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Effects of Atorvastatin on Human C Reactive Protein Metabolism

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Abstract

Objective—Statins are known to reduce plasma C-reactive protein (CRP) concentrations. Our goal was to define the mechanisms by which CRP was reduced by maximal dose atorvastatin.

Methods—Eight subjects with combined hyperlipidemia (5 men and 3 postmenopausal women) were enrolled in a randomized, placebo-controlled double-blind, cross over study. Subjects underwent a 15-hour primed-constant infusion with deuterated leucine after 8 weeks of placebo and 80 mg/day of atorvastatin. CRP was isolated from lipoprotein deficient plasma, (density >1.21 g/ml) by affinity chromatography. Isotopic enrichment was determined by gas chromatography/mass spectrometry. Kinetic parameters were determined using compartmental modeling. Paired *t* test and Wilcoxon signed ranks test were used to compare differences between placebo and atorvastatin.

Results—Compared with placebo, atorvastatin decreased median CRP pool size by 28.4% (13.31 ± 3.78 vs 10.26 ± 3.93 mg; $p=0.16$), associated with a median CRP fractional catabolic rate increase of 39.9% (0.34 ± 0.06 vs 0.50 ± 0.11 pools/day; $p=0.09$), with no significant effect on median CRP production rate (0.050 ± 0.01 vs 0.049 ± 0.01 mg/kg/day; $p=0.78$).

Conclusion—Our data indicate that maximal doses of atorvastatin lower plasma CRP levels by substantially decreasing the median CRP plasma residence time from 2.94 days to 2.0 days, with no significant effect on the median CRP production rate.

Keywords

Atorvastatin; C-reactive protein; lipoprotein; metabolism

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It is widely accepted that inflammation plays a clinically significant role in the development of atherosclerosis [1]. Clinical studies also support a link between chronic inflammation and coronary heart disease (CHD) [2, 3]. Furthermore, inflammatory markers have been proposed as emerging risk factors for CHD [4]. C-reactive protein (CRP) is a major acute phase reactant produced by hepatocytes. The concentration rises rapidly (within 4–6 hours), and markedly (as much as 1000-fold) after acute tissue injury or inflammation. As a marker of inflammation, CRP has been thought to have an important role in cardiovascular risk stratification and treatment decision [5]. In a 2010 meta-analysis, high sensitivity CRP (hs-CRP) was found to be an independent predictor of cardiovascular disease including CHD, ischemic stroke, and deaths due to several common cancers [6].

Statins have anti-inflammatory effects and reduce plasma CRP concentrations [7–9]. Reductions of both low density lipoprotein cholesterol (LDL-C) and CRP are important in decreasing the risk of cardiovascular events [7]. The magnitude of risk reduction obtained from statins is greater when baseline CRP levels are elevated [10, 11]. The JUPITER (Justification for the Use of Statins in Primary Prevention: an Intervention Evaluating Rosuvastatin) trial reported an association between the degree of CRP lowering achieved and the risk of cardiovascular disease. A 79% reduction in vascular events was observed in subjects who achieved both an LDL-C <1.8 mmol/l (70 mg/dl) and CRP <1 mg/l compared with placebo [12]. In addition to this primary prevention trial, the reduction of CRP levels itself or as a statin-related pleiotropic effect has also been assessed in other scenarios, including the acute phase of myocardial infarction; secondary prevention of cardiovascular disease, special groups of patients such as diabetic patients and chronic kidney disease [13]. Statin-mediated lowering of CRP levels appears to be unrelated to the magnitude of LDL-C reduction [8]. Atorvastatin significantly decreased hsCRP concentrations in subjects with or without diabetes or the metabolic syndrome [14]. In addition, the CRP reduction by atorvastatin has been reported to be dose-dependent with higher doses being more effective in decreasing CRP concentrations [14]. Atorvastatin 80 mg/day has been shown to reduce CRP concentration by 34–40% from baseline in subjects with hyperlipidemia [14, 15], and 36.4% in those with coronary heart disease who had normal-range lipid profile [16].

