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Associations between autoantibodies against apo B-100 peptides and vascular complications in patients with type 2 diabetes

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Abstract

Aims/hypothesis. Oxidation of LDL in the arterial extracellular matrix is a key event in the development of atherosclerosis and autoantibodies against oxidized LDL antigens reflect disease severity and risk for development of acute cardiovascular events. Since type 2 diabetes is associated with increased oxidative stress we tested the hypothesis that autoantibodies against oxidized LDL antigens are biomarkers for vascular complications in diabetes.

Methods. We studied 497 patients with type 2 diabetes without clinical signs of coronary heart disease. Oxidized LDL autoantibodies were determined by ELISA detecting IgG and IgM specific for native and malondialdehyde (MDA)-modified apolipoprotein (apo) B-100 peptides p45 and p210. The severity of coronary disease was assessed by coronary artery calcium score.

Results. Patients affected by retinopathy had significantly higher levels of IgG against MDA-p45 and MDA-p210. In contrast, high levels of autoantibodies against the corresponding native peptides were associated with less coronary calcification and a lower risk for progression of coronary disease.

Conclusions/interpretation. Our observations suggest that LDL oxidation is involved in the pathogenesis of diabetic retinopathy and that autoantibodies

against apo B peptides may act as biomarkers for both micro- and macrovascular complications in diabetes.

Key words: Retinopathy, peripheral neuropathy, LDL, oxidation, antibodies

Abbreviations

Apo B	Apolipoprotein B
BMI	Body mass index
CAC	Coronary artery calcium score
CRP	C reactive protein
LDL	Low density lipoprotein
MDA	Malondialdehyde
MPS	Myocardial perfusion scintigraphy
IMT	Intima-media thickness

Introduction

The role of immune responses against modified self antigens in atherosclerosis has attracted increasing attention during recent years [1, 2]. Hypercholesterolemic mice deficient for components of the innate [3, 4] and adaptive [5-8] immune systems are generally characterized by decreased plaque formation suggesting that immune responses against self antigens modified by hypercholesterolemia are pro-atherogenic. Oxidized LDL is considered as the most important self antigen generated by hypercholesterolemia and autoantibodies against oxidized LDL are abundant in both hypercholesterolemic animals and in humans [9-11]. However, unexpected findings of decreased atherosclerosis development in hypercholesterolemic animals immunized with oxidized LDL have demonstrated that athero-protective immune responses also exist [12-16]. Collectively these studies imply that the initiation and progression of atherosclerosis is modulated by the balance between protective and pro-atherogenic immunity [17].

The immunogenic properties of oxidized LDL is dependent on the formation of oxidized phospholipids, fragmentation of the LDL protein apo B-100 as well as aldehyde-modification of these apo B fragments [10, 18]. We have

previously identified several native and aldehyde-modified apo B peptide sequences that are targeted by autoimmune responses in humans [19]. Immune responses against the apo B amino acids 661-680 (p45) and 3136-3155 (p210) have been found to be of particular importance and pilot vaccines based on these peptides significantly inhibit atherosclerosis development in experimental animals [20, 21]. Subjects with high levels of IgG against the native forms of p45 [22] and p210 [23] have a lower risk for development of acute myocardial infarction while high levels of IgM against aldehyde-modified p45 and p210 are associated with more advanced carotid atherosclerosis [19, 24]. An inverse relation between IgG against native p210 and severity of coronary atherosclerosis has recently also been reported [23].

Type 2 diabetes is associated with both an increased vascular oxidative stress and presence of LDL with an increased susceptibility to oxidation [25]. However, the possible relations between autoimmune responses to modified self-antigens in oxidized LDL and vascular disease in diabetes remain largely unexplored. The aim of the present studies was to determine if there is an association between autoantibodies against the apo B antigens p45 and p210 and vascular complications in patients with type 2 diabetes.

Methods

Patients

The subjects included in the present analysis were derived from a cohort of type 2 diabetic patients without history or symptoms of coronary heart disease participating in a study of the ability of coronary calcium score to predict silent myocardial ischemia and short term cardiovascular events [26]. The patient recruitment, inclusion and exclusion criteria have previously been reported [26]. The study patients were prospectively recruited from 4 diabetes clinics in secondary care. The study was approved by the local research ethics committees of participating institutions and the Administration of Radioactive Substances Advisory Committee and all subjects gave informed consent. A total of 510 subjects were prospectively enrolled into the study. Symptoms of peripheral neuropathy were recorded and sensation (using 10g monofilament), knee and ankle reflexes were assessed. Retinopathy was assessed by annual retinal examination according to national guidelines [27]. Fasting blood and urine samples were obtained for DCCT-aligned HbA_{1c}, lipid profile, urea, creatinine, and urine albumin/creatinine ratio. Microalbuminuria was defined as a urine

albumin/creatinine ratio > 2.5 mg/mmol for men and > 3.5 mg/mmol for women.

