography (PPG).[7] This optical technique enables detecting blood volume changes in microvascular beds during hyperaemia.[7] We sought to evaluate the correlates of digital PPG pulse amplitude hyperaemic responses as a measure of peripheral arterial function in a sample of a general population.

MATERIALS AND METHODS

Design and sample

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).[8] From August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address,

were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS random function. Households with a number matching the list were invited. The initial participation rate was 78.0%.

The FLEMENGHO study is on-going longitudinal population study and, therefore, the participants were repeatedly visited at home and examined at a local examination centre. From January 2010 until March 2012 a scheduled follow-up examination included also measurement of **digital vascular function** with the PPG technique. From 444 invited participants for this examination, we obtained informed written consent from 378 subjects (response rate 85.1%). We excluded 43 subjects with cardiac dysrhythmias, such as atrial fibrillation, pacemaker and frequent extrasystole. Because the PPG pulse amplitude was of insufficient quality to assess vascular function (n=14) or because the hyperaemic test was discontinued (n=10) we discarded a further 24 subjects. Thus, the number of participants statistically analysed totaled 311.

Determination of PPG pulse amplitude

The participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 3 hours before the test. No medication was taken on the day of the examination. We studied **digital vascular function** in an air-conditioned room at constant temperature around 22°C. To attain a cardiovascular steady-state before starting the test, the subjects had rested for at least 20 minutes in the supine position. Since peripheral vasoconstriction is correlated with the surrounding temperature, before the test, special care was taken to keep fingertips temperature around 35°C. The blood pressure was the average of 5 auscultatory readings, obtained with a standard sphygmomanometer. The blood pressure measurement was performed on the arm that served as control.

Digital pulse amplitude was measured with a PPG device (FLOMEDI Company, Brussels) transmitting infrared light at a wavelength of 940 nm and positioned on the tip of each index finger. Digital output from the PPG device was recorded through an analogue-to-digital converter (10 bit, sampling frequency 250 Hz). We expressed the amplitude of the PPG PWA signal in arbitrary units. To determine the amplitude changes of the digital pulse curve in response to hyperaemia, we used a protocol as described by Hamburg *et al*.[6] As shown in Figure 1, panel C and D, baseline PPG pulse amplitude was registered at each of the two index fingertips for at least 5 minutes to ensure a stable baseline PPG signal. For the analysis, we used PPG pulse amplitude that was measured for last 2 min 20 sec. Next, arterial flow was interrupted for 5 minutes by an inflatable cuff placed on the proximal forearm with an occlusion pressure of 200-220 mmHg (around 50 mmHg above the participant's systolic pressure). Complete cessation of blood flow to the hand is verified by the absence of a PPG signal from the occluded arm. After cuff deflation, we analysed the PPG pulse amplitude at both fingers using a computerised, automated algorithm (FLOMEDI Company, Brussels) that provided the averaged pulse amplitude for each 30-second interval up to 4 minutes (see the PPG pulse tracking in Figure 1).

For each 30-second interval, the response of the PPG pulse wave amplitude to hyperaemia was calculated from the hyperaemic fingertip as the ratio of the post-deflation PPG pulse amplitude to the baseline amplitude (PA_{ht}/PA_{h0}, where PA is the pulse amplitude, h is the hyperaemic finger, t is time interval, and 0 is baseline). To obtain the PPG pulse amplitude ratio we divided PA_{ht}/PA_{h0} ratio by the corresponding ratio at the control hand (PA_{ct}/PA_{c0}, where c is the control finger).

To determine the inter-session reproducibility of the hyperaemic response, we analysed PPG ratios measured on two different occasions in 5 subjects. We determined the

absolute and relative biases of the averaged PPG pulse amplitude ratios per each 30-second time interval between the two sessions as well as 95% limits of agreement between sessions. Absolute and relative biases between the two sessions were calculated according to Bland and Altman's method as (x1 – x2) vs averaged and (100*(x1 – x2)/averaged) vs averaged, respectively. The absolute and relative biases of the averaged PPG pulse amplitude ratios at each time interval between the two sessions were 0.062 (95% confidence interval [CI]: –0.10 to 0.23) and 3.29% (95% CI: –8.8% to 15.4%), respectively.

Other measurements

At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each subject's medical history, smoking and drinking habits, and intake of medications. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic (average of 5 consecutive auscultatory readings at the examination centre) or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Overweight was a body mass index between 25 and 30 kg/m². Obesity was a body mass index of 30 kg/m² or higher.

Statistical methods

For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute, Cary, NC). The central tendency and the spread of the data are reported as mean ± SD. Departure from normality was evaluated by Shapiro-Wilk's statistic and skewness by computation of the coefficient of skewness, *i.e.*, the third moment about the mean divided by the cube of the standard deviation. We compared means and proportions by means of a sample t-test and by the χ^2-test, respectively. Significance was $P < 0.05$ on two-sided test.

We performed single and stepwise multiple regression to assess the independent correlations of the PPG pulse amplitude ratio during each 30-second interval with sex, age,

systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. We set the *P*-values for variables to enter and to stay in the regression models at 0.10.

RESULTS

Characteristics of participants and PPG pulse amplitude

The participants included 154 (53.5%) women, and 134 (43.1%) hypertensive patients of whom 78 (25.1 %) were on antihypertensive drug treatment. Table 1 shows the clinical characteristics and PPG pulse amplitude measures of the study participants by sex. In this cohort, women had lower systolic and diastolic blood pressure and higher heart rate than men, less often reported alcohol consumption and had no history of ischaemic heart disease.

The geometric means of the baseline PPG amplitude were 7.3 (5%-95% percentiles: 2.7 to 25.9) and 9.3 (5%-95% percentiles: 3.9 to 25.3) at the hyperaemic and control finger, respectively. We observed a high correlation between values of the baseline PPG amplitude recorded at both fingers (r=0.89, *P*<0.0001). As shown in Figure 2, after forearm cuff deflation, the ratio of the PPG pulse amplitude to baseline rose rapidly in the hyperaemic fingertip, with maximal response occurring in the 30- to 60-second interval, whereas the changes of PPG amplitude in the control finger were minimal. Table 1 lists the mean values of the post-deflation PPG pulse amplitude ratio at each 30-second interval by sex. The hyperaemic response at each 30-second interval was significantly higher in women compared to men (Table 1). In both women and men, the maximal hyperaemic response was detected in the 30- to 60-second interval.

