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THE EFFECT OF A MEDITERRANEAN DIET MODEL ON SERUM *BETA*-CAROTENE CONCENTRATION. A PRELIMINARY ASSESSMENT

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ABSTRACT

Background. Some of the main nutritional reasons for recommending a Mediterranean diet is to prevent metabolic diseases arising through free radical formation. A key constituent compound is β-carotene which, amongst the carotenoids, displays the greatest provitamin A activity as well as possessing significant antioxidant properties.

Objectives. Principally, to determine the relationship between serum β-carotene levels and the effect of Mediterranean diet guidelines in a selected group of women.

Materials and Methods. The subject group consisted of 26 women aged 19-22 years. A nutritional assessment was performed using 3 day repeats of 24-hour recall interviews. A 9-point aMED (alternate Mediterranean Diet) score was used to study dietary habits. Serum β-carotene was measured by liquid chromatography with photodiode array detection (HPLC-PDA).

Results. β-carotene dietary intake was highly variable, ranging from 734 to 14476 μg/day (median 3022 μg/day). Serum β-carotene concentration ranged between 0.071-1.905 μmol/L (median 0.519 μmol/L) and was significantly associated with the Mediterranean Diet model (*Spearman* r=0.633, p<0.001). Out of the dietary sources of β-carotene, consuming carrots had the most significant impact on its serum concentration. Other dietary factors positively affecting serum β-carotene were: consumption of nuts and seeds, pulses, a favourable ratio of mono-unsaturated fatty acids to saturated fatty acids and eating fruit and wholegrain cereal products.

Conclusions. Adopting a Mediterranean-based diet had a positive effect on increasing serum beta-carotene levels.

Key words: *Mediterranean model of consumption, beta-carotene, serum*

STRESZCZENIE

Wprowadzenie. Sposób żywienia oparty o zalecenia diety śródziemnomorskiej rekomendowany jest w celu prewencji chorób metabolicznych, w patomechanizmie których uczestniczą wolne rodniki. β-karoten jest związkiem charakteryzującym się największą spośród karotenoidów aktywnością prowitaminy A, a także wykazuje właściwości antyoksydacyjne.

Cel badań. Zasadniczym celem pracy było zbadanie zależności pomiędzy stężeniem β-karotenu w surowicy krwi a stosowaniem zaleceń diety śródziemnomorskiej w wybranej grupie kobiet.

Materiał i metody. W badaniach uczestniczyło 26 kobiet w wieku 19-22 lata. Dane dotyczące spożycia produktów spożywczych uzyskano przy pomocy wywiadu 24-godzinnego, który przeprowadzono 3-krotnie. Nawyki żywieniowe badanych kobiet oceniane były przy pomocy 9-punktowej skali a-MED (*alternate Mediterranean Diet Score*). Stężenie β-karotenu w surowicy krwi oznaczono metodą chromatografii cieczowej z detekcją fotodiodową (HPLC-PDA).

Wyniki. Pobranie -karotenu z dietą przez badane kobiety charakteryzowało się dużą zmiennością i zawarte było w przedziale wartości od 734 do 14476 μg/dobę (mediana 3022 μg/dobę). Stężenie beta-karotenu w surowicy krwi badanych mieściło się w zakresie 0,071-1,905 μmol/l (mediana 0,519 μmol/l). Stężenie β-karotenu w surowicy krwi było w znaczący sposób powiązane z przyjętym modelem diety śródziemnomorskiej (R w teście *Spearmana*=0,633, p<0,001). Spośród żywieniowych źródeł β-karotenu wpływ na stężenie tej prowitaminy w surowicy krwi wywierało spożycie marchwi. Do innych czynników żywieniowych mających dodatni wpływ na stężenie β-karotenu w surowicy krwi należało spożycie orzechów i nasion oraz roślin strączkowych, a także korzystny stosunek kwasów tłuszczowych jednonienasyconych do nasyconych, oraz spożycie owoców i produktów zbożowych pełnoziarnistych.

Wnioski. Sposób odżywiania zbliżony do śródziemnomorskiego modelu spożycia dodatnio wpływał na stężenie β-karotenu w surowicy krwi badanych kobiet.

Słowa kluczowe: *śródziemnomorski model spożycia, β-karoten, surowica*

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INTRODUCTION

Amongst the carotenoids, β-carotene is a compound demonstrating physiological provitamin A activity [15]. It is an immuno-modulator and shows detoxifying properties through inducing the activity of certain liver enzymes [11]. It has a dual role in participating for both oxidative and reduction reactions. Depending on how β-carotene is taken and its dose, it can either act as an antioxidant or pro-oxidant [11]. As an antioxidant, it removes singlet oxygen, scavenges peroxyl and hydroxyl radicals as well as inactivating anionic superoxide compounds. Rat studies have shown its pro-oxidative action, particularly in conjunction with limiting the tocopherol supply; a substance that protects the β-carotene molecule from auto-oxidation [8].

