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Time course of forearm arterial compliance changes during reactive hyperemia

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Baldassarre, Damiano, Mauro Amato, Carlo Palombo, Carmela Morizzo, Linda Pustina, and Cesare R. Sirtori. Time course of forearm arterial compliance changes during reactive hyperemia. *Am J Physiol Heart Circ Physiol* 281: H1093–H1103, 2001.—Ultrasonic studies have shown that arterial compliance increases after prolonged ischemia. The objective of the present study was to develop an alternative plethysmographic method to investigate compliance, exploring validity and clinical applicability. Forearm pulse volume (FPV) and blood pressure (BP) were used to establish the FPV-BP relationship. Forearm arterial compliance (FAC) was measured, and the area under the FAC-BP curve (FAC_{AUC}) was determined. The time course curve of compliance changes during reactive hyperemia was obtained by continuous measurements of FAC_{AUC} for 20 s before and for 300 s after arterial occlusion. This technique allows us to effectively assess compliance changes during reactive hyperemia. Furthermore, the selected measurement protocol indicated the necessity for continuous measurements to detect "true" maximal FAC_{AUC} changes. On multivariate analysis, preischemic FAC_{AUC} was mainly affected by sex, peak FAC_{AUC} was affected by sex and systolic BP, percent changes were affected by plasma high-density and low-density lipoprotein cholesterol, peak time was affected by age and body mass index, and descent time was affected by plasma triglyceride levels. The proposed technique is highly sensitive and well comparable with the generally accepted echotracking system. It may thus be considered as an alternative tool to detect and monitor compliance changes induced by arterial occlusion.

plethysmography; endothelial dysfunction

ARTERIAL COMPLIANCE IS BECOMING an increasingly important clinical parameter. When reduced, it may be a potentially useful indicator of the presence of arterial disease (30). Functional changes in the arterial wall leading to reduced compliance may precede the onset of clinically apparent disease (clinical manifestations and/or arterial wall structural changes) and may identify individuals at risk before disease onset. A variety of techniques evaluating different arterial wall functions (arterial diameter, blood flow, and cross-sectional area) have been developed to measure this parameter (3, 10, 13, 14, 31). Some of them allow the assessment of large vessel compliance only, whereas others allow the assessment of large and small vessel compliance, i.e., resulting from changes at the arteriolar level. At present, there is no consensus as to the "best" method for measuring peripheral compliance, but it is important to note that a number of available techniques do not take into account that compliance is a pressure-dependent parameter.

Independent of the technique, most of the studies published so far have generally shown that differences among cases are not detectable in unstimulated conditions but become clearly evident when measured after the administration of organic nitrates (endothelium-independent vasodilators) or cholinergic stimuli (endothelium-dependent vasodilators). Because these vasoactive substances must be administered via a catheter inserted into the brachial artery, this may become an invasive, costly, and time-consuming procedure, associated with potential risk and discomfort, thus severely limiting clinical applicability.

A possible alternative endothelium-dependent stimulus useful for identifying endothelial dysfunction in humans in a noninvasive way is reactive hyperemia (20, 27, 34). This stimulus, obtained with a more or less prolonged arterial occlusion, induces an increased flow (27, 34) causing endothelium-mediated vasodilatation (20, 36) and increased compliance (17, 19).

In studies evaluating arterial compliance changes during reactive hyperemia, the postischemic modifications have been generally measured at arbitrary time intervals of 20–30 s, and the time course curve of postischemic variations was generally ignored.

We propose a new plethysmographic method useful for measuring forearm arterial compliance (FAC) [the area under the FAC-blood pressure (BP) curve (FAC_{AUC})]. This method, which takes into account nonlinear compliance/BP dependence and allows continuous measurements, was previously validated by evaluating its capacity to detect acetylcholine-induced changes as well as differences between hypercholesterolemic patients and age- and sex-matched normal controls (2).

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The present study was designed to *1*) evaluate the ability of this method to appreciate FAC_{AUC} changes occurring during reactive hyperemia; *2*) assess the validity of the aforementioned noncontinuous postischemic compliance measurement procedures; *3*) further validate the plethysmographic method vis-à-vis measurements made with an echotracking device; *4*) assess whether apart from the maximal compliance changes, known to be impaired in the presence of several pathological conditions (e.g., hypercholesterolemia) (17), other time-dependent postischemic parameters may be related to plasma lipids and other laboratory and clinical variables.

METHODS

Subjects. The experimental group consisted of 95 volunteers (53 men and 42 women, age range: 18–80 yr) recruited from the medical staff of our institutions as well as from patients attending the E. Grossi Paoletti Center for the Study of Metabolic Diseases. In view of the methodological nature of the study, subjects were enrolled without any specific selection criteria. Oral informed consent was obtained from all subjects, and the study was approved by the Internal Review Board. The characteristics of the studied population are shown in Table 1.

At the time of the investigation, 44 subjects (47.3%) were found to be normolipidemics [low-density lipoprotein cholesterol (LDL-C) $\leq$ 130 mg/dl], 17 subjects (18.3%) had borderline hypercholesterolemia (130 $<$ LDL-C $\leq$ 160 mg/dl), and 32 subjects (34.4%) had clear-cut hypercholesterolemia (LDL-C $>$ 160 mg/dl).

Sixteen percent of the patients were found to be moderately hypertensive [systolic BP (SBP) $>$ 160 or diastolic BP (DBP) $>$ 90]; two patients were suffering from cardiovascular diseases (myocardial infarction or angina), three patients from peripheral vascular disease, and two patients had had at least one acute cerebrovascular event [transient ischemic attack (TIA) or stroke]. The other subjects were free from established atherosclerotic lesions (no myocardial infarction, angina, claudication, or cerebrovascular ischemia) or from arterial occlusive disease (no murmurs or decreased vascular pulses and absence of ultrasound-detected evidence of overt carotid atherosclerosis: mean intima-media thickness $<$ 1.3 mm and single maximum intima-media thickness $<$ 1.5 mm) (39). In addition, none had diabetes mellitus and, excluding the seven patients with cardio-, cerebro-, or peripheral vascular disease, none had been taking, in the 2 mo before the study, lipid-lowering, antihypertensive, or any other medication known to affect arterial distensibility. In the whole group there were 27 smokers (29%); they abstained from smoking for the 12 h before the test.

Table 1. *Characteristics of the studied subjects*

n	95
Age, yr	44.1 ± 16.9
Body mass index, kg/m^2	24.4 ± 3.7
Heart rate, beats/min	74.7 ± 13.5
SBP, mmHg	142.3 ± 19.9
DBP, mmHg	81.6 ± 10.9
Total cholesterol, mg/dl	217.1 ± 53.2
HDL-C, mg/dl	42.0 ± 20.4
LDL-C, mg/dl	144.7 ± 43.6
LDL-C/HDL-C	4.09 ± 4.87
Triglycerides, mg/dl	130 (29–594)

Results are expressed as means ± SD except for triglycerides, which are expressed as median and range; *n*, no. of subjects. SBP and DPB, systolic and diastolic blood pressures (BP), respectively; HDL-C and LDL-C, high- and low-density lipoprotein cholesterol, respectively.

