Prediabetic and diabetic *in vivo* modification of circulating low-density lipoprotein attenuates its stimulatory effect on adrenal aldosterone and cortisol secretion

Steffi Kopprasch*, Jens Pietzsch^{1,*}, Ishrath Ansurudeen*, Juergen Graessler, Alexander W Krug, Monika Ehrhart-Bornstein and Stefan R Bornstein

Department of Internal Medicine 3, Pathological Biochemistry, Carl Gustav Carus Medical School, University of Technology Dresden, Fetscherstrasse 74, D-01307 Dresden, Germany

¹Department of Radiopharmaceutical Biology, Institute of Radiopharmacy, Research Center Dresden-Rossendorf, D-01314 Dresden, Germany

(Correspondence should be addressed to S Kopprasch; Email: steffi.kopprasch@uniklinikum-dresden.de)

*(S Kopprasch, J Pietzsch, and I Ansurudeen contributed equally to this work)

Abstract

Modification of low-density lipoprotein (LDL) and abnormal aldosterone and cortisol metabolism have been implicated in the pathogenesis of type 2 diabetes (DM2) and diabetic vascular disease. Since LDL serves as a major cholesterol source for adrenal steroidogenesis, we investigated whether LDL modification in prediabetic and diabetic subjects influences adrenocortical aldosterone and cortisol release. LDL was isolated from 30 subjects with normal glucose tolerance (NGT-LDL), 30 subjects with impaired glucose tolerance (IGT-LDL), and 26 patients with DM2 (DM2-LDL). Oxidation and glycoxidation characteristics of LDL apolipoprotein B100 of each individual was assessed by gas chromatography–mass spectrometry analysis. Human adrenocortical cells (NCI-H295R) were incubated for 24 h with 100 µg/ml LDL and after removal of supernatants stimulated for

a further 24 h with angiotensin II (AngII). In supernatants, aldosterone and cortisol secretion was measured. IGT-LDL and DM2-LDL were substantially more modified than NGT-LDL. Each of the five measured oxidation/glycoxidation markers was significantly positively associated with glycemic control, measured as HbA_{1c}. LDL from all subjects stimulated both the basal and AngII-induced aldosterone and cortisol release from adrenocortical cells. However, hormone secretion was significantly inversely related to the degree of LDL oxidation/glycoxidation. We conclude that LDL modifications in IGT and DM2 subjects may have significant clinical benefits by counteracting prediabetic and diabetic overactivity of the renin–angiotensin–aldosterone system and enhanced cortisol generation.

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Introduction

Biochemical modification of low-density lipoprotein (LDL) and the subsequent increase in their uptake by monocytes/macrophages and foam cell formation have been implicated in the pathogenesis of diabetic vascular disease including atherosclerosis, the major cause of death in diabetic patients (Brown *et al.* 2005, Rader & Daugherty 2008). As measured by ELISA techniques, enhanced circulating levels of oxidized LDL (oxLDL) have been found in both impaired glucose tolerance (IGT; Kopprasch *et al.* 2002) and type 2 diabetes (DM2; Toshima *et al.* 2000) subjects. In addition to oxidation, LDL can also be modified by glycoxidation processes, thus forming advanced glycation end products like N^ε-(carboxymethyl)lysine (CML) and N^ε-(carboxyethyl)lysine (CEL) under hyperglycemic conditions (Veiraiyah 2005). Using the highly sensitive and specific technique of gas chromatography–mass spectrometry (GC–MS) analysis, we could demonstrate the enhanced oxidation and glycoxidation of circulating LDL apolipoprotein

B100 (apoB100) isolated from IGT subjects compared with LDL from normal glucose tolerance (NGT) subjects (Graessler *et al.* 2007).

In addition to LDL modification, both overactivity of the renin–angiotensin–aldosterone system (RAAS; Henriksen 2007, Ostergren 2007) and dysregulation of the hypothalamic–pituitary–adrenal axis with resulting increments in basal plasma cortisol levels (Bruehl *et al.* 2007) and enhanced tissue sensitivity to cortisol (Andrews *et al.* 2002) have been causally related to the pathogenesis of DM2 and its adverse cardiovascular consequences. A direct relationship between plasma aldosterone levels and markers of insulin resistance has been demonstrated in normotensive and hypertensive subjects (Colussi *et al.* 2007, Kidambi *et al.* 2007).

