Fasting and postprandial glycoxidative and lipoxidative stress are increased in women with type 2 diabetes mellitus

Received for publication 21 December 2006 and accepted in revised form 18 April 2007.

Roger K. Schindhelm, BSC, MD; Marjan Alssema, MSC; Peter G. Scheffer, PHD; Michaela Diamant, MD, PHD; Jacqueline M. Dekker, PHD; Rob Barto, PHD; Giel Nijpels, MD, PHD; Piet J. Kostense, PHD; Robert J. Heine, MD, PHD; Casper G. Schalkwijk, PHD; Tom Teerlink, PHD

1Endocrinology, 3Clinical Chemistry, 4General Practice, 5Clinical Epidemiology and Biostatistics, 2EMGO Institute, VU University Medical Center, Amsterdam, The Netherlands, 6Internal Medicine, Academic Hospital Maastricht, Maastricht, The Netherlands

Corresponding author:
T. Teerlink, PhD
VU University Medical Center
Clinical Chemistry
P.O. Box 7057
1007 MB Amsterdam, The Netherlands
E-mail: t.teerlink@vumc.nl
Abstract

Objective We studied acute changes in markers of glycoxidative and lipoxidative stress, including oxidized low-density-lipoprotein, Nε-(carboxyethyl)-lysine (CEL), Nε-(carboxymethyl)-lysine (CML) and 3-deoxyglucosone (3DG), following two consecutive meals.

Research Design and Methods Post-menopausal women (27 with normal glucose metabolism [NGM]; 26 with type 2 diabetes [DM2]), received two consecutive fat-rich meals and two consecutive carbohydrate-rich meals on two occasions. Glucose and triglyceride concentrations were measured at baseline and 1,2,4,6 and 8h following breakfast; lunch was given at 4h. Oxidized-LDL-to-LDL-cholesterol ratio (oxLDL/LDL-C), CEL, CML and 3DG were measured at baseline and at 8h.

Results Fasting oxLDL/LDL-C, 3DG and CML were higher in women with DM2 compared to women with NGM and were comparable to the postprandial values at 8 hours in NGM. Postprandial rises in oxLDL/LDL-C and 3DG were similar in both groups. However, oxLDL/LDL-C increased more after the fat-rich meals, whereas CML and 3DG increased more after the carbohydrate-rich meals. After the fat-rich meals, the increase in oxLDL/LDL-C was correlated with postprandial triglycerides, whereas the increase in 3DG was correlated with postprandial glucose.

Conclusions The acute changes in markers of glycoxidative and lipoxidative stress in both DM2 and NGM suggest that post-absorptive oxidative stress may partly underlie the association of postprandial derangements and cardiovascular risk.
Patients with type 2 diabetes mellitus (DM2) have an increased risk of cardiovascular disease (CVD) (1), which can only partly be explained by the classical CVD risk factors such as hypertension, high LDL-cholesterol, low HDL-cholesterol and smoking (2). In post-menopausal women, as compared to men, the relative risk of CVD conferred by DM2 is even higher (3). Zilversmit postulated in 1979 that disturbances in postprandial metabolism may contribute to the excess risk of CVD (4), due to postprandial elevations of glucose and triglyceride-enriched lipoproteins (5,6).

Oxidative stress is regarded as a common pathway by which many of the classical CVD risk factors and postprandial dysmetabolism may initiate and promote atherosclerosis (7). Indeed, elevated levels of oxidized LDL (oxLDL) are associated with an increased risk for CVD (8). Prolonged exposure to a high-fat diet has been shown to result in an increase in plasma levels of oxLDL (9). Another mechanism that might link postprandial dysmetabolism and the risk of CVD in patients with DM2 includes the formation of advanced glycation endproducts (AGEs), which are related to micro- and macrovascular complications (10). Two of the most studied AGEs, Nε-(carboxyethyl)lysine (CEL) and Nε-(carboxymethyl)lysine (CML), can be formed on proteins by both glycoxidation and lipid-peroxidation pathways. α-Dicarbonyl compounds such as 3-deoxyglucosone (3DG), glyoxal and methylglyoxal, are reactive intermediates in the formation of AGEs (11). 3DG is a relatively stable intermediate, whereas glyoxal and methylglyoxal convert readily into CML and CEL, respectively. Because the latter are stable compounds, their quantification may yield more relevant information than quantification of their reactive dicarbonyl precursors.

