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Randomized Control Trials

Diet-induced weight loss reduces postprandial dicarbonyl stress in abdominally obese men: Secondary analysis of a randomized controlled trial

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SUMMARY

Aims: Dicarbonyl compounds contribute to the formation of advanced glycation endproducts (AGEs) and the development of insulin resistance and vascular complications. Dicarbonyl stress may already be detrimental in obesity. We evaluated whether diet-induced weight loss can effectively reverse dicarbonyl stress in abdominally obese men.

Materials and methods: Plasma samples were collected from lean ($n = 25$) and abdominally obese men ($n = 52$) in the fasting state, and during a mixed meal test (MMT). Abdominally obese men were randomized to 8 weeks of dietary weight loss or habitual diet, followed by a second MMT. The α-dicarbonyls methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG) and AGEs were measured by UPLC-MS/MS. Skin autofluorescence (SAF) was measured using the AGE reader. T-tests were used for the cross-sectional analysis and ANCOVA to assess the treatment effect.

Results: Postprandial glucose, MGO and 3-DG concentrations were higher in obese men as compared to lean men ($p < 0.05$ for all). Fasting dicarbonyls, AGEs, and SAF were not different between lean and obese men. After the weight loss intervention, fasting MGO levels tended to decrease by 25 nmol/L (95%-CI: -51-0.5; $p = 0.054$). Postprandial dicarbonyls were decreased after weight loss as compared to the control group: iAUC of MGO decreased by 57% (5280 nmol/L·min; 95%-CI: 33–10526; $p = 0.049$), of GO by 66% (11,329 nmol/L·min; 95%-CI: 495–22162; $p = 0.041$), and of 3-DG by 45% (20,175 nmol/L·min; 95%-CI: 5351–35000; $p = 0.009$). AGEs and SAF did not change significantly after weight loss.

Conclusion: Abdominal obesity is characterized by increased postprandial dicarbonyl stress, which can be reduced by a weight loss intervention.

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Abbreviations: 3-DG, 3-deoxyglucosone; AGEs, Advanced glycation endproducts; AKR1B1, Aldo-Keto Reductase Family 1 Member B; ALDH2, Aldehyde dehydrogenase; CEL, N^{ε}-(1-carboxyethyl)lysine; CML, N^{ε}-(carboxymethyl)lysine; GLO1, Glyoxalase 1; GO, Glyoxal; (i)AUC, (Incremental) Area under the curve; MG-H1, N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; MGO, Methylglyoxal; SAF, Skin autofluorescence; UPLC-MS/MS, Ultra-performance liquid chromatography tandem mass spectrometry.

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1. Introduction

The ever-increasing rise in obesity contributes to the increasing incidence of type 2 diabetes and cardiovascular disease [1]. The formation of dicarbonyl compounds in obesity may be a key driver in the development of type 2 diabetes and cardiovascular disease [2]. Dicarbonyl compounds are reactive metabolites that are mainly formed as a byproduct of glycolysis, especially in the postprandial state. Dicarbonyl compounds may react with protein residues to form advanced glycation endproducts (AGEs) which are linked to the detrimental effects on cellular and vascular function [3,4]. In addition, in animal models of obesity, increased levels of dicarbonyls are linked to adipose tissue dysregulation [5,6].

Methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) are the major dicarbonyl compounds [7]. Recently, we demonstrated that individuals with type 2 diabetes had higher plasma levels of MGO, GO and 3DG during an oral glucose load [8] or a meal [9] compared to healthy individuals. As MGO is the major precursor in the formation of AGEs, it is a key player in the development of age-related diseases [10]. Moreover, MGO has been associated with the development of diabetic complications [3], hypertension [11], atherosclerosis [12], cancer [13], and neurodegenerative disorders [14]. In addition, we recently found MGO to be associated with incident cardiovascular disease in both type 1 and type 2 diabetes [15,16]. The glyoxalase system, with glyoxalase I (GLO1) as the rate-limiting enzyme, is responsible for the enzymatic detoxification of MGO. It has been demonstrated that GLO1 expression and activity is decreased in adipose tissue and liver in animal models of obesity [5,6]. Downregulation of GLO1, as well as increased MGO formation may contribute to dicarbonyl stress in obesity and the health impairment of the obese phenotype.

In obese women with type 2 diabetes we demonstrated that postprandial dicarbonyl stress could be reduced by a very-low calorie diet [9]. As we hypothesize that dicarbonyl stress is an early contributor to incident T2D and its (cardiovascular) complication, we thus investigated the effect of a dietary weight loss intervention on dicarbonyl stress and AGEs in abdominally obese individuals. We addressed this in two ways. First, we investigated whether abdominally obese individuals had higher fasting and postprandial plasma levels of dicarbonyls and AGEs, as well as skin autofluorescence (SAF). The latter is considered to be a reflection of tissue AGEs. Second, we investigated whether a dietary weight loss intervention could reduce plasma levels of dicarbonyls and AGEs, as well as SAF.

2. Materials and methods

2.1. Study population

The methods of this randomized controlled trial have been described in detail previously [17–19]. Briefly, we included 52 abdominally obese men, as well as 25 lean men. All 74 individuals were between 18 and 65 years, nonsmoking, nondiabetic, and without cardiovascular disease. A stable body weight (weight difference <3 kg for ≥3 months) was required, and inclusion was based on waist circumference <94 cm for the lean men and between 102 and 110 cm for abdominally obese men.

