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Case Report

Effect of amaranth consumption on diabetes-related biomarkers in patients with diabetes

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Abstract

Amaranth has been claimed as an excellent source of bioactive compounds; however, the action of these compounds has not been tested *in vivo*. The aim of this study was to evaluate the effect of amaranth consumption in Mexican patients with diabetes. Selected patients have been pharmacologically treated for at least five years without showing any health improvement. Patients with diabetes were divided in four groups according to their obesity degree: normal weight (n=15), overweight (n=22), obesity I (n=13), and obesity II and III (n=12). Participants consumed amaranth daily (20 g) for three months. After the trial, it was observed a decrease in weight (W) in obesity I group (85.8 to 84.2 kg) as well as a reduction of body mass index (BMI, 32.5 to 31.8 kg/m^2). Levels of serum markers related with obesity such as leptin, resistin, and visfatin decreased in all groups. The cardiovascular risk biomarker plasminogen activator inhibitor 1 (PAI-1) also decreased after the three months of treatment. This study suggests that amaranth consumption, a food containing bioactive compounds, is a promising complementation for diabetes and obesity health improvements.

Keywords: Amaranth, DPPIV activity, Serum biomarkers, Obesity, Type 2 diabetes

Introduction

Diabetes and obesity are chronic disorders that are reaching epidemic proportions leading to a dramatic increase in incidence of secondary diseases such as low-grade chronic inflammation, atherosclerosis, fatty liver, and some psychopathologies [1-4]. Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by high blood glucose levels and defective carbohydrate utilization due to a relative or absolute deficiency of insulin or insulin resistance with impairment of β-cell function [4]. The World Health Organization (WHO) has estimated that 347 million people worldwide have diabetes and 3.4 million people died from consequences of high fasting blood sugar [5]. From those deaths, 80% occur in low- and middle-income countries [6]. In Mexico there are currently 10.6 million people who suffer from T2DM and its complications [7, 8].

Changes related to nutrition and the increasingly sedentary lifestyles are two of the main factors that have contributed to the increased rates of obesity, diabetes, and heart diseases [8]. Improving eating habits with the introduction of foods containing bioactive compounds may be an effective primary prevention strategy to address these rising disease rates [8, 9]. Actually the

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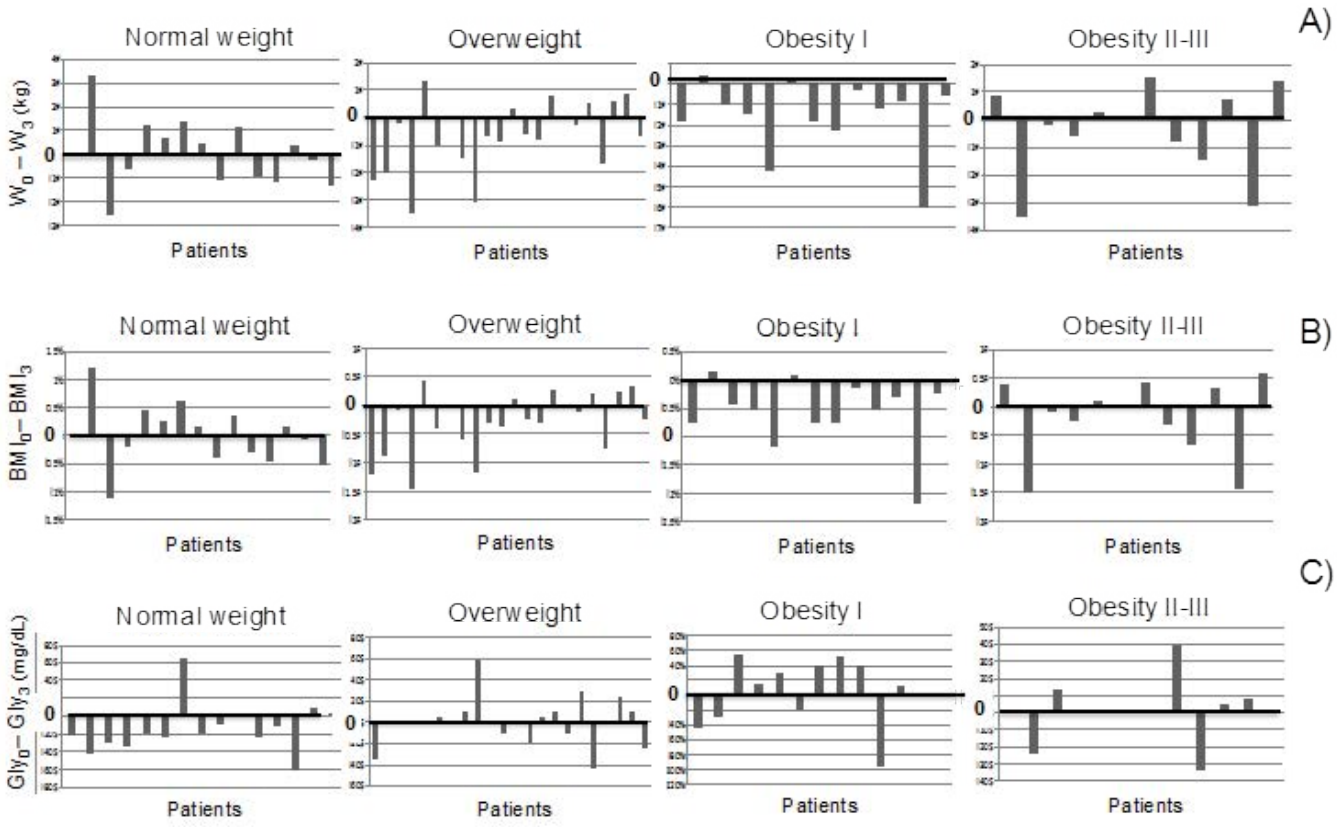

