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1 **Effect of three consecutive meals on the physicochemical properties of HDL and LDL in**  
2 **individuals with the metabolic syndrome and patients with type 2 diabetes**

3

4

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13

14 Running title; Meal related changes to HDL and LDL

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22

1 **Abstract**

2

3 **Background/Objectives:** Postprandial hyperlipidemia, which is exaggerated and prolonged  
4 in insulin resistant individuals, has been associated with cardiovascular disease. The objective  
5 of the present study was to investigate whether and how the composition, size, and function of  
6 HDL and LDL particles are affected in the postprandial state among males with the metabolic  
7 syndrome (MetS) or type 2 diabetes (T2DM), compared to controls.

8 **Subjects/Methods:** Fourteen males with T2DM, 14 with the MetS , and 14 age-matched  
9 controls were given 3 standardized high-fat mixed meals (900 kcal; 50-g fat, 75-g  
10 carbohydrate, and 35-g protein) as breakfast, lunch and dinner. Blood sampling was  
11 performed just before each meal, and 4 and 8 hours after the last meal. HDL and LDL were  
12 isolated by ultracentrifugation and analyzed for their composition, particle diameter, and  
13 functional properties.

14 **Results:** Postprandial triglycerides levels in plasma, HDL and LDL particles increased  
15 significantly in all groups ( $P<0.01$ ). Compared to the control subjects, patients with T2DM  
16 had smaller LDL particles, and in agreement, a lower cholesterol-to-protein content in both  
17 fasting and postprandial samples. A prolonged increase in susceptibility of LDL to oxidation  
18 was found in all subjects, but was most evident in T2DM. The postprandial effect on LDL  
19 oxidation was associated with an increase in LDL triglyceride ( $r=0.29$ ,  $P<0.05$ ). In T2DM the  
20 anti-oxidative capacity of HDL trended to impairment after the third meal.

21 **Conclusions:** Postprandial increases in triglycerides, especially in T2DM, are accompanied  
22 by pro-atherosclerotic functional changes in HDL and LDL particles.

23

24 **Keywords:** atherogenesis; diabetes; HDL; LDL; metabolic syndrome; postprandial

25

1 **Introduction**

2

3 Postprandial hyperlipidemia is considered to be an important risk factor for atherosclerosis  
4 (Lopez-Miranda *et al.*, 2007; O'Keefe *et al.*, 2007). In clinical practice, however, triglycerides  
5 are traditionally measured in the fasting state, typically the lowest triglyceride level of the  
6 day. Recent studies have shown that measurement of non-fasting triglycerides levels may be  
7 superior to fasting concentrations because the former are clearly associated with increased  
8 risk of cardiovascular disease (CVD) and mortality (Bansal *et al.*, 2007; Mora *et al.*, 2008;  
9 Nordestgaard *et al.*, 2007). Postprandial responses related to triglyceride metabolism may  
10 trigger a number of pro-atherosclerotic processes including leukocyte activation (Schindhelm  
11 *et al.*, 2008), up-regulation of pro-inflammatory genes (van Oostrom *et al.*, 2003), decrease in  
12 circulating antioxidant defense (Cardona *et al.*, 2008), and oxidative stress (Bowen *et al.*,  
13 2004; O'Keefe *et al.*, 2007; Ursini *et al.*, 2002). Furthermore, reactive oxygen species (ROS)  
14 peroxidize constituents of lipoproteins, leading to modifications, including oxidized LDL and  
15 HDL, that may alter their functional properties. Individuals with the metabolic syndrome  
16 (MetS) and particularly patients with type 2 diabetes mellitus (T2DM) have a postprandial  
17 hyperlipidemia which is associated with an increased CVD risk. It is likely that the  
18 lipoprotein composition and physicochemical properties in MetS and T2DM may become  
19 postprandially more affected in comparison to healthy normolipidemic subjects. The  
20 postprandial effects on lipoprotein metabolism have been described in literature, but in  
21 contrast to our study most studies used just one meal and restricted to one or two study  
22 groups. To the best of our knowledge, the present study is the first to examine the influence of  
23 three consecutive meals on both LDL and HDL properties in men with the MetS or T2DM  
24 compared to controls. So, the aim of the present study was to investigate whether postprandial

