# Dietary advanced glycation end products restriction diminishes inflammation markers and oxidative stress in patients with type 2 diabetes mellitus

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**The augmented consumption of dietary advanced glycation end products (dAGEs) has been associated with increased oxidative stress and inflammation, however, there is insufficient information over the effect on insulin resistance. The objective of the present study is to investigate the effect of dAGEs restriction on tumor necrosis factor-α (TNF-α), malondialdehyde, C-reactive protein (CRP), and insulin resistance in DM2 patients. We carried out a randomized 6 weeks prospective study in two groups of patients: subjects with a standard diet (*n* = 13), vs low dAGEs (*n* = 13). At the beginning and the end of study, we collected anthropometric measurements, and values of circulating glucose, HbA1c, lipids, insulin, serum AGEs, CRP, TNF-α and malondialdehyde. Anthropometric measurements, glucose, and lipids were similar in both groups at base line and at the end of the study. Estimation of basal dAGEs was similar in both groups; after 6 weeks it was unchanged in the standard group but in the low dAGEs group decreased by 44% ($p$<0.0002). Changes in TNF-α levels were different under standard diet (12.5 ± 14.7) as compared with low dAGEs (–18.36 ± 17.1, $p$<0.00001); changes in malondialdehyde were different in the respective groups (2.0 ± 2.61 and –0.83 ± 2.0, $p$<0.005) no changes were found for insulin levels or HOMA-IR. In conclusion, The dAGEs restriction decreased significantly TNF-α and malondialdehyde levels.**

***Key Words***: **advanced glycation end products, malondialdehyde, TNF-α, diabetes mellitus, diet**

The accumulation of AGEs in tissues induces oxidative stress and promotes inflammation, two important factors in the physiopathology of diabetes complications. The endogenous AGEs production jointly with compounds from the diet, promote a systemic glycoxidant burden, oxidant stress and cell activation, enhancing vulnerability of target tissues to diabetes injury.[1–3] The variety and the amount of dietary AGEs (dAGE) depend on food nutrients, the heating used in food processing,[3–5] pH conditions, presence of some metal ions ($Cu^{++}$, $Fe^{++}$) and water content.[6] *N*-ε-carboxymethyllysine (CML), has been typically used as a marker of dAGEs.[6,7] Several studies have shown that dAGEs intake modifies the circulating AGEs levels in human subjects and animals, with or without diabetes or renal disease, providing experimental evidences that food rich in AGEs is an important contributor to the total pool of AGEs in the body.[1,8–11] Furthermore, a recent paper proposed that according to animal models a simple reduction of basal oxidative stress by dietary AGEs restriction sharply reduced the risk of diabetes mellitus and its complications.[12]

In a group of healthy subjects, dAGEs restriction was associated with significant reduction of inflammation markers, plasma C-reactive protein, tumor necrosis factor-α (TNF-α) and VCAM-1.[10,13] In patients with type 2 diabetes mellitus (DM2), meals with high-dAGEs induced a pronounced acute impairment of vascular function. It suggests that chemical modifications of foods induced by heat treatment could contribute to the development of vascular dysfunction. A study in normal human subjects on self-selected diets with low or high dAGEs content showed a strong correlation of AGEs intake and serum levels of these compounds with the markers of inflammation and insulin resistance.[14] Recently a research in diabetic patients with AGE-restriction showed diminished insulin, insulin resistance, TNF-α and serum AGEs.[15] This suggests a role of high AGEs in diet on the inflammatory response and insulin resistance.

The aim of this work was to investigate the effect of decreasing dAGEs intake on inflammation markers, oxidative stress and insulin resistance in patients with DM2. Presently, there is insufficient evidence on the effect of AGEs restriction on insulin resistance in DM2 patients.

## Materials and Methods

We carried out a randomized prospective study in DM2 patients recruited at the Clinical Services of the Health Department, and referred to our research facilities at the Department of Medical Science of The University of Guanajuato. The research group was not involved on the medical treatment or on the prescription of medication for diabetes control. The Institutional Ethics Committee approved the protocol, and all subjects signed an informed consent.

