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Associations between obesity and serum lipid soluble micronutrients among premenopausal women

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Abstract

Elucidating potential pathways that micronutrients may reduce/promote chronic disease may contribute to our understanding of the underlying etiology of disease and their utility as markers of risk. In the current study, we examined associations of serum lipid soluble micronutrients with body mass index (BMI). We hypothesized that obesity may differentially influence serum micronutrient levels, thereby affecting risk for chronic disease incidence and mortality. Baseline serum samples from 180 premenopausal women from a nutritional trial were analyzed for leptin, C-reactive protein (CRP), 25-hydroxyvitamin D (25(OH)D), carotenoids, and tocopherols. Participants were stratified into normal weight (18.5-24.9), overweight (25-29.9), and obese (≥ 30) subgroups by BMI (kg/m^{12}). Differences in serum biomarkers among BMI subgroups were adjusted for Asian ethnicity and smoking status. As expected, obese individuals had significantly higher serum levels of leptin and CRP (P's < 0.05) compared to normal weight women. γ-Tocopherol levels were significantly higher in obese individuals (P < 0.05), while α-tocopherol levels did not differ among BMI subgroups. Serum levels of 25(OH)D and carotenoids (except lycopene) were significantly lower in obese than in normal weight women (P's < 0.05). The associations between BMI and carotenoids were independent of dietary intake. The obesity-associated reduction for total pro-vitamin A carotenoids (45%) was approximately 3-fold greater than that observed for non pro-vitamin A carotenoids (16%). Our results indicate potential influences of obesity on serum levels of lipid soluble micronutrients and suggest that metabolism of pro-vitamin A carotenoids may contribute to the differences observed.

Keywords

Obesity; BMI; lipid soluble micronutrients; CRP; premenopausal women

1. Introduction

Obesity is associated with increased risk of heart disease [1], diabetes [2], and cancer [3]. The mechanism(s) by which obesity may alter cancer risk is not clearly understood, however, research has suggested that fat accumulation could contribute to chronic systemic inflammation [4] which may be a critical component of tumor progression [5]. Modifiers such as micronutrients may reduce the damaging effects of inflammation and, therefore reduce the risk

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associated with obesity. Suggestive associations between BMI (body mass index) and lipid soluble micronutrients, such as vitamin D, carotenoids, and tocopherols, have been reported previously [4,6-8]. These micronutrients play critical roles in metabolism and the maintenance of tissue functions and have been linked with cancer incidence in epidemiologic studies [9, 10]. Understanding the potential determinants of serum levels of lipid soluble micronutrients is essential to elucidating their function with respect to reducing/promoting chronic diseases as well as their utility as markers of risk.

There has been considerable research effort into the potential protective effects of carotenoids and vitamin D against chronic diseases [11,12]; however, little is known about the regulation of these micronutrients *in vivo*. While serum levels of carotenoids partially reflect dietary intake [13], other physiologic factors may also determine the absorption, transportation, storage, and utilization of carotenoids. 25-hydroxyvitamin D (25(OH)D) levels in plasma have been used to assess vitamin D status, yet determinants of its status independent of sun exposure and dietary intake are poorly understood. Lower levels of 25(OH)D have been observed among obese adults and children [14,15]; however, no clear explanation for this association has been published.

The primary objective of the current study was to assess the associations of serum lipid soluble micronutrients (particularly vitamin D, carotenoids, α- and γ-tocopherols) with BMI to indentify unique attributes of serum micronutrients responsible for observed associations. We hypothesized that obesity may differentially influence serum micronutrient levels, thereby affecting risk for disease incidence and mortality.

2. Methods and Materials

2.1. Subjects and study design

The study protocol was approved by the institutional Review Board of the University of Hawaii. Written informed consent and permission for using stored samples for future analysis were obtained from all participants. As described previously [16], 220 females aged 35 to 46 years were randomly assigned to either the soy intervention or the control group. A validated food frequency questionnaire [17] completed by participants at baseline was used for dietary assessment. The baseline data and serum samples were used in the current cross sectional study as the effect of soy intervention was not of interest; BMI and biomarkers were available for 180 participants (we excluded 3 underweight participants from the study population).