- 1 hypertriglyceridemia affects the physicochemical properties of HDL and LDL in individuals
- 2 with T2DM and with the MetS, in comparison to controls.
- 3

1 **Methods**

2

3 *Study design*

4 In total 42 nonsmoking Caucasian male subjects were enrolled; 14 patients with  
5 uncomplicated T2DM, 14 subjects with the MetS, and 14 age-matched volunteers (i.e.  
6 ‘control group’). Diet, sulphonylurea and/or metformin were the only glucose-lowering  
7 treatments allowed in the T2DM group. Males with the MetS had to meet 3 out of 5 inclusion  
8 criteria based on NCEP/ATP III-criteria (Cleeman *et al.*, 2001), without having  
9 hyperglycemia during a 75-g oral glucose tolerance test (OGTT). Inclusion criteria for control  
10 males included a BMI > 25 kg/m<sup>2</sup>, normoglycemia during a 75-g OGTT, fasting triglycerides  
11 < 1.7 mmol/l, and blood pressure < 145/90 mmHg. Exclusion criteria for all were excess  
12 alcohol intake (>20 units/week), history of hepatitis and/or pancreatitis, abnormal liver and  
13 renal function tests (>2 times upper limits of normal), recent (<3 months) changes in weight  
14 (≥5%) and/or medication, history or current use of glucocorticosteroids, insulin and/or  
15 thiazolidinediones. Since it has been demonstrated that statins reduce postprandial lipid  
16 excursions in several patients groups, including MetS (Hajer *et al.*, 2009), we excluded  
17 subjects using statins or other lipid-lowering drugs. Participants were instructed to omit their  
18 medication during the examination and to refrain from heavy physical activities during the  
19 previous 24-h. After an overnight fast, participants received three consecutive, isocaloric (900  
20 kcal) mixed meals (75-g carbohydrates, 50-g fat [60% saturated], and 35-g protein) at time  
21 points t = 0-h (breakfast), 4-h (lunch), and 8-h (dinner) and were studied during a 16-h period  
22 (Tushuizen *et al.*, 2007). Venous blood collections were performed before and 4 hours  
23 following every single meal (t = 0, 4, 8, 12, and 16-h) for measurements of plasma lipids,  
24 glucose, insulin and for measurement of the physicochemical properties of HDL and LDL.  
25 Plasma aliquots were obtained after centrifugation (1550g, 20 min at 4°C), snap frozen in

1 liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until assay. Informed consent was obtained from all  
2 participants and the study was approved by the local ethics committee.

3

#### 4 *Routine biochemical analyses*

5 Glucose was measured enzymatically (Roche, Mannheim, Germany) and insulin with a  
6 double-antibody radioimmunoassay (Linco Research, St. Louis, MO, USA). HbA1c was  
7 analyzed by ion-exchange high-performance liquid chromatography (Bio-Rad, Veenendaal,  
8 The Netherlands). Total-cholesterol, HDL-cholesterol and triglycerides were measured by  
9 standard enzymatic methods (Roche, Mannheim, Germany). LDL-cholesterol was calculated,  
10 but only in fasting plasma samples (Friedewald *et al.*, 1972). The protein content of LDL and  
11 HDL was measured by the Lowry method, modified to allow measurement in lipoproteins as  
12 described by Markwell (Markwell *et al.*, 1981). The concentration of cholesterol and  
13 triglycerides in isolated lipoprotein fractions were measured by standard enzymatic methods  
14 (Roche, Mannheim, Germany).

15

#### 16 *HDL and LDL particle size and function*

17 HDL and LDL were isolated by ultracentrifugation and afterwards the average particle size  
18 was determined by high performance gel-filtration chromatography, using fibrinogen (22.2  
19 nm), thyroglobulin (17.0 nm) and bovine serum albumin (7.1 nm) as calibrators of known  
20 diameter (Scheffer *et al.*, 1997). Intra- and inter-assay CVs were 0.1% and 0.2%, respectively.  
21 The susceptibility of LDL to *in vitro* oxidation, expressed as lag time, was determined by  
22 monitoring the conjugated dienes formation using copper (II) ions as pro-oxidant at a final  
23 concentration of  $18\ \mu\text{mol/L}$  (Scheffer *et al.*, 2003). Experiments were performed at  $30^{\circ}\text{C}$  on a  
24 HTS 7000 plate reader (Perkin Elmer, Norwalk, CT, USA). The intra- and inter-assay CVs for  
25 lag time determination were 1.6% and 3.6%. The anti-oxidative function of HDL was

