Long-term treatment with low-dose metoprolol CR/XL is associated with increased plaque echogenicity: The Beta-blocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS).

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Long-term treatment with low-dose metoprolol CR/XL is associated with increased plaque echogenicity: The beta-blocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS)

Östling: Metoprolol and carotid plaque echogenicity.

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Abstract

Objectives: To examine whether the decrease in IMT progression rate in the carotid bulb induced by metoprolol CR/XL treatment (25 mg once daily) observed in the β-blocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS) was accompanied by an effect on carotid plaque echogenicity.

Methods: Gray scale median (GSM) in carotid plaques, used as a score of echogenicity, was measured at baseline and after 36 months in those 341 subjects (aged 49 to 69 years) with an asymptomatic moderate- to large-sized carotid plaque present at baseline and at follow-up. Participants were in a factorial design assigned to treatment with metoprolol CR/XL (25 mg once daily), fluvastatin (40 mg once daily) or corresponding placebo.

Results: After 36 months plaques were more echogenic in participants treated compared to those not treated with metoprolol CR/XL (57.3±16.8 versus 51.8±20.0, p=0.006). GSM had increased more from baseline in the metoprolol CR/XL treated subjects (25±15 versus 18±20, p<0.001), and plaques that had become more echolucent were less frequent in the metoprolol CR/XL treated subjects (3.6 versus 17.0%, p<0.001).

Conclusions: Long-term treatment with low dose metoprolol CR/XL in clinically healthy subjects with moderate-sized carotid plaques was associated with increase in plaque echogenicity, suggesting a potential beneficial effect of the β-blocker treatment on plaque stability.

Key words: plaque, echogenicity, ß-blocker, ultrasound

1. Introduction

B-mode ultrasound of the carotid arteries is a valid method for non-invasive assessment of markers of atherosclerosis, such as intima-media thickness (IMT), and plaques. Carotid IMT has been shown to be an important cardiovascular risk factor in numerous population-based studies [1]. Clinical trials have frequently used change in IMT over time (i.e. IMT progression rate) as a surrogate variable for vascular risk [2-5]. However, it has become increasingly evident that the risk of an atherosclerotic plaque to rupture and to give rise to an acute clinical event is not only dependent on plaque size but more on the composition of the plaque. High-risk plaques (also referred to as vulnerable plaques) are typically characterized by a thin fibrous cap and increased accumulation of lipids and inflammatory cells. The possibility to assess carotid plaque morphology in terms of echogenicity expressed as gray scale median (GSM) on ultrasound images has been explored during recent years. The results of these studies indicate that echolucent plaques are associated with an increased risk for ischemic cerebrovascular events independent of degree of stenosis [6, 7]. Echolucent plaques have been shown to contain more lipids [8], macrophages [9], and elastin [10] in comparison to more echogenic lesions.

We have reported beneficial effects on early stages of carotid atherosclerosis development (IMT thickness) in humans with metoprolol succinate controlled release/extended release (CR/XL) in two long-term placebo controlled studies [3, 11]. In the randomized placebo controlled ß–blocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS) three-year low dose metoprolol treatment (25 mg daily) was associated with reduced rate of progression of IMT in the carotid bifurcation, the vessel region where carotid plaques are most frequently found. Similar results have been reported from several statin studies [12-14] although there

was no effect of the statin used in BCAPS (fluvastatin, 40 mg once daily) on bifurcation IMT progression rate [3].

High-dose treatment with statins increases echogenicity in human plaques [15-17], but whether this can also be achieved by ß–blocker treatment is unclear. However, results from an animal study showed that metoprolol could inhibit atherosclerosis development and stabilize vulnerable plaques [18].

Thus, the aim of the present post-hoc analysis of BCAPS was to explore whether long-term, low dose metoprolol treatment affected echogenicity, as measured by GSM, in moderate- to large-sized carotid plaque.