2.2 Sample analysis

Serum samples were extracted and subsequently analyzed for carotenoids (α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lycopene, lutein, lutein/zeaxanthin, and anhydrolutein) and tocopherols (α-tocopherol and γ-tocopherol) by reverse phase high pressure liquid chromatography (HPLC) with photodiode array detection between 220 – 600 nm as previously described [18,19]. In brief, an aliquot of 0.30 ml of serum was mixed with 0.30 ml ethanol containing butylate hydroxytoluene as antioxidant and 3 internal standards (tocol, retinyl laurate, and n-butyl-*β*-apo-8′-carotenoate), followed by partitioning into 0.8 ml hexane. The hexane layer was evaporated in amber vials at room temperature under a stream of nitrogen. The dry extracts were re-dissolved in 0.15 ml HPLC mobile phase. The separation for carotenoids and tocopherols was performed on a Spherex ODS analytical and guard column with a mobile phase consisting of MeOH, $CHCl_2$, MeCN, Bis-trispropane, and butylated hydroxytoluene. Accuracy was regularly validated through the analysis of external standards within each sample batch and through participation in the quality assurance program organized by U.S. National Institute of Standards and Technology (Gaithersburg, MD) [20].

Serum 25(OH)D was measured according to the manufacturer's directions using a double-antibody enzyme-linked immunosorbent essay (ELISA) kit (Immunodiagnostic Systems, Ltd., Fountain Hills, AZ). ELISA kits purchased from R&D System (Minneapolis, MN) were used to analyze leptin levels. The C-reactive protein (CRP) assay was based on a latex particle enhanced immunoturbidimetric method using a Cobas MiraPlus clinical autoanalyser and a kit from Point Scientific, Inc (Lincoln Park, MI).

2.3 Statistical analyses

Participants were stratified into the following subgroups by BMI (kg/m^2): normal weight (18.5 – 24.9); overweight (25-29.9); and obese (≥ 30). Differences in serum levels of micronutrients, CRP, leptin, and dietary energy and carotenoid intakes between BMI subgroups were tested using analysis of variance (ANOVA). In order to have statistical power = 80%, a total of 159 subjects were needed for ANOVA with a three-group variable, assuming a medium effect size (f=0.25) and alpha=0.05 (two-tailed). The model was adjusted for Asian ethnicity and smoking status (current smoker versus current non-smoker). BMI subgroup differences in serum carotenoids were additionally adjusted for nutrient density (dietary intake of respective carotenoids / dietary total energy intake x 1000; [21]) to determine whether the differences could be partially explained by dietary intake.

The associations between serum biomarkers and BMI were also assessed by Pearson correlation (r). Student's t-test was used to assess ethnicity differences (Asians versus Caucasians) and a BMI-adjusted model was also examined. SAS software (SAS Institute, Cary, NC) was used for all statistical analyses and all P values < 0.05 were considered statistically significant.

3. Results

Participants mean age and BMI were 43.0 ± 2.9 years and 26.3 ± 5.7 kg/m^2, respectively. 40.6 % were Asians, 36.7 % were Caucasians, and only 6.1% were current smokers. BMI, leptin, CRP, and 25(OH)D were significantly lower in Asians than in Caucasians. Asians also had significantly higher serum levels of α- and β-cryptoxanthin and total pro- and non provitamin A carotenoids compared to Caucasians (Table 1). After adjusting for BMI, significant ethnic differences remained for CRP (P < 0.05) and 25(OH)D (P < 0.05).

BMI was positively associated with serum levels of leptin (r = 0.78, P < 0.0001), CRP (r = 0.51, P < 0.0001), and γ-tocopherol (r = 0.25, P = 0.0008), and inversely associated with 25 (OH)D (r = −0.24, P = 0.0009) and carotenoids [α-carotene (r = −0.26, P = 0.0004), β-carotene (r = −0.31, P < 0.0001), α- cryptoxanthin (r = −0.40, P < 0.0001), β-cryptoxanthin (r = −0.29, P = 0.0002), and *trans* lutein (r = −0.23, P = 0.002)]. As shown in Table 2, significantly higher leptin, CRP, and γ-tocopherol, and lower 25(OH)D and carotenoids (α-carotene, β-carotene, α- cryptoxanthin, β-cryptoxanthin, and *trans* lutein) were also observed in obese individuals after adjustment for Asian ethnicity and smoking status (P's < 0.05).