A Mediterranean diet is recommended in preventing metabolic diseases which may arise through free radical formation. The diet itself is very varied and contains high amounts of vegetables, fruit, and wholegrain cereals but limited levels of saturated fats and meat at the expense of vegetable oil and fish. Such foodstuffs contain many natural antioxidants, including the carotenoids [4]. Dietary uptake of β-carotene depends on many factors, both endo- and exogenic ones. For this reason one of the main study aims was to determine the relation between serum β-carotene concentration and the guidelines of Mediterranean diet in a group of women with an intent of subsequently expanding the study.

MATERIAL AND METHODS

The study subjects were 26 women, aged 19-22, students of dietetics at the Medical University of Bialystok. They had to be of good health, neither overweight nor obese (BMI $\leq$ 25kg/m^2) and not be taking any dietary supplements containing β-carotene. Data about consumed foodstuffs were obtained from 3 day repeats of 24-hour recall interviews in the spring season. The quantities eaten were assessed either by actual weighing or with the help of an album with photographed foodstuffs and products [12]. The data so gathered were then analysed by the 'Dieta 2.0' computer programme. Dietary habits, according to the Mediterranean Model, were assessed by a 9 point aMED (alternate Mediterranean Diet) score [5,14]. For the final points values, foodstuffs consumed were divided into 8 groups: vegetables - without potatoes, pulses, fruit, nuts and seeds, wholegrain cereal products, red meat and products, fish and alcohol. This included data on the ratio of monounsaturated to saturated oils. The amounts eaten was evaluated by the number of portions of a given food product. Results above the median were given one point, whilst those for red meat and products were awarded 1 point if they fell below the median. In addition 1 point was also allocated for consuming 5-25 g alcohol per day. Fasting blood samples were taken the next morning after the study had been performed. Blood was collected from the antecubital vein into Vacutainer tubes (Becton-Dickinson, France) with a clot activator. After half an hour, the blood was centrifuged at 1800 x g for 10 minutes. Serum thus obtained, was stored at -18º C until analysis. β-carotene was measured in serum extracts by HPLC according to the modified procedure of *Thurnham* et al [13]. The extracts were prepared by taking 100 µl of serum and first mixing for 1 min. with 200 µl of 0.1 µmol/L SDS (sodium dodecyl sulphate). Next, 200 µl of 42 µmol/L of tocopherol octane in ethanol was added as an internal standard, followed by 1 ml n-heptane containing 0.5 g/L butylhydroxy--toluene (BHT). The mixture was intensively mixed for 2.5 min. and then centrifuged at 1500 x g for 10 min. The heptane fraction was transferred into glass tubes and evaporated in a water bath at 40ºC. Residues were then resuspended in 100 µl of mobile phase consisting of acetonitrile, methanol and dichloromethane in a 4:4:1 proportion after which this sample was passed through a Chromofil syringe filter (Macherey-Nagel, Germany) of pore size 0.45 µm. Levels of β-carotene were then determined using liquid chromatography on a Shimadzu Prominence HPLC system (Japan). A 20µl volume of sample was injected through a Rheodyne valve, (7725 model), and isocratic separation was performed on a C18 Luna 4.6 x 250 mm column, of pore size 5µm (Phenomenex, USA), at a flow rate of 1ml/min using a LC-20AD Shimadzu pump. Spectrophotometric detection was by a Shimadzu photodiode array SPD-M20A detector at 450nm. All samples were measured in triplicate. β-carotene was purchased from Sigma, USA which was used to produce a standard curve. Permission for the studies was granted by the Bioethics Commission of the Medical University of Bialystok. Statistical analysis was performed by the Statistica 10.0 package (StatSoft Inc). The distribution of 26 variables was confirmed by the *Kolmogorov-Smirnov* test. A *Pearson's* correlation was used on those variables normally distributed. The *Student's* t-test assessed the significance of differences between dietary energy values, contents of protein, carbohydrate and fats and aMED values. A *Spearman's* rank correlation was used for assessing the relationship between the latter and other defined variables. The influence of diet on serum β-carotene was evaluated by stepwise forward multiple regression taking $p<0.05$ as the critical value.

RESULTS AND DISCUSSION

There were no significant differences between either dietary caloric values or the basic nutritional components relative to the aMED categories (Table 1). The amounts consumed of these basic nutritional components were independent of the dietary model. β-carotene dietary intakes were however very variable, ranging 734 - 14476 µg/day (median 3033 µg/day). These were similar to a USA study also performed on women aged 19-24 years, whose diet contained 3149 µg/day β-carotene [9]. The present study showed a serum β-carotene range of 0.071-1.905 µmol/L (median 0.519 µmol/L), which was however similar to other European population groups [10].

