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Two years of smoking cessation does not reduce arterial wall thickness and stiffness

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ABSTRACT

Background: Smoking cessation rapidly reduces cardiovascular risk. The pathophysiological mechanisms involved are still being debated. We measured structural and functional arterial wall properties of the femoral and carotid arteries after smoking cessation to investigate their possible role in cardiovascular risk reduction.

Methods: Out of 127 smokers, 33 proved to stop smoking for two years. They were compared with 50 nonsmokers and 55 persistent smokers in a prospective study. Cross-sectional compliance and distensibility coefficients as well as intima-media thickness of both carotid arteries and of the right common femoral artery were measured ultrasonographically at baseline and 3, 6, 12 and 24 months after smoking cessation. The nonsmoking and persistent smokers group were measured twice at an interval of 24 months.

Results: Persistent smoking and two years of smoking cessation did not affect cross-sectional compliance and distensibility coefficients. Although at baseline intima-media layers were thicker in smokers, the change over time in intima-media thickness did not differ significantly between all three groups.

Conclusion: Two years of smoking cessation was not accompanied by a slower progression or a regression in intima-media thickness nor by an improved cross-sectional compliance or distensibility coefficient. Nevertheless, smoking cessation should be recommended as it reduces cardiovascular risk rapidly after smoking cessation.

Introduction

Smoking cessation reduces the increased cardiovascular risk, as convincingly demonstrated in large cohort studies.¹⁻³ A considerable risk reduction has been described already shortly after quitting.⁴ Rosenberg *et al.*^{5,6} reported that the risk of myocardial infarction declines to a level almost indistinguishable from that of never-smokers within three years. The disease processes involved in the increased cardiovascular risk as well as those explaining the risk reduction after smoking cessation are still under debate. Besides changes in haemostatic factors,⁷ endothelial function⁸ and blood lipids,⁹ alteration of arterial wall structure and function may also play a role.

Indeed, the intima-media layers of large peripheral arteries are thicker in smokers.¹⁰⁻¹⁴ Results concerning the progression of intima-media thickening in smokers are inconclusive¹⁵⁻¹⁷ and depend on the population studied, the presence of concomitant cardiovascular risk factors and the applied measurement protocols.

The effects of smoking on dynamic vessel wall function are variable. Acute smoking is associated with a temporary increase in arterial wall stiffness.¹⁸⁻²⁰ However, the results in chronic smoking are equivocal and vary with the site of measurement.^{19,21,22} Again, differences in study populations with respect to age and concomitant cardiovascular risk, as well as variability in the methods used, seem to be responsible.²²⁻²⁵

There are no prospective longitudinal studies in which the effect of smoking cessation on arterial wall structure and function is investigated. Therefore we investigated the effect of two years of smoking cessation on intima-media thickness and on dynamic vessel wall properties of the common

femoral artery as well as of both carotid arteries. To study the effect of smoking and its cessation exclusively, the study population consisted of subjects free from symptomatic atherosclerotic disease and without further concomitant cardiovascular risk factors.

METHODS

Subjects

Altogether, 367 applicants responded to three advertisements in daily and weekly papers in the surroundings of Nijmegen, a medium-sized city (150,000 inhabitants) in the Netherlands. From this group 127 smokers with the intention to quit smoking during the study (QS), 60 persistent smokers (PS) and 56 nonsmokers (NS) met the inclusion criteria and agreed to participate.

The QS still smoked from the time of inclusion to the first measurement. Smokers smoked at least five cigarettes a day for at least five years. Nonsmokers had either never smoked or had smoked previously, but not in the preceding five years. To be sure that we exclusively studied the effect of smoking, much attention was paid to include only subjects free of symptomatic atherosclerotic disease and without additional cardiovascular risk factors. Therefore, the following criteria for exclusion were applied: 1) demonstrated cardiovascular diseases; 2) irregular heart rhythm disturbances other than sporadic premature beats on electrocardiograms; 3) use of antihypertensives, lipid- or glucose-lowering medications or hormonal medicines including oral contraceptives; 4) absence of cardiovascular risk factors defined as obesity (body mass index $>30 \text{ kg/m}^2$), hypertension (systolic blood pressure exceeding 160 mmHg and/or diastolic blood pressure exceeding 95 mmHg) defined at two separate occasions, diabetes mellitus (history or symptoms of diabetes mellitus or nonfasting plasma glucose $>11.1 \text{ mmol/l}$), hypercholesterolaemia (nonfasting total serum cholesterol $>6.5 \text{ mmol/l}$) or a decreased ankle-arm pressure index (ankle-arm pressure index <0.80).

