Article Title:

Metabolic syndrome and serum carotenoids: findings of a cross-sectional study in Queensland, Australia

Short running title

Metabolic syndrome and serum carotenoids

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Several components of the metabolic syndrome, particularly diabetes and cardiovascular disease, are known to be oxidative stress-related conditions and there is research to suggest that antioxidant nutrients may play a protective role in these conditions. Carotenoids are compounds derived primarily from plants and several have been shown to be potent antioxidant nutrients. The aim of this study was to examine the associations between metabolic syndrome status and major serum carotenoids in adult Australians. Data on the presence of the metabolic syndrome, based on International Diabetes Federation 2005 criteria, were collected from 1523 adults aged 25 years and over in six randomly selected urban centers in Queensland, Australia, using a cross-sectional study design. Weight, height, BMI, waist circumference, blood pressure, fasting and 2-hour blood glucose and lipids were determined, as well as five serum carotenoids. Mean serum

alpha-carotene, beta-carotene and the sum of the five carotenoid concentrations were significantly lower ($p<0.05$) in persons with the metabolic syndrome (after adjusting for age, sex, education, BMI status, alcohol intake, smoking, physical activity status and vitamin/mineral use) than persons without the syndrome. Alpha, beta and total carotenoids also decreased significantly ($p<0.05$) with increased number of components of the metabolic syndrome, after adjusting for these confounders. These differences were significant among former smokers and non-smokers, but not in current smokers. Low concentrations of serum alpha-carotene, beta-carotene and the sum of five carotenoids appear to be associated with metabolic syndrome status. Additional research, particularly longitudinal studies, may help to determine if these associations are causally related to the metabolic syndrome, or are a result of the pathologies of the syndrome.

Introduction

Although there is no universal definition of the metabolic syndrome, it is generally described as a constellation of pathologies or anthropometric conditions, which include central obesity, glucose intolerance, lipid abnormalities, and hypertension. It is, however, universally accepted that the presence of the metabolic syndrome is associated with increased risk of type 2 diabetes and cardiovascular disease [1, 2].

The prevalence of the metabolic syndrome in developed countries varies widely depending upon definitions used and age ranges included, but is estimated to be 24% among adults 20 years and over in the US [3]. Given the impending worldwide epidemic of obesity, diabetes and cardiovascular disease, strategies aimed at greater understanding of the pathology of the syndrome, as well as strategies aimed at preventing or treating persons with the syndrome are urgently required.

Both diabetes and cardiovascular disease are known to be oxidative stress-related conditions and some researchers [4] suggest that antioxidant nutrients may play a protective role in these conditions. Several cross–sectional surveys [5-7] have found lower levels of serum carotenoids among those with impaired glucose metabolism or type 2 diabetes. Carotenoids are compounds derived primarily from plants and several have been shown to be potent antioxidants [8]. There is considerable epidemiological evidence that some serum carotenoids may play a protective role against the development of chronic diseases such as atherosclerosis [9, 10], stroke [11], hypertension [12], certain cancers [10, 13], inflammatory diseases [14] and diabetic retinopathy [15]. The primary carotenoids found in human serum are alpha (α)-carotene, beta (β)-carotene, beta (β)-cryptoxanthin, lutein/zeaxanthin and lycopene [16]

Several studies have found an association between fruit and vegetable intake, or dietary patterns rich in fruit and vegetables, with a lower risk of the metabolic syndrome. [17-20]. Other studies, however, have failed to find such associations [21] Results of clinical trials testing supplements of these nutrients with chronic diseases, however, have not been promising [22].

Few studies have investigated associations of antioxidant nutrients and the metabolic syndrome. Ford and colleagues [23] reported lower levels of several carotenoids and vitamins C and E among those with metabolic syndrome present compared with those without the syndrome in the Third National Health and Nutrition Examination Survey. Sugiura et al [24] suggested that several carotenoids may exert a protective effect against the development of the metabolic syndrome, especially among current smokers. Confirming these findings in another population may add strength to these associations.

In this study, we investigated the relationships between these five primary serum carotenoids and the metabolic syndrome in a cross-sectional population-based study in Queensland, Australia.