Twelve additional normal subjects (normolipidemic, normotensive, nonsmokers) were enrolled to examine whether the shape of the time course postischemic curve might depend on ischemic duration. These underwent two complete compliance examinations separated by a 1-h interval carried out by performing two different durations of ischemia (see below). The protocol was decided after a preliminary study in healthy subjects (n = 6), which confirmed that 15 min are sufficient to obtain a complete vessel recovery after either 3 or 12 min of arterial occlusion (data not shown). For the assessment of repeatability of the methodology, nine more normal subjects underwent two plethysmographic investigations 1 mo apart.

Finally, an additional group of 12 subjects was recruited, without any specific selection criteria, to evaluate the agreement between the plethysmographic method proposed here and an echotracking device, the technological "gold standard" for arterial compliance measurements (35). The ultrasound images were taken from longitudinal views of the radial arteries as close as possible to the side were the strain gauge was positioned. All the measurements of arterial wall movements were performed automatically by the ultrasound instrument and processed to obtain the diameter-BP curve as well as the compliance-BP curve. To match the parameters obtained with the plethysmographic method, the area under the compliance-BP curve, defined over a standard range of blood pressures (70–130 mmHg), was determined and defined as "radial artery compliance" (RAC_{AUC}). In three of these subjects, the between-method agreement was tested considering the compliance changes during hyperemia, and, in five other subjects, it was tested by evaluating the compliance changes induced by glyceryl trinitrate (GTN) administration. In these last experiments, after the baseline FAC_{AUC} and RAC_{AUC} evaluations, two doses of sublingual GTN were administered, and FAC_{AUC} and RAC_{AUC} were recorded after 4 min. In three of these subjects, the doses of GTN used were 25 and 50 μg; in the fourth subject, they were 50 and 100 μg; and in the fifth subject, they were 200 and 400 μg.

Lipids. At the time of blood sampling for lipid analysis, the subjects were on a free diet. Venous blood was collected from the antecubital vein after an overnight fast and anticoagulated with EDTA (1 mg/ml). Total cholesterol and triglycerides were determined by enzyme methods (7, 29). High-density lipoprotein cholesterol (HDL-C) was separated by selective precipitation of ApoB-containing lipoproteins with dextran-sulfate-$MgCl_2$ (38). Plasma LDL-C levels were calculated according to the Friedewald formula (15).

BP measurements. Heart rate, DBP, mean BP, and SBP were continuously recorded from the middle finger of the dominant arm using a Finapress instrument (2300 Finapress blood pressure monitor, Ohmeda). This method provides accurate continuous BP measurements comparable with intra-arterial recordings (19, 26).

FAC measurements. The FAC of the nondominant arm was measured by a previously described plethysmographic method, which allows direct assessment of the nonlinear "FAC-BP curve" relative to each single cardiac cycle (2). Briefly, measurements of forearm pulse volume (FPV) were made by means of the extensimetric hemofluximeter Angiomed (Microlab Electronic; Padua, Italy). A double-stranded loop of fine-gauge silastic rubber tubing (internal diameter 0.5 mm, external diameter 2.1 mm) containing gallium, indium, and

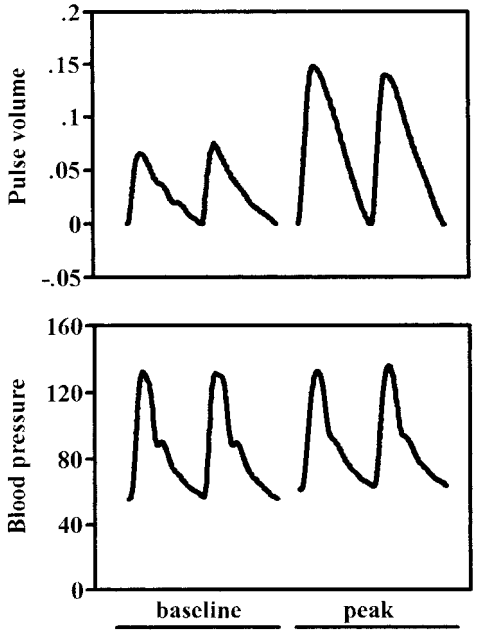

Fig. 1. Simultaneous recordings of the pulsatile change in the forearm pulse volume (*top*) and hand finger arterial blood pressure (BP; *bottom*) in the preischemic condition and at the peak value during reactive hyperemia.

tin was placed around the upper forearm. Simultaneous recordings of FPV (Fig. 1, *top*) and BP (Fig. 1, *bottom*) were used to establish the relationship between FPV and BP (Fig. 2, *top*). The mathematical model that fits best with these experimental points is a logarithmic function mathematically expressed as

$$y = a \times \ln(x) - b \quad (1)$$

with correlation coefficients ranging from $r = 0.72$ to 0.99. In 72.7% of the cases, the correlation coefficient was >0.9 (see Fig. 2, *top*). a and b are the coefficients that fit best with the experimental points. x and y are BP and FPV, respectively.

The slope of this function represents the nonlinear FAC-BP curve (Fig. 2, *bottom*), mathematically calculated as the first derivative of *Eq. 1*

$$y = a \times 1/x \quad (2)$$

Thus, in our model, compliance is confirmed to have a nonlinear dependence on pressure, decreasing with increasing BP.

The distance between the site where the strain gauge was positioned and the site where pulse pressure was recorded creates a phase shift in the systodiastolic volume-pressure relationship. It has been demonstrated that hysteresis related to the viscoelasticity of the vessel wall is negligible: the observed phase shift is mainly due to the distance mismatch and can be mathematically corrected (35), as performed by us.

To obtain a comparison among patients in isobaric conditions, FAC_{AUC}, defined over a standard range of BP (70–130 mmHg), was determined by calculating the integral, between 70–130 mmHg, of *Eq. 2*

$$y = a \times \ln(130/70) \quad (3)$$

After baseline measurements (average measurement of 16–25 beats obtained in 20 s), an ischemia occlusive test was carried out by upper arm pressure cuff inflation, 30 mmHg above the systolic pressure for 3 min. (In the group of 12 subjects enrolled to examine the possibility that the shape of the time course curve may depend on the duration of ischemia, the ischemia occlusive test was carried out by cuff inflation for both 3 and 12 min.)

After cuff deflation, the FPV and BP were continuously recorded for an additional 5 min. The time course of postischemic compliance changes was visualized by plotting the postischemic FAC_{AUC} measurements (each FAC_{AUC} value is the average of 4–5 beats) versus time (Fig. 3). Postischemic compliance parameters such as peak FAC_{AUC}, percent change, peak time, descent time, time of peak maintenance, and reserve area were then calculated (24). The procedures and formulas for the derivation of the postischemic parameters are detailed in the APPENDIX.

A single observer performed all examinations after patients had rested supine for 15 min in a temperature-controlled room at 26 ± 2°C.