Figure 1: Anthropometric measurements of patients in the present study. Bars represents the changes in A) weight (W), B) body mass index (BMI), and C) glycaemia (Gly) values in each patient within each group at the beginning (subscript letter 0) and after 3 months (subscript letter 3) of amaranth consumption. The ordinate with value 0 means no change. Positive values (bars up) indicate increase and negative values (bars down) a decrease.

incretin-based therapy is used to lower the hyperglycemic state in the patient with diabetes [10-11]. Incretins are peptidic hormones released by the intestinal enteroendocrine cells to the bloodstream in response to nutrient intake where they stimulate the insulin secretion. Incretins, especially the hormone glucagon-like peptide 1 (GLP-1), are responsible for over 50% of postprandial insulin secretion [12]. However, the half-life of incretins is very short (<2 min) due to the cleavage and its inactivation by dipeptidyl peptidase IV (DPPIV). Hence, DPPIV inhibitors allow a prolonged action of incretins [13] and currently several synthetic DPPIV inhibitors are used as antidiabetic drugs [14]. However, when the enzyme is strongly inhibited, side effects may occur because DPPIV can also inactivate chemokines, neuropeptides, and other peptidic hormones leading to a wide range of physiological effects. Aside from its insulinotropic response, DPPIV inhibition can cause angioedema, pancreatitis, among others secondary effects [15]. Therefore, it is necessary to discover alternative agents that would exert hypoglycemic activity but with fewer or no secondary effects.

Amaranth (*Amaranthus* spp.) is a highly nutritious and non-allergenic pseudocereal with remarkable nutraceutical properties [16]. The interest in amaranth has increased due to the presence of encrypted peptides with inhibitory activity against the angiotensin-converting enzyme [17], and peptides that are able to inhibit up to 50% of DPPIV activity [18]. Although the high potential of amaranth, as a source of bioactive compounds, until date no human clinical trials are conducted to evaluate the effects of amaranth consumption. Hence, the goal of the present study was to evaluate the effect of amaranth consumption in Mexican patients with diabetes and obesity. The selected patients have been treated for at least five years without showing any health improvement. Changes in anthropometric measurements such, as weight (W), body mass index (BMI) and glycemic status, as well diabetes serum biomarkers and serum DPPIV activity were evaluated at the beginning and after three months of amaranth consumption (Figure 1).