LDL serves as a major source of cholesterol for adrenal steroidogenesis. LDL-derived cholesteryl esters can be delivered to adrenocortical cells by receptor-mediated endocytotic uptake via the LDL receptor or scavenger receptors class B type I. Additionally, cholesterol can be

obtained from intracellular sources including endogenous *de novo* synthesis and release from stored lipid droplets (Toth *et al.* 1997, Kraemer 2007).

In the present study, we investigated whether oxidative/glycooxidative modifications of LDL isolated from IGT and DM2 individuals influence adrenocortical aldosterone and cortisol synthesis that might potentially contribute to diabetic hormonal dysregulation.

Research design and methods

Subjects

Sixty subjects (30 with NGT and 30 with IGT) who were at risk for the development of diabetes owing to a family history of DM2, obesity, and/or hyper-/dyslipoproteinemia and 26 patients with clinically overt diabetes mellitus were examined. The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki. All subjects consented to participate in the study, which was approved by the local ethics committee.

The diagnosis of NGT and IGT was confirmed by an oral glucose tolerance test according to World Health Organization guidelines and criteria. Plasma triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured as described previously (Pietzsch *et al.* 1995). LDL cholesterol was calculated using the Friedewald formula. HbA_{1c} was measured by HPLC on a Diamat analyzer (Bio-Rad).

LDL isolation and biochemical characterization of apoB100 modification

Immediately before oral glucose challenge EDTA plasma for LDL preparation was obtained. To 1.5 ml of plasma 15 µl conservation medium was added containing 70 g sucrose, 50 mg phenylmethylsulfonyl fluoride, 20 mg streptomycin sulfate, 20 mg DL-dithiothreitol, and 20 mg sodium azide per 100 ml water. After addition of conservation medium, plasma samples were shock frozen in liquid nitrogen and stored at -80 °C until analysis. LDL (density 1.006–1.063 g/ml) was isolated from thawed samples by sequential, very fast ultracentrifugation (VFU) as described previously by us (Pietzsch *et al.* 1995). In brief, for VFU, we used the Optima TLX ultracentrifuge with rotor TLA-120.2 and thick-walled polycarbonate tubes (Beckman Instruments Inc., Palo Alto, CA, USA). Run conditions were full speed (625 000 g) and 18 °C temperature. In the first step, the tubes were filled with 0.5 ml conserved plasma, which was overlaid with 0.5 ml medium of density 1.006 kg/l. The densities and periods chosen for flotation were 1.006 g/ml and 100 min for VLDL and 1.063 g/ml and 100 min for total LDL. Flotated LDL was aspirated using capillary pipettes. Blood plasma and LDL were all processed in subdued light to prevent the photooxidation of LDL. All buffers and solutions were made oxygen free by degassing and purging with argon. Of note, considering the final

dilution of the conservation medium of ~1:40 000 to 1:80 000 in the LDL fractions applied to the cells, adverse effects of constituents of the plasma conservation medium are very unlikely.

The extent of LDL apoB100 modification was evaluated by GC-MS analysis of three highly specific oxidation products (5-hydroxy-2-aminovaleric acid (HAVA), 6-hydroxy-2-aminocaproic acid (HACA), and 3-chlorotyrosine) and two glycooxidation products (CML, CEL) as described previously in detail (Graessler *et al.* 2007). The content of oxidation/glycooxidation markers analyzed in each individual LDL sample was normalized to the content of LDL apoB100 (as mol of measured analyte per mol of apoB100) and is expressed in the results as modified residues per 10 000 residues of the corresponding parent amino acid.

