

## Insulin Resistance and Adiposity in Relation to Serum $\beta$ -Carotene Levels

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### Objective

To determine the effects of placebo vs an encapsulated supplement of fruit and vegetable juice concentrate (FVJC) on serum  $\beta$ -carotene levels, insulin resistance, adiposity, and subclinical inflammation in boys.

### Study design

Thirty age-matched prepubertal boys (9 lean and 21 overweight (OW); age range, 6-10 years) were studied. All participants received nutrition counseling and were randomized to receive FVJC or placebo capsules for 6 months. Total cholesterol, triglycerides, lipid corrected  $\beta$ -carotene, serum retinol, glucose, insulin, retinol binding protein-4, leptin, adiponectin, leptin-to-adiponectin ratio, high-sensitivity C-reactive protein, and interleukin-6 were measured before and after the 6-month intervention. Homeostasis model assessment-insulin resistance (HOMA-IR), acute insulin response to intravenous glucose, along with abdominal fat mass (dual-energy x-ray absorptiometry) were also determined.

### Results

Baseline  $\beta$ -carotene concentrations correlated inversely with HOMA-IR, leptin-to-adiponectin ratio, and abdominal fat mass ( $P \leq .01$ ). FVJC intake increased  $\beta$ -carotene concentrations ( $P \leq .001$ ) but did not influence retinol or retinol binding protein-4. Retinol insufficiency  $<1.047 \mu\text{M}$  was present in 18% of the entire cohort at baseline and in 37% at 6 months. HOMA-IR decreased after supplementation in the OW cohort, when adjusted for percent weight change ( $P = .014$ ). The percent change in abdominal fat mass increased in the placebo group and decreased in the FVJC group ( $P = .029$ ).

### Conclusions

A 6-month supplementation with FVJC in the presence of nutritional counseling was associated with an increase in serum  $\beta$ -carotene concentrations and a reduction in adiposity in conjunction with an improvement in insulin resistance in OW boys.

The US Department of Health and Human Services objectives for *Healthy People 2010*<sup>1</sup> and the latest Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report<sup>2</sup> include a diet high in fruits and vegetables to promote health. Despite these and other public health recommendations, few children meet the daily recommended intake of fruits and vegetables for various reasons.<sup>3</sup> In this context, supplementation of essential nutrients as an adjuvant to nutritional counseling therapy may be of importance. Cross-sectional studies in obese children and adults report reduced lipophilic nutrients, including  $\beta$ -carotene and serum retinol (SR) concentrations, presumably from either reduced intake of fruits and vegetables or entrapment in adipose tissue.<sup>4</sup> Increased consumption of green leafy vegetables, which are important dietary sources of carotenoids, has been associated with reduced risk for type 2 diabetes.<sup>5</sup> Although the results of supplementation with single or a few combined vitamins have been mixed for disease prevention in adults,<sup>6</sup> the issue in children remains understudied.

Recent studies also suggest that there is a relationship between retinol binding protein-4 (RBP4), which carries all- *trans* retinol to its target tissues, and obesity-related insulin resistance (IR) in both adults and children, <sup>78910</sup> whereas others have reported conflicting data. <sup>1112</sup> RBP4 is produced primarily in the liver, and about 20% is produced in the adipocytes. <sup>13</sup> In the liver, RBP4 secretion is dependent on retinoid availability, such that it is blocked in times of liver retinol deficiency and restored on repletion. <sup>13</sup> Despite SR being a major biological determinant of RBP4, few studies have assessed these levels concurrently in nutritional intervention studies. <sup>910</sup>

This double-blind placebo-controlled study was designed to determine the effects of supplementation with an encapsulated fruit and vegetable juice concentrate (FVJC) along with nutritional counseling on serum  $\beta$ -carotene, retinol, RBP4, and adiposity in lean and overweight (OW) boys. Change in serum  $\beta$ -carotene was considered the primary outcome measure, and IR and factors related to subclinical inflammation were studied as secondary outcomes.

## Methods

The study was approved by the Institutional Review Committee at Wolfson Children's Hospital, Jacksonville, Florida, and conducted in accordance with the Declaration of Helsinki. Written parental informed consent and child's assent were obtained for all participants on enrollment in the study. Subjects received nominal monetary compensation for their participation. The study was registered at INTER REF ClinicalTrials.gov (NCT00842543).

