

Randomized Study of the Effects of Statin on Inflammatory and Prothrombotic States in Obese Adolescents

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Abstract

Background: Increased inflammatory cytokines, C-reactive protein (CRP) and prothrombotic parameters are potential markers of cardiovascular risks in childhood obesity.

Objectives: We investigated whether a statin treatment can reverse these early cardiovascular risk indicators. The primary outcome was the effect of sixteen weeks of statins on the inflammatory cytokines, CRP and the pro-thrombotic state in obese adolescents.

Methods: This randomized controlled double-blind study conducted at the University Hospital of Geneva included 28 obese adolescents aged 12-16 years. Subjects received either placebo (P) or atorvastatin (A) for sixteen weeks. Levels of monocyte chemoattractant protein 1 (MCP-1), interleukin-6, interferon- γ -inducible protein, interleukin-10, interleukin-1 receptor antagonist and CRP were measured at baseline, after sixteen weeks and after one year. Coagulation parameters were evaluated by prothrombin time, activated partial thromboplastin time, fibrinogen levels and endogenous thrombin potential (ETP) at each visit.

Results: The MCP-1 level was stable in group A after sixteen weeks of treatment, while it tended to increase in group P (median evolution, 0.2 pg/mL in A vs 34.3 pg/mL in P). The ETP levels decreased in group A and increased in group P (median evolution, -7.0 mA in A vs 12.9 mA in P). These differences of the evolution were not statistically different (MCP-1 $p=0.09$, ETP $p=0.07$), but after adjustment for age and BMI the ETP evolution reached significance (ETP adjusted, $p=0.01$). The baseline fibrinogen levels were already high in both groups (median 3.6 g/L, norm < 3.0). No prolonged effects were detected after one year.

Conclusions: Increased levels of fibrinogen are already observed in obese adolescents reflecting the procoagulant risk. ETP decreased significantly with atorvastatin treatment in comparison to the non-treated group. Atorvastatin did however not decrease inflammation parameters.

Trial registration number: 2006DR3104. Swissmedic, Swiss Agency for Therapeutic Products.

Keywords: Obesity; Adolescent; Statin; Inflammation; Hypercoagulability

List of abbreviations: CRP: C-reactive protein; P: Placebo; A: Atorvastatin; MCP-1: Monocyte chemoattractant protein 1
ETP: Endogenous thrombin potential; CVD: Cardiovascular disease; HOMA: Homeostasis model assessment of insulin resistance; MCP-1: MCP-1 induced protein; IL-6: Interleukin-6; IP-10: Interferon- γ -inducible protein; IL-10: Interleukin-10; IL-1-Ra: Interleukin-1 receptor antagonist; PT: Prothrombin time; aPTT: activated partial thromboplastin time; tLag: lag time; tMax: time-to-peak; cMax: the peak height; TNF- α : Tumor Necrosis Factor- α ; BMI: Body Mass Index; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; Tg: Triglycerides; V1: Visit 1; V2: Visit 2; V3: Visit 3; OGTT: Oral glucose tolerance test; CK: Creatine kinase; CK-MB: CK muscular fraction; DMT2: Diabetes mellitus type 2; C-vx: Cardiovascular; CCR2: Chemokine receptor 2; NIH: National Institutes of Health; BP: Blood pressure

Introduction

Obesity is a major public health concern in many countries. In Europe 4.1% of the girls and 5.4% of the boys aged 10-12 years are obese according to the International Obesity Task Force criteria [1,2]. Cardiovascular disease (CVD) manifests itself generally in adulthood, but atherosclerotic process can begin early in childhood and be accelerated by obesity, increased insulin resistance, dyslipidemia and high blood pressure, thus increasing the future adult morbidity and mortality [3,4]. For example, severe obesity in children is already associated with arterial wall stiffness and endothelial dysfunction [5]. Furthermore, an increased HOMA index (Homeostasis model assessment of insulin resistance) per se was shown to worsen the endothelial function in childhood

[6]. Glucose intolerance and a positive family history of diabetes in obese young patients account for increased cardiovascular risks; even the characteristics of the mothers (weight, height, diabetes, smoking,...) and of birth (gestational age, length, head circumference, ...) were shown to determine early alterations of the aorta in term neonates [7].

