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# Dietary Antioxidants Present in Eggs May be Related to Lower Concentrations of Biomarkers of Inflammation in Type-2 Diabetic Patients

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## Abstract

We previously reported that compared to an oatmeal breakfast, one egg per day did not alter plasma lipids, plasma glucose, glycosylated hemoglobin or C reactive protein in individuals with Type 2 Diabetes Mellitus (T2DM) (n = 29). However, plasma tumor necrosis factor-α (TNF-α) and the liver enzyme Aspartate Amino Transferase (AST) were lower during the egg period ($P < 0.05$). The objective of this study was to investigate whether those observed reductions in biomarkers of inflammation were due to nutrients present in eggs. Therefore, we measured plasma concentrations of lutein and zeaxanthin and conducted detailed dietary analysis of all vitamins and minerals to determine if higher intake of certain micronutrients would support these observed differences. In agreement with dietary intake, no significant differences were observed in plasma lutein (0.39 ± 0.16 vs 0.36 ± 0.15 µmol/L) or zeaxanthin (0.144 ± 0.076 vs 0.125 ± 0.048 µmol/L) for eggs and oatmeal, respectively. However, intake of vitamin A, vitamin E and selenium were higher ($P < 0.0001$) during the egg period. We also observed that during the oatmeal period, subjects consumed more calcium and magnesium ($P < 0.001$), possibly associated with the inclusion of lactose-free milk in the corresponding prescribed breakfast. These results suggest that although no differences were observed in plasma lutein or zeaxanthin, intake of other antioxidants present in eggs might have contributed to the observed reductions in inflammation in this population with T2DM.

**Keywords:** Antioxidants; Diabetes Type 2; Dietary Records; Eggs; Oatmeal

## Introduction

In spite of the removal of the upper limit for cholesterol intake from the dietary guidelines [1], there is still uncertainty regarding egg intake and increased risk for heart disease in individuals with Type 2 Diabetes Mellitus (T2DM) [2,3]. The literature is quite controversial in this topic; some studies report that dietary cholesterol is associated with diabetes risk [4,5] while other studies do not find this connection [6,7]. Although there are reports on clinical interventions which have shown positive effects on the biomarkers of heart disease upon egg consumption in diabetic populations [8,9], there is still a need for more clinical trials to better establish a connection between egg intake and biomarkers of heart disease.

In contrast, oatmeal, a good source of β-glucan, has been characterized as heart healthy due to the effectiveness of this dietary fiber component in lowering plasma LDL cholesterol [10]. Oatmeal has also been shown to be beneficial in the case of uncontrolled T2DM [11]. In our previous crossover study, we demonstrated that compared to an oatmeal-based breakfast, one egg per day did not alter the biomarkers for heart disease or glucose metabolism [9]. Further, egg intake resulted in lower plasma Tumor Necrosis Factor- α (TNF-α) and Aspartate Aminotransferase (AST) values compared to the oatmeal indicating positive effects of eggs on these biomarkers of inflammation [9].

Individuals with diabetes have other conditions that further increase their risk for heart disease including dyslipidemias, oxidative stress and low-grade inflammation [12]. Numerous studies have shown that antioxidants exert a protective effect against oxidative stress and inflammation [13]. Eggs are a very good source of antioxidants including carotenoids, vitamins and minerals. The aim of this study was to investigate further what nutrients and antioxidants present in egg could be responsible for the observed changes in inflammation in diabetic patients when compared to an oatmeal breakfast. For that purpose, we measured plasma carotenoids present in eggs and conducted a careful dietary analysis of other vitamins and minerals present in eggs that could be responsible for the lower concentrations of TNF-α and AST. We hypothesized that plasma lutein and zeaxanthin would be higher following the egg breakfast and that Vitamin A, Vitamin E and selenium would be consumed in higher concentrations during the egg breakfast and therefore contribute to the observed results.

## Methods

### Experimental Design

Thirty-three individuals with type-2 diabetes were recruited to participate in this study. The age was between 35-65 years. We estimated that 25 subjects would be enough to observe changes in inflammatory markers based on previous studies [14]. We recruited 33 subjects to allow for attrition. The study took place between June-December 2013. The exclusion criteria were uncontrolled diabetes, retinopathy, heart disease, cancer, or renal problems. In addition, participants had to have glycosylated Hemoglobin (HbA1c) < 9%. Participants were randomly allocated by one of two breakfasts to consume either one egg or 40 g of oatmeal with 2 cups (472 mL) of lactose-free milk/day for 5 weeks. After a 3-week washout, subjects were allocated to the alternate breakfast for an additional 5 weeks. Subjects were 98% compliant with this intervention. A more detailed description of the experimental design has been reported previously [9].

