Resistin, Leptin and Antioxidant Vitamins A and E Status in a Tunisian Group of Obese Men Diabetics
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ABSTRACT
Introduction: Our work is a comparative study that aims to evaluate vitamins A and E, leptin and Resistin serum levels in a population of obese diabetic males and to identify any correlations that may exist between these parameters. Materials and Methods: A measurement of vitamins A and E, leptin, Resistin, glucose, insulin and some lipid parameters serum levels was performed in 30 obese diabetic men free of all other metabolic and/or physical diseases with a mean age of \((47,97 \pm 4.32)\) years old compared to 30 healthy subjects. Results: Our results showed a significant difference between the two groups as for the vitamins A and E, leptin and Resistin levels. Indeed, vitamin A level in our obese diabetic patients is \((0.50 \pm 0.46 \text{mg/l})\) against \((1.98 \pm 0.97 \text{mg/l})\) for the control group and vitamin E level is \((7.92 \pm 2.94 \text{mg/l})\) against \((12.21 \pm 1.57 \text{mg/l})\) for the control group. Moreover, leptin level (which is \(18.22 \pm 4.35 \text{ng/ml}\)) and Resistin level (which is \(8.55 \pm 2.86 \text{ng/ml}\)) of obese diabetic males are significantly higher than those observed for healthy individuals (respectively \(4.51 \pm 1.94 \text{ng/ml}\) and \(3.72 \pm 1.20 \text{ng/ml}\)). Correlations showed that there is a significant negative association between leptin and vitamin A \((r = -0.58; p = 0.001)\) also leptin and vitamin E \((r = -0.501; p = 0.005)\). As well, an inverse significant relationship was found between resistin and vitamin A \((r = -0.42; p = 0.021)\) likewise Resistin and vitamin E \((r = -0.534; p = 0.002)\). Discussion: Our results are consistent with those of several studies demonstrating a significant reduction in rates of vitamins A and E and a remarkable increase in leptin and resistin serum levels in obese diabetic subjects. This confirms the inverse relationship of vitamins A and E with leptin and Resistin. Other studies have reported contradictory results. Conclusion: The antioxidant status of the obese diabetic subject is altered and vitamins A and E rates are lowered. Given the inverse significant correlation with leptin and Resistin, vitamins A and E may exert a specific effect on the secretion and expression of these two adipokines.

Keywords: Obesity, Diabetes, Resistin, Leptin, BMI, Antioxidants
INTRODUCTION

Obesity is a multifactorial chronic disease that constitutes a major public health problem because of its prevalence and the frequency and severity of its complications. The current epidemic of obesity concerns many countries, developed and developing, including Tunisia that seems to be at an advanced stage. The status of adipose tissue as an endocrine organ secreting biologically active molecules (such as leptin and resistin), involved in energy balance and glucose and lipid metabolism, helped to better understand the mechanisms involved in the pathophysiology of obesity and its complications. The development of insulin resistance associated with type 2 diabetes is one of the common complications of obesity. Indeed, in obese people moved uncontrolled oxidative stress, cause or consequence of insulin resistance, playing an important role in the development of type 2 diabetes and the genesis of its complications. Low levels of antioxidants in such individuals could further aggravate the situation and increase cellular and vascular damage.

In this context, we carried out a comparative study to evaluate serum levels of resistin, leptin and antioxidant vitamins (A and E) and establish any correlation that may exist between both leptin and resistin and the other between these two adipokines and these two vitamins in a population of men obese diabetics.

METHODOLOGY

Study population

Our study included 2 groups. The first group is composed of 30 obese men with type 2 diabetes (BMI > 30 kg/m² and fasting glucose ≥ 7 mmol/l), exempt from other metabolic and physical diseases whose Diabetes age is between 3 and 5 years, recruited from the External Consultation of the service “C” of Diabetology and Nutrition disease and therapeutic Dietetics at the National Institute of Nutrition. The second group consists of 30 non-obese men, recruited from volunteers accompanying the patients to the said consultation or among our entourage. They were matched to the men from the first group by age. All these men of this control group were well informed about the objectives of our study and gave their participation agreements. For men in the control group, they have benefited from the results of biochemical tests and taking blood pressure.

Anthropometry

For taking the weight, each respondent was weighed twice by the dietician and a nurse of the service instead of the survey with a scale calibrated person being lightly clad. The average of the two measurements was calculated. Taking the size was carried out using a micro fathom. The same measurement procedure used for weight was used. These two parameters have enabled us to calculate the Body Mass Index (BMI = weight (kg) / Size² (m²)). A man is considered obese if his BMI is over 30 kg/m².

