Low Plasma Antioxidants and Normal Plasma B Vitamins and Homocysteine in Patients with Severe Obesity

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Abstract

Background: Obesity is among the well-established risk factors for cardiovascular morbidity and mortality. However, the exact mechanisms are not well understood. Low concentrations of vitamins (fat soluble antioxidants and B vitamins) are linked to accelerated atherosclerosis through increased oxidative stress and homocysteine.

Objective: To compare plasma antioxidant vitamins (carotenoids and vitamin E), B vitamins (folic acid and B12) and homocysteine – all linked to increased cardiovascular morbidity – between patients with severe obesity and lean control subjects.

Methods: We investigated plasma carotenoids, vitamin E, folic acid, B12, and homocysteine in 25 obese patients and their age-matched controls (body mass index 38 ± 3 vs. 21 ± 2 kg/m^2), respectively), related to BMI and plasma insulin.

Results: Patients with obesity had normal B vitamins and a non-significant decrease in plasma homocysteine as compared to controls (9.4 ± 2.6 vs. 11.4 ± 4.8 μmol/L, $P = 0.07$). There was a significant decrease in both plasma carotenoids and vitamin E (0.69 ± 0.32 vs. 1.25 ± 0.72 and 24 ± 10 vs. 33 ± 14 μg/ml, respectively; $P < 0.01$). Both vitamins were inversely related to BMI and plasma insulin, which was significantly increased in patients with obesity (22 ± 21 vs. 6 ± 2 μU/ml, $P < 0.01$).

Conclusions: Obese patients with BMI above 35 kg/m^2 show low plasma antioxidants (carotenoids and vitamin E). This may result in increased oxidative stress and consequently enhanced atherosclerosis in these patients.

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Recent epidemiologic studies have confirmed the well-known positive relationship between body mass index above 27 kg/m^2 (BMI in kg/m^2 body surface) and cardiovascular morbidity. Obesity behaves as an independent and strong risk factor especially above BMI 35 kg/m^2 [1]. Traditional risk factors explaining excess cardiovascular mortality in obesity include: hypertension, dyslipidemia with high triglycerides and low high density lipoprotein-cholesterol related to central adiposity, insulin resistance and hyperinsulinemia, which affect blood vessels and contribute to hypertension and coronary artery disease [2]. There is ample evidence linking increased concentration of plasma homocysteine to premature coronary and carotid atherosclerosis. Homocysteine is controlled both by mutations in its regulating enzymes and by the B vitamins, folic acid, B12 and B6 (pyridoxine) [3,4]. The relationship between homocysteine and obesity has not been investigated before. Some evidence suggests an increase in plasma homocysteine concomitant to weight loss, apparently due to a decrease in the regulatory B vitamins [5,6]. Low plasma antioxidants have been linked to oxidative modification of LDL, a process necessary for the generation of atherosclerotic plaque [7]. There is an inverse relationship between plasma antioxidant vitamin carotenoids and vitamin E and cardiovascular diseases [8,9]. Indeed, some studies have shown enhanced oxidative stress and low concentrations of plasma antioxidants in patients with obesity [10,11].

We therefore measured plasma carotenoids, vitamins E and B12, folic acid, and plasma homocysteine in patients with severe obesity, compared the results with a normal weight control group and then related them to plasma insulin and BMI.

Patients and Methods

We investigated 25 obese patients, 4 men and 21 women aged 26–52 (mean 44 ± 8 years), with BMI 35–45 (mean 38 ± 3 kg/m^2). None of the patients had cardiac or renal disease, diabetes or hypothyroidism. No patient was taking any vitamin or food supplements, nor was any patient on a weight reducing diet at the time of the study. The obese patients were compared with a control group of 25 (4 men and 21 women) age-matched normal weight subjects with BMI 19.5–24.5 (mean 21 ± 2 kg/m^2). All subjects signed an informed consent approved by the Helsinki Committee of HaEmek Medical Center.

Blood was taken after a 14 hour fast. Plasma was separated and kept at –20°C until analyzed. The following tests were done: plasma lipids (cholesterol, triglycerides and high density lipoprotein-cholesterol), glucose, albumin, renal and liver function, by a Hitachi 747 (Japan) autoanalyzer. Plasma vitamin B12, thyroid-stimulating hormone and insulin were analyzed in the AXSYM system by microparticle enzyme immunoassay. Folic acid was analyzed in the red blood cells by the AXSYM system. Plasma homocysteine was analyzed in an Abbott IMX system (USA) according to fluorescence polarization immunoassay technology [12]. The biochemical tests were determined in the clinical biochemistry laboratories of HaEmek Medical Center, which are operated under strict quality control standards. The antioxidants, vitamins A, E and carotenoids were determined by high performance liquid chromatography analysis in the National Oceanographic Research Institute, as previously described [13].