Twenty-nine subjects finished both arms of this dietary intervention. The intervention protocol was approved by the University of Connecticut Institutional Review Board, the Ethical Committee from Centro de Investigacion en Alimentacion y Desarrollo (CIAD), and the Review Board from Hospital Ignacio Chávez in the city of Hermosillo, Sonora, Mexico. All subjects gave written informed consent prior to initiating the study. This study is registered at Clinicaltrials.gov (trial # NCT02181244)

### Dietary Records

Participants were asked to follow their usual diet during both breakfast dietary periods. Four-day weighed food records on alternate days [15] were used to evaluate dietary intake of vitamins and minerals during both breakfast periods for a total of 8 records. Subjects were provided with food scales (Ohaus CS 2000) and a chart to record daily intake and researchers in the study worked very closely with participants to ensure reliable food assessment during the day. Dietary intake was analyzed using the ESHA Food processor program (ESHA, Food Processor, 7.20, ESHA Research Editor, 2007). Regional foods typically consumed by these subjects are also included in this database [16].

### Plasma Lutein and Zeaxanthin

Lutein and zeaxanthin were extracted from plasma as described previously [17] using a 2:1 (v:v) chloroform: methanol mixture followed by hexane. A known concentration of internal standard (trans-β-apo-8'-carotenal, Sigma-Aldrich, St. Louis, MO) was added to each sample prior to extraction to calculate carotenoid recovery.

## Results

### Dietary Records

Dietary records indicate substantial differences in vitamin and mineral intake between the two breakfasts. After the egg period there was a 326% increase in vitamin A ($p = 0.002$), 127% in vitamin E ($p < 0.001$) and 159% in selenium ($p< 0.001$). In contrast, calcium concentrations were two-fold and magnesium 1.2-fold higher during the oatmeal period due to the addition of 2 glasses of lactose-free milk (Table 1).

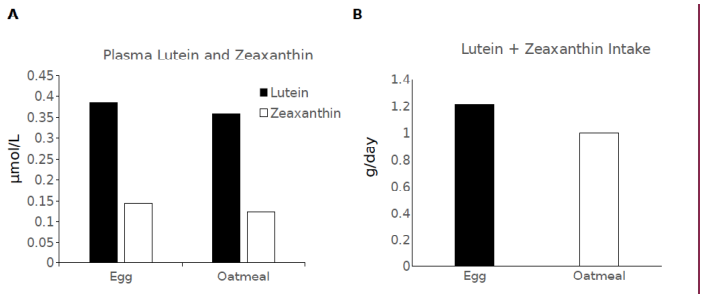
| Nutrient | Oatmeal | Eggs | P value |
|---|---|---|---|
| Vitamin A (RE/day) | 117.8 ± 137.2 | 384.3 ± 388.3 | 0.002 |
| Vitamin D (mcg/day) | 7.4 ± 2.5 | 3.5 ± 2.1 | < 0.001 |
| Vitamin E (mg/d) | 5.1 ± 1.7 | 6.5 ± 2.3 | < 0.001 |
| Vitamin K (mcg/d) | 35.3 ± 48.6 | 35.7 ± 27.0 | 0.944 |
| Vitamin C (mg/d) | 9.2 ± 3.9 | 23.1 ± 8.2 | < 0.001 |
| Thiamine (mg/d) | 1.05 ± 0.29 | 0.98 ± 0.33 | 0.346 |
| Riboflavin (mg/d) | 1.0 ± 0.4 | 1.5 ± 0.5 | < 0.001 |
| Vitamin $B_6$ (mg/d) | 23.5 ± 8.4 | 27.5 ± 8.5 | 0.029 |
| Biotin (mcg/d) | 2.3 ± 1.8 | 5.0 ± 4.2 | 0.001 |
| Pantothenic acid (mg/d) | 2.8 ± 1.0 | 4.1 ± 0.9 | 0.001 |
| Folic Acid (mcg/d) | 423 ± 188 | 499 ± 215 | 0.013 |
| Vitamin $B_{12}$ (mcg/d) | 1.6 ± 0.7 | 1.8 ± 1.0 | 0.101 |
| Selenium (mcg/d) | 94.6 ± 42.9 | 59.2 ± 30.7 | < 0.001 |
| Calcium (mg/d) | 2423 ± 3344 | 1176 ± 1463 | 0.002 |
| Phosphorus (mg/d) | 783 ± 272 | 1084 ± 308 | < 0.001 |
| Magnesium (mg/d) | 215 ± 65 | 260 ± 79 | 0.001 |
| Iron (mg/d) | 12.1 ± 3.8 | 13.7 ± 4.4 | 0.046 |
| Potassium (mg/d) | 3409 ± 611 | 2568 ± 804 | <0.001 |

| | | | |
|---|---|---|---|
| Iodine (mcg/d) | 20.6 ± 17.4 | 73.0 ± 32.5 | < 0.001 |
| Values are presented as mean ± SD. Differences in nutrient intake were assessed by student's paired t-test. | | | |

**Table 1:** Vitamin and mineral intake of Type 2 Diabetic subjects (n = 29) during the oatmeal and the egg periods.

### Plasma Antioxidants

Plasma lutein and zeaxanthin (Figure 1A) and dietary lutein + zeaxanthin (Figure 1B) were not different between breakfast periods.




