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 intimal-medial 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. A considerable risk reduction has been described already shortly after quitting. Rosenberg et al. 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, endothelial function and blood lipids, 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. Results concerning the progression of intima-media thickening in smokers are inconclusive 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. However, the results in chronic smoking are equivocal and vary with the site of measurement. 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.

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 advertise-
ments 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 per-
sistent smokers (PS) and 36 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 kg/m²), 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 dia-
betes mellitus or nonfasting plasma glucose >11.1 mmol/l),
hypercholesterolaemia (nonfasting total serum cholesterol
>6.5 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 differences subsequently 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. MIIMT 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. 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 super-
ficial 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%. Images
were analysed using a semi-automatic software
programme (Eurequa; TSA Company, Meudon, France) as
previously described. 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%.  

Measurement of dynamic vessel wall properties
Cross-sectional compliance (CC) and distensibility coeffi-
cients (DC) of separate arteries were assessed by measuring
diameters (D) and diameter changes (ΔD) ultrasono-
graphically using a vessel wall movement detector (Wall
Track System®, Maastricht, the Netherlands) as described
by the groups of Hoeks and Reneman. 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. 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 suppletion 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.21,22 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>
<thead>
<tr>
<th>GROUP</th>
<th>QS (N=33)</th>
<th>PS (N=55)</th>
<th>NS (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)†</td>
<td>43 ± 8</td>
<td>47 ± 11</td>
<td>46 ± 11</td>
</tr>
<tr>
<td>Number of males (%)</td>
<td>18 (55)/15</td>
<td>34 (62)/21</td>
<td>26 (52)/24</td>
</tr>
<tr>
<td>Alcohol intake (units/week)</td>
<td>15 ± 12*</td>
<td>15 ± 17*</td>
<td>6 ± 10</td>
</tr>
<tr>
<td>Number of pack-years*</td>
<td>19 ± 10*</td>
<td>28 ± 17</td>
<td></td>
</tr>
<tr>
<td>Body mass index at inclusion (kg/m²)</td>
<td>23.3 ± 2.3</td>
<td>23.0 ± 3.0</td>
<td>23.6 ± 2.5</td>
</tr>
<tr>
<td>Body mass index after 24 months (kg/m²)</td>
<td>25.2 ± 2.5*</td>
<td>23.6 ± 4.3</td>
<td>24.0 ± 2.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 ± 12</td>
<td>129 ± 14</td>
<td>130 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 ± 7</td>
<td>79 ± 8</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>68 ± 10</td>
<td>70 ± 11</td>
<td>65 ± 11</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.22 ± 0.93</td>
<td>5.04 ± 0.75</td>
<td>4.87 ± 0.92</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>5.16 ± 0.97</td>
<td>5.18 ± 0.81</td>
<td>4.95 ± 0.85</td>
</tr>
</tbody>
</table>

* 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 ± SD.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PARAMETER</th>
<th>QS (N=33) BASELINE CHANGE IN 24 MONTHS</th>
<th>PS (N=55) BASELINE CHANGE IN 24 MONTHS</th>
<th>NS (N=50) BASELINE CHANGE IN 24 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCCA: IMT (mm)</td>
<td>0.73 ± 0.13 (32) -0.05 ± 0.12 (32)</td>
<td>0.77 ± 0.17 (53) -0.04 ± 0.13 (53)</td>
<td>0.71 ± 0.12 (48) -0.02 ± 0.13 (48)</td>
<td></td>
</tr>
<tr>
<td>LCCA: IMT (mm)</td>
<td>0.70 ± 0.15 (31) -0.04 ± 0.11 (31)</td>
<td>0.72 ± 0.17 (54) -0.01 ± 0.13 (52)</td>
<td>0.73 ± 0.13 (50) -0.01 ± 0.12 (48)</td>
<td></td>
</tr>
<tr>
<td>RB : IMT (mm)</td>
<td>1.01 ± 0.31 (21) † -0.10 ± 0.28 (19)</td>
<td>1.00 ± 0.54 (44) -0.10 ± 0.26 (10)</td>
<td>0.82 ± 0.29 (38) -0.06 ± 0.21 (28)</td>
<td></td>
</tr>
<tr>
<td>LB : IMT (mm)</td>
<td>1.05 ± 0.31 (12) † -0.06 ± 0.21 (10)</td>
<td>0.84 ± 0.34 (57) ‡ -0.04 ± 0.20 (31)</td>
<td>0.74 ± 0.26 (34) -0.04 ± 0.19 (29)</td>
<td></td>
</tr>
<tr>
<td>RICA : IMT (mm)</td>
<td>0.74 ± 0.24 (28) † 0.004 ± 0.20 (23)</td>
<td>0.71 ± 0.23 (44) -0.04 ± 0.13 (17)</td>
<td>0.61 ± 0.19 (43) -0.04 ± 0.15 (11)</td>
<td></td>
</tr>
<tr>
<td>LICA : IMT (mm)</td>
<td>0.70 ± 0.18 (15) † -0.01 ± 0.13 (14)</td>
<td>0.63 ± 0.20 (42) -0.03 ± 0.11 (14)</td>
<td>0.57 ± 0.20 (35) 0.01 ± 0.18 (12)</td>
<td></td>
</tr>
<tr>
<td>RCFA : IMT (mm)</td>
<td>1.01 ± 0.24 (29) † -0.10 ± 0.23 (24)</td>
<td>1.18 ± 0.48 (50) † -0.01 ± 0.28 (18)</td>
<td>0.88 ± 0.29 (49) -0.03 ± 0.21 (39)</td>
<td></td>
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</tbody>
</table>