Additional exclusion criteria were: fasting plasma glucose >7.0 mmol/L, HbA1c >6.5%, serum total cholesterol >8.0 mmol/L, serum triglycerides >4.5 mmol/L, systolic blood pressure >160 mmHg. Contraindications for MRI were not allowed and neither was medication use affecting glucose metabolism, blood pressure, or serum lipids.

The study was conducted in accordance with the Declaration of Helsinki, approved by the Ethics Committee of Maastricht University Medical Centre, and registered at ClinicalTrials.gov (NCT01675401). Written informed consent was obtained from all participants before study enrollment.

2.2. Study design and intervention

Abdominally obese men were randomly assigned to either an 8-week weight loss program or maintaining their habitual diet for 8 weeks. Measurements took place before and after the 8-week period. Lean individuals were studied at baseline only.

Individuals assigned to the weight-loss program consumed a very-low-calorie diet (VLCD; Modifast; Novartis Nutrition, The Netherlands) providing 2.1 MJ (500 kcal) per day for 4–5 weeks. Hereafter, a mixed-solid, calorie-restricted diet, which provided 4.2 MJ/day for 1–2 weeks, was prescribed as a transition period. The composition of this diet was in agreement with Dutch dietary guidelines, and was described in detail earlier [17]. In weeks 7 and 8, individuals consumed daily menus based on the estimated energy requirements based on their newly achieved body weight in order to retain a stable weight.

Individuals assigned to the weight-stable control group were asked to maintain their habitual diet throughout the study period and were monitored to avoid fluctuations in weight.

In addition, individuals from all groups were instructed not to change their physical activity levels and write down any protocol deviations in a daily diary. The day before the measurements, individuals were asked not to consume high-fat foods or alcohol or to perform any strenuous physical exercise. After a 12-h overnight fast, volunteers arrived at the research facilities by public transport or by car to standardize pre-measurements conditions. All measurements were conducted in a temperature-controlled room at 24 ± 0.5 °C.

2.3. Mixed meal test

A mixed meal test was performed at baseline and after the intervention period. After an acclimatization period and the placement of an intravenous cannula, a fasting venous blood sample was drawn. Within 10 min, all participants had to consume a standardized mixed meal. Blood samples were collected at regular intervals as described previously [19]. Samples were analyzed for glucose and dicarbonyl postprandial effects, at 0 min (fasted), 60 min, 120 min, and 240 min after consumption of the mixed meal.

The mixed meal consisted of two muffins and 300 mL low-fat milk (0% fat milk; Friesland-Campina, Woerden, The Netherlands). The mixed meal had an energy content of 1100 kcal and provided 26.5 g protein (9.6 En%), 121.0 g carbohydrates (44 En %) and 56.6 g fat (46.6 En%), as previously described [19].

For the plasma dicarbonyls MGO, GO, 3-DG, and for the glucose levels measured during the mixed meal test, we calculated the incremental area under the curve (iAUC) using the trapezoidal rule [20]. To compare the postprandial changes in plasma dicarbonyl or glucose levels, the incremental area under the curve (iAUC) was calculated by subtracting the respective fasting levels from each individual data point. The iAUC of the dicarbonyl levels is expressed as nmol/l x min and the iAUC for glucose is expressed as mmol/l x min.

2.4. Baseline characteristics

Plasma glucose concentrations were determined with a YSI2300 glucose analyzer (YSI). Serum fasting samples were analyzed for total cholesterol (CHOD-PAP method; Roche Diagnostics, Mannheim, Germany), HDL cholesterol (precipitation method; Roche Diagnostics, Mannheim, Germany), triacylglycerol with correction

for free glycerol (GPO Trinder; Sigma–Aldrich Corp., St. Louis, MO, USA), HbA1c (Bio-Rad), and insulin (RIA; Millipore, Billerica, MA, USA) concentrations. Twenty-four-hour ambulatory blood pressure measurements were performed (Mobil-O-Graph; I.E.M.). Blood pressure was recorded every 15 min during daytime and every 30 min at night. Subjects were asked not to change their daily activities during this period except the avoidance of intensive physical exercise. From these recordings 24-h mean ambulatory blood pressure was calculated.

2.5. Measurement of plasma dicarbonyls, AGEs and skin autofluorescence

EDTA and NaF tubes were centrifuged at 1300×g for 15 min at 4 °C to obtain plasma. The supernatants were transferred into 1.5 mL Eppendorf tubes and stored at −80 °C until further analysis. Plasma glucose concentrations were measured in NaF-plasma (Horiba ABX, Montpellier, France). Plasma levels of dicarbonyls and AGEs were measured in EDTA plasma samples.

Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to determine the plasma levels for MGO, GO and 3-DG, as previously described [21]. Plasma levels of the free and protein-bound AGEs N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(1-carboxyethyl)lysine (CEL), and N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), were determined with UPLC-MS/MS. Protein-bound pentosidine was determined with high performance liquid chromatography with fluorescent detection [22]. Coefficients of variation for all MS/MS analyses were below 10%.

Prior to each mixed meal test, skin autofluorescence (SAF) was measured using the AGE reader (DiagnOptics Technologies, Groningen, The Netherlands). The AGE reader is a device that uses the fluorescent properties of certain protein modifications and AGEs, to estimate their level of accumulation in the skin. Technical details have been described elsewhere [23,24].

2.6. Measurement of ketone bodies

Serum samples were collected at the start and end of the intervention period. Ketone body concentrations were measured using nuclear magnetic resonance (NMR). Details about this analysis have been described in detail previously [25,26].