Subjects and Methods

Subjects

The study was conducted in Queensland, Australia between October and December 2000 as part of a national study, the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), to determine the prevalence of diabetes and associated cardiovascular risk factors among adults aged 25 years and over. Details of the sampling framework, overall study design, methods and central findings have been published elsewhere [25, 26]. The study was approved by the International Diabetes Institute and The University of Queensland ethics committees. Six urban sites were randomly selected from census collector districts (CDs) in Queensland. The CDs were selected without replacement and with probability proportional to size. Non-institutionalised adults aged 25 years and over residing in private dwellings were included in the survey if they had resided permanently at the address for a minimum of 6 months prior to the survey. Persons with physical or intellectual disabilities that precluded participation in the study were not included.

Trained interviewers conducted house-to-house interviews and eligible participants were invited to attend a biomedical examination which included collection of blood samples, blood pressure determinations, anthropometric measurements and the administration of standardised questionnaires related to diet as well as socio-demographic, lifestyle and health related characteristics. All respondents gave informed consent to participate in the survey upon arrival

at the testing site. A total of 1536 persons (890 females and 646 males) in Queensland completed the physical examination. Although the overall response rate in the study was low (70% of eligible adults took part in the initial household survey, and of those, 55% completed the physical examination), the internal validity and quality control of the data collection were of high quality [26].

Methods

Study participants arrived for the examination having fasted for at least 12 hours. Blood pressure measurements were taken using Dinamap sphygmomanometer. Blood was drawn for fasting glucose. Participants not taking hypoglycaemic medication completed an oral glucose tolerance test (OGTT) (two hours after consuming a 75-gram glucose drink). Fasting and two-hour (2h) plasma glucose were measured enzymatically (glucose oxidase) on an Olympus AU600 analyser. Participants were also classified as having diabetes if they were receiving treatment for diabetes in the form of tablets or insulin at the time of the study.

Lipids; total cholesterol (chol), high-density lipoprotein cholesterol (HDL), and triglyceride were measured enzymatically on an Olympus AU 600. Low density lipoprotein (LDL) cholesterol was calculated from the equation of Friedwald et al [27]: LDL=total cholesterol-[HDL+ (triglyceride/5)]

Blood was drawn for the carotenoid determinations at the time of the 2h OGTT, or 2 hours after the fasting sample for those who did not have the OGTT. Serum samples were meticulously handled and protected from light at each stage of processing to prevent deterioration and degradation. The serum was pipetted, frozen, packed in dry ice and shipped to the laboratory in Brisbane and were analyzed within three weeks of collection. The five serum carotenoids were assayed simultaneously according to the high performance liquid chromatography (HPLC) procedure described by Talwar et al. [28]. The intrabatch coefficient of variations by this method were 6.5%, 7.6%, 7.3% 6.9% and 9.0% for α-carotene, β-carotene, β-cryptoxanthin, lutein and lycopene respectively and the interbatch (analyzed after stored at -70 C over a period of 8 weeks) CVs were 13%, 9.6%, 8.7%, 8.5 and 11% respectively.

The diagnostic criteria for the metabolic syndrome are based on the 2005 International Diabetes Federation (IDF) definition [29]. According to the IDF, to be identified as having the metabolic syndrome a person must have *central obesity* (defined as waist circumference ≥94 cm for Europid men and ≥80 cm for Europid women, with ethnicity specific values for other groups) plus any two of the following four factors: *raised serum triglyceride level* (≥1.7 mmol/L), *reduced serum HDL cholesterol* (<1.03 mmol/L in males and <1.29 mmol/L in females – or

specific treatment for these lipid abnormalities), *raised blood pressure* (systolic blood pressure ≥130 mmHg or diastolic ≥85 mmHg - or treatment of previously diagnosed hypertension, *impaired fasting glycemia* (fasting plasma glucose ≥5.6 mmol/L or previous diagnosed type 2 diabetes).

Of those diagnosed with diabetes, 2.5% were classified as having type 1 diabetes and were excluded from the analysis because they do not share common aetiological factors such as obesity, increasing age, nutrition or physical activity with metabolic syndrome. Participants were defined as having Type 1 diabetes if insulin treatment had been started within 2 years of diagnosis and, if they were 40 years of age or older when diagnosed, their current BMI was < 27.