A total of 39 prepubertal boys (age range, 6-10 years) were enrolled, and 30 completed the study (9 lean boys with body mass index [BMI]  $\leq 85\%$  and 21 OW boys with BMI of  $>85\%$ ). Subjects were recruited from the Nemours Endocrinology and Metabolism Clinic in Jacksonville, Florida, and through approved advertising sent to neighboring pediatric clinics.

Subjects with a history of chronic illness or chronic medications were excluded from the study. To avoid illness-related acute changes in the markers of interest, subjects were studied only if they had no history of recent illness or bone fracture within 2 weeks of their blood draw. They were instructed not to consume any medications, including vitamins, herbal remedies, or anti-inflammatory drugs, within 30 days of the anticipated blood draw.

The intent-to-treat principle was applied to 39 subjects who were randomized using a randomization scheme generated with use of the website INTER REF Randomization.com ( INTER REF <http://www.randomization.com>) and received either active supplement or identical placebo capsules provided by the manufacturer (Juice Plus+; NSA, LLC, Collierville, Tennessee) in conjunction with 6 months of nutritional counseling.

The study protocol is summarized in [Figure 1](#) (available at INTER REF [www.jpeds.com](http://www.jpeds.com)). History and physical exams were performed including sitting blood pressure measured 3 times with an automated sphygmomanometer. Tanner staging based on pubic hair and genitalia was performed in all subjects. Waist circumference was measured to the nearest centimeter with a flexible steel tape while the subjects were standing, after gently exhaling, at the minimal circumference measurable on the horizontal plane between the lowest portion of the rib cage and iliac crest. A digital scale and Harpenden stadiometer were used to measure height and body mass and then to calculate BMI ( $\text{kg}/\text{m}^2$ ). All subjects underwent a modified rapid intravenous glucose tolerance test at baseline and at the end of the study using 0.5 g/kg glucose (25% dextrose, maximum 25 g) infused over 3 minutes, and blood was obtained at baseline and 3 and 5 minutes after glucose administration.

A dual-energy x-ray absorptiometry scan was performed to measure body composition (Hologic Discovery A 45903; Hologic Inc, Bedford, Massachusetts). Percent body fat, total body fat, trunk fat, and abdominal fat mass values were obtained. The abdominal region of interest was defined manually by adjusting the lines of the right rib box (standard software option) between the upper L1 and lower L4 border and the inner costal margin of the whole body scan following the protocol used by Svendsen et al. <sup>14</sup> The coefficients of variation of the total body, trunk, and abdominal fat mass have been reported at 1.4%, 3.6%, and 4.7%, respectively. <sup>15</sup>

All subjects had nutrition counseling sessions at baseline and 3 months with a registered research dietician, targeted to achieve improvement in the consumption of fruits and vegetables and physical activity. All participants were instructed to take 1 study capsule daily with breakfast and dinner. Two active capsules of FVJC provided approximately 3.75 mg of  $\beta$ -carotene, 117 mg of vitamin C, 22.5 IU of vitamin E, 210  $\mu\text{g}$  of folate, 30 mg of

calcium, and 21 kJ per day. The FVJC consist of *orchard blend* with apple, orange, pineapple, cranberry, peach, acerola cherry, and papaya and *garden blend* with carrot, parsley, beet, kale, broccoli, cabbage, spinach, and tomato. Dietary composition and micronutrient intake were assessed using a modified 122-item, validated food frequency questionnaire administered by the dietician.<sup>16</sup> Estimates of nutritional intake were quantified with the use of the nutrition analysis program Food Processor (version 9.3.0) developed by ESHA Research (Salem, Oregon). Physical activity was quantified based on the previous week's activity levels at school or home or during leisure time.

Blood was collected after 10 hours of fasting and processed under orange lights immediately after collection—aliquots of serum and plasma were frozen in opaque tubes at  $-80^{\circ}\text{C}$  until analysis. Methods for measurement were as follows: glucose (hexokinase), insulin (chemiluminescent immunometric assay), total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations (colorimetric assay),  $\beta$ -carotene and SR (reverse-phase high-performance liquid chromatography with photodiode array detection between 220-600 nm as previously described<sup>17</sup>), RBP4 and interleukin-6 (IL-6) (enzyme-linked immunosorbent assay), high-sensitivity C-reactive protein (HS-CRP) (immunonephelometry; Siemens Healthcare Diagnostics, Deerfield, Illinois), and adiponectin and leptin (radioimmunoassay; Linco Research, St Charles, Missouri).

The homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the formula: fasting glucose (mM)  $\times$  fasting insulin ( $\mu\text{U}/\text{mL}$ )/22.5. The quantitative insulin sensitivity check index (QUICKI) was calculated as  $1/[\log_{10}(\text{fasting insulin in } \mu\text{U}/\text{mL}) + \log_{10}(\text{fasting glucose in mg/dL})]$ .<sup>18</sup> The acute insulin response (AIR) was defined as the mean incremental rise in plasma insulin at 3 and 5 minutes after a rapid intravenous glucose load. To adjust AIR for the effects of insulin sensitivity, a glucose disposal index (GDI) was calculated as  $\log_{10}(\text{AIR} \times \text{fasting glucose concentration}/\text{fasting insulin concentration})$  in SI units.<sup>19</sup>

$\beta$ -carotene concentration is closely correlated with major lipid distribution and was corrected for lipid status (LCBC) by dividing by the sum of total cholesterol and triglycerides expressed in mM.<sup>20</sup> The molar ratio of RBP4-to-retinol was calculated by dividing the serum concentrations of RBP4 ( $\mu\text{M}$ ) by retinol ( $\mu\text{M}$ ). The molar ratio of leptin-to-adiponectin (L/A) was calculated by dividing leptin (in ng/mL) by adiponectin (in  $\mu\text{g}/\text{mL}$ ). The fat mass index was calculated by dividing the total body fat (in kg) from dual-energy x-ray absorptiometry by the height (in  $\text{m}^2$ ).

Weight gain velocity for all participants was calculated as grams per month at 3-month intervals. Actual weight velocity minus the expected weight velocity for age- and weight-matched normalized data<sup>21</sup> was used to identify changes. The data are presented in quartiles of percent change in actual weight gain from expected weight gain (0%) at 3 months and 6 months for all subjects in [Figure 2](#) (available at INTER REF [www.jpeds.com](http://www.jpeds.com)).

## Statistical Analysis

The intention-to-treat principle was applied to all subjects included in the primary analysis. Baseline, demographic, and other clinical characteristics are presented in [Table I](#) and the same for the intervention groups in [Table II](#) (available at INTER REF [www.jpeds.com](http://www.jpeds.com)). Quantitative variables are presented using either mean with SD or, in the case of substantially skewed distribution, median and IQR. Categorical variables are presented using frequencies and percentages. Two-sample *t* test or a nonparametric Mann-Whitney *U* test, whichever is appropriate, is used to compare quantitative variables, and  $\chi^2$  test/Fisher exact test are used to compare the percentages between 2 groups. Wilcoxon signed-rank test was used for paired sample comparisons of nonparametric variables.

Table I

Baseline clinical and biochemical characteristics of study subjects by BMI group

	Lean group (n = 13)	OW group (n = 26)	P value
Age (y)	9.36 $\pm$ 1.31	9.04 $\pm$ 1.43	NS
BMI Z-score	-0.08 $\pm$ 1.43	2.14 $\pm$ 0.47	<.05
Waist/height ratio	0.42 $\pm$ 0.03	0.59 $\pm$ 0.07	<.05