Determinants of PPG pulse amplitude ratio

We performed stepwise regression to assess the independent correlations of the hyperaemic response for each 30-second interval after cuff deflation with sex, age, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease. With age forced in the models, the explained variance for the PPG pulse amplitude ratio ranged from 9.7% at 0-30 second-interval to 22.5% at 60-90-second time interval (Table 2). The PPG PWA changes throughout 0-240-second intervals significantly decreased with male sex ($P \leq 0.0004$) and with body mass index ($P \leq 0.017$). The hyperaemic response at 0- to 90-second intervals decreased with current smoking ($P \leq 0.0007$). These associations with sex, body mass index and current smoking were mutually independent. In addition, the PPG pulse amplitude ratio at 30- to 60-second interval decreased with total cholesterol, but this association only reached borderline significance ($P=0.045$;Table 2). Blood glucose was also selected as an independent determinant of the PPG ratio (Table 2), but overall impact of this covariable is relatively small (explained about 1,5% of total variability). Moreover, blood glucose was not a significant determinant of the maximal peak of hyperaemic response which occurs at 30- to 60 second and 60- to 90-second intervals.

Figure 3 illustrates the hyperaemic responses by the smoking status while adjusted for important covariables. The maximal hyperaemic response in the 30-to 60-second interval was significantly lower in current smokers compared to non-smokers (1.37 vs 1.76; $P<0.0001$). Figure 4 shows the adjusted PPG pulse amplitude hyperaemic responses in subjects, divided into 3 categories according to their body mass index. In overweight (n=134; 1.51±0.060) and obese (n=64; 1.44±0.082) subjects the maximal hyperaemic response was significantly lower compared to lean participants (n=113; 1.71±0.064).

DISCUSSION

In our cohort recruited from a population study, we evaluated the relationship between PPG pulse amplitude hyperaemic response, a noninvasive measure of peripheral microcirculation, and cardiovascular risk factors. We observed a time-dependent increase in digital PPG pulse amplitude that peaked in the 30- to 60-second interval after induction of reactive hyperaemia. In keeping with the literature,[6, 9-11] we found that PPG pulse amplitude hyperaemic response was higher in women than in men and in nonsmokers than smokers. Moreover, digital vasodilator function as measured by the PPG technique inversely correlated with body mass index.

Endothelial function is often **estimated** non-invasively by vascular reactivity tests. Several methods are available to study endothelial function in the peripheral macrocirculation (conduit arteries) and microcirculation (resistance arteries and arterioles).[2, 12] Measurement of the brachial artery diameter before and after 5 minutes of occlusion of the arterial flow to the forearm is the most widely used test to assess endothelium-dependent vasodilation.[4, 13, 14] The change in arterial diameter gives a measure of flow-mediated vasodilatation (FMD). This technique, however, is operator dependent, is costly and requires a long post-processing time. Measurement of microcirculatory reactive hyperaemia can be assessed by digital pulse amplitude measured by applanation tonometry[5, 6] or photoplethysmography.[15, 16] Lund[17] described the potential of the PPG technique for the assessment of vasodilation by using this technique to measure haemodynamic response to nitroglycerin. Moreover, Theunissen *et al*[18] observed in divers an increase in circulating NO after successive breath-hold dives. This increase in circulating NO level was associated with higher hyperaemic response measured using the same PPG device as in our study.

Both techniques for assessment of digital vascular function are non-operator-dependent, and the equipment is an order of magnitude less expensive than for ultrasonography. However, the tonometry method might be more expensive as compare to the PPG technique because of additional costs associated with changeable plethysmographic probes. Furthermore, the digital tonometry procedure is more complicated and less comfortable for patients because it requires attachment of a pneumo-electrical tube to an additional pneumatic digital cuff which should be constantly inflated during the test.

We observed similar digital PPG pulse amplitude changes during the hyperaemic response compared with results from studies using the finger applanation tonomtery based method.[6, 10, 11] In the Framingham study,[6, 10] similar to our study, the ratio of the digital pulse amplitude to baseline rose rapidly in the hyperaemic fingertip after forearm cuff deflation, and then slowly decreased towards baseline. However, we detected the maximal hyperaemic response in the 30- to 60-second interval, whereas in the Framingham study the pressure amplitude ratio was highest in the 60- to 90-second interval. The difference in the time of maximal hyperaemic response between the Framingham study and our report might be related to the fact that finger PAT measures pressure changes while photoplethysmography measures changes of the relative amount of blood volume.

We observed the relations between the hyperaemic PPG pulse amplitude response and cardiovascular risk factors. In our current study and in other community-based studies,[6, 10, 11] men had a less pronounced hyperaemic response than women, which is probably in part attributable to physiological differences in vessel diameter and wall thickness between the sexes. In line with other studies,[6, 10, 11] which used the PAT technique to evaluate **digital vascular function**, we demonstrated a significant inverse associations between PPG amplitude changes and smoking, obesity and total cholesterol. The mechanism underlying these associations might be related to the fact that exposure to cigarette smoke and

metabolic risk factors cause impairment of nitric oxide production and an increase of oxidative stress and proinflammatory reaction that leads to endothelial dysfunction and atherosclerosis.[19] We also tested the influences of antihypertensive and antihyperlipidemic treatment on the hyperaemic PPG pulse amplitude response, which were not significant (results not shown). The observed association between the PPG PWA ratio and blood glucose during some time intervals might be related to the optic technique which we used in our study.