Cell culture

Human adrenocortical tumor cells (NCI-H295R) were cultured in DMEM/F12 supplemented with insulin (66 nmol/l), hydrocortisone (10 nmol/l), 17β-estradiol (10 nmol/l), apo-transferrin (10 µg/ml), sodium selenite (30 nmol/l), penicillin (100 units/ml), streptomycin (100 µg/ml), and 2% FBS at 37 °C in a humidified atmosphere of 5% CO₂/95% air. Cells seeded at a density of 70 000 cells per cm² were used for experiments. Eighty percent confluent cells were incubated with 100 µg/ml isolated LDL in serum-free and hydrocortisone-free DMEM/F12 media for 24 h. After collection of medium, the cells were treated for a further 24 h with the physiological stimulant of adrenal aldosterone synthesis angiotensin II (AngII; 100 nmol/l) again in serum-free and hydrocortisone-free DMEM/F12 medium. Aldosterone release into the medium after 24-h and 48-h incubation was determined in three replicates for each cell treatment by RIA (Diagnostic Systems Laboratories, Sinsheim, Germany), and the means were used in the analyses. Accordingly, cortisol release was determined by a competitive luminometric assay (Diasorin, Saluggia, Italy). In control experiments, we performed trypan blue exclusion tests of cell viability. During a 24-h incubation period with LDL, cell viability varied between 79 and 86% and did not significantly differ from PBS controls.

Statistical analysis

All data are expressed as means ± S.E.M. The means of the three subject groups NGT, IGT, and DM2 were compared by one-way ANOVA followed by *post hoc* Sidak test. Spearman's rank correlation coefficients (ρ) were used to express the relationships between hormone release from adrenocortical cells and oxidative/glycooxidative LDL parameters and between systemic HbA_{1c} levels and oxidative/glycooxidative LDL markers. All data were analyzed using the SPSS statistical package (version 12.0 for Windows; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

The clinical details and baseline metabolic parameters of the NGT, IGT, and DM2 subjects are given in Table 1. When compared with NGT and IGT individuals, patients with long-lasting DM2 were older and had a greater BMI. DM2 subjects had more macrovascular complications including myocardial infarction, stroke, and limb amputations. In addition, the majority of DM2 subjects suffered from an increased risk of developing microvascular diseases, e.g., retinopathy, nephropathy, and neuropathy. HbA_{1c} levels tended to be higher in IGT subjects and were significantly increased in DM2 patients. HDL cholesterol levels were significantly lower in both the IGT and DM2 groups. Total cholesterol, triglycerides, and LDL cholesterol did not differ significantly between the groups (Table 1).

After LDL preparation from the plasma of each study participant, apoB100 oxidation and glycoxidation characteristics from each individual LDL sample were assessed. As shown in Table 2, protein moieties of LDL isolated from IGT and DM2 subjects were substantially more oxidized and glycoxidized when compared with LDL samples from NGT subjects. HAVA, a specific oxidation product of apoB100 arginine and proline residues, was significantly increased by 2.9-fold in IGT-LDL and by 3.6-fold in DM2-LDL respectively. Similarly, HACA, a primary oxidation marker of lysine side-chain residues, was enhanced by 4.4-fold in IGT-LDL and by 5.9-fold in DM2-LDL respectively. Moreover, 3-chlorotyrosine, a highly specific oxidation product of tyrosine residues indicating the presence of myeloperoxidase-derived hypochlorous acid, was elevated by 2.7-fold in LDL from DM2 patients compared with those from NGT subjects. As expected, the most pronounced differences were observed in CML and CEL contents, being 8.4- and 8.1-fold higher in IGT-LDL than in NGT-LDL and increasing further in DM2-LDL.

To determine whether the high systemic glucose levels promote LDL protein oxidation/glycoxidation, we assessed the relationship between glycemic control, measured as HbA_{1c}, and protein oxidation/glycoxidation markers. Correlation analysis revealed a significant association between HbA_{1c} and CML ($\rho=0.442$), CEL ($\rho=0.455$), HACA ($\rho=0.430$), and 3-chlorotyrosine ($\rho=0.434$); all $P<0.001$. HAVA levels correlated to a lesser extent ($\rho=0.355$, $P<0.01$) with glycated hemoglobin. Together, these observations support the hypothesis that glucose could directly stimulate the formation of both apoB100 oxidation and glycoxidation products *in vivo*.