To date, the mechanisms by which statins reduce CRP concentrations have not been studied in humans. Stable isotope methodology is the standard method for studying *in vivo* kinetics of plasma proteins in humans. The goals of this study were to define the effects of atorvastatin 80 mg/day, relative to placebo, on the kinetics of CRP.

Subjects and Methods

Study Subjects and Design

Nine subjects with combined hyperlipidemia, five men and four postmenopausal women without hormonal replacement therapy, were recruited in the study. CRP could not be isolated in one female subject due to extremely low plasma CRP concentration after atorvastatin treatment. Therefore, kinetic analyses were based on 5 male and 3 female subjects. Plasma lipid criteria for enrollment were plasma LDL-C levels 160 mg/dl, triglyceride (TG) levels 150 mg/dl, and low high density lipoprotein cholesterol (HDL-C) levels (40 mg/dl in men, and 50 mg/dl in women). This was a randomized, double-blind,

crossover study. Subjects were instructed to follow the therapeutic lifestyle changes diet as recommended by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) throughout the study. After 3 weeks of a lead-in therapeutic lifestyle changes diet phase, subjects were randomized to receive either placebo or atorvastatin 80 mg/day treatment for 8 weeks. Each phase was separated by a washout period of at least 4-weeks. During weeks 7 and 8 of each phase, 12 hour fasting blood samples were obtained for the measurement of plasma lipids and CRP concentrations. Plasma was separated at 1000 *g* for 30 minutes at 4°C and stored at –80° C until used. During week 8 of each phase, subjects were admitted to the General Clinical Research Center of Tufts Medical Center to undergo a stable isotope study. Subjects were fed hourly for 20 hours with small identical meals starting 5 hour before and continuing throughout the infusion. A primed-constant infusion of 10 µmol/Kg body weight/hour deuterated leucine ([5,5,5-²H₃] L-leucine; C/D/N Isotopes, Inc., Pointed-Claire, Quebec, Canada) was carried out for 15 hours. Blood samples were collected in EDTA tubes just before the infusion (0 hr) and at 30, 35, 45 minute and 1, 1.5, 2, 3, 4, 6, 9, 12, 14, 15 hour during the infusion. The details of the study design were published previously [17]. All study participants provided written informed consent, and the study protocol was approved by the Institutional Review Board of Tufts University-School of Medicine and Tufts Medical Center.

Lipid and apolipoprotein measurements

Plasma total cholesterol (TC) and TG were measured by automated enzymatic assays standardized through the Centers for Disease Control [18]. Plasma LDL-C and HDL-C concentrations were measured directly with kits from Equal Diagnostics (Exton, PA) and Roche Diagnostics (Indianapolis, IN), respectively. Sequential density ultracentrifugation in a Beckman ultracentrifuge (Beckman, Palo Alto, CA) was used to separate each lipoprotein fraction from 5 ml of plasma from each infusion time point as previously described; triglyceride rich lipoproteins (TRL) $d < 1.006$ g/ml, intermediate density lipoprotein (IDL) $d = 1.006$ – 1.019 g/ml, low density lipoprotein (LDL) $d 1.019$ – 1.063 , and high density lipoprotein (HDL) $d 1.063$ – 1.21 g/ml. Apolipoprotein B (apoB) concentrations in plasma, and in the TRL and IDL fractions were measured by ELISA (BioDesign, Saco, ME). LDL apoB concentrations were calculated by subtracting TRL and IDL apoB from total plasma apoB levels. Plasma CRP concentrations were measured using a high-sensitivity immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, WA). Fasting samples from week 7 of each phase were measured for CRP concentrations in duplicate. Fasting blood samples were available for CRP measurements in 7 subjects in the placebo phase, and 6 subjects in the atorvastatin phase. Non-fasting plasma samples during week 8 infusion of each phase were used for mass measurement of kinetic data. The averages of 8 infusion timepoints (1, 2, 3, 4, 6, 9, 12, and 15 hour) were used in the calculations.