Similar to other studies, in which finger applanation tonometry was used to assess the **digital vascular function**,[10] we did not observe a significant relation between hyperaemic PPG pulse amplitude changes and age. On the other hand, previous studies reported lower hyperaemic response as assessed by FMD with advancing age.[13, 14] Differences in the age-related hyperaemic responses between microcirculatory and macrocirculatory reactivity might explain these divergent findings.[9] Moreover, recent studies demonstrated that brachial and digital measures of vascular function were uncorrelated with each other.[10, 20] It was suggested that FMD and PAT provide distinct information regarding vascular function in conduit versus smaller digital vessels. **In our study we also did not observe the difference in hyperaemic response between patients with hypertension and normotensive participants. Similar finding was observed in other epidemological study[10] that used applanation tonometry in assessing microvascular function. We could speculate that the PPG reactive hyperaemia index (microvasculature) is more sensitive to metabolic factors, especially body mass index, smoking and total cholesterol and less sensitive to systemic hemodynamic factors such as high blood pressure.**

The present study must be interpreted within the context of its potential limitations and strengths. First, PPG pulse amplitude registration is prone to measurement error due to

higher variability in comparisons with the FMD technique.[21] On the other hand, assessment of the hyperaemic PPG pulse wave amplitude changes requires little training and is operator independent. Moreover, under strictly controlled conditions, we were able to demonstrate a good inter-session reproducibility of the hyperaemic response as measured by the PPG techniques. Second, placing the occlusion cuff above the site of hyperaemic response measurement might evoke a dilatory response that is related in part to ischaemia and, therefore, is not entirely mediated by NO. **Moreover, the sex difference in the hyperaemic response observed in our study might be in part attributable to physiological differences in vessel diameter (allometric differences) and, therefore, could not also entirely explained by low NO realize in men. Further studies should account for the differential response to hyperaemia between men and women.** Third, our sample size was smaller compared to other studies.[10,11] On the other hand, the correlates of hyperaemic response were as expected and constitute an internal validation of the PPG techniques in assessment of digital vascular function. Forth, as shown in Table 2, in our study, we could explain only around 20% of variability of the PPG PWA ratios by traditional cardiovascular risk factors. The remaining variability might be influenced by genetic factors, inflammatory processes or other confounders that we did not consider in our study. Moreover, in our opinion, it is important to demonstrate in prospective studies that the hyperaemic response as assessed by the PPG technique might be an independent predictor of cardiovascular events.

In conclusion, our study is the first to implement the PPG technique to measure digital pulse amplitude hyperaemic changes in a sample of a general population. We demonstrated that measurement of the hyperaemic response by the PPG technique might be a useful tool in the detection of peripheral microvascular dysfunction associated with smoking and obesity, while accounting for the differential hyperaemic response between men and women. Further

research including clinical and prospective epidemiological studies are required to validate the PPG technique for non-invasive assessment of endothelial function and prediction of cardiovascular outcome, respectively.

Acknowledgments

The authors gratefully acknowledge the expert assistance of Linda Custers, Marie-Jeanne Jehoul, Dais Thijs and Hanne Truyens (Leuven, Belgium).

Contributors

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis and interpretation of the data. TK, EVV, JK drafted the manuscript. All authors gave final approval of the final version.

Funding

The European Union (grants HEALTH-2011-278249-EU-MASCARA, HEALTH-F7-305507-HoMAGE, and ERC Advanced Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grants G.0734.09, G.0880.13 and G. 0881.13) gave support to the Research Unit Hypertension and Cardiovascular Epidemiology.

Disclosures

GC and DJ work at FLOMEDI, a spin-off company (Spin Off In Brussels - Innoviris) of the Technical Department of the Haute Ecole Paul Henri Spaak. The company designs and develops software and electronic medical devices in order to facilitate, simplify, and increase accuracy of non-invasive assessment of vascular stiffness.

None of the other authors declares a conflict of interest.

REFERENCES

1. Vita JA. Endothelial function. *Circulation* 2011;124:e906-e912.
2. Flammer AJ, Anderson T, Celermajer DS, *et al.* The assessment of endothelial function: from research into clinical practice. *Circulation* 2012;126:753-767.
3. Nohria A, Gerhard-Herman M, Creager MA, *et al.* Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006;101:545-548.
4. Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J* 2003;145:943-951.
5. Kuvin JT, Patel AR, Sliney KA, *et al.* Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2013;146:168-174.
6. Hamburg NM, Keyes MJ, Larson MG, *et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467-2474.
7. Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiol Meas* 2007;28:R1-R39.
8. Kuznetsova T, Herbots L, López B, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009;2:105-112.
9. Brunner H, Cockcroft JR, Deanfield J, *et al.* Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:233-246.
10. Hamburg NM, Palmisano J, Larson MG, *et al.* Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011;57:390-396.

11. Schnabel RB, Schulz A, Wild PS, *et al.* Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. *Circ Cardiovasc Imaging* 2011;4:371-380.
12. Lekakis J, Abraham P, Abraham P, *et al.* Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil* 2011;18:775-789.
13. Benjamin EJ, Larson MG, Keyes MJ, *et al.* Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.
14. Nawrot TS, Staessen JA, Fagard RH, *et al.* Endothelial function and outdoor temperature. *Eur J Epidemiol* 2005;20:407-410.
15. Zahedi E, Jaafar R, Ali MA, *et al.* Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. *Physiol Meas* 2008;29:625-637.
16. Millasseau SC, Ritter JM, Takazawa K, *et al.* Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* 2006;24:1449-1456.
17. Lund F. Digital pulse plethysmography (DPG) in studies of the hemodynamic response to nitrates--a survey of recording methods and principles of analysis. *Acta Pharmacol Toxicol (Copenh)* 1986;59:79-86.
18. Theunissen S, Guerrero F, Sponsiello N, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med* 2013;40:135-144.
19. Steffen Y, Vuillaume G, Stolle K, *et al.* Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide* 2012;27:176-184.
20. Martin BJ, Gurtu V, Chan S, *et al.* The relationship between peripheral arterial tonometry and classic measures of endothelial function. *Vasc Med* 2013;18:13-18.

21. Donald AE, Charakida M, Cole TJ, *et al.* Non-invasive assessment of endothelial function: which technique? *J Am Coll Cardiol* 2006;48:1846-1850.

Legend to figures

Figure 1. Panel A shows a system incorporating two PPG devices transmitting infrared light, analogue-to-digital converter and forearm pressure cuff. Panel B shows the position of cuff and two PPG devices during the test. Panel C and D show recorded pulse amplitude tracing. In the arm undergoing hyperemia (panel C, top tracing, and panel D), baseline amplitude is recorded. During cuff inflation, flow is occluded and restores after cuff release (hyperaemic period). In the contralateral control finger (panel C, bottom tracing), flow continues throughout, and pulse amplitude undergoes minimal changes.