Coronary Calcium Imaging and Myocardial Perfusion scintigraphy

The protocol for CAC imaging and myocardial perfusion scintigraphy (MPS) have been previously described [28]. In the present study CAC scores were classified into two categories; low to moderate coronary calcification (≤ 400 Agatston units) and severe to extensive (> 400 Agatston units) [28, 29]. MPS was performed on a 2-day stress-rest ^{99m}Tc sestamibi protocol using symptom-limited treadmill exercise.

ELISA against the MDA-modified apo B-100 peptide

Polypeptide corresponding to the amino acids 661 and 680 in human apo B-100 (p45; IEIGLEGKGFEP TLEALFGK) and 3136-3155 amino acid sequence of human apo B-100 (p210; KTTKQSF DLSVKAQYKKNKH) were synthesized (KJ Ross Petersen AS, Horsholm, Denmark) and modified by 0.5 M MDA for 3 h at 37 °C. They were subsequently used in ELISAs for determination of peptide-specific antibodies as previously described [19].

Statistical analysis

Differences regarding baseline characteristics were tested by *t*-test and chi-square test, as applicable. Skewed variables were log-transformed before statistical tests. Student's *t*-test was used to test for significance between group means. Spearman correlation coefficients were used to examine the relationship among continuous variables. A logistic regression model was used to determine independent associations of various risk factors with retinopathy, peripheral neuropathy and coronary calcification, respectively. A p-value of <0.05 was considered as significant.

Results

The subjects included in the present analysis were recruited from a cohort of patients with type 2 diabetes participating in a study of the ability of coronary calcium score to predict silent myocardial ischemia and short term cardiovascular events [26]. Of the original 510 subjects enrolled, 13 were excluded from the present studies because plasma samples were no longer available. Of the remaining 497 diabetic patients 114 (22.9%) suffered from retinopathy, 90 (18.1%) from peripheral neuropathy and 71 (14.4%) had microalbuminuria. Since patients with clinically manifest coronary artery disease, cerebrovascular disease and peripheral artery disease were excluded

from the initial study we used coronary calcium scores and ischemia as assessed by myocardial perfusion scintigraphy as surrogate markers for diabetic macrovascular complications. The clinical characteristics of the study cohort have been previously published [26, 30].

Autoantibodies against native and MDA-modified apo B peptides p45 and p210 were detected by custom-made ELISAs. The highest antibody levels were found against the p210 peptide. Antibody levels against MDA-modified peptides were generally higher than against the corresponding native peptide. This was particularly evident for p210 for which both IgG and IgM levels were three-fold higher for the MDA-peptide (figure 1).

Autoantibodies against apo B peptides and microvascular complications

Patients with retinopathy had significantly higher levels of IgG against MDA-p45 and MDA-p210 than patients without retinopathy (figure 2). They also had significantly higher levels of IgG against native p210 and a similar trend could be observed for IgG against native p45 (figure 2). There were no differences in p45 or p210 IgM levels between patients with retinopathy and patients without retinopathy (data not shown). Patients that had developed retinopathy were older, had a longer duration of diabetes, increased HbA1c,

moderately elevated HDL and were more frequently on insulin treatment (Table 1). When all of these variables were entered into a logistic regression model MDA-p210 IgG (β -coefficient 1.98, $p < 0.001$), age (β -coefficient 0.04, $p = 0.005$), duration of diabetes (β -coefficient 0.06, $p = 0.001$) and HbA1c levels (β -coefficient 0.22, $p = 0.001$) remained as independent predictors of the presence of retinopathy. To further control for the possible influence of the diabetes duration we restricted the analysis to subjects with duration of disease less than 10 years ($n = 333$). Increased levels of MDA-p210 IgG was found in patients with retinopathy ($n = 53$) also in this subgroup (1.05 ± 0.24 versus 0.89 ± 0.26 abs units, $p < 0.001$).

MDA-p210 IgG levels increased with age, higher blood pressure and higher LDL levels (Table 2). High levels of MDA-p210 IgG were also associated with low triglycerides and HDL unexpectedly suggesting that antibody levels are increased in states of good metabolic control. However, there was no association between MDA-p210 IgG and HbA1c levels or duration of diabetes.

Patients with clinical manifestations of peripheral neuropathy had higher levels of MDA-p210 IgG than patients without signs of neuropathy

(1.02 ± 0.26 versus 0.89 ± 0.27 , $p < 0.001$), whereas there were no differences between patients with or without neuropathy for any of the other apo B peptide autoantibodies analyzed. Patients with neuropathy were also characterized by a longer duration of diabetes and were more often on insulin treatment (Table 3). When entered into a logistic regression model only MDA-p210 IgG remained as independent predictor of the presence of neuropathy. Again when the analysis was restricted to subjects with duration of diabetes of less than 10 years increased levels of MDA-p210 IgG was found in patients with neuropathy ($n=54$; 1.05 ± 0.22 versus 0.89 ± 0.27 abs units, $p < 0.001$).

There were no differences in autoantibodies against p45 and p210 in patients with or without presence of microalbuminuria.