**Criteria for inclusion and exclusion.** We recruited 34 non-smoking DM2 patients with the following inclusion criteria: Aged 30 to 65 years with less than 10 years since diagnosis, serum creatinine lower to 1.2 mg/dl, stable weight on the previous 2 months, no food allergies and no clinical evidence of infections, any serious condition such as cancer, heart or liver disease. Subjects who needed changes in the medication for diabetes, or reported unsatisfactory adherence to diet were excluded from the study.

**Study design.** At the initial interview of the patient clinical

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data and a detailed 2 days dietary history were collected. After a follow-up period of 6 weeks without any diet change, they were randomized by means of a random number selection (pair or odd) for each subject after acceptance of inclusion to either a low-dAGEs (L-dAGEs) ($n = 17$) or standard-dAGEs (S-dAGEs) ($n = 17$) diet during 6 weeks. A physician blinded for the group of study carried out the clinical evaluation of patients. Anthropometric measurements weight, height to obtain body mass index (weight/height$^2$), fasting venous blood and urine samples were taken at the beginning and at the end of the study.

**Dietary prescription.** Diets were designed according to the American Diabetes Association recommendations, containing 50–55% carbohydrate, 20% protein, and 25–30% fat. S-dAGEs participants were advised to prepare meals as usual. The L-AGEs meal plan was prepared as recommended by Goldberg *et al.*[4] For the quantification of micro- and macronutrients, Food Processor software Nutripac was used. The AGEs content of each food item was measure according the database of 250 food reported by Goldbert *et al.*[4] The amount of CML expressed as AGEs kilounits (KU) and the intake was reported as AGE/day. A registered dietitian reviewed all the analyses.

The volunteers in this group received written indications for food preparation at appropriate cooking times and temperatures. They also received instructions to boil and steam the food, to avoid fried entrees and reheat food indirectly using steam in a double boiler.

**Biochemical measurements.** Serum glucose and lipids were measured by enzymatic method (GODPAP, Lakeside, México City, and Enzymatic techniques, respectively); HbA1c was measured by a method of ion exchange chromatography (Sigma, St. Louis, MO). A morning urine sample was obtained to detect a possible urinary tract infection. These samples were processed the same day. Serum samples were stored at –70°C until further determinations of the metabolic markers. Serum fluorescent AGEs (mainly pentosidine) were measured according to our previous report,[16] C-reactive protein by inmunoturbidimetric high density (BioSystems, S.A., Barcelona, Spain), TNF-α by ELISA (BioSource Europe S.A. Belgium), and malondialdehyde (MDA) by extraction-spectrophotometric procedure with a variation coefficient of 3.5%.[17]

**Follow-up.** After assignation to a diet group, subjects were followed weekly during 6 weeks. At each visit, body weight was registered, a urine sample collected, and adherence to diet reviewed on basis of a daily food log.

**Statistical analyses.** Data are shown as means ± standard deviation (SD). We compared groups of treatment with the *t* test for independent samples or with the Mann-Whitney *U* test. Significant difference was considered for $p<0.05$.

## Results

The study included 34 patients, 17 in the group with the standard-dAGEs and 17 in the group with low-dAGEs; we excluded five of them because of clinical evidence of an infectious disease, and 3 because they required modification in the hypoglycemic treatment. The 13 remaining patients in each group did not report any associated problem. In the S-dAGEs group 11 patients were treated with hypoglycemic agents (2 with metformin, 3 with sulfonylureas, and 6 with both), and 2 patients with diet only. In the group with L-dAGEs 9 patients were treated with hypo-

