

Short-Term Low Calorie Diet Intervention Reduces Serum Advanced Glycation End Products in Healthy Overweight or Obese Adults

Alejandro Gugliucci^a Kazuhiko Kotani^b Jennifer Taing^a Yukiyo Matsuoka^b
Yoshiko Sano^b Makiko Yoshimura^c Kahori Egawa^c Chika Horikawa^c
Yoshinori Kitagawa^c Yoshinobu Kiso^c Satoshi Kimura^d Naoki Sakane^b

^aGlycation, Oxidation and Disease Laboratory, Touro University-California, Vallejo, Calif., USA; ^bDivision of Preventive Medicine, Clinical Research Institute for Endocrine and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto; ^cInstitute for Health Care Science, Suntory Ltd. Research Center, Osaka; ^dDepartment of Laboratory Medicine and Central Clinical Laboratory, Showa University Northern Yokohama Hospital, Yokohama, Japan

Key Words

Weight reduction · Body mass index · Advanced glycation · Caloric restriction

Abstract

Background: Obesity is a metabolic and cardiovascular risk factor. A low calorie diet (LCD) is one of the treatment modalities for weight loss. Serum advanced glycation end products (AGEs) are linked to increased atherogenicity and inflammation in diseases such as diabetes and renal failure. Obesity has an inflammatory component, but interestingly there are no studies on serum AGE levels in obesity or on the effects of LCD as a therapeutic measure on these markers of glycation. **Aim:** We hypothesized that weight loss by caloric restriction has a beneficial effect on serum AGE levels. We investigated the prospective effects of a sole LCD intervention for weight loss on serum AGEs in a cohort of overweight and non-morbidly obese but otherwise healthy subjects. **Methods:** A total of 37 Japanese subjects (30 females, 7 males, mean age 48.2 ± 9.3 years) with a mean BMI of 28.3 ± 3.2 participated in this study. During the intervention

period of 2 months, they were placed on an LCD (Diet's™; 5,023 kJ/day) with meal replacement every dinner. The following data were evaluated pre- and post-intervention: AGEs, BMI, waist circumference, blood pressure, serum glucose, cholesterol, triglycerides, HDL- and LDL- cholesterol. **Results and Discussion:** After the intervention, BMI levels were clearly reduced by 6.3% ($p < 0.001$), waist circumference by 5.7% ($p < 0.002$) and triglycerides by 11.9 % ($p < 0.002$). At baseline, AGEs levels were 63 ± 11 AU for obese subjects and 63 ± 14 for control subjects (not significant). After intervention, AGEs were reduced by 7.21% (range 0–35%, $p < 0.001$). The percent change in AGEs was significantly and positively correlated with that of triglycerides ($r = 0.42$, $p < 0.009$), waist circumference ($r = 0.40$, $p < 0.011$), and BMI ($r = 0.42$, $p < 0.007$). We show for the first time that serum AGEs can be reduced by an LCD intervention on weight loss, a change that correlates with the reduction in triglycerides. This may plausibly be a reflection of a reduction in glycation/lipoxidation due to the caloric restriction and its metabolic consequences, or it may be due to the decreased intake of food containing glycotoxins, or a combination of both.

Copyright © 2009 S. Karger AG, Basel

Introduction

Obesity is a crucial risk factor in the development of its co-morbid diseases such as metabolic syndrome and cardiovascular disease, and the increased prevalence of obesity is a major problem in many countries [1]. Low level inflammation seems to be a constant finding in obesity and it is one of the perpetuating factors [2]. Serum advanced glycation end products (AGEs) are linked to increased atherogenicity and inflammation [3–5]. AGE adducts are formed on long and short-lived cellular and extracellular proteins. A role for AGE-associated receptors is likely in human diseases, of which the best studied are AGE receptor 1 (AGE R1) and the receptor for AGE (RAGE). AGE R1 blocks oxidative stress and inflammatory responses, whereas RAGE increases them and its deleterious actions seem to predominate [6]. The toxicity of AGEs resides mainly in AGE receptor-mediated responses, which induce inflammatory pathways, chiefly in diseases such as diabetes and renal failure [6]. In diabetes, they are produced in increased amounts as a result of hyperglycemia and its metabolic consequences [3–5]. In renal failure, excretion is impaired.

Indeed, the kidney plays a key role in AGE catabolism. Cellular proteolysis forms AGE-peptides and free AGEs from these proteins, which are released into plasma for urinary excretion. Free AGE moieties are the chief molecular form by which AGEs are excreted in urine [7, 8]. They display a high renal clearance, which distinctly declines in chronic renal failure patients, leading to accumulation of plasma AGE adducts. Finally, another source of circulating AGEs are those present in foods rich in Maillard reaction products, some of which are absorbed and are collectively called glycotoxins [9]. Intervention with diets low in Maillard reaction products have reduced circulating AGEs [9, 10].