Figure 2. PPG pulse amplitude response for the hyperaemic (closed symbols) and control (open symbols) finger in women (circles) and men (squares). Women had more pronounced responses than men. Symbols are means, dashed line – 95% confidence interval.

Figure 3. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in smokers and nonsmokers subjects. Smokers had significantly lower response throughout the 0- to 120-second postdeflation intervals. Symbols are means and SE. Models are adjusted for sex, age, body mass index, total cholesterol and blood glucose. ***P<0.001 vs nonsmokers.

Figure 4. PPG ratio of pulse amplitude for each 30 second time interval after cuff deflation to the baseline pulse amplitude divided by the corresponding ratio in the control finger in subjects with normal body mass index (BMI), overweight (25 kg/m²≤BMI<30 kg/m²) and obesity (BMI≥30 kg/m²). Symbols are means and SE. Models are adjusted for sex, age, smoking, total cholesterol and blood glucose. *P<0.05, **P<0.01, ***P<0.001 vs lean participants. $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001 vs lean participants.

Table 1. Characteristics of participants

Clinical measurements				PPG pulse amplitude measures			
Characteristic	Women (*n* =154)	Men (*n* =157)	P-value	Characteristic	Women (*n* =154)	Men (*n* =156)	*P-value*
Anthropometrics				PPG ratio			
Age, y	53.51±12.2	51.8±14.5	0.26	Time interval (sec)			
Body mass index, kg/m^2	26.3±4.0	27.3±3.7	0.03	0-30	1.43 (0.87 to 2.02)	1.27 (0.83 to 1.84)	0.002
Systolic pressure, mm Hg	125.5±15.4	131.4±14.3	0.0006	30-60	1.93 (1.08 to 2.86)	1.46 (1.00 to 2.13)	<0.0001
Diastolic pressure, mm Hg	80.4±8.0	84.5±9.5	<0.0001	60-90	1.84 (1.10 to 2.50)	1.37 (0.97 to 1.93)	<0.0001
Heart rate, beats/minute	66.5±10.2	62.4±9.9	0.0003	90-120	1.64 (1.09 to 2.16)	1.27 (0.93 to 1.79)	<0.0001
Questionnaire data				120-150	1.49 (1.06 to 2.01)	1.20 (0.92 to 1.59)	<0.0001
Current smoking, n (%)	28 (18.2)	18 (11.5)	0.10	150-180	1.38 (1.00 to 1.84)	1.16 (0.89 to 1.46)	<0.0001
Alcohol, n (%)	39 (25.3)	94 (59.9)	<0.0001	180-210	1.30 (0.98 to 1.65)	1.14 (0.87 to 1.43)	<0.0001
Diabetes, n (%)	5 (3.3)	4 (2.6)	0.72	210-240	1.24 (0.95 to 1.65)	1.11 (0.87 to 1.33)	<0.0001
Treated for hypertension, n (%)	38 (24.7)	40 (25.5)	0.87				
Beta-blockers, n (%)	18 (11.7)	23 (14.7)	0.44				
ACE or ARB, n (%)	12 (7.8)	15 (9.6)	0.58				
Diuretics or CCB, n (%)	22 (14.3)	19 (12.1)	0.57				
Previous history of IHD, n (%)	0 (0)	7 (4.5)	0.008				
Total cholesterol, mmol/l	5.2±1.00	5.0±0.96	0.037				
Lipid lowering agents, n (%)	10 (6.5)	8 (5.1)	0.60				

Values are mean (±SD), mean (10%-90%), or number of subjects (%). PPG indicates photoplethysmography, ACE indicates angiotensin-converting enzyme; ARB indicates angiotensin receptor blockers, CCB indicates calcium channel blockers, IHD indicates ischemic heart disease.

Table 2. Correlates of PPG ratios selected by stepwise regression

Parameter	PPG ratio							
	Time Intervals (sec)							
	0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
Regression statistic								
Model R^2 (%)	9.7	21.4	22.5	19.8	19.2	16.3	13.2	12.2
Age (+10 years)*								
ß±SE	0.014±0.020	-0.0007±0.028	0.005±0.025	0.004±0.019	0.007±0.017	-0.0008±0.014	0.004±0.012	0.002±0.011
	P=0.45	*P*=0.98	*P*=0.85	*P*=0.85	*P*=0.68	*P*=0.95	*P*=0.72	*P*=0.91
Partial r^2 (%)	0.02	0	0.01	0.04	0.06	0	0.04	0.01
Female (0,1)								
ß±SE	0.16 ± 0.05	0.49 ± 0.68	0.46 ± 0.061	0.35 ± 0.050	0.26 ± 0.041	0.20 ± 0.035	0.14 ± 0.031	0.12 ± 0.03
	P=0.0004	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Partial r^2 (%)	3.9	13.6	16.0	15.1	13.5	12	7.7	6.1
Current smoking (0,1)								
ß±SE	-0.30 ± 0.07	-0.39 ± 0.09	-0.29 ± 0.085	-	-	-	-	-
	P=0.0004	*P*<0.0001	*P*=0.0007					
Partial r^2 (%)	4.0	3.7	3.1	-	-	-	-	-
Body mass index (kg/m²)								
ß±SE	-0.014±0.007	-0.027±0.009	-0.032±0.008	-0.025±0.007	-0.022±0.005	-0.018±0.005	-0.015±0.004	-0.013±0.004
	P=0.017	*P*=0.003	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*=0.0002	*P*=0.0008
Partial r^2 (%)	1.7	3.0	3.4	3.4	4.5	4.5	4.1	3.5
Total Cholesterol (+1mmol/l)								
ß±SE	-	-0.068 ±0.034	-	-	-	-	-	-
		P = 0.045						
Partial r^2 (%)	-	1.1	-	-	-	-	-	-
Blood Glucose (+1mmol/l)								
ß±SE	0.10 ± 0.04	-	-	0.07 ± 0.04	0.06 ± 0.03	-	0.05 ± 0.02	0.06 ± 0.02
	P=0.013			*P*=0.026	*P*=0.034		*P*=0.027	*P*=0.006
Partial r^2 (%)	1.9	-	-	1.3	1.2	-	1.4	2.2

Values are mutually adjusted partial regression coefficients ±SE. Age was forced into all models. The covariables considered in stepwise models included sex, systolic and diastolic blood pressures, heart rate, body mass index, current smoking, total cholesterol, LDL cholesterol, haematocrit, blood glucose, antihypertensive and lipid-lowering drug treatment, and previous history of ischaemic heart disease.