Data on acute meal-induced changes in oxLDL, 3DG and CML and CEL are limited. To date, one study in healthy young men demonstrated an increase in the oxLDL-to-LDL-cholesterol-ratio after two consecutive fat-rich mixed meals (12), and one study in patients with coronary artery disease (CAD) found that postprandial oxLDL was associated with the severity and extent of CAD (13).

In the present study, 26 women with DM2 and 27 women with normal glucose metabolism (NGM) received two consecutive (breakfast and lunch) fat-rich or carbohydrate-rich meals, in order to study meal-related changes in oxidative stress (oxLDL), AGE-compounds (CML and CEL), and a reactive AGE-precursor (3DG). In addition to the effects of meal composition and the presence of diabetes, we also studied associations between postprandial changes of these markers and changes in glucose and triglycerides.

Methods

Subjects

The present study was a sub-study of a cross-sectional study to assess the effect and relative contributions of two consecutive fat-rich and carbohydrate-rich meals on markers of CVD risk in women with NGM (fasting glucose<6.1 mmol/l and 2-h post-load glucose<7.8 mmol/l) (n=76) and DM2 (n=79). Women with DM2 (n=522), recruited from the registry of the Diabetes Care System in the city of Hoorn, the Netherlands, and women who were randomly selected from the municipal registry of Hoorn (n=541), aged 50-65 years at the beginning of the study, were invited to participate in the study. Of these 1,063 women, 431 women were complete non-responders, 258 women were not willing to participate and 220 women did not meet the inclusion criteria. Inclusion criteria were: post-menopausal status, non-smoking, no untreated endocrine disorder other than DM2, no use of short acting insulin.
analogue, no use of PPAR-α and -γ agonists, no use of oral corticosteroids, no use of hormone replacement therapy, no statin use (for NGM-subjects only), HbA1c<9.0%, fasting cholesterol<8.0 mmol/l, fasting triglycerides<4.0 mmol/l, systolic blood pressure<190 mmHg, no liver impairment or renal impairment. For the present study, a sub-sample of 26 women with DM2 (no use of statins or insulin analogues) and 27 women with NGM matched for age, were studied. All participants gave written informed consent, and the study protocol was approved by the ethics committee of the VU University Medical Center in Amsterdam.

**Study design**
The study consisted of a screening visit and two visits for the test-meals. On the screening visit, fasting blood samples were drawn after a 12h overnight fast. Blood pressure was measured thrice with an oscillometric blood pressure measuring device (Collin Press-mate BP-8800, Colin, Komaki-City, Japan) after a 15-minute supine rest. Weight and height were measured in participants wearing light clothes only. Smoking and alcohol consumption were assessed by a questionnaire (14).

**Test meals**
Postprandial meal responses were examined following two standardized consecutive test meals (breakfast and lunch). On one day, both meals were high in fat content and on the other occasion, both meals were high in carbohydrate content. The test meal occasions were performed in random order. The fat-rich meals consisted of 2 croissants, 10 g of butter, 40 g of high-fat cheese and 300 ml of high-fat milk (3349kJ; 50 g fat; 56 g carbohydrates). The carbohydrate-rich meals consisted of 2 slices of bread, 25 g of marmalade, 30 g of cooked chicken breast, 50g of ginger bread and 300ml of drinking yogurt fortified with 45 g of soluble carbohydrates (3261kJ; 4 g fat, 162 g carbohydrates). Both meals were eaten within 10 minutes. Apart from the test meals and water (ad libitum), participants were not allowed to eat.

**Laboratory analysis**
Serum total cholesterol, HDL-cholesterol and triglycerides were measured by enzymatic colorimetric assays (Roche, Mannheim, Germany). Fasting and postprandial LDL-cholesterol was calculated according to the Friedewald-formula if triglycerides<4.5 mmol/l (15). Plasma glucose levels were determined with a glucose oxidase method (Granutest, Merck, Darmstadt, Germany) and HbA1c was measured with cation-exchange chromatography (Menarini Diagnostics, Florence, Italy). OxLDL was measured in EDTA-plasma in duplicate with a competitive ELISA (Mercodia, Uppsala, Sweden), with inter-assay and intra-assay coefficients of variation of 7.8% and 4.8%, respectively. Because a fairly strong correlation between oxLDL and LDL-cholesterol is a consistent finding in several studies (16,17), we expressed oxLDL as a ratio per LDL-cholesterol (oxLDL/LDL-C) as proposed by Holvoet et al (18).