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 ± SD.

<table>
<thead>
<tr>
<th>GROUP</th>
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<th>QS (N=33) BASELINE CHANGE IN 24 MONTHS</th>
<th>PS (N=55) BASELINE CHANGE IN 24 MONTHS</th>
<th>NS (N=50) BASELINE CHANGE IN 24 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCCA: CC (mm²/kPa)</td>
<td>0.70 ± 0.25 (33) -0.002 ± 0.17 (33)</td>
<td>0.69 ± 0.18 (55) -0.03 ± 0.16 (55)</td>
<td>0.72 ± 0.23 (50) -0.05 ± 0.17 (50)</td>
<td></td>
</tr>
<tr>
<td>DC (10⁻³/kPa)</td>
<td>19.06 ± 7.07 (33) -0.40 ± 5.22 (33)</td>
<td>18.67 ± 5.50 (55) -0.48 ± 4.04 (55)</td>
<td>19.85 ± 6.88 (50) -0.75 ± 4.83 (50)</td>
<td></td>
</tr>
<tr>
<td>LCCA: CC (mm²/kPa)</td>
<td>0.64 ± 0.24 (33) -0.04 ± 0.17 (33)</td>
<td>0.64 ± 0.19 (55) -0.04 ± 0.13 (55)</td>
<td>0.62 ± 0.26 (50) -0.02 ± 0.12 (50)</td>
<td></td>
</tr>
<tr>
<td>DC (10⁻³/kPa)</td>
<td>17.66 ± 6.19 (33) -1.05 ± 5.02 (33)</td>
<td>17.55 ± 6.12 (55) -0.81 ± 0.37 (55)</td>
<td>16.98 ± 6.74 (50) -0.15 ± 3.96 (50)</td>
<td></td>
</tr>
<tr>
<td>RCFA: CC (mm²/kPa)</td>
<td>0.71 ± 0.31 (36) -0.09 ± 0.35 (36)</td>
<td>0.65 ± 0.22 (55) -0.06 ± 0.24 (55)</td>
<td>0.70 ± 0.35 (48) 0.08 ± 0.31 (48)</td>
<td></td>
</tr>
<tr>
<td>DC (10⁻³/kPa)</td>
<td>11.23 ± 4.59 (36) 0.91 ± 4.82 (36)</td>
<td>10.74 ± 4.05 (55) 1.08 ± 4.19 (55)</td>
<td>11.04 ± 5.27 (48) 1.45 ± 4.72 (48)</td>
<td></td>
</tr>
</tbody>
</table>

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 ± 0.48 mm and 0.88 ± 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 ± 0.06 mm²/kPa (p=0.064) and 0.37 ± 4.62 10⁻³ kPa, respectively, on the right side and 0.03 ± 0.14 mm²/kPa (p=0.002) and 0.63 ± 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 ± 0.29 mm²/kPa (p=0.046) and 1.15 ± 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 1 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.
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. 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 groups 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. 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. 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 50% respectively. Crouse et al. reported 37% of missing values when IMT of the internal carotid artery was measured. Other studies also encountered the problem of missing values to the same degree as we did the present study. 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. 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. 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. 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. 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. Studies in which the effect of weight on dynamic vessel wall properties was evaluated yielded conflicting results. 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.

ACKNOWLEDGEMENTS

This research project was financially supported by grant NHS 94.035 from the Dutch Heart Foundation, the Netherlands.

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