Figure 1

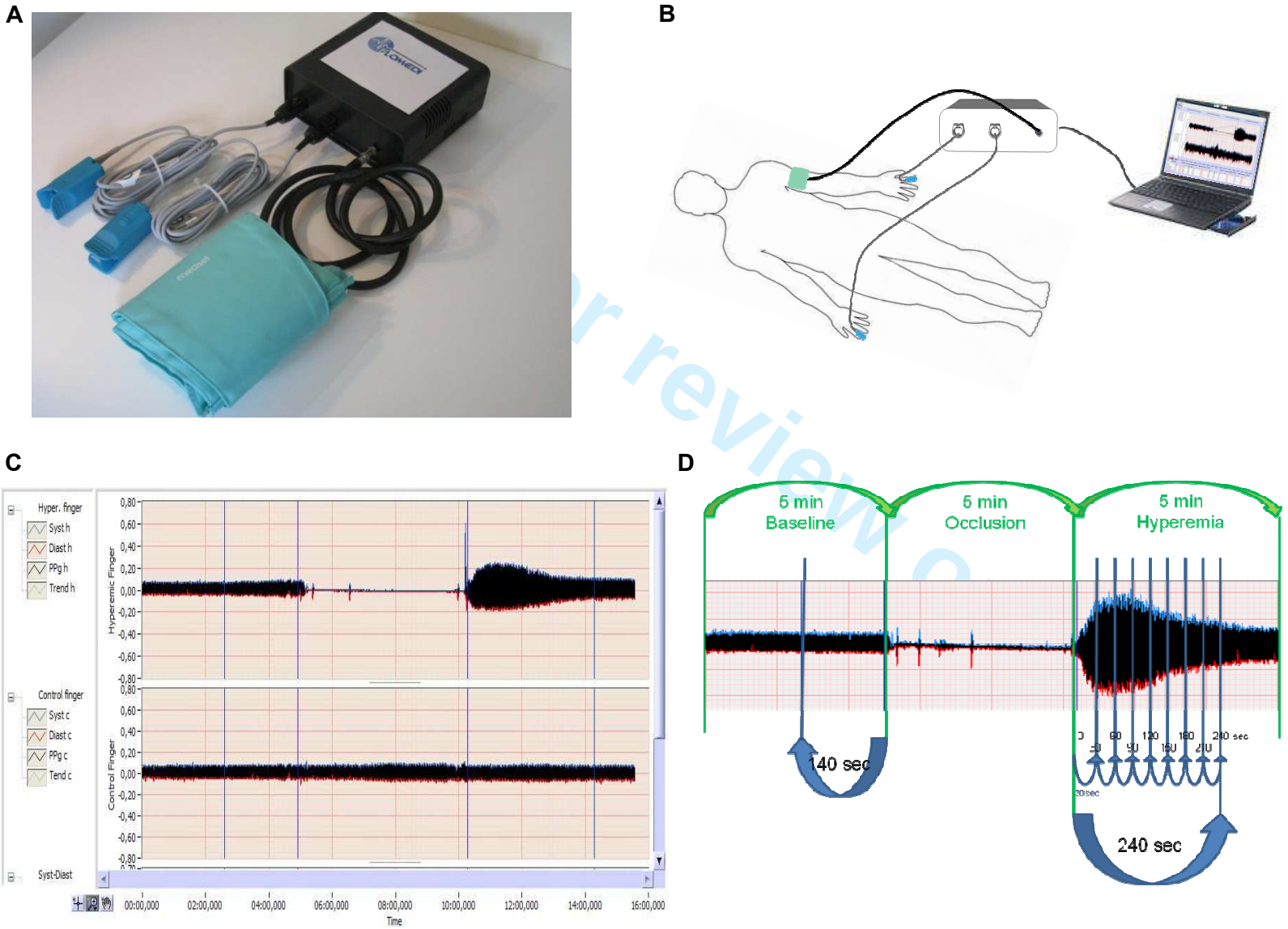


Figure 2

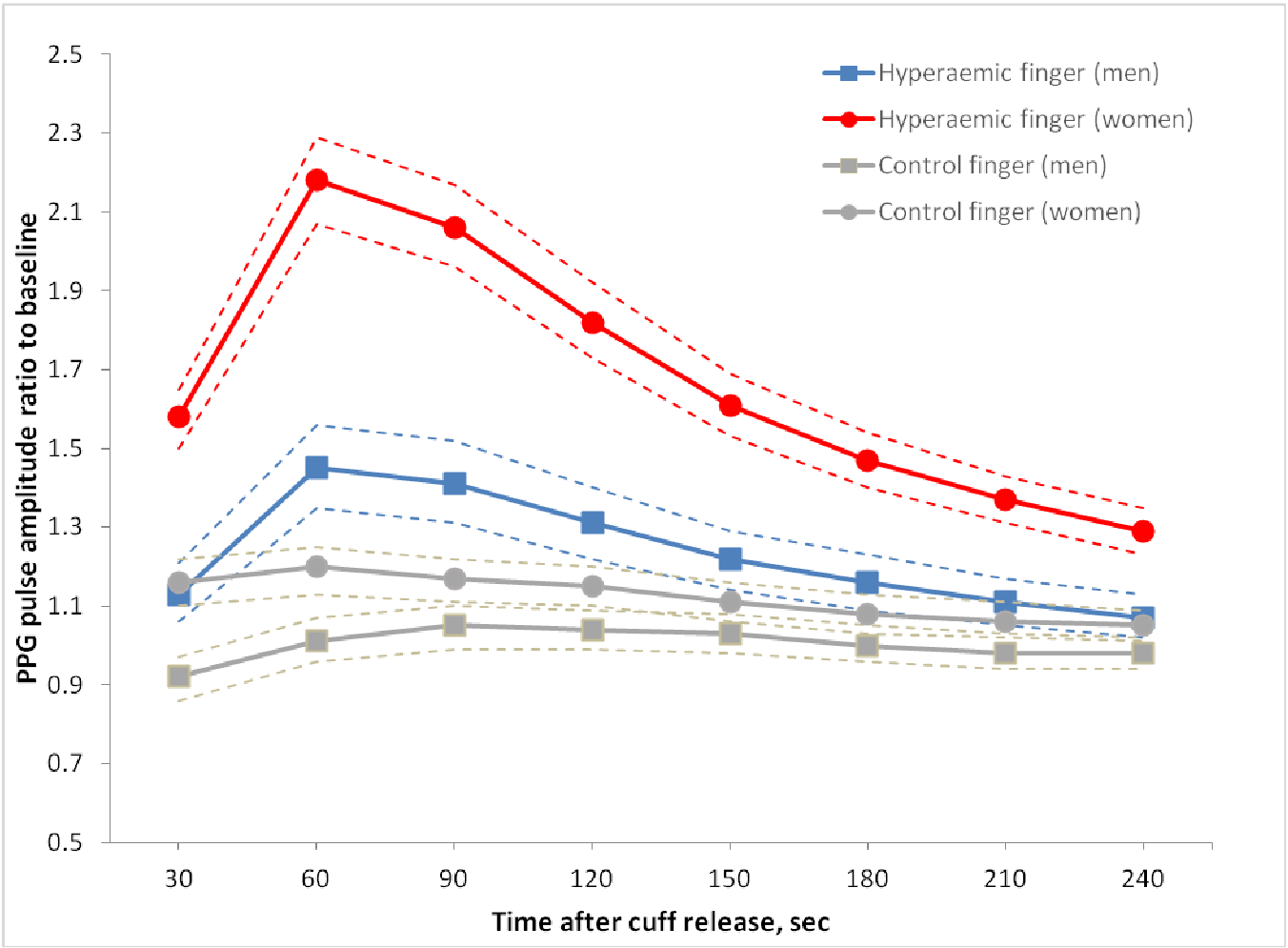

Hyperaemic finger (men)
Hyperaemic finger (women)
Control finger (men)
Control finger (women)
PPG pulse amplitude ratio to baseline
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
30
60
90
120
150
180
210
240
Time after cuff release, sec