Statistical analyses. Mean ± SD values were used as descriptive measures of normally distributed variables; in other cases, the median and range were used. Pre- and postischemic FAC_{AUC} comparisons were done by a Wilcoxon signed-rank test. Correlation analyses were performed using parametric methods (Pearson's moment correlation) after log transformation of triglycerides and plethysmographic variables. Multiple stepwise regression analysis was used to determine the relative importance of each variable (total cholesterol, HDL-C, LDL-C, triglycerides, age, and body mass index) in predicting pre- and postischemic FAC_{AUC} parameters. Statistical significance was accepted at a value of $P < 0.05$.

The method repeatability was evaluated by estimating the coefficient of variation (CV) between plethysmographic values obtained during the initial and the respective replicate measurements obtained after ~1 mo. CV was calculated after the log transformation of the variables considered.

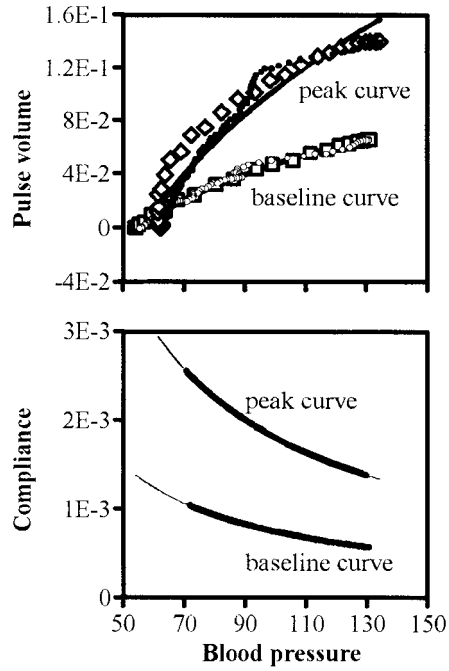

Fig. 2. Example of the pulse volume-BP relationship (*top*) and compliance-BP curve (*bottom*) in the preischemic condition and at the peak value during reactive hyperemia.

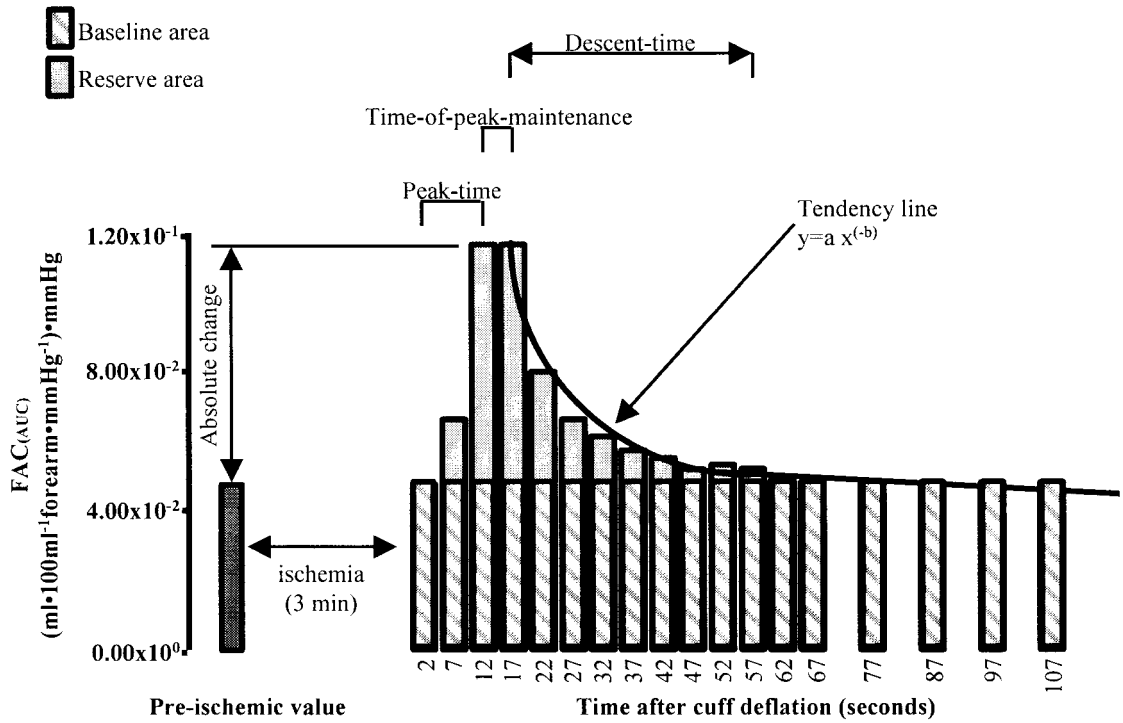

Fig. 3. Typical example of a time course of compliance changes during reactive hyperemia showing the "true" area under the forearm arterial compliance (FAC)-BP curve (FAC_{AUC}) maximal change not otherwise detectable with noncontinuous measurements. Each FAC_{AUC} value is the average of 4–5 beats. *a* and *b* are coefficients of the mathematical function that best fit with the descent time.

The agreement between the plethysmographic and ultrasonic technologies was evaluated by estimating the consistent bias between at least 36 compliance readings obtained with the plethysmographic method versus the same readings obtained with the echotracking device as recommended by Bland and Altman (4). Each point considered in this analysis was the mean compliance value obtained from at least 12 cardiac cycles. The coefficient of repeatability was calculated according to the British Standards Institution (6) and corresponds to 2 SD of the relative differences between replicate measurements. In addition, correlation coefficients, CV (in %), and the percent error between measurements were also calculated.

RESULTS

FAC and reactive hyperemia. During reactive hyperemia, arterial BP did not change, whereas FPV increased (Fig. 1). The rise of FPV produced a higher slope of the FPV-BP curve and, as a consequence, an increase in the FAC_{AUC} (Fig. 2).

Before arterial occlusion, FAC_{AUC} ranged between 1.85×10^{-2} and 15.2×10^{-2} with a median of 5.77×10^{-2} ml·100 ml forearm^{-1}·mmHg^{-1}·mmHg. After cuff deflation, FAC_{AUC} rose in all the subjects, reached a maximum, and then tended to return to the baseline levels in an asymptotic manner (Fig. 3). The median percent change was rather variable from subject to subject, ranging from 16.3 to 265.5%, with a median of 84% ($P < 0.0001$ vs. preischemia; Table 2).

Time course of FAC_{AUC} changes during reactive hyperemia. To assess the FAC_{AUC} closest to the real peak value ("true peak value"), FAC_{AUC} measurements were carried out continuously, and the time course of FAC_{AUC} changes was defined. This protocol allowed analyzing the shape of the time course curve, thus providing the opportunity to understand whether the peak FAC_{AUC} value was maintained for a time period long enough to be detectable with noncontinuous measurements. The time-dependent plethysmographic characteristics of the 93 subjects studied are shown in Table 2. FAC_{AUC} reached its maximal value ~17 s

Table 2. *Postischemic compliance characteristics of the studied subjects*

Peak $FAC_{AUC} \times 10^{-2}$	
Mean ± SD	11.0 ± 4.1
Median (range)	10.7 (3.80–23.40)
Percent change	
Mean ± SD	91.9 ± 49.7
Median (range)	84.0 (16.3–265.5)
Peak time, s	
Mean ± SD	16.0 ± 10.6
Median (range)	17.0 (2.0–52.0)
Time of peak maintenance, s	
Mean ± SD	5.2 ± 6.3
Median (range)	2.5 (2.5–41.7)
Descent time, s	
Mean ± SD	67.8 ± 36.8
Median (range)	63.4 (8.2–196.5)
Reserve area, au	
Mean ± SD	1.9 ± 1.1
Median (range)	1.7 (0.2–4.9)

FAC_{AUC}, area under the forearm arterial compliance (FAC)-BP curve (in ml·100 ml forearm^{-1}·mmHg^{-1}·mmHg). au, Arbitrary units.