All participants filled in a questionnaire concerning their health, smoking behaviour and underwent a thorough physical examination to check if inclusion criteria were fulfilled, including a blood survey and electrocardiogram. The Medical Ethics Committee of the University Medical Centre of Nijmegen gave their approval to this study. All participants gave written informed consent.

Study design

The PS and NS were invited twice at an interval of 24 months for measurements of intima-media thickness (IMT) and arterial wall dynamics. The QS were measured before and 3, 6, 12 and 24 months after smoking cessation. The inclusion procedure for all participants as well as all dynamic vessel wall measurements were performed by

the same person (FvdB) to aim at maximal accuracy. To avoid diurnal differences²⁶ subsequent measurements were performed at the same time of day. Subjects were not allowed to smoke or to drink caffeine-containing beverages for one and ten hours prior to investigation, respectively. At each visit participants were asked about their health, smoking behaviour and use of medication. Morning urine samples were collected 3, 6, 12 and 24 months after smoking cessation for cotinine measurements.

Measurement of intima-media thickness

All scans were performed using a Biosound Phase 2 real-time scanner equipped with a 10 MHz transducer according to a validated protocol.^{14,27} IMT can only be determined accurately in the far wall position, because only the far wall IMT is defined by leading edges which enables correct ultrasonographic representation.²⁷⁻²⁹ Therefore, the far walls of the following sites were measured: 1) the distal 1 cm of the straight part of both common carotid arteries; 2) the right and left carotid bulb (from 1 cm proximal to the level of the flow divider); 3) the proximal 1 cm of both internal carotid arteries and 4) the right common femoral artery, 1 cm proximal of the bifurcation into the deep and superficial femoral artery.

All ultrasound scans were performed by three well-trained sonographers who regularly participate in quality control measurement sessions. The inter-sonographer variability that was determined in a group of subjects with normal IMT ranged from 2.5 to 6.4%. The coefficient of variation for subjects with increased IMT varied between 2.5 and 8.2%.²⁷ Images were analysed using a semi-automatic software programme (Eurequa; TSA Company, Meudon, France) as previously described.²⁷ Three measurements were made at each scan site. The average of the measurements was taken. The measurements were calculated by two readers with an inter-reader variability of less than 2%.²⁷

Measurement of dynamic vessel wall properties

Cross-sectional compliance (CC) and distensibility coefficients (DC) of separate arteries were assessed by measuring diameters (D) and diameter changes (ΔD) ultrasonographically using a vessel wall movement detector (Wall Track System[®], Maastricht, the Netherlands) as described by the groups of Hoeks and Reneman.^{30,31}

The system used in this study consisted of an ultrasound device with a 7.5 MHz transducer (Scanner 200, Pie Medical) and a data acquisition system, connected to a personal computer. All measurements were performed by one sonographer (FvdB), according to a protocol validated in our clinic.^{22,32} Tracings were recorded at the following sites: 1) the right and left common carotid arteries (2 cm proximal of the bulb), 2) the right common femoral artery (at least 1 cm proximal of the bifurcation into the deep and superficial femoral artery).

Smoking cessation

Smokers were not allowed to use nicotine replacement therapy during the 24-month cessation period. Smokers who had initially stopped were phoned weekly during the first three months for support. Thereafter they were contacted fortnightly.

Smoking cessation was considered successful when the participant stated being completely abstinent in addition to urinary cotinine concentrations below 300 ng/ml.

Quantitative measurements of metabolites of nicotine in urine

Concentrations of cotinine and 3-hydroxycotinine in urine samples were determined by the commercially available Double Antibody Nicotine Metabolite kit (EURO/DPC Ltd, United Kingdom: quality system ISO 9001/EN29001/BS 5750 part I) using liquid-phase radioimmunoassay.

Subjects with urinary nicotine metabolite concentrations exceeding 300 ng/ml were considered smokers. The cut-off value was determined by EURO/DPC Ltd, United Kingdom.

Data analysis

Statistical differences in baseline characteristics and structural and functional vessel wall variables between groups were analysed using a Mann-Whitney U test.

The effect of smoking on dynamic vessel wall properties and intima-media thickness was analysed firstly by Friedman tests followed by Wilcoxon signed-rank tests. P values less than 0.05 (two-sided) were considered significant.