3DG was measured with a newly developed LC-MS/MS method after pre-column derivatization according to a published procedure (19). In brief, 0.5 ml of whole blood was mixed with 1.0 ml of 1.2 mol/l perchloric acid and immediately stored at -80 °C until assayed. After thawing, the samples were centrifuged (5 min at 20,000 g) and 80 µl of the supernatant was mixed with 100 µl of the internal standard solution (1 µmol/l 2,3-pentanedione dissolved in ethanol). After addition of 20 µl of the derivatization reagent (10 mmol/l 2,4-dinitrophenylhydrazine dissolved in 1.2 mol/l perchloric acid), the samples were incubated for 16 h at room temperature. Chromatographic separation was performed on a Xterra MS C18-column (4.6x50 mm; 3.5 µm particle size) from Waters (Milford, MA, USA) using a linear gradient from 40% to 95% acetonitrile in water over 9 min at a
flow rate of 1 ml/min. Mass transitions of 521.1 → 431.0 and 521.1 → 182.1 for 3DG and 459.1 → 182.1 for the internal standard were monitored in negative-ion mode. Intra-assay and inter-assay coefficients of variation for 3DG were 8% and 12%, respectively. Two subjects with NGM had missing 3DG because of missing blood samples. In a pilot study applying the same study-protocol, we assessed the time-course of 3DG in 9 DM2 and 8 NGM subjects and measured 3DG with an HPLC-method with fluorescence detection as described previously (20). The highest 3DG concentration was observed at 8 hours after the first meal (21).

Unbound CML and CEL were measured in EDTA-plasma by HPLC with stable-isotope-dilution tandem mass spectrometric detection (LC-MS/MS) using a procedure developed for the analysis of protein-bound CML and CEL (22), with a modified sample preparation procedure. Briefly, after centrifugation of the plasma samples (5 min at 20,000 g), 50 µl of the supernatant was mixed with 150 µl of a combined internal standard solution (containing deuterated CML and CEL) and proteins were removed by centrifugation (20 min at 15,000 g) in a 10 kDa cut-off ultrafiltration device (Vivaspin 500 PES from Vivascience AG, Hannover, Germany). A 90 µl aliquot of the ultrafiltrate was mixed with 10 µl of a 50 mmol/l nonafluoropentanoic acid solution and 25 µl of this mixture was subjected to LC-MS/MS analysis as described before (22). Intra-assay and inter-assay coefficients of variation for CML were 3% and 4%, respectively and for CEL 5% and 9%, respectively.

To assess the cumulative effect of both consecutive meals we measured oxLDL, 3DG, CML, and CEL at baseline and at 8h.

Statistical analyses
Analyses were performed with SPSS for Windows 11.01 (SPSS Inc., Chicago, IL, USA). Data are presented as mean values (standard deviation), and in case of a skewed distribution, as median values (interquartile range). Data were ln-transformed before testing in case of a skewed distribution. Differences between women with NGM and DM2 were tested with Student’s t-test for continuous variables and χ²-test for dichotomous variables. Differences between meals were tested with paired samples t-tests. Postprandial changes in glucose and triglycerides were calculated as incremental area under the curve (iAUC) by the trapezoid method. Correlations between postprandial responses were calculated by Spearman’s correlation-coefficients. We considered a two-sided P<0.05 to indicate statistical significance.

Results
Characteristics of the study population
The clinical and biochemical characteristics of the participants at the screening visit are presented in Table 1. Figure 1 shows the 8 h-time courses of triglycerides and glucose after two consecutive fat-rich and after two consecutive carbohydrate-rich meals. At both meal visits, fasting triglyceride and glucose levels were higher in women with DM2 compared to the women with NGM (both P<0.001). Triglycerides-iAUC was higher following the fat-rich meals than after the carbohydrate-rich meals in both women with DM2 and NGM (both P<0.001), but was similar in both groups. Following the carbohydrate-rich meals, glucose-iAUC was higher in women with DM2 compared to NGM (P<0.001), whereas this difference was borderline significant after the fat-rich meals (P=0.06).