BIOCHEMICAL ANALYSES

Venous blood is collected in the morning after fasting for 12 hours. The levy is, in fact, three types of tubes by a specialized laboratory technician for both diabetics obese patients than for controls. EDTA tubes (of ethylene diamine tetraacetic) and dry tubes for hormonal study (insulin + + leptin Resistin) and heparin lithium for lipid and glucose balance.

The dosage of vitamin A and vitamin E is carried out by high-performance liquid chromatography according to isocratic reverse-polarity phase. The blood glucose is done by an enzymatic method with glucose oxidase kit "Beckman" adapted analyzer on "Beckman synchronizaton CX9." The insulin was determined by IRMA (Immunoradiometric Assay). The triglyceride level is determined by an enzymatic method, kit "Beckman synchronization Cx7". The Cholesterol is determined by an enzymatic method using cholesterol oxidase kit "Beckman" adapted on analyzer "Beckman SYNCHRON CX9". The dosage of leptin is performed by the radioimmunological method with competition "RIA Millipore # HL-81HK with a sensitivity of 0.5 ng/ml to 100 ng/ml." The dosage of the Resistin was performed by the ELISA method "Millipore # EZHR-95K with a sensitivity ranging from 0.16 ng/ml to 10 ng/ml."

STATISTICAL ANALYSIS

We calculated simple frequencies and relative frequencies (percentages) for categorical variables. We calculated averages and standard deviations (SDs) and determined the extent (range = minimum and maximum) for quantitative variables. The Comparisons of 2 means of series were performed using the Student's t-test for independent groups. The comparisons of percentages from Independent sets were made with the test chi-square Pearson, and for non-validity of this test using Fisher's exact bilateral test. The links between two quantitative variables were investigated by the Pearson correlation coefficient and in the case of invalidity by the correlation coefficient of Spearman rank.

To Research thresholds of vitamin A and vitamin E considered of risk factors for obesity, we have established a ROC curve (Receiver Operating Curves) studying; sensitivity = f (1 specificity). After verifying that the area under the curve was significantly > 0.50, we have chosen as the threshold value of the variable that gives the best value of the difference [(sensitivity) - (1 specificity)]. We calculated the odds ratio (OR), which represents the number of times that the probability (risk) of an event multiplied by exposure to a factor compared to the non-exposure. In all statistical tests, the significance level was set at 0.05.

RESULTS

The average age of the diabetic obese group and witnesses was respectively (47.9 ± 4.3) years and (48.6 ± 5.2) years (p = 0.63). BMI was significantly higher in obese male subjects with diabetes than in those with normal weight without diabetes, respectively (33.71 ± 5.99) kg/m² vs (20.04 ± 1.74) kg/m². The Biochemical characteristics of the two study groups are illustrated in Table 1 below.
Table 1: Comparison of biochemical parameters between diabetic obese and control group

<table>
<thead>
<tr>
<th></th>
<th>obese diabetic</th>
<th>controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A (mg/l)</td>
<td>0.50 ± 0.46</td>
<td>1.98 ± 0.97</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>Vit E (mg/l)</td>
<td>7.92 ± 2.94</td>
<td>12.21 ± 1.57</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>10.26 ± 1.81</td>
<td>5.48 ± 0.85</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>insulinemia (µU/l)</td>
<td>10.10 ± 2.28</td>
<td>4.44 ± 1.43</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.68 ± 1.04</td>
<td>4.33 ± 0.67</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>triglyceridemia (mmol/l)</td>
<td>1.70 ± 0.98</td>
<td>0.98 ± 0.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>18.22 ± 4.35</td>
<td>4.51 ± 1.94</td>
<td>&lt; 10⁻³</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>8.55 ± 2.86</td>
<td>3.72 ± 1.20</td>
<td>&lt; 10⁻³</td>
</tr>
</tbody>
</table>

Table 2: Correlations of vitamin A and vitamin E with different biochemical parameters, BMI in the group of diabetic obese.