BMI = body mass index

LDL = low density lipoprotein

Statistical analysis

All measurements are presented as mean ± SD. Student's *t*-test was used for non-paired group analysis. Pearson's correlation coefficients were used as well as bivariant analysis. *P* less than 0.05 represents statistical significance. The SPSS/PS statistical package was used by a biostatistician from the Faculty of Medicine.

Results

Table 1, illustrating the metabolic characteristics of the two groups, shows that the obese patients had significantly higher plasma glucose, hemoglobulin A_1C, insulin and triglyceride concentrations with lower HDL-cholesterol concentrations. Also, there was a significant increase in plasma TSH, although within normal values. Regarding routine blood tests, plasma urea, calcium, phosphate, protein, albumin, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase hormone, Na, K, Cl, Hb and platelets were not different and were within normal range in all subjects. There was a significant decrease in plasma creatinine (0.84 ± 0.13 vs. 0.91 ± 0.11 mg/dl, $P < 0.05$), a significant increase in plasma uric acid (6.4 ± 1.0 vs. 4.3 ± 0.1 mg/dl, $P < 0.01$) and alanine aminotransferase (24 ± 13 vs. 16 ± 8 U/L, $P < 0.05$), and an increase in white blood cells (7.888 ± 2.012 vs. 6.304 ± 1.334, $P < 0.01$) in the obese patients compared to the controls, respectively.

Table 2 shows B vitamin and plasma homocysteine levels. There were no differences between the groups, with normal range of B vitamins. There was a decrease in plasma homocysteine, which was 21% lower in the obese patients though it did not reach significance ($P = 0.07$).

Table 3 shows the levels of plasma antioxidant vitamins in patients with obesity compared to controls. There was a significant drop in plasma carotenoids and vitamin E in the obese patients whereas vitamin A was not changed. As both carotenoids and vitamin E are fat soluble and transported by plasma lipids (mainly LDL), repeated measurements were done by the correction of carotenoids and vitamin E against plasma lipids (cholesterol plus triglycerides). These equations showed the same significance ($P < 0.01$) as the uncorrected values [Table 4].

Correlation tests revealed a significant correlation between plasma vitamin E and carotenoids ($r = 0.72$, $P < 0.01$). Also, there was a significant correlation between BMI and plasma insulin ($r = 0.44$, $P < 0.01$). There was a significant inverse correlation between BMI and plasma carotenoids ($r = –0.43$, $P < 0.01$) and vitamin E ($r = –0.32$, $P < 0.05$), and between plasma insulin and carotenoid concentrations ($r = –0.31$, $P < 0.05$).

Discussion

This study demonstrated that levels of the fat-soluble antioxidants, vitamin E and carotenoids were significantly lower in patients with severe obesity as compared to normal weight subjects, whereas no differences were observed in the concentrations of the B vitamins, folic acid and B12. We excluded patients with metabolic disorders (hypothyroidism, diabetes mellitus and nephropathy), which could have influenced the results. Also, patients were eating their ordinary diet without any food supplements. Consequently, our results are suggestive of true differences in antioxidant vitamins, which are not simply explained by confounding factors related to obesity.

There is a growing amount of clinical evidence indicating that mild to moderate fasting hyperhomocysteinemia is an independent risk factor for atherosclerosis [14]. Our review of the literature relating plasma homocysteine, a non-traditional coronary risk factor, to obesity revealed some interesting findings. Hyperhomocysteinemia occurred 1 year after gastroplasty for severe obesity [5]. Also, vitamin B12 deficiency was observed after gastric surgery for

Table 1. Metabolic tests in patients with obesity

	Obesity (n=25)	Control (n=25)	*P*
BMI (kg/m^2)	38 ± 3 **	21 ± 2	<0.0001
Glucose (mg/dl)	97 ± 11 **	85 ± 7	<0.0001
HbA_1C (%)	5.6 ± 0.4 *	5.4 ± 0.3	0.01
Insulin (μU/ml)	22 ± 21 **	6 ± 2	<0.0001
TSH (μU/ml)	2.1 ± 1.1 *	1.5 ± 0.7	0.035
Cholesterol (mg/dl)	209 ± 35	203 ± 28	0.64
Triglycerides (mg/dl)	158 ± 72 **	90 ± 71	0.001
LDL-C (mg/dl)	124 ± 34	120 ± 23	0.50
HDL-C (mg/dl)	51 ± 12 **	63 ± 13	0.001

All results are mean ± SD
* $P < 0.05$, ** $P < 0.01$

Table 2. Plasma homocysteine and B vitamins in patients with obesity

	Obesity (n=25)	Control (n=25)	*P*
Plasma B12 (μg/ml)	294 ± 101	331 ± 131	0.27
RBC-FA (μg/ml)	357 ± 151	309 ± 110	0.21
Plasma homocysteine (μmol/L)	9.4 ± 2.6	11.4 ± 4.8	0.07