The chronic low-grade adipose and liver inflammation that is present in obesity is a major cause of the secondary systemic insulin resistance, increasing the risk of diabetes [8].

The pro-inflammatory cytokines activated by obesity contribute to the atherosclerotic development. For example, MCP-1, the MCP-1 receptor and the recently discovered MCP-1 induced protein (MCPIP), all play central roles in the monocyte-endothelial cell interactions in atherogenesis [9,10]. MCP-1 seems to be important for the monocyte/macrophage accumulation in adipose tissue, which accelerates inflammatory processes [11].

The MCP-1 level is significantly higher in obese children than in lean ones, independent of pubertal stage [12,13]. CRP and interleukin-6 (IL-6) levels are also positively linked with BMI, total body fat percentage and trunk fat mass [12]. Interferon- γ -inducible protein (IP-10) plays roles in monocytes/macrophage and T-cell chemotaxis, promotes T-cell adhesion to endothelial cells, and has an angiostatic effect [14]. IP-10 levels are also increased in chronic inflammatory arthritis [15].

It has already been shown that successful lifestyle intervention with weight loss in obese children can help to reduce the low-grade inflammation [16].

On the other hand, anti-inflammatory cytokines like interleukin-10 (IL-10) exert major inhibitory effects in that they suppress macrophage function and repress the production of pro-inflammatory cytokines. The protective role of IL-10 in atherosclerosis has been shown in animal studies and mutations affecting the IL-10 receptor have been identified in inflammatory bowel disease [17]. Interleukin-1 receptor antagonist (IL1-Ra) also negatively modulates the IL-1 inflammatory pathway [18].

Inflammation increases procoagulant factors, leading to an increased thrombotic risk. Conventional coagulation tests, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), do not clearly reflect the hypercoagulability state associated with obesity. This is why we used the endogenous thrombin potential, a global method, that evaluates the entire process of coagulation [19]. When the coagulation system is triggered, there is a transient wave of thrombin after a certain lag time (tLag), which corresponds to the clotting time. The activity level is proportional to the concentration of thrombin and to the time that it is active, i.e. to the area under the concentration curve. This area is called the endogenous thrombin potential (ETP) and reflects the function of the hemostatic system. The time-to-peak is defined by tMax and the peak height by cMax; the earlier and higher the peaks, the greater the tendency to clot. Fibrinogen levels are also markers of atherosclerotic-thrombotic risk [20] and fibrinogen values $> 5\text{g/L}$ increase the risk of thrombosis four-fold compared with the reference levels of $< 3\text{g/L}$ [21].

Beyond their effects on lipoprotein concentrations, statins have well-known anti-inflammatory actions that could account for their reduction of cardiovascular risks [22]. An *in vitro* study showed dose-dependent inhibition of MCP-1 production by human endothelial cells exposed to statins [23]. In diabetic patients, however, atorvastatin treatment for 12 weeks resulted in no significant decrease in the CRP, TNF- α (Tumor Necrosis Factor- α) or MCP-1 levels [24].

Overall, childhood obesity is associated with increased inflammatory cytokines and a hypercoagulability state, which contribute to early vascular and cardiac alterations. Reducing these factors by therapeutic interventions would contribute to reduce the risk for developing later metabolic complications.

The aim of this study was to investigate whether a therapeutic intervention with atorvastatin can reverse the inflammatory and prothrombotic markers present in obese adolescents [13].

The primary outcome was to evaluate the effect of sixteen weeks of statin treatment on pro-inflammatory cytokines, CRP and the pro-thrombotic state. The secondary outcome was the effect of statins on insulin resistance and BMI (Body Mass Index).

Methods

Participants

Over a three-year period, we recruited 33 children who were obese as defined by the international standard curve of Cole [2]. All were 12-16 years old and were outpatients at the Pediatric Endocrine clinic at the Children's University Hospital of Geneva. The local ethics committee approved the study protocol and written informed consent was obtained from the parents and the children. The exclusion criteria included having diabetes mellitus type 1 or 2, infections or use of anti-inflammatory drugs at the time of recruitment. One patient was excluded for overt diabetes type 2 requiring oral antidiabetic treatment after diabetes was discovered when the first blood test was performed. Four patients were lost during follow-up and did not fulfill the study protocol. This randomized clinical trial was analyzed by the 'per protocol' approach and 28 patients were included in the final analysis.

