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# **Atorvastatin Normalizes Endothelial Function in Healthy Smokers**

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## ABSTRACT

Endothelial function predicts cardiovascular disease. The aim of this study was to examine whether endothelial function in smokers with normal cholesterol levels could be normalized by treatment with 80 mg atorvastatin in comparison with a control group. Healthy smokers (n=20) and non-smokers (n=20) were randomized to 80 mg atorvastatin or to placebo in an open, randomized cross-over study. Endothelial functional (Flow-mediated dilatation; FMD) was measured. At baseline smokers had a lower FMD compared to the non-smoking group,  $2.2 \% \pm 0.5$  and  $4.5 \% \pm 0.9$ , respectively ( $p < 0.05$ ). In the smoker group FMD increased to  $4.0 \% \pm 0.8$  during treatment with atorvastatin ( $p < 0.05$ ) and returned to basal level during placebo,  $2.3 \% \pm 0.6$ . In the non-smoking group FMD was unaffected by both atorvastatin and placebo. The net change of total cholesterol or LDL cholesterol was not associated with the net change of FMD when the group was considered as a whole or the smoking group was considered separately. In conclusion, an improved endothelial function was observed in a group of smokers, when they were on 80 mg atorvastatin, whereas atorvastatin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the LDL decrease during atorvastatin treatment. The results show that poor endothelial function in smokers with normal lipid levels can be improved by a statin treatment.

## INTRODUCTION

Both primary and secondary prevention studies have demonstrated that statin treatment reduces cardiovascular end points [1-3]. There are several ultrasound and angiographic studies showing that a lipid-lowering treatment is associated with a reduction of the intima-media thickness or improvement of the lumen area compared with placebo [4-6]. However, the clinical benefit of the drugs used in the clinical studies [3] was manifest early in the course of lipid-lowering therapy before regression of atherosclerosis could occur. Therefore, other mechanisms than cholesterol-lowering may be responsible for the positive outcome after treatment with statins. Modifications of endothelial function, inflammatory responses and plaque stability have been suggested as possible mechanisms.

Impaired endothelium-dependent relaxation has been observed in subjects with atherosclerosis [7]. Moreover, studies have demonstrated that the presence of cardiovascular risk factors such as cigarette smoking [8] and hypercholesterolaemia [9-11] is associated with endothelial dysfunction. Furthermore, cholesterol lowering has been shown to improve the endothelial function [9-11]. In one study an improvement of the endothelial function after 1 month on simvastatin treatment was demonstrated in hypercholesterolemic subject [10], whereas most other studies have reported an improvement after 6 months or more [9,11]. However, there are also studies failing to show a positive effect on endothelial function with statin treatment in certain populations [12-13].

The underlying pathophysiological explanation behind the improved endothelial function after treatment with statins in subjects with hypercholesterolaemia is not known. However, it has been observed that statins activate endothelial nitric oxide synthase independent of its cholesterol-lowering actions and this has been suggested to be a possible explanation for the

positive outcome after treatment with statins [14-15]. It is unclear if different cardiovascular risk factors, mediate endothelial dysfunction via the same pathophysiological pathway. Recently, it has been shown that pretreatment with vitamin C attenuates the endothelial dysfunction after acute cigarette smoking [16]. Cigarette smoking is also associated with increased monocyte-endothelial cell adhesion and in a study, L-arginine (the physiologic substrate for nitric oxide), but not antioxidants reversed that abnormality [17]. Thus, there is a possibility that an agent, such as a statin, which activates nitrate oxide formation, may improve the endothelial function in smokers, independent of a cholesterol-lowering effect.

The aim of this study was to examine whether endothelial function in smokers with normal cholesterol levels could be normalized by treatment with 80 mg atorvastatin in comparison with a non-smoking group.