2.7. Measurement of GLO1, AKR1B1, and ALDH2

PBMC's were isolated from the blood samples drawn in the fasted state at baseline and after the 8-week period by using BD Vacutainer Cell Preparation Tubes. RNA was isolated (RNeasy Micro kit, Qiagen, Venlo, The Netherlands) and quantified (Nanodrop ND 1000, Nanodrop Technologies, Wilmington, DE, USA); an Agilent 2100 Bioanalyser with RNA 6000 microchips was used to check integrity. Samples were included for microarray analysis if the RNA integrity number was >7. PBMC samples from 15 lean and 29 abdominally obese individuals yielded enough RNA of sufficient quality to perform microarray analysis. The expression of glyoxalase 1 (GLO1), aldo-keto reductase family 1 member B (AKR1B1), and aldehyde dehydrogenase (ALDH2) between groups was tested with an independent samples t-test with empirical Bayes correction. Details regarding the microarray analysis have been described elsewhere [25].

2.8. Statistics

Normally distributed variables are presented as means ± standard deviations or as median with interquartile range, where appropriate. Cross-sectional differences between lean and abdominally obese men, were analyzed by an independent Student's t-test. Treatment effects of the weight loss intervention compared to the weight stable control group were analyzed by one-way ANCOVA with adjustment for baseline values. Differences in postprandial changes between the study groups were analyzed using mixed ANOVA with intervention or obesity as between-subjects factor and time within-subjects factor. For the mixed ANOVA intervention analysis, we subtracted the baseline postprandial curve from the postprandial curve at follow-up. Non-normally distributed values were log transformed prior to analysis. A P value ≤ 0.05 was considered to be significant. All statistical analyses were performed with IBM SPSS Statistics Software version 20.0.

3. Results

A CONSORT flow diagram of the intervention design and inclusion is shown in Supplemental Figure S1. At baseline, 25 normal-weight and 52 abdominally obese participants were analyzed. From the abdominally obese participants, 49 men completed the study and were included in the analyses; 23 in the weight loss intervention and 26 in the control group [18].

The baseline characteristics are shown in Table 1. Though age was not different between the subgroups, most anthropometric and metabolic indices were significantly different between the lean and obese groups (Table 1). Further details have been described previously [18,19].

3.1. Cross-sectional analysis

In the fasting state, the dicarbonyls MGO, GO, and 3-DG were not significantly different between lean and abdominally obese men (Table 2). We found lower levels of protein-bound CML in the abdominally obese men compared to lean men ($p = 0.025$). Plasma concentrations of protein-bound CEL, protein-bound MG-H1 and pentosidine, and free CML, CEL and MG-H1, did not show a significant difference between lean and obese men (Table 2). SAF was not different between lean and obese individuals (Table 2).

Postprandial concentrations of glucose, MGO and 3-DG, as assessed by the iAUC during the mixed meal test, were significantly higher in obese compared to lean men (Fig. 1, Table S3). Results from the mixed ANOVA analyses of the mixed meal data showed that postprandial increases in MGO ($p = 0.039$) and 3-DG ($p = 0.001$) were significantly different between lean and obese individuals, this was not the case for GO concentrations ($p = 0.29$).

Two hours after the mixed meal, glucose (lean 5.87 ± 0.91 mmol/L vs obese 6.78 ± 1.10 mmol/L, $p = 0.001$), MGO (lean 321 ± 51 nmol/L vs obese 357 ± 70 nmol/L, $p = 0.025$) and 3-DG (lean 1064 ± 163 nmol/L vs obese 1238 ± 203 nmol/L, $p < 0.001$) concentrations were higher in obese compared to lean men; there was no significant difference after 4 h.

3.2. Effect of the weight loss intervention

After an 8-week dietary weight loss intervention, BMI decreased by 10% on average and multiple cardiometabolic variables were improved, as described previously [17–19]. Most notably, there was a decrease in fasting plasma glucose of 0.24 mmol/L (95%-CI: 0.06–0.42; $p < 0.05$), a decrease in HOMA-IR of 1.11 (95%-CI: 0.66–1.56; $p < 0.001$), and a significant increase in whole body glucose disposal of 1.36 mg/kg/min (95%-CI: 0.81–1.92; $p < 0.001$).

Fasting MGO, but not other dicarbonyls, tended to decrease after weight reduction compared to the weight stable control group by 25 nmol/L (95%-CI: -51-0.5; $p = 0.054$; Table 3). SAF was unaffected

Table 1
Study population characteristics.