Demographic and lifestyle variables were collected using standardised questionnaires. Height and weight were measured by trained personnel and body mass index (BMI) calculated as weight (in kilograms)/height (in meters squared). BMI was categorised as: *obese* (BMI ≥30), *overweight* (BMI ≥25 to <30) and *normal weight* (BMI <25). As there were only 18 participants classified as underweight (BMI<18.5), they were grouped with the normal weight group.

Waist circumference was measured half-way between the lower border of the ribs and the iliac crest on a horizontal plane. Using a steel measuring tape, two measurements were recorded. If the measurements varied by more than 2 cm, a third measurement was taken. The mean of the two closest measurements was calculated.

Blood pressure measurement was performed in a seated position after participants had rested for at least 5 minutes, using a Dinamap semiautomatic oscillometric recorder. An appropriate cuff size was used and the arm was supported by a table at heart level. Three readings were taken at 1-minute intervals. The mean of the first two readings was recorded. If the difference between the three readings was greater than 10mmHg, the mean of the two closest measurements was used.

Physical activity beneficial to health was categorised as: *sufficiently active (*greater than 150 minutes "physical activity time" in the previous week), *insufficiently active but not sedentary* (less than 150 minutes "physical activity time" in the previous week), and *sedentary* (no participation in physical activity in the previous week). "Physical activity time" was calculated as the sum of the time spent walking, or performing moderate activity plus double the time spent in vigorous activity to reflect its greater intensity.

Vitamin supplement use during the previous 24 hours was categorised as *yes* for respondents who indicated that they took any vitamin or mineral supplements on the previous day and *no* for respondents who indicated they did not.

Criteria used to assess the number of metabolic syndrome components present in a participant using the 2005 International Diabetes Federation [29] definition are as follows: Components = 0 -none of the metabolic syndrome components (i.e. abdominal obesity, raised triglyceride, reduced HDL-cholesterol, raised blood pressure, and impaired fasting plasma glucose) are present; Components = any 1 one of the five metabolic syndrome components is present ; Components = 2 - any two of the five components are present; Components = 3 any three of the components are present; Components = 4 - any four of the components are present; Components = 5 = all five metabolic syndrome components are present.

Participants were asked, "How many serves of vegetables do you usually eat each day? Including fresh, frozen or tinned vegetables (A serve = ½ cup cooked vegetables or 1 cup of salad vegetables)." Usual consumption of fruit was assessed by the question, "How many serves of fruit do you usually eat each day? Including fresh, frozen or tinned fruit (A serve = 1 medium piece or 2 small pieces of fruit or 1 cup of diced pieces of fruit.)." Participants were categorised into three groups according to their responses to both questions; *≤1 serve, 2-3 serves, ≥4 serves.*

Statistical analyses

Data were analysed using the survey (svy) commands in STATA statistical software version 8 [30]. These commands take into account the complex cluster survey design in the calculation of estimates, variance, standard errors and confidence intervals. Age-adjusted means or percentages for selected baseline characteristics were weighted for age and sex to the Queensland population aged over 25 for the survey year.

Pearson's chi-square statistic was used to assess the relationship between presence or absence of the metabolic syndrome and selected categorical variables. Student's t-test was used to compare differences in means between two groups; analysis of variance was used to assess overall differences in means between serum carotenoids and variables with more than two groups.

Distributions of serum carotenoids were skewed and therefore natural logarithmically transformed to better approximate the normal distribution for regression analyses. Association between log transformed serum carotenoids as dependent variables and metabolic syndrome status were assessed using multiple linear regression analysis. Results are reported as back-transformed geometric means. Analysis was performed for each serum carotenoid separately, and the sum of the five carotenoids, adjusting for the following potential confounders: age, sex, education, BMI, smoking, alcohol intake, physical activity, and vitamin use. We further

performed stratified analysis by smoking status to further investigate if the association between serum carotenoids and metabolic syndrome is modified by smoking status.

The confounders were included simultaneously into the model. Due to missing values, the sample size is not the same for all analyses.

