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CHAPTER 5

Biomarkers of metabolic syndrome during periodontal therapy: a 12-month observational study.

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Submitted

Abstract

Context: Periodontitis patients often suffer from undiagnosed diseases and conditions.

Objective: To observe parameters of metabolic syndrome (MetS) during periodontal therapy.

Design: 12-month observational study with outcomes measured at baseline, 3, 6 and 12 months.

Settings: Department of Periodontology of the Academic Centre for Dentistry Amsterdam, The Netherlands.

Patients: 110 periodontitis patients without known comorbidities, underwent periodontal therapy.

Main outcome measures: Parameters of MetS: waist circumference (WC), systolic and diastolic blood pressure (BP), HDL cholesterol, triglycerides, glucose. Diagnosis of MetS was also assessed.

Results: Apparent healthy subjects with periodontitis may have MetS. After periodontal therapy patients showed a reduction in systolic BP after 3 ($p = 0.004$), 6 ($p < 0.001$) and 12 months ($p = 0.042$) and of triglycerides after 3 ($p < 0.001$), 6 ($p < 0.001$) and 12 months ($p = 0.018$). Despite the absence of known comorbidities, 27.2% ($n=30$) of the periodontitis patients were diagnosed with MetS. After periodontal treatment this proportion changed to 14.5% ($p = 0.007$) at 3 months, to 17.3% ($p = 0.017$) at 6 months and to 21.8% at 12-month follow-up ($p = 0.383$).

Conclusions: During periodontal therapy, periodontitis patients show significantly decreased levels for two out of five parameters of MetS. For about half of those patients with MetS, this condition was no longer present at 3 months after periodontal therapy but, for some of them, re-appeared over-time. Improving periodontal health can lead to an improvement of the metabolic status and, as a consequence, of the general health.

Introduction

Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth and it is also considered a condition that may increase the risk for cardiovascular diseases (CVD) (Tonetti et al. 2013), increase systemic inflammation (Teeuw et al. 2014) and can affect the glycemic control in diabetic patients (Lalla & Papapanou 2011, Preshaw et al. 2012). In the Western countries, the incidence of periodontitis varies from 6 to 50% depending on the geographic area and the classification used, but 6-13% of the population suffers from a severe form of this disease (Albandar 2011, Eke et al. 2012, Hugoson & Norderyd 2008). It has been calculated that patients affected by severe periodontitis have ulcerated lesions (pockets) with a Periodontal Inflamed Surface Area (PISA) of up to 39 cm² (Nesse et al. 2008). Through this ulcerated pocket wall, bacterial pathogens and their antigens (lipopolysaccharides) cause daily short-lived bacteraemias, which can induce an increase in the systemic inflammatory burden. Two reviews from a joint workshop by the European Federation of Periodontology and the American Academy of Periodontology, indicated that a pro-inflammatory state in periodontitis can contribute to an increased risk for insulin resistance, and subsequently type 2 diabetes mellitus (T2DM), and CVD (Schenkein & Loos 2013, Taylor et al. 2013).

Although there is substantial evidence about the associations between periodontitis on the one hand and T2DM and CVD on the other hand, evidence supporting a relationship between periodontitis and Metabolic syndrome (MetS) is much more limited. MetS is acquiring attention as a systemic condition, which can increase the risk for CVD by 2 fold and T2DM by 5 fold (Alberti et al. 2009, Gami et al. 2007). MetS is a combination of metabolic disturbances defined by central obesity and any of two of the following additional factors: hypertension, dyslipidemia (raised triglycerides, reduced high-density lipoproteins [HDL]) and raised fasting plasma glucose (Alberti et al. 2006). It is a condition that often remains undiagnosed. Recent large cross-sectional studies showed a mild but significant higher prevalence of MetS in patients with periodontitis in comparison to individuals with gingivitis or healthy controls (Nibali et al. 2013, Tu et al. 2013). To date, several clinical trials investigated the effect of periodontal therapy on a variety of markers for the risk of CVD, both in patients with or without known comorbidities (Teeuw et al. 2014). These included mainly markers of systemic inflammation, dyslipidemia, glucose and hypertension. However studies focusing specifically on the accepted biomarkers of MetS, after periodontal treatment, are scarce. Acharya and co-workers (Acharya et al. 2010) evaluated in a periodontal treatment

study systemic markers of inflammation and dyslipidemia in periodontitis patients with and without MetS. Their results showed only in patients with MetS a significant decline in serum levels of triglycerides and HDL cholesterol. In contrast, Lopez and co-workers (2012), in a treatment study of periodontitis patients with MetS, found no change in the serum levels of the biomarkers of MetS.