RESULTS

Initially 127 QS (mean age: 43 ± 8 years) joined the study. Of the 44 subjects (35%) who completed the study, 11 were excluded because of: 1) admitting cigarette use (n=2), 2) urinary cotinine level exceeding 300 ng/ml (n=5), 3) use of oral contraceptives (n=2), 4) use of beta-adrenoceptor antagonists (n=2). Finally, 29% (n=37) of the smoking cessation attempts could be considered successful but 26% (n=33) were evaluable. From the PS and the NS, five and six subjects, respectively, were excluded due to the following reasons: 1) use of hormonal supplementation therapy or oral contraceptives (3 NS), 2) use of cholesterol-lowering medication (2 NS), 3) occurrence of a transient ischaemic attack (1 PS), 4) smoking cessation (1 PS), 5) death from unknown cause (1 PS), 6) lost from follow-up (1 NS and 2 PS).

Demographic data of the initial groups have been described earlier.^{14,22} The baseline characteristics of the eligible subjects are shown in *table 1*. Groups were very similar except for weekly alcohol consumption which was significantly higher in both smoking groups. PS had significantly more pack-years compared with QS (p=0.007). To assess the effect of smoking cessation on IMT and arterial wall stiffness we first compared the changes of these parameters after 24 months between groups. In *table 2* baseline values and the changes of IMT of all investigated arteries are shown.

IMT of both bulbs (right: mean difference 0.23 mm, left: mean difference 0.31 mm), of both internal carotid arteries (right and left: mean difference 0.13 mm) and of the right

Table 1

Baseline characteristics of the groups of smokers who quit (QS; n=33), persisting smokers (PS; n=55) and nonsmokers (NS; n=50) who successfully completed the study.

PARAMETER	GROUP		
	QS (N=33)	PS (N=55)	NS (N=50)
Age (years) [†]	43 ± 8	47 ± 11	46 ± 11
Number of males (%) / females	18 (55) / 15	34 (62) / 21	26 (52) / 24
Alcohol intake (units/week) [*]	15 ± 12 [‡]	15 ± 17 [§]	6 ± 10
Number of pack-years ^{†*}	19 ± 10 [¶]	28 ± 17	
Body mass index at inclusion (kg/m ²) [*]	23.3 ± 2.3	23.0 ± 3.0	23.6 ± 2.5
Body mass index after 24 months (kg/m ²) [*]	25.2 ± 2.5 [¶]	23.6 ± 4.3	24.0 ± 2.6
Systolic blood pressure (mmHg) [*]	127 ± 12	129 ± 14	130 ± 14
Diastolic blood pressure (mmHg) [*]	77 ± 7	79 ± 8	79 ± 8
Heart rate (beats/min) [*]	68 ± 10	70 ± 11	65 ± 11
Serum cholesterol (mmol/l) [*]	5.22 ± 0.93	5.04 ± 0.75	4.87 ± 0.92
Blood glucose (mmol/l) [*]	5.16 ± 0.97	5.18 ± 0.81	4.95 ± 0.85

^{*} Data are presented as mean ± S.D. [†] Calculations are performed with the assumption that one pack contains 25 cigarettes; [‡] p < 0.001, [§] p < 0.01, ^{||} p < 0.05 compared with NS; [¶] p < 0.01 compared with PS (Mann-Whitney U test).

Table 2

Baseline intima-media thickness (IMT) of both carotid arteries and of the right femoral artery and changes in these parameters in 24 months. Data are presented as mean \pm SD.

PARAMETER	GROUP					
	QS (N=33) BASELINE CHANGE IN 24 MONTHS		PS (N=55) BASELINE CHANGE IN 24 MONTHS		NS (N=50) BASELINE CHANGE IN 24 MONTHS	
RCCA: IMT (mm)	0.75 \pm 0.13 (32)	-0.05 \pm 0.12 (32) *	0.77 \pm 0.17 (53)	-0.04 \pm 0.13 (53) *	0.71 \pm 0.12 (48)	0.02 \pm 0.13 (48)
LCCA: IMT (mm)	0.76 \pm 0.15 (31)	-0.04 \pm 0.11 (30)	0.72 \pm 0.17 (54)	-0.01 \pm 0.13 (52)	0.73 \pm 0.13 (50)	-0.01 \pm 0.12 (48)
RB : IMT (mm)	1.03 \pm 0.31 (21) *	-0.10 \pm 0.28 (19)	1.00 \pm 0.54 (44)	-0.10 \pm 0.26 (30)	0.80 \pm 0.29 (38)	-0.06 \pm 0.21 (28)
LB : IMT (mm)	1.05 \pm 0.31 (12) †	-0.06 \pm 0.21 (10)	0.84 \pm 0.34 (37) ‡	-0.04 \pm 0.20 (31)	0.74 \pm 0.26 (34)	-0.04 \pm 0.19 (29)
RICA : IMT (mm)	0.74 \pm 0.24 (28) *	0.004 \pm 0.20 (23)	0.71 \pm 0.23 (44)	-0.04 \pm 0.15 (37)	0.61 \pm 0.19 (43)	-0.04 \pm 0.15 (31)
LICA : IMT (mm)	0.70 \pm 0.18 (15) †	-0.01 \pm 0.13 (14)	0.63 \pm 0.20 (42)	0.03 \pm 0.11 (34)	0.57 \pm 0.20 (35)	0.01 \pm 0.18 (32)
RCFA : IMT (mm)	1.01 \pm 0.24 (29) *	-0.10 \pm 0.23 (24)	1.18 \pm 0.48 (50) †	-0.01 \pm 0.28 (38)	0.88 \pm 0.29 (49)	-0.03 \pm 0.21 (39)