Fat mass index (kg/m <sup>2</sup> )	3.39 $\pm$ 0.68	9.83 $\pm$ 2.68	<.05
Abdominal fat mass (kg)	1.249 $\pm$ 0.391	4.144 $\pm$ 0.141	<.05
Systolic blood pressure (mm Hg)	110 $\pm$ 10	109 $\pm$ 23	NS
$\beta$ -carotene ( $\mu$ M)	0.29 (0.16-0.36)	0.22 (0.12-0.26)	NS
SR ( $\mu$ M)	1.17 (1.0-1.27)	1.41 (1.12-1.58)	.019
LC $\beta$ C ( $\mu$ M)	0.055 (0.038-0.093)	0.035 (0.023-0.066)	.04
RBP4 ( $\mu$ M)	1.02 (0.89-1.31)	1.26 (1.01-1.45)	NS <sup>‡</sup>
Insulin (pM)	43.9 (30.5-65.2)	98.7 (66.7-133.1)	<.001
HDL (mM)	1.27 (1.07-1.45)	1.06 (0.85-1.29)	.034
Triglycerides (mM)	0.52 (0.37-0.75)	0.96 (0.61-1.20)	.004
HOMA-IR	1.34 (0.8-1.82)	3.68 (1.98-4.01)	<.001
QUICKI	0.37 $\pm$ 0.03	0.33 $\pm$ 0.04	.003
AIR	345 (206-597)	687 (402-1014)	.014
GDI	1.67 $\pm$ 0.30	1.50 $\pm$ 0.25	NS
HS-CRP (mg/L)	0.36 (0.16-0.39)	0.93 (0.38-0.93)	.01
IL-6 (pg/mL)	0.69 (0.64-1.07)	1.35 (0.92-2.3)	.018
Leptin (ng/mL)	3.76 (2.5-4.8)	21.3 (10.6-26.5)	<.001
Adiponectin ( $\mu$ g/mL)	14.7 (10.8-20.9)	10.7 (7.6-12.4)	.04
L/A	0.23 (0.19-0.31)	1.85 (1.02-2.67)	<.001

\*Mean  $\pm$  SD (all appropriate values).

†Median (IQR) (all appropriate values).

‡*P* = .084.

The percent changes in concentrations from baseline at 3 and 6 months are presented in [Table III](#) (available at INTER REF [www.jpeds.com](http://www.jpeds.com)). Repeated-measures subgroup analysis was performed among OW children to compare the mean changes in natural log-transformed HOMA-IR and QUICKI over time (3 months and 6 months) as response variables, and the intervention group was used as the independent variable. Both models were adjusted for a principal component of baseline values of the corresponding variable and the percent change in weight presented in [Table IV](#). All tests were 2 tailed at the level of significance of .05. The statistical software SPSS version 19.0 (SPSS, Chicago, Illinois) was used for analyses.

Table IV

Subgroup analysis of treatment effects of FVJC vs placebo in OW group for HOMA-IR and QUICKI at 3 and 6 months

	FVJC group (n = 11)			Placebo group (n = 10)			<i>P</i> value
	0 mo <sup>‡</sup>	3 mo	6 mo	0 mo <sup>‡</sup>	3 mo	6 mo	
Log HOMA-IR	1.268 $\pm$ 0.1	1.205 $\pm$ 0.07	1.204 $\pm$ 0.07	1.339 $\pm$ 0.09	1.441 $\pm$ 0.08	1.429 $\pm$ 0.08	.014
QUICKI	0.336 $\pm$ 0.01	0.341 $\pm$ 0.01	0.341 $\pm$ 0.01	0.326 $\pm$ 0.01	0.313 $\pm$ 0.01	0.315 $\pm$ 0.01	.017

\*Least squared mean  $\pm$  SE (all appropriate values).

†Adjusted for percent change in body weight.

‡*P* value between FVJC and placebo at 0 mo is not significant.

## Results

Of the 246 children screened for eligibility, 74 did not meet criteria, 35 refused to participate, 98 were not randomized for various reasons, 39 were randomized, and 9 dropped out before completion of the study. Pill count data were available for 88% of all supplement bottles dispensed. At 3 months, 60% of the lean and 55% of the OW took >75% of the active supplement capsules, and at 6 months 80% of the lean and 50% of the OW children had >75% compliance. However, the groups were not statistically different.

The clinical and biochemical characteristics of the study participants by BMI and treatment groups are presented in [Tables I](#) and [II](#), respectively. By design, significant differences between the lean and OW groups exist at baseline in terms of adiposity including BMI Z-score, waist circumference, waist-to-height ratio, fat mass index, and abdominal fat mass ( $P \leq .05$ ; [Table I](#)). There were no differences between the treatment and placebo groups at baseline ([Table II](#)).

None of the study subjects at baseline met the definition for vitamin A deficiency (SR levels  $<0.7 \mu\text{M}$ ); however, vitamin A insufficiency (all-*trans* retinol  $<1.047 \mu\text{M}$ ) was present in 18% at baseline and 37% at 6 months for the entire cohort. LC $\beta$ C was lower ( $P = .04$ ) and SR was higher ( $P = .019$ ) in the OW children compared with the lean children. RBP4 tended to be lower in lean vs OW children ( $P = .084$ ), whereas the ratio of RBP4 to retinol was not different between these groups.