Autoantibodies against apo B peptides and coronary heart disease

Patients were stratified as low to moderate coronary calcification (≤ 400 Agatston units, $n=438$) and severe to extensive (>400 Agatston units, $n=59$) [28]. Patients with low to moderate coronary calcification had higher plasma levels of both IgG and IgM against native p45 (0.26 ± 0.31 versus 0.19 ± 0.23 abs units, $p < 0.05$ and 0.062 ± 0.11 versus 0.048 ± 0.111 abs units,

p=0.005, respectively) and native p210 (0.29±0.174 versus 0.24±0.14 abs units, p<0.01 and 0.48±0.24 versus 0.39±0.18 abs units, p=0.005, respectively; figure 3). When controlling for age, systolic blood pressure, duration of diabetes, LDL, HDL, triglycerides and HbA1c in a logistic regression analysis IgG against p210, but not against p45, remained independently associated with the severity of coronary calcification. Follow-up CAC imaging was performed in 398 subjects (age 52±8 years, 61% male and HbA1c 8±1.5%) [30]. The mean follow-up time was 2.5±0.4 years. Progression of CAC was defined as a change of ≥ 2.5 between the square root transformed values of baseline and follow-up volumetric scores. Patients with coronary calcium progression (n=119) had lower plasma levels of IgG against native p45 than patients without progression (0.19±0.21 versus 0.29±0.33 abs units, p<0.001), whereas there was no association between antibodies against p210 and CAC progression. Native p210 IgG levels were significantly related to higher HDL and lower triglyceride levels suggesting an association with good metabolic control, while there was no significant association between IgG against native p45 and age or metabolic measurements (Table 2).

During the follow-up studies twenty events occurred (two cardiac deaths, nine non-fatal MIs, three non-hemorrhagic strokes and three late revascularizations) [30]. No differences were observed in antibody levels between patients that suffered from a cardiovascular event and those that remained event-free (data not shown). MPS data was available for 174 patients. There were no significant differences in antibody levels among patients with normal myocardial perfusion and those with varying degrees of myocardial ischemia (data not shown).

Discussion

In the present studies we identified increased levels of IgG against the MDA-modified apo B epitopes p45 and p210 in type 2 diabetes patients suffering from retinopathy and peripheral neuropathy. This suggests that oxidation of LDL may be involved in the development of diabetic microvascular complications and that autoantibodies against MDA-p210 represent potential biomarkers for diabetic retinopathy and peripheral neuropathy. We also demonstrate that subjects with high antibody levels against the native form of these peptides have less coronary calcification as well as less progression of coronary disease. This observation is well in line with previous studies demonstrating a lower cardiovascular risk in patients

with high levels of autoantibodies against native apo B peptides [22, 23].

Our findings provide evidence for a role of immune responses against LDL-associated antigens in diabetic complications but suggest that these may be different between micro- and macrovascular complications.

The possibility that oxidation of lipoproteins entrapped in the extracellular matrix of retinal vessels may contribute to the development of diabetic retinopathy remains largely unexplored. Oxidized LDL is pro-inflammatory and potentially cytotoxic for endothelial cells [31, 32]. It may also activate autoimmune responses that cause further damage to the affected tissue [1]. Dysregulation of lipoprotein metabolism in type 2 diabetes leads to the formation of small dense LDL particles with increased susceptibility to oxidation [33]. Accordingly, the present observations of increased levels of autoantibodies against the oxidized LDL-specific antigens MDA-p45 and MDA-p210 in patients with diabetic retinopathy is compatible with the notion that the combination of increased retinal endothelial oxidative stress and presence of LDL particles with increased oxidation susceptibility contributes to an increased local oxidation of LDL. This possibility is in line with epidemiological data demonstrating that hyperlipidemia increases the risk for development of maculopathy in diabetes as well as with the

observation that statin therapy may protect against the development of age-related macula degeneration [34-36]. The possibility that autoimmune responses against modified self antigens in oxidized LDL is of functional importance for the development of diabetic retinopathy requires evaluation in appropriate animal models.

Diabetic neuropathy has many different clinical manifestations and is one of the most common complications of diabetes [37]. Like proliferative retinopathy its severity parallels the degree and duration of hyperglycemia and the pathophysiology is believed to involve an increased intracellular stress. Its possible association with LDL oxidation remains essentially unexplored. Again, the present observations argue that this concept should be explored in appropriate animal models.

Another possibility is that the elevated levels of MDA apo B peptide autoantibodies in patients with diabetic retinopathy and peripheral neuropathy only act as markers of the disease. There are relatively few studies of the association of oxidized LDL autoantibodies with diabetes and its complications. Festa et al [38] have reported increased anti-oxidized LDL IgG levels in patients with type 1 diabetes as compared to controls, but also that antibody levels were decreased in patients with a long duration of

diabetes and high HbA1c levels. The latter observation was explained by an increased formation of oxidized LDL-specific immune complexes. Increased levels of oxidized LDL-specific immune complexes has also been linked to both micro- and macrovascular complications in diabetes [39-41].