LDL-C significantly decreased from baseline to 8h in both groups and after both meal types (from 3.6±0.8 mmol/l to 3.2±0.7 mmol/l and 3.7±0.8 mmol/l to 3.3±0.7 mmol/l, fat-rich meals and carbohydrate-rich meals, respectively in NGM and from 3.3±0.7 mmol/l to 2.9±0.7 mmol/l and 3.3±0.7 mmol/l to 3.0±0.7 mmol/l, fat-rich
meals and carbohydrate-rich meals, respectively in DM2 (all \( P < 0.001 \)).

**Glycoxidative and lipoxidative stress markers**

The oxLDL/LDL-C, 3DG, CML and CEL values in women with NGM and DM2 at baseline and at 8h are listed in Table 2. Baseline oxLDL/LDL-C, 3DG and CML were significantly higher in DM2 compared to NGM. The increase of oxLDL/LDL-C during the fat-rich meals was higher than the increase during the carbohydrate-rich meals (17±10% versus 10±7% in NGM and 15±13% versus 7±9% in DM2, both \( P < 0.05 \)). Of interest, the fasting values of oxLDL/LDL-C, 3DG and CML in women with DM2 were similar to the postprandial values at 8h in women with NGM (all \( P > 0.1 \)).

Baseline oxLDL/LDL-C was correlated with fasting triglycerides in women with DM2 (\( r = 0.39, P < 0.05 \)), but not in women with NGM. After the fat-rich meals, changes in oxLDL/LDL-C correlated with changes in triglycerides, either expressed as triglycerides-iAUC (\( r = 0.32, P < 0.05 \); Figure 2a) or as increase over baseline at 8h (\( r = 0.50, P < 0.001 \)).

The mean increase of 3DG after the carbohydrate-rich meals was higher than after the fat-rich meals (29±21% versus 13±24%, \( P < 0.05 \) in NGM, and 36±17% versus 15±20%, \( P < 0.001 \) in DM2). Fasting plasma glucose concentration was correlated with baseline 3DG in women with DM2 (\( r = 0.50, P < 0.01 \)), but not in women with NGM. Postprandial changes in 3DG correlated to glucose-iAUC after the fat-rich meals (\( r = 0.37, P < 0.01 \); Figure 2b), but not with glucose-iAUC after the carbohydrate-rich meals.

The changes of CML and CEL were neither correlated to triglycerides-iAUC nor to glucose-iAUC, irrespective of meal composition.

**Discussion**

In the present cross-sectional study in postmenopausal women with NGM and DM2, we studied the postprandial changes of oxLDL/LDL-C, 3DG, CML and CEL, following two consecutive fat-rich or carbohydrate-rich meals. The main findings are that the fasting values of oxLDL/LDL-C, 3DG and CML are higher in women with DM2 compared to women with NGM. In women with DM2, the fasting values of oxLDL/LDL-C, 3DG and CML were similar to the postprandial values at 8h in women with NGM. Overall, the postprandial rises of oxLDL/LDL-C and 3DG were of similar magnitude in DM2 and NGM. OxLDL/LDL-C showed a greater increase after the fat-rich meals, whereas CML and 3DG increased more after the carbohydrate-rich meals. The increase in oxLDL/LDL-C was correlated with the rise in postprandial triglycerides, whereas the increase in 3DG was correlated with the postprandial rise of glucose.