	Lean (n = 25)	Obese (n = 52)	Weight Loss Baseline (n = 23)	Weight Stable Baseline (n = 26)
Age [Years]	53.7 [25.0–61.6]	51.8 [45.7–60.7]	52.4 [46.8–61.7]	52.0 [45.4–61.1]
Body weight [kg]	74.9 ± 8.3	96.9 ± 8.4***	98.2 ± 8.1	95.9 ± 8.9
Fat Free Mass [kg]	60.8 ± 6.4	69.6 ± 6.1***	69.7 ± 5.9	69.2 ± 6.6
Fat Mass [kg]	14.1 ± 4.8	27.2 ± 4.6***	28.5 ± 4.2	26.7 ± 4.6
BMI [kg/m^2]	23.3 ± 1.8	30.1 ± 2.1***	30.2 ± 1.5	29.9 ± 2.5
Waist Circumference [cm]	84.9 ± 6.3	106.5 ± 3.6***	106.8 ± 3.4	106.2 ± 3.8
Hip Circumference [cm]	96.6 ± 4.3	107.5 ± 5.2***	108.1 ± 4.4	107.2 ± 6.0
HOMA-IR	1.65 ± 0.46	2.84 ± 1.38***	2.64 ± 1.21	2.90 ± 1.40
Fasting Plasma Glucose [mmol/L]	5.35 ± 0.29	5.64 ± 0.48**	5.49 ± 0.37	5.75 ± 0.53
Fasting Plasma Insulin [mIU/L]	7.1 ± 1.9	11.6 ± 5.4***	11.2 ± 5.0	11.5 ± 5.6
HbA1c [%]	5.18 ± 0.37	5.30 ± 0.37	5.24 ± 0.35	5.33 ± 0.39
Total Cholesterol [mmol/L]	4.55 ± 0.78	5.56 ± 0.97***	5.60 ± 1.09	5.63 ± 0.86
LDL-Cholesterol [mmol/L] [†]	2.82 ± 0.70	3.68 ± 0.89***	3.70 ± 0.96	3.71 ± 0.85
HDL-Cholesterol [mmol/L]	1.26 ± 0.26	1.11 ± 0.21**	1.15 ± 0.17	1.08 ± 0.23
Triacylglycerol [mmol/L]	0.95 [0.67–1.11]	1.66 [1.17–2.19]***	1.43 [0.94–2.02]	1.75 [1.20–2.38]
24-h Systolic BP [mmHg]	117.5 ± 8.8	123.4 ± 8.7**	120.2 ± 9.2	125.6 ± 7.6
24-h Diastolic BP [mmHg]	72.5 ± 9.4	80.4 ± 7.3***	78.3 ± 7.6	82.0 ± 6.7

Data presented as mean ± SD or median [IQR]. Baseline differences between obese and lean men were assessed by means of independent Student's T-test or Mann–Whitney U test where appropriate; **p < 0.01, ***p < 0.001 compared to baseline values of the lean men [†] LDL Cholesterol analyzed in 25 lean and 50 obese men [18].

Table 2
Cross-sectional analysis of fasting dicarbonyls, AGEs, SAF, and postprandial dicarbonyls.

	Lean (n = 25)	Obese (n = 52)	P value
Fasting concentrations			
MGO (nmol/L)	293 ± 52	297 ± 54	0.75
GO (nmol/L)	755 ± 195	686 ± 139	0.079
3-DG (nmol/L)	958 ± 74	993 ± 117	0.17
CML (nmol/L)	94 [81–115]	96 [83–114]	0.84
CEL (nmol/L)	48 [44–60]	53 [46–69]	0.12
MG-H1 (nmol/L)	130 [87–179]	124 [94–182]	0.34
Protein-bound CML (nmol/L)	81 [71–91]	74 [66–84]	0.025*
Protein-bound CEL (nmol/L)	31 [25–40]	35 [28–51]	0.059
Protein-bound MG-H1 (nmol/L)	241 [223–278]	237 [207–269]	0.47
Pentosidine (nmol/lysine)	0.40 [0.34–0.50]	0.35 [0.30–0.43]	0.29
SAF (Skin autofluorescence)	1.96 ± 0.40	2.08 ± 0.45	0.25

Data are presented as mean ± SD or median [IQR]. Baseline differences between obese and lean men were assessed by means of an independent samples t-test (*p < 0.05). Skewed variables (free and protein-bound AGEs) were Log transformed prior to analysis.
Dicarbonyls: MGO: methylglyoxal, GO: glyoxal, 3-DG: 3-deoxyglucosone.
Advanced glycation end products (AGEs): CML: N$^{\varepsilon}$-(carboxymethyl)lysine, CEL: N$^{\varepsilon}$-(1-carboxyethyl)lysine, MG-H1: N$^{\delta}$-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

by weight loss (p = 0.9; Table 3). Similarly, fasting concentrations of plasma AGE in the free and protein-bound form were largely unaltered by the weight loss intervention (Table 3). Only protein-bound CEL levels were significantly reduced by weight loss as compared to the control group by 16 nmol/L (95%-CI: 4–28; p = 0.002).

Postprandial dicarbonyl levels in the weight reduction group were reduced as compared to the weight stable group (Fig. 2, Table S4). The postprandial iAUC of MGO decreased by 57% (5280 nmol/L x min; 95%-CI: 33–10526; p = 0.049), the iAUC of GO decreased by 66% (11,329 nmol/L x min; 95%-CI: 495–22162; p = 0.041), and the iAUC of 3-DG decreased by 45% (20,175 nmol/L x min; 95%-CI: 5351–35000; p = 0.009). Results from the mixed ANOVA analyses of the intervention data showed that postprandial increases in MGO (p = 0.007) and 3-DG (p = 0.043) were significantly decreased after weight loss as compared to the weight stable group. This was not the case for postprandial GO concentrations (p = 0.144).

Postprandial dicarbonyl iAUC levels were reduced by weight loss to values similar to those of the lean subgroup (Supplementary Figure S2). After adjustment for glucose iAUC levels, the treatment effect on postprandial dicarbonyls was strongly attenuated (Supplementary Table S5).

3.3. GLO1, AKR1B1, ALDH2, and ketone bodies

The expression of GLO1, AKR1B1, and ALDH2, i.e. enzymes potentially involved in the degradation of MGO, did not differ between lean and obese individual in this study (Supplementary table S1). Furthermore, there was no change in GLO1, AKR1B1, and ALDH2 expression by the weight loss intervention.