Results

The prevalence of the metabolic syndrome by demographic and health-related characteristics is provided in Table 1. The overall prevalence of the syndrome was 24% and was significantly higher among males than females. As would be expected, significant differences in prevalence of the syndrome were seen with body mass index, waist circumference, systolic and diastolic blood pressure and blood lipids. Significant differences were also evident by age group, smoking status, educational status and income. Income was marginally inversely associated. The prevalence increased with age, and was lower in those with post graduate education. No significant differences were seen by alcohol intake, physical activity levels, vitamin usage, or fruit intake. There was actually an inverse relationship between vegetable intake (not fruit) and serum carotenoids. Those who consumed 4 serves or more of vegetable were less likely to have the metabolic syndrome compared to those who consumed 1 serve or less of vegetables.

(Insert Table 1 about here)

Table 2 provides the adjusted geometric mean of each of the serum carotenoids by metabolic syndrome status. The mean concentrations of serum α-carotene, β-carotene and the sum of the five carotenoids were significantly lower for participants with the metabolic syndrome present compared with those without the syndrome, after adjusting for potential confounding variables including age, sex, educational status, BMI, smoking, alcohol intake, physical activity and vitamin use.

(Insert Table 2 about here).

Table 3 shows that concentrations of α-carotene, β-carotene and the sum of the five carotenoids decreased significantly as the number of components of the metabolic syndrome increased after adjusting for potential confounding variables as above.. Table 3 also indicates the percentage of participants with none, one, two, three, four or all five of the components.

(Insert Table 3 about here)

Similarly there was an inverse association between quartiles of individual and total serum carotenoids and metabolic syndrome status and each of its components as shown in Table 4. The

strength of these associations was strongest for α-carotene, β-carotene and the sum of the five carotenoids.

(Insert Table 4 about here)

Table 5 looks at the effect of smoking status on carotenoid concentrations and metabolic syndrome. No significant differences in serum carotenoid concentrations by metabolic syndrome status were found among 'current smokers'. However, among 'former' and 'never' smokers serum α-carotene and β-carotene were significantly lower among those with metabolic syndrome than those without. In addition, among 'former' smokers serum lycopene was marginally lower in those with metabolic syndrome, while among 'never' smokers total serum carotenoids was marginally lower in those with metabolic syndrome compared to those without.

We also found no significant interaction between smoking status and serum carotenoid concentrations in relation to the metabolic syndrome status. The following are the p values for interaction after adjustment for the confounding variables in relation to: α-carotene (p for interaction=0.25), β-carotene (p for interaction=0.15), β-cryptoxanthin (p for interaction=0.40), lutein/zeaxanthin (p for interaction=0.25), lycopene (p for interaction=0.41), and total serum carotenoid (p for interaction=0.15).

(Insert Table 5 about here)

Discussion

This study was designed to investigate the association between several serum carotenoids and the metabolic syndrome. The data from the present population study suggest that several serum carotenoids are inversely related to the metabolic syndrome. Our study showed significantly lower concentrations of α-carotene, β-carotene and the sum of the five carotenoids among those with the metabolic syndrome present compared to those without. We also found decreasing concentrations of all the carotenoids tested as the number of the metabolic syndrome components increased. This was significant for α-carotene, β-carotene, β-cryptoxanthin and total carotenoids. These findings are consistent with data reported by Ford et al [23] from the third National Health and Nutrition Examination Survey (NHANES III). In the NHANES III study, significantly lower concentrations of all the carotenoids, except lycopene, were found among persons with the metabolic syndrome compared with those without, after adjusting for confounding factors similar to those in our study. The NHANES III study [19] also found decreasing concentrations of all the carotenoids (except lycopene) and total carotenoids with increasing number of metabolic syndrome components. Our study used the criteria for metabolic syndrome as suggested by the International Diabetes Federation [31], which requires

central obesity as the major component. In the NHANESIII study, central obesity is a component of the syndrome, but it is not a required component. Also we adjusted for BMI in all of our analyses, whereas the NHANES study did not. This adjustment for BMI may have attenuated the strength of the association of several carotenoids in our study as compared with NHANES III.

We did find that all the serum carotenoids decreased as BMI status increased and this was significant for all of the carotenoids. This is consistent with findings of a prospective study by Anderson and colleagues [32] in which a high BMI status was strongly associated with lower concentrations of total carotenoids.

Since serum carotenoids are considered reliable markers of vegetable and fruit intake, low serum concentrations could conceivably be due to lower intakes. Our previous study [33] did find statistically significant associations between vegetable and fruit intake and serum levels of α-carotene, β-carotene, β-cryptoxanthin and lutein-zeaxanthin. We also found fewer individuals with the highest vegetable intake with the metabolic syndrome compare with those with the lowest intakes of vegetables. However, we found no association with intake of fruit and the syndrome.