CRP isolation

CRP was isolated from the $d > 1.21$ g/ml fraction by affinity chromatography and gel electrophoresis. Briefly, EDTA was removed from the samples by an overnight dialysis against Tris-buffered saline. The EDTA-free $d > 1.21$ g/ml protein fractions were then incubated with immobilized *p*-aminophenyl phosphoryl choline resin (Thermo Scientific, Rockford, IL) in a chromatography column for 1 hour at room temperature. The unbound

fraction, which contained CRP, was eluted with Tris buffer containing 2 mM EDTA. The CRP monomer unit was separated by 12% monogradient SDS-PAGE gels for 17 hours at 50 volts, transferred to PVDF membranes, and visualized with 0.1% Coomassie blue R250. Purified human CRP (Meridian Life Science, Inc, ME) and a molecular weight standard were used to identify the isolated CRP monomer bands. CRP immunoblotting and protein identification by LC/MS/MS, using in-gel digestion and A Sequest search of the NCBI non-redundant protein database, confirmed the presence and purity of the isolated CRP proteins.

Isotopic enrichment measurements and Kinetic analysis

Isolated CRP bands were excised from the membrane, hydrolyzed in 12 N HCl at 110 °C for 24 hour, and evaporated to dryness. Amino acids were converted to heptafluorobutyramide derivatives and analyzed by a gas chromatography/mass spectrometry (Agilent Technologies 6890/5973N). Selected ion monitoring at m/z 349 (derivatized leucine – HF⁻) and m/z 352 (derivatized d₃-leucine – HF⁻) was used to determine the areas under the chromatographic peaks of each ion. Mole percentage enrichment for each sample was calculated from the areas under the curve and converted to tracer-tracee ratio as previously described [19]. The Simulation Analysis and Modeling II (SAAM II) program was used for determination of CRP kinetic parameters using multi-compartmental modeling. TRL apoB-100 plateau was used as the tracer plateau for CRP kinetic analysis. The fractional catabolic rate (FCR) of CRP was estimated after fitting the model to the CRP tracer data. The production rate (PR) of CRP was calculated as the product of FCR and pool size, which equal the plasma CRP concentration multiplied by plasma volume. Plasma volume was estimated as 4.5% of body weight in kg.

Statistical analyses

All continuous variables were checked for their distributions. Changes of parameters between placebo and atorvastatin treatment were compared using paired *t* test if they were normally distributed, and Wilcoxon Signed Ranks test if they were non-normally distributed, which was the case for all CRP related parameters. Fasting and non-fasting CRP concentrations were also compared using Wilcoxon Signed Ranks test. P values <0.05 were considered statistically significant. All analyses were performed using the SPSS statistical Package (SPSS, Chicago, IL).

Results

The 8 subjects analyzed had a mean age of 55.4±8.4 years and a mean body mass index (BMI) of 28.3±3.3 kg/m². Non-fasting plasma lipids, apolipoproteins and CRP levels of the study participants during placebo and atorvastatin 80 mg/day treatment phase are shown in table 1. As previously reported, atorvastatin treatment significantly decreased plasma levels of TC, TG, LDL-C, IDL apoB, and LDL apoB. Plasma LDL-C levels decreased by 54.3%, while HDL-C levels increased by 4.78% as compared to values on placebo. Plasma CRP levels decreased by 28.4% after 8 weeks of atorvastatin 80 mg/day treatment; however, this did not reach statistical significance [2.70 (1.43, 5.73) vs 1.76 (0.89, 3.16) mg/l; p=0.12].