Recently, the ketone body acetoacetate has been identified as a potential scavenger of MGO [27]. Serum concentrations of acetoacetate and β-hydroxybutyrate were not different between lean and obese individuals (Supplementary table S2). β-hydroxybutyrate, but not acetoacetate, was significantly increased by weight loss as compared to the weight stable control group (Supplementary table S2; median changes in the weight loss group and control group were +20.8 μmoL/l and −8.0 μmoL/l, respectively; p = 0.044).

4. Discussion

We showed that in apparently healthy abdominally obese men, postprandial plasma dicarbonyl levels were significantly higher compared to lean men. This difference was not manifest in the fasting state. Furthermore, in the fasting state we found no significant difference in SAF and a panel of specific plasma AGEs between lean and obese individuals, except for protein-bound CML, which was lower in the abdominally obese subgroup compared to lean men.

Most importantly, a diet-induced weight loss intervention reduced postprandial dicarbonyl levels in abdominally obese men as compared to the control group. Fasting levels of MGO were marginally decreased after weight loss, but not 3DG, GO, AGEs, and SAF.

4.1. Cross-sectional analysis

We found a higher iAUC of postprandial dicarbonyls in abdominally obese men, compared to lean men. This observation is in line with our previous finding in obese women with type 2

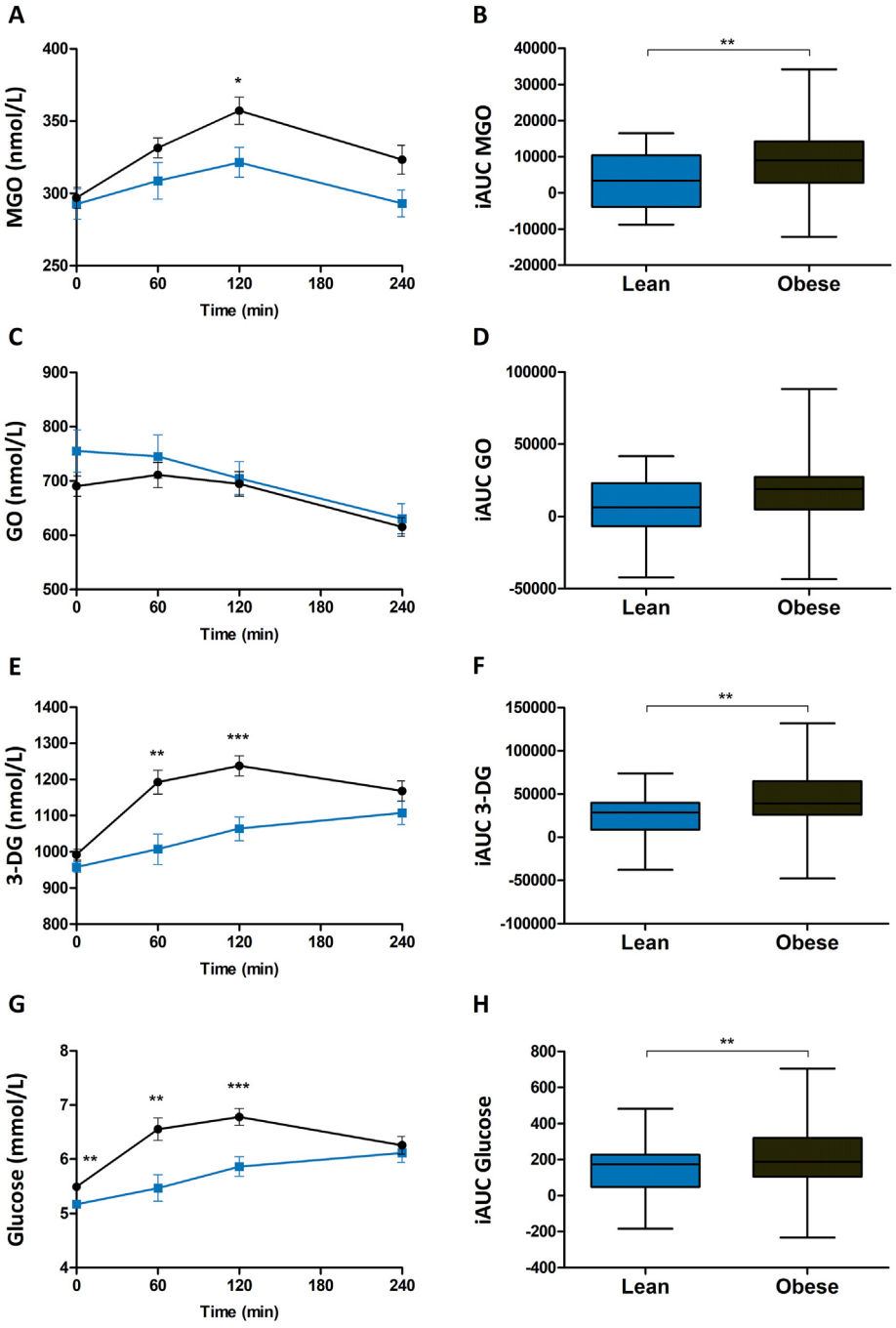

Fig. 1. Cross-sectional analysis of postprandial dicarbonyls and glucose, Graphs (A–B) show MGO concentrations in nmol/L during the mixed meal test, and the respective iAUC data in nmol/L x min (C–D) show the same for GO and (E–F) for 3-DG (G–H) show glucose concentrations in mmol/L and the respective iAUC in mmol/L x min. Black circle = obese, blue square = lean. Graphs are presented as mean with SEM, boxplots indicate median, 25th and 75th percentile, and min to max whiskers. Differences between obese and lean men were assessed by means of an independent samples t-test. P-values on top of the curves show the difference between both groups per time point; p-values on the boxplots indicate the iAUC difference (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). iAUC: incremental area under the curve, MGO: methylglyoxal, GO: glyoxal, 3-DG: 3-deoxyglucosone. Data shown in supplementary table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

diabetes [9]. However, we previously reported higher fasting plasma concentration of MGO in obese women with normal glucose tolerance compared to lean individuals, while we did not find a significant difference in the fasting state in men in the current study. This could potentially be explained by the degree of obesity since the average BMI in our previous study was larger than 40 kg/m^2, as compared to a BMI of 30.1 kg/m^2 in our current study. Most importantly, we found a higher iAUC of postprandial dicarbonyls in abdominally obese men, compared to lean men.