Several studies have investigated fruit and vegetable intake in relation to the metabolic syndrome with mixed results. The NHANES study [19] reported significantly lower intakes of fruit and vegetables among those with the syndrome present compared to those without the syndrome. To estimate the number of monthly servings of fruit and vegetables, they summed 21 items on the NHANES food frequency questionnaire. Esmaillzadeh [18] reported a significant inverse association between fruit and vegetable intake and the metabolic syndrome among Tehrani female teachers. Fruit and vegetable intake was estimated from a semiquantitative food frequency questionnaire. Several studies [17, 34] have suggested that a 'healthy' dietary pattern, or one of lower nutritional risk is associated with lower risk of the metabolic syndrome. Another study [21] of dietary patterns, however, showed no association with a 'prudent' dietary pattern, typified by a higher intake of vegetables, fruit, fish and poultry, and the metabolic syndrome, but did show a positive association with a 'western' pattern characterised by high intakes of refined grains, processed meat, fried foods and red meat.

Although the assessment of fruit and vegetable intake in this study was relatively crude (i.e. how many serves of vegetables do you usually eat in a day?) and therefore may not accurately reflect true fruit and vegetable intake, our findings of vegetable intake does appear to be consistent with those of other studies suggesting a positive relationship between vegetable intake and the risk of the metabolic syndrome. Also our study was conducted from October

through December in Queensland, Australia. These months are spring and early summer and as most of Queensland is tropical and subtropical the availability of fruit and vegetables is abundant year round. Thus the time of year in which the study was conducted would have had little effect on results.

Smokers have been found to have significantly lower serum carotenoid concentrations compared with non-smokers [35]. Overall, we found significantly lower concentrations of total carotenoids and the individual carotenoids, except lycopene, in smokers compared with non-smokers (data not shown). However, in relation to metabolic syndrome status, we found no significant difference in serum carotenoid levels by metabolic syndrome status among current smokers. But among former and never smokers serum alpha-carotene and beta-carotene were relatively lower among those with metabolic syndrome than those without. Serum lycopene was marginally lower for those with metabolic syndrome compared to those without among former smokers, while total serum carotenoids was marginally lower for those with metabolic syndrome compared to those without among never smokers. We also found no significant interaction between smoking status and serum carotenoids in relation to the metabolic syndrome.

Our findings were partly similar to that of Sugiura and co-workers [24] who found inverse associations between serum β-carotene among non-smoking adults. Our findings were also similar to those of Hozawa and co-investigators [36], how found an inverse association of total carotenoids with incidence of diabetes and several markers of glucose tolerance only among non- smokers, but no association among current smokers.

There were several limitations in our study. We did not have accurate data on the intake of any of these carotenoids from supplements. Approximately a third of our participants reported taking a vitamin, mineral or herbal supplement in the 24 hours prior to the examination. Although we collected information about the supplements, there was no data base available that could estimate the content of these carotenoids in the supplement. From investigation of a variety of supplements that were on the market at the time, few of them contained any of these carotenoids. Approximately 57% of women in the study were post menopausal,and as might be expected, had higher percentage of women with the syndrome. We did not account for menopause status in our analyses,however,

The cross-sectional design of our study does not permit us to draw inferences regarding causality. It is not possible to conclude whether low serum concentrations of carotenoids found in participants with the metabolic syndrome in our study are the result of increased utilisation of these antioxidants due to the oxidative stress effects of the disease or whether the lower

concentrations are the result of lower intakes of dietary carotenoids and play a role in the pathogenesis of the syndrome.

Oxidative stress is an imbalance, which produces free radicals that overwhelm the body's antioxidant defences and high levels of oxidative stress are known to deplete the body's reserves of antioxidants. Oxidative stress has been implicated in the pathogenesis of several components of the metabolic syndrome including glucose or insulin abnormalities [4], hypertension [37] and obesity [38]. Recent studies [39] have reported elevated markers of oxidative stress among subjects with metabolic syndrome compared with those without the syndrome. Thus the oxidative stress nature of the metabolic syndrome may cause greater utilisation of antioxidants resulting in lower concentrations of antioxidants such as carotenoids.