RCCA = right common carotid artery, LCCA = left common carotid artery, RB = right bulb, LB = left bulb, RICA = right internal carotid artery, LICA = left internal carotid artery, RCFA = right common femoral artery; number of successful observations is noted between brackets; * $p < 0.05$, † $p < 0.01$ vs NS; ‡ $p < 0.05$ vs QS (Mann-Whitney U test).

Table 3

Baseline cross-sectional compliance (CC) and distensibility coefficients (DC) of both carotid arteries and of the right femoral artery and changes in these parameters in 24 months. Data are presented as mean \pm SD.

PARAMETER	GROUP					
	QS (N=33) BASELINE CHANGE IN 24 MONTHS		PS (N=55) BASELINE CHANGE IN 24 MONTHS		NS (N=50) BASELINE CHANGE IN 24 MONTHS	
RCCA: CC (mm ² /kPa)	0.70 \pm 0.25 (33)	-0.002 \pm 0.17 (33)	0.69 \pm 0.18 (55)	-0.03 \pm 0.16 (55)	0.72 \pm 0.23 (50)	-0.05 \pm 0.17 (50)
: DC (10 ⁻³ /kPa)	19.06 \pm 7.07 (33)	-0.40 \pm 5.22 (33)	18.67 \pm 5.50 (55)	-0.48 \pm 4.04 (55)	19.85 \pm 6.68 (50)	-0.75 \pm 4.83 (50)
LCCA: CC (mm ² /kPa)	0.64 \pm 0.24 (33)	-0.04 \pm 0.17 (33)	0.64 \pm 0.19 (55)	-0.04 \pm 0.13 (55)	0.62 \pm 0.26 (50)	-0.02 \pm 0.12 (50)
: DC (10 ⁻³ /kPa)	17.66 \pm 6.19 (33)	-1.05 \pm 5.02 (33)	17.55 \pm 6.12 (55)	-0.81 \pm 0.37 (55)	16.98 \pm 6.74 (50)	-0.15 \pm 3.96 (50)
RCFA: CC (mm ² /kPa)	0.71 \pm 0.31 (33)	0.09 \pm 0.35 (33)	0.65 \pm 0.22 (55)	0.06 \pm 0.24 (55)	0.70 \pm 0.35 (48)	0.08 \pm 0.31 (48)
: DC (10 ⁻³ /kPa)	11.23 \pm 4.59 (33)	0.81 \pm 4.82 (33)	10.74 \pm 4.05 (55)	1.08 \pm 4.19 (55)	11.04 \pm 5.27 (48)	1.45 \pm 4.72 (48)

RCCA = right common carotid artery, LCCA = left common carotid artery, RCFA = right common femoral artery; number of successful observations is noted between brackets

common femoral artery (mean difference 0.13 mm) were significantly thicker in QS compared with NS. Similar trends were seen when the PS were compared with the NS, although only significantly different in the right common femoral artery with values of 1.18 \pm 0.48 mm and 0.88 \pm 0.29 mm ($p = 0.001$).