The aim of the present one-year follow-up study was to monitor possible changes in the five parameters of MetS after periodontal therapy in a population with periodontitis without known comorbidities.

Material and methods

Study population

Consecutive subjects, who were referred to the Department of Periodontology of the Academic Centre for Dentistry Amsterdam (ACTA) for treatment of periodontitis in the period 2008-2013, were screened for eligibility. Patients were recruited if they fulfilled the following inclusion criteria: presence of chronic periodontitis, self-reported good general health and not being aware of any form of diabetes, CVD, (auto)immune disease or any other systemic or metabolic disease, not receiving any medication for hypertension, dyslipidemia of hyperglycemia. A periodontal case was defined if he/she had proximal attachment loss of at least ≥ 3 mm in ≥ 2 non-adjacent teeth (Tonetti & Claffey 2005). For this study a patient was included if he/she presented $\geq 30\%$ alveolar bone loss at ≥ 2 teeth per quadrant and presence of ≥ 2 teeth per quadrant with periodontal pockets ≥ 5 mm with clinical evidence of attachment loss and at least 50% of all sites in the mouth with bleeding on probing (BOP). Further exclusion criteria were: regular use of medications, use of antibiotics in the past 6 months, periodontal treatment in the last 2 years, pregnancy or lactation, presence of implants or orthodontic appliances and presence of < 20 natural teeth. The subjects who agreed to participate in this research, signed a written informed consent. The study protocol was approved by the Medical Ethical Committee of the Academic Medical Centre of Amsterdam, The Netherlands (MEC 07/264). The study was part of a trial registered in Current Controlled Trials (ISRCTN36043780).

General, medical and periodontal examinations

A recent and past medical history, general patient characteristics, alcohol consumption and smoking habits were recorded by means of a questionnaire. A patient was defined as a smoker if he/she was currently smoking or quit ≤ 6 months before intake, and as a non-smoker if he/she had never smoked or quit smoking > 6 months before the baseline examination. Measurements for general health and MetS were taken: height and weight to calculate the body mass index (BMI) and the waist circumference (WC) was measured, after exhaling, at the umbilicus level. Blood pressure (BP) was measured between 09:00-11:00 a.m., with a calibrated automated device (Omron M10-IT, Omron Healthcare, Japan) at least 10 minutes after that the patient sat on the dental chair. Blood pressure was recorded on both arms and the mean of both measurements was calculated. Thereafter, fasting blood was collected and

periodontal measurements were recorded. Periodontal assessment included: dental plaque (presence/absence), bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment level (CAL) after which PISA was calculated (Nesse et al. 2008). All baseline measurements of general health and MetS as well as periodontal measurements were repeated at 3, 6 and 12 months after treatment by the same calibrated clinician.

Periodontal therapy

For all patients, basic periodontal therapy (BPT) was carried out by 3 experienced and specifically trained dental hygienists of the Department of Periodontology of ACTA in 3 appointments within one week. Oral hygiene instructions were provided with a powered toothbrush (Philips Oral Healthcare, Bothell, WA, USA) and interdental aids. Participants were randomized in four treatment modalities. Patients received either BPT alone or, in addition to this, systemic antibiotics and/or subgingival disinfection with 0.5% sodium hypochlorite (NaOCl) (Table 1). All subjects were subsequently enrolled in a 3-monthly maintenance program at the Department of Periodontology until the end of the follow-up (1-year)

Laboratory measures

0.5 ml aliquots of EDTA plasma were prepared after whole blood centrifugation (4000 rpm for 10 minutes) and stored at -80° C until further analysis. In one batch, HDL, glucose and triglycerides were analyzed with the enzymatic colorimetric methods on a modular analyzer Roche Cobas 8000 c502 (Roche Diagnostics Corp, Germany).