Another hypothesis is that lower intakes of antioxidant-rich fruit and vegetables result in lower serum concentrations and thus are involved in the pathogenesis of these conditions. Epidemiologic studies have found lower antioxidant intakes with components of the metabolic syndrome such as diabetes status [40]. Suzuki and co-workers, [7] found a significantly lower odds ratio for high HbA1c among those with the highest intakes of carrots and pumpkin compared with a low intake group. The large EPIC-Norfolk study found that persons with higher intakes of vegetables and fruit have higher serum carotenoid concentrations and lower risk of type 2 diabetes [41].

While numerous observational studies have shown an inverse relationship between antioxidant intakes and serum concentrations and the risk of several of these components, clinical trials providing single or combination antioxidants as supplements have not resulted in any beneficial reduction in these conditions [22]. Further research, particularly longitudinal studies may shed light on whether low levels of carotenoids are causally involved in the development of the syndrome, or if they are a result of these pathologies. Clinical trials based on diets high in carotenoid-rich fruit and vegetables may also provide important insights not only in relation to the prevention of components of the metabolic syndrome, but also in reducing the risk of developing the syndrome itself.

Obesity and the other components of the metabolic syndrome are increasing in most countries of the world today and will continue to increase. As populations age, and as overweight and obesity continue to escalate, especially among children, these conditions will become an increasing burden on the health system. Lifestyle interventions have been able to show dramatic reductions in risk of diabetes among those with IGT [42, 43]. However, strategies for both primary and secondary prevention will be necessary to reduce the burden of obesity,

diabetes and the metabolic syndrome in future years, in future generations in both developed and developing countries.

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518 Table 1. Age-adjusted means or percentages for selected baseline characteristics of AusDiab participants
519 aged 25 years and over by metabolic syndrome status (N=1523[1])

Selected characteristics (%, mean)	Metabolic syndrome status				
	Present		Absent		P value[2]
	N	Percent or Mean (SE)[3]	n	Percent or Mean (SE)[3]	
Gender (%)[4]	N	Percent	n	Percent	0.05
Male	195	26.7	451	73.3	
Female	229	21.3	661	78.7	
All persons	424	24.0	1112	76.0	
Age group (yrs) (%)[4]					<0.01
25-34	19	11.0	161	89.0	
35-44	48	14.6	275	85.4	
45-54	114	29.4	270	70.6	
55-64	120	38.4	199	61.7	
65-74	88	38.0	145	62.0	
75+	35	36.2	62	63.8	
Educational status (%)					0.04
Secondary school or less	191	27.5	415	72.5	
Trade certificate, bachelor's degree	214	23.0	598	77.0	
Post-graduate qualification	19	15.8	95	84.2	
Income (%)					0.06
$800-1500+/week	136	19	465	81	
$400-799/week	133	24	327	76	
$0-399/week	147	30	299	70	
Body Mass Index (BMI) (%)					<0.01
Obese (BMI>30)	208	57.2	149	42.8	
Overweight (BMI≥25 to <30)	189	28.3	379	71.7	
Normal (BMI<25)	27	3.2	584	96.8	
Smoking status (%)					0.05
Current	59	25.1	147	74.9	
Former smokers	142	29.8	289	70.2	
Never smoked	218	20.8	660	79.2	
Alcohol intake (%)					0.12
None	105	28.0	233	72.0	
≤ 60 std drinks/month	265	22.1	761	77.9	
> 60 std drinks/month	54	27.8	118	72.2	
Physical activity beneficial to health (%)					0.07
Sufficiently active	179	22.0	542	78.0	
Insufficiently active	134	22.2	380	77.8	