There was a highly significant overall treatment effect between FVJC and placebo in the percent change in  $\beta$ -carotene ( $P = .001$ ) for the entire cohort but not for the percent change in SR, RBP4, or RBP4-to-SR ratio. At 6 months, serum  $\beta$ -carotene increased by  $303\% \pm 85\%$  in the FVJC-treated lean group, which was similar to the  $334\% \pm 57\%$  increase in the OW group. However, in the placebo group, the percent change in  $\beta$ -carotene increased only  $23\% \pm 94\%$  in the lean and it decreased  $30\% \pm 57\%$  in the OW group ([Table III](#)). Surprisingly, the rise in  $\beta$ -carotene was not accompanied by a concomitant rise in SR or RBP4 levels in either the lean or OW group ([Table III](#)). After 6 months of treatment, the OW children showed a 1.9% decrease in RBP4 with FVJC as opposed to a 9.5% increase with the placebo, which did not reach statistical significance ( $P = .187$ ; [Table III](#)).

The dietary intake of vitamin A as  $\beta$ -carotene, obtained from the food frequency questionnaire (FFQ), directly correlated with log<sub>10</sub>LC $\beta$ C at baseline ( $r = 0.322$ ,  $P \leq .05$ ). FFQ data at baseline and 6 months were analyzed by repeated measures (ANOVA) for differences in the percent intake of fat, vitamin A as retinol equivalents, vitamin A as  $\beta$ -carotene, physical activity, and screen time. There were no differences in these measures between the two groups when adjusted for the total daily caloric intake (data not shown).

Baseline fasting glucose was similar in both lean and OW children, whereas fasting insulin was higher in the OW cohort, as expected ( $P \leq .05$ ). Also, markers of IR (HOMA-IR and QUICKI) and  $\beta$ -cell function (AIR) were different between the lean and OW groups at baseline ( $P < .05$ ; [Table I](#)). The GDI, which is an integrated measure of insulin secretion and insulin action, was similar in lean and OW subjects ( $P = .2$ ) at baseline. FVJC supplementation reduced HOMA-IR ( $P = .014$ ) and enhanced QUICKI ( $P = .016$ ) in the OW group compared with placebo group, after adjusting for percent change in weight ([Table IV](#)). FVJC treatment effect at 6 months compared with placebo increased the GDI ( $P = .037$ ) for all subjects, but the significance was lost when the OW subgroup was analyzed separately.

Triglyceride concentrations were higher ( $P = .004$ ) and HDL concentrations were lower ( $P = .034$ ) in the OW group ([Table I](#)). FVJC had no effects in lipid concentrations in the lean group but significantly lowered triglyceride concentrations ( $P = .032$ ) in the OW group. The FVJC groups showed no change in free fatty acid ( $P = .301$ ) or HDL cholesterol concentrations ( $P = .533$ ).

Treatment groups had lower rates of weight gain during the first 3 months of the study, but this effect was lost in the second 3 months of the study ([Figure 2](#)).

There were no significant treatment effects between groups for BMI Z score, waist circumference, waist-to-height ratio, fat mass index, percent body fat, total body fat, and trunk fat. Treatment effect by univariate ANOVA on the percent change in abdominal fat mass (in kg) for the entire cohort at 6 months showed that the placebo group had a 11.2% increase (95% CI, 4.16 to 18.23) as opposed to a 1.47% (95% CI, -8.31 to 5.37) decrease in the FVJC supplement group ( $P = .029$ ).

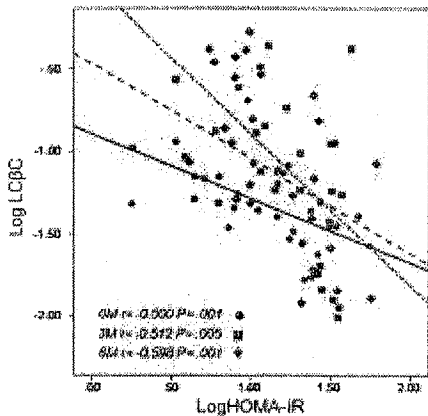
The OW cohort showed higher HS-CRP ( $P = .01$ ) and IL-6 ( $P = .018$ ) levels than the lean controls at baseline ([Table I](#)). However, there were only negligible changes in HS-CRP and IL-6 in the OW children at 3 and 6

months in both the FVJC and placebo groups.