Comparison of individual fasting and non-fasting plasma CRP concentrations in the study participants during placebo and atorvastatin phases is shown in Figure 1. Non-fasting CRP concentrations were significantly higher than fasting CRP concentrations [2.26 (1.27,3.21) vs. 1.70 (0.97,4.21) mg/l; $p=0.046$]. The median percent increase was 8.43% (2.80%, 19.19%) However non-fasting CRP concentrations at 1 hr and the average of 8 timepoints during the infusion were comparable [3.03 (1.23, 3.11) vs 2.26 (1.27, 3.21) mg/l; $p=1.0$] indicating that CRP levels did not change significantly during the stable isotope infusion study. CRP kinetic parameters during placebo and atorvastatin treatments are shown in table 2. CRP Tracer/Tracee ratio during a 15-hour primed-constant infusion with deuterated leucine of the study subject (A9) was presented in online supplemental material figure 1.

Atorvastatin 80 mg/day decreased median CRP pool size (PS) by 28.38% (13.31 ± 3.783 vs 10.263 ± 3.934 mg; $p=0.09$). Median CRP FCR increased by 39.91% (0.343 ± 0.056 vs 0.500 ± 0.111 pools/day; $p=0.09$), while CRP PR remained unchanged (0.050 ± 0.012 vs 0.049 ± 0.013 mg/kg/day; $p=0.78$). However, none of these parameters reached statistical significance (online supplemental material figure 2).

Discussion

It is well known that statins decreases plasma LDL-C and inflammatory markers [7]. Plasma CRP reductions have been reported to be variable according to statin type, statin dose and population studied [8, 20]. We have found that atorvastatin 80 mg/day significantly decreased plasma lipids and apolipoproteins concentrations, and decreased median CRP concentrations by 28.4%. The magnitude of CRP reduction in our study was lower than that reported in previous studies (34–40%) using the same dose of atorvastatin [14–16]. This could be explained by differences in study population characteristics, as well as the 8 week duration of atorvastatin treatment in our study.

The mechanism by which statins reduce CRP concentration in humans has not been previously evaluated. CRP is an acute phase reactant protein produced mostly by the liver in response to cytokines IL-6 and IL-1 β . A previous study has demonstrated that statins reduce the IL-1 β -inducible CRP expression in hepatocytes [21]. Arnaud, et al [22] studied the mechanism of CRP reduction by statins using human hepatoma cell line (Hep3B). They reported that statins reduced IL-6-induced CRP production at both the protein and mRNAs level in hepatocytes. This occurred via inhibition of protein geranylgeranylation, and reduced CRP gene expression by decreasing the activation of the transcription factor STAT3 [22]. A study using rosuvastatin also reported that rosuvastatin inhibit IL-6 induced CRP expression in hepatoma cells [23] However these data are all based on tissue culture experiments, and such data do not always correspond to *in vivo* observations in animals or humans. We were not able to identify any such studies in the literature.

Although *in vitro* studies provide clues to the mechanism of drugs in humans, *in vivo* kinetic studies are the standard method to examine plasma protein metabolism. To date, only one study had examined the metabolism of CRP in humans using stable isotope methodology [24]. The authors found that plasma CRP concentration significantly correlated with both CRP production rate and fractional catabolic rate. Moreover, the production rate appeared to

be the main determinant of CRP concentrations, and showed associations with features of the metabolic syndrome and IL-6 [24]. Our study is the first, to our knowledge, to study the effect of a statin on CRP kinetics in humans using stable isotope methodology. We found that atorvastatin enhanced the fractional catabolism of CRP by 39.9%. Although an *in vitro* study suggested that statins inhibit CRP and IL-6 production, we did not find any reductions in CRP production rate after 8 weeks of atorvastatin 80 mg/day treatment. In fact we noted that atorvastatin treatment was associated with a modest increase in CRP production as compared to placebo. In addition, a recent large population-based study examining the associations between statin use, CRP and inflammatory cytokines showed that statins users had significantly lower CRP levels as compared with statin non-users [25]. However there was no significant associations between statin use and IL-6, IL-1 β , and TNF- α levels [25].