All results are mean ± SD.
RBC-FA = folic acid in red blood cells

Table 3. Plasma antioxidant vitamins in patients with obesity

	Obesity (n=25)	Control (n=25)	*P*
Vitamin A (μg/ml)	0.30 ± 0.11	0.34 ± 0.20	0.30
Carotenoids (μg/ml)	0.69 ± 0.32 **	1.25 ± 0.72	0.001
Vitamin E (μg/ml)	24 ± 10 **	33 ± 14	0.001

** $P < 0.01$
All results are mean ± SD.

Table 4. Corrected plasma antioxidant vitamins in patients with obesity

	Obesity (n=25)	Control (n=25)	*P*
Vitamin A	0.08 ± 0.03	0.11 ± 0.07	0.20
Carotenoids	0.19 ± 0.08	0.43 ± 0.25	<0.001
Vitamin E	6.5 ± 2.7	11.3 ± 4.8	<0.001

All results are mean ± SD (μg/mg)
Corrected values represent plasma antioxidants divided into cholesterol + triglycerides

HDL = high density lipoprotein
TSH = thyroid-stimulating hormone

obesity [6]. An adequate oral vitamin supplementation protected against hyperhomocysteinemia during weight reduction [15]. Thus, low B vitamins may follow weight-reducing regimes. Consequently, an increase in plasma homocysteine is anticipated and can be prevented by vitamin supplementation

Epidemiologic studies have demonstrated an association between increased intake of antioxidant vitamins such as vitamin E, carotenoids and vitamin C and reduced morbidity and mortality from coronary artery disease. This association has been explained on the basis of the LDL-oxidative modification process, which involves the initiation of atherogenesis and its protection by oxidation of the LDL lipids [16]. Extensive studies have shown an inverse association between CAD events and vitamin E and carotenoid intake [8,9]. Based on these data, high intake of fruits and vegetables has been included in guidelines against CAD [17].

While recent studies fail to show such an association between carotenoids and CAD [7], we and others demonstrated the ability of β-carotene to protect LDL against oxidation both in healthy subjects and in diabetic patients, with an emphasis on β-carotene derived from natural sources [13,18,19]. Thus, our observation showing a significant reduction in plasma antioxidants in obese patients suggests a so far unrecognized CAD risk factor in these patients. Only a limited number of studies have dealt with antioxidants and obesity. Lower serum levels of vitamin E were demonstrated in subjects with abdominal obesity [11]. Vitamin E and β-carotene were significantly lower in plasma of obese boys [20] and LDL of obese girls [21]. In a recent study comparing vitamin E and β-carotene in 6,139 children aged 6–10 years old, both vitamins were significantly lower in obese children, while no differences were observed in the intake of these antioxidants from the diet [22]. Furthermore, few studies have shown enhanced oxidation, as reflected by increased oxidation of end-products in the plasma of obese patients [10,23].

The mechanisms responsible for altered antioxidant levels in obesity are obscure. A recent study showed a decreased bioavailability of fat-soluble vitamin D in obesity, apparently due to its deposition in body fat compartments [24]. We can only speculate that the mechanism is the same, with redistribution of fat-soluble vitamins into fatty tissues, thus leaving fewer antioxidants available for plasma and other essential sites. Such a mechanism may be supported by studies showing different dose-level responses of lean and obese subjects to standardized antioxidant supplementation.

Another mechanism that potentially relates obesity to low antioxidants and enhanced oxidation involves hyperinsulinemia. Physiologically, severe obesity is characterized by hyperinsulinemia, as demonstrated in our obese subjects whose insulin level was very high and just below the upper limit for insulin blood level (still not defined as diabetes). A recent study in healthy subjects investigated the relationship between insulin resistance and plasma concentrations (lipid hydroperoxides), carotenoids and tocopherols (vitamin E). There was a significant direct relationship between plasma insulin and plasma oxidation (lipid hydroperoxide concentrations). A significant inverse relationship was found between plasma insulin and carotenoids and tocopherols [25]. Another study showed an inverse relationship between plasma vitamin E and insulin in obese children. Thus, such mechanisms linking hyperinsulinemia to low plasma antioxidants may operate in patients with severe obesity.

In conclusion, we have shown a significant drop in plasma antioxidant vitamins, carotenoids and vitamin E in patients with severe obesity and hyperinsulinemia as compared to lean subjects. Plasma B vitamins, folic acid and B12 were not different, with a non-significant drop in plasma homocysteine. Addressing the etiology of the evident increased cardiovascular morbidity in patients with severe obesity, we suggest that low antioxidants can be added to the well-known list of traditional risk factors for CAD in obese patients. Further studies are required to define the mechanisms underlying this association. In the meantime, the intake of fruits and vegetables, which serve as the major source of dietary antioxidant vitamins, should be strongly advised in patients with obesity.

CAD = coronary artery disease

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