IMT progression was not significantly different between groups except for the far wall of the right common carotid artery. Compared with the NS (in whom the far wall IMT of the right common carotid artery increased by 2.8%), a significant decrease of 6.7 and 5.2% in the IMT of the right common carotid artery was found in the PS ($p = 0.039$) and in the QS ($p = 0.012$), respectively, after 24 months. As shown in table 3, baseline as well as 24-month changes of CC and DC were not different between the three groups. When we compared CC and DC in the three groups taken together, values of the left common carotid artery significantly decreased (fall of 0.03 \pm 0.06 mm²/kPa ($p = 0.064$)

and 0.37 \pm 4.62 10⁻³/kPa, respectively, on the right side and 0.03 \pm 0.14 mm²/kPa ($p = 0.002$) and 0.63 \pm 4.12 10⁻³/kPa ($p = 0.017$) on the left side) after 24 months while at the same time those of the right common femoral artery indicated less arterial stiffening (increase in CC and DC of 0.07 \pm 0.29 mm²/kPa ($p = 0.046$) and 1.15 \pm 4.51 10⁻³/kPa ($p = 0.025$), respectively).

Secondly, we studied IMT, CC and DC changes after 3, 6, 12 and 24 months of smoking cessation in the QS who succeeded in their effort to quit smoking. In figure 1a the percentual changes in IMT (compared with baseline) of the right and left common carotid artery, right and left bulb, right and left internal carotid artery and the right common femoral artery are shown after 3, 6, 12 and 24 months of smoking cessation. Again, there were no significant changes in IMT. There were no significant differences when the results on the right side were compared with the measurements on the left side.

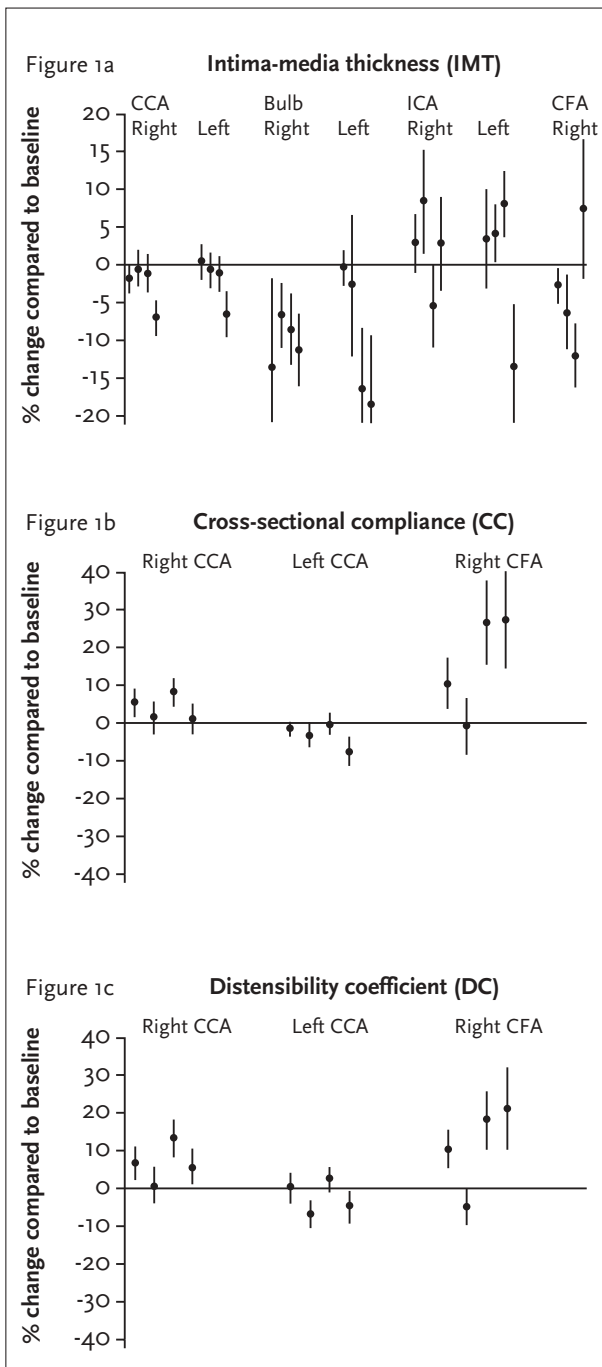


Figure 1 Change in intimal-medial thickness, cross-sectional compliance and distensibility coefficient after successful smoking cessation ($n=33$). IMT is shown for the right and left CCA, right and left bulb, right and left internal carotid artery (ICA) and the right CFA (figure 1a), while CC (figure 1b) and DC (figure 1c) are presented for the right and left common carotid artery (CCA) and for the right common femoral artery (CFA).

The four consecutive lines (each consisting of a vertical line and black dot) under the name of the arterial segment represent the data after 3, 6, 12 and 24 months of smoking cessation, respectively. The black dots represent the mean change with the vertical lines as the standard error.