1 assessed by monitoring its capacity to inhibit the oxidation of dichlorodihydrofluorescein  
2 (Invitrogen, Carlsbad, CA, USA) by oxidized 1-palmitoyl-2-arachidonoyl-sn-  
3 phosphatidylcholine (Avanti Lipids, Alabaster, AL, USA) (Navab *et al.*, 2001). Briefly, in a  
4 total volume of 790  $\mu$ L phosphate buffered saline (pH 7.4), 35  $\mu$ L of a normal LDL solution  
5 (final concentration of 18  $\mu$ mol/L cholesterol), 35  $\mu$ L of test HDL-cholesterol (final  
6 concentration of 11  $\mu$ mol/L cholesterol), 20  $\mu$ L oxidized 1-palmitoyl-2-arachidonoyl-sn-  
7 phosphatidylcholine (final concentration of 50  $\mu$ mol/L), and 10  $\mu$ L of  
8 dichlorodihydrofluorescein solution (final concentration of 40  $\mu$ mol/L) were incubated in  
9 glass tubes for 2 hours at 37°C. Afterwards the fluorescence intensity was determined with a  
10 HTS 7000 plate reader at 485 and 530 nm (excitation and emission wavelength, respectively).  
11 Values for fluorescence intensity at t=0 for each group were normalized to 1.0, and  
12 consequently HDL function > 1.0 in postprandial samples may indicate dysfunctionality. The  
13 intra-assay CV was 3.4%.

14

#### 15 *Statistical analysis*

16 Data are presented as mean (SD) or median (interquartile range). Non-normally distributed  
17 data were log transformed before analysis. Correlations were performed using Spearman's  
18 rank correlation test and differences between groups were tested using ANOVA and  
19 Bonferroni post hoc analyses. To estimate the overall changes during 16-h, the area under  
20 curve (AUC) of metabolic parameters against time was calculated according to the linear  
21 trapezoidal method. In order to test whether the changes of the physicochemical properties of  
22 HDL and LDL over time are affected by group, i.e. controls, MetS and T2DM, we performed  
23 analysis of variance (ANOVA) for repeated measurements with the interaction-term time x  
24 group.  $P < 0.05$  was considered to indicate statistical significance. All analyses were performed  
25 using SPSS software, version 15 (SPSS Inc, Chicago, IL, USA).

26

1 **Results**

2

3 *Baseline characteristics*

4 Subject characteristics at baseline divided by group are illustrated in Table 1. No significant  
5 differences between the groups were noted in age, fasting total cholesterol and LDL-  
6 cholesterol. Individuals with either MetS or T2DM had higher values for waist circumference,  
7 blood pressure, insulin and plasma triglycerides. BMI, fasting glucose and HbA1c were  
8 significantly higher in the T2DM group. HDL-cholesterol was significantly lower in MetS  
9 and T2DM. Table 2 shows the physicochemical properties of HDL and LDL in the three  
10 study groups before the meals. The triglyceride content of lipoprotein particles was higher in  
11 the T2DM group, but reached statistical significance only in HDL. At baseline, T2DM  
12 patients, relative to controls, had smaller LDL particles ( $P<0.05$ ) and in agreement a lower  
13 cholesterol-to-protein content in their LDL ( $P<0.05$ ). Likewise, the cholesterol content of  
14 HDL was significantly lower in patients with MetS and T2DM (both  $P<0.001$ ), but  
15 differences in fasting HDL particle size did not reach statistical significance ( $P=0.22$  and  
16  $P=0.08$ , respectively for MetS and T2DM compared to controls).

17

18 *Effects of meals on plasma lipids, glucose, insulin and lipoprotein properties*

19 The postprandial responses following three consecutive meals were shown in Figures 1 and 2  
20 and were also presented as AUC in Table 3. Plasma triglyceride peaked 4 hours ( $t=4$ -h) after  
21 the first meal in controls, whereas in MetS and T2DM the highest levels were observed just  
22 before dinner ( $t=8$ -h; Figure 1). The increase in plasma triglyceride was similar in MetS and  
23 T2DM. Postprandially, plasma HDL-cholesterol decreased somewhat in all groups. In MetS  
24 and T2DM compared to the control group, AUCs for plasma triglycerides concentrations  
25 were higher, whereas AUCs for HDL-cholesterol were lower. Postprandial glucose increased

1 in all groups at t=12-h, but a marked rise was observed only in T2DM. Insulin concentrations  
2 were elevated following each meal, reaching the highest concentrations after the third meal  
3 (t=12-h) in all groups, with the highest responses in MetS.