## **METHODS**

### *Subjects*

Healthy smokers (> 10 cigarettes daily), under the age of 40 were recruited from the department and by advertisement (n=20). An aged matched non-smoking group (n=20) was recruited in the same way. Exclusion criteria: signs of cardiovascular disease, pharmacological treatment, diabetes mellitus and pregnancy. Contraception was necessary for included women. Snuff and nicotine replacement treatment were not allowed. All subjects gave informed consent after written and oral information. The ethics committee of the Karolinska Institute at Huddinge University Hospital approved the study.

## *Design*

An open, randomized cross-over study. The subjects were, after an initial physical examination, examined with a flow-mediated dilatation examination (FMD) and randomized to active treatment with 80 mg atorvastatin daily or to no treatment. The non-smoking group underwent the same procedure. After 4 weeks each subject came back for a second examination of the endothelial function. There was a cross-over between the treatment arms and the subjects were re-examined after another 4 weeks with a last examination of the endothelial function. See flow-chart in Figure 1.

## *Measurements*

Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, plasma-glucose, Hb, CK, ASAT, ALAT) were controlled at baseline, 4 and 8 weeks. Resting blood pressure was measured in the right arm after 10 min supine rest. Smoking was assessed by a questionnaire. No subject had any side effect of the drug or elevated CK, ASAT or ALAT.

### *Flow mediated dilatation (FMD) and intima-media thickness of the brachial artery*

The ultrasound procedures for assessing endothelium-dependent FMD were performed as previously described [18-19]. The subjects were examined in the morning after fasting since midnight. A high-resolution ultrasound scanner (System Five, GE Vingmed, Horten, Norway) with a 10.0-MHz linear array transducer was used. After 10-minute equilibration period at rest in the recumbent position, a single dedicated ultrasonographer performed measurements of the left brachial artery FMD. Scans of the brachial artery were taken proximal to the antecubital fossa and saved on videotape. The transducer was fixed with a stand during the FMD examinations. Baseline diameter recordings were obtained after which arterial occlusion was performed by inflating a forearm blood pressure cuff (12,5 cm wide) to

250 mmHg for 4.5 min. After cuff-release, diameter recordings were repeated during the post-occlusive increase in brachial artery blood flow. Arterial flow velocity recordings were obtained using pulsed wave Doppler at 60 ° to the vessel with the sample volume (1.5 mm) in the centre of the artery. The complete experimental sequence was performed twice at 30 min interval.

Images were digitally acquired from the videotape and measured in random order by a single observer blinded to the conditions under which the ultrasonic images were obtained.

Measurement of the brachial artery diameter was defined as a distance from the leading edge of the near wall intima-lumen echo to the leading edge of the far wall lumen-intima echo along a line perpendicular to the artery's long axis. A computer system [20] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 5 mm long section of the brachial artery was used. Brachial artery diameter was calculated in diastolic frames taken coincidentally with the R wave on the electrocardiogram twice at rest and then 45, 60 and 75 seconds after cuff deflation. A mean of the diameters after 45, 60 and 75 seconds was calculated. Diameter changes were expressed as the percentage change relative to the mean baseline value. The mean of two FMD examinations was used.

The intima-media thickness was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo.

### **Statistical analysis**

Descriptive measures are reported as means and standard deviations of the mean.

Experimental measures are reported as means and SE of the mean. Changes within a study group were compared with a paired t test, whereas comparisons between the study groups were made using an unpaired t test. Pearson's correlation coefficients were calculated. In a

clinical trial using this technique a mean improvement in flow mediated dilatation of at least 2 % would usually be required to detect a treatment benefit. In a cross-over trial design, with 80 % power and 95 % confidence interval, a less than 1.5 % change in flow-mediated dilatation will be detected with 20 subjects [21]. With the technique in our hands the coefficient of variation is 2 % and a significant enhancement of 1.7 % induced by a treatment was detected in a crossover design in 12 subjects [19].

## RESULTS

Baseline characteristics of all individuals enrolled are shown in Table 1. The only observed difference between the study groups was a higher HDL cholesterol in non-smoking subjects compared to the smokers ( $p < 0.05$ ). The ultrasound data at baseline is given in Table 2. The lumen and IMT of the brachial artery did not differ between the study groups, whereas smokers had a lower FMD compared to the non-smoking group,  $2.2 \% \pm 0.5$  and  $4.5 \% \pm 0.8$ , respectively ( $p < 0.05$ ).