Procedures

A total of 28 patients were included in this randomized double-blinded study. Patients were randomized into two groups: one group received placebo for sixteen weeks (group P) and the other group received atorvastatin (group A; 0.2-0.3 mg/kg/d, max 20 mg). The tablets were prepared by the hospital pharmacy and the pharmacist revealed the randomization key at the end of the study.

Fasting glucose, insulin, total cholesterol, LDL-cholesterol (Low Density Lipoprotein-cholesterol), HDL-cholesterol (High Density Lipoprotein-cholesterol), triglycerides (Tg), cytokines (IL-1Ra, IL-6, IL-10), chemokines (MCP-1, IP-10), CRP, PT, aPTT, fibrinogen, ETP and D-dimer levels were determined at baseline (Visit 1; V1) and after sixteen weeks of treatment (Visit 2; V2). The same parameters were determined again after 1 year (Visit 3; V3) to evaluate whether any effect of statins was sustained.

Parents provided information about their family history, especially history of diabetes mellitus and cardiovascular diseases like premature coronary artery disease in first degree relatives. Children were evaluated in the morning. Their weight and height were measured using standard anthropometric techniques, and the BMI was calculated. Blood pressure was measured after the subject was lying on a bed for 10 minutes. The physical examination included determination of the pubertal stage according to the criteria of Tanner and the assessment of striae, signs of hyperandrogenism and acanthosis nigricans. All subjects were asked to fast overnight (12 hours), blood samples were obtained from subjects by a standard phlebotomy, without pressure on the arm for the ETP sample.

The oral glucose tolerance test (OGTT) was performed after overnight fasting. Blood samples for glucose and insulin determination were drawn through an intravenous line, 0 and 30 minutes before and 30, 60, 90 and 120 minutes after an oral glucose load of 1.75g/kg body weight (maximum 100g). Total creatine kinase (CK), the CK muscular fraction (CK-MB), aminotransferases, ALAT and ASAT were measured at each blood draw to document potential side effects like, myositis or hepatitis.

Laboratory analysis

Blood glucose was measured using the glucose oxidase method on a Beckman-Coulter DXC800 analyzer. Serum insulin was determined with an immunoassay on a DPC Immulite 2500 (Siemens, Marburg, Germany). CRP ultra sensitive measurements were carried out using a turbidimetric method on the DXC800 Beckman analyzer; normal level < 4 mg/L. PT, aPTT, fibrinogen, and ETP were measured with Innovin, Actin, Multifibren, and ETP kits, respectively, on a BCS analyzer (reagents and analyzer from Siemens, Marburg, Germany). D-dimer levels were determined with the VIDAS Exclusion Assay (BioMérieux, Marcy-l'Etoile, France).

Cytokines (IL-1Ra, IL-6, IL-10) and chemokine's (MCP-1, IP-10) were measured using a commercially available multiplex beads immunoassay that is based on the Luminex platform (Fluorokine MAP Multiplex Human Cytokine Panel, R&D Systems, Minneapolis, MN, USA) according to the supplier's instructions.

All measurements were performed in the same run assay to avoid inter-assay variability. All the intra-assay variation coefficients were below 8%.

The degree of insulin resistance was determined using HOMA-IR (fasting plasma insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5) [25]. Insulin resistance was defined as HOMA-IR > P95 for age and sex according to the values defined by Allard, *et al.* [26]. For the OGTT, glucose intolerance and diabetes were assessed according to WHO criteria values.

Total CK and CK-MB were measured using a DXC800 analyzer with Beckman Coulter kits.

Statistical analysis

Statistical analyses were performed with the computer program S-plus 8.0 for Windows (Insightful Corp., Seattle, USA). Data were reported as median and minimum/maximum values. The variation of the parameters from baseline Visit 1 to Visit 2 was compared between groups P and A using the Mann-Whitney test. Age and BMI at baseline were slightly unbalanced in the two groups; we therefore also tested the difference with adjustment for age and BMI, using a multivariate linear regression model. The normality of residuals was graphically inspected and only normally distributed models were interpreted. P-values less than 0.05 were considered significant. The sample size allowed the detection of large effect sizes (1.2 or more) between group P and group A with a power of 80% and a two-sided risk α of 0.05.