Investigation

Ethical approval for the trial was obtained from the Ethics Committee Research of ISSSTE-SLP, Mexico (Reg. COFEPRIS 123301538X0054) with reference MX-M453N. Participant's

recruitment and follow-up was conducted from August to October 2013, all participants gave written informed consent. Patients (n=62), were selected according to the following inclusion criteria: Patients with T2DM (at least 5 years since diagnosis) controlled by hypoglycemic and antihypertensive drugs and patients who did not consume any kind of amaranth product before the trial. All patients followed up the treatment as indicated by the clinical team from the beginning of the diagnosis. Exclusion criteria included those patients with complications associated to diabetes (the use of insulin, significant liver and renal, cardiovascular or psychiatric illness), which indicates a bad attachment to the treatment.

Participants were allocated into four groups depending on their BMI values (normal weight, over weight, obesity I, and obesity II and III). Patients were followed-up for three months; during this time patients had a normal diet but part of their calories intake was substituted for 20 g of amaranth. Amaranth was supplemented in a wide range of products such as bread prepared with 20% amaranth flour, traditional Mexican candies "alegrias", amaranth flour for milkshakes, and popped amaranth as cereal. Compliance checks and possible incidences (adverse effects) were monitored with questionnaires and through meetings along the study. Patients were followed-up by the Hospital staff (nutritionists, psychologists), and doctors in charge of patients.

Physical measurements were height (H), weight (W), waist circumference (WC), systolic and diastolic blood pressure. All measurements were assessed using standardized methods, with participants dressed in light clothing and barefoot. The Body Mass Index (BMI) was calculated using the averages of the H and W, with the formulae as: [weight (kg)]/[height (m^2)]. Hypertension was defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg. Patients with values of SBP and DBP under the defined limits but who reported taking antihypertensive treatment for at least two weeks before the survey onset were classified as hypertensive.

Fasting capillary glucose (FCG) was measured using the Accu-Check Performa glucometer (Roche Diagnostics GmbH, GE) after an overnight fast. Following the Mexican norm NOM-015-SSA2-2010, patients with an abnormal FCG ≥ 126 mg/dL were classified as persons with diabetes [19].

Blood samples (20 mL) were collected into vacutainers (Becton, Dickinson & Company, New Jersey, USA) from all patients in a 10 h fasting state. Blood collection was carried out at the beginning and after three months of consuming a diet supplemented with amaranth. Serum was separated from whole blood by centrifugation at 300g for 20 min and stored at -80 °C until analysis.

Serum diabetes-related biomarkers (insulin, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin, and visfatin) were measured by using the Human Bio-Plex Pro Diabetes Assay (Bio-Rad, Hercules CA, USA) and the Bio-Plex MAGPIX multiplex reader (Bio-Rad). All samples were run in triplicates following the manufacturer´s protocol. Controls were used to monitor coefficients of variation, which were <10%. Biomarker concentration was calculated as pg/mL using standards supplied by manufacturer.

Serum DPPIV activity was determined in a continuous monitoring assay in a Multiskan Go microplate reader at 415 nm (Thermo Scientific, Waltham, MA, USA) at 37 °C [18]. Briefly, 15 µL of serum were mixed with 45 µL assay buffer (10 mM Tris-HCl, pH 7.6) and 50 µL of a 1 mM substrate Gly-Pro-p-nitroanilide hydrochloride (Gly-Pro-PNA, Sigma-Aldrich) into each microplate well. Enzyme activity was calculated at 30 min and expressed in nmol/mL/min (U/L) of Gly-Pro-PNA hydrolyzed.

All analyses were performed in triplicate and values were expressed as the mean ± standard error (SE). Comparisons at initial time and after three months within and between groups were determined using one-way analysis of variance (ANOVA). Results were considered statistically significant if a p-value was found less than 0.05. GraphPad Prism v6.0 software (La Jolla, CA, USA) was used for the statistical comparisons. A Pearson´s correlation was carried out using Xlstat software and Bonferroni test was used for correcting for multiple comparisons.