4 HDL and LDL were postprandially enriched in triglyceride-to-protein ratio in all three  
5 study groups (Figure 2) reaching a maximum concentration between 8 and 12 hours after  
6 breakfast. The lipoprotein triglyceride responses, expressed as AUC, were similar amongst  
7 the groups (Table 3). Postprandial HDL and LDL particles derived from T2DM and MetS  
8 versus controls had a decreased cholesterol content when expressed as AUC (Table 3),  
9 reaching the lowest values 8h after breakfast (Figure 2). Postprandial HDL and LDL particles,  
10 especially those from T2DM, were larger compared to fasting diameters (Figure 2). In the  
11 T2DM group, the HDL size peaked at t=8 and LDL at t=12. Postprandial changes in HDL  
12 size correlated significantly to changes in LDL size ( $r=0.47$ ;  $P<0.01$ ; all groups). The anti-  
13 oxidative capacity of HDL was hardly effected in the postprandial state, except for an change  
14 towards impairment after the third meal in T2DM (t=12-h; Figure 2), compared to both other  
15 groups. Subsequently, at the next time-point measured, HDL functionality returned to  
16 baseline level. The postprandial effect on HDL function was positively correlated to changes  
17 in HDL-cholesterol content and HDL particle size ( $r=0.50$  and  $r=0.46$ , respectively; both  
18  $P<0.01$ ; all groups). LDL lag time was postprandially decreased in all groups after breakfast  
19 and subsequently returned toward baseline levels at t=4-h in controls, at t=8-h in MetS, and at  
20 t=12-h in T2DM. The most important correlate of postprandial changes in LDL lag time was  
21 the increase of plasma triglyceride concentration ( $r=0.29$ ,  $P<0.05$ ; all groups).

22 The P-values of the time x group interaction terms for the physicochemical properties  
23 of LDL and HDL were all statistically non-significant (all  $P>0.05$ ), except for the triglyceride  
24 to protein ration in HDL ( $P<0.05$ ). However the differences of the AUC of the triglyceride to  
25 protein ration in HDL was non-significant (all  $P>0.05$ ).

26

1 **Discussion**

2

3 Our results show that plasma triglycerides increased significantly after breakfast and lunch,  
4 but remarkably no further increase was found after dinner. The prolonged  
5 hypertriglyceridemia following three consecutive meals in males with the MetS and patients  
6 with T2DM results in the formation of triglyceride-enriched HDL and LDL particles and an  
7 concomitant increased postprandial susceptibility of LDL to oxidation, especially in those  
8 with T2DM. Furthermore, enlarged lipoprotein particles and an impairment of the anti-  
9 oxidative capacity of HDL were postprandially observed in T2DM.

10 Insulin resistant states, including the MetS and T2DM, are associated with decreased  
11 plasma HDL-cholesterol concentration, whereas LDL-cholesterol concentration is usually  
12 normal. However, in T2DM the metabolism of LDL is modified, rendering the lipoprotein  
13 particles more atherogenic (Scheffer *et al.*, 2005). Furthermore, it has been shown that high-  
14 fat intake is associated with oxidative stress. For example, monocytes polymorphic nuclear  
15 cells release more superoxide anion when they are exposed to plasma from patients with  
16 hypertriglyceridemia (Hiramatsu *et al.*, 1988; Pronai *et al.*, 1991). In addition, whipping  
17 cream (75-g of fat) resulted in an increase in nitrotyrosine, a marker of peroxynitrite and  
18 nitrosative stress generation, in normal subjects and T2DM patients (Ceriello *et al.*, 2004).  
19 The ensuing postprandial oxidative stress may trigger a number of atherogenic changes  
20 including increases in inflammation, vasoconstriction and oxidation of LDL (O'Keefe *et al.*,  
21 2007). In the present study we have focused on (oxidative) modifications to both HDL and  
22 LDL, like changes in composition, function, and particle size.