Statistical analysis

Statistical analysis was performed with a software package (SPSS 21.0, IBM Statistics, USA). Analysis was performed on an intention-to-treat basis (ITT). Data were explored with Little's Missing Completely At Random test (Groenwold et al. 2012). Missing data were imputed using the Expectation-Maximization method (Elashoff et al. 2008). Outcome measures were the biomarkers of MetS (WC, systolic and diastolic BP, triglycerides, HDL, glucose). To test the change in primary outcomes, ANOVA for repeated measures was used and the treatment modalities were used as fixed factor and gender, age and smoking as covariates. The same tests were used to investigate the changes of periodontal outcomes (PPD, CAL, Plaque, BOP and PISA). In order to test the interaction between AB and disinfection with NaOCl, an

additional analysis was used, employing a two-way ANOVA (factorial design) as described in chapter 4. This latter analysis was employed for both periodontal parameters and biomarkers of MetS. These statistical tests yielded adjusted p values (p_{adj}).

An analysis on the presence of MetS in this population was performed. Diagnosis of MetS was based on the presence of central obesity (WC ≥ 102 cm in men or ≥ 88 cm in women) plus ≥ 2 of the following risk determinants: triglycerides ≥ 1.7 mmol/l, HDL < 1.03 mmol/l in males or < 1.29 mmol/l in females, BP $\geq 130/85$ mmHg, fasting glucose ≥ 5.6 mmol/l (Grundy 2008). A distribution of the patients positive (MetS+) or negative (MetS-) for a diagnosis of MetS at baseline and at 3-, 6-, and 12-month follow-up was performed and differences in prevalence were tested with McNemar test.

$P < 0.05$ was considered statistically significant.

Results

A total of 134 individuals met the inclusion criteria and 110 patients accepted to take part in this research. The main reasons for not being eligible were having comorbidity, usage of any medication and not meeting periodontal case definition. At the end of the study, a total of 11 patients were lost at follow-up and 99 patients completed the study. Two patients started taking anti-hypertensive medication during the follow-up.

The average age of the patients was 47.8 years, and the population was composed mainly of high-educated, Dutch-Caucasian patients. There were slightly more males and smokers, and the average BMI was 25.2 kg/m² (Table 1).

BPT was effective in reducing periodontal inflammation already at 3 months and the reduction remained stable at 6 and 12 months ($p_{\text{adj}} < 0.001$ for all periodontal parameters at all follow-up times in comparison to baseline) (Table 2). For the total population, it was observed that periodontal therapy led to an improved periodontal condition at 12 months. The factorial design analyses on periodontal parameters showed a significant better effect of antibiotics on PPD and PISA (see Table 4 in chapter 4). Pooled data, adjusted for treatment modalities and other potential confounding factors (see M&M), showed a significant reduction in PPD (mean difference 1.1 mm; [95% C.I. = 0.92,1.18] $p_{\text{adj}} < 0.001$), CAL (mean difference 0.60 mm [95% C.I. = 0.45,0.74] $p_{\text{adj}} < 0.001$), BOP (mean difference 44.1% [95% C.I. = 39.9,48.4] $p_{\text{adj}} < 0.001$), Plaque (mean difference 42.4% [95% C.I. = 36.8,48.1] $p_{\text{adj}} < 0.001$) and PISA (mean difference 13.06 cm² [95% C.I. = 11.54,14.48] $p_{\text{adj}} < 0.001$) (Table 2).

Biomarkers of MetS

In Figure 1 and Table S1 we present the data for the 5 biomarkers of MetS. The adjunctive treatments with antibiotics and/or NaOCl did not account for any significant difference between the groups for any of the five parameters of MetS at any follow-up point. Therefore all patients were pooled for data presentation of the parameters of MetS. WC did not show a significant difference at 12 months compared to the baseline values. However, a significant increase at 12 months in comparison with 3 and 6 months ($p_{\text{adj}} = 0.010$ and $p_{\text{adj}} = 0.027$, respectively) was noted (Fig. 1A, Table S1). Two of the four additional parameters of MetS showed a significant change after periodontal treatment (Fig. 1, Table S1): a decrease in levels of triglycerides was found already at three months after treatment ($p_{\text{adj}} < 0.001$) and