Table 3. *Reproducibility of change in FAC during reactive hyperemia*

	Baseline (n = 9)	1 Mo (n = 9)	CV, %
Preischemic $FAC_{AUC} \times 10^{-2}$			
Means ± SD	5.83 ± 1.48	5.43 ± 1.71	7.9
Median (range)	5.94 (4.03–7.61)	5.77 (2.83–7.57)	
Peak $FAC_{AUC} \times 10^{-2}$			
Means ± SD	10.7 ± 1.85	10.4 ± 4.02	8.4
Median (range)	11.6 (7.91–12.6)	8.37 (6.68–16.2)	
Percent change			
Means ± SD	90.0 ± 41.6	96.3 ± 57.7	8.8
Median (range)	76.2 (55.1–170.2)	98.8 (28.3–178.8)	
Peak time, s			
Means ± SD	8.97 ± 8.12	4.82 ± 5.97	32.2
Median (range)	7.37 (1.9–22.2)	2.45 (1.2–16.9)	
Time of peak maintenance, s			
Means ± SD	5.7 ± 7.8	3.9 ± 3.4	47.3
Median (range)	2.5 (2.5–21.7)	2.5 (2.5–10.8)	
Descent time, s			
Means ± SD	78.1 ± 23.4	54.6 ± 25.1	16.2
Median (range)	77.0 (43.7–111.5)	61.8 (8.9–76.3)	
Reserve area, au			
Means ± SD	2.12 ± 1.06	1.34 ± 0.81	62
Median (range)	1.95 (0.93–3.82)	1.23 (0.37–2.65)	

n, No. of subjects. FAC_{AUC} was measured in ml·100 ml $forearm^{-1}$·$mmHg^{-1}$·mmHg. CV, coefficient of variation.

after cuff deflation (range, 2–52 s) and, in 77% of subjects, was maintained for not more than 2.5–7 s, a time interval too short for legitimate noncontinuous measurements. Thus, in most subjects, descent starts almost immediately, and FAC_{AUC} returns to its respective preischemic values in ~63 s (descent time range, 8–196 s).

Repeatability of pre- and postischemic plethysmographic variables. Table 3 shows the pre- and postischemic plethysmographic variables obtained at the initial visit and after 1 mo in a subset of nine subjects; the CVs are also reported. Whereas the CV was <10% for preischemic FAC_{AUC}, peak FAC_{AUC} and percent change, it ranged between 16.2 and 62% for the time-related variables.

Agreement between the plethysmographic method and echotracking device in compliance determination. The 12 subjects recruited for the agreement study had a mean age (±SD) of 37 ± 11 yr (range, 27–55 yr). Eleven (92%) were males, and, with the exception of two smokers and two hypertensives, none was exposed to significant cardiovascular risk factors (e.g., blood pressure, lipids, or diabetes).

Data included in the between-method analysis were obtained by pooling all the compliance measurements obtained in stimulated and unstimulated conditions, with a total of 36 data analyzed. The area under the compliance-BP curve ranged from 2.41×10^{-2} to 6.73×10^{-2} ml·100 ml $forearm^{-1}$·$mmHg^{-1}$·mmHg, with a mean value of $4.15 \times 10^{-2} \pm 1.10 \times 10^{-2}$ ml·100 ml $forearm^{-1}$·$mmHg^{-1}$·mmHg, when measured with the plethysmographic method and from 1.06×10^{-2} to 5.64×10^{-2} mm^2·$mmHg^{-1}$ ·10^{-3}·mmHg, with a mean value of $3.27 \times 10^{-2} \pm 1.12 \times 10^{-2}$ mm^2·$mmHg^{-1}$·10^{-3}·mmHg, when measured with the echotracking device. The bias between readings was $-0.87 \times 10^{-2} \pm 0.61 \times 10^{-2}$, with a repeatability coefficient of 2.13×10^{-2} and limits of agreement between the two systems ranging from -2.1×10^{-2} to 0.35×10^{-2} (Fig. 4, *top*). The mean absolute difference between the two measurements was $0.93 \times 10^{-2} \pm 0.53 \times 10^{-2}$, with a CV of 11.7% and a correlation coefficient of 0.85 ($y = 0.014 + 0.83x$; $P < 0.0001$; Fig. 4, *bottom*).

Agreement between plethysmography and echotracking in assessment of compliance changes after GTN administration. The FAC_{AUC} and RAC_{AUC} responses to the two doses of sublingual GTN in the five subjects selected for the GTN study are illustrated in Fig. 5. In the three subjects treated with low doses of GTN (25 and 50 μg) and in the single subject treated with 50 and 100 μg of GTN, a dose-dependent increase of RAC_{AUC} was observed. In contrast, in the single subject treated with the higher doses of GTN (from 200 to 400 μg), a dose-dependent reduction of RAC_{AUC} was

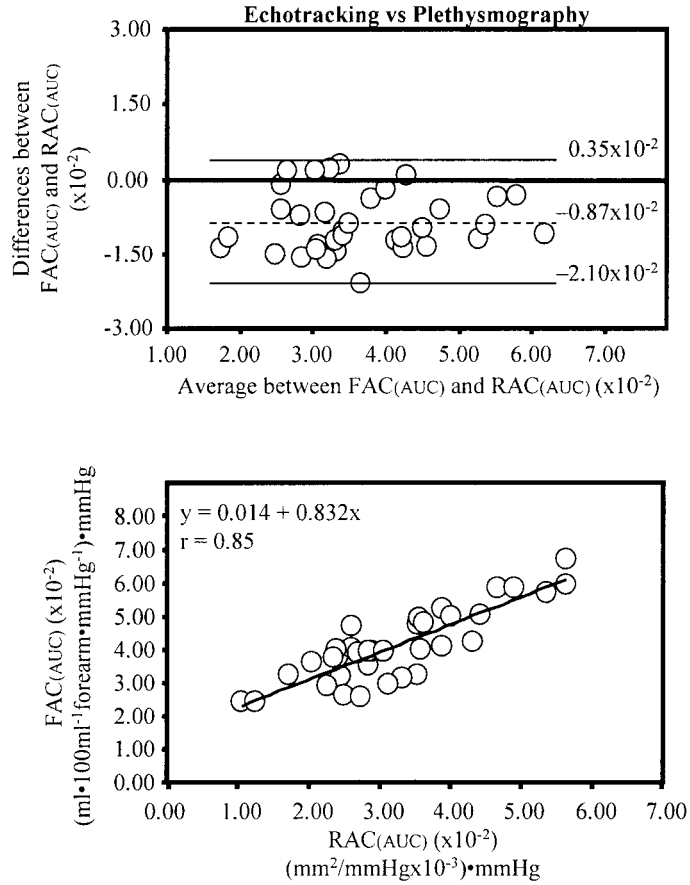

Fig. 4. *Top*: agreement between the plethysmographic and ultrasound technologies. The differences between measurements obtained with the plethysmographic and with the ultrasound methods are plotted versus their means. The mean difference and the limits of agreement are also indicated. *Bottom*: relationship between measurements obtained with the plethysmographic system and with ultrasound technology. RAC_{AUC}, area under the radial artery compliance (RAC)-BP curve.