The associations between oxidized LDL autoantibodies and cardiovascular disease have been extensively studied but with inconsistent results [42-47]. The reason for this remains to be elucidated but may involve technical difficulties in standardizing ELISAs based on a complex and poorly defined antigen such as oxidized LDL. One approach to circumvent this problem has been to use more precisely characterized oxidized LDL antigens such as native and MDA-modified peptide fragments of apo B [19]. The limitation of this approach is that only some of the antibodies generated against oxidized LDL are detected, but at the same time it offers the advantage of improved specificity and reproducibility. Here we demonstrate that high antibody levels against the native p45 and p210 apo B peptides are associated with less severe coronary calcification. Moreover, we show that patients with high baseline plasma levels of native p45 IgG have reduced progression of coronary calcification. These observations are in good agreement with previous studies demonstrating that subjects with high levels

of IgG against native p45 and p210 are at lower risk of developing acute cardiovascular events [22, 23]. An adverse association has also been found between IgG against native p210 and coronary atherosclerosis [23]. Studies evaluating the functional role of immune responses against native apo B peptides have shown that immunization of hypercholesterolemic mice with the p45 and p210 peptides inhibits the development of atherosclerosis suggesting that it may be possible to develop apo B peptide-based vaccines for prevention of cardiovascular disease. The present observations provide some indirect support for the notion that such vaccines could be effective also in patients with diabetes.

There has been an increasing interest in identifying novel biomarkers that can be used to predict the risk for development and to monitor treatment for diabetic vascular complications. The present observations suggest that autoantibodies against the MDA-modified apo B peptide p210 are possible biomarkers for diabetic retinopathy and peripheral neuropathy, while antibodies against the corresponding native peptides are potential biomarkers for macrovascular complications. These possibilities need to be addressed in appropriate animal models as well as in large prospective clinical studies.

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Disclosures

None.

Figure legends

Figure 1. Box plots showing plasma levels of (A) IgG and (B) IgM against native and MDA-modified apo B peptides p45 and p210 in the study cohort.

Figure 2. Box plots showing plasma levels of IgG against (A) native p45, (B) MDA-p45, (C) native p210 and (D) MDA-p210 in diabetic subjects with or without retinopathy.

Figure 3. Box plots showing plasma levels of (A) IgG against native p45, (B) IgM against native p45, (C) IgG against native p210 and (D) IgM against native p210 in diabetic subjects with low to moderate coronary calcification (≤ 400 Agatston units) and severe to extensive (> 400 Agatston units). CAC; Coronary artery calcium score.

References

- [1] Binder CJ, Chang MK, Shaw PX, et al. (2002) Innate and acquired immunity in atherogenesis. *Nat Med* 8: 1218-1226
- [2] Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 6: 508-519
- [3] Bjorkbacka H, Kunjathoor VV, Moore KJ, et al. (2004) Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* 10: 416-421
- [4] Michelsen KS, Wong MH, Shah PK, et al. (2004) Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proc Natl Acad Sci U S A* 101: 10679-10684
- [5] Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C (1997) IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. *J Clin Invest* 99: 2752-2761
- [6] Lee TS, Yen HC, Pan CC, Chau LY (1999) The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 19: 734-742.
- [7] Tupin E, Nicoletti A, Elhage R, et al. (2004) CD1d-dependent Activation of NKT Cells Aggravates Atherosclerosis. *J Exp Med* 199: 417-422
- [8] Zhou X, Robertson AK, Rudling M, Parini P, Hansson GK (2005) Lesion development and response to immunization reveal a complex role for CD4 in atherosclerosis. *Circ Res* 96: 427-434
- [9] Glass CK, Witztum JL (2001) Atherosclerosis: The Road Ahead. *Cell* 104: 503-516
- [10] Palinski W, Witztum JL (2000) Immune responses to oxidative neopeptides on LDL and phospholipids modulate the development of atherosclerosis. *J Intern Med* 247: 371-380.
- [11] Hulthe J (2004) Antibodies to oxidized LDL in atherosclerosis development--clinical and animal studies. *Clin Chim Acta* 348: 1-8
- [12] Palinski W, Miller E, Witztum JL (1995) Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci U S A* 92: 821-825.
- [13] Ameli S, Hultgardh-Nilsson A, Regnstrom J, et al. (1996) Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 16: 1074-1079
- [14] Nilsson J, Calara F, Regnstrom J, et al. (1997) Immunization with homologous oxidized low density lipoprotein reduces neointimal formation after balloon injury in hypercholesterolemic rabbits. *J Am Coll Cardiol* 30: 1886-1891
- [15] George J, Afek A, Gilburd B, et al. (1998) Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis* 138: 147-152.
- [16] Zhou X, Caligiuri G, Hamsten A, Lefvert AK, Hansson GK (2001) LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. *Arterioscler Thromb Vasc Biol* 21: 108-114.
- [17] Nilsson J, Hansson GK (2008) Autoimmunity in atherosclerosis: a protective response losing control? *J Intern Med* 263: 464-478