In Figure 2 the effect of 80 mg atorvastatin on total and LDL cholesterol is presented. Total cholesterol decreased from  $4.2 \pm 0.4$  mmol/l to  $2.6 \pm 0.6$  mmol/l in the smoking group during treatment with 80 mg atorvastatin ( $p < 0.05$ ), whereas the corresponding levels in the non-smoking group was  $4.4 \pm 0.7$  mmol/l and  $2.7 \pm 0.6$  mmol/l ( $p < 0.05$ ). LDL cholesterol decreased from  $2.4 \pm 0.5$  mmol/l to  $1.0 \pm 0.5$  mmol/l in the smoking group during treatment with 80 mg atorvastatin ( $p < 0.05$ ). In the non-smoking group LDL cholesterol was  $2.4 \pm 0.6$  mmol/l at baseline and  $1.0 \pm 0.4$  mmol/l during atorvastatin treatment ( $p < 0.05$ ).

None of the measured lipid levels during the placebo phase differed from the baseline values. The triglyceride level decreased from  $1.0 \pm 0.4$  mmol/l at baseline to  $0.6 \pm 0.2$  mmol/l during atorvastatin treatment in the non-smoking group ( $p < 0.05$ ) (Figure 3).



During the open-labelled placebo all lipid levels return to the basal levels in both study groups.

The FMD measurements are presented in Table 1 and in Figure 4. In the smoker group FMD was  $2.2 \% \pm 0.5$  at baseline. FMD increased to  $4.0 \% \pm 0.8$  during treatment with atorvastatin ( $p < 0.05$ ) and returned to basal level during placebo,  $2.3 \% \pm 0.6$ . In the non-smoking group FMD was unaffected by both atorvastatin and placebo. Thus, FMD increased 1.8 % in the smoker group during atorvastatin treatment whereas

The net change of total cholesterol or LDL cholesterol was not correlated with the net change of FMD when the group was considered as a whole or the smoking group was considered separately.

## DISCUSSION

In this study we confirm that cigarette smokers have a worse flow-mediated dilatation compared to an aged matched non-smoking group. In the smoking group, endothelial function was improved to the same level as in a non-smoking group, when they were on 80 mg atorvastatin, whereas atorvastatin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the LDL decrease during atorvastatin treatment.

Despite a clearly low FMD, the intima media-thickness of the brachial artery was not enlarged in the smoker group. It is more common to use intima-media thickness of the common carotid artery as a surrogate measure for atherosclerosis, however we have

previously reported that the brachial artery intima media thickness is significantly associated with the artery intima media thickness in the common carotid artery [18]. In a recent autopsy study Sorensen et al [22] the grade of lesion severity in the brachial and the left anterior descending coronary artery was significantly correlated and so was the severity in the brachial and carotid arteries. Thus, the finding in that autopsy study supports that also intima media thickness of the brachial artery may serve as a surrogate variable when studying atherosclerosis. However it is important to know that it is not possible to differentiate between intimal and medial thickening with ultrasound technique. Wiesmann et al [23] found, using high-resolution MRI, a poor central vascular distensibility in both the common carotid arteries and at multiple sites in the aorta in a group of smokers compared to non-smokers. The discordance between this study and the study by Wiesmann is probably explained by different examination methods and examination of different vessel beds.

The underlying mechanism why cigarette smoking deteriorates endothelial function is not completely known. FMD gives us an indirect measure of NO bioavailability in the brachial artery. Since also snuff and nicotine spray [24-25] decrease FMD, nicotine has been suggested to be responsible for this action. However, because there are numerous known and unknown components of cigarette smoke whose metabolic fate in the human body is unknown, an appropriate in vitro model of cigarette smoking exposure remains to be established. Previous reports suggest statins activate endothelial nitric oxide synthase [14-15] and our observation of an improved FMD in the smoker group only, might be explained by a deactivated NO synthase among smokers, which returns back to a normal activation level during statin treatment, whereas the NO synthase activity among non-smokers already is activated and therefore the statin treatment has no effect. In the present study we found no relationship between improvement of FMD in the smoking group and the improved lipid

profile. This observation supports the hypothesis that statins activate endothelial nitric oxide synthase independent of the cholesterol-lowering actions.