Sedentary	111	33.1	185	66.9	
Vitamin use (%)					0.81
No	265	23.4	705	76.6	
Yes	116	22.8	340	77.2	
Fruit intake (serves/day) %					0.68
≤ 1 serve	72	25.6	177	74.4	
1-3 serves	219	23.4	586	76.6	
≥ 4 serves	123	24.5	329	75.5	
Vegetable intake (serves/day) %					0.02
≤ 1 serve	168	25.0	426	75.0	
1-3 serves	204	24.3	522	75.7	
≥ 4 serves	41	20.1	141	79.9	
Blood Pressure (mmHg)		Mean (SE)		Mean (SE)	
Systolic BP (mean, SE)	420	137.4 (1.9)	1104	121.6 (1.2)	<0.01
Diastolic BP (mean, SE)	421	73.4 (1.2)	1105	65.8 (1.1)	<0.01
Blood Lipids (mmol/L)					
HDL-cholesterol (mean, SE)	424	1.15 (0.03)	1112	1.46 (0.01)	<0.01
Triglyceride (mean, SE)	424	2.54 (0.14)	1112	1.13 (0.03)	<0.01
LDL-cholesterol (mean, SE)	377	3.70 (0.07)	1106	3.37 (0.04)	0.02
Fasting plasma glucose (mmol/L, mean, SE)	424	5.74 (0.08)	1112	5.10 (0.02)	<0.01
Waist circumference in (cm, mean, SE)					
Males	195	106.5 (0.91)	451	91.1 (1.09)	<0.01
Females	229	97.4 (1.42)	661	78.9 (1.33)	<0.01

[1] N is not the same because of missing values

[2] Chi square statistic test of association with adjustment for cluster design; T-test for differences in means for variables with 2 groups

[3] Weighted for age and sex to the Queensland population aged over 25 for the survey year; SE=Standard Error

[4] Unadjusted

Table 2. Adjusted[1] geometric mean concentrations of serum carotenoids by metabolic syndrome for adults 25 years and over who participated in the Queensland AusDiab study, 2000 (N=1523)

Serum Carotenoid (mmol/L)	Metabolic Syndrome present (n=420)		Metabolic Syndrome absent (n=1103)		P value[2]
	Mean[3]	95% CI	Mean[3]	95% CI	
α-carotene	0.10	0.07-0.15	0.13	0.10-0.18	0.02
β-carotene	0.45	0.33-0.62	0.59	0.47-0.73	0.01
β -cryptoxanthin	0.20	0.16-0.24	0.21	0.18-0.25	0.19
Lutein/zeaxanthin	0.41	0.36-0.46	0.41	0.34-0.50	0.89
Lycopene	0.40	0.34-0.47	0.43	0.39-0.49	0.31
Sum of serum carotenoids	1.82	1.62-2.04	2.02	1.76-2.33	0.04

[1] Multivariable linear regression model adjusting for potential cofounders including: age (25-34, 35-44, 45-54, 55-64, 65-74, 75+); sex (male, female); education (post-graduate qualification, trade-certificate and bachelor degree, secondary school); BMI (obese, overweight, normal); alcohol intake (>60drinks per month, <60 drinks per month, none); smoking (current, former, never); physical activity (sedentary, insufficiently active, sufficiently active); and vitamin use during 24hrs (yes, no),

[2] Serum carotenoids were log transformed for regression analyses. Back transformed geometric mean reported in table.

Table 3. Mean (SE) age and sex adjusted[1] concentrations of serum carotenoids by metabolic syndrome components among adults 25 years and over who participated in the Queensland AusDiab study, 2000 (N=1523)

	Number of Metabolic Syndrome components[2] present in participants					
	0	1	2	3	4	5
	n=344	(n=367)	(n=344)	(n=250)	(n=155)	(n=63)
Proportion of participants with metabolic syndrome components (%)	22	24	23	17	10	4
Serum Carotenoid[4] (mmol/L)	Mean (SE)[5]	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
α-carotene	0.21 (0.02)	0.18, (0.03)	0.17, (0.02)	0.15, (0.03)	0.12, (0.02)	0.10, (0.01)
β-carotene	0.83, (0.09)	0.73, (0.10)	0.68, (0.09)	0.60, (0.07)	0.48 (0.06)	0.48 (0.08)
β-cryptoxanthin	0.29, (0.03)	0.31, (0.04)	0.28, (0.02)	0.26, (0.03)	0.23, (0.02)	0.25, (0.06)
Lutein/zeaxanthin	0.46, (0.03)	0.44, (0.03)	0.46, (0.03)	0.43, (0.03)	0.46, (0.02)	0.38, (0.03)
Lycopene	0.62, (0.04)	0.60, (0.04)	0.56, (0.03)	0.49, (0.02)	0.48, (0.03)	0.38, (0.02)
Total serum carotenoids	2.42, 0.16	2.27, 0.21	2.14, 0.13	1.93, 0.15	1.78, 0.10	1.59, 0.16

[1] Multivariable logistic regression model adjusted for age (25-34, 35-44, 45-54, 55-64, 65-74, 75+); sex (male, female); education (post-graduate qualification, trade-certificate and bachelor degree, secondary school); BMI (obese, overweight, normal); alcohol intake (>60drinks per month, <60 drinks per month, none); smoking (current, former, never); physical activity (sedentary, insufficiently active, sufficiently active); and vitamin use (yes, no).