Research on the relationships between obesity and circulating AGEs is limited at present and data are scarce. One clinical study showed decreased levels of serum AGEs after treatment with orlistat in women with polycystic ovary syndrome [11] and an animal study showed increased circulating and visceral fat AGEs in rats fed a high fat diet [12]. There has been no study on serum AGE levels in obese humans and the effects of low calorie diets (LCD) as a therapeutic strategy on these markers.

LCD is one of the treatment modalities adopted as a part of lifestyle management for weight loss, leading to the improvement of metabolic and cardiovascular conditions [13–15]. We hypothesized that weight loss by caloric restriction has a beneficial effect on serum AGE lev-

els. We investigated the prospective effects of an LCD intervention for weight loss on serum AGEs in a population of overweight and non-morbidly obese but otherwise healthy subjects.

Subjects and Methods

In total, 37 otherwise healthy and free-living Japanese subjects (30 female, 7 male), aged 32–63 years (mean 48.2 ± 9.3), with a mean BMI of 28.3 ± 3.2 (range 25.1–38.6) participated in this study. Exclusion criteria were as follows: the use of medications and nutrient supplements; pregnancy; alcohol abuse; psychological contraindications as determined by study investigators, or known hypersensitivity to any of the ingredients of the formula. The study was approved by the ethics committee of Kyoto Medical Center, and all subjects gave informed consent. The study was powered for a standardized effect size of 1 (a 5% change over baseline in paired samples), with a 2-sided $\alpha = 0.01$ and a $\beta = 0.10$ and required an $n = 32$.

During the study intervention period, subjects were placed on an LCD (5,023 kJ/day, consisting of 47% protein, 7% lipid and 40% carbohydrate) with meal replacement (Diet's™; Suntory Co. Ltd., Osaka, Japan), encompassing a standardized group of food items. The volunteers were asked to change one of the meals (dinner) for the meal replacement above. The subjects were also instructed by trained dietitians about the planning of nutritionally balanced and constant diet for their other meals, and compliance was checked through daily dietary records. The average reduction in calorie intake was 20%. They were encouraged not to change usual physical activity during the intervention.

The subjects' data at baseline (pre-intervention) and the end of 2 months' study (post-intervention) were used. After an overnight fast, the subjects' BMI, blood pressure (measured using a mercury sphygmomanometer), plasma glucose (enzymatic kinetic method; Shino-Test Co. Ltd., Tokyo, Japan) and lipids such as total cholesterol (enzymatic kinetic method; Wako Pure Chemical Industries Co. Ltd., Tokyo, Japan) and triglyceride (enzymatic kinetic method; Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) were measured. Both HDL and LDL cholesterol were measured using a homogeneous assay (Daiichi Pure Chemicals). For serum AGE measurement, a fasting blood sample from both subsets was obtained by venipuncture and collected in evacuated dry tubes. Blood was centrifuged at 800 g at 4°C for 15 min and separated serum or plasma was immediately analyzed or frozen at –80°C until use. Serum AGEs were measured by fluorescence intensity recorded at 440 nm (emission maximum) upon excitation at 350 nm using a Spectramax Gemini XPS spectrofluorometer with Softmax Pro software (Molecular Devices, Sunnyvale, Calif., USA), as previously described [3–16]. Fluorescence intensity is expressed in arbitrary units (AU).

Statistical Analyses

Results for Gaussian-distributed continuous variables are expressed as the mean value \pm SD, except for triglycerides. Due to the latter's non-Gaussian distribution, the median and the interquartile interval was used. For difference analyses, 1-tailed paired *t* test for Gaussian variables and Mann-Whitney test for non-

Table 1. Clinical and metabolic variables pre- and post-intervention

Variables	Pre-intervention	Post-intervention	Change %	p value (vs. baseline)
BMI	28.3 ± 3.2	26.4 ± 3.1	-6.3	<0.001*
Systolic blood pressure, mm Hg	129.1 ± 15.2	124.4 ± 14.9	-3.25	0.012*
Diastolic blood pressure, mm Hg	80.8 ± 11.0	75.7 ± 10.28	-5.64	<0.001*
Glucose, mmol/l	5.01 ± 0.52	4.71 ± 0.51	-4.65	<0.001*
Total cholesterol, mmol/l	5.72 ± 1.03	5.21 ± 1.01	-7.21	<0.001*
Triglyceride, mmol/l	1.16 (0.45–1.76)	0.92 (0.45–1.53)	-11.94	0.002*
HDL cholesterol, mmol/l	1.57 ± 0.36	1.54 ± 0.28	-2.21	0.103
LDL cholesterol, mmol/l	3.23 ± 0.77	2.88 ± 0.77	-8.62	<0.001*
AGE, AU	62.6 ± 11.5	57.9 ± 10.7	-7.21	<0.001*

Data are means ± SD, except for triglyceride results, which are medians and interquartile intervals.