There is increasing evidence that postprandial glucose and triglycerides may play an important role in macro- and microvascular complications (23). However, their relative contributions and the mechanisms leading to the increased CVD risk are not yet fully understood (5). Prospective studies have shown that postprandial or post-load glucose levels are more strongly associated with increased CVD than fasting glucose (24,25). Oxidative stress is hypothesized to be an important pathway linking postprandial glucose and triglyceride responses to CVD (26,27). Indeed, in a recent study it was found that postprandial oxLDL was a determinant of the extent of coronary atherosclerosis in patients with CAD (13). A limitation of most previous studies is that responses after a single meal were studied, which might not reflect the “real life” burden of the postprandial state, possibly leading to an underestimation of the associations between postprandial responses and CVD risk. A recent study from our
group that applied two consecutive meals (breakfast and lunch) in healthy young men, demonstrated that even slightly increased levels of triglycerides and glucose were associated with endothelial dysfunction and increased markers of oxidative stress, including oxLDL (12). We concluded that these findings might be relevant for insulin resistant subjects and DM2 individuals who have a more pronounced (exaggerated and prolonged) postprandial elevation of glucose and triglycerides, possibly leading to more oxidative stress. Our present findings support this conjecture, as we found that baseline oxLDL was higher in DM2 than in NGM, with a greater increase of oxLDL after the fat-rich meals compared to the carbohydrate-rich meals. Interestingly, although fasting oxLDL/LDL-C was higher in the DM2 group, we found no difference in the postprandial increase in oxLDL/LDL-C between NGM and DM2.

There are multiple sources and mechanisms in the formation of AGEs in vivo, involving both oxidative and non-oxidative reactions of reducing carbohydrates and other metabolic intermediates with amino acid residues (28). The AGE precursor 3DG, which is increased in patients with diabetes (29), is formed by non-oxidative modifications of Amadori adducts and from fructose-3-phosphate (30). In line with these observations, we found that 3DG increased more after the carbohydrate-rich meals than after the fat-rich meals. In addition, postprandial changes in 3DG were significantly correlated to changes in glucose, but surprisingly, only after the fat-rich meals. In patients with type 1 diabetes Beisswenger et al observed an increase in 3DG within two hours (31), whereas in our pilot study in patients with DM2 we did not observe a rise in 3DG after the first meal (21).

Some studies have shown that total serum CML is higher in DM2 than in healthy individuals (32,33), although in other studies no difference was observed (22). We chose not to measure total CML and CEL, but rather the unbound forms of these compounds, that are continuously released during degradation of intracellular proteins with a rapid turn-over. Because oxidative stress not only enhances formation of protein-bound CML and CEL, but also triggers proteasomal degradation of damaged proteins (34,35), the unbound fraction of CML and CEL may better reflect formation of AGEs by oxidative stress. We observed that CML was affected by both meal types. However, possibly due to a relatively large biological variation, as reflected by differences in the baseline CML and CEL values at the two meal visits, these changes were less consistent than for oxLDL and 3DG. Future studies should assess whether changes in the protein-bound forms of CML and CEL are more informative.

The major precursor of CML is glyoxal and oxidative stress seems to enhance the formation of glyoxal from glucose (36), which is consistent with our finding of a greater increase in CML after the carbohydrate-rich meals compared to the fat-rich meals.

Potential limitations
First, both groups were not matched for BMI, lipids, blood pressure and alcohol intake, all of which may have independent effects on the parameters measured. However, we chose not to match for these variables, to avoid selection of relatively healthy DM2 patients. Second, our study was performed in elderly postmenopausal women and the changes observed may be different in younger women as well as in men.

Conclusion
In summary, we found significant elevations of postprandial oxLDL/LDL-C, 3DG and CML in postmenopausal women with DM2 and NGM. Since postprandial increases in α-dicarbonyls and associated AGEs are
related to cellular damage, therapies aimed at reducing postprandial glycemia and triglyceridemia, may contribute to the reduction in micro- and macrovascular complications.

Acknowledgements
Supported by Novartis AG Switzerland and a grant from Dutch Diabetes Research Foundation (grant no.2001.00.052). We thank Danielle van Assema for her assistance in organization and Rick Vermue for his excellent technical assistance.
References