2. Methods

2.1 Study population

The design and main results of BCAPS have been previously published [3]. In short, B-mode ultrasound examination of the carotid artery was performed on a random 50 % (n=6103) of those, aged 46-69 years, who participated between November 1991 and February 1994 in the "Malmö Diet and Cancer" Study (MDCS) [19]. The objective in this cardiovascular subcohort of MDCS was to study the epidemiology of carotid artery disease [20]. Because of the large numbers to investigate it was decided to investigate only one of the carotid arteries (i.e. right side). Of the examined subjects, 44 % (n=2585) had at least one plaque, defined as a focal thickening of the IMT >1.2 mm. These subjects were invited to participate in BCAPS; 1548 came to enrolment which included a new ultrasound examination of the carotid artery. The aim was to include clinically healthy subjects therefore subjects with history of myocardial infarction, history of surgical intervention in the carotid artery, regular use of β-blockers or

statins, blood pressure equal or above 160/95 mmHg, total cholesterol above 8 mmol/L or hyperglycemia suspected to require insulin treatment were excluded. In total 793 subjects (54% women) accepted and provided informed consent. The trial was conducted between November 1994 and February 1999, with the primary aim to evaluate the effect of metoprolol (25 mg once daily) and fluvastatin (40 mg once daily) on 3-year change in mean IMT in the common carotid artery (CCA) and corresponding change in maximum IMT in the bulb [3]. Subjects were randomly allocated to 1 of 4 treatment groups according to a factorial design: placebo/placebo, metoprolol 25 mg/placebo, fluvastatin 40 mg/metoprolol 25 mg or fluvastatin 40 mg/placebo, all tablets given once daily in the morning.

The present analysis includes participants who: 1) had completed all follow-up visits including ultrasound in BCAPS, and 2) had a measurable moderate- to large-sized carotid plaque at enrolment and at the final follow-up visit after 36 months treatment. To differentiate between small and moderate-sized plaque the cut-off point of 10 mm^2, previously defined [21], was used. Among those included in BCAPS, 151 (19%) had a small plaque (<10 mm2) either at baseline (n=125) or at the 36-months follow-up examination. In that group, 41 were treated with placebo/placebo, 34 with metoprolol/placebo, 37 with fluvastatin/metoprolol and 39 with fluvastatin/placebo. Another 133 participants could not be included in GSM analysis since the plaque images were not feasible for GSM measurement (i.e. 36, 32, 30 and 35 subjects respectively, in the four treatment groups). Finally, 168 participants were not included due to GSM protocol violation (i.e. 30, 46, 52 and 40 subjects, respectively, in the four treatment groups). The reason for exclusion was withdrawal (n=68), did not attend all visits (n=53) or missing 36 months follow up ultrasound examination (n=47). Thus, the present study population consists of 341 patients (48 % women), aged 49 to 69 years (see flow chart in Figure 1).

2.2 Clinical data

Assessment of laboratory and other clinical parameters in BCAPS have previously been described in detail [3]. In short, blood pressure and heart rate were obtained after 5 minutes rest with the subject in the semi supine position. Body mass index (BMI) was measured as kg/m^2. Fasting blood samples, including total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), triglycerides and glucose were determined every year and analyzed at the local Department of Clinical Chemistry according to standard procedures at the laboratory.

2.3 B-Mode ultrasound

Carotid ultrasound investigation was performed at baseline with a last follow-up after 36 months treatment. An Acuson XP (Acuson, Mountain View, Calif.) with a 7 MHz transducer was used. All investigations were done with the same setting on the machine, except for the overall gain, transmit zone and depth gain compensation (DGC), used to optimize image quality for measurement of area and height of plaques and of IMT.

All patients were examined in a supine position with the head turned approximately 45 degrees away from the examined side. The carotid artery was scanned for presence of plaque in a pre-defined window consisting of three centimeters of the common carotid artery (CCA), the bulb and one centimeter of the internal- and external carotid artery. All images for measurement of IMT, plaque area, plaque thickness and GSM in the plaque were obtained in the longitudinal projection showing the thickest intima-media complex. Plaque was defined as a focal thickening of the IMT exceeding 1.2 mm. All measurements were performed by three specially trained and well experienced sonographers, without knowledge of allocation group.

2.4 Measurement of IMT

The examination and reading procedures have been described previously [22]. In short, all images for IMT measurement were taken in end-diastole, verified by simultaneous ECG registration. IMT in CCA (CCA IMT_{mean}) is presented as the mean value over a one centimeter long segment just proximal to the beginning of the bulb. Maximal IMT in the bulb (bulb IMT_{max}) was measured at the site showing the thickest intima-media complex. Plaque thickness (plaque IMT_{max}) was measured as maximal IMT at the site of each plaque. Measurements were done in the far wall according to the leading edge principle using a specially designed, computer-assisted image analyzing system based on automated detection of the echo structures but with the option for manual corrections of the operator [23].