23 Lipoprotein particles have been demonstrated to be heterogeneous in size. Several  
24 studies measured LDL particle diameter using non-denaturing polyacrylamide gel  
25 electrophoresis in the postprandial state with inconsistent results. Small, but significant,

1 reductions of LDL size of up to 0.1 nm were observed in myocardial infarction patients 6-h  
2 after an oral fat tolerance test (Koba *et al.*, 2005), and in apparently healthy men 4-h after a  
3 breakfast with high-fat (Blackburn *et al.*, 2003). A decrease in LDL size of 0.4 nm was  
4 observed in hypertriglyceridemic patients 6-h after an oral fat load (Noto *et al.*, 2006). The  
5 group of Taskinen measured LDL size 6-h after a high-fat meal in patients with coronary  
6 artery disease (Graner *et al.*, 2006), and 8-h after a mixed fat-rich meal in subjects with  
7 diabetes (Vakkilainen *et al.*, 2002). In both studies, LDL particles size remained statistically  
8 unchanged. In contrast to these studies, but in agreement to our study, a significant increase in  
9 LDL size (0.73 nm;  $P < 0.05$ ) was found in patients with primary mixed hyperlipidemia after  
10 an oral fat-tolerance test (Foger *et al.*, 1995). In the present study we used high-performance  
11 gel filtration chromatography for measurement of HDL and LDL particle size, which is  
12 regarded as a very precise technique (CV's  $< 0.2\%$ ) (Scheffer *et al.*, 1997), and as such, small  
13 changes in diameter will be easily detected. The mean size of HDL and LDL in T2DM was  
14 postprandially enlarged probably due to the increase in triglyceride content. Triglyceride in  
15 HDL and LDL is a good substrate for hepatic lipase, which hydrolyses triglycerides and thus  
16 ultimately converts lipoprotein particles smaller and denser. Since our follow-up duration was  
17 16-h, it is possible that we have missed this latter part. Interestingly, HDL and LDL size  
18 peaked at different time points. In T2DM, HDL particle size peaked 8-h after breakfast and  
19 the largest mean LDL size was observed 4-h later, at  $t=12$ -h. These results suggest that the  
20 exchange in lipids, mediated by cholesterol-ester transfer protein (CETP), is faster in HDL  
21 than in LDL.

22 T2DM and MetS are associated with low-grade inflammation and oxidative stress.  
23 Therefore, it is likely that the antioxidant and anti-inflammatory properties of HDL, in which  
24 both the protein and lipid components are involved, are altered especially in postprandial  
25 conditions. Although the postprandial anti oxidative properties of HDL derived from T2DM

1 was somewhat impaired after 3 consecutive meals at t=12-h, the current study observed no  
2 significant changes between- and within groups. The anti-inflammatory/oxidative properties  
3 of HDL in the postprandial state have been studied earlier in two relatively small studies with  
4 healthy subjects. Nicholls et al. showed that the anti-inflammatory potential of HDL was  
5 reduced after consumption of saturated fat and improved following the consumption of  
6 polyunsaturated fat (Nicholls *et al.*, 2006). Patel and coworkers demonstrated an impaired  
7 anti-inflammatory capacity of HDL in response to 20% Intralipid (Patel *et al.*, 2009). In these  
8 studies, but in contrast to our study, the anti-inflammatory activity of HDL were measured  
9 using postprandial endothelial cells expression of intracellular cell adhesion molecules-1  
10 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1). It is currently not known  
11 whether this approach for HDL functionality is in agreement with the procedure that has been  
12 used in the present study.

13 In the postprandial state, LDL may become oxidized by an increase of free radical  
14 production (eg. superoxide anion and hydroperoxyl radical) or nonradical oxidants (eg.  
15 hydrogen peroxide and hypochlorite). Oxidation of LDL is an important early event in the  
16 development of atherosclerosis. Oxidatively modified LDL is taken up by macrophages,  
17 which may become foam cells, leading to the formation of atherosclerotic plaques. In  
18 agreement with an earlier study (Scheffer *et al.*, 2003), fasting LDL lag times of T2DM and  
19 controls were not different, but interestingly the present study showed that postprandial LDL,  
20 especially derived from T2DM, is more susceptible to *in vitro* oxidation. Diwadkar et al.  
21 demonstrated that postprandial samples of diabetic subjects had a significantly shorter lag  
22 phase following one physiological meal (85-g fat), but unfortunately they did not measure the  
23 lipid content of this lipoprotein class (Diwadkar *et al.*, 1999). Of note, we demonstrated that  
24 the postprandial increase of LDL towards oxidation was positively associated with the

1 triglyceride-to-protein ratio in LDL. In agreement, the highest triglyceride content in LDL and  
2 the most pronounced change in LDL oxidation was found in T2DM.