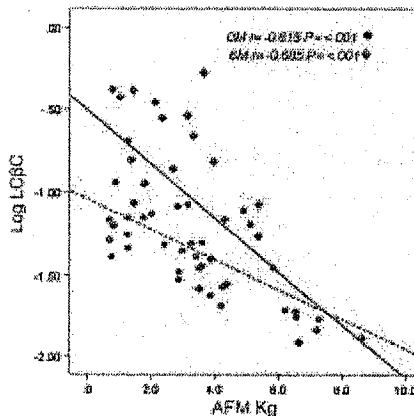
OW children also showed higher leptin ( $P < .001$ ) and lower adiponectin ( $P = .04$ ) and L/A ( $P \leq .001$ ) than their lean counterparts at baseline ([Table I](#)). Although there was no treatment effect of FVJC on leptin and adiponectin per se, L/A ratio showed a lowering trend ( $P = .071$ ), when analyzed by repeated-measures ANOVA adjusting for percent change in weight for the entire cohort.

There was a strong inverse correlation between LC $\beta$ C and HOMA-IR ( $r = -0.500$ ,  $P = .001$ ), which strengthened over time at 3 and 6 months, in contrast to SR and RBP4, which did not correlate with HOMA-IR ([Figure 3](#), A-C). There were inverse correlations between LC $\beta$ C and abdominal fat mass at baseline ( $r = -0.615$ ,  $P \leq .001$ ) and 6 months ( $r = -0.685$ ,  $P \leq .001$ ) ([Figure 3](#), E). A direct correlation between SR and abdominal fat mass was significant only at baseline ( $r = 0.429$ ,  $P \leq .013$ ), but this significance vanished at 6 months ( $r = 0.262$ ,  $P = .178$ ) for the entire cohort ([Figure 3](#), F). In contrast, the concentration of RBP4 did not correlate with abdominal fat mass in either group ([Figure 3](#), G). There was an inverse correlation between LC $\beta$ C and L/A ratio ( $r = -0.596$ ,  $P = .001$ ) for the entire cohort, which persisted at 3 and 6 months ([Figure 3](#), D). The L/A ratio also directly correlated ( $P = .001$ ) with the abdominal fat mass ([Figure 3](#), H). Wilcoxon signed ranks test determined inverse correlations between the percent change in LC $\beta$ C and percent change in leptin at 3 months ( $Z = -2.497$ ,  $P = .013$ ) and 6 months ( $Z = -2.573$ ,  $P = .010$ ) as well as the percent change in abdominal fat mass at 3 months ( $Z = -2.497$ ,  $P = .013$ ) and 6 months ( $Z = -2.378$ ,  $P = .017$ ) for the entire cohort.

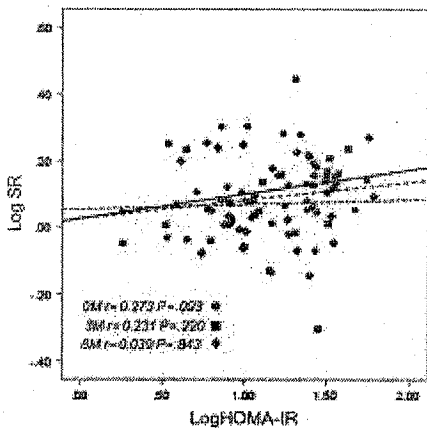
A) Correlation between Log Lipid Corrected  $\beta$ -carotene and LogHOMA-IR



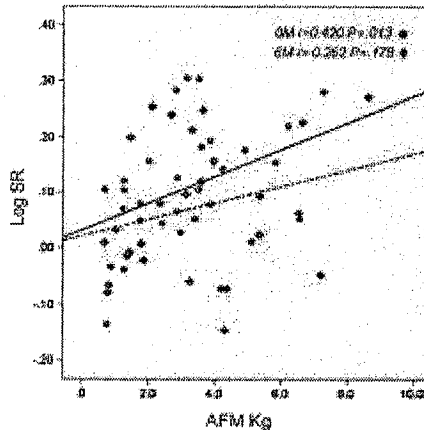
E) Correlation between Log Lipid Corrected  $\beta$ -carotene and Abdominal Fat Mass (Kg)