Other studies have suggested damage of NO by free radicals [26] as the potential mechanisms through which smoking impairs endothelial function. This theory was supported by an observation by Papamichael et al [27], who found that simultaneous consumption of red wine when smoking, partly decreased the harmful acute effect of smoking through its effect on FMD. Furthermore, treatment with vitamin C attenuates the endothelial dysfunction after acute cigarette smoking [16].

We found a lower HDL cholesterol in the smoking group, which is a known phenomena since before [28]. The mechanisms responsible are not clearly elucidated, and the role of dietary differences between smokers and non-smokers is unknown. As expected we observed dramatic decrease of total and HDL cholesterol in both study groups but the improved FMD level in the smoking group was independent of these lipid level changes.

In this study FMD was used as a surrogate variable and one can question the relevance of this observation. However, we know from numerous studies that FMD is associated with cardiovascular risk factors including cigarette smoking [8-11] and we know that FMD predicts a poor outcome in different patient groups [29-30]. Our results are supported in a previous study [31]. The results may suggest the cardiovascular risk in smokers with normal lipid levels can be reduced by a statin treatment. However, to confirm that information a large study with hard end-points is needed.

In summary, we observed an improved endothelial function in a group of smokers, when they were on 80 mg atorvastatin, whereas atorvastatin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the LDL decrease during atorvastatin treatment.

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**Table 1.** Baseline demographics

| <b>Parameters</b>          | <b>Smokers (n=20)</b> | <b>Non-smokers (n=20)</b> |
|----------------------------|-----------------------|---------------------------|
| Age (year)                 | 30 ± 6                | 30 ± 5                    |
| Blood pressure (mm Hg)     | 119 / 71 ± 15 / 9     | 116 / 72 ± 12 / 7         |
| Total cholesterol (mmol/L) | 4.2 ± 0.4             | 4.4 ± 0.6                 |
| LDL cholesterol (mmol/L)   | 2.4 ± 0.5             | 2.4 ± 0.6                 |
| HDL cholesterol (mmol/L)   | 1.3 ± 0.3             | 1.6 ± 0.4 *               |
| Triglycerides (mmol/L)     | 1.1 ± 0.6             | 1.0 ± 0.5                 |
| Glucose (mmol/L)           | 4.9 ± 0.3             | 4.7 ± 0.3                 |

Values are means ±SD.