**Table 1.** General characteristics of both groups at baseline

| | S-dAGEs mean ± SD $n = 13$ | L-dAGEs mean ± SD $n = 13$ | t/*Z** | *p* |
|---|---|---|---|---|
| Gender (M/F) | 2/11 | 1/12 | | |
| Age (years) | 48.5 ± 6.2 | 46.0 ± 5.0 | 1.14 | 0.26 |
| Year since diagnosis of DM | 6.3 ± 4.9 | 4.1 ± 3.5 | 1.31 | 0.2 |
| Weight (kg) | 69.7 ± 14 | 72 ± 10.4 | 1.82 | 0.31 |
| BMI | 28.8 ± 4.76 | 29.8 ± 4.0 | 1.34 | 0.62 |
| SBP (mmHg) | 130 ± 19 | 121 ± 21 | 1.26 | 0.69 |
| DBP (mmHg) | 82 ± 11 | 75 ± 11 | 1.01 | 0.99 |
| **Biochemical data** | | | | |
| Glucose (mg/dl) | 151.3 ± 55.8 | 147.7 ± 64.9 | 1.36 | 0.6 |
| HbA1c (%) | 9.08 ± 2.28 | 8.05 ± 1.46 | 1.5 | 0.14 |
| Total-cholesterol (mg/dl) | 185.6 ± 35.2 | 162.4 ± 35.2 | 1.03 | 0.96 |
| Triglycerides (mg/dl) | 239.1 ± 97.4 | 203.7 ± 106.3 | 1.11 | 0.86 |
| LDL-C (mg/dl) | 104.4 ± 34.4 | 88.9 ± 31.3 | 1.24 | 0.71 |
| HDL-C (mg/dl) | 44.1 ± 11.6 | 42.5 ± 8.9 | 1.61 | 0.42 |
| **Metabolic markers** | | | | |
| Fluorescent serum AGEs (arbitrary units) | 6.91 ± 1.44 | 7.1 ± 2 | 1.9 | 0.29 |
| Malondialdehyde (μmol/l) | 3.5 ± 1.1 | 4.3 ± 2 | 3.42 | <0.04 |
| PCR (mg/l) | 10.2 ± 6.2 | 6.9 ± 6.1 | 1.01 | 0.98 |
| TNF-α (pg/ml) | 14.4 ± 8.10 | 34.9 ± 18.7 | 2.95* | <0.002 |
| Insulin (μU/ml) | 18.6 ± 17.2 | 14.4 ± 6.8 | 6.33 | <0.003 |
| Homa-IR | 6.9 ± 6.6 | 5.3 ± 3.2 | 4.15 | <0.02 |
| **Dietary variables** | | | | |
| Kilocal/day | 1737 ± 393 | 1619 ± 324 | 1.47 | 0.51 |
| Carbohydrates (g/day) | 243.4 ± 75.7 | 240 ± 67.5 | 1.26 | 0.7 |
| Fats (gr/day) | 50.7 ± 14.7 | 40.5 ± 13.4 | 1.21 | 0.74 |
| Proteins (gr/day) | 73.1 ± 11.4 | 70.4 ± 20.7 | 3.37 | <0.04 |
| dAGEs (as CML, KU/day) | 9910 ± 4164 | 8956 ± 3587 | 1.35 | 0.61 |

We included 34 patients, 17 in the group with the S-dAGEs and 17 the group with L-dAGEs; 5 of them were excluded because during the follow-up they showed clinical evidence of an infectious disease, and 3 because they required modification in the hypoglycemic treatment. *Mann-Whitney *U* test.


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**Table 2.** Changes in metabolic variables after six weeks, as compared with basal levels in the group of study

| | S-dAGEs | L-dAGEs | T/Z* | p |
|---|---|---|---|---|
| **Biochemical data** | | | | |
| Glucose (mg/dl) | 4.55 ± 35.6 | −18 ± 56.7 | −1.21 | 0.24 |
| Total-cholesterol (mg/dl) | −9.0 ± 44.9 | 5.7 ± 43.3 | 0.84 | 0.41 |
| Triglycerides (mg/dl) | −3.7 ± 80.2 | 5.4 ± 59.0 | 0.33 | 0.74 |
| HDL-cholesterol (mg/dl) | –4.2 ± 10.3 | −2.5 ± 7.4 | 0.48 | 0.64 |
| HbA1c (%) | –0.11 ± 1.9 | 0.19 ± 1.3 | 0.46 | 0.65 |
| **Metabolic markers** | | | | |
| Fluorescent serum AGEs (arbitrary units) | 0.68 ± 2.1 | 0.29 ± 1.3 | −0.57 | 0.57 |
| Malondialdehyde (μmol/l) | 2.0 ± 2.61 | −0.83 ± 2.0 | −3.12 | <0.005 |
| PCR (mg/l) | −1.21 ± 5.5 | −1.69 ± 5.4 | 0.23 | 0.82 |
| TNF-α (pg/ml) | 12.5 ± 14.7 | −18.36 ± 17.1 | –4.92 | <0.00001 |
| Insulin (μU/ml) | −7.0 ± 17.0 | –4.81 ± 7.3 | −0.67 | 0.5 |
| HOMA-IR | −2.5 ± 6.1 | −2.29 ± 3.7 | 0.13 | 0.89 |
| **Dietary variables** | | | | |
| Kilocal/day | −222 ± 415.1 | −110 ± 307 | 0.78 | 0.44 |
| Carbohydrates (g/day) | −32.0 ± 73.2 | −23.6 ± 61.9 | 0.3 | 0.76 |
| Fats (g/day) | −6.1 ± 16.2 | 0.13 ± 20.6 | 0.85 | 0.4 |
| Proteins (g/day) | −9.6 ± 14.6 | −3.63 ± 22.0 | 0.82 | 0.42 |
| dAGEs (as CML, KU/day) | 2304 ± 4169 | –4990 ± 3380 | –4.9 | <0.00005 |