- [18] Nilsson J, Hansson GK, Shah PK (2005) Immunomodulation of atherosclerosis: implications for vaccine development. *Arterioscler Thromb Vasc Biol* 25: 18-28
- [19] Fredrikson GN, Hedblad B, Berglund G, et al. (2003) Identification of immune responses against aldehyde-modified peptide sequences in apo B-100 associated with cardiovascular disease. *Arterioscler Thromb Vasc Biol* 23: 872-878
- [20] Fredrikson GN, Soderberg I, Lindholm M, et al. (2003) Inhibition of Atherosclerosis in ApoE-Null Mice by Immunization with ApoB-100 Peptide Sequences. *Arterioscler Thromb Vasc Biol* 23: 879-884
- [21] Fredrikson GN, Andersson L, Soderberg I, et al. (2005) Atheroprotective immunization with MDA-modified apo B-100 peptide sequences is associated with activation of Th2 specific antibody expression. *Autoimmunity* 38: 171-179
- [22] Fredrikson GN, Schiopu A, Berglund G, Alm R, Shah PK, Nilsson J (2007) Autoantibody against the amino acid sequence 661-680 in apo B-100 is associated with decreased carotid stenosis and cardiovascular events. *Atherosclerosis* 194: e188-192
- [23] Sjogren P, Fredrikson GN, Samnegard A, et al. (2008) High plasma concentrations of autoantibodies against native peptide 210 of apoB-100 are related to less coronary atherosclerosis and lower risk of myocardial infarction. *Eur Heart J* 29: 2218-2226
- [24] Fredrikson GN, Hedblad B, Berglund G, et al. (2007) Association between IgM against an aldehyde-modified peptide in apolipoprotein B-100 and progression of carotid disease. *Stroke* 38: 1495-1500
- [25] Nilsson J, Bengtsson E, Fredrikson GN, Bjorkbacka H (2008) Inflammation and immunity in diabetic vascular complications. *Curr Opin Lipidol* 19: 519-524
- [26] Anand DV, Lim E, Hopkins D, et al. (2006) Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. *Eur Heart J* 27: 713-721
- [27] Harding S, Greenwood R, Aldington S, et al. (2003) Grading and disease management in national screening for diabetic retinopathy in England and Wales. *Diabet Med* 20: 965-971
- [28] Anand DV, Lahiri A, Lim E, Hopkins D, Corder R (2006) The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. *J Am Coll Cardiol* 47: 1850-1857
- [29] Shaw LJ, Raggi P, Schisterman E, Berman DS, Callister TQ (2003) Prognostic value of cardiac risk factors and coronary artery calcium screening for all-cause mortality. *Radiology* 228: 826-833
- [30] Anand DV, Lim E, Darko D, et al. (2007) Determinants of progression of coronary artery calcification in type 2 diabetes role of glycemic control and inflammatory/vascular calcification markers. *J Am Coll Cardiol* 50: 2218-2225
- [31] Berliner J, Leitinger N, Watson A, Huber J, Fogelman A, Navab M (1997) Oxidized lipids in atherogenesis: formation, destruction and action. *Thromb Haemost* 78: 195-199
- [32] Leitinger N (2003) Oxidized phospholipids as modulators of inflammation in atherosclerosis. *Curr Opin Lipidol* 14: 421-430
- [33] Krauss RM (2004) Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 27: 1496-1504

- [34] Chowdhury TA, Hopkins D, Dodson PM, Vafidis GC (2002) The role of serum lipids in exudative diabetic maculopathy: is there a place for lipid lowering therapy? *Eye* (London, England) 16: 689-693
- [35] Chew EY, Klein ML, Ferris FL, 3rd, et al. (1996) Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy. Early Treatment Diabetic Retinopathy Study (ETDRS) Report 22. *Archives of ophthalmology* 114: 1079-1084
- [36] Hall NF, Gale CR, Syddall H, Phillips DI, Martyn CN (2001) Risk of macular degeneration in users of statins: cross sectional study. *BMJ* (Clinical research ed 323: 375-376
- [37] Clark CM, Jr., Lee DA (1995) Prevention and treatment of the complications of diabetes mellitus. *N Engl J Med* 332: 1210-1217
- [38] Festa A, Kopp HP, Schernthaner G, Menzel EJ (1998) Autoantibodies to oxidised low density lipoproteins in IDDM are inversely related to metabolic control and microvascular complications. *Diabetologia* 41: 350-356.
- [39] Orchard TJ, Virella G, Forrest KY, Evans RW, Becker DJ, Lopes-Virella MF (1999) Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 48: 1454-1458.
- [40] Lopes-Virella MF, Virella G, Orchard TJ, et al. (1999) Antibodies to oxidized LDL and LDL-containing immune complexes as risk factors for coronary artery disease in diabetes mellitus. *Clin Immunol* 90: 165-172.
- [41] Atchley DH, Lopes-Virella MF, Zheng D, Kenny D, Virella G (2002) Oxidized LDL-anti-oxidized LDL immune complexes and diabetic nephropathy. *Diabetologia* 45: 1562-1571
- [42] Lehtimäki T, Lehtinen S, Solakivi T, et al. (1999) Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler Thromb Vasc Biol* 19: 23-27.
- [43] Inoue T, Uchida T, Kamishirado H, Takayanagi K, Hayashi T, Morooka S (2001) Clinical significance of antibody against oxidized low density lipoprotein in patients with atherosclerotic coronary artery disease. *J Am Coll Cardiol* 37: 775-779
- [44] Tornvall P, Waeg G, Nilsson J, Hamsten A, Regnström J (2003) Autoantibodies against modified low-density lipoproteins in coronary artery disease. *Atherosclerosis* 167: 347-353
- [45] Boullier A, Hamon M, Walters-Laporte E, et al. (1995) Detection of autoantibodies against oxidized low-density lipoproteins and of IgG-bound low density lipoproteins in patients with coronary artery disease. *Clin Chim Acta* 238: 1-10.
- [46] Rossi GP, Cesari M, De Toni R, et al. (2003) Antibodies to oxidized low-density lipoproteins and angiographically assessed coronary artery disease in white patients. *Circulation* 108: 2467-2472
- [47] Karvonen J, Paivansalo M, Kesäniemi YA, Horkko S (2003) Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. *Circulation* 108: 2107-2112