Results

Control group P included 12 patients, 7 females and 5 males, while the treated group A included 16 patients, 8 females and 8 males (Table 1). The two groups showed similar clinical characteristics at baseline: median age was 13.2 vs 14.3 years (p-value = 0.29), and the median BMI was 30.1 vs 33.1 kg/m² in groups P and A, respectively (p-value = 0.30). The median systolic and diastolic blood pressures were below the 90th percentile. Lipid levels, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides, were all within the normal range at baseline (V1) (Table 2). 18 adolescents had a positive family history for diabetes mellitus type 2 (DMT2) (7 in group P and 11 in group A), and 14 had a positive family history for cardiovascular disease (C-vx) (5 in group P and 9 in group A) (Table S1, supplementary data). The oral glucose tolerance test performed at baseline was normal in two patients according to WHO criteria. Of all patients, 6 were glucose intolerant and 20 showed hyperinsulinemia as defined by an insulin level > 50 mU/L. Nine patients were insulin-resistant at baseline as defined by a HOMA-IR > P95. The fibrinogen levels were already high in both groups of obese adolescents at baseline (Table 2), reflecting an increased risk of thrombosis in this population [20].

Variables	P (n=12) Median [min; max]	A (n=16) Median [min; max]	p-value °
Age, years	13.2 [12.1;15.6]	14.3 [12;16.5]	0.29
Female, n [%]	7 [58.3]	8 [50.0]	0.72
Male, n [%]	5 [41.7]	8 [50.0]	
Weight, kg	83.3 [67;107]	87.2 [75.6;128.5]	0.34
Height, cm	165.3 [155;172.5]	166 [154;177]	0.73
BMI, kg/m ²	30.1 [27.2;38.9]	33.1 [30.1;43.9]	0.30
Systolic BP, mmHg	115.5 [107;123]	116.5 [95;140]	0.76
Systolic BP p90	122.5 [120;127]	123.5 [120;130]	
Diastolic BP, mmHg	62 [48;70]	63.5 [44;79]	0.40
Diastolic BP p90	78 [77;79]	78.5 [77;80]	
Heart rate, pulse/min	68.5 [50;91]	69.5 [50;93]	0.59

Data are reported as median values with minimal and maximal values [min; max] for continuous factors and as values with percentages for categorical factors.

P: placebo group; A: atorvastatin-treated group; BMI: body mass index; BP: blood pressure; BP p90: percentile 90 adapted for sex and age

° Mann-Whitney test for continuous characteristics and Fisher exact test for gender

Table 1: Clinical characteristics of the two groups

	P (n=12) Median [min; max]	A (n=16) Median [min; max]
HOMA	3.1 [1.4;6.9]	3.2 [0.4;5.4]
MCP-1, pg/mL	137.3 [80.5;358.1]	172.2 [44.6;280.4]
IL-6, pg/mL	0	0
IP-10, pg/mL	4.3 [1.9; 9.5]	2.6 [1.3;12]
IL1-Ra, pg/mL	1330.2 [780.5;2690.4]	1219.5 [558.9;2956.1]
IL-10, pg/mL	0	0
CRP, mg/L	1.4 [0.5;5.6]	2.7 [0.5;18.5]
PT, %	96.5 [84;100]	100 [77.3;100]
aPTT, sec	26.6 [23.5;30]	26.4 [24;31.2]
Fibrinogen, g/L	3.6 [2.7;4.4]	3.6 [2.6;5.6]
D-Dimers, ng/mL	267 [124;665]	279 [131;627]
ETP, mA	366.2 [323.9;422.2]	397.1 [329.4;468.3]
tLag, sec	16.6 [15.2;18.1]	16.4 [12.9;20.4]
tMax, sec	45.2 [37.6;58]	48.6 [25.6;66.2]
cMax, nM	144.1 [127.1;161.7]	139.6 [127;183]
Cholesterol, mmol/L	3.7 [3.1;4.7]	3.4 [2.6;5]
HDL, mmol/L	1.0 [0.5;1.5]	0.95 [0.6;1.7]
LDL, mmol/L	2.4 [1.6;3.2]	2.1 [1.2;3.4]
Tg, mmol/L	0.8 [0.49;1.6]	0.7 [0.5;2.1]