*Mann-Whitney *U* test.

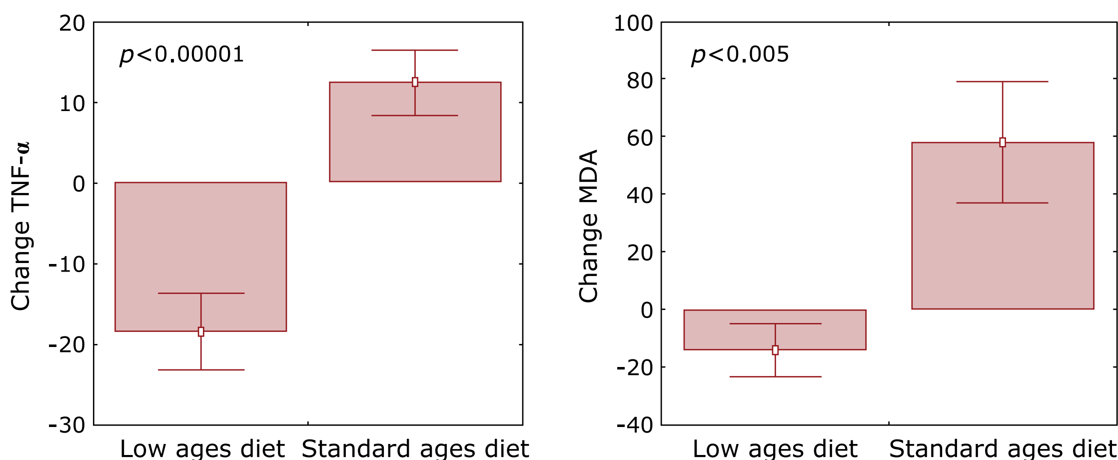


**Fig. 1.** Changes in TNF-α and MDA in type 2 diabetic patients. Changes in TNF-α and MDA in type-2 diabetic patients after 6 weeks with low AGEs diet as compared with those under Standard AGEs diet.

glycemic agents (1 with metformin, 1 with sulfonylureas, and 7 with both), and 3 patients only with diet. No patient reported changes in physical activity. 3 patients in each group received treatment for hypertension.

Table 1 shows the comparison of clinical and metabolic characteristics for both groups at baseline. Age and years since diagnosis were similar in both groups. The S-dAGEs had higher insulin levels ($p<0.003$) but lower TNF-α levels ($p<0.007$) and marginally higher protein intake ($p<0.04$) as compared with the group of L-dAGE. After 6 week of dietary intervention the AGE intake in the standard group showed a non significantly increase ($p = 0.07$) and in the low dAGEs the intake of AGEs decrease by 44% ($p<0.0002$); dAGEs change was +2304 ± 4169 for the Standard diet and –4990 ± 3380 for the low AGEs diet ($p<0.00005$) (Table 2). Also MDA and TNF-α significantly decreased in the group with L-dAGEs with respect to the group under S-dAGEs (Fig. 1). Other biochemical data, dietary variables, insulin levels or HOMA-IR did not show significant changes.