was retained up to the 12-month follow-up ($p_{\text{adj}} = 0.018$). Systolic BP also decreased significantly from baseline at 3 months after treatment ($p_{\text{adj}} = 0.004$), at 6 months ($p_{\text{adj}} < 0.001$) and at 12 months ($p_{\text{adj}} = 0.042$). HDL and glucose did not significantly change after treatment. In the statistical model, smoking was the only covariate, which showed a significant association with change of triglycerides. No other association was found between the covariates and the biomarkers of MetS (Figure 1, Table S1).

Patients with diagnosis of MetS

30 patients (27.2%), 20 men and 10 females, were diagnosed at baseline with MetS (MetS+). The ITT analysis showed that the number of MetS+ patients decreased to 16 (14.5%, $p = 0.007$) at the 3-month follow-up, to 19 patients (17.3%, $p = 0.017$) at 6-month follow-up, but there was an increase to 24 patients (21.8%, $p = 0.383$) at 12-month follow-up (Table 3). More specifically at 12 months, from the 30 MetS+ at baseline, 13 had no longer MetS (43.3% reduction), while 9 patients (33%) changed their metabolic status only temporarily. However, from the 80 MetS- patients at baseline, 8 (10%) became MetS+. Data from per-protocol analysis showed similar results, this is presented in Supplemental Table 2, with frequencies of patients per time point of analysis.

Discussion

The current study aimed to investigate changes in the 5 parameters of MetS during periodontal therapy in 110 patients with chronic periodontitis. Basic periodontal therapy resulted in an improved periodontal condition, which remained stable for the subsequent year. From the 5 parameters of MetS, a significant reduction of levels of triglycerides and the systolic BP could be assessed. The additional use of systemic antibiotics and/or local disinfection with sodium hypochlorite did not lead to any significant additional change for the 5 parameters of MetS. The reduction of triglycerides in a periodontitis population corroborate a recent study, which also showed a reduction of triglycerides up to one year after periodontal therapy (Mourao et al. 2014), however two other studies did not find similar reductions (D'Aiuto et al. 2006, Kamil et al. 2011). Contrasting results are also reported regarding BP; some studies, not specifically studying MetS, showed no effect of periodontal treatment on BP (Higashi et al. 2008, Lopez et al. 2012), whereas in other studies a decrease of BP was found in periodontitis patients having comorbidities (Chen et al. 2012, D'Aiuto et al. 2006, Sun et al. 2011, Vidal et al. 2013). In the end, variations in severity of periodontal status, initial levels of the MetS parameters, age, length of follow-up and BMI of the study population, may be explanatory reasons for discrepancies between those results.

The results of the current study are in line with the hypothesis that the presence of periodontal inflammation is associated with the presence of a systemic pro-inflammatory state, which can be reduced with periodontal therapy (D'Aiuto et al. 2013, Paraskevas et al. 2008, Teeuw et al. 2014). The reduction of this pro-inflammatory state can lead to an improvement of systemic conditions, such as endothelial function, flow mediated dilatation and arterial stiffness (Orlandi et al. 2014, Tonetti et al. 2007). We suggest therefore that, in our cohort, the observed reduction of triglycerides and BP is related to the decrease of the periodontal inflammation and that improving periodontal health can lead to an improvement of the metabolic status and, as a consequence, of the general health.

Interestingly, in our periodontitis population, 27.2% of the subjects, who were unaware of their condition, were diagnosed at baseline with MetS. This prevalence is slightly below the data reported by D'Aiuto et al. (2008), who reported in a large cross sectional investigation in periodontitis patients (N = 13,994) a prevalence of MetS of 34-37%. Notably, there was a significant reduction of the number of MetS+ patients up to 6-months after periodontal therapy (from 30 to 19). But at 12 months, 24 patients were MetS+. At the 12 month time

point, of the 30 patients, who at baseline were diagnosed with metabolic syndrome, 43.3% improved their metabolic condition. We cannot exclude that these participants changed their lifestyle. We could not find any appreciable change in their smoking habits, however, modifications in other factors as physical activity or diet, which have not been recorded, may have contributed to the change of their metabolic status. 9 of the 30 patients with MetS+ at baseline had only a temporary improvement of their metabolic status and in 10% of patients, who were MetS- at baseline, the metabolic status deteriorated..