Since IGT and DM2 subjects suffer more frequently from macrovascular disease and the adrenocortical hormones, especially aldosterone, have been implicated in the pathogenesis of atherosclerosis (Takai *et al.* 2005, Krug *et al.* 2007), we investigated whether the *in vivo*-modified LDL influences adrenal aldosterone synthesis. Since H295R cells are pluripotent cell lines, we additionally examined whether adrenocortical cortisol synthesis was affected by modified LDL.

As shown in Fig. 1A, 24-h treatment of human adrenocortical cells with LDL isolated from NGT, IGT, and DM2 subjects stimulated aldosterone release significantly by about twofold. Of note, the observed LDL-induced aldosterone release was in the range evoked by the physiological stimulus AngII and it was comparatively less in the IGT and DM2 groups. The differences in aldosterone release between the groups were even more prominent when the LDL-pretreated H295R cells were subsequently stimulated with AngII for the next 24 h (Fig. 1B). In this experimental set-up, the greatest aldosterone release was found in the NGT group, declining gradually in the IGT and DM2 groups. The 24-h pretreatment with LDL from NGT and IGT subjects sensitized the NCI-H295R cells to

Table 1 Baseline clinical characteristics and metabolic parameters of normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (DM2) subjects

	NGT (n=30)	IGT (n=30)	DM2 (n=26)	P value	
				IGT/NGT	DM2/NGT
Clinical parameter					
Age (years)	40±1	41±1	60±3	NS	<0.001
Sex (F/M)	21/9	21/9	11/15		
BMI (kg/m ²)	26.6±0.8	28.2±1.2	29.6±1.3	NS	NS
Macrovascular complications (n)	0	0	18		
Microvascular complications (n)	0	0	19		
Hypertension (n)	5	9	19		
Insulin therapy (n)	0	0	15		
Diabetes duration (years)	0	0	22±2		
Metabolic parameter					
Total cholesterol (mmol/l)	5.49±0.17	5.44±0.15	4.97±0.28	NS	NS
Triglycerides (mmol/l)	1.29±0.13	1.72±0.16	1.97±0.47	NS	NS
LDL cholesterol (mmol/l)	3.22±0.16	3.30±0.82	3.02±0.22	NS	NS
HDL cholesterol (mmol/l)	1.67±0.09	1.35±0.07	1.35±0.09	<0.05	<0.05
HbA _{1c} (%)	4.8±0.1	5.0±0.1	6.7±0.3	NS	<0.001

Data are means±S.E.M. Comparison between groups was performed by one-way ANOVA followed by *post hoc* Sidak test.

Table 2 Oxidation and glycooxidation parameters of low-density lipoprotein (LDL) apolipoprotein B100 isolated from normal glucose tolerance (NGT; *n*=30), impaired glucose tolerance (IGT; *n*=30), and type 2 diabetes (DM2; *n*=26) subjects

Parameter	NGT-LDL	IGT-LDL	DM2-LDL	P value		
				NGT versus IGT	NGT versus DM2	IGT versus DM2
HAVA ^a	0.41±0.52	1.18±0.10	1.46±0.10	<0.001	<0.001	NS
HACA ^b	0.20±0.02	0.88±0.07	1.17±0.08	<0.001	<0.001	0.01
3-Chlorotyrosine ^c	0.11±0.01	0.15±0.02	0.30±0.03	NS	<0.001	<0.001
CML ^b	1.53±0.15	12.85±1.24	15.73±0.76	<0.001	<0.001	NS
CEL ^b	0.16±0.02	1.30±0.15	1.55±0.12	<0.001	<0.001	NS

Data are means±S.E.M.; they were compared by one-way ANOVA followed by *post hoc* Sidak test.

^aResidues per 10 000 arginine plus proline residues.

^bResidues per 10 000 lysine residues.

^cResidues per 10 000 tyrosine residues.

subsequent stimulation with AngII. By contrast, such an increase could not be observed with LDL from DM2 subjects (Fig. 1B).