Results

One of the important considerations for the selection of patients was that all of them have been under treatment for diabetes for at least five years without showing any health improvement. Our results have shown that amaranth consumption had a beneficial effect in health. After only three months of consuming a diet containing amaranth a decrease in W was observed, mainly in the obesity I group (85.8 to 84.2 kg, p=0.00675), which resulted in a BMI reduction (32.4 to 31.8 kg/m^2, p=0.00456). A WC reduction was observed in normal weight group (93.1 to 90.6 cm, p=0.01093). However, because metabolism responses in humans are dependent of several factors and variability is common among individuals, we look at the individual changes. In this sense, it was observed that 46.6%, 68.2%, 84%, and 50% of the patients with normal weight, overweight, obesity I, and obesity II-III groups, respectively, had significant decrease in W (Figure 1A). As a result, the BMI reduction was also observed (Figure 1B). It was noteworthy that 73% of patients in normal weight group showed a reduction of glycemic levels, whereas in the other three groups the 25% to 30% of patients showed a reduction of this value (Figure 1C). Interestingly, in some patients, the doctor in charge reduced their medication. The metformin dose was decreased in one patient with normal weight, while the reduction of medication in one or two drugs (metformin, sitagliptin, or glibenclamide) was observed in 22%, 16% and 30% of patients in groups overweight, obesity I, and obesity II-III, respectively. In this study, it was detected that some patients presented hypertension but after three months of amaranth consumption, some of this hypertensive patients improved their blood pressure. However, longer treatments should be done to see this improvement in all patients.

Marker	Values at initial time				Values after three months			
	Normal weight	Over weight	Obesity I	Obesity II-III	Normal weight	Over weight	Obesity I	Obesity II-III
Insulin (pg/mL)	59.9 ±3.4	161 ±5.7	145 ±15.5	212 ±9.7	63.6 ±3.8 p=0.22442	157 ±5.6 p=0.47301	120 ±3.6 p=0.02237	530 ±21.8 **p< 0.00001**
Leptin (pg/mL)	1717 ±37.9	2698 ±65.9	3988 ±108.2	6484 ±223.0	1593 ±42.8 **p=0.00881**	2422 ±75.2 **p=0.00107**	3313 ±88.7 **p= 0.00002**	5221 ±47.5 **p=0.00032**
PAI-1 (pg/mL)	9105 ±127.1	12315 ±509.1	10152 ±221.8	12357 ±838.1	7604 ±291.9 **p=0.00042**	9495 ±436.0 **p=0.00008**	8014 ±108.5 **p< 0.00001**	9366 ±287.3 **p=0.00328**
Resistin (pg/mL)	1385 ±40.2	1666 ±69.7	1163 ±41.4	1723 ±47.8	1027 ±17.8 **p=0.00009**	1031 ±18.1 **p=0.00003**	823 ±11.0 **p=0.00004**	971 ±24.0 **p<0.00001**
Visfatin (pg/mL)	875 ±12.6	2190 ±54.2	950 ±25.1	1799 ±62.4	761 ±31.0 **p=0.00161**	1611 ±43.8 **p=0.00007**	812 ±17.9 **p=0.00002**	1360 ±45.3 **p=0.00068**
DPPIV (U/L)	26.9 ±1.7	29.9 ±0.3	33.8 ±1.3	30.7 ±1.2	31.0 ±0.9 p=0.19642	30.2 ±0.4 p=0.02189	28.5 ±2.1 p=0.05976	28.1 ±0.7 p=0.02452

All the patients completed the study. Values are expressed as mean ± SE. Significant differences (p<0.05) after three months within and among groups are in bold numbers. Threshold for significance p = 0.05/4 = 0.0125 for Bonferroni correction. Significant p-values according with Bonferroni test are bold-faced.

Table 1: Serum diabetes-related biomarkers in patients with type 2 diabetes mellitus before and after three months of amaranth consumption.