It is important to note that a successful periodontal therapy requires the patients to be highly compliant and motivated to perform on a daily basis an excellent oral hygiene. The compliance of the current population is reflected by the decrease in the periodontal parameters and by a reduction of dental plaque, which can be considered as a measure of daily self-performed care. It could be speculated that the improved compliance of patients to the high oral hygiene level required for a successful periodontal therapy may have had a positive influence to the general health behavior. Thus, we speculate that the improvement of periodontal conditions and the reduction of the systemic pro-inflammatory state, together with a possible improvement with patients' health behavior may have contributed to improve their metabolic conditions.

As a point of discussion it is important to mention that on purpose we did not include a control group. For a true control group for this study we should have included patients with moderate to severe periodontitis who should have been left untreated for one year. Besides the fact that we considered that unethical, it has been noted, from similar intervention trials in periodontitis with >3-month follow-up, that a control group, composed of "community treated" or "untreated" subjects, suffers from high drop-out rate, a lack of compliance with the study protocol while seeking periodontal treatment outside the study (Couper et al. 2008).

Conclusions

The results of the current study showed that after basic periodontal therapy there was a reduction of levels of triglycerides and of systolic BP and no significant change in the other three parameters of MetS. Also, we observed a reduction of number of patients with MetS, that, however, rebounded partly over time.

Future investigations should focus on those factors, which can contribute to maintain at longer term the metabolic improvements after periodontal therapy observed in our study.

Source of funding

This study was funded by several sources: by the authors' institution , by an unconditional grant from Philips Oral Healthcare, by a grant from the University of Amsterdam for the focal point "Oral infection and inflammation" and by a grant from the Stichting NVvP.

Figure 1. Changes of markers of metabolic syndrome during 12 months follow-up. Error bars represent standard errors of means.

* $p_{\text{adj}} < 0.05$ in comparison with baseline (repeated measures ANOVA including Bonferroni correction), adjusted for gender, age, smoking and treatment modalities.

† $p_{\text{adj}} < 0.05$ in comparison to 3 and 6 months (repeated measures ANOVA including Bonferroni correction, males and females combined), adjusted for gender, age, smoking and treatment modalities.