**Figures 1(A-B): (A)** Concentration of plasma lutein and zeaxanthin after 5 weeks of consuming one egg per day or a cup of oatmeal with lactose-free milk and **(B)** intake of lutein and zeaxanthin during the egg and the oatmeal periods.

## Discussion

### Dietary Records

The intention of this short communication was to determine if antioxidants present in eggs could be associated with the lower plasma concentrations of AST and TNF-α compared to oatmeal as found in our previous study [9]. We were able to demonstrate, by use of dietary records, that consumption of vitamin A, vitamin E, and selenium - nutrients present in eggs - was much higher during the egg breakfast. We also have shown that both dietary and plasma lutein and zeaxanthin were not higher in the egg compared to the oatmeal period.

### Biomarkers of Inflammation and Lutein and Zeaxanthin

Diabetic patients are exemplified by having elevated concentrations of biomarkers of inflammation [12]. TNF-α contributes to the modulation of immune and inflammatory responses [18] and the primary source of this cytokine is activated macrophages and monocytes [19]. High concentrations of liver enzymes, such as AST, is indicative of liver inflammation [20]. Thus, the observed reductions in plasma concentrations of AST and TNF-α in our previous study following egg [9] deserves further consideration. Originally, we hypothesized that these decreases might be related to the presence of lutein and zeaxanthin in the egg yolk.

Previous studies in our laboratory have shown significant increases in plasma lutein and zeaxanthin after consumption of 2-3 eggs for an extended period of time in children [21], young populations [17], elderly [22], and obese [23] individuals and in subjects classified with metabolic syndrome [24]. Although in this study we did not observe any changes in dietary lutein and zeaxanthin, other studies have reported a consistent increase in plasma lutein and zeaxanthin with egg intake, which is not directly associated to total dietary intake [14,25] but is due to the fact that lutein and zeaxanthin are highly bioavailable in eggs [26]. Although eggs are not an excellent dietary source for lutein and zeaxanthin, the egg matrix possesses the ideal milieu for effective absorption of these carotenoids in the small intestine [24]. However, as previously shown, one egg per day is not enough to result in significant increases of plasma lutein and zeaxanthin. As reported by DiMarco et al. [17] in subjects consuming 0, 1, 2, and 3 eggs for 4 weeks each, significant differences in plasma lutein and zeaxanthin compared to 0 eggs were observed with the intake of 2 and 3 eggs but not with only 1 egg per day. Missimer et al. [27] also reported higher concentrations of plasma lutein and zeaxanthin compared to an oatmeal breakfast after the consumption of 2 eggs per day. It appears that the concentration of lutein and zeaxanthin in one egg is not sufficient to increase plasma concentrations in most cases. Although the concentrations of lutein and zeaxanthin were higher after egg consumption, these numbers did not reach significance suggesting that the observed reductions in inflammatory biomarkers might hot have been related to these carotenoids

## Biomarkers of Inflammation and Antioxidants in Eggs

In contrast to lutein and zeaxanthin intake, analysis of dietary records showed that the concentrations of micronutrients varied widely depending on the assigned breakfast. It is clear that those individuals in the egg group consumed higher concentrations of antioxidants including vitamin A, vitamin E, and selenium, which could be associated with previously observed decreases in TNF-α and AST.

Retinoic acid has been demonstrated to attenuate the inflammatory response and reduce the production of inflammatory mediators including TNF-α in various cell types of peripheral tissues [28]. Vitamin A protects against inflammation when provided in reasonable amounts as a supplement or as part of the diet. For example, it has been shown that when great amounts of vitamin A are provided as a supplement, this results in increases in inflammation and liver enzymes in obese women [29] while in cells studies, addition of vitamin A ameliorates cholestatic liver

injury and decreases the concentrations of liver enzymes [30]. Vitamin E has been shown to inhibit nuclear factor-κβ, reduce the production of TNF-α [31], and reduce concentrations of TNF-α during inflammatory conditions [32]. In addition, a recent meta-analysis also concluded that vitamin E supplementation optimizes liver enzyme levels for those patients identified with non-alcoholic fatty liver disease and hepatic cirrhosis [33] clearly indicating the protective effect of vitamin E against inflammation.

Eggs are an important source of selenium and studies have shown that selenium deficiency exacerbates the inflammatory response [34]. Further, selenium, due to its unique role of incorporation into selenoproteins, has been identified to have multiple health effects [35]. Selenium has also been reported to decrease liver enzymes in diabetic rats [36]. However, it has also been indicated that excess selenium supplementation can lead to insulin resistance and oxidative stress [34], thus obtaining selenium from dietary sources would be a good practice rather than using selenium supplementation.

## Conclusions

These studies [27-35] support that the higher concentrations of dietary vitamin A, vitamin E, and selenium during the egg period could be associated with the observed decreases in TNF-α and AST compared to the oatmeal breakfast [9]. In addition, this study further confirms that inclusion of 1 egg/d in people with T2DM increases those nutrients with antioxidant properties, which could be beneficial in mitigating inflammation in this high-at risk population.

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