To obtain more information about the patient's response at molecular level, serum markers were measured. As shown in Table 1, after three months of treatment, insulin concentration increased significantly only in obesity II-III (212 pg/mL to 530 pg/mL. Leptin, the hormone related with obesity, increased depending on the obesity degree and correlating with the group classification (Table 1). PAI-1, resistin and visfatin showed a lower concentration in normal weight group followed by obesity I group, overweight and the highest levels were detected in obesity II-III group. These four markers showed a decreased after three months of amaranth consumption indicating, at molecular level, the health improvement in all patients (Table 1).

Serum DPPIV activity was higher in overweight group (33.8 U/L), while the lowest value was detected in normal weight group (26.92 U/L) but no significant changes were observed in this activity after three months of amaranth consumption (Table 1).

Discussion

Amaranth has higher protein content (15-22%) than traditional cereals (8-14%) and is a good source of high quality proteins, containing more methionine and lysine than legumes and cereals, respectively [16]. In addition, amaranth contains encrypted peptides with inhibitory activity against ACE [17]; and DPPIV [18]. However clinical trials to evaluate the antihypertensive and antidiabetic actions of amaranth are scarce. Amaranth supplementation represents an increase in protein intake, and it is well known that diets rich in proteins produce greater satiety resulting in weight loss [22].

MAGPIX assay is an accurate and valid system for markers detection [23] and we used this technology to analyze the changes in ten of T2DM-related markers. Particularly a reduction in leptin, PAI-1, resistin and visfatin was observed in all the patients after three months of amaranth consumption. Leptin may function as a part of a signaling pathway and regulate the size of the body fat depot. Leptin acts directly or indirectly on the central nervous system to inhibit food intake and/or regulates energy expenditure as part of a homeostatic mechanism to maintain consistency of the adipose mass [24, 25]. PAI-1 is a glycoprotein that may play important pathological roles in immune and inflammatory responses [26]. Moreover, PAI-1 is an important inhibitor of the fibrinolytic system, so elevated levels could suppress fibrinolysis and result in an increased risk of thrombosis [27]. It was observed that PAI-1 levels decreased in all groups after three months of amaranth consumption. It is well known that amaranth has anti-inflammatory actions by suppressing NF-κB pathway [28] and the antihypertensive properties of amaranth have been also reported [17]. Our findings suggest that these properties could also be mediated through PAI-1 levels.

Visfatin circulating levels are increased in obesity, T2DM, and in atherothrombotic disease, turning this protein in a promising therapeutic target in metabolic-related cardiovascular diseases. Visfatin can directly increase insulin secretion in β-cells [29] and is considered an adipokine-enzyme that acts as a proinflammatory cytokine capable to induce TNF-α, IL-6, and IL-1β [30,31]. In this study, visfatin levels were decreased in all patients with diabetes after amaranth consumption, which supports the known anti-inflammatory activity of amaranth [28].

There are a few clinical studies examining serum DPPIV activity in patients with T2DM, all of them with contrasting results: unchanged [32], increased [33], and decreased [34] plasma

activity. The real DPPIV effect at present is unclear; nonetheless, hyperglycemia does not appear to affect DPPIV enzymatic activity *per se*, but in contrast, it can increase DPPIV gene expression [35]. Our results showed that there was no correlation between DPPIV activity in serum and the obesity degree. This observation illustrates the caveat that has previously been suggested that the measurement of DPPIV activity in the plasma may not necessarily reflect the *in vivo* degree of degradation of incretin hormones by DPPIV [36]. The explanation of this is because the N-terminal truncation of both GLP-1 and GIP *in vivo* proceeds much more rapidly than can be explained solely by the activity of the soluble form of the enzyme in plasma. In addition, the half-life of both incretin hormones in human plasma is much longer *in vitro* than that found *in vivo* [35-37].

Conclusions

After three months of amaranth consumption, a seed containing bioactive peptides, some health improvements were observed in patients with diabetes and obesity. At molecular level it was observed a trend in all groups to decrease serum markers related with obesity such as leptin, resistin, and visfatin. Amaranth consumption also induced the decrease the cardiovascular risk as indicated by PAI-1 blood levels. As far we know, this is the first report on human clinical trial to test the health benefits of amaranth consumption, results are promising, most of the patients expressed feeling good and healthier but certainly, it is necessary to follow clinical trial for longer times to see more evident effects in all patients.

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