2.5 Measurement of GSM

GSM analysis was performed on a longitudinal image showing the largest plaque present in the predefined window. Measurement of GSM was done using Adobe Photoshop 8.0 with the method described by Elatrozy et al [24]. In brief, the method is based on the standardization of images where black, represented by lumen, and white, represented by the adventitia, is given the values 0 and 190 respectively. After adjustment of the grey scale in the images according to these values, the plaque was outlined. The median value of the grey scale, the GSM value, in this outlined area was used to define the degree of echogenicity in the plaque. GSM was measured in the largest plaque within the predefined window. An intravariability study (n=14) for measurement of GSM showed that the method had good reproducibility, coefficient of variation was 6 % and absolute difference between measurements was 3.7 units

(7.8 %). Since one single sonographer performed all measurements no intervariability study was performed.

2.6 Statistics

SPSS 17.0 was used for the statistical analyses. CCA IMT_{mean}, plaque $IMT_{max,}$ bulb IMT_{max}, glucose and triglycerides were skewed and therefore log-transformed. Student's t-test was used for continuous variables to test differences between mean levels at baseline in the four treatment groups. The primary effect variable, i.e. change in GSM, were analyzed for each patient in a linear model. Change in GSM was defined as the 36-month value, while simultaneously considering GSM at baseline. An initial model was formulated with GSM values at 36 months as the dependent variable, including baseline GSM and treatment (metoprolol versus not metoprolol) as covariates. This model was also used for fluvastatin treatment. Secondly, we also examined the association between change in GSM during follow-up and baseline values for blood lipids, blood pressure, heart rate, BMI, glucose, CCA IMT_{mean}, plaque $IMT_{max,}$ bulb IMT_{max} and treatment, while simultaneously considering GSM at baseline in a multivariate linear regression model. We also explored in a multivariate model whether change in GSM was related to change in risk factors (i.e. total cholesterol, LDL, HDL, triglycerides, glucose, blood pressure and BMI) during the 36-months follow-up period. Finally, we adjusted the initial model for those variables that were found statistically significant associated with change in GSM in a univariate linear regression model, i.e. bulb IMT_{max} and systolic blood pressure at baseline and change in plaque IMT_{max} during the follow-up period. A two-tailed value of $p<0.05$ was considered significant.

3. Results

Mean plaque area was 19.6 ±9.3 mm^2 (range: 10-65 mm^2). Only two participants had a plaque causing an increase in blood flow velocity, thus indicating stenosis >40% (45 and 75 % respectively). Baseline characteristics in treatment groups are presented in table 1. LDL-cholesterol at baseline, in this subgroup of the BCAPS cohort, was significantly higher in those randomized to metoprolol (n=165) in comparison to subjects not randomized to metoprolol (n=176), 4.4±0.9 versus 4.1±0.9 mmol/L, p=0.039.

3.1 Associations between change in GSM and risk factors

GSM at follow-up was significantly associated with GSM at baseline. Change in GSM was significantly associated with bulb $IMT_{max,}$ and systolic blood pressure at baseline (beta= -0,128, p=0,009 and beta=0,139, p=0,004 respectively) and with change in Plaque IMT_{max} (beta=-0,193, p<0,001). In a multivariate linear regression analysis, adjusting for blood lipids, blood glucose, blood pressure and BMI, only GSM at baseline was associated with change in GSM (beta=0.405, p<0.001). When adjusting for change in risk factors (i.e. total cholesterol, LDL, HDL, triglycerides, glucose, blood pressure and BMI) during the 36-months follow-up period none of the risk factors was independently associated with change in GSM.

3.2 Change in GSM during follow-up

In all participants (n=341) mean GSM increased during the follow-up period from 33 to 54 units. After 36 months plaques were more echogenic in participants treated with metoprolol

than in those not treated with metoprolol (57.3±16.8 versus 51.8±20.0 units, p=0.006); GSM had increased significantly more in the metoprolol treated participants (25±15 versus 18±20, p<0.001; Figure 2), and plaques that had become more echolucent were less frequent in the metoprolol treated subjects (3.6 versus 17.0%, p<0.001; Figure 3). The difference in change in GSM between metoprolol and non-metoprolol treated participants remained significant after further adjustment for those variables significantly associated with change in GSM, i.e. SBP, GSM and bulb IMT_{max} at baseline and with change in plaque thickness during follow-up. Adding LDL at baseline to the model did not affect the significant differences in GSM change between groups.