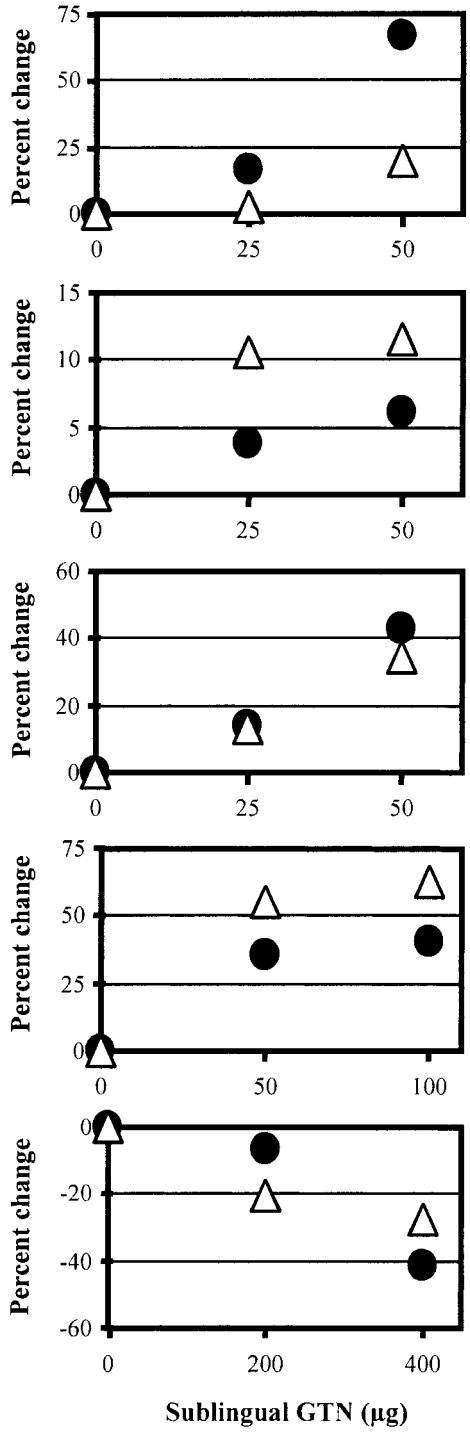

Fig. 5. Changes in area under the compliance-BP curve induced by two doses of sublingual glyceryl trinitrate (GTN) as measured using the plethysmographic method proposed here (△) as well as echotracking technology (●) in 5 control subjects.

detected. All the compliance changes detected with the echotracking system were also appreciated using the plethysmographic device. Interestingly, although quite different in terms of absolute values, the trends of the compliance changes are substantially identical with the plethysmographic method and with the echotracking device (Fig. 5).

Agreement between plethysmography and echotracking in assessment of compliance changes during reactive hyperemia. The FAC_{AUC} and RAC_{AUC} responses to reactive hyperemia in three subjects are shown in Fig. 6. Although not identical in absolute terms, the kinetics of the compliance changes during reactive hyperemia are clearly appreciable using both the plethysmographic method and the echotracking device (Fig. 6).

Duration of ischemic test and shape of the time course curve. In the 12 subjects enrolled to investigate whether duration of arterial occlusion may influence the shape of the time course curve, two complete plethysmographic examinations were performed at a 1-h interval. The FAC_{AUC} changes obtained after 3 and 12 min of arterial occlusion were then compared. As shown in Table 4, a prolonged arterial occlusion clearly produced higher values for each parameter considered. However, the time required to reach the peak was again highly variable (range, 2–27 s), and the time of peak maintenance was again very brief, not exceeding, in 83% of the cases, 14 s (range, 5–45 s).

Correlation between plethysmographic parameters and laboratory and clinical variables. To investigate whether the different postischemic plethysmographic

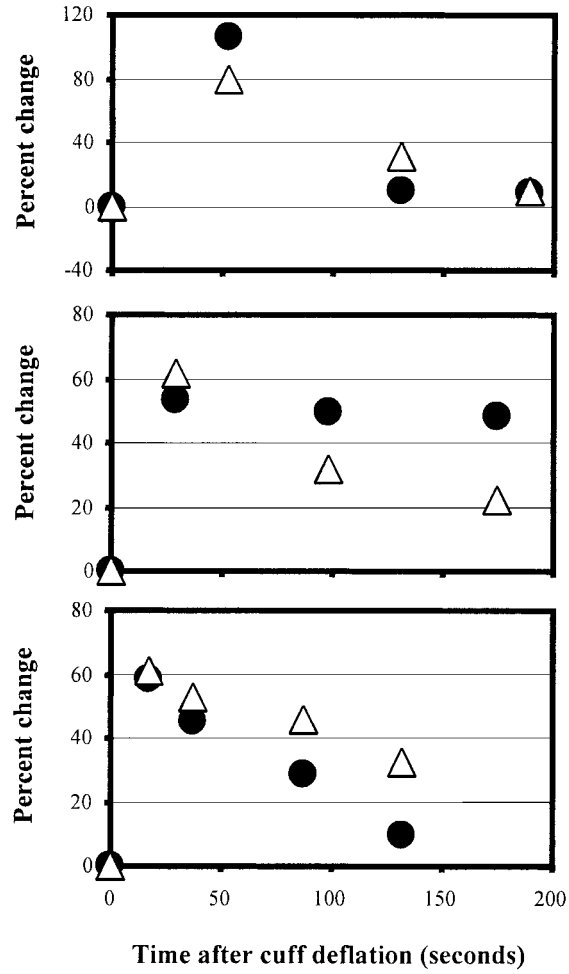

Fig. 6. Time-course curve of compliance changes during reactive hyperemia in 3 control subjects as measured using plethysmography (△) and echotracking technology (●).