Similar, but lesser pronounced, tendencies were observed for LDL- and AngII-stimulated adrenocortical cortisol release (Fig. 2A and B). Basically, incubation with LDL stimulated subsequent adrenal cortisol release by about twofold. Subsequent treatment of cells with AngII, known to stimulate adrenal cortisol synthesis besides its natural stimulus ACTH (Liang *et al.* 2007), evoked an increase in cortisol secretion, which, however, did not significantly differ between NGT, IGT, and DM2 groups. Overall, these data suggest that LDL-mediated modulation in steroidogenesis is not restricted to mineralocorticoids but also includes adrenal cortisol synthesis.

To substantiate the impact of oxidative and glycooxidative LDL modifications on adrenocortical hormone synthesis, we correlated the levels of specific oxidative/glycooxidative markers with adrenal aldosterone and cortisol release in all subjects investigated. As shown in Table 3, each of the apoB100 amino acid modifications was significantly negatively associated with aldosterone but not cortisol release. After 24-h incubation with LDL plus 24-h incubation with AngII, the glycooxidation products CML and CEL showed a stronger negative correlation with aldosterone release than the oxidation markers HAVA and HACA (Table 3).

Discussion

The present study confirms our previous finding of increased HAVA, HACA, 3-chlorotyrosine, CML, and CEL levels in LDL apoB100 in IGT subjects (Graessler *et al.* 2007), and extends it by the demonstration of significantly elevated oxidative/glycooxidative protein modification markers in circulating LDL obtained from DM2 patients. The LDL biomarkers investigated by us are highly specific protein oxidation/glycooxidation products that most probably arise from the two metabolic hallmarks of DM2, namely oxidative stress and hyperglycemia (Pennathur & Heinecke 2007). In the present study, the possible role of hyperglycemia as a

substantial factor triggering LDL protein modification was supported by the finding that HbA_{1c}, a parameter of glycemic control, was significantly positively associated with each of the five investigated biomarkers.

Figure 1 Aldosterone release from human adrenocortical cells after treatment with (A) LDL and (B) angiotensin II. NCI-H295R cells were preincubated for 24 h with 100 µg/ml LDL isolated from NGT (*n*=30), IGT (*n*=30), and DM2 (*n*=26) subjects. After removal of supernatants, the cells were stimulated for further 24 h with AngII. **P*<0.05, ****P*<0.001 versus controls (C), #*P*<0.001 NGT versus DM2, §*P*<0.01 IGT versus DM2. Values are means±S.E.M.

Figure 2 Cortisol release from human adrenocortical cells after treatment with (A) LDL and (B) angiotensin II. NCI-H295R cells were preincubated for 24 h with 100 µg/ml LDL isolated from NGT ($n=30$), IGT ($n=30$), and DM2 ($n=26$) subjects. After removal of supernatants, the cells were stimulated for further 24 h with AngII. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus controls (C). Values are means $\pm$ S.E.M.

Hypertension is a common clinical feature of diabetes and primarily contributes to cardiovascular disease. Recent studies have implicated the RAAS as a key mediator of cardiovascular dysfunction in diabetes (Sowers & Stump 2004). AngII has been shown to have direct effects on endothelial dysfunction and aldosterone is considered to be a cardiovascular risk factor, promoting cardiac fibrosis and atherosclerosis (Krug & Ehrhart-Bornstein 2008). In addition, hyperaldosteronism has been recognized as one of the possible causes of glucose intolerance. However, clinical studies that have assessed insulin sensitivity in patients with primary aldosteronism show substantial inconsistencies (Catena *et al.* 2006).

Multiple, partially species-specific systems are suggested to be involved in cellular cholesterol delivery for adrenocortical steroidogenesis. Cholesterol can be derived from the uptake of cholesterol-rich lipoproteins, as well as from endogenous cholesterol synthesis and by the mobilization of stored cholesteryl esters (Azhar *et al.* 2003). Accumulating evidence suggests that plasma lipoproteins, namely LDL and HDL, are the major sources of cholesterol for steroid production in the

Table 3 Correlation analysis of oxidation and glycooxidation parameters of low-density lipoprotein (LDL) apolipoprotein B100 isolated from normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (DM2) subjects and LDL-induced hormone release from adrenocortical cells