There was no difference in change in GSM during follow-up between subjects treated or not treated with fluvastatin (20±16 versus 22±20), in any of the used models.

3.3 Change in risk factors during follow-up

Change in systolic blood pressure and heart rate differed significantly between metoprolol and not metoprolol treated subjects (-0.2 versus 2.9 mmHg, p=0.043 and -3.5 versus -0.3 bpm, p=0.005 respectively). Also, metoprolol treated subjects had an increase in BMI (0.62 versus 0.29 kg/m^2, p=0.032) as compared to not treated subjects, whereas HDL was significantly more increased in not metoprolol treated subjects (0.15 versus 0.07 mmol/ L, p= 0.002). In the fluvastatin group a significant decrease in total cholesterol (-0.96 versus -0.20 mmol/ L, p<0.001) and LDL (-1.09 versus -0.35 mmol/ L, p<0.001) was observed as compared to no fluvastatin treatment.

4. Discussion

This post hoc analysis of echogenicity of carotid artery plaques in BCAPS showed that low dose treatment with metoprolol (25 mg once daily) for 36 months increased echogenicity of atherosclerotic plaques in the carotid bifurcation. Several lines of evidence suggest that this could possibly reflect a stabilizing effect on the lesion. Previous studies have shown that echogenic plaques have a more stable phenotype and are associated with a lower risk for development of clinical events [6, 7].

To our best knowledge this is the first study addressing change in echogenicity in relation to long-term β-blockade treatment in humans. Furthermore, studies on possible anti-atherosclerotic mechanisms of ß-blockers in humans are scarce. However, in animal models several mechanisms have been suggested. One mechanism is that an increase in shear stress and decrease in pressure-related cyclic stretching, induced by a decrease in heart rate and pulse pressure as effect of ß-blockers, leads to a positive effect on atherogenesis, including reduced endothelial injury [25]. It has been suggested that an endothelial injury caused by activation of the sympathetic nervous system also leads to increased permeability to LDL particles in the arterial wall [26]. Additionally, Hirsch and co-workers showed that treatment with propanolol prevented the increase in heart rate and blood pressure and extreme increase of endothelial cell replication rate (a sign of endothelial damage) induced by massive sympathetic nervous system response to physical restraint in laboratory rats [27]. In the present study metoprolol led to a significant, although small, decrease in SBP and heart rate after 3 years of treatment, compared with controls. This suggests that an attenuation of the sympathetic activation induced by ß-blockers could be a possible explanation to the positive effect of metoprolol on plaque echogenicity, by decreasing LDL influx in the arterial wall

Another possible link between echogenicity and ß-blockers could be that ß-blockers induce an increase in production of prostacyclin, a potent vasodilator. Prostacyclin was found to prevent both growth of fibrous tissue and cholesterol accumulation in the vessel wall. Furthermore, an increase in prostacyclin has been reported in metoprolol-treated rabbits compared to placebo treated animals, when exposed to high sympathetic activity [25].

Besides the effect on the ß-receptor, it is possible that metoprolol affects other targets in the cells, particularly when considering that it is a lipophilic compound with great accessibility to the cells. An animal study showed that treatment with metoprolol did attenuate the expression of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1ß) [28]. In another study an increase in the concentration of nitric oxide (NO) and a decrease in the concentration of peroxynitrite ($ONOO^-$) was found in metoprolol-treated rats as compared to both atenolol treated animals and also untreated controls, indicating that metoprolol succinate (but not atenolol) was capable of reversing endothelial dysfunction, a condition known to contribute to the atherosclerotic process [29].

Liang et al has recently demonstrated that metoprolol-treated rabbits had lower levels of ICAM-1 and VCAM-1 in abdominal plaques compared with controls. Additionally metoprolol treatment decreased a histological vulnerability index, based on percent macrophages and lipids divided by percent of smooth muscle cells and fibers, in abdominal aortic plaques in rabbits [18]. Previous studies on human plaques have shown that echolucent plaques contain more lipids [8] and macrophages [9], while calcium [8, 10] and fibrous tissue [8] are more frequently found in echogenic subtypes. These reports are in accordance with the findings of the present study that metoprolol treatment is associated with an increase in plaque echogenicity in humans.

In BCAPS statin treatment (fluvastatin 40 mg once daily) was significantly associated with IMT progression rate in the CCA, however not with progression rate of IMT in the bulb [3].