Here, we also investigated plasma levels of AGEs, as well as SAF. We showed that abdominally obese men neither had higher fasting plasma levels of AGEs, nor SAF. The only exception was the decreased concentration of CML in abdominally obese men, which is in line with our previous observations, and is likely due to the

Table 3
Effect of weight loss on fasting dicarbonyls, AGEs, SAF, and postprandial dicarbonyls.

	Weight loss (n = 23)		Weight stable (n = 26)		Treatment effect	
	Baseline	Follow-up	Baseline	Follow-up	Mean change (95% CI)[†]	P value[‡]
Fasting concentrations						
MGO (nmol/L)	291 ± 52	275 ± 52	300 ± 55	303 ± 42	−25 (−51, 0.5)	0.05
GO (nmol/L)	674 ± 152	607 ± 136	700 ± 135	654 ± 140	−35 (−106, 36)	0.32
3-DG (nmol/L)	950 ± 105	938 ± 86	1022 ± 102	1023 ± 114	−37 (−85, 10)	0.12
CML (nmol/L)	95 [82–130]	129 [84–148]	96 [82–114]	103 [86–123]	24 (−1, 49)	0.09
CEL (nmol/L)	54 [46–69]	51 [40–76]	53 [47–72]	56 [47–71]	−1 (−17, 14)	0.48
MG-H1 (nmol/L)	138 [95–190]	130 [102–284]	129 [90–177]	136 [99–199]	55 (−37, 147)	0.52
Protein-bound CML (nmol/L)	80 [70–86]	77 [67–84]	73 [64–81]	75 [68–83]	−3 (−8, 3)	0.29
Protein-bound CEL (nmol/L)	39 [29–57]	30 [26–39]	34 [26–44]	44 [32–59]	−16 (−28, −4)	0.002**
Protein-bound MG-H1 (nmol/L)	235 [207–275]	245 [221–285]	242 [210–258]	241 [210–259]	21 (−8, 49)	0.17
Pentosidine (nmol/lysine)	0.38 [0.31–0.45]	0.42 [0.36–0.48]	0.33 [0.31–0.40]	0.33 [0.29–0.39]	0.02 (−0.07, 0.11)	0.36
SAF (Skin autofluorescence)	2.07 ± 0.43	2.10 ± 0.39	2.16 ± 0.44	2.16 ± 0.39	0.01 (−0.10, 0.11)	0.90

Data are presented as mean ± SD or median [IQR]. Differences in changes between the weight loss treatment and weight stable control treatment, were tested using one-way ANCOVA with adjustment for baseline values [(†)]Adjusted mean changes (estimated marginal means) with 95% CI are given [(‡)]All P values are obtained from the ANCOVA analysis with adjustment for baseline values (**p < 0.01). Skewed variables (free and protein-bound AGEs) were Log transformed prior to analysis.
Dicarbonyls: MGO: methylglyoxal, GO: glyoxal, 3-DG: 3-deoxyglucosone.
Advanced glycation end products (AGEs): CML: N^{ε}-(carboxymethyl)lysine, CEL: N^{ε}-(1-carboxyethyl)lysine, MG-H1: N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

uptake and accumulation of CML in the expanded adipose tissue of obese individuals, with a consequently lower amount of circulating free levels of CML [4].

4.2. Effect of the weight loss intervention

We found that weight loss by caloric restriction reduced fasting plasma MGO and postprandial iAUC of MGO, GO, and 3-DG in abdominally obese individuals. After weight loss, postprandial dicarbonyl stress was reduced to values comparable to those at baseline in the lean group. These data are in line with a reduction in postprandial dicarbonyls in obese women after a 3 weeks caloric restriction [9]. Whilst we previously found the postprandial improvement was largely dependent on decreased fasting levels, we now show that weight reduction by caloric restriction improves postprandial dicarbonyl iAUCs.

Weight reduction by means of caloric restriction did not reduce free and protein-bound AGEs in plasma, except for protein-bound CEL, which was significantly decreased. CEL is an MGO-derived lysine modification, thus, the decrease in MGO levels by the weight loss intervention could partly explain this finding.

Weight loss did not affect the accumulation of AGEs in skin tissue, as determined by SAF. It is known that modifications of long-lived matrix proteins and cross-links, most notably pentosidine, are generally formed over a long period of time; and that short-term changes in plasma concentrations and AGEs in tissue often do not change in parallel [28]. Indeed, also the plasma concentration of the crosslink pentosidine was not affected by weight loss. Our finding is in accordance with a recent study in morbidly obese individuals where bariatric surgery did not change SAF in the 5 years follow-up period [29].