Table 1. The energy value and contents of macronutrients in diets of the women studied in relation to the aggregated aMED indices*

	Range of aMED index (Number of participants)		
	0-1 (N=8)	2-3 (N=8)	4-6 (N=10)
Energy (kcal)	1827±425	1740±535	1803±386
Protein (g)	66.7±18.6	63.3±20.2	67.6±11.7
Fat (g)	65.0±19.1	63.1±25.0	54.6±23.6
Carbohydrates (g)	260±69	246±76	274±55

* The mean values did not differ significantly in *Student*'s t-test.

Serum β-carotene was correlated with the amount consumed in the diet, r=0.493 p<0.01 (Figure 1). This moderate r value reflects that although dietary beta-carotene influences, to a large degree, serum levels of this provitamin it is not the only factor responsible for this effect.

The aMED index, by whose means the association of the women's diet with the Mediterranean diet model was assessed, had values ranging 0-6 compared to the accepted score of 0-9 [5, 14] (Figure 2). Thus the dietary habits of the women did not fully comply with the aforementioned model. Furthermore, in 4 cases the women's diet completely differed from the Mediterranean diet model (aMED=0). The serum β-carotene levels were significantly associated with the Mediterranean diet model (*Spearman* r=0.633, p<0.001) (Figure 3). This was consistent with other findings [1]. There was however no correlation between β-carotene consumption with the test dietary model (*Spearman* r=0.272, p=0.154), which may be due to other factors affecting

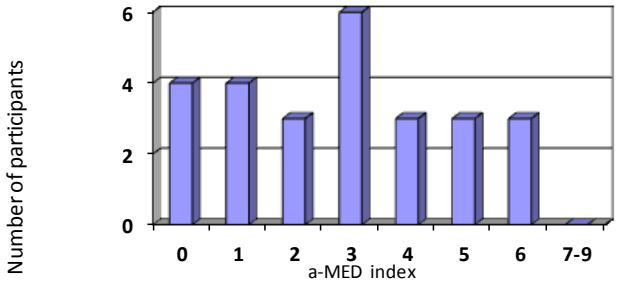

Figure 2. Distribution of aMED index among the women studied

serum β-carotene, amongst which dietary intake is included. In foodstuffs, β-carotene is found in various forms such as being complexed with protein or in crystalline forms localised within plant cells. Thus during digestion, absorption and assimilation of β-carotene is dependent on its release from the plant matrix as well as any heat treatment, which may lead to the break-up of β-carotene complexes. In addition, the absorption of β-carotene from vegetables is limited to only 5-30% compared to the absorption of purified β-carotene from dietary supplements [3]. Other significant dietary factors affecting β-carotene absorption are fat content

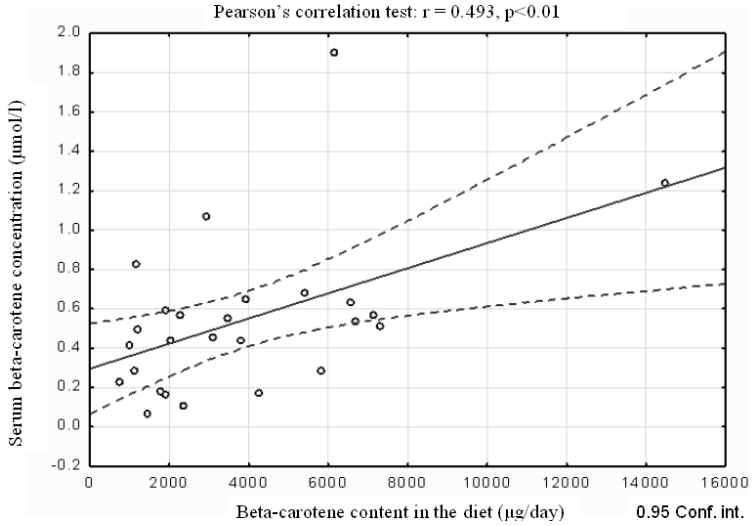

Figure 1. Correlation between the serum *beta*-carotene concentration and its contents in the diets of the women studied