Table 4. *Plethysmographic characteristics before and after 3 or 12 min of arterial occlusion*

	Arterial Occlusion	
	3 Min	12 Min
Preischemic $FAC_{AUC} \times 10^{-2}$		
Means ± SD	6.46 ± 3.22	6.42 ± 3.02
Median (range)	5.51 (2.21–14.1)	6.21 (2.43–13.5)
Peak $FAC_{AUC} \times 10^{-2}$		
Means ± SD	16.6 ± 8.74	25.9 ± 14.8
Median (range)	13.4 (4.66–35.7)	21.6 (9.58–62.1)*
Percent change		
Means ± SD	160 ± 70	301 ± 100
Median (range)	171 (56–261)	314 (157–441)*
Peak time, s		
Means ± SD	4 ± 3	12 ± 9
Median (range)	3 (2–12)	8 (2–27)*
Time of peak maintenance, s		
Means ± SD	5.7 ± 8.7	11.4 ± 13.6
Median (range)	2.5 (2.5–32.5)	4.7 (2.5–42.0)*
Descent time, s		
Means ± SD	64 ± 29	130 ± 50
Median (range)	66 (19–109)	109 (73–209)*
Reserve area, au		
Means ± SD	2.79 ± 1.94	11.44 ± 6.56
Median (range)	1.84 (0.65–5.56)	11 (2.33–23.84)*

FAC_{AUC} was measured in ml·100 ml forearm^{-1}·mmHg^{-1}·mmHg. *$P < 0.01$ by Wilcoxon signed-rank test.

parameters were related to laboratory and clinical variables, a series of correlation analyses was performed (Table 5). The peak FAC_{AUC} correlated inversely with SBP, whereas percent change correlated inversely with LDL-C and the LDL-C-to-HDL-C ratio and directly with HDL-C. Peak time correlated inversely with age and HDL-C, whereas descent time and the reserve area correlated inversely with age and log-transformed triglyceride content.

Multiple regression analyses. Table 6 shows a series of five forward stepwise multiple regression analyses performed using log-transformed plethysmographic parameters as dependent variables and laboratory/clinical variables as independent variables. In this series of analyses, sex was the only parameter independently associated with preischemic FAC_{AUC} ($R^2 = 0.19$, $P = 0.001$). Sex (directly) and SBP (inversely) significantly predicted the peak FAC_{AUC} value ($R^2 = 0.26$, $P = 0.0001$ and $P = 0.01$, respectively), whereas HDL-C (directly) and total cholesterol (inversely) correlated independently with the percent change ($R^2 = 0.10$, $P = 0.01$ and $P = 0.04$, respectively). Finally, age (inversely) and body mass index (directly) correlated with the peak time ($R^2 = 0.13$, $P = 0.003$ and $P = 0.02$, respectively), whereas log-transformed triglyceride content was the only independent predictor of descent time ($R^2 = 0.09$, $P = 0.005$).

Plethysmographic parameters and lipid tertiles. To further investigate the impact of plasma lipid/lipoprotein levels in predicting pre- and postischemic plethysmographic variables, the data were stratified into tertiles according to HDL-C, LDL-C, triglycerides, and the LDL-C-to-HDL-C ratio (Table 7). According to what was observed in the correlation analysis, the percent changes increased and peak time decreased with rising HDL-C levels. In contrast, the percent changes decreased with rising LDL-C levels and LDL-C-to-HDL-C ratios. As far as time-related variables are concerned, descent time and the reserve area significantly decreased with rising triglyceride values.

DISCUSSION

A number of studies performed using high-resolution ultrasound methods to assess arterial compliance (measurements of finger BP and radial artery diameter, allowing the analysis of the diameter-pressure curve) have shown that compliance increases markedly after prolonged ischemia, thus allowing the study of reserve above baseline (18). In the present study, it is also clearly substantiated that with the proposed plethysmographic method, based on continuous measurements of finger BP and FPV (FPV is the result of changes at the level of large and small vessels level), postischemic compliance modifications occurring during reactive hyperemia can be detected even after only 3 min of arterial occlusion. Despite the high interindividual variability in the preischemic FAC_{AUC} value, all patients show, after cuff deflation, a significant increase in FAC_{AUC}, with percent changes ranging from 16.3 to 265.5%.

To the best of our knowledge, this is also the first study attempting to explore the kinetics of compliance modification using a continuous measurement approach, thus allowing the evaluation of the validity of noncontinuous measurement procedures often used, by others, to study compliance changes in the course of reactive hyperemia. The present findings clearly show that FAC_{AUC} changes during reactive hyperemia are strongly related to the time factor. Thus, according to the present results, a noncontinuous measurement procedure may be questionable. These findings, in fact, not only show that the time required to reach the peak value (peak time) is quite variable, ranging from subject to subject from 2 to 52 s, but also, and more important, that the peak value is maintained for a time period too short (<7 s in ~77% of cases) to be assessed by measurements carried out at arbitrary time intervals. After a defined interval from cuff deflation, i.e., 30 s, measured values may not be representative of the

Table 5. *Correlation coefficients among log-transformed postischemic compliance parameters and lipid and anamnestic variables*

	Peak FAC_{AUC}	Percent Change	Peak Time	Descent Time	Reserve Area
Age	NS	NS	−0.20*	−0.20*	−0.25†
SBP	−0.20*	NS	NS	NS	NS
DBP	NS	NS	NS	NS	NS
Total cholesterol	NS	NS	NS	NS	NS
Log triglycerides	NS	NS	NS	−0.31†	−0.27†
HDL-C	NS	0.23*	−0.20*	NS	NS
LDL-C	NS	−0.22*	NS	NS	NS
LDL-C/HDL-C	NS	−0.32†	NS	NS	NS

NS, not significant. *$P < 0.05$; †$P < 0.01$.

Table 6. *Forward stepwise multiple regression analysis*

Correlated Independent Variables	R^2	R^2 Adjusted	*b*	*b* (SE)	95% Confidence Interval		*P*
			Preischemic FAC_{AUC}				
Sex	0.1919	0.1604	0.26990	0.07798	0.11462	0.42518	0.001
			Peak FAC_{AUC}				
Sex	0.2586	0.2195	0.33875	0.07654	0.18632	0.49119	0.0001
SBP			−0.00728	0.00274	−0.01274	−0.00183	0.01
			Percent change				
HDL-C	0.1027	0.0797	0.00817	0.00315	0.00190	0.01444	0.011
LDL-C			−0.00254	0.00121	−0.00494	−0.00014	0.038
			Peak time				
Age	0.1296	0.1073	−0.01979	0.00632	−0.03237	−0.00721	0.003
Body mass index			0.07203	0.02931	0.01367	0.13038	0.016
			Descent time				
Log triglycerides	0.0947	0.0832	−0.00197	0.00068	−0.00333	−0.00060	0.005

The following dependent variables were log transformed: preischemic FAC_{AUC}, peak FAC_{AUC}, percent change, peak time, and descent time. The following laboratory and clinical variables were considered in the models: sex, age, body mass index, DBP, SBP, total cholesterol, log triglycerides, HDL-C, and LDL-C. *b*, Coefficient of the mathematical function that best fits with the descent time.

true peak but, more likely, provide a simple estimate of the ascent or descent phases of the time course curve (Fig. 7). The concept that a noncontinuous measurement procedure may be questionable is also underlined by the fact that methodological repeatability is clearly lower for time-related variables versus FAC_{AUC} and percent change values (Table 3).