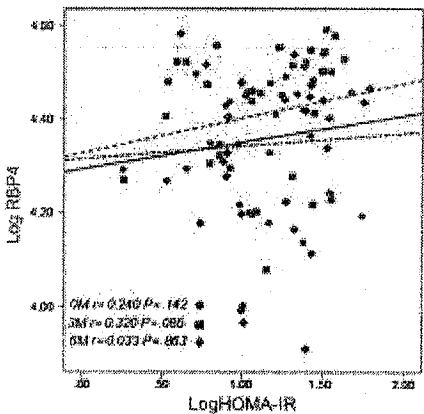
B) Correlation between Log SR and LogHOMA-IR



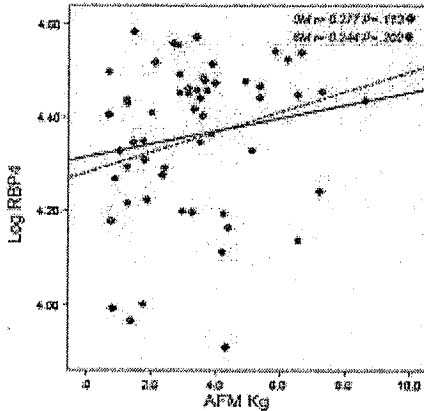
F) Correlation between Log SR and Abdominal Fat Mass (Kg)



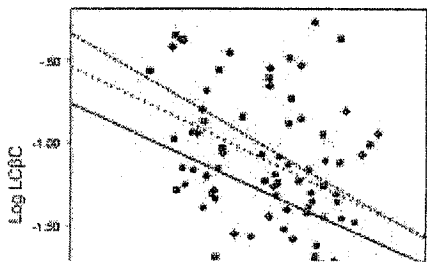
C) Correlation between Log RBP4 and LogHOMA-IR



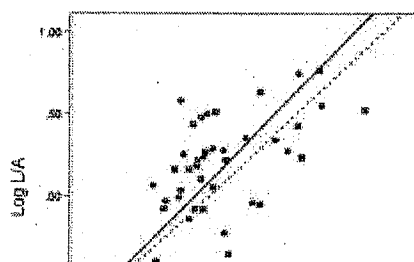
G) Correlation between Log RBP4 and Abdominal Fat Mass (Kg)



D) Pearson correlation Log LCβC vs. Log L/A



H) Pearson correlation Log L/A vs. AFM (Kg)



Pearson correlations between A, D, E, LC $\beta$ C B, F, SR, and C, G, RBP4, vs A-C, HOMA-IR and D, L/A at baseline (—), 3 mo (---), and 6 mo (---) and E-H, abdominal fat mass at baseline (—) and 6 mo (---). AFM, abdominal fat mass.

## Discussion

A previous intervention study using carrot juice, which is rich in  $\beta$ -carotene, has reported a doubling of plasma all-*trans* retinoic acid levels without significant increase in retinol.<sup>22</sup> Nutritional counseling targeted to increase the consumption of fruits and vegetables in our study showed an increase in serum  $\beta$ -carotene in the lean control group, but quite intriguingly, its level decreased in the OW placebo group. On the other hand, the 6-month supplementation with FVJC capsules enhanced the serum  $\beta$ -carotene similarly in both lean and OW children. In sharp contrast, the levels of retinol unexpectedly decreased in all groups by 6 months of the intervention (Table III). Our results suggest that the absorptive capacity of  $\beta$ -carotene in the supplement is equivalent in both groups, but the differences in dietary response in the lean control vs OW boys could not be reconciled. Because carotenoid bioavailability and bioconversion are heavily influenced by numerous extrinsic and intrinsic factors, including high interindividual differences due to common genetic variations in the enzymes responsible for cleavage and absorption, we cannot speculate on the nature of this observation.<sup>23</sup> Analysis of the FFQ data was only predictive of serum LC $\beta$ C at baseline and was not predictive of the changes in  $\beta$ -carotene, retinol, or LC $\beta$ C at 6 months.