In figures 1b and 1c the percentual changes after 3, 6, 12 and 24 months of smoking cessation in CC and DC of both common carotid arteries and of the right femoral artery when compared with baseline values are presented. Smoking cessation of a varied duration was not accompanied by significant changes in these parameters.

DISCUSSION

To our surprise, two years of smoking cessation was not accompanied by significant changes in arterial wall structure and in arterial wall function and therefore these changes cannot explain the rapid reduction in cardiovascular risk, as reported in the literature.^{1,3}

This is the first longitudinal long-term study in which the effect of smoking cessation on structural and functional vessel wall properties is prospectively evaluated in a large cohort of QS. Moreover, the cohort consisted of a well-defined population sample in which most confounding factors were conscientiously excluded and smoking cessation was maximally objectified.

Demographic characteristics, IMT and arterial wall dynamics of the initial groups²² did not differ from the data of the reduced QS group (from 127 to 33 subjects) described here. In both the initial and the final QS and PS groups, the weekly alcohol consumption was more than twice that in NS, which reflects different lifestyles which in turn can affect vascular wall properties. The association between smoking and drinking alcohol has been noted before.³³⁻³⁶ IMT was thicker in smokers as reported earlier.¹⁴ Although PS smoked heavier at baseline, IMT was less enlarged in this group compared with the QS. Perhaps latent smoking-related disease was already present in the QS which intentionally motivated this group to stop smoking (all subjects participated voluntarily). Only 'healthy' smokers who did not suffer from cardiovascular disease or cardiovascular risk factors other than smoking were studied to examine the influence of smoking as a single factor on vascular wall properties. The deliberate selection of healthy smokers might provide another explanation of this remarkable finding.

Except for the IMT of the right common carotid artery no significant differences in IMT progression were seen between groups. Right common carotid artery IMT showed significant regression of similar magnitude in both the PS and QS when compared with the NS. As this was an isolated observation which could not be explained properly, we considered it accidental and due to multiple comparisons. Our data are in accordance with a six-year follow-up study performed by Belcaro *et al.*¹⁶ who could not detect increased IMT progression of the carotid bulbs and of the femoral bifurcation segment in persistent smokers of similar age. The percentage of missing values due to measurement

difficulties at the site of the internal carotid arteries and the carotid bulbs were 34 and 59% respectively. Crouse *et al.* reported 37% of missing values when IMT of the internal carotid artery was measured.³⁷ Other studies also encountered the problem of missing values to the same degree as we did the present study.^{27,38}

We included 27 ex-smokers in the nonsmoking group which may have affected outcome. It has been described that former smokers have IMT values intermediate to those measured in smokers and never-smokers.^{13,39} However, comparisons between 27 ex-smokers and the 23 never-smokers in the NS group revealed no difference.

Analysis of measurements performed at variable points of time after smoking cessation in the successfully QS (n=33) yielded similar findings. Although percentual changes compared to baseline were negative in the common carotid arteries and in the bulbs on both sides (shown in *figure 1a*), no significant differences nor trends were found. As we performed quantitative measurements (thickness) to describe arterial wall structure, changes in qualitative parameters (plaque morphology), which are difficult to quantify, could still be involved in the decreased cardiovascular risk in smokers.

We found that dynamic vessel wall properties of carotid and femoral arteries were not affected by chronic smoking, which is in accordance with one other study.¹⁹ However, as the arterial tree is heterogeneous no conclusions concerning other arteries can be drawn.

Age has a major influence on CC and DC of the elastic common carotid arteries but not on CC and DC of the muscular femoral arteries.⁴⁰ Indeed, two years of aging was associated with significant decreases in CC and DC of the carotid arteries, while CC and DC of the femoral artery increased during the same time period. A possible explanation for the significant increase in femoral arterial wall dynamics (pointing to less stiffening) might be an improvement in lifestyle factors, which are known to affect functional femoral arterial wall properties.^{41,42} Participation in lifestyle intervention studies such as the present one inevitably attracts people who care about their health. Possible positive changes in our study may have been counteracted by the significant increase in body weight after smoking cessation.^{22,43,44}

Studies in which the effect of weight on dynamic vessel wall properties was evaluated yielded conflicting results.^{45,46} Yet, it is difficult to believe that a minor weight gain (8%) caused a complete attenuation of the positive effects of smoking cessation.