The reasons for the elevated between-patient variability in the time course compliance parameters reported here are not clear. Variability may reflect prominent influences not controlled for, such as neural effects, humoral stimuli, or regional metabolic changes. In addition, biological variability may, of course, be partially contributory. The presence of vascular risk factors, as well as a possible influence of flow-mediated vasodilatation on the time course compliance parameters, may further contribute to the described variability. Independent of the mechanism(s) of a such striking variability, the present results strongly advocate that, to determine true maximal changes of arterial compliance during reactive hyperemia, a continuous measurement is more appropriate. A possible criticism to this conclusion might be that only 3 min of arterial occlusion are too brief a stimulus to induce maximal compliance changes. Ischemia was limited to 3 min because the subject's pain, frequently resulting from a more prolonged ischemia, was a clear limiting factor. However, in the 12 subjects in whom 3 and 12 min of arterial occlusion were compared, we observed that, although 12 min of arterial occlusion effectively produced higher values for each parameter considered, the peak time was again too variable and the time of peak maintenance too brief to allow acceptance of noncontinuous measurements. Thus, in performing 12 min of arterial occlusion, a continuous measurement procedure also seems to be essential (at least for the first 1 or 2 min after cuff deflation) to identify the true peak FAC_{AUC} value.

Another important reason to determine the time course curve derives from the fact that this procedure may provide, in addition to maximal FAC_{AUC}, other time-dependent compliance parameters potentially useful to better characterize possible endothelial dysfunction. This hypothesis is supported by previous studies (20, 34) in human peripheral conduit arteries suggesting that, whereas nitric oxide is minimally involved in regulating forearm blood flow at peak reactive hyperemia, it plays a significant role in maintaining vasodilatation during the post-peak phase. On the basis of these findings, one may assume that the marked increases in forearm blood flow during the early phases of reactive hyperemia can induce increased shear stress, which may release nitric oxide from the endothelium, thus contributing to vasodilatation and possibly compliance modulation during the mid to late phase of reactive hyperemia. The mechanisms by which the endothelium, or perhaps endothelium-derived nitric oxide, can modulate the postischemic time-dependent compliance parameters were not investigated in this study. One may, however, speculate that local factors contributing to flow-mediated vasodilatation of microvessels, such as changes in interstitial potassium and hydrogen ions, osmolality, carbon dioxide, catecholamines, prostaglandins, and adenosine (1, 5, 8, 12, 20, 23, 25), can also be involved in the regulation of the parameters considered here. Although we cannot be sure about the endothelium-dependent nature of these parameters, it is interesting to note that some of them are markedly correlated with well-known traditional risk factors for endothelial dysfunction, such as age (9, 11, 16), high cholesterol (17, 33, 37), and triglycerides (28) (Table 5); such findings were also confirmed after stratification of the studied group into tertiles for lipid variables (Table 7).

Most of the studies published so far evaluating the effects of vascular risk factors on vasoactivity after

Table 7. *Postischemic compliance characteristics after stratification of subjects into tertiles of HDL-C, LDL-C, triglycerides, and LDL-C/HDL-C ratio*

	HDL-C		LDL-C		Triglycerides		LDL-C/HDL-C	
	1st Tertile	3rd Tertile	1st Tertile	3rd Tertile	1st Tertile	3rd Tertile	1st Tertile	3rd Tertile
HDL-C, mg/dl	18.1 ± 6.5	64.6 ± 8.8	40.3 ± 21.9	43.5 ± 17.7	51.6 ± 18.9	32.3 ± 17.2	61.9 ± 11.1	21.1 ± 10.2
LDL-C, mg/dl	134.6 ± 48.1	142.8 ± 41.4	97.7 ± 16.7	192.0 ± 28.3	130.0 ± 37.3	158.0 ± 45.9	122.7 ± 31.2	156.2 ± 48.2
Triglycerides, mg/dl	197.0 ± 115.3	100.2 ± 41.4‡	130.2 ± 105.5	169.3 ± 91.6	74.1 ± 17.6	254.3 ± 109.8	118.7 ± 103.0	206.8 ± 109.8‡
LDL-C/HDL-C	8.6 ± 5.1	2.3 ± 0.8	3.6 ± 2.5	6.0 ± 5.2	2.0 ± 1.5	7.2 ± 5.7	2.0 ± 0.5	8.9 ± 4.9
Peak $FAC_{AUC} \times 10^{-2}$								
Means ± SD	10.8 ± 3.5	10.5 ± 4.4	11.4 ± 4.3	10.8 ± 3.8	10.8 ± 4.6	10.9 ± 3.7	11.6 ± 4.5	11.4 ± 3.5
Median	9.8	9.3	10.6	11.2	9.3	10.7	11.7	10.9
(range)	(5.0–19.7)	(4.2–22.8)	(5.0–22.8)	(3.8–21.3)	(5.0–22.8)	(3.8–19.2)	(5.2–22.8)	(6.6–19.7)
Percent change								
Means ± SD	72.9 ± 41.4	104.7 ± 57.3*	104.1 ± 60.1	77.1 ± 36.0*	91.0 ± 35.6	80.9 ± 47.4	113.7 ± 56.2	73.5 ± 39.4†
Median	59.0	94.0	84.0	76.0	84.0	74.0	105.0	65.0
(range)	(21.9–169.3)	(16.3–265.5)	(32.1–165.5)	(16.3–176.6)	(35.1–169.3)	(21.9–221.5)	(32.1–265.5)	(21.9–176.6)
Peak time, s								
Means ± SD	17.7 ± 10.9	13.8 ± 12.8*	13.2 ± 9.8	16.5 ± 9.7	17.4 ± 12.2	15.2 ± 8.3	12.4 ± 11.4	17.1 ± 9.8
Median	17	7	12	17	17	17	7	17
(range)	(2–52)	(1–47)	(2–42)	(2–47)	(2–47)	(2–37)	(1–42)	(2–52)
Time of peak maintenance, s								
Means ± SD	5.7 ± 4.9	3.1 ± 1.6	4.9 ± 4.4	6.3 ± 8.7	4.3 ± 4.6	6.7 ± 8.3	3.3 ± 1.8	5.1 ± 4.4
Median	3	3	3	3	3	3	3	3
(range)	(3–18)	(3–8)	(3–18)	(3–42)	(3–22)	(3–42)	(3–8)	(3–18)
Descent time, s								
Means ± SD	63.8 ± 38.4	67.12 ± 33.7	68.3 ± 34.2	64.0 ± 31.3	77.5 ± 29.1	53.9 ± 28.6‡	66.7 ± 32.4	57.3 ± 33.6
Median	70	58	69	54	78	53	63	53
(range)	(8–140)	(13–128)	(8–140)	(13–132)	(14–123)	(8–112)	(14–128)	(8–139)
Reserve area, au								
Means ± SD	1.54 ± 0.89	1.83 ± 1.21	1.87 ± 0.94	1.66 ± 1.03	2.13 ± 1.06	1.52 ± 1.04*	2.07 ± 1.28	1.48 ± 0.88
Median	1.34	1.67	1.79	1.25	2.27	1.22	1.88	1.33
(range)	(0.34–3.59)	(0.38–4.88)	(0.38–3.70)	(0.36–3.82)	(0.50–4.38)	(0.34–3.81)	(0.45–4.88)	(0.34–3.35)

Second Tertiles have been omitted to exclude patients with borderline values of the variables listed. FAC_{AUC} was measured in ml·100 ml forearm^{-1}·mmHg^{-1}·mmHg. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ vs. 1st tertiles by Student's *t*-test after log transformation of plethysmographic variables.