Data are reported as median values, with minimal and maximal values [min; max]. P: placebo group; A: atorvastatin-treated group; HOMA: homeostatic model assessment; MCP-1: monocyte chemoattractant protein 1; IL-6: interleukin-6; IP-10: interferon- γ -inducible protein; IL1-Ra: interleukin-1 receptor antagonist; IL-10: interleukin-10; CRP: C-reactive protein; PT: prothrombin time; aPTT: activated partial thromboplastin time; ETP: endogenous thrombin potential; tLag: lag time; tMax: time to peak; cMax: peak value; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; Tg: triglycerides; sec: seconds; mA: milli-absorbance units
0 indicates values < 1 pg/mL (values below the detection level of 1 pg/mL)

Table 2: HOMA, cytokines, CRP, coagulation parameters and lipid profile at baseline V1

The second visit (V2) took place after sixteen weeks of placebo or atorvastatin treatment. The MCP-1 level was stable in group A while it tended to increase in group P (median evolution 0.2 pg/mL vs 34.3 pg/ml) (Table 3). However, the comparison of the evolution between V1 and V2 of the two groups showed that this difference was not significant (p-value = 0.09) (Table 3). The same trend was observed for the ETP, which decreased in group A and increased in group P (median evolution -7.0 mA vs 12.9 mA) (Table 3); however, the comparison of the evolution over time in the two groups did not reach statistical significance (p-value = 0.07) (Table 3); for ETP the normal distribution allowed an adjustment for age and BMI, the evolution of ETP reached then significance (p-value= 0.01) (Table 3).

We observed no changes in the levels of IP-10, IL-1Ra, IL-6 and IL-10 during follow-up; however IL-6 and IL-10 values were below detection level (Table 2). The comparison of the evolution in both groups was not significant for any of these parameters (IP-10 p-value = 0.13, IL-1Ra p-value = 0.92) (Table 3).

The BMI and the HOMA did not show significant modifications over the sixteen weeks follow-up (BMI p-value = 0.28, HOMA p-value = 0.28) (Table 3). The total cholesterol and the LDL-cholesterol decreased in group A between visit V1 and V2 and the comparison of the evolution in both groups was clearly significant for these parameters (Cholesterol p-value= 0.009, LDL-cholesterol p-value= 0.007); regarding the LDL-cholesterol which was normally distributed the significance was maintained even after the adjustment for age and BMI (LDL-cholesterol p-value= 0.01) (Table 3). This was in line with the expected effect of the statins.

All inflammation and coagulation parameters were measured again after one year at V3, i.e. eight months after the end of treatment with placebo or atorvastatin. There were no prolonged or significant effects on any of the parameters at V3 (Tables S2, S3, S4, supplementary data).

	P (n=12) Median [min; max]	A (n=16) Median [min; max]	p-value ^o	p-value ^{oo}
BMI, kg/m ²	0.6 [-0.2;3.1]	0.3 [-2.9;2.6]	0.28	
HOMA	1.0 [0;4.5]	0.6 [-1.2;5.0]	0.28	
MCP-1, pg/ml	34.3 [-135.7;96.2]	0.2 [-48.4;33.9]	0.09	
IP-10, pg/ml	-0.4 [-2.0;2.8]	0.4 [-10.0;16.1]	0.13	
IL1-Ra, pg/mL	-48.2 [-628.4;1008.6]	-23.2 [-584.1;300.9]	0.92	
CRP, mg/L	0.3 [-1.2;4.2]	0.5 [-4.4;3.5]	0.48	
PT, %	0.5 [-2.0;10.0]	0 [-11.3;18.0]	0.17	
aPTT, sec	0 [-2.4;2.3]	-0.2 [-2.1;1.8]	0.56	
Fibrinogen, g/L	0.5 [-0.1;1.6]	0.5 [-0.6;1.5]	0.36	0.44
D-Dimers, ng/ml	-14 [-175.0;88.0]	-1 [-255.0;232.0]	0.91	0.77
ETP, mA	12.9 [-19.1;35.9]	-7.0 [-63.9;43.8]	0.07	0.01*
tLag, sec	-0.2 [-2.4;2.0]	0.6 [-2.8;5.0]	0.10	0.09
tMax, sec	-6.7 [-20.3;8.7]	-4.4 [-23.4;13.4]	0.60	
cMax, nM	8.6 [-11.7;30.7]	2.0 [-46.1;31.4]	0.17	
Cholesterol, mmol/L	0.1 [-0.5;1.5]	-0.6 [-1.2;1.5]	0.009*	
HDL, mmol/L	0.1 [-0.1;0.4]	0.1 [-0.3;0.3]	1.00	0.90
LDL, mmol/L	0.2 [-0.5;1.0]	-0.4 [-1.2;1.1]	0.007*	0.01*
Tg, mmol/L	0 [-0.5;0.5]	-0.2 [-1.4;0.3]	0.27	