* $p < 0.05$ (statistical significance).

Gaussian ones were performed. Differences were considered to be statistically significant if the null hypothesis could be rejected with 95% confidence. Correlations between continuous variables were assessed with the Pearson test. The NCSS statistical software package (NCSS, Kaysville, Utah, USA) was employed for all calculations.

Results

All subjects completed the LCD intervention. The data on respective variables in pre- and post-intervention are listed in table 1. During the intervention, most variables were significantly reduced. BMI levels were clearly reduced by 6.3% ($p < 0.001$), waist circumference by 5.7% ($p < 0.002$) and triglycerides by 11.9% ($p < 0.002$). Initially, AGE levels were 63 ± 11 AU for obese subjects, and following intervention they were reduced by 7.21% (range 0–35%, $p < 0.001$). As listed in table 2, the percent change in AGEs was significantly and positively correlated to that of triglycerides ($r = 0.42$, $p < 0.009$), waist circumference ($r = 0.40$, $p < 0.011$) and BMI ($r = 0.42$, $p < 0.007$).

Discussion

To the best of our knowledge, this is the first report to demonstrate that an LCD intervention for weight loss reduces serum AGEs in a population of overweight/obese, but otherwise healthy, subjects. A previous study had shown an AGE-reducing effect of orlistat treatment on a cohort of women with polycystic ovary syndrome [11]. In

Table 2. Correlation between the percent change in AGEs with percent change in BMI, triglycerides and waist circumference

Variables	r (p value)
BMI	0.424 (0.007)
Triglycerides	0.423 (0.009)
Waist circumference	0.405 (0.011)

r = Pearson's rank correlation coefficient.

comparison, our study has several strengths: we selected a homogeneous population of healthy individuals who were overweight or (non-morbidly) obese and who were under no pharmacological treatment, and the intervention was a well-controlled diet alone (removing co-morbidities and the pharmacological treatment as confounding factors; orlistat decreases lipid absorption and lipids participate in AGE formation).

The lowering of AGE levels may be explained by more than one factor and may be considered, a priori, as another positive effect of low calorie intake and weight loss.

At least 4 mechanisms may be postulated. First, our data show that changes in AGEs correlate with the changes both in BMI and in waist circumference, suggesting that the loss of visceral and peripheral adipose tissue may play a mechanistic role. AGEs, and their specific receptor, RAGE, are involved in inflammation and vascular com-

plications [6–17]. The soluble form of RAGE (sRAGE) appears to act as an AGE quencher and may have a protective effect [17]. Interestingly, a recent report showed that sRAGE is negatively and significantly correlated with BMI and waist/hip circumference ratio [18]. Hence, the first mechanism for serum AGE reduction in our cohort may be an increase in sRAGE produced by lowering BMI, which in turn acts by reducing the circulating load of AGEs.

The second mechanism is that low caloric intake and its consequent lowering of substrate availability reduces production of reactive oxygen species and α -dicarbonyl formation, key factors in AGE production [8–19].

The third mechanism may involve reduced intake of exogenous AGEs which is usually a consequence of reduced food intake. Indeed, a recent study in mice seems to indicate that one of the factors mediating reduced oxidative stress in caloric restriction is the reduction in the intake of Maillard products in the food [20]. The reduction in both carbohydrates and lipids in the diet (both can enhance formation of AGEs) and an increase in protein (which may increase the availability of anti-glycation agents like carnosine) may have acted synergistically to produce this effect. A limitation of our study is that AGE content of the food was not measured.

Finally, the fourth mechanism for AGE reduction could be linked to lipid changes. Indeed, another conse-

quence of the metabolic shifts induced by caloric restriction is a change in lipid metabolism and improved insulin sensitivity. We show here that there is a significant positive correlation between the lowering of serum AGEs and that of triglycerides (but not of glycemia). Advanced glycation and lipoxidation reactions produce some common end products, collectively called advanced lipoxidation end products [3, 8–21]. In our study we measured fluorescent AGEs, which account for only a fraction of these adducts. More studies are needed to further confirm our results, measuring specific AGE compounds, such as free adducts and AGE peptides, by LC-MS/MS and assessing the AGE content in the diet.