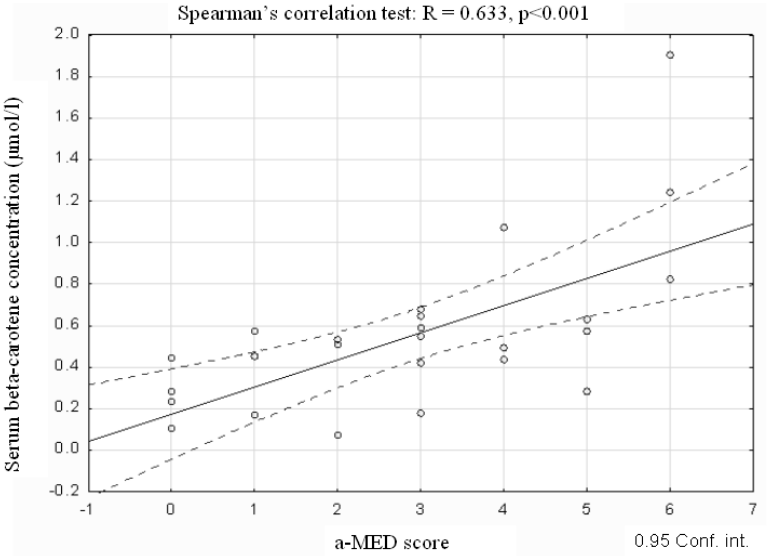

Figure 3. Diagram of correlation between the serum *beta*-carotene concentration and values of the aMED index

[15], the presence of fibre together with plant sterols and phospholipids. Normal digestive functions are also crucial [15].

Foodstuffs containing the most β-carotene are green vegetables, carrots, red pepper and certain fruit [7]. Recent studies showed that the main sources of β-carotene in the adult diets in Poland were in 60% fresh vegetables, of which 40% were carrots [6]. The current study showed that the sources of β-carotene found in the women's diets were carrots, red pepper, lettuce, tomatoes, sorrel, broccoli and grapefruit. The impact of dietary β-carotene on its serum concentration depended on only one factor, ie. consumption of carrots (Table 1), however this accounted for just 10% of the variability (R^2=0.099 for the model). Nevertheless, the consumption of carrots remains an important factor affecting serum β-carotene levels.

Vegetables, fruit, wholegrain cereal products, pulses and other seeds and nuts are the dominant foodstuffs in a Mediterranean diet. Eating fish and poultry but limiting red meat consumption is also recommended [14]. Another factor is a moderate alcohol consumption, mostly wine. Even though vegetables are the key component of the Mediterranean diet, this variable was automatically excluded from the regression analysis of the model tested, because all women in fact consumed regular 1-3 daily portions of vegetables (raw or cooked). An association between serum β-carotene was found with dietary pulses, nuts and seeds (Table 2). The latter two contain 40-70% fatty acids, mainly mono- and polyunsaturated, whereas they do not contain trans fatty acids [2]. This high unsaturated fatty acid content and their biological quality may facilitate β-carotene absorption from the digestive tract. Nuts and seeds are a source of tocopherol, which protects the β-carotene molecule. To a lesser degree, serum β-carotene levels are influenced by an advantageous ratio of monounsaturated to saturated fatty acids and a large consumption of fruit and wholegrain cereal products. In summary, it was found that 5 variables of the Mediterranean diet model accounted for almost half the association between nutrition (R^2=0.447) and serum β-carotene concentrations; these being dietary consumption of nuts and seeds, pulses,

Table 2. The influence of the dietary β-carotene sources on its serum concentration assessed in a forward stepwise selection of the multiple linear regression test [1)]

	Beta	Std. error Beta	p	R^2 for model
Intercept			0.001	
Carrot	0.547	0.221	0.046	0.099

[1)] Variables listed: red pepper, lettuce, tomatos, sorrell, broccoli, and grapefruits, were excluded from the model of computation.

Table 3. The influence of dietary factors contained in the model of Mediterranean diet on the serum - β-carotene concentration assessed in a forward stepwise selection of the multiple linear regression test [1)]

	Beta*	Std. error Beta	p	R^2 for model
Intercept			0.130	
Nuts and seeds	0.385	0.151	0.019	
Pulses	0.355	0.157	0.034	
Monounsaturated fatty acids: saturated fatty acids ratio	0.300	0.151	0.060	0.447
Fruits	0.264	0.154	0.102	
Wholemeal cereals	0.221	0.158	0.176	

[1)] Variables listed: vegetables, red meats and the red meat products, fish, and alcohol, were excluded from the model of computation
* Sequence according to the main effects of variables

fruit, wholegrain cereal products as well as the ratio of monounsaturated to saturated fatty acids.

CONCLUSIONS

1. Dietary habits which were close to the Mediterranean dietary model positively affected serum β-carotene levels in the studied group of women.
2. Concentration of serum β-carotene depended not only on its dietary sources but it was also a multifactorial effect, in which the general content of the diet was substantial.
3. The presented study is preliminary in determining the nutritional factors that affect serum β-carotene levels. This requires continuation in a more extensive population group with participation of both genders and subjects of different ages.

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