	Aldosterone	
	48-h levels (pmol/l) ^a	Incremental changes induced with AngII (pmol/l) ^b
HAVA		
ρ	-0.217	-0.209
P value	0.045	0.054
HACA		
ρ	-0.258	-0.270
P value	0.016	0.012
3-Chlorotyrosine		
ρ	-0.299	-0.411
P value	0.005	0.000
CML		
ρ	-0.311	-0.333
P value	0.004	0.002
CEL		
ρ	-0.320	-0.349
P value	0.003	0.001
	Cortisol	
	48 h levels (nmol/l) ^a	Incremental changes induced with AngII (nmol/l) ^b
HAVA		
ρ	-0.054	-0.149
P value	0.632	0.187
HACA		
ρ	-0.060	-0.150
P value	0.599	0.184
3-Chlorotyrosine		
ρ	-0.119	-0.170
P value	0.294	0.132
CML		
ρ	-0.069	-0.103
P value	0.545	0.363
CEL		
ρ	-0.084	-0.142
P value	0.458	0.210

ρ , Spearman's rank correlation coefficient. Twenty-four hour incubation with LDL did not yield significant correlation coefficients for cortisol and aldosterone.

^aAldosterone and cortisol levels were measured in the supernatants of adrenocortical NCI-H295R cells after pretreatment with isolated LDL (100 µg/ml, $n=86$) for 24 h and post-treatment with angiotensin II (100 nmol/l) for a further 24 h.

^bHormone levels in the supernatant after 24 h were subtracted from hormone levels obtained after 48-h incubation (24 h with LDL plus 24 h with AngII).

adrenal gland. However, although a potential involvement of LDL as a source for aldosterone biosynthesis in glomerulosa cells was reported several years ago, marked species differences and contradictory results have hampered the building of a coherent understanding of this process (Capponi 2002). While LDL-derived cholesteryl esters can be delivered by receptor-mediated endocytotic uptake via the LDL receptor

or by scavenger receptors class B type I (Kraemer 2007), HDL cholesteryl esters are preferentially delivered by scavenger receptor BI-mediated selective-uptake pathways to steroidogenic cells (Connelly & Williams 2003).

In our present study, we tested whether prediabetic and diabetic *in vivo* modification of LDL influences aldosterone synthesis in adrenocortical NCI-H295R cells, thereby potentially modifying RAAS activity. The hypothesis of oxidative stress-mediated modulation of adrenal aldosterone secretion has been supported by the recent results from Goodfriend *et al.* (2004), who showed that oxidized derivatives of polyunsaturated fatty acids may stimulate adrenal aldosterone production in humans and mediate some of the deleterious effects of obesity and oxidative stress, especially in black individuals.

The results of the present study demonstrate that the addition of LDL to adrenocortical cells stimulated aldosterone secretion irrespective of the LDL source from NGT, IGT, or DM2 subjects. This underlines the importance of LDL as a source of adrenal steroidogenesis. However, the ability of LDL to stimulate adrenal aldosterone secretion decreased with its increasing degree of oxidation/glycooxidation. This conclusion is based on two results of the present study. First, stimulation of aldosterone release from adrenocortical cells by IGT-LDL and DM2-LDL was lower when compared with NGT-LDL, especially after post-treatment with the physiological stimulus AngII (Fig. 1). Secondly, each of the apoB100 oxidation/glycooxidation parameters quantified by us showed a significantly negative correlation with aldosterone release from adrenocortical NCI-H295R cells (Table 3).

Glucocorticoid hormones (mainly cortisol in man) play a key role in water and electrolyte metabolism, regulating blood pressure, immune function, and glucose metabolism. Cortisol excess is characterized by hypertension, visceral obesity, and glucose intolerance (Andrews & Walker 1999). In addition to LDL-induced changes in basal and AngII-induced mineralocorticoid release, we found in the present study a significantly increased AngII-induced cortisol secretion (Fig. 2B) that gradually, but not significantly, decreased with the increasing degree of LDL oxidation/glycooxidation. Of note, we observed that the level of cortisol was about 1000-fold higher than the level of aldosterone in the supernatants of H295R cells, suggesting that cortisol may be also critical for contributing to the pathophysiology of modified LDL and AngII.