Table 1. Clinical characteristics of diabetic patients with and without retinopathy

	No retinopathy N=383	Retinopathy N=114	P value
Age	52.2 (8.2)	55.2 (8.3)	<0.001
Male gender (%)	60.6	61.4	0.87
Duration of diabetes (years)	7.4 (5.7)	10.4 (6.5)	<0.001
HbA1c (%)	8.1 (1.6)	8.6 (1.6)	0.001
BMI (kg/m ²)	28.5 (4.8)	28.5 (5.4)	0.97
Systolic BP (mmHg)	136 (16)	138 (18)	0.29
Diastolic BP (mmHg)	83 (12)	85 (14)	0.15
Total chol (mmol/L)	4.8 (0.9)	4.8 (1.0)	0.99
LDL chol (mmol/L)	2.7 (0.8)	2.7 (0.9)	0.94
HDL chol (mmol/L)	1.2 (0.4)	1.4 (0.4)	0.019
Triacylglycerol (mmol/L)	1.9 (1.1)	1.8 (1.2)	0.33
CRP (mg/L)	8.2 (33.2)	8.1 (14.8)	0.98
Current smokers (%)	20.9	14.0	0.17
Insulin therapy (%)	18.5	29.8	0.01
Statin therapy (%)	39.9	36.8	0.55

Values are mean (standard deviation) or percentage.

Table 2. Correlations between risk factors and plasma IgG levels against p45 and p210

	p45	MDA-p45	P210	MDA-p210
Age (years)	-0.01	0.03	0.06	0.12**
Duration of diabetes (years)	0.04	0.01	0.01	-0.05
HbA1c (%)	0.05	-0.01	-0.02	-0.07
BMI (kg/m ²)	-0.06	-0.02	0.01	0.02
Systolic BP (mmHg)	0.03	0.04	0.02	0.09*
Diastolic BP (mmHg)	0.07	0.07	0.02	0.13***
Total chol (mmol/L)	0.00	0.02	0.00	0.08
LDL chol (mmol/L)	0.02	0.01	0.05	0.11*
HDL chol (mmol/L)	-0.02	0.03	0.12*	0.16**
Triaglycerol (mmol/L)	0.01	0.33	-0.16***	-0.14***
CRP (mg/L)	0.01	0.01	0.01	-0.01

Chol; cholesterol. Values are Spearman Rank correlation coefficients.

*p<0.05, **p<0.01 and ***p<0.005

Table 3. Clinical characteristics of diabetic patients with and without peripheral neuropathy

	No neuropathy N=407	Neuropathy N=90	P value
Age (years)	52.4 (8.5)	53.9 (7.9)	0.14
Male gender (%)	60.4	62.2	0.75
Duration of diabetes (years)	7.8 (5.7)	9.2 (7.1)	0.04
HbA1c (%)	8.2 (1.8)	8.2 (1.5)	0.73
BMI (kg/m ²)	28.3 (4.8)	29.4 (5.6)	0.06
Systolic BP (mmHg)	137 (16)	136 (18)	0.52
Diastolic BP (mmHg)	83 (12)	85 (13)	0.18
Total chol (mmol/L)	4.8 (0.9)	4.8 (0.9)	0.56
LDL chol (mmol/L)	2.7 (0.8)	2.8 (0.7)	0.48
HDL chol (mmol/L)	1.3 (0.4)	1.3 (0.4)	0.63
Triacycerol (mmol/L)	1.9 (1.1)	1.9 (1.3)	0.64
CRP (mg/L)	8.6 (33.9)	6.2 (7.5)	0.50
Current smokers (%)	18.9	21.1	0.56
Insulin therapy (%)	18.7	47.5	0.004
Statin therapy (%)	38.8	41.1	0.69

Values are mean (standard deviation) or percentage.