Previous studies reported significant variations in circulating inflammatory markers concentrations after food intake in male subjects [26–30]. Consumption of a high fat meal resulted in significant rise in IL-6 and decreased TNF- α concentrations [26, 31]. However, data on postprandial CRP responses are equivocal. Carroll, et al reported a significant rise in serum CRP concentrations after high fat meal in diabetic patients [32] while Payette, et al reported no significant changes in serum CRP concentration 4 and 8 hours after consuming a high fat meal [31]. Nowadays, most experts recommend non-fasting blood samples for CRP assessment because CRP levels are stable over a long time and demonstrate almost no circadian rhythm [33]. We found that non-fasting CRP concentration were significantly higher than fasting CRP concentrations. This indicates that food intake significantly raised CRP concentrations. This is consistent with our earlier study showing that feeding increased median CRP levels by 22% in normal subjects [20]. Furthermore, atorvastatin 40 mg/day decreased both fasting and postprandial CRP to a similar extent [20]. Despite significant increases in CRP levels with food intake, the magnitude of absolute CRP increases was relatively small, and might not influence changes in cardiovascular risk criteria based on CRP levels.

Our study has some limitations. The sample size was small and the magnitude of CRP reduction after atorvastatin treatment was somewhat less than previously reported with the same dose of atorvastatin. Therefore, the reduction in CRP concentration and CRP PS were not statistically significant in this study. Metabolic studies with a larger sample size should be carried out to confirm our findings. Moreover, we cannot address if the finding of increasing CRP fractional catabolism by statin was due to the anti-inflammatory effects of statins or rather due to the fact that statins enhance the clearance of apoB containing lipoproteins. We have recently documented a significant association between CRP metabolism and TRL apoB-100 and apoB-48 catabolism. Why this linkage occurred is unclear except that both CRP and TRL apoB are catabolized by the liver, and their catabolism appears to be regulated by common mechanisms [34].

In conclusion, our data is consistent with the novel concept that statins reduce plasma CRP levels, not by lowering production, but by enhancing CRP fractional catabolic rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* 2003; 111:1805–1812. [PubMed: 12813013]
2. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *Jama.* 1998; 279:1477–1482. [PubMed: 9600484]
3. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* 2004; 351:2599–2610. [PubMed: 15602020]
4. Genest J. C-reactive protein: risk factor, biomarker and/or therapeutic target? *Can J Cardiol.* 2010; 26 (Suppl A):41A–44A.
5. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003; 107:499–511. [PubMed: 12551878]
6. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet.* 2010; 375:132–140. [PubMed: 20031199]
7. Quist-Paulsen P. Statins and inflammation: an update. *Curr Opin Cardiol.* 2010; 25:399–405. [PubMed: 20421792]
8. Asher J, Houston M. Statins and C-reactive protein levels. *J Clin Hypertens (Greenwich).* 2007; 9:622–628. [PubMed: 17673884]
9. Biasucci LM, Biasillo G, Stefanelli A. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. *Clin Chem Lab Med.* 2010; 48:1685–1691. [PubMed: 20868311]
10. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med.* 2001; 344:1959–1965. [PubMed: 11430324]
11. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1998; 98:839–844. [PubMed: 9738637]
12. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet.* 2009; 373:1175–1182. [PubMed: 19329177]
13. Martinez VB, Gonzalez-Juanatey JR. Markers of inflammation and cardiovascular disease: clinical applications of C-reactive protein determination. *Am J Cardiovasc Drugs.* 2009; 9 (Suppl 1):3–7. [PubMed: 20000881]
14. Gensini GF, Gori AM, Dilaghi B, Rostagno C, Gaw A, Blanco-Colio LM, et al. Effect of atorvastatin on circulating hsCRP concentrations: a sub-study of the achieve cholesterol targets

fast with atorvastatin stratified titration (ACTFAST) study. *Int J Cardiol.* 2010; 142:257–264. [PubMed: 19217176]