<table>
<thead>
<tr>
<th>Variables</th>
<th>NGM</th>
<th>DM2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>27</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.3 (4.0)</td>
<td>60.4 (3.2)</td>
<td>0.93</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (3.1)</td>
<td>31.8 (5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.5 (0.3)</td>
<td>7.6 (1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6 (0.3)</td>
<td>6.5 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.0 (1.0)</td>
<td>5.6 (0.9)</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.93 (0.57)</td>
<td>1.47 (0.30)</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.6 (0.9)</td>
<td>3.3 (0.9)</td>
<td>0.22</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)*</td>
<td>0.9 (0.8-1.6)</td>
<td>2.0 (1.4-2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 (12)</td>
<td>143 (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>69 (8)</td>
<td>79 (7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (former)</td>
<td>56%</td>
<td>39%</td>
<td>0.16</td>
</tr>
<tr>
<td>Alcohol-intake (g/week)</td>
<td>78 ± 12</td>
<td>24 ± 7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>0</td>
<td>58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes treatment (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- diet alone</td>
<td>-</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>- metformin</td>
<td>-</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>- sulfonylureas</td>
<td>-</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>- acarbose</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or median (interquartile range) in case of skewed data. NGM= normal glucose metabolism, DM2= type 2 diabetes mellitus. *In-transformed before analysis.
Table 2.

Fasting and postprandial (8 hours) levels of oxLDL/LDL-C, 3DG, CML and CEL in women with NGM compared to women with DM2*

<table>
<thead>
<tr>
<th></th>
<th>NGM</th>
<th>DM2</th>
<th>NGM</th>
<th>DM2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Postprandial</td>
<td>Fasting</td>
<td>Postprandial</td>
</tr>
<tr>
<td>OxLDL/LDL-C (U/mmol)</td>
<td>Fat</td>
<td>21.6 (3.8)</td>
<td>25.1 (4.8)‡</td>
<td>25.4 (4.6)‡</td>
</tr>
<tr>
<td></td>
<td>Carb</td>
<td>21.6 (2.8)</td>
<td>23.7 (3.8)‡</td>
<td>25.7 (4.3)‡</td>
</tr>
<tr>
<td>3DG (nmol/l)</td>
<td>Fat</td>
<td>164 (41)</td>
<td>183 (45)‡</td>
<td>208 (60)§</td>
</tr>
<tr>
<td></td>
<td>Carb</td>
<td>164 (37)</td>
<td>208 (40)§</td>
<td>210 (49)§</td>
</tr>
<tr>
<td>CML (nmol/l)</td>
<td>Fat</td>
<td>39.6 (10.1)</td>
<td>45.1 (10.6)‡</td>
<td>52.2 (18.1)‡</td>
</tr>
<tr>
<td></td>
<td>Carb</td>
<td>43.3 (10.7)</td>
<td>54.7 (9.4)§</td>
<td>48.2 (16.2)‡</td>
</tr>
<tr>
<td>CEL (nmol/l)</td>
<td>Fat</td>
<td>62.7 (31.2)</td>
<td>67.4 (15.1)</td>
<td>67.0 (27.4)</td>
</tr>
<tr>
<td></td>
<td>Carb</td>
<td>56.4 (13.5)</td>
<td>72.1 (12.8) §</td>
<td>65.8 (23.3)</td>
</tr>
</tbody>
</table>

*Abbreviations: NGM: normal glucose metabolism, DM2: type 2 diabetes mellitus, Fat: fat-rich meals, Carb: carbohydrate-rich meals, OxLDL/LDL-C: ratio of oxidized LDL to total LDL-cholesterol, 3DG: 3-dexoxyglucosone, CML: Nepsilon-(carboxymethyl)-lysine, CEL: Nepsilon-(carboxyethyl)-lysine
† P < 0.05 DM2 baseline versus NGM baseline or 8h versus baseline
‡ P < 0.01 DM2 baseline versus NGM baseline or 8h versus baseline
§ P < 0.001 DM2 baseline versus NGM baseline or 8h versus baseline
Legends

Figure 1. Time course of triglycerides and glucose concentrations (mean±SD) following two consecutive fat-rich or carbohydrate-rich meals in women with normal glucose metabolism (open circles) and type 2 diabetes mellitus (black circles).
Figure 2. Relations between postprandial changes of the oxLDL-to-LDL-cholesterol ratio and 8h incremental area under the curve for plasma triglycerides (A) and between changes of 3-deoxyglucosone and 8h incremental area under the curve for glucose (B) after two consecutive fat-rich meals in women with normal glucose metabolism (open circles) and type 2 diabetes mellitus (black circles). Strengths of the associations are indicated by Spearman’s correlation coefficients.