Data are reported as median values with minimal and maximal values [min; max].

Group P: placebo group; Group A: atorvastatin-treated group; V1: Visit 1 at baseline; V2: Visit 2 after sixteen weeks of placebo or atorvastatin treatment; BMI: body mass index; HOMA: homeostatic model assessment; MCP-1: monocyte chemoattractant protein 1; IP-10: interferon- γ -inducible protein; IL1-Ra: interleukin-1 receptor antagonist; CRP: C-reactive protein; PT: prothrombin time; aPTT: activated partial thromboplastin time; ETP: endogenous thrombin potential; tLag: lag time; tMax: time to peak; cMax: peak value; HDL: High Density Lipoprotein; LDL: Low Density lipoprotein; Tg: triglycerides; sec: seconds; mA: milli-absorbance units

The change in each parameters from baseline to the sixteenth weeks are shown for Group P and Group A. The change since baseline of each parameter has been compared between the two arms using Mann-Whitney tests. Multivariate regression analyses to compare the parameters between arms with adjustment for age and BMI were applied to the parameters with a normal distribution (Fibrinogen, D-Dimers, ETP, tLag, HDL, LDL).

^oMann-Whitney test

^{oo}Multivariate regression test

*p-values < 0.05 were considered statistically significant.

Table 3: Comparison of the evolution between group P and group A over the sixteen weeks of treatment

Side effects

We monitored all patients for potential side effects during the sixteen weeks treatment period and saw a transient increase in total CK in 9 subjects (32%); in 2 subjects, 1 in group P and the other in group A, the total CK increased slightly at V2. For the other 7 patients total CK was already increased at baseline or several months after the treatment had been stopped at V3. This was attributed to exercise.

One patient had four times increased aminotransferases at V3 more than eight months after the statin treatment had been stopped; this was attributed to a probable infection. Two patients had short-lived abdominal pain at the end of the statin treatment but showed no increase in the aminotransferase level.

Discussion

This prospective randomized double-blind study evaluated the effect of atorvastatin on inflammatory and coagulation markers and on the evolution of the BMI and insulin resistance in obese adolescents. This young population is very important to study, because early therapeutic interventions may have long-term benefits and decrease the adult morbidity and mortality.

Statins and inflammatory parameters

Statins tended to stabilize the pro-inflammatory MCP-1 level in group A. In contrast, the MCP-1 level increased over time in the untreated group P. It was recently demonstrated in rats that simvastatin significantly reduces MCP-1 and TNF- α levels as well as body mass index [27]. MCP-1 expression is increased in obese mice and is an important recruiter of macrophage precursors into atherosclerotic tissues [28]. Notably, mice with genetic inactivation of MCP-1 and its CC-chemokine receptor 2 (CCR2) are particularly resistant to atherosclerosis [9]. An MCP-1 competitor, that inhibits monocyte chemotaxis or transendothelial migration, also reduces inflammatory monocyte recruitment, limits neo-intimal hyperplasia and attenuates myocardial ischemia-reperfusion injury in mice [29]. MCP-1 is also the most increased pro-inflammatory parameter in obese children compared to lean ones and correlates positively with the degree of obesity [13,30].

In vitro studies show dose-dependent inhibition of MCP-1 production by human endothelial cells exposed to statins [23,31]. The tendency of statins to lower MCP-1 in obese adolescents, as observed in group A, could be beneficial in preventing atherosclerosis and further myocardial ischemia.