15. Thongtang N, Ai M, Otokozawa S, Himbergen TV, Asztalos BF, Nakajima K, et al. Effects of maximal atorvastatin and rosuvastatin treatment on markers of glucose homeostasis and inflammation. *Am J Cardiol.* 2011; 107:387–392. [PubMed: 21257003]
16. Bonnet J, McPherson R, Tedgui A, Simoneau D, Nozza A, Martineau P, et al. Comparative effects of 10-mg versus 80-mg Atorvastatin on high-sensitivity C-reactive protein in patients with stable coronary artery disease: results of the CAP (Comparative Atorvastatin Pleiotropic effects) study. *Clin Ther.* 2008; 30:2298–2313. [PubMed: 19167589]
17. Lamon-Fava S, Diffenderfer MR, Barrett PH, Buchsbaum A, Matthan NR, Lichtenstein AH, et al. Effects of different doses of atorvastatin on human apolipoprotein B-100, B-48, and A-I metabolism. *J Lipid Res.* 2007; 48:1746–1753. [PubMed: 17526934]
18. McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta.* 1987; 166:1–8. [PubMed: 3608193]
19. Cobelli C, Toffolo G, Bier DM, Nosadini R. Models to interpret kinetic data in stable isotope tracer studies. *Am J Physiol.* 1987; 253:E551–564. [PubMed: 3688225]
20. Schaefer EJ, McNamara JR, Asztalos BF, Tayler T, Daly JA, Gleason JL, et al. Effects of atorvastatin versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A2 in patients with coronary heart disease versus control subjects. *Am J Cardiol.* 2005; 95:1025–1032. [PubMed: 15842965]
21. Kleemann R, Verschuren L, de Rooij BJ, Lindeman J, de Maat MM, Szalai AJ, et al. Evidence for anti-inflammatory activity of statins and PPARalpha activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. *Blood.* 2004; 103:4188–4194. [PubMed: 14976045]
22. Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D, et al. Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. *Arterioscler Thromb Vasc Biol.* 2005; 25:1231–1236. [PubMed: 15790934]
23. Mayer C, Gruber HJ, Landl EM, Pailer S, Scharnagl H, Truschnig-Wilders M, et al. Rosuvastatin reduces interleukin-6-induced expression of C-reactive protein in human hepatocytes in a STAT3- and C/EBP-dependent fashion. *Int J Clin Pharmacol Ther.* 2007; 45:319–327. [PubMed: 17595889]
24. Mauger JF, Levesque J, Paradis ME, Bergeron N, Tchernof A, Couture P, et al. Intravascular kinetics of C-reactive protein and their relationships with features of the metabolic syndrome. *J Clin Endocrinol Metab.* 2008; 93:3158–3164. [PubMed: 18492753]
25. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: A population-based ColaUS study. *Atherosclerosis.* 2011
26. Blackburn P, Despres JP, Lamarche B, Tremblay A, Bergeron J, Lemieux I, et al. Postprandial variations of plasma inflammatory markers in abdominally obese men. *Obesity (Silver Spring).* 2006; 14:1747–1754. [PubMed: 17062804]
27. Gill JM, Caslake MJ, McAllister C, Tsofliou F, Ferrell WR, Packard CJ, et al. Effects of short-term detraining on postprandial metabolism, endothelial function, and inflammation in endurance-trained men: dissociation between changes in triglyceride metabolism and endothelial function. *j.gill@bio.gla.ac.uk. J Clin Endocrinol Metab.* 2003; 88:4328–4335. [PubMed: 12970306]
28. Manning PJ, Sutherland WH, Hendry G, de Jong SA, McGrath M, Williams SM. Changes in circulating postprandial proinflammatory cytokine concentrations in diet-controlled type 2 diabetes and the effect of ingested fat. *Diabetes Care.* 2004; 27:2509–2511. [PubMed: 15451929]
29. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol.* 2002; 39:1145–1150. [PubMed: 11923038]
30. Orban Z, Remaley AT, Sampson M, Trajanoski Z, Chrousos GP. The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. *J Clin Endocrinol Metab.* 1999; 84:2126–2133. [PubMed: 10372721]