## Discussion

Several reports support a beneficial health effect of AGEs restriction in diet of diabetic patients, mainly by a reduction of circulating markers of inflammation and vascular dysfunction.[1,13] Uribarri *et al.*[14] proposed that this favorable outcome requires only a 50% decrease in dietary AGEs, evaluated as CML. In our study, L-dAGEs group the amount the AGEs reduction, as calculated with the tables of Golberg *et al.*[14] was about 44%. The result was a significant diminution of TNF-α levels and MDA but not AGEs serum levels. We attributed the reduction of TNF-α and MDA in our study to lower d-AGEs, in spite of the fact that AGEs serum levels did not decrease significantly after dAGEs reduction. Chao *et al.*[18] studied subjects with type 2 DM and found a positive correlation between high dietary AGEs intake and TNF-α and levels of oxidation markers 8-isoprostanes. A follow up study by 4 months in healthy subjects reported that low diet AGEs reduced 8-isoprostanes and TNF-α.[19] The latter 2 reports, carried out in diabetic and non diabetic subjects show the association of AGEs intake with TNF-α levels, and inflammation levels.



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These findings are consistent with our reports, although the marker we used was Malondialdehyde. Furthermore, the consumption of AGEs-rich diets by mice is associated with elevated circulating and tissue AGEs and conditions such as atherosclerosis.[20] The reduction of TNF-α and Malondialdehyde found in our study after l-AGEs diet, is important because it supports the benefit of simple instructions to reduce d-AGEs modifying food preparation in order to improve health conditions in diabetic patients.

In our group of study after d-AGEs reduction, metabolic control, lipid profile, or energy intake did not change. Evidence in the literature indicate that simple restriction of d-AGEs, without altering nutrients or caloric intake can lead to a significant reduction in oxidative stress and inflammatory markers supporting the conclusion that AGEs in food are toxins for patients with DM and healthy individuals.[12]

The absence of change of serum fluorescent AGEs after dietary AGEs restriction are puzzling. We considered that different turnover rates of diverse molecular types of AGEs might hinder changes with diet. It is also possible that different estimation procedures in foods and serum make the comparison biased. The d-AGEs estimation is based on the CML content by means of ELISA,[4,7] while our assessment of serum fluorescent compounds detects mainly pentosidine[16] and a large amount of non fluorescent AGEs are not detected.[21] CML is synthesized from hexoses, pentoses and from non-carbohydrate sources such as lipids and amino acids, and is considered a versatile food marker.[22] On the other hand, pentosidine is a specific advanced glycation end product, whose fluorescence is due to cross-links with arginine and lysine residues in proteins. Fluorescent AGEs are useful for the evaluation of cumulative damage to proteins; their correlation with serum HbA1c has been previously reported.[16,23,24]

In our study insulin or HOMA-IR, did not decrease. In contrast, Uribarri *et al.*[15] reported that DM2 subjects with very high basal intake of AGEs (20000 KU), after four months AGEs restriction decreased hyperinsulinemia, oxidative stress and TNF-α levels. We explain differences because in our study d-AGEs restriction lasted only six weeks, and the group submitted to a low AGEs diet, had a limited basal AGEs intake (8956 KU). We propose that to demonstrate an effect on insulin levels, a higher basal consumption of AGEs and longer time for observation may be needed.

C-reactive protein (CRP), a marker of inflammation may also decrease, under a low AGEs diet.[1,8,13,14] In our study, the group with L-dAGEs showed a trend to decrease in CRP values as compared to S-dAGEs group. Since dAGEs are evaluated on basis of CML content, our results support previous reports indicating that this particular AGE contributes to lipid peroxidation thus promoting oxidative stress.[14,25,26] In both diabetic and, non-diabetic adults at low dAGEs diet showed lower levels of oxidative stress markers.[15] In this context, our finding offers the first prospective evidence on the relationship between dietary AGEs and an oxidative stress marker (MDA) in DM2 patients.

Another adverse effect of AGEs is the induction of reactive oxygen species, thus increasing oxidative stress that stimulates the synthesis and release of proinflammatory cytokines in diabetic patients and in healthy subjects. In this study, only 6 weeks of d-AGEs restriction resulted in the reduction of inflammation markers, and malondialdehyde in DM2 patients.

These findings urge for further studies in order to examine in more detail the effect of low d-AGEs meals on diabetes control and on the potential risk of complications. This would support formal recommendations for food preparation to delay and/or to prevent diabetes and its complications.

In summary, lower d-AGEs intake resulted in decreased TNF-α and malondialdehyde concentrations. Presumptively simple recommendations regarding adequate food preparation may improve the conditions of diabetes mellitus. The potential inclusion of dietary recommendation for restriction of AGEs intake in clinical guidelines should have an impact on cultural traditions for food preparation.

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## Conflict of Interest

No potential conflicts of interest were disclosed.

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