The FVJC supplementation significantly lowered triglycerides in the OW boys, and this may have played an important role in improving insulin sensitivity. Animal studies propose a diet-responsive regulatory network that tightly controls  $\beta$ -carotene absorption, dietary lipid transport, and intracellular retinoid production through all-*trans* retinoic acid negative feedback regulation of the enzyme Bcmo1.<sup>24</sup> An increase in all-*trans* retinoic acid is thought to downregulate intestinal lipid absorption by reducing the expression of the SR-BI (scavenger receptor class B type 1). All-*trans* retinoic acid via retinoic acid receptors induces the expression of the intestinal transcription factor intestine specific homeobox. Intestine specific homeobox in turn repressed the expression of SR-B1 and Bcmo1, thus preventing accumulation of excess  $\beta$ -carotene.<sup>25</sup>

The current study also shows inverse correlations among  $\beta$ -carotene, IR, and adipokine ratios at baseline for the entire cohort, and this correlation strengthened with FVJC supplementation over time. This is in agreement with a recent cross-sectional analysis of the National Health and Nutrition Examination Survey 2001-2006 data in adults that report lower serum carotenoid concentrations in those individuals with higher HOMA-IR and features of the metabolic syndrome, even after controlling for total cholesterol and triglycerides among other potential confounders.<sup>26</sup>

Although the present study showed a trend toward higher RBP4 levels in lean vs OW children ( $P = .084$ ), serum RBP4 was not correlated with HOMA-IR, AIR, GDI, inflammatory markers, and/or measures of adiposity. Intriguingly, FVJC supplementation for 6 months did not raise retinol or lower RBP4 levels in the OW cohort despite a reduction in HOMA-IR as we had initially hypothesized. This suggests that  $\beta$ -carotene may play a more important role in metabolic gene regulation than RBP4 or retinol itself. Previous studies in insulin-resistant obese children have shown a reduction in the elevated RBP4 by lifestyle intervention, which includes exercise, even without marked weight loss, but with improvements in markers of inflammation and insulin sensitivity.<sup>8</sup> On the other hand, others have suggested that the reduction in RBP4 is dependent on the degree of weight loss.<sup>10</sup> In the present study, none of the subjects achieved significant weight loss ( $\geq -0.5$  BMI Z-score) or increased exercise. However, the improvement in IR in the FVJC vs placebo in the OW children reached significance only after adjusting for the percent change in body weight and correlated with the enhanced levels of  $\beta$ -carotene. This suggests that changes in body weight along with measurements of carotenoid and retinoid status are crucial in RBP4 studies for accurate interpretation of the results. The beneficial improvements in IR, triglyceride levels, and abdominal fat attenuation with juice concentrate supplementation opens up new avenues of research to determine which combination of nutrients may be responsible for the effects and underscores the need to enhance intake of whole fruits and vegetables with high carotenoid value and bioavailability. A single antioxidant vitamin given at high doses in subjects with high risk of type 2 diabetes may not have substantial benefits and could even have negative consequences, as seen in previous studies.<sup>6</sup>

Strengths of the study include the randomized double-blind placebo-controlled nature of the intervention, the use of serum markers such as  $\beta$ -carotene to document dietary compliance, and simultaneous measurement of serum



$\beta$ -carotene, retinol, and RBP4 along with markers of adiposity, IR, and subclinical inflammation. There are also limitations to the study because of the complex nature of the supplement, and consequently, we cannot draw definite conclusions in terms of causality. Other limitations include the fact that  $\beta$ -carotene is only one of numerous carotenoids and other phytonutrients present in natural fruits and vegetables, and this precludes us from drawing definitive conclusions on ascribing a role for  $\beta$ -carotene as solely responsible for the changes observed in adiposity and IR in the present study. The relatively small sample size, low compliance, and use of surrogate markers for IR emphasize the pilot nature of this study. Compliance with the supplement did not differ significantly within groups. Further, the current study was conducted only in prepubertal boys, and therefore, extrapolation of the data to the general pediatric and/or adult population should be done with caution. Future studies with more sophisticated measures of IR and abdominal fat distribution in a larger cohort would be needed to confirm the findings.

Serum  $\beta$ -carotene, and not retinol and/or RBP4, appears to be promising as a potential marker of insulin sensitivity and nutritional status, but additional large-scale studies are needed to validate its usefulness. Although our study suggests beneficial effects of FVJC in the presence of nutritional counseling on adiposity and IR in prepubertal boys, we caution that FVJC capsules should not be considered as substitutes to the daily intake of fresh fruits and vegetables in children.

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