The effect of statins on the atherosclerotic process has been explored in several studies. Andersen et al [13] found that fluvastatin decreased IMT in CCA as well as in the carotid bulb in hypertensive patients aged 41-50 years. In the METEOR study rosuvastatin treatment in middle-aged subjects with Framingham risk score lower than 10% and evidence of subclinical atherosclerosis significantly reduced rate of progression of maximum carotid IMT over 2 years versus placebo [14]. The effect of statins on plaque echogenicity has also been evaluated previously. Pravastatin increased GSM in carotid plaques [15-17] while atorvastatin increased GSM both in carotid and coronary plaques [15, 16]. Treatment with high dose pravastatin has also been shown to stabilize carotid plaques [30]. In the present study we found no association between change in plaque echogenicity and treatment with 40 mg fluvastatin once daily.

One limitation of the study was that only clinically healthy subjects with moderate- to large-sized plaques were studied. The mean plaque area was 19 mm^2 (range 10-65 mm^2). Only two of the included subjects had a stenosis > 40 %. Whether the same effect of ß–blockers on plaque echogenicity could be seen on large plaque causing higher degrees of stenosis is still unknown and unethical to test in this type of randomized clinical trial design. Another short-coming is that only a subsample (341 out of 793 participants) of the BCAPS cohort could be included in the GSM study. Although, the proportion of excluded subjects was similar in the 4 treatment groups, it is unclear to what extent plaque echogenicity was similar in included or excluded subjects and whether this could have any impact on the results.

Additionally the design of the study as a post hoc analysis could be criticized. However, BCAPS was a randomized placebo controlled double blind study, and all GSM analyses were blinded to treatment group. Moreover, the size and the risk factor distribution at baseline of the four treatment groups were rather similar to that in the whole BCAPS cohort (3).

The present post hoc analysis of BCAPS, a randomized, double-blind, placebo-controlled, single center study ultrasound study, demonstrated that low dose metoprolol treatment (25 mg once daily) increased carotid plaque echogenicity compared with subjects without corresponding treatment. Previously published results from this study showed that low dose metoprolol treatment reduced the progression rate of carotid bulb IMT. This finding suggests a potential beneficial effect of metoprolol on plaque stability, and a mechanism that may contribute to the cardiovascular protective effects of β-blockers. Further studies are warranted to confirm these results.

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Disclosure statement

John Wikstrand was former a senior medical adviser at AstraZeneca, at present professor emeritus at the Wallenberg Laboratory for Cardiovascular Research at Sahlgrenska Academy at Gothenburg University, Sweden. There are no other conflicts of interest.

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Figure 1. Flow chart of the study population.

Figure 2. Difference in gray scale median (GSM) after 36 months treatment in groups with and without metoprolol, $p<0.001$.

Figure 3. Number of plaques that had become more echolucent (open bars) or more echogenic (filled bars) after 36 months treatment with and without metoprolol, $p<0.001$.

Figure 1

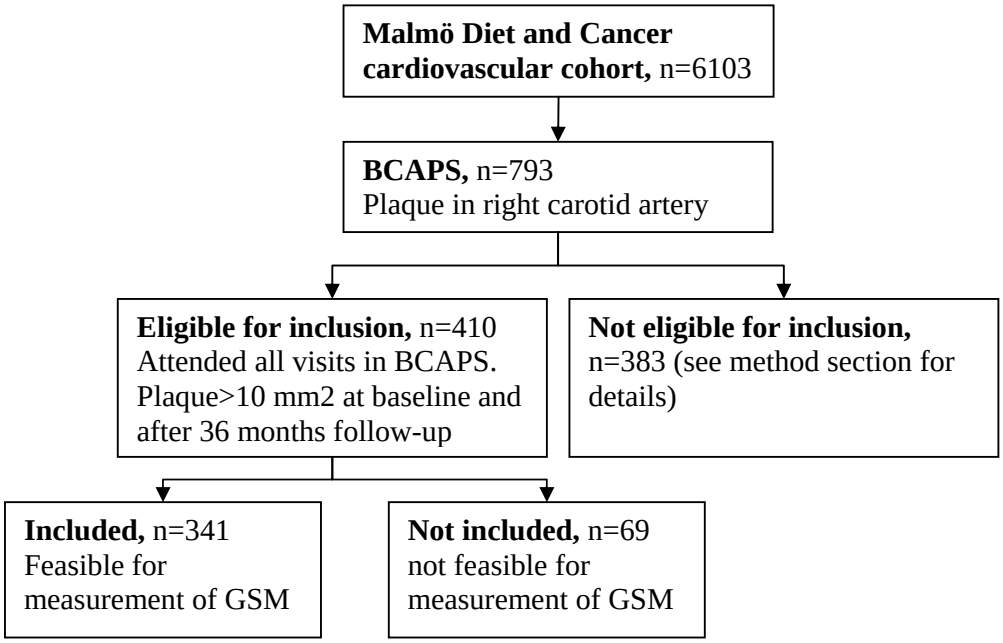
Malmö Diet and Cancer cardiovascular cohort, n=6103
BCAPS, n=793
Plaque in right carotid artery
Eligible for inclusion, n=410
Attended all visits in BCAPS.
Plaque>10 mm2 at baseline and after 36 months follow-up
Not eligible for inclusion, n=383 (see method section for details)
Included, n=341
Feasible for measurement of GSM
Not included, n=69
not feasible for measurement of GSM