In conclusion, in a cohort of healthy overweight/obese subjects we showed reduced serum AGE levels during an LCD intervention on weight loss and a significant positive correlation with the reduction in triglyceride levels. As AGEs are implicated and are biomarkers for oxidative stress, our results show another positive effect of caloric restriction. The question of the clinical significance of this finding deserves further exploration.

Acknowledgments

The expert support of Mr. John Schulze in technical aspects of the work, and of Ms. Claire Trias in editorial assistance are greatly appreciated.

References

- Haffner SM: Relationship of metabolic risk factors and development of cardiovascular disease and diabetes. *Obesity* 2006;14:S121–S127.
- Shin MJ, Hyun YJ, Kim OY, Kim JY, Jang Y, Lee JH: Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. *Int J Obes* 2006;30:1529–1534.
- Monnier VM, Kohn RR, Cerami A: Accelerated age-related browning of human collagen in diabetes mellitus. *Proc Natl Acad Sci USA* 1984;81:583–587.
- Dyer DG, Blackledge JA, Thorpe SR, Baynes JW: Formation of pentosidine during non-enzymatic browning of proteins by glucose: identification of glucose and other carbohydrates as possible precursors of pentosidine in vivo. *J Biol Chem* 1991;266:11654–11660.
- Csiszar A, Ungvari ZI: Endothelial dysfunction and vascular inflammation in type II diabetes: interaction of AGE/RAGE and TNF- α signaling. *Am J Physiol Heart Circ Physiol* 2008;295:H475–H476.
- Soro-Paavonen A, Watson AM, Li J, Paavonen K, Koitka A, Calkin AC: RAGE deficiency attenuates the development of atherosclerosis in diabetes. *Diabetes* 2008;57:2461–2469.
- Gugliucci A, Bendayan M: Renal fate of circulating advanced glycosylated end products (AGE): evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. *Diabetologia* 1996;39:149–160.
- Rabbani N, Sebekova K, Sebekova K, Heidland A, Thornalley PJ: Accumulation of free adduct glycation, oxidation, and nitration products follows acute loss of renal function. *Kidney Int* 2007;72:1113–1121.
- Vlassara H, Striker G: Glycotoxins in the diet promote diabetes and diabetic complications. *Curr Diab Rep* 2007;7:235–241.
- Uribarri J, Peppas M, Cai W, Goldberg T, Lu M, He C, Vlassara H: Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. *J Am Soc Nephrol* 2003;14:728–731.
- Diamanti-Kandarakis E, Katsikis I, Piperi C, Alexandraki K, Panidis D: Effect of long-term orlistat treatment on serum levels of advanced glycation end-products in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2007;66:103–109.
- Li SY, Liu Y, Sigmon VK, McCort A, Ren J: High-fat diet enhances visceral advanced glycation end products, nuclear O-Glc-Nac modification, p38 mitogen-activated protein kinase activation and apoptosis. *Diabetes Obes Metab* 2005;7:448–454.
- Hill JO, Wyatt H: Outpatient management of obesity: a primary care perspective. *Obes Res* 2002;2:S124–S130.

- 14 Berkel LA, Poston WS, Reeves RS, Foreyt JP: Behavioral interventions for obesity. *J Am Diet Assoc* 2005;105:S35–S43.
- 15 Barnard RJ: Effects of life-style modification on serum lipids. *Arch Intern Med* 1991;151:1389–1394.
- 16 Gugliucci A, Mehlhaff K, Kinugasa E, Ogata H, Hermo R, Schulze J, Kimura S: Paraoxonase-1 concentrations in end-stage renal disease patients increase after hemodialysis: correlation with low molecular AGE adduct clearance. *Clin Chim Acta* 2007;377:213–220.
- 17 Clynes R, Moser B, Yan SF, Ramasamy R, Herold K, Schmidt AM: Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. *Curr Mol Med* 2007;7:743–751.
- 18 Norata GD, Garlaschelli K, Grigore L, Tibolla G, Raselli S, Redaelli L: Circulating soluble receptor for advanced glycation end products is inversely associated with body mass index and waist/hip ratio in the general population. *Nutr Metab Cardiovasc Dis* 2008;19:129–139. DOI: [10.1016/j.numecd.2008.03.004](https://doi.org/10.1016/j.numecd.2008.03.004).
- 19 Vander Jagt DL: Methylglyoxal, diabetes mellitus and diabetic complications. *Drug Metabol Drug Interact* 2008;23:93–124.
- 20 Cai W, He JC, Zhu L, Chen X, Zheng F, Striker GE, Vlassara H: Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. *Am J Pathol* 2008;173:327–336.
- 21 Januszewski AS, Jenkins AJ, Baynes JW, Thorpe SR: Lipid-derived modifications of plasma proteins in experimental and human diabetes. *Ann NY Acad Sci* 2005;1043:404–412.