<table>
<thead>
<tr>
<th>Glycemia</th>
<th>Insulin</th>
<th>Total Cholest</th>
<th>triglyceridemia</th>
<th>Leptin</th>
<th>Resistin</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A</td>
<td>r = -0.36</td>
<td>r = -0.43</td>
<td>r = -0.004</td>
<td>r = -0.13</td>
<td>r = -0.58</td>
<td>r = -0.43</td>
</tr>
<tr>
<td></td>
<td>p = 0.04</td>
<td>p = 0.02</td>
<td>p = NS</td>
<td>p = NS</td>
<td>p = 0.001</td>
<td>p = 0.021</td>
</tr>
<tr>
<td>Vit E</td>
<td>r = -0.54</td>
<td>r = -0.64</td>
<td>r = -0.015</td>
<td>r = -0.09</td>
<td>r = -0.51</td>
<td>r = -0.53</td>
</tr>
<tr>
<td></td>
<td>p = 0.002</td>
<td>p &lt; 10⁻³</td>
<td>p = NS</td>
<td>p = NS</td>
<td>p = 0.005</td>
<td>p = 0.002</td>
</tr>
</tbody>
</table>

Table 1 shows that the antioxidant status is better in subjects with normal weight without diabetes than in diabetic obese resulting in levels of vitamins A and E significantly higher. It also appears from these results that the lipid parameters, leptin, insulin, and resistin were significantly higher in diabetic obese. We found in the diabetic obese group that resistin is significantly and positively correlated with leptin (r = 0.77; p < 10⁻³). In addition, in this group, there are significant correlations between antioxidant vitamins A and E with BMI, blood glucose, leptin, insulin, and resistin. Table 2 summarizes our main results.

The results obtained, show that vitamins A and E are significantly and negatively correlated with different biochemical parameters measured except for Total Cholesterol and serum triglycerides. The concordance of vitamin E with these parameters is better compared to that of vitamin A. We also conclude that vitamin E was significantly and negatively correlated with BMI, while vitamin A is not significantly correlated with BMI. That during these two antioxidant vitamins are negatively and significantly correlated with resistin which is adipokine incriminated in the genesis of diabetes among obese. The following figures illustrate schematically these correlations.
Figure 1: Correlation Vitamin A = f (BMI) in diabetic obese

![Graph showing correlation between BMI and Vitamin A with r = -0.33, p = 0.07.]

Figure 2: Correlation Vitamin A = f (Resistin) in diabetic obese

![Graph showing correlation between Resistin and Vitamin A with r = -0.43, p = 0.02.]

Figure 3: Correlation Vitamin E = f (BMI) in diabetic obese

![Graph showing correlation between BMI and Vitamin E with r = -0.74, p < 10^-3.]

BMI (kg/m²)

Vitamin A (mg/l)

Resistin (ng/ml)

Vitamin E (mg/l)
**Figure 4:** Correlation Vitamin E = f (Resistin) in diabetic obese

![Graph showing correlation between Vitamin E and Resistin in diabetic obese individuals](image1)

- $r = -0.54$
- $p = 0.002$

**Figure 5:** Correlation Resistin = f (BMI) in diabetic obese

![Graph showing correlation between Resistin and BMI in diabetic obese individuals](image2)

- $r = 0.72$
- $p < 10^{-3}$
In addition, we have tried to find the risk thresholds for vitamin A and E below which the likelihood of developing type diabetes associated with obesity is increased. We used the curve ROC (Receiver Operating Curves) which is obtained by studying sensitivity = f (1-specificity) after verification of the application conditions. (Figures 6 and 7)
The results of the analysis gave two thresholds for vitamin E and A, respectively 10.54 mg / l and 0.63 mg / l. The Tables 3 and 4 illustrate the distribution of cases in the survey, according to the threshold values of the two vitamins and give the corresponding ch-2 and OR values. These results show that the risks are very highly significant. Thus, a patient with a blood level of vitamin A <0.63 mg / l is at a very high risk of developing type 2 diabetes associated with obesity, whereas vitamin E <10.54 mg / l Run a patient 20 times more likely to develop a diabetes type 2 associated with obesity.

### Table 3: Distribution of the number of surveyed patients according to the vitamin A threshold

<table>
<thead>
<tr>
<th>Threshold value of vitamin A</th>
<th>Obese diabetic</th>
<th>Control</th>
<th>( \chi^2 )</th>
<th>OR</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.63 mg/l</td>
<td>25</td>
<td>1</td>
<td>39.1; p &lt; 10^{-3}</td>
<td>145</td>
<td>[15.8; 1325.3]</td>
</tr>
<tr>
<td>≥ 0.63 mg/l</td>
<td>5</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Distribution of the number of surveyed patients according to the vitamin E threshold