31. Payette C, Blackburn P, Lamarche B, Tremblay A, Bergeron J, Lemieux I, et al. Sex differences in postprandial plasma tumor necrosis factor- α , interleukin-6, and C-reactive protein concentrations. *Metabolism*. 2009; 58:1593–1601. [PubMed: 19604525]
32. Carroll MF, Schade DS. Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation*. 2003; 108:24–31. [PubMed: 12821556]
33. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003; 107:363–369. [PubMed: 12551853]
34. Thongtang N, Diffenderfer MR, Ooi EM, Asztalos BF, Dolnikowski GG, Lamon-Fava S, et al. Linkage between C-reactive protein and triglyceride-rich lipoprotein metabolism. *Metabolism*. 2012 Sep 24. pii: S0026-0495(12)00312-5. Epub ahead of print. doi: 10.1016/j.metabol.2012.08.008

Highlights

- Statins are known to reduce plasma C-reactive protein (CRP) concentrations
- We aim to define the effects of atorvastatin 80 mg/day vs placebo on CRP kinetics
- Atorvastatin lower plasma CRP levels by substantially increasing CRP catabolism
- There was no significant effect of atorvastatin on CRP production rate

Fig 1.
Comparison between fasting and non-fasting plasma CRP concentrations of the study participants during placebo and atorvastatin treatment(N=13).

TABLE 1

Non-fasting Plasma lipids, Apolipoprotein B, and CRP Concentrations of the Study Participants (n=8)

Parameter	Placebo	Atorvastatin 80 mg	Absolute change	Percent change	P values
Total cholesterol (mg/day)	233.9±24.5	134.8±21.8	-99.2±21.1	-42.3%	<0.001
Triglyceride (mg/dl)*	254.5(235.5,362.4)	175.0(158.7,246.2)	-79.8(-166.6,-27.5)	-33.3%	0.025
LDL-C (mg/dl)	147.5±25.0	67.5±15.4	-80.0±16.0	-54.3%	<0.001
HDL-C (mg/dl)	34.9±5.93	36.3±5.71	+1.34±3.47	+4.78%	0.312
VLDL apoB	9.90±2.46	8.27±1.56	-1.64±2.84	-11.1%	0.147
IDL apoB	3.30±1.00	2.07±0.43	-1.23±0.86	-32.8%	0.005
LDL apoB	96.6±12.2	51.1±9.32	-45.5±10.2	-54.3%	<0.001
CRP (mg/L)*	2.70(1.43,5.73)	1.76(0.89,3.16)	-0.42(-1.74,-0.02)	-28.4%	0.123

Values are expressed as mean ± SD or *median (interquartile range)

P values comparing differences between placebo and atorvastatin 80 mg/day (paired T test and Wilcoxon signed ranks test for non-normally distributed data)

LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; VLDL, very low density lipoprotein; apoB, apolipoprotein B; IDL, intermediate density lipoprotein

TABLE 2

CRP Kinetic Parameters During the Placebo and Atorvastatin 80 mg/day Phases

Parameters	Placebo	Atorvastatin 80 mg/day	Median percent change	P values
CRP PS (mg)	13.31±3.783	10.263±3.934	-28.38%	0.086
CRP FCR (pools/day)	0.343±0.056	0.500±0.111	+39.91%	0.093
CRP PR (mg/Kg/D)	0.050±0.012	0.049±0.013	+14.63%	0.779
CRP PR (mg/day)	4.392±1.146	4.258±1.251	+15.43%	0.674

Values expressed as mean ± SEM

P values comparing placebo and atorvastatin treatment (Wilcoxon signed ranks test)

PS, pool size; FCR, fractional catabolic rate; PR, production rate