3         There are several limitations of this study. In each group a relatively small number of  
4 subjects was examined and because our study has been conducted in men, the results may  
5 require replication in women. In addition, the exact peak of the highest postprandial  
6 concentrations is not known because a 4-h interval was used. Finally, we did not measure  
7 systemic oxidative stress levels.

8  
9         Altogether, in subjects with the MetS and in patients with T2DM, the exaggerated and  
10 prolonged postprandial concentrations of plasma triglycerides lead to an increased triglyceride  
11 content of HDL and LDL and in agreement, a lower cholesterol-to-protein content in  
12 postprandial lipoprotein samples. The cholesterol-to-protein ratio and particle size of HDL  
13 have been shown to be important correlates of the anti oxidative properties of HDL, whereas  
14 the LDL triglyceride content was a determinant of LDL susceptibility to *in vitro* oxidation. In  
15 both fasting and postprandial samples, subjects with MetS and T2DM had smaller HDL and  
16 LDL particles. A prolonged period of increased LDL oxidisability was seen in T2DM. In  
17 conclusion, our study demonstrated that the exposure to 3 high-fat mixed consecutive meals  
18 results into pro-atherosclerotic changes of lipoprotein particles, especially in T2DM.

19

1 **Conflict of interest**

2 The authors declare no conflict of interest.

3

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8

9

10

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1 **Figure legends**

2

3 ***Figure 1***

4 Time courses during 16-h of the postprandial response following 3 consecutive meals of  
5 plasma triglyceride, HDL-cholesterol, glucose, and insulin (mean  $\pm$  SEM) in control subjects  
6 (closed circles), individuals with the metabolic syndrome (open squares) and patients with  
7 type 2 diabetes (open circles). B, breakfast; L, lunch; D, diner.

8

9 ***Figure 2***

10 Time courses during 16-h of the postprandial response following 3 consecutive meals of high-  
11 density lipoprotein and low-density lipoprotein constituents (triglyceride and cholesterol) and  
12 size and of the anti inflammatory function of high-density lipoprotein and the susceptibility of  
13 low-density lipoprotein to oxidation (mean  $\pm$  SEM) in control subjects (closed circles),  
14 individuals with the metabolic syndrome (open squares) and patients with type 2 diabetes  
15 (open circles). The concentration of cholesterol and triglyceride in lipoprotein fractions are  
16 expressed as mmol per gram protein. B, breakfast; L, lunch; D, diner.

17

18

1 **Table 1**

2 **Baseline clinical and biochemical characteristics of the study-population**

3

Variable	Controls	MetS	<i>P</i> -value <sup>a</sup>	T2DM	<i>P</i> -value <sup>b</sup>
<i>n</i>	14	14		14	
Age, years	54.3 (7.5)	56.4 (6.5)	NS	55.5 (4.0)	NS
BMI, kg/m <sup>2</sup>	27.5 (2.9)	30.7 (3.2)	NS	32.7 (4.1)	<0.001
Waist circumference, cm	101.2 (8.4)	111.8 (9.8)	0.012	112.1 (9.2)	0.009
SBP, mmHg	121 (6)	139 (13)	<0.001	136 (13)	<0.001
DBP, mmHg	74 (5)	84 (6)	<0.001	83 (4)	<0.001
Fasting glucose, mmol/L	5.4 (0.2)	5.6 (0.4)	NS	8.8 (2.2)	<0.001
HbA1c, %	5.6 (0.4)	5.9 (0.4)	NS	7.2 (1.1)	<0.001
Insulin, pmol/L	27 (22-37)	51 (36-85)	0.007	66 (41-84)	<0.001
Total cholesterol, mmol/L	5.1 (0.9)	5.4 (0.9)	NS	5.1 (0.5)	NS
HDL-cholesterol, mmol/L	1.49 (0.40)	1.10 (0.31)	0.006	1.06 (0.18)	0.002
LDL-cholesterol, mmol/L	3.1 (0.8)	3.2 (0.8)	NS	3.1 (0.5)	NS
Triglycerides, mmol/L	1.0 (0.7-1.2)	2.0 (1.7-2.6)	0.002	1.8 (1.3-2.7)	0.002

4

5 MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus; BMI, body mass index; DBP,  
6 diastolic blood pressure; SBP, systolic blood pressure. Data are presented as mean (SD), or  
7 median (interquartile range).