Lipoprotein receptor expression and AngII-mediated regulation of lipoprotein metabolism in H295R adrenocortical cells have been studied previously (Martin *et al.* 1999, Cherradi *et al.* 2001, Pilon *et al.* 2003). Cherradi *et al.* (2001) and Pilon *et al.* (2003) reported that AngII stimulated SR-BI, but not LDL receptor expression. Recently, it has been shown that AngII induced uptake of LDL in primary human adrenocortical cells and stimulated aldosterone and cortisol synthesis (Liang *et al.* 2007). Leitersdorf *et al.* (1985) reported that AngII stimulated receptor-mediated LDL uptake and aldosterone, but not cortisol synthesis in primary bovine adrenocortical cells. From these studies, it follows that not

only post-treatment with AngII (as in the present study) but also pretreatment with AngII is able to substantially modify adrenocortical hormone synthesis and release.

The results of the present study show, for the first time, that oxidative/glycooxidative LDL modification opposes basal and AngII-induced steroidogenesis. A possible explanation for the current observations may be a decreased receptor-mediated uptake of biochemically modified LDL particles, resulting in decreased intracellular cholesteryl ester availability. In this context, Wiklund *et al.* (1987) observed a significantly lower *in vivo* uptake of glycated LDL compared with native LDL in the adrenal glands in rabbits using radioiodinated tyramine-cellobiose-labeled LDL particles. In their study, *in vivo* uptake and degradation of glycated LDL in adrenal glands was inversely related to the degree of modification, and, in the case of strongly modified LDL, the uptake was reduced by up to 16% compared with native LDL. More recently, we could demonstrate a significantly lower *in vivo* uptake of oxLDL in the adrenal glands of rats using fluorine-18-radiolabeled LDL particles and dynamic small animal positron emission tomography studies (Pietzsch *et al.* 2005). In this study, the uptake of oxLDL was reduced by up to 3% compared with native LDL. However, the degree of glycativ modification of LDL apoB100 in the Wiklund *et al.* (1987) study (measured as the percentage of modified lysine residues) and of oxidative modification of LDL apoB100 in our study (measured as HAVA and HACA concentration respectively) was substantially higher when compared with IGT-LDL and DM2-LDL. Very recently, dynamic small animal positron emission tomography studies in rats using both *in vitro*-modified LDL (glycated and glycooxidized LDL) and LDL obtained from IGT and DM2 subjects respectively also revealed a lower *in vivo* uptake in the adrenal glands (Pietzsch *et al.* 2007). In this study, the reduction in adrenal uptake still amounted to ~55% for *in vitro*-modified LDL and to about 35% for LDL obtained from IGT and DM2 subjects. Of note, the degree of the LDL *in vitro* modification used in this study was comparable with *in vivo* modification observed in IGT and DM2 subjects. In addition to decreased uptake of modified LDL, a LDL-mediated reduction in adrenocortical cell sensitivity towards the physiological stimulus AngII could be a further mechanistic explanation for altered aldosterone release evoked by IGT-LDL and DM2-LDL.

Attenuation of adrenal mineralocorticoid release in diabetic patients may have important clinical implications. Even in the absence of overt hypoaldosteronism, which may be rare in diabetic patients, a subclinical impairment of aldosterone release may contribute to dangerous hyperkalemia frequently seen in these patients. Patients with significant LDL modifications and potential impaired aldosterone response should therefore not be treated with aldosterone antagonists.

In conclusion, our investigation demonstrates that oxidative and glycooxidative apoB100 modification of circulating LDL increases with the increasing degree of hyperglycemia in prediabetic and diabetic subjects. LDL obtained from NGT,

IGT, or DM2 subjects was able to stimulate basal and AngII-induced aldosterone and cortisol release from human adrenocortical cells. However, adrenal aldosterone and, to a lesser extent, cortisol secretion was inversely related to LDL *in vivo* modification. Thus, oxidative and glycoxidative modifications of circulating LDL in IGT and DM2 subjects might represent a novel physiological adaptive response to preserve adrenocortical hormonal integrity in IGT and DM2 subjects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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