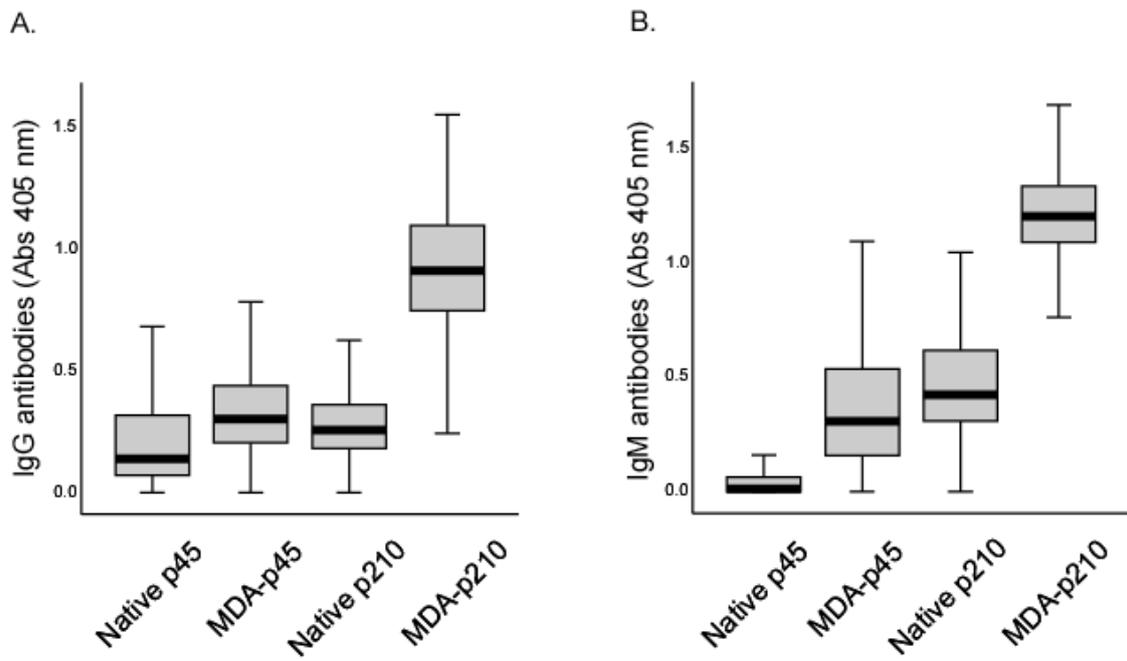


Figure 1.