8 <sup>a</sup>MetS syndrome compared with control group

9 <sup>b</sup>T2DM compared with control group

10

1 **Table 2**  
 2 **Fasting lipoprotein properties of the study-population**

3

Variable	Controls	MetS	P-value <sup>a</sup>	T2DM	P-value <sup>b</sup>
<i>n</i>	14	14		14	
<i>HDL constituents and size:</i>					
Triglyceride-to-protein, mmol/g	0.07 (0.02)	0.08 (0.03)	NS	0.09 (0.03)	0.018
Cholesterol-to-protein, mmol/g	0.93 (0.15)	0.70 (0.12)	<0.001	0.67 (0.11)	<0.001
Particle size, nm	8.61 (0.35)	8.42 (0.18)	NS	8.38 (0.21)	NS
<i>LDL constituents, size and susceptibility to oxidation:</i>					
Triglyceride-to-protein, mmol/g	0.27 (0.07)	0.25 (0.05)	NS	0.30 (0.11)	NS
Cholesterol-to-protein, mmol/g	4.31 (0.33)	4.06 (0.30)	NS	3.93 (0.41)	0.018
Particle size, nm	21.23 (0.55)	20.59 (0.47)	0.016	20.69 (0.62)	<0.05
Oxidisabilty, min	63.2 (7.4)	56.8 (7.6)	NS	62.8 (8.7)	NS

4

5 MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus.

6 Data are presented as mean (SD).

7 <sup>a</sup>MetS syndrome compared with control group

8 <sup>b</sup>T2DM compared with control group

9

1 **Table 3**  
 2 **Postprandial response of 3 consecutive meals on plasma lipids, glucose, insulin,**  
 3 **lipoprotein properties, during a 16-h period expressed as area under the curve (AUC)**  
 4

Variable	Controls	MetS	<i>P</i> -value <sup>a</sup>	T2DM	<i>P</i> -value <sup>b</sup>
<i>n</i>	14	14		14	
<i>AUC plasma variables</i>					
Triglycerides, mmol/L	25.3 (11.3)	45.2 (13.6)	0.014	46.1 (24.8)	0.010
HDL-cholesterol, mmol/L	23.1 (6.4)	15.7 (4.1)	<0.001	14.9 (2.6)	<0.001
Glucose, mmol/L	88.2 (6.9)	91.2 (5.0)	NS	145.8 (48.1)	<0.001
Insulin, nmol/L	1.3 (0.7)	3.3 (2.4)	<0.001	2.7 (0.7)	<0.001
<i>AUC HDL constituents and size:</i>					
Triglyceride-to-protein, mmol/g	1.4 (0.3)	1.7 (0.5)	NS	1.8 (0.6)	NS
Cholesterol-to-protein, mmol/g	14.6 (2.8)	10.8 (1.8)	<0.001	10.2 (1.8)	<0.001
Particle size, nm	145 (6)	142 (4)	NS	142 (4)	NS
<i>AUC LDL constituents, size and oxidation</i>					
Triglyceride-to-protein, mmol/g	5.0 (1.1)	4.5 (1.0)	NS	5.3 (1.8)	NS
Cholesterol-to-protein, mmol/g	69.4 (5.1)	64.1 (5.1)	NS	62.4 (7.1)	0.009
Particle size, nm	341 (8)	330 (7)	0.005	332 (10)	0.042
Oxidisabilty, min	987 (105)	898 (112)	NS	951 (133)	NS

5  
 6 AUC, area under the curve over a 16h time period; MetS, metabolic syndrome; T2DM, type 2  
 7 diabetes mellitus.  
 8 Data are presented as mean (SD).  
 9 <sup>a</sup>MetS syndrome compared with control group  
 10 <sup>b</sup>T2DM diabetes compared with control group