In conclusion, although IMT is thickened in chronic smokers no difference in IMT progression could be shown after two years of smoking cessation when compared with results in persistent smokers and in nonsmokers. Furthermore, neither chronic smoking nor two years of smoking cessation has an effect on arterial wall stiffness in a

carefully selected population of smokers without additional cardiovascular risk factors. Therefore, the improvement in arterial vessel wall structure and function does not explain the rapid cardiovascular risk reduction after smoking cessation. The risk reduction should, more likely, to be ascribed to haemostatic and/or endothelial factors.

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REFERENCES

1. Doll R, Peto R, Wheatley K, Gray R, Sutherland I. Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ* 1994;309:901-11.
2. Freund KM, Belanger AJ, D'Agostino RB, Kannel WB. The health risks of smoking. The Framingham Study: 34 years of follow-up. *Ann Epidemiol* 1993;3:417-24.
3. Wolf PA, D'Agostino RB, Kannel WB, Bonita R, Belanger AJ. Cigarette smoking as a risk factor for stroke. The Framingham Study. *JAMA* 1988;259:1025-9.
4. Samet JM. The health benefits of smoking cessation. *Med Clin North Am* 1992;76:399-414.
5. Rosenberg L, Kaufman DW, Helmrich SP, Shapiro S. The risk of myocardial infarction after quitting smoking in men under 55 years of age. *N Engl J Med* 1985;313:1511-4.
6. Rosenberg L, Palmer JR, Shapiro S. Decline in the risk of myocardial infarction among women who stop smoking. *N Engl J Med* 1990;322:213-7.
7. Simpson AJ, Gray RS, Moore NR, Booth NA. The effects of chronic smoking on the fibrinolytic potential of plasma and platelets. *Br J Haematol* 1997;97:208-13.
8. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993;88:2149-55.
9. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. *BMJ* 1989;298:784-4.
10. Howard G, Burke GL, Szklo M, et al. Active and passive smoking are associated with increased carotid wall thickness. The Atherosclerosis Risk in Communities Study. *Arch Intern Med* 1994;154:1277-82.
11. Tell GS, Polak JF, Ward BJ, Kittner SJ, Savage PJ, Robbins J. Relation of smoking with carotid artery wall thickness and stenosis in older adults. The Cardiovascular Health Study. The Cardiovascular Health Study (CHS) Collaborative Research Group. *Circulation* 1994;90:2905-8.
12. Smilde TJ, Berkmortel FWPJ van den, Boers GJ, et al. Carotid and femoral artery wall thickness and stiffness in patients at risk for cardiovascular disease, with special emphasis on hyperhomocysteinemia. *Arterioscler Thromb Vasc Biol* 1998;12:1958-63.