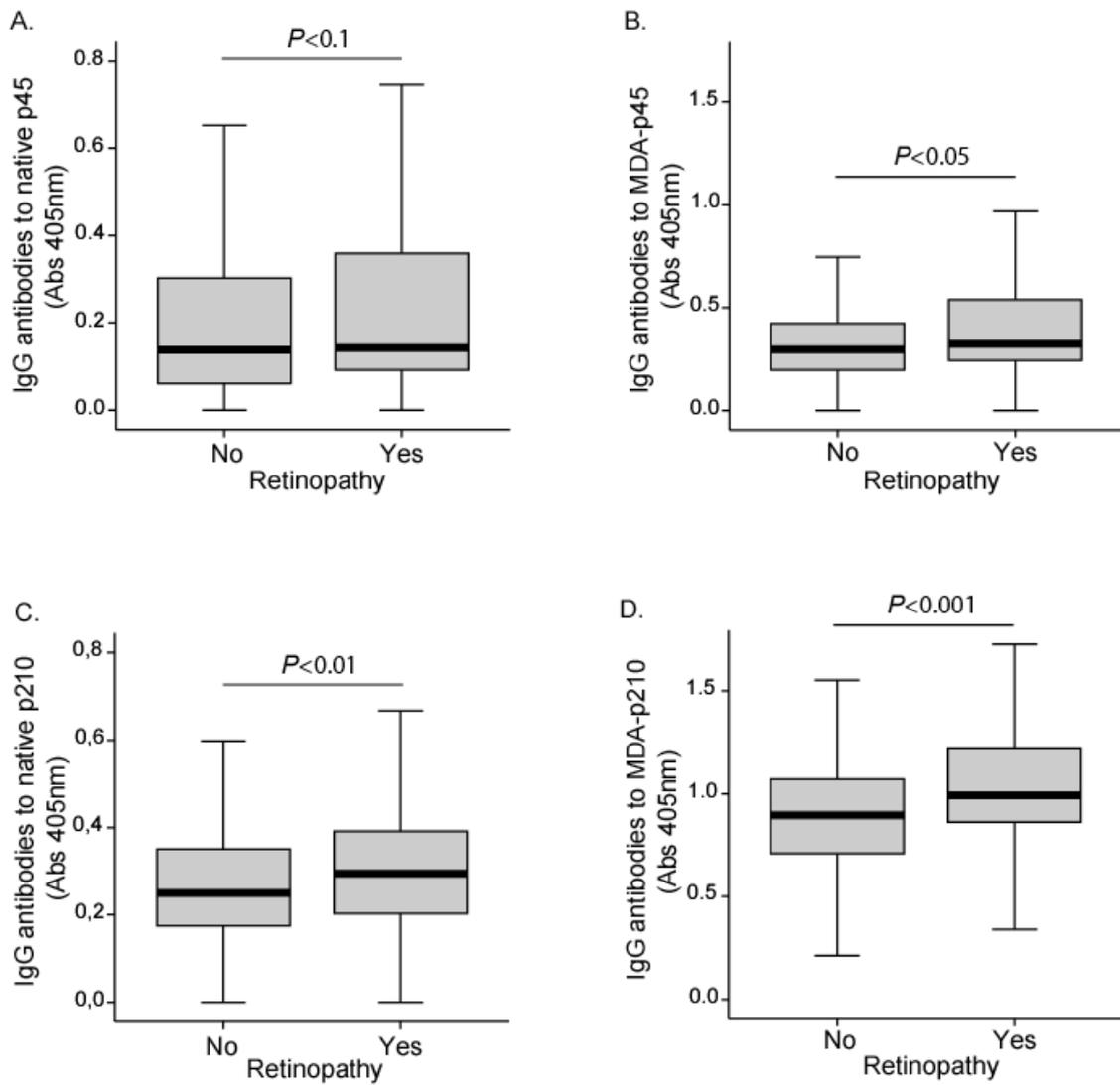


Figure 2.

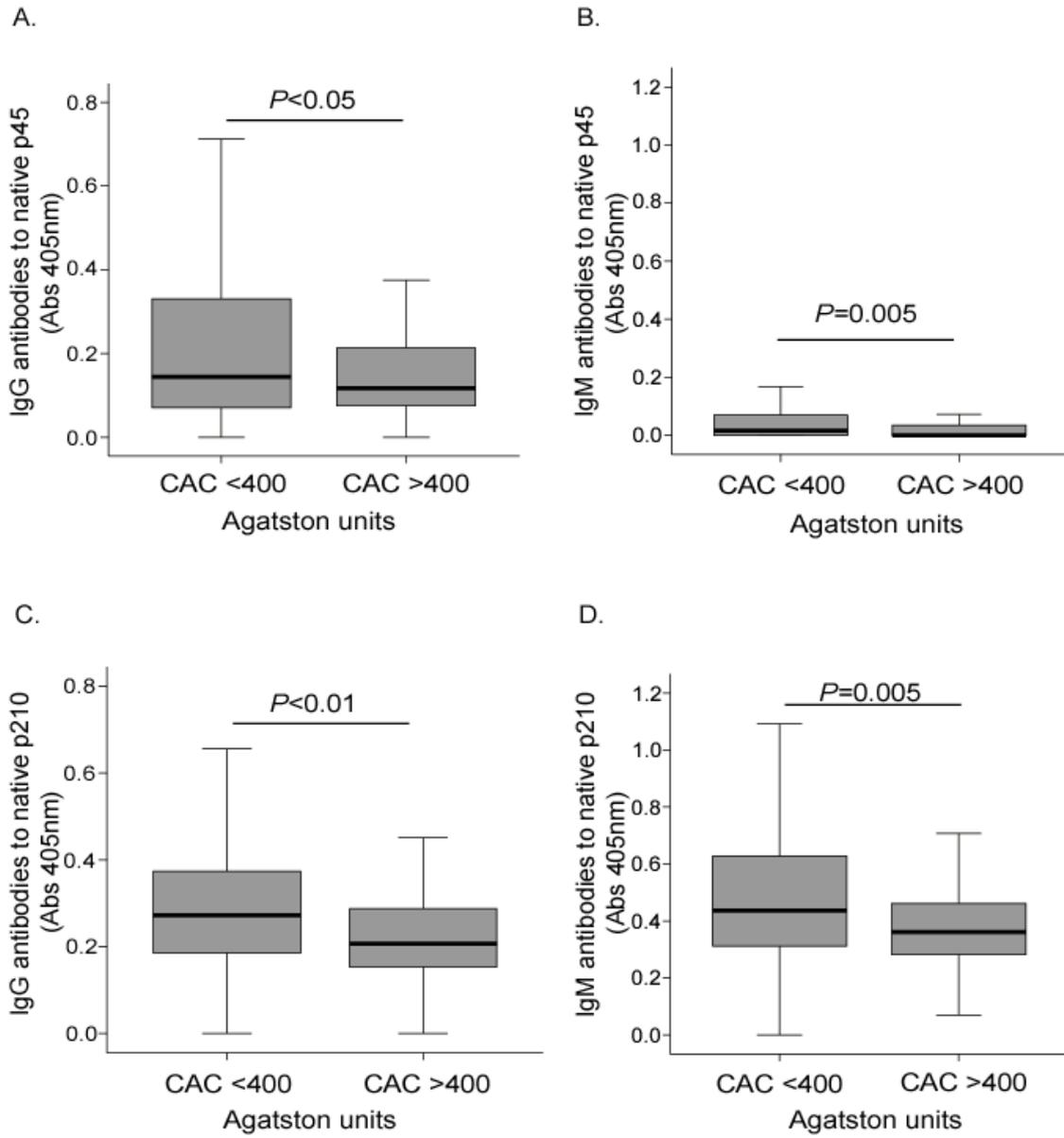


Figure 3.