Statins have no detectable effect on other pro-inflammatory cytokines such as IL-6 and IP-10 in this study. IL-6 levels have been shown to decrease in patients with chronic heart failure with dyslipidemia during 6 months of statin therapy, and this also ameliorated cardiac function [32]. Simvastatin also significantly decreases IL-6 levels in healthy men after just 14 days [33]. It is possible that the low levels of IL-6 at baseline in our study did not allow us to detect changes in this parameter.

The anti-inflammatory cytokine IL1-Ra positively correlates with BMI and HOMA, but is decreased in patients with longstanding DMT2 [34]. We did not observe any effect of statins on IL1-Ra in the present study.

IL-10 correlates positively with BMI and has been proposed to be a negative predictive factor for the development of diabetes. We chose to measure IL-10, because it is secreted by human white adipose tissue and is a major inhibitor of pro-inflammatory cytokine synthesis and induces the release of IL-1Ra [35]. *In vivo* experiments, in apolipoprotein E-deficient mice showed that simvastatin significantly increase local expression of IL-10 in the induced atherosclerotic plaques [36]. In our study, however, we did not detect systemic induction of IL-10 with statins.

In this study, statin treatment had no effect on CRP levels. The basal CRP level was quite different between the two study groups, due to a single outlier patient in group A who had CRP values > 10 mg/L as well as high fibrinogen levels (> 5 g/L) at all time points. This patient was not excluded from the study because the high parameters were obesity related, there was no sign of a concomitant infection. CRP is increased in obese children and adolescents [13], and is also an independent major risk marker of cardiovascular complications [37]. The effects of statins on CRP levels remain controversial. Kinlay, *et al.* [38] concluded that the within-person variability of CRP level is about the same as the reduction in CRP levels from intensive statin therapy and another group found no significant changes in CRP levels by statins in type 2 diabetic patients after 12 weeks of treatment [24]. Furthermore, pharmacogenetic determinants seem to play an important role in the response to statin treatment [39].

Statins and coagulation parameters

ETP levels tended to increase in group P and the basal fibrinogen level was already high in both groups. Fibrinogen levels are markers of atherosclerotic-thrombotic risk [20] and fibrinogen values > 5g/L increase the risk of thrombosis four-fold compared with the reference of levels < 3g/L [21]. The values observed in the adolescents in this study were already predictive of vascular disease.

It is well established that clotting factors and lipid infiltration of vascular walls are important in the development of atherosclerosis. ETP is increased in obese children as young as six years of age [40] and hemostatic alterations can normalize after weight loss due to successful lifestyle intervention [41]. Patients with peripheral arterial occlusive disease treated for 8 weeks with atorvastatin showed a significant reduction of thrombin generation [42]. The mechanisms by which statins reduce thrombosis risk are unclear, but a recent review by researchers at the National Institutes of Health (NIH) confirmed that the anti-thrombotic effects of statins are likely related to their anti-inflammatory properties [43]. Our study showed a significant decrease of ETP after sixteen weeks of statins.

Statins, BMI and insulin resistance

We noted no effects of statins on body weight or on insulin resistance index HOMA. Insulin resistance, diabetes or even a positive family history of diabetes are related to early alterations of vascular and heart functions leading to the so-called diabetic cardiomyopathy [44]. In order to prevent such systemic complications, normalization of the altered glucose homeostasis is warranted. It also has to be taken into account, that statins modestly raise blood glucose levels, increasing the risk of development of diabetes mellitus [45]. Statins should therefore be used with caution in obese adolescents, especially if there is an acute risk for diabetes.

Some of the strengths of this study include the fact that the study was randomized and longitudinal in a population difficult to recruit.

For several outcomes, the p-value was above the significance level. This lack of significance may be partially due to a lack of power because the study was designed to detect large effect sizes. Limitations of this study include a relatively small number of participants.

Conclusion

In this study treatment of sixteen weeks with statins tended to stabilize the pro-inflammatory MCP-1 levels and to improve the prothrombotic state as reflected by the ETP.

Inflammatory markers predict future atherosclerotic events, and therapies that modify their concentrations can confer cardiovascular protection and prevent additional metabolic complications. Thus, modulating MCP-1 chemotactic signaling and the hypercoagulability state associated with obesity might be interesting therapeutic approaches to prevent atherosclerosis and cardio-vascular complications.

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supplementary data

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