Although total levels of both pro-vitamin A (α-carotene + β-carotene + β-cryptoxanthin) and non pro-vitamin A (lycopene + lutein/zeaxanthin + α-cryptoxanthin + anhydrolutein) carotenoids were significantly lower in obese individuals, the difference (obese versus normal weight) was considerably greater for pro-vitamin A carotenoids with 45% reduction observed in the obese subgroup. The reduction for non pro-vitamin A carotenoids was only 16% (Table 2). α-Tocopherol and lycopene levels did not differ among BMI subgroups.

No significant differences were detected in dietary intakes of total energy (P = 0.32), α-carotene (P = 0.14), β-carotene (P = 0.25), lycopene (P = 0.31), β-cryptoxanthin (P = 0.37), and lutein (P = 0.09) across BMI subgroups. The differences in serum α-carotene, β-carotene, β-

cryptoxanthin, and *trans* lutein between BMI subgroups persisted after adjustment for nutrient density (Table 2).

4. Discussion

In our study, serum levels of γ-tocopherol were significantly higher, and 25(OH)D and carotenoids, particularly pro-vitamin A carotenoids were significantly lower in the obese subgroup. The differences in serum carotenoids between BMI subgroups persisted after adjustment for nutrient density, indicating the effect of BMI on serum caroteniods was independent of dietary intake. Furthermore, the ethnic differences disappeared (except for CRP and 25(OH)D) after adjusting for BMI, suggesting that BMI may be more relevant to serum levels of lipid soluble micronutrients than ethnicity. Our results are consistent with the hypothesis that obesity differentially influences serum micronutrient levels.

Our findings were in agreement with Andersen et al. [6] who reported a strong inverse association between BMI and carotenoids (α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin) among non-smokers, both cross-sectionally and longitudinally. Obesity is associated with chronic inflammation generating oxidative stress [4], and this was confirmed by our observation that serum CRP (indicator of systemic inflammation) levels were elevated in the obese subgroup. The state of chronic inflammation may be an important determinant of serum levels of lipid soluble micronutrients. With respect to serum carotenoids, interestingly, the obese subgroup had an average concentration of total pro-vitamin A carotenoids (α-carotene + β-carotene + β-cryptoxanthin) that was 45% lower than that observed for normal weight subgroup. In contrast, serum levels of total non pro-vitamin A carotenoids (lycopene + lutein/zeaxanthin + α-cryptoxanthin + anhydrolutein) were reduced only by 16%. Since there were no significant differences in dietary intakes of total energy and carotenoids across BMI subgroups, it is unlikely that the differential pattern observed for pro- and non-provitamin A carotenoids was due to lack of intake. Vitamin A has been recognized for the favorable effect on immune response to infections and antioxidant action [22]. Thus, chronic inflammation associated with obesity (indicted by elevated CRP levels in obese individuals in our study) may lead to a greater requirement for vitamin A, resulting in enhanced metabolism of pro-vitamin A carotenoids. The smaller changes in serum levels for the non pro-vitamin A carotenoids may reflect the loss of carotenoids due to higher oxidation in obese individuals. As a consequence, the lower levels of carotenoids observed in obese individuals may be a reflection of two separate processes, metabolism of carotenoids to vitamin A for immune function and the more generalized function of carotenoids as antioxidants.

The levels of serum γ-tocopherol were significantly higher in the obese subgroup in our study, whereas no significant BMI subgroup differences were observed in serum α-tocopherol levels. γ-Tocopherol, unlike α-tocopherol, possesses anti-inflammatory activity [23] and its levels are observed to rise in response to inflammatory signals [24,25]. Therefore, the elevated serum γ-tocopherol levels in OB individuals may signify a response to systemic inflammation associated with obesity.