<table>
<thead>
<tr>
<th>Threshold value of vitamin A</th>
<th>Obese diabetic</th>
<th>Control</th>
<th>( \chi^2 )</th>
<th>OR</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10.54 mg/l</td>
<td>25</td>
<td>6</td>
<td>24.1; p &lt; 10^{-3}</td>
<td>20</td>
<td>[5.4; 74.3]</td>
</tr>
<tr>
<td>≥ 10.54 mg/l</td>
<td>5</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results of our work revealed a significant difference in the levels of liposoluble antioxidants, vitamins A and E, between the two study groups. Indeed, the vitamin A level was (0.50 ± 0.46 vs. 1.98 ± 0.97 mg / l), respectively, for obese diabetic men and controls with (p <10^{-3}) and the rate of vitamin E was (7.92 ± 2.94 vs. 12.21 ± 1.57 mg / l) respectively for obese diabetic men and controls with (p <10^{-3}). This comparison shows that the status of antioxidant vitamins in diabetic obese is altered. Several studies confirm this result and show low plasma concentrations of α-tocopherol and carotenoids in obese patients. These observations are largely attributed to an increase in oxidative stress in these subjects.

The Oxidative stress, which is the consequence of an imbalance between free radicals and antioxidant defenses, is one of the main factors responsible for the development of insulin resistance and type 2 diabetes. Indeed, a longitudinal study of adult males followed for 27 years revealed that serum concentrations and dietary intake of β-carotene and α-tocopherol independently predicted insulin resistance and the incidence of diabetes Type2. This finding is an argument in favor of the protective role of vitamins A and E in the development of non-insulin dependent diabetes, which has been confirmed by other studies such as Montonen et al.

In addition, contradictory results have been found in other studies and intervention trials. For example, in a double-blind, placebo-controlled randomized trial, supplementation with α-tocopherol or β-carotene has been shown not to inhibit the development of diabetes Type2 in male smokers and the levels of these two vitamins are not associated with the risk of this disease. This lack of effect could be given, inter alia, to a trial period that is too short or an unsuitable time for intervention.

Similarly, two different studies in type 2 diabetic subjects demonstrated that supplementation of the mixture of tocopherols for 6 weeks reduces the plasma but not the urinary concentration of F2-isoprostanes which is a marker of oxidative stress in vivo. The exact reason for this phenomenon is unknown. This may be due to impair renal function. Finally, a recent meta-analysis of the role of carotenoids in diabetes Type2 showed that lycopene decreases oxidative stress in diabetic patients but there is no evidence of an association between caroteone consumption and risk reduction of diabetes.

The results of our study showed that leptinemia in the diabetic obese group was significantly higher than in non-diabetic normopondorol subjects. Indeed, obesity is rarely the consequence of a deficiency in leptin but on the contrary, it is often associated with a hyperleptinemia logically proportional to the adipose mass. The high rate of leptin can be explained by the acquisition of a leptin resistance characteristic of obesity developed under a maladaptive diet called "Diet-Induced Obesity" (DIO). This leptin resistance is mainly due to a defect in the transport of leptin to the hypothalamus and / or to deficiencies in the signaling of leptin receptors.

In turn, the relationship between leptin and type 2 diabetes was the goal of many studies. A Chinese study published in 2013 of 1,234 people concluded that there is a significant correlation between serum leptin concentrations and insulin resistance. Indeed, leptin levels in insulin-resistant subjects were almost double that of subjects who had a normal sensitivity to insulin at the same level of adiposity. The rate of resistinemia in the diabetic obese patients was significantly higher than that observed in the control group (8.55 ± 2.86 ng / ml vs 3.72 ± 1.20 ng / ml; p <10^{-3}). This result is similar to that of Lu and his collaborators.

\( \chi^2 \) and OR = 20
indeed, resistin is a protein secreted by adipocytes capable of altering glucose tolerance and the action of insulin which will most likely lead to insulin resistance. this assumes that there is a possible link between obesity, diabetes and high plasma level of resistin [22,23]. it has also been shown that resistin may play a role in obesity and that there is a significant correlation between plasma levels of this adipokine, BMI, waist circumference and fat mass in a study of 66 obese diabetic subjects [24].

on the other hand, our results showed a negative and significant correlation between vitamin A and blood glucose (r = -0.36, p = 0.048) and vitamin A and insulinemia (r = -0.43; p = 0.02). this is in agreement with the results of several studies that have demonstrated an inverse relationship between plasma vitamin A concentrations and the incidence of diabetes [5,25]. ylönén and his team found in a study of 81 men and 101 women at increased risk of developing diabetes that high intakes of carotenoids (α and β-carotene, lycopene) were associated with low levels of fasting glucose and that the increase in vitamin A levels is correlated with a lower risk of developing insulin resistance and therefore diabetes type 2 [26]. these observations can be attributed to the antioxidant properties of carotenoids capable of counteracting oxidative stress and to improve insulin sensitivity, thus inducing a hypoglycemic effect [25-27].