This study confirmed the effect of weight loss on dicarbonyl stress in a male abdominally obese population. Nonetheless, further research is required to investigate which tissues or cell types contribute most to the increased postprandial dicarbonyl stress in obesity, and to assess the mediating factors by which dicarbonyls were reduced. Multiple mechanisms can contribute to postprandial dicarbonyl stress in obese individuals. Glucose is a major substrate for dicarbonyl compounds [8]. Indeed, the treatment effect was attenuated when correcting for the postprandial iAUC of glucose, and therefore it is likely that glucose is the main substrate for postprandial dicarbonyl formation and that weight loss reduces dicarbonyl stress mainly by improving insulin sensitivity. In other studies, we repeatedly found that higher plasma levels of dicarbonyls, MGO in particular, were associated with incident cardiovascular disease, mortality and kidney disease independently of plasma glucose levels or even HbA1c, in individuals with- and without diabetes [15,16]. This indicates the importance of dicarbonyl compounds as ethiological factors in the development of obesity-related complications. While postprandial glucose levels may largely explain the observed increase in dicarbonyls, lipid peroxidation and the formation of reactive oxygen species [30] could also contribute to the amount of dicarbonyls after a mixed meal. Furthermore, interventions like caloric restriction could influence the detoxification rate of MGO, by induction of GLO1. GLO1 is the key rate-limiting enzyme of the glyoxalase system, converting MGO to D-lactate. Caloric restriction could induce the activation of the stress-responsive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a known inducer of GLO1, by binding to a functional antioxidant-response element in the GLO1 gene [31]. However, we did not find a change in GLO1 expression between lean and obese individual. Furthermore, there was no change in GLO1 expression by the weight loss intervention. The expression of two other genes known to influence MGO metabolism, AKR1B1 and ALDH2 [32], were unchanged as well.

Recently, the ketone body acetoacetate has also been identified as a potential scavenger of MGO [27]. Possibly, higher levels of acetoacetate seen with caloric restriction could scavenge circulating MGO. Acetoacetate and β-hydroxybutyrate are metabolically interconvertible ketone bodies. Under normal conditions, the ratio of β-hydroxybutyrate and acetoacetate is stable around 1:1. During ketosis this ratio is shifted toward higher concentration of β-hydroxybutyrate due to increased fatty acid oxidation [33]. Indeed, after our two-weeks weight stable phase, we found an increase in β-hydroxybutyrate. Hence, we cannot exclude the possibility that ketones after caloric restriction are, at least partly, involved in the decrease of MGO. However, a shift in ketone body and acetoacetate concentrations on their own are unlikely to explain the reduced dicarbonyl stress after weight loss given the available data.

4.3. Strengths and limitations

Our study has some limitations. Using strict inclusion criteria, we tried to achieve as little variation in our population as possible.