\* p<0.05

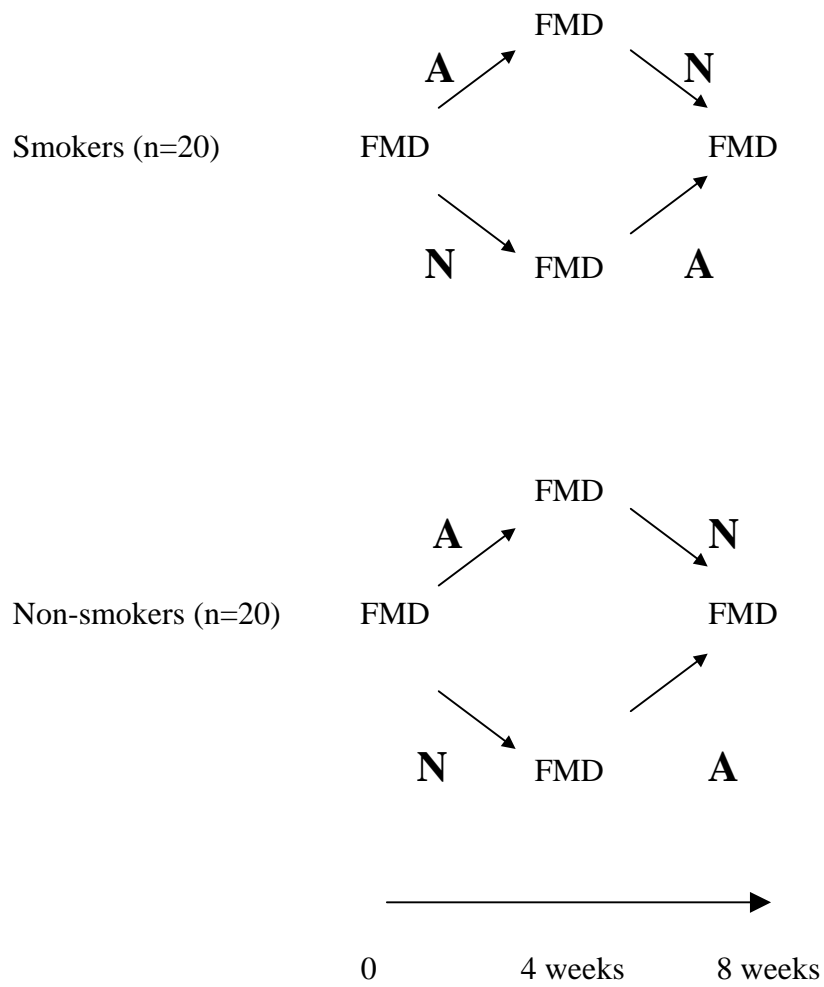
**Table 2.** Baseline ultrasound data of the subjects

| <b>Brachial examination</b>  | <b>Smokers (n=20)</b> | <b>Non-smokers (n=20)</b> |
|------------------------------|-----------------------|---------------------------|