Figure 3

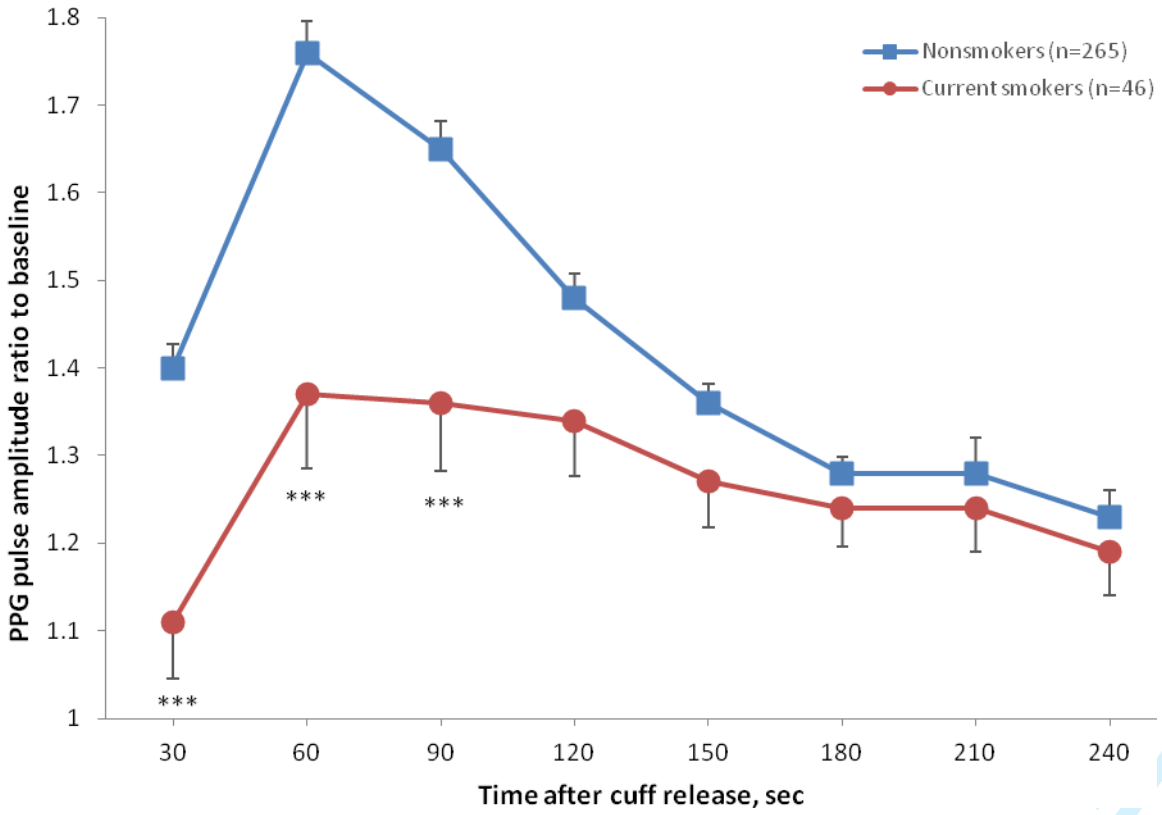
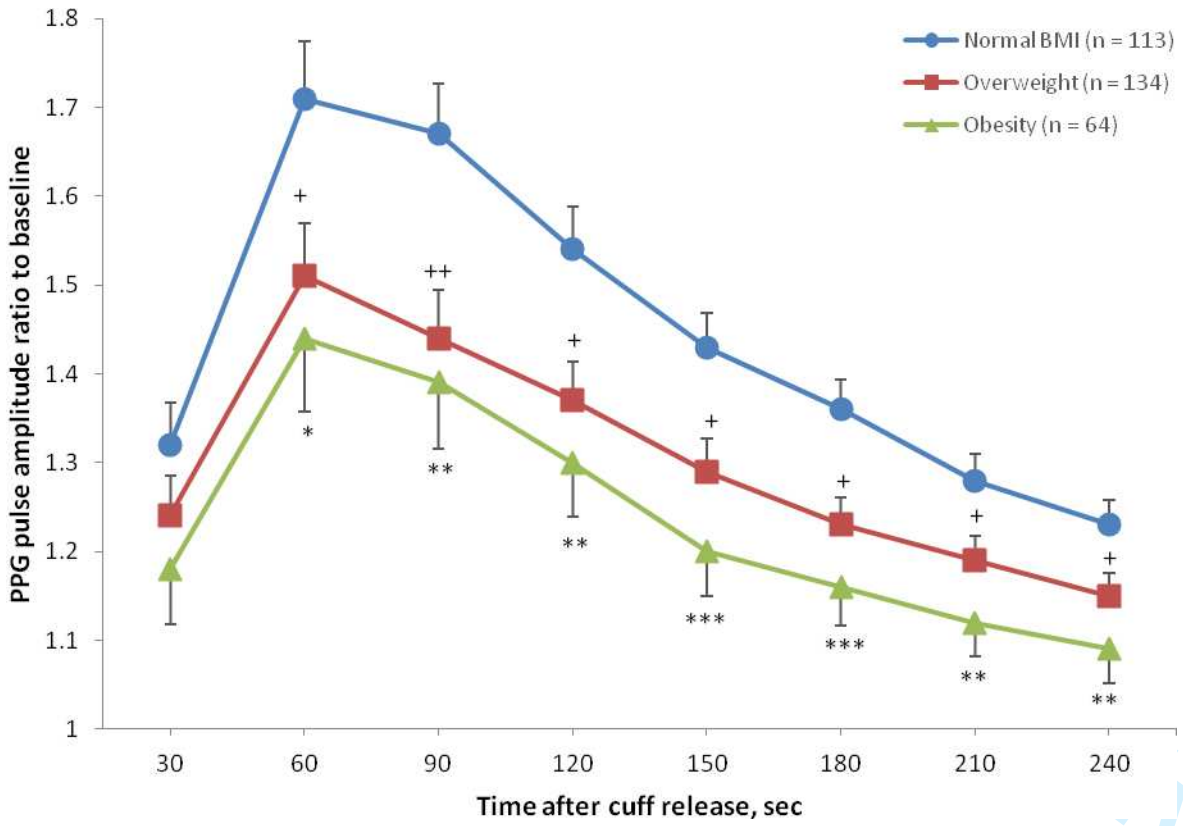