Figure 2

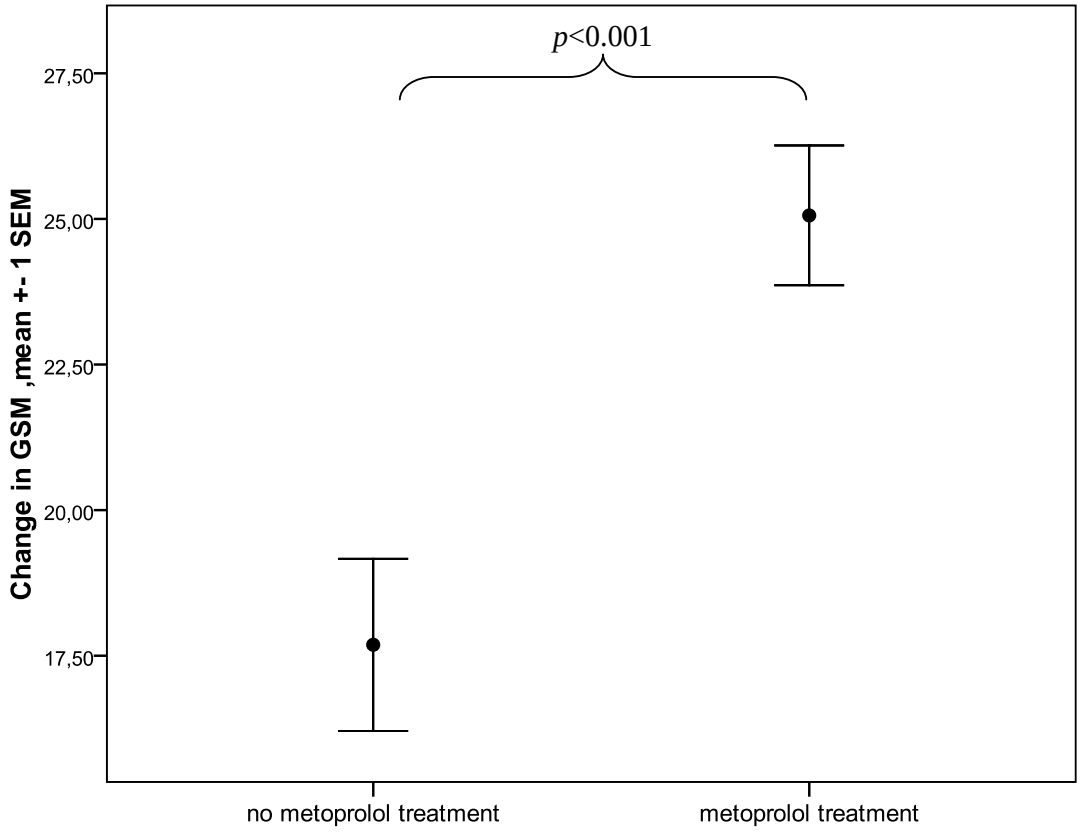
p<0.001
27,50
25,00
22,50
20,00
17,50
Change in GSM ,mean +- 1 SEM
no metoprolol treatment
metoprolol treatment