Consistent with previous reports [14,15], we observed significantly lower mean serum 25(OH)D levels in the obese subgroup. Obese individuals may be exposed to less sunlight, and therefore, have lower levels of vitamin D. In a random population based sample of 367 subjects aged 25-70 years, Kull et al. [26] found lower sunbathing habits in obese than non-obese individuals. On the other hand, the lower vitamin D may be attributed to metabolic differences. Wortsman et al. [27] found substantially lower circulating 25(OH)D levels in obese adults taking supplemental vitamin D and exposed to UV light than non-obese matched controls, suggesting different pathways in the metabolism of vitamin D and/or its precursors by obesity level. Our results for pro-vitamin A carotenoids suggest that there may be an increased need

for vitamin A in obese individuals due to inflammation. Vitamin A and D receptors are structurally related [28] and both molecules have been shown to have crucial effects on the immune response [29]; therefore, another possible explanation is that obese individuals may also have an increased need for vitamin D, metabolizing it to the active form more readily, and depleting plasma levels of the precursor vitamin D species.

Our study was limited due to its cross sectional design, preventing us from determining unequivocally the causations of the observed associations. However, our results demonstrated that BMI was positively associated with serum levels of γ-tocopherol, and inversely associated with 25(OH)D and carotenoids, particularly pro-vitamin A carotenoids, suggesting possible influences of obesity on metabolism of these micronutrients. The differential association of BMI for the tocopherols and for pro- and non pro-vitamin A carotenoids also argues against generalized fat effects and oxidative effects alone as determinants of serum levels of these micronutrients.

Future epidemiologic studies of serum levels of lipid soluble micronutrients should consider obesity-related physiologic effects on circulating levels, in addition to dietary exposure in any analysis of their associations with disease risk.

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Abbreviations

BMI	Body mass index
CRP	C-reactive protein
25(OH)D	25-hycroxyvitamin D
HPLC	high performance liquid chromatography
ELISA	enzyme-linked immunosorbent assay
ANOVA	Analysis of variance

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Table 1

Characteristics of study participants by ethnicity.

	Asians	Caucasians	All subjects
N	73	66	180
Body mass index (kg/m^2)	24.7 (23.7, 25.7)*	27.1 (25.4, 28.8)	26.3 (25.4, 27.1)
Age (year)	43.5 (42.9, 44.1)	42.8 (42.1, 43.6)	43.0 (42.6, 43.5)
Leptin (ng/mL) †	11.8 (10.1, 13.9)*	17.6 (14.4, 21.5)	14.7 (13.2, 16.6)
C-reactive protein (mg/L) †	0.61 (0.44, 0.84)*	1.42 (1.00, 2.01)	0.92 (0.74, 1.13)
25(OH)D (nM)	68.4 (63.5, 73.4)*	78.4 (70.6, 86.2)	72.2 (68.4, 76.1)
α-Tocopherol (μg/mL)	14.4 (12.9, 15.9)	13.2 (11.7, 14.7)	13.6 (12.7, 14.4)
γ-Tocopherol (μg/mL)	1.6 (1.4, 1.9)	1.7 (1.5, 1.9)	1.7 (1.5, 1.8)
Pro-vitamin A carotenoids			
α-Carotene (ng/mL)	74.1 (61.7, 86.5)	67.0 (54.7, 79.2)	68.1 (60.8, 75.3)
β-Carotene (ng/mL)	427 (348, 506)	358 (293, 423)	386 (343, 430)
β-Cryptoxanthin (ng/mL)	288 (232, 343)*	207 (172, 242)	254 (221, 287)
Total pro-vitamin A carotnoids (ng/mL)	789 (671, 906)*	632 (536, 728)	708 (639, 777)
Non pro-vitamin A carotenoids			
α-Cryptoxanthin (ng/mL)	48.8 (42.8, 54.7)*	39.5 (36.3, 42.6)	43.4 (40.5, 46.2)
Lycopene (ng/mL)	360 (326, 395)	339 (305, 373)	349 (328, 371)
Trans lutein (ng/mL)	251 (223, 280)	221 (196, 246)	232 (214, 249)
Total non pro-vitamin A carotenoids (ng/mL)	1133 (1041, 1225)*	1001 (924, 1077)	1049 (997, 1100)

Values are presented as mean (95% confidence interval for mean).

*P value < 0.05 (Student's t-test) for comparison to Caucasians.

†Geometric mean and 95% confidence interval for geometric mean. Log transformed value were used for P-value.