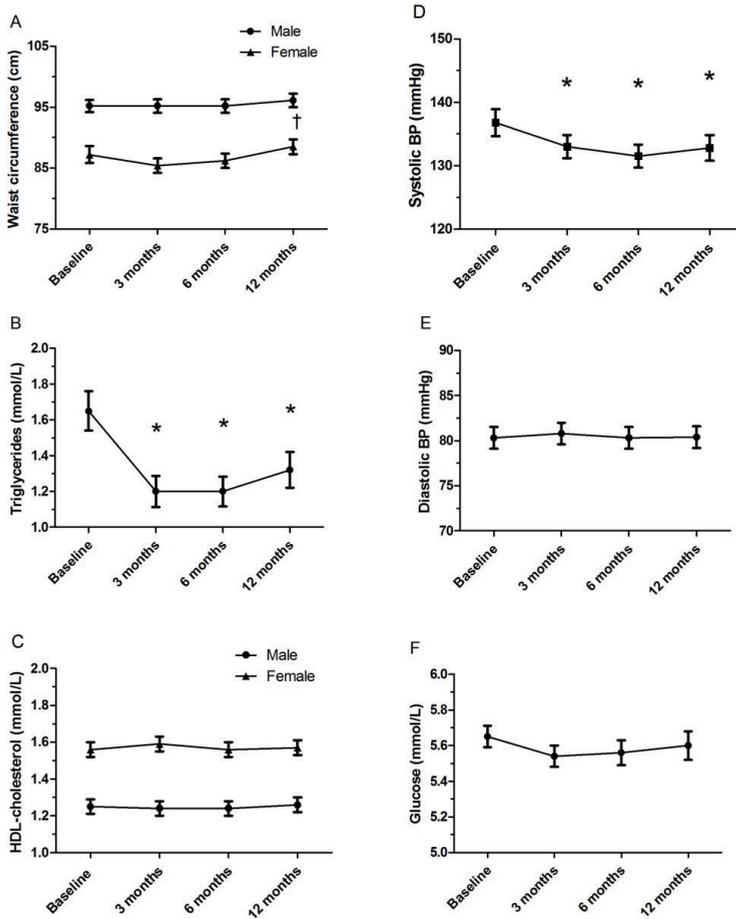


Table 1. Baseline Characteristics

	Total
No of subjects	110
Age (years)	47.8 ± 9.2
Gender	
Male	57 (63)
Female	42 (47)
Ethnicity	
Dutch Caucasian	81 (74)
other	29 (26)
Education	
< high school	27 (25)
≥ high school	83 (75)
Smoking	
non-smokers	48 (44)
current smokers	62 (56)
Alcohol	
≥2 units/day	33 (30)
<2 units/day	77 (70)
BMI (kg/m ²)	25.2 ± 3.6
Treatment allocation	
BPT+S	29
BPT+S+AB	29
BPT+NaOCl	27
BPT+NaOCl+AB	25

Values are mean ± standard deviation or number of subjects (%).

Abbreviations: BMI, body mass index. BPT, basic periodontal therapy. S, saline. AB, antibiotics (amoxicillin 375mg + metronidazole 250mg, 3 x day x 7 days). NaOCl, disinfection with 0.5% sodium hypochlorite.

Table 2. Effect of Periodontal Therapy on Periodontal parameters

		Total
No of subjects		110*
PPD (mm) ¹	Baseline	3.9 ± 0.6 ^a
	3 months	2.8 ± 0.5
	6 months	2.8 ± 0.4
	12 months	2.8 ± 0.5
CAL (mm)	Baseline	4.2 ± 1.0 ^a
	3 months	3.6 ± 1.0
	6 months	3.6 ± 1.0
	12 months	3.6 ± 1.0
BOP (%)	Baseline	67.3 ± 15.3 ^a
	3 months	22.0 ± 13.5
	6 months	21.9 ± 13.9
	12 months	23.2 ± 14.8
PISA (cm ²) ¹	Baseline	17.7 ± 5.7 ^a
	3 months	4.3 ± 5.8
	6 months	4.4 ± 3.3
	12 months	4.7 ± 3.6
Plaque (%)	Baseline	64.7 ± 24.1 ^a
	3 months	20.2 ± 18.1
	6 months	23.8 ± 20.9
	12 months	22.3 ± 18.0

Values are means ± standard deviation.

* Based on intention-to-treat analysis.

^a $p < 0.001$ different in comparison with the other follow-up points (ANOVA for repeated measures including Bonferroni correction, adjusted for age, gender, smoking and treatment modalities).

¹ Factorial design analysis showed a significant higher reduction of PPD and PISA in the groups using systemic antibiotics (see Table 4 in chapter 4)

Abbreviations: PPD, Probing Pocket depth; CAL, Attachment Level; BOP, Bleeding On Probing; PISA, Periodontal Inflamed Surface Area.

Table 3. Distribution of Individuals with Diagnosis of Metabolic Syndrome

	Baseline	3 months	6 months	12 months
No of subjects *	110	110	110	110
MetS+	30 (27.2)	16 (14.5) ^a	19 (17.3) ^b	25 (21.8)
MetS-	80 (72.8)	94 (85.5) ^a	91 (82.3) ^b	85 (78.2)

Values are number of subjects (%).

* Based on intention-to-treat analysis.

^a $p = 0.007$, ^b $p = 0.017$ (Mc Nemar test) In comparison with baseline.

Diagnosis of Metabolic Syndrome: presence of central obesity (WC ≥ 102 cm in men or ≥ 88 cm in women) and ≥ 2 of the following parameters: TR ≥ 1.7 mmol/l, HDL < 1.03 mmol/l in men or < 1.29 mmol/l in women, BP $\geq 130/85$ mmHg, fasting glucose ≥ 5.6 mmol/l

Abbreviations: MetS+, patients with diagnosis of Metabolic Syndrome; MetS-, patients without diagnosis of Metabolic syndrome; WC, waist circumference; TR, triglycerides; HDL, high density lipoproteins; BP, blood pressure.