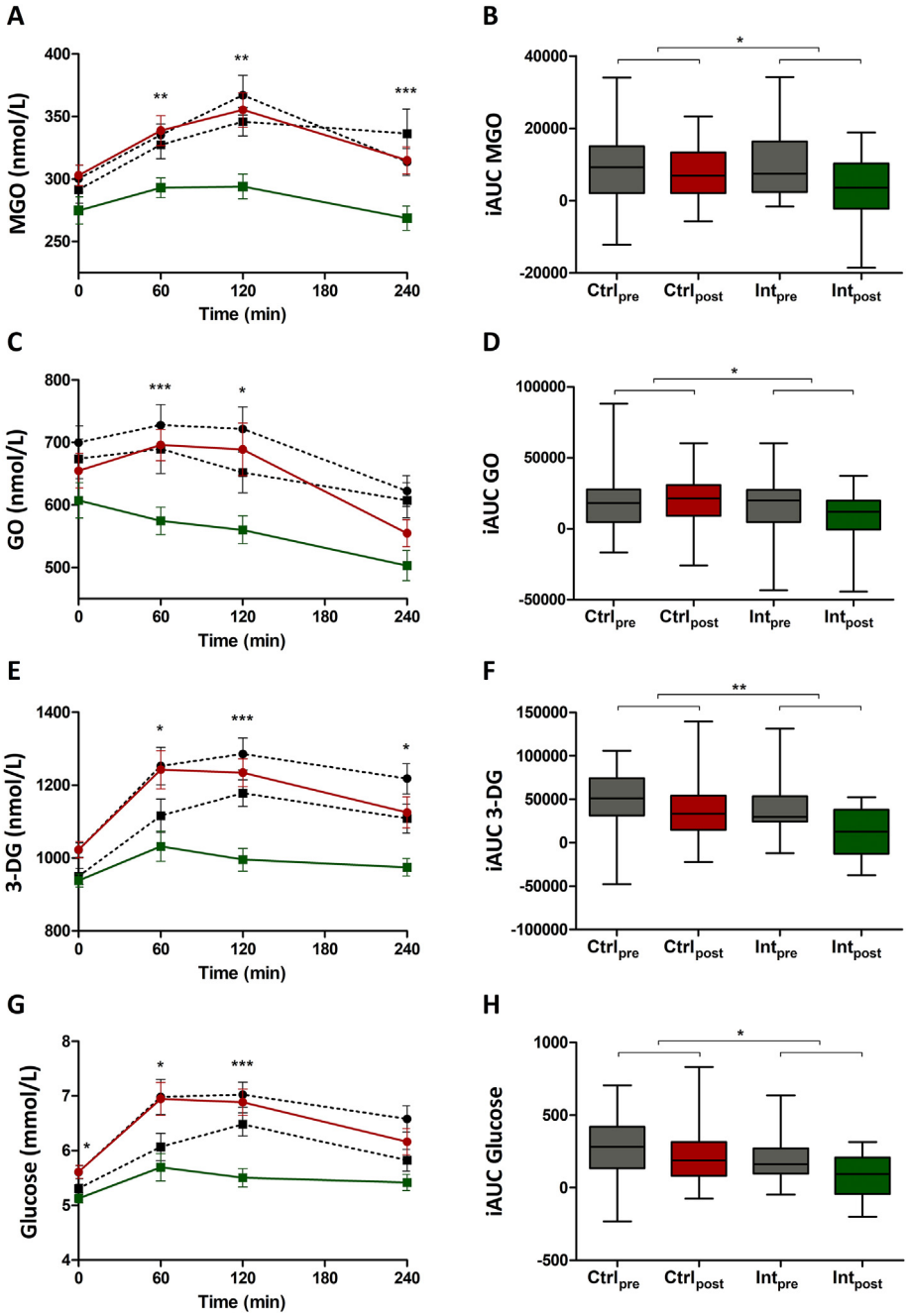

Fig. 2. Effect of a weight loss intervention on postprandial dicarbonyls and glucose, Graphs (A–B) show MGO concentrations in nmol/L during the mixed meal test, and the respective iAUC data in nmol/L x min (C–D) show the same for GO and (E–F) for 3-DG (G–H) show glucose concentrations in mmol/L and the respective iAUC in mmol/L x min. Black circle, dotted curve = $Ctrl_{pre}$ = control at baseline. Red cirlce = $Ctrl_{post}$ = control at follow-up. Black square, dotted curve = Int_{pre} = weight loss intervention at baseline. Green square = Int_{post} = weight loss intervention at follow-up. Graphs are presented as mean with SEM, boxplots indicate median, 25th and 75th percentile, and min to max whiskers. Differences in changes between the weight loss intervention and weight stable control were tested using one-way ANCOVA with adjustment for baseline values. P-values on top of the curves show the difference between both groups per time point; p-values on the boxplots indicate the iAUC difference (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$). iAUC: incremental area under the curve, MGO: methylglyoxal, GO: glyoxal, 3-DG: 3-deoxyglucosone. Data shown in supplementary table S4. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

However, selection bias could be a complicating factor for this RCT with participation on a voluntary basis. Furthermore, we only performed measurements at baseline and follow-up. Because of this, we lack information on temporal improvements and improvements taking place during the first weeks of caloric restriction. Although this does not diminish our findings, additional time points could help to elucidate the physiological changes during weight loss. Our study lacks tissue biopsies; we cannot exclude that PBMCs are not fully representative of other tissues with regard to gene expression of GLO1, AKR1B1 and ALDH2. Finally, we only included men, which may reduce the external validity of our results. Given the similarities we found in our previous study with only women included (9), we do not expect that a difference in sex would change the overall conclusion.

The strengths of our study include its RCT design, blinded analyses, intensive monitoring of the participants by a research dietician, very low-dropout rate, use of validated UPLC-MS/MS measurements, and the inclusion of a large panel of AGEs and dicarbonyl compounds. The inclusion of a control group in this trial, which remained weight stable over the course of the intervention, provides a solid baseline when assessing the effects of the intervention. It is also important to note that the dietary program ended with a weight maintenance phase of two weeks after attaining the intended weight loss.

4.4. Clinical implications

Our current findings are of potential clinical importance, since dicarbonyl compounds, and MGO in particular, have been identified as potential mediators of cardio-metabolic disease in obesity [34]. We recently demonstrated that higher plasma concentrations of MGO are associated with an accumulation of MGO in adipose tissue [5]. Although the consequences of MGO in adipose tissue are largely unknown, it has been shown that direct incubation of adipocytes with MGO results in increased proliferation indicating that MGO may be involved in the expansion of the adipose tissue. In addition, in an animal model of obesity, elevated MGO levels in serum and adipose tissue were closely associated with the decreased adipose tissue capillarization, adipose blood flow, and the development of insulin resistance [35,36]. In an animal model of obesity, we recently demonstrated that the MGO scavenger pyridoxamine reduced increased pro-inflammatory gene expression levels in visceral adipose tissue, whereas the expression of the anti-inflammatory cytokine adiponectin was increased [5]. Thus, the accumulation of MGO in adipose tissue increases adipose tissue inflammation and may contribute to insulin resistance and the development of type 2 diabetes. Hence, the rapid formation of MGO in the postprandial state could play a role in the association between obesity and consequent metabolic complications. Therefore, it could be valuable to target this early increase in postprandial dicarbonyl stress in obese individuals, for example by actively quenching dicarbonyls or the induction of the glyoxalase system. These treatment strategies are currently under investigation. This is challenging, as compounds investigated so far either lacked efficacy or were linked to potential toxicity [37]. However, compounds that showed potential efficacy, such as the dicarbonyl quenchers pyridoxamine, carnosine or carnosinol, and the co-formulation trans-resveratrol/hesperetin as inducer of GLO1, have a more favorable pharmacologic profile and are under active investigation [5,38,39].

5. Conclusion

Postprandial dicarbonyl stress potentially forms an important risk for obesity-related complications. We showed that postprandial dicarbonyl stress is present in obesity, and can be reversed by a dietary weight loss intervention. Future work should investigate whether targeting postprandial dicarbonyl stress in obesity can prevent obesity-related complications, type 2 diabetes and cardiovascular disease.

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Clinical trial registry

The current study was approved by the Ethics Committee of Maastricht University Medical Center (METC123040), and registered on August 21st, 2012 at ClinicalTrials.gov as NCT01675401.

Conflicts of interest

The authors declare no conflict of interest. JvD is employed by a company that manufactures and markets food products.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.03.042.

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