Table 2

Serum levels of biomarkers by body mass index (BMI) among 180 premenopausal women*

	BMI subgroup					
	Normal weight (18.5 – 24.9 kg/m^2)	**Overweight (25 – 29.9 kg/m^2)**	**Obese (≥ 30 kg/m^2)**	**P value**	**Absolute difference between obese and normal weight****	**% Difference between obese and normal weight*****
N	91	48	41			
Leptin (ng/mL)†	9.0 (8.2, 10.0)^a	16.4 (14.9, 20.1)^b	33.1 (30.0, 40.4)^c	< 0.001	24.1	268
C-reactive protein (mg/L)†	0.52 (0.41, 0.68)^a	1.17 (0.83, 1.67)^b	2.29 (1.55, 3.35)^c	<0.0001	1.77	340
α-Tocopherol (μg/mL)	13.4 (12.2, 14.7)	14.1 (12.4, 15.8)	13.3 (11.5, 15.0)	0.74	−0.1	−0.7
γ-Tocopherol (μg/mL)	1.5 (1.3, 1.7)^a	1.8 (1.5, 2.1)^{ab}	2.0 (1.7, 2.3)^b	0.01	0.5	33
25(OH)D (nM)	79.1 (73.7, 84.4)^a	68.5 (61.3, 75.8)^{ab}	61.6 (53.6, 69.5)^b	0.001	−17.5	−22
Pro-vitamin A carotenoids						
α-Carotene (ng/mL)	79.2 (69.3, 89.1)^a	64.0 (50.2, 77.7)^{ab}	49.6 (35.3, 63.9)^b	0.009	−29.6	−37
β-Carotene (ng/mL)	470 (412, 529)^a	350 (269, 431)^b	251 (167, 335)^b	0.0002	−219	−47
β-Cryptoxanthin (ng/mL)	306 (261, 352)^a	234 (170, 297)^{ab}	169 (103, 235)^b	0.003	−137	−45
Total pro-vitamin A carotenoids (ng/mL)	856 (765, 947)^a	648 (522, 774)^b	469 (338, 601)^b	<0.0001	−387	−45
Non pro-vitamin A carotenoids						
α-Cryptoxanthin (ng/mL)	48.6 (44.8, 52.3)^a	40.4 (35.0, 45.3)^b	36.1 (30.7, 41.5)^b	0.0005	−12.5	−26
Lycopene (ng/mL)	363 (333, 394)	315 (273, 358)	356 (312, 400)	0.18	−7	−2
Trans lutein (ng/mL)	255 (231, 279)^a	224 (191, 258)^{ab}	192 (157, 227)^b	0.02	−63	−25
Total non pro-vitamin A carotenoids (ng/mL)	1126 (1055, 1196)^a	1001 (903, 1098)^{ab}	944 (843, 1045)^b	0.01	−182	−16
Additionally adjusted for nutrient density‡						
α-Carotene (ng/mL)	77.4 (68.2, 86.6)^a	62.5 (49.4, 75.2)^{ab}	54.9 (41.5, 68.3)^b	0.02	−22.5	−29
β-Carotene (ng/mL)	468 (411, 525)^a	339 (260, 419)^{ab}	267(184, 350)^b	0.003	−201	−43
β-Cryptoxanthin (ng/mL)	304 (263, 344)^a	217 (161, 274)^b	191 (131, 249)^b	0.003	−113	−37
Lycopene (ng/mL)	363 (333, 394)	316 (273, 358)	356 (312, 400)	0.19	−7	−2
Trans lutein (ng/mL)	258 (234, 281)^a	215 (182, 248)^{ab}	196 (162, 230)^b	0.01	−62	−24

*Values are presented as mean (95% confidence interval for mean) adjusted for ethnicity and smoking status. P values were calculated from ANOVA for BMI subgroups. Mean values within a row with different superscript letters (^{a,b,c}) are significantly different (Tukey test; $P < 0.05$).

** Absolute difference: mean for obese – mean for normal weight

*** % difference: [(mean for Obese – mean for normal weight) / mean for normal weight] × 100%.

† Geometric mean and 95% confidence interval for geometric mean. Log transformed values were used for P value calculation.

‡ Nutrient density = (dietary intake of respective carotenoids / dietary total energy intake) × 1000.