Supplemental table 1: Effect of Basic Periodontal Therapy on Markers of Metabolic Syndrome

		All patients	Male	Female
No of subjects		110*	63	47
WC (cm)	Baseline	91.8 ± 12.3	95.2 ± 9.8	87.2 ± 13.7
	3 months	91.0 ± 12.2	95.2 ± 10.7	85.4 ± 11.9
	6 months	91.3 ± 11.9	95.2 ± 10.7	86.2 ± 11.7
	12 months	92.9 ± 11.7 ^{cd}	96.1 ± 10.6	88.5 ± 11.9
Triglycerides (mmol/l)	Baseline	1.65 ± 1.16		
	3 months	1.20 ± 0.87 ^a		
	6 months	1.20 ± 0.87 ^a		
	12 months	1.32 ± 1.00 ^b		
HDL (mmol/l)	Baseline	1.38 ± 0.43	1.24 ± 0.38	1.56 ± 0.42
	3 months	1.39 ± 0.44	1.24 ± 0.38	1.59 ± 0.44
	6 months	1.37 ± 0.44	1.24 ± 0.40	1.56 ± 0.41
	12 months	1.39 ± 0.41	1.26 ± 0.36	1.57 ± 0.41
Blood pressure				
Systolic (mm/Hg)	Baseline	136.8 ± 20.0		
	3 months	133.0 ± 17.3 ^b		
	6 months	131.5 ± 17.3 ^a		
	12 months	132.8 ± 19.6 ^b		
Diastolic (mmHg)	Baseline	80.3 ± 11.7		
	3 months	80.8 ± 11.2		
	6 months	80.3 ± 11.7		
	12 months	80.4 ± 11.6		
Glucose (mmol/l)	Baseline	5.65 ± 0.60		
	3 months	5.54 ± 0.52		
	6 months	5.56 ± 0.57		
	12 months	5.55 ± 0.72		

Values are means ± standard deviation.

* Based on intention-to-treat analysis (ANOVA for repeated measures + Bonferroni, adjusted for age, gender, smoking and treatment modalities).

^a $p_{\text{adj}} < 0.001$ in comparison to baseline

^b $p_{\text{adj}} < 0.05$ in comparison to baseline

^c $p_{\text{adj}} < 0.05$ in comparison to 3 months

^d $p_{\text{adj}} < 0.05$ in comparison to 6 months

Smoking was a significant covariate associated with triglycerides $p < 0.05$

Abbreviations: WC, Waist Circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplemental table 2A. Distribution of Individuals with Diagnosis of Metabolic Syndrome

	Baseline	3 months	6 months	12 months
No of subjects*	110	105	99	99
MetS+	30 (27.2)	16 (15.2) ^a	16 (16.2) ^b	21 (21.2)
MetS-	80 (72.8)	89 (84.8) ^a	83 (83.8) ^b	78 (78.8)

Supplemental table 2B. Distribution of Individuals with Diagnosis of Metabolic Syndrome

	MetS+		MetS-	
No of subjects*				
Baseline (110)	30		80	
	MetS+	MetS-	MetS+	MetS-
3 months (105)	11	18	5	71
6 months (99)	12	16	4	67
12 months (99)	16	13	5	65

Values are number of subjects (%).

* Based on per-protocol analysis.

^a $p = 0.011$, ^b $p = 0.012$ (Mc Nemar test).

Diagnosis of Metabolic Syndrome: presence of ≥ 3 of the following parameters: WC ≥ 102 cm in men or ≥ 88 cm in women, TR ≥ 1.7 mmol/l, HDL < 1.03 mmol/l in men or < 1.29 mmol/l in women, BP $\geq 130/85$ mmHg, fasting glucose ≥ 5.6 mmol/l

Abbreviations: MetS+, patients with diagnosis of Metabolic Syndrome; MetS-, patients without diagnosis of Metabolic syndrome; WC, waist circumference; TR, triglycerides; HDL, high density lipoproteins; BP, blood pressure.

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