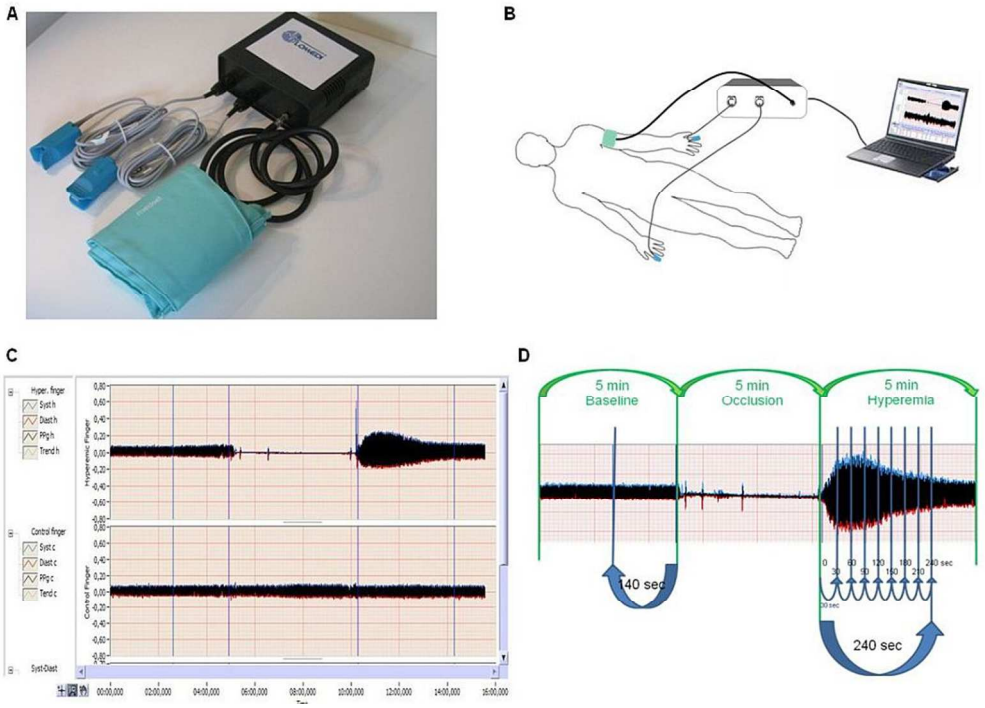
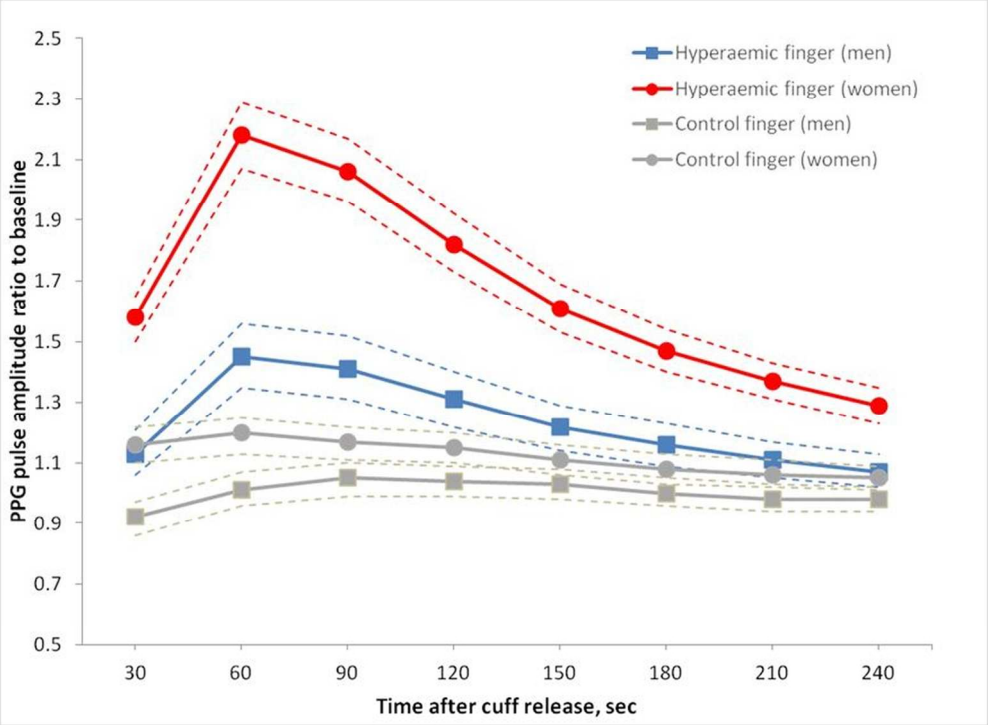

PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
***
***
***
30
60
90
120
150
180
210
240
Time after cuff release, sec
Nonsmokers (n=265)
Current smokers (n=46)

Figure 4

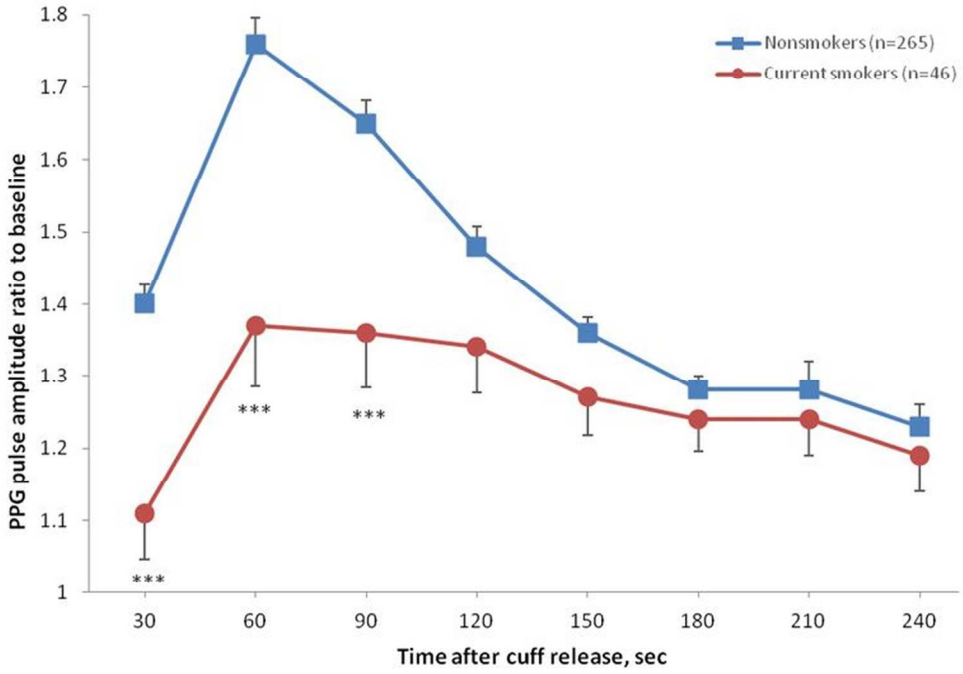

Normal BMI (n = 113)
Overweight (n = 134)
Obesity (n = 64)
PPG pulse amplitude ratio to baseline
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
30
60
90
120
150
180
210
240
Time after cuff release, sec
+
++
+
+
+
+
+
*
**
**
***
***
**
**



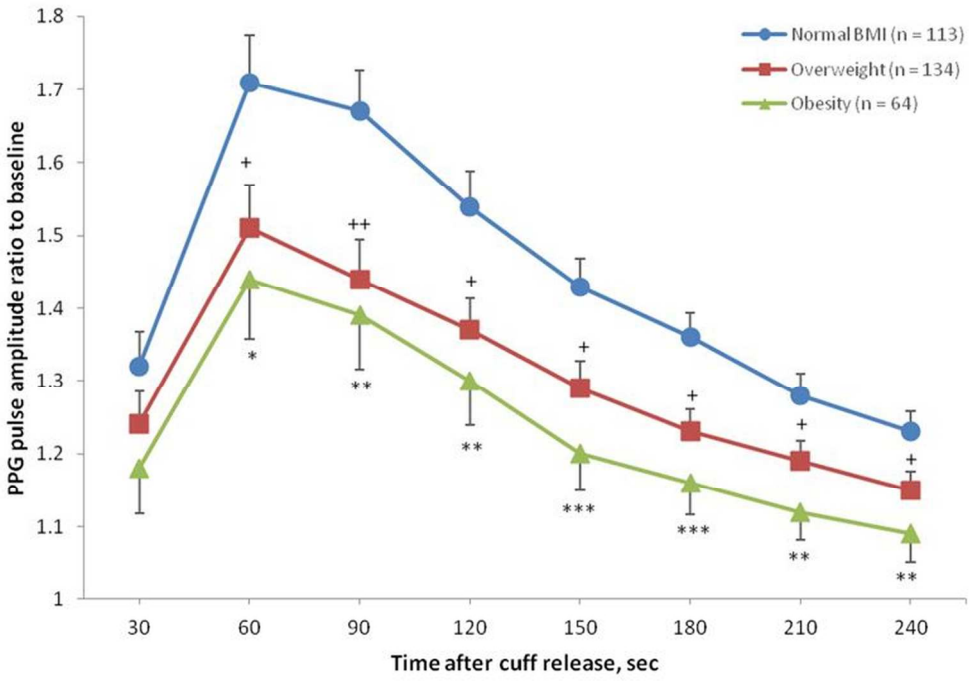
90x64mm (300 x 300 DPI)



121x90mm (300 x 300 DPI)



127x90mm (300 x 300 DPI)



127x90mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**Done**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**Done**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**Done**
Objectives	3	State specific objectives, including any prespecified hypotheses	**Done**
Methods			
Study design	4	Present key elements of study design early in the paper	**Done**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**Done**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	**Done**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**Done**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**Done**
Bias	9	Describe any efforts to address potential sources of bias	**Done**
Study size	10	Explain how the study size was arrived at	**Done**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**Done**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**Done**
		(*b*) Describe any methods used to examine subgroups and interactions	**Done**
		(*c*) Explain how missing data were addressed	NA

(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**Done**
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Done**
		(b) Indicate number of participants with missing data for each variable of interest	**Done**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*NA*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	**Done**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Done**
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Done**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Done**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Done**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Done**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Done**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**Done**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.