| Intima-media thickness, (mm) | 0.24 $\pm$ 0.004      | 0.25 $\pm$ 0.007          |
| Lumen diameter (mm)          | 3.6 $\pm$ 0.09        | 3.5 $\pm$ 0.13            |

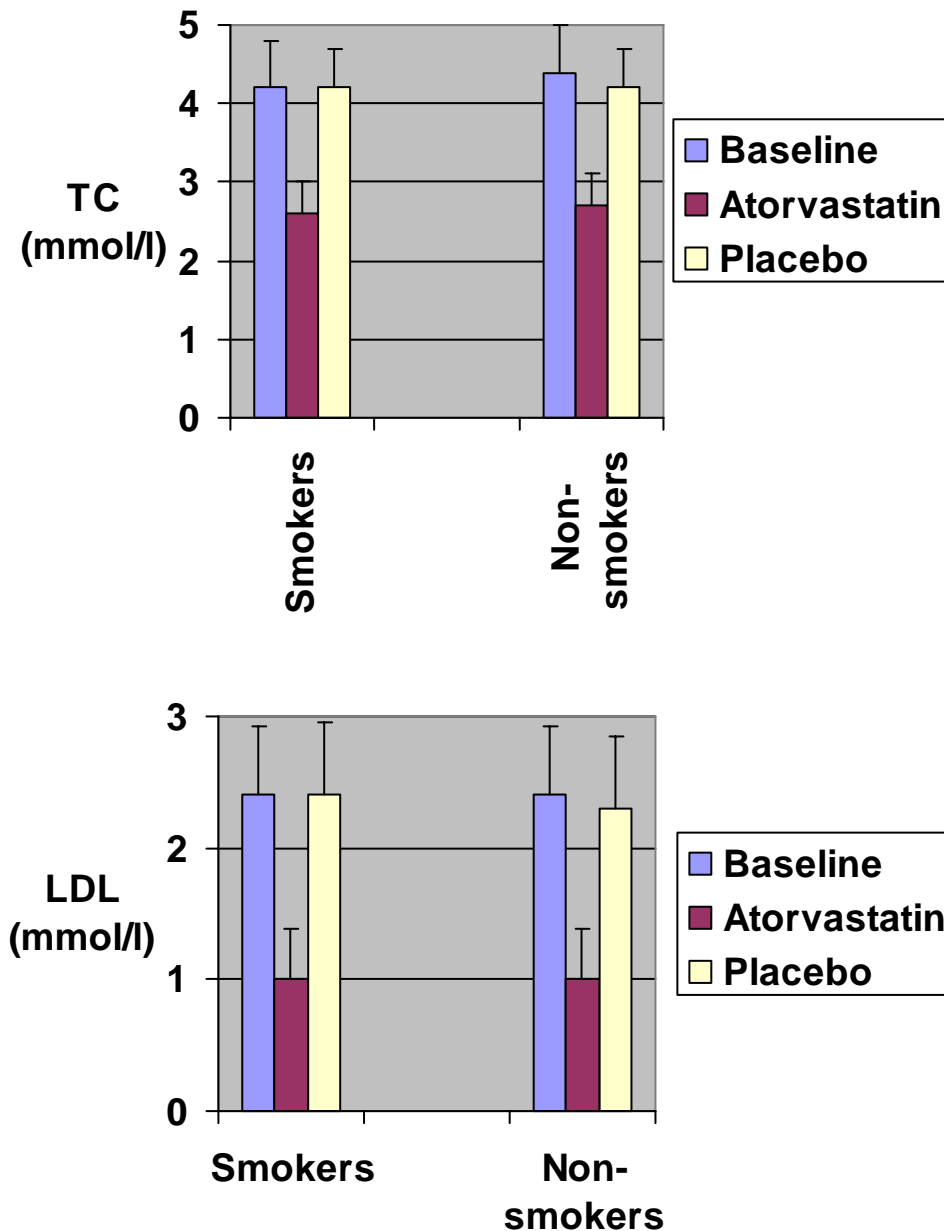
Values are means  $\pm$ SEM.