13. Diez-Roux AV, Nieto FJ, Comstock GW, Howard G, Szklo M. The relationship of active and passive smoking to carotid atherosclerosis 12-14 years later. *Prev Med* 1995;24:48-55.
14. Berkmortel FWJ van den, Smilde TJ, Wollersheim H, Langen H van, Boo T de, Thien T. Intima-media thickness of peripheral arteries in asymptomatic cigarette smokers. *Atherosclerosis* 2000;250:397-401.
15. Salonen R, Salonen JT. Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. *Atherosclerosis* 1990;81:33-40.
16. Belcaro G, Laurora G, Cesarone MR, De Sanctis MT, Incandela L, Barsotti A. Progression of subclinical atherosclerosis in 6 years. Ultrasound evaluation of the average, combined femoral and carotid bifurcation intima-media thickness. *Vasa* 1995;24:227-32.
17. Howard G, Wagenknecht LE, Burke GL, et al. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. *JAMA* 1998;279:119-24.
18. Giannattasio C, Mangoni AA, Stella ML, Carugo S, Grassi G, Mancia G. Acute effects of smoking on radial artery compliance in humans. *J Hypertens* 1994;12:691-6.
19. Kool MJ, Hoeks AP, Struijker Boudier HA, Reneman RS, Bortel LM van. Short- and long-term effects of smoking on arterial wall properties in habitual smokers. *J Am Coll Cardiol* 1993;22:1881-6.
20. Failla M, Grappiolo A, Carugo S, Calchera I, Giannattasio C, Mancia G. Effects of cigarette smoking on carotid and radial artery distensibility. *J Hypertens* 1997;15:1659-64.
21. Wollersheim H, Firestone G, Fronck A. Selective increase in popliteal artery wall stiffness in long-term smokers. *J Hypertens* 1993;11(Suppl 5):S84-5.
22. Berkmortel FWJ van den, Wollersheim H, Langen H van, Boo T de, Thien T. Dynamic vessel wall properties of large conduit arteries in habitual cigarette smokers. *Eur J Int Med* 1999;10:159-65.
23. Sonesson B, Ahlgren AR, Lazer L, Lanne T. Does long-term smoking affect aortic stiffness more in women than in men? *Clin Physiol* 1997;17:439-47.
24. Jonason T, Henrikssen E, Kangro T, Nilsson H, Vessby B, Ringqvist I. Stiffness of the common carotid artery in healthy 50-year-old subjects. *Clin Physiol* 1997;17:569-77.
25. Taquet A, Bonithon Kopp C, Simon A, et al. Relations of cardiovascular risk factors to aortic pulse wave velocity in asymptomatic middle-aged women. *Eur J Epidemiol* 1993;9:298-306.
26. Kool MJ, Wijnen JA, Hoeks AP, Struyker Boudier HA, Bortel LM van. Diurnal pattern of vessel-wall properties of large arteries in healthy men. *J Hypertens* 1991;9(Suppl 6):S108-9.
27. Smilde TJ, Wollersheim H, Langen H van, Stalenhoef AF. Reproducibility of ultrasonographic measurements of different carotid and femoral artery segments in healthy subjects and in patients with increased intima-media thickness. *Clin Sci* 1997;93:317-24.
28. Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand J. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol* 1991;11:565-77.
29. Wong M, Edelstein J, Wollman J, Bond MG. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. *Arterioscler Thromb* 1993;13:482-6.
30. Reneman RS, Merode T van, Hick P, Hoeks AP. Cardiovascular applications of multi-gate pulsed Doppler systems. *Ultrasound Med Biol* 1986;12:357-70.
31. Hoeks AP, Brands PJ, Smeets FA, Reneman RS. Assessment of the distensibility of superficial arteries. *Ultrasound Med Biol* 1990;16:121-8.
32. Berkmortel F van den, Wollersheim H, Langen H van, Thien T. Dynamic vessel wall properties and their reproducibility in subjects with increased cardiovascular risk. *J Hum Hypertens* 1998;12:345-50.
33. Thornton A, Lee P, Fry J. Differences between smokers, ex-smokers, passive smokers and non-smokers. *J Clin Epidemiol* 1994;47:1143-62.
34. Margetts BM, Jackson AA. Interactions between people's diet and their smoking habits: the dietary and nutritional survey of British adults. *BMJ* 1993;307:1381-4.
35. Preston AM. Cigarette smoking-nutritional implications. *Prog Food Nutr Sci* 1991;15:183-217.
36. Knoflach M, Kiechl S, Kind M, et al. Cardiovascular risk factors and atherosclerosis in young males: ARMY study (Atherosclerosis Risk Factors in Male Youngsters). *Circulation* 2003;108:1064-9.
37. Crouse JR, Byington RP, Bond MG, et al. Pravastatin, lipids, and atherosclerosis in the carotid arteries: design features of a clinical trial with carotid atherosclerosis outcome. *Control Clin Trials* 1992;13:495-506.
38. Salonen JT, Salonen R. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. *Arterioscler Thromb* 1991;11:1245-9.
39. Tell GS, Howard G, McKinney WM, Toole JF. Cigarette smoking cessation and extracranial carotid atherosclerosis. *JAMA* 1989;261:1178-80.
40. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME. Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb* 1993;13:90-7.
41. Kool MJ, Struijker Boudier HA, Wijnen JA, Hoeks AP, Bortel LM van. Effects of diurnal variability and exercise training on properties of large arteries. *J Hypertens* 1992;10(Suppl 6):S49-52.
42. Cameron JD, Dart AD. Exercise training increases total systemic arterial compliance in humans. *Am J Physiol* 1994;266:H693-701.
43. Williamson DF, Madans J, Anda RF, Kleinman JC, Giovino GA, Byers T. Smoking cessation and severity of weight gain in a national cohort. *N Engl J Med* 1991;324:739-45.
44. Mizoue T, Ueda R, Tokui N, Hino Y, Yoshimura T. Body mass decrease after initial gain following smoking cessation. *Int J Epidemiol* 1998;27:984-8.
45. Mangoni AA, Giannattasio C, Brunani A, et al. Radial artery compliance in young, obese, normotensive subjects. *Hypertension* 1995;26:984-8.
46. Toto Moukoko JJ, Achimastos A, Asmar RG, Hugues CJ, Safar ME. Pulse wave velocity in patients with obesity and hypertension. *Am Heart J* 1986;112:136-40.