Figure 3

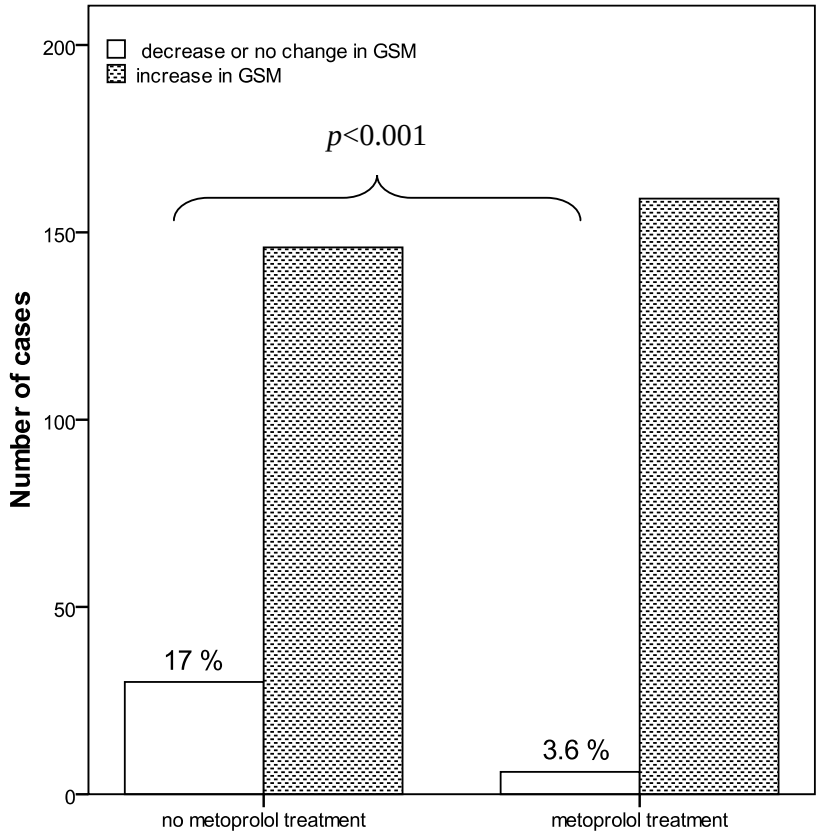
decrease or no change in GSM
increase in GSM
p<0.001
Number of cases
200
150
100
50
0
17 %
3.6 %
no metoprolol treatment
metoprolol treatment

Table 1. **Baseline characteristics by treatment groups**

	Treatment groups			
	Placebo/placebo n=92	Metoprolol/placebo n=87	Metoprolol/fluvastatin n=78	Fluvastatin/placebo n=84
Gray scale median	33.4 (12.5)	32.0 (13.0)	32.5 (16.6)	34.8 (16.4)
SBP, mmHg	139.8 (13.15)	140.9 (15.19)	141.7 (13.7)	139.8 (14.1)
DBP, mmHg	84.7 (6.39)	85.0 (6.55)	85.7 (5.77)	85.3 (6.59)
Heart rate, bpm	69 (12)	69 (12)	69 (10)	68 (10)
BMI, kg/m^2	25.5 (3.4)	25.3 (3.5)	25.0 (2.8)	25.3 (3.0)
Cholesterol, mmol/l	6.04 (1.00)	6.20 (1.06)	6.42 (1.05)	6.16 (0.94)
HDL, mmol/l	1.36 (0.37)	1.28 (0.31)	1.41 (0.34)	1.36 (0.35)
LDL, mmol/l	4.06 (0.87)	4.31 (0.97)	4.40 (0.93)	4.24 (0.87)
Triglycerides, mmol/l[#]	1.22 (0.90-1.77)	1.17 (0.93-1.58)	1.17 (0.87-1.75)	1.22 (0.86-1,38)
Glucose, mmol/l[#]	5.00 (4.60-5.40)	5.00 (4.70-5.50)	5.00 (4.70-5.50)	4.98 (4.60-5.20)
CCA IMT_{mean}, mm[#]	0.90 (0.80-0.98)	0.92 (0.78-1.03)	0.87 (0.79-1.00)	0.87 (0.77-0.97)
Bulb IMT_{max}, mm[#]	1.96 (1.60-2.28)	1.81 (1.64-2.30)	2.03 (1.63-2.38)	2.00 (1.67-2.29)
Plaque IMT_{max}, mm[#]	2.05 (1.83-2.34)	2.07 (1.77-2.53)	2.08 (1.83-2.35)	2.12 (1.76-2.30)