\*  $p < 0.05$

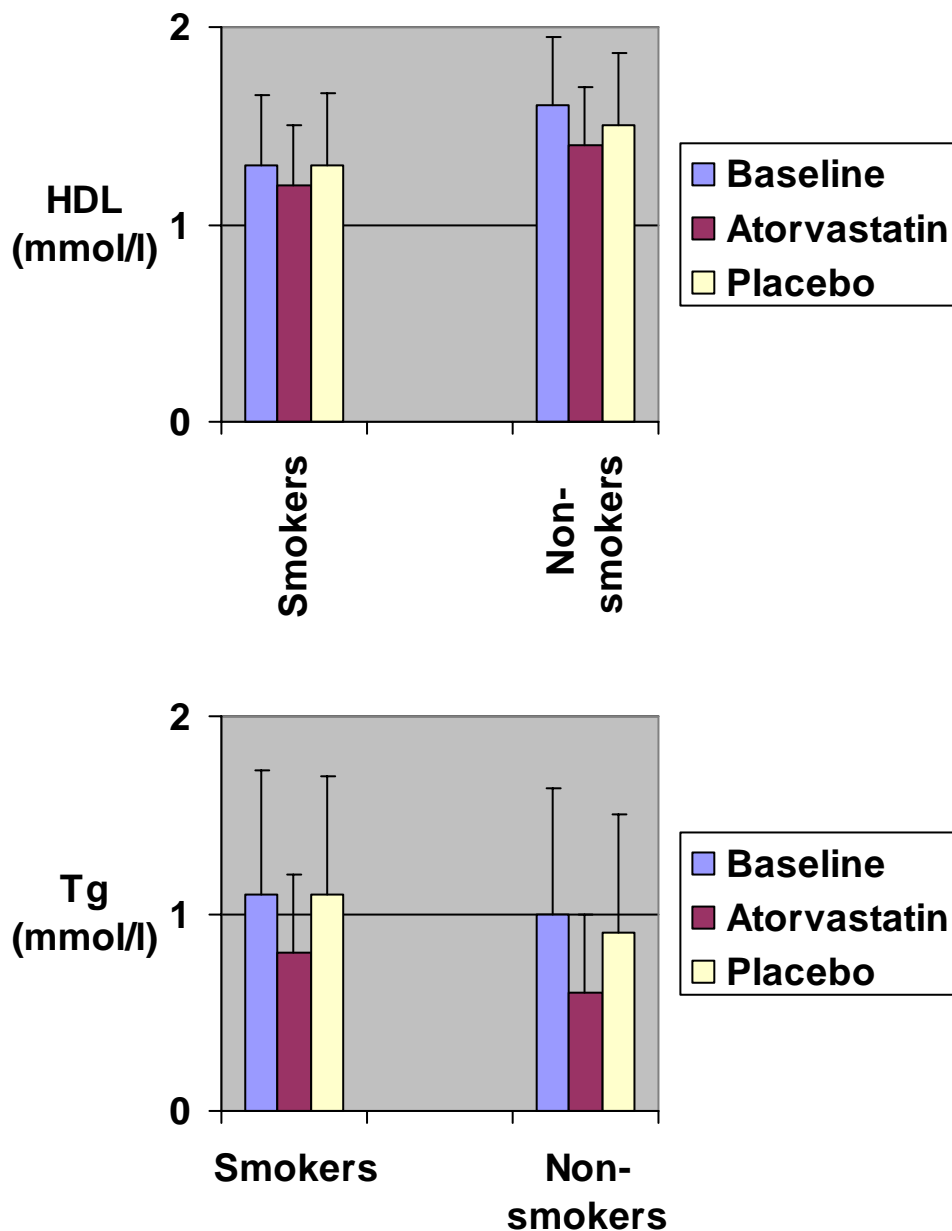
**Figure 1: Flow-chart**



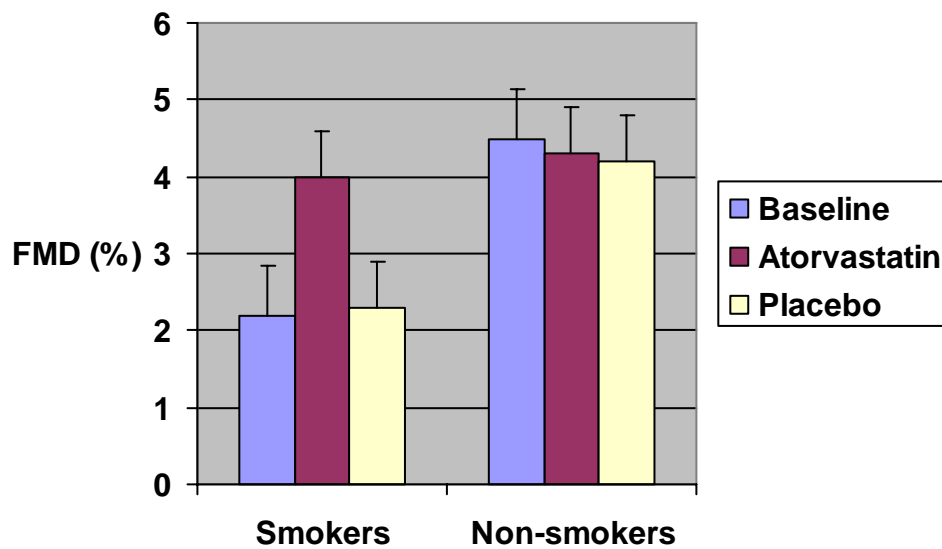
**A**; atorvastatin 80 mg daily  
**N**, no treatment  
 FMD; flow-mediated dilatation



**Figure 2.** Total cholesterol (above) and LDL cholesterol (below) in smokers and non-smokers at baseline and during atorvastatin and placebo. (Means and SD)



**Figure 3.** HDL cholesterol (above) and triglycerides (below) in smokers and non-smokers at baseline and during atorvastatin and placebo. (Means and SD)



**Figure 4.** FMD) in smokers and non-smokers at baseline and during atorvastatin and placebo. treatment in healthy smokers. (Means and SEM)