in our results, we found a negative correlation tangent to the significance between vitamin A and BMI (r = -0.33, p = 0.078). chai and his team in their study of 220 women between the ages of 35 and 46 showed a significantly lower rate of carotenoids in obese compared to a control group and independent associations of dietary consumption between BMI and carotenoids [28].

this inverse relationship can be explained by the existence of a positive association between BMI and markers of systemic oxidative stress in obese people [29, 30]. we found a positive and non-significant correlation between vitamin A and lipid parameters (total cholesterol and triglycerides) (p> 0.05). this result is very close to that obtained by devaraj et al. [31] and ali salem and his team in a study of 30 rats showing that the level of β-carotene is associated with a slight increase in serum triglycerides [32].

this positive association between vitamin A and serum lipid concentrations is largely attributed to the transport of carotenoids by lipoproteins in the blood [30,32]. the results of our study showed that there was a negative and significant correlation between vitamin E and blood glucose (r = -0.54; p = 0.002) and vitamin E and insulinemia (r = -0.64; p <10-3). indeed, it has been shown that vitamin E is able to block the oxidation of lipids to attenuate the gene expression of the proteins involved in inflammatory processes due to oxidative stress and associated with obesity and insulin resistance. this suggests a beneficial role of vitamin E in the prevention of the risk of diabetes and the reduction of its complications by opposing oxidative stress [30, 34, 35, 36].

our results also showed a negative and significant correlation between vitamin E levels and BMI (r = -0.74, p <10-3). these results are in agreement with those of other studies [34,37]. indeed, reitman et al. had demonstrated that vitamin E levels were inversely related to BMI in a study of obese and non-obese subjects [38]. similarly, a study published in 2003 in 72 subjects in Thailand with a BMI greater than 25 kg / m2 found that serum α-tocopherol was lower in obese and overweight compared to control group [36]. a negative and significant correlation was found between vitamin A and leptin in our study population (r = -0.58, p = 0.001). the study of aeberli and his team in 2006 which concerned obese children indicated that increased consumption of β-carotene was associated with low levels of leptin [39]. regarding the relationship between vitamin E and leptin, a negative and significant association (r = -0.501; p = 0.005) was also obtained in our study group. this result is in line with that of ben slama and his team in 2009 in a population of diabetic obese. the authors hypothesized that vitamin E may have an indirect influence on leptin secretion since an inverse relationship has been observed between insulin and tocopherol and in turn insulin is a stimulant of secretion of leptin [7].

indeed, leptin could induce the production of reactive oxygen species while increasing the oxidation of fatty acids, which is in favor of oxidative stress and therefore a decrease in antioxidant power [40,41]. the results of our work showed a negative and significant correlation between the serum level of vitamin A and resistinemia (r = -0.42, p = 0.021). these findings can be explained by what has been demonstrated in the discovery of felipe and his team who have demonstrated that retinoic acid through these two 9-cis and all-trans forms is capable of inhibiting expression of resistin in white and brown adipose tissue of mice [42].

the correlation between plasma vitamin E and resistinemia was negative and significant (r = -0.534, p = 0.002). the inverse correlation between resistin and antioxidants has been taken up in other works. indeed, kougias et al. indicate that selenomethionine, an antioxidant responsible for the increase in glutathione peroxidase activity in endothelial cells, completely blocks resistin-induced vasomotor dysfunction in pork arteries [43]. bo and his team find that vitamin E supplementation is associated with a significant decrease in resistin levels in healthy individuals. these observations are in favor of a direct and specific effect of antioxidant substances on the expression of resistin [44].

conclusion

to conclude, the levels of liposoluble antioxidants (vitamin A and E), leptin and resistin have been shown to vary significantly between male diabetic obese and controls. indeed, cause or consequence of the increase of the oxidative stress, the antioxidant status of the diabetic obese subject is altered and the serum levels of vitamins A and E are lowered. in contrast, leptinemia and resistinemia are increased suggesting an involvement of these two adipokines in obesity, insulin resistance and the risk of diabetes. the correlation study revealed that serum concentrations of vitamins A and E were inversely and significantly associated with leptin and resistin levels. this could be an argument in favor of a protective role for liposoluble antioxidants, vitamins A and E, resulting in a specific effect on the secretion and expression of leptin and resistin.

conflict of interest

- the authors confirm that there is no conflict of interest in this work.
- the authors reassure that this article is not submitted to any other scientific journal for a possible publication.

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