arterial occlusive stimuli have been carried out completely ignoring time factors. To the best of our knowledge, the only study (32) that evaluated the effects of a major vascular risk factor (cigarette smoking) on vasoactivity using continuous measurements showed improved measurement accuracy and an improved possibility of uncovering arterial functional abnormalities in smokers not otherwise detectable when the "time" factor is ignored. Thus vascular risk factors may modulate postischemic compliance changes, affecting not only the degree of reactive response, but also overall duration, or even both of these aspects. The present results provide further support to this hypothesis. In fact, when analyzing the cardiovascular risk factors of the examined patient series, it becomes apparent that, whereas reduced HDL-C levels may affect vasoactivity by reducing percent changes and delaying the time necessary to reach peak values, age seems to act only by affecting time-dependent parameters. Thus, according to Stadler et al. (32, 37), considering time-dependent parameters, one can appreciate the effects of vascular risk factors not otherwise detectable by considering percent changes only. The direct correlation between percent changes and HDL-C and the inverse correlation between percent changes and LDL-C or LDL-C-to-HDL-C ratios confirm, also in this nonselected group of subjects, a positive role for HDL-C (21, 22, 28) and a negative role for hypercholesterolemia on endothelial function (2, 17, 33). After adjustment of data for the other variables considered, in multiple stepwise regression analysis (Table 6), sex was the only variable independently related to the preischemic FAC_{AUC} thus explaining ~19% ($R^2 = 0.19$) of variability. Sex and SBP independently correlated with peak

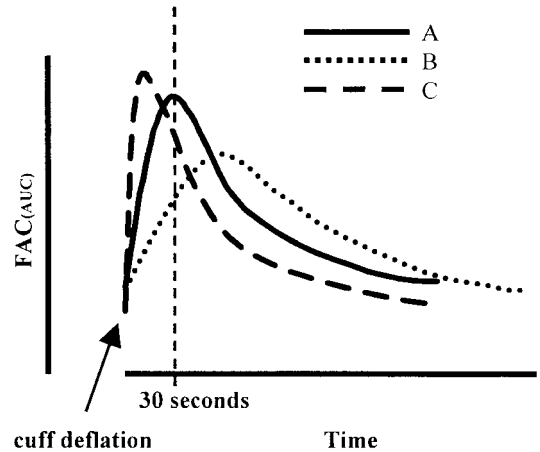

Fig. 7. Time course curves of 3 subjects. After a definite time interval (i.e., 30 s), the measured compliance value may correspond to the true maximal compliance change (*curve A*) but also to a simple value of the ascent (*curve B*) or descent phase (*curve C*).

FAC_{AUC} and together explained ~26% ($R^2 = 0.26$) of variability. Peak time was mainly affected by age and body mass index ($R^2 = 0.13$), whereas lipid variables affected mainly the percent change, significantly and independently correlated with plasma levels of HDL-C (directly) and LDL-C (inversely), and descent time, this last mainly affected by plasma levels of triglycerides. As far as the reserve area is concerned, the correlations observed with the linear regression analysis did not reach statistical significance after data adjustment for the other variables considered. Another interesting finding that can be deduced from our results is that descent time seems to be related to the presence of pathological conditions when it is reduced, whereas the same is true for the prolongation of peak time. This suggestion is well supported not only by the inverse correlation between peak time and HDL-C, a well-known protective lipoprotein parameter (21, 22, 28), but also by the same negative correlation with percent changes of arterial compliance (-0.38, $P < 0.001$), known to be reduced when vascular risk factors or pathological conditions are present.

All of these results strongly advocate the use of the plethysmographic method proposed here as a useful tool to measure arterial compliance in vivo. Because more extensive background information was needed to validate its ability to provide measurements applicable to clinical trials, a study was performed comparing the method proposed here with the echotracking system, a more widely accepted ultrasonic approach often applied to clinical trials (17, 19).

A high between-methods correlation coefficient was observed ($r = 0.85$, Fig. 4); however, by following the analytical approach proposed by Bland and Altman (4), the most appropriate method for evaluating the consistency of a new method of measurement versus an established one, it became apparent that the two methods can result in a discrepancy in arterial compliance of about -0.87×10^{-2}. Thus, whereas the plethysmographic method may not be considered as interchangeable with the echotracking one, both provide essentially the same kind of information. Indeed, as documented in Figs. 5 and 6, although different in terms of absolute values, the trends of compliance changes during reactive hyperemia as well as those induced by sublingual GTN are essentially identical for the two methods, thus indicating that the plethysmographic method described here provides reliable technology for arterial compliance measurements applicable to clinical trials.

In conclusion, the proposed technique appears to be highly sensitive and may be considered, as an alternative to ultrasound, as a potentially useful tool to detect and monitor in vivo compliance changes induced by arterial occlusion. In addition, the study strongly suggests that the time factor should not be underestimated when postischemic variables are considered. It may, in fact, provide further time-dependent markers enabling the objective assessment of the effects of risk factors, and perhaps of risk factor modifications, on compliance changes during reactive hyperemia, a stimulus often used in pharmacological and clinical trials to investigate, in a completely noninvasive way, arterial endothelial dysfunction in humans.

APPENDIX

The peak area under the forearm arterial compliance (FAC)-blood pressure (BP) curve (peak FAC_{AUC}) was the maximal FAC_{AUC} value obtained after cuff deflation, and the peak time was the time required to reach the peak value.

Time of peak maintenance was calculated as the difference between the time where the descent phase starts and peak time, whereas the descent time was the time required to return within 10% of the baseline value calculated using the following formula

$$\text{descent time}_{10\%} = (FAC_{(AUC)10\%}/a)^{(1/b)} \qquad (A1)$$

where a and b are the coefficients of the mathematical function that best fits with the experimental points of the descent phase (see Fig. 3), mathematically expressed as

$$y = ax^{(-b)} \qquad (A2)$$

The percent change was calculated as follows: (peak FAC_{AUC}/preischemic $FAC_{AUC} \times 100$) $- 100$, whereas the reserve area was the area under the time course curve minus the baseline area.

The area under the time course curve was calculated by adding the areas under the graph between each pair of consecutive FAC_{AUC} measurements (24) with the measurements y_1 and y_2 at times t_1 and t_2; the area under the curve between those two times was the product of the time difference and the average of the two measurements. Thus we get $[(t_2 - t_1)(y_1 + y_2)]/2$, i.e., fitting with the trapezium rule. If we have $n + 1$ measurements of y_1 at time t_i ($i = 0, \ldots, n$), then the area under the curve (AUC) can be calculated as

$$\text{AUC} = \frac{1}{2}\sum_{i=0}^{n-1}[(t_{i+1} - t_i)(y_i + y_{i+1})] \qquad (A3)$$

The baseline area was calculated as the product of the preischemic FAC_{AUC} value and total time, calculated by adding peak time, time of peak maintenance, and descent time (Fig. 3), i.e., baseline area = preischemic $FAC_{AUC} \times$ (peak time + time of peak maintence + descent time).

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