[2] Components = 0 - none of the components of metabolic syndrome are present;

[2] Components =1 - any 1 of the metabolic syndrome components is present

[2] Components=2 - any 2 of the 5 metabolic syndrome components are present

[2] Components =3 - any 3 of the 5 metabolic syndrome components are present

[2] Components =4 - any 4 of the 5 metabolic syndrome components are present

[2] Components = 5 - all 5 metabolic syndrome components are present

[3] ANOVA used to test for differences in means for variables with more than 2 groups

[4] Serum carotenoids not log transformed.

[5] SE=Standard Error

Table 4 (NEW). Odds Ratios (OR†) with 95% Confidence Intervals (CI) for Metabolic syndrome and its components according to quartiles of plasma serum carotenoids.

Serum carotenoids	Metabolic syndrome (n=424)		Abdominal obesity (n=858)		High triacylglycerol (n=474)		Low HDL cholesterol (n=4690		High fasting blood glucose (n=297)		High blood pressure (n=6670	
	OR† 95% CI		OR† 95% CI		OR† 95% CI		OR† 95% CI		OR† 95% CI		OR† 95% CI	
Alpha carotene (μg/day)												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Q2	0.76	0.53, 1.08	0.74	0.37, 1.48	0.83	0.59, 1.18	0.65	0.37, 1.13	0.89	0.65, 1.21	0.81	0.57, 1.17
Q3	0.59	0.40, 0.89	0.69	0.55, 0.87	0.53	0.36, 0.78	0.78	0.50, 1.19	0.72	0.47, 1.10	0.56	0.35, 0.91
Q4	0.36	0.21, 0.63	0.47	0.33, 0.65	0.55	0.36, 0.83	0.46	0.24, 0.92	0.54	0.33, 0.90	0.50	0.42, 0.59
P for trend	= 0.005		= <0.01		= 0.011		= 0.06		= 0.011		= <0.01	
Beta-carotene												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Q2	0.63	0.51, 0.77	0.87	0.33, 2.27	0.70	0.37, 1.33	0.64	0.38, 1.06	0.94	0.47, 1.90	0.47	0.27, 0.80
Q3	0.56	0.35, 0.89	0.58	0.24, 1.36	0.46	0.31, 0.69	0.60	0.31, 1.40	0.91	0.71-1.17	0.48	0.31, 0.73
Q4	0.36	0.24, 0.55	0.57	0.31, 1.06	0.37	0.21, 0.64	0.39	0.20, 0.76	0.72	0.45, 1.17	0.35	0.23, 0.52
P for trend	=0.003		=0.03		= 0.004		= 0.03		= 0.11		= 0.002	
Beta-cryptoxanthin												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Q2	1.00	0.62, 1.63	0.77	0.43, 1.37	1.07	0.61-1.89	0.97	0.49, 1.91	1.07	0.75, 1.52	0.93	0.41, 2.09
Q3	0.68	0.35, 1.32	0.82	0.29-2.29	0.59	0.46, 0.77	0.58	0.37, 0.90	1.09	0.33, 3.59	0.80	0.44, 1.46
Q4	0.78	0.51, 1.20	0.88	0.47-1.64	0.71	0.46, 1.10	0.59	0.35, 1.01	0.69	0.40, 1.20	0.91	0.43, 1.94
P for trend	=0.07		=0.74		= 0.03		= 0.05		= 0.41		= 0.69	
Lutein/zeaxanthin												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Q2	1.13	0.55, 2.33	0.91	0.67, 1.25	1.00	0.53, 1.90	0.90	0.45, 1.80	0.95	0.75, 1.20	0.97	0.61, 1.55
Q3	1.12	0.64, 1.95	1.17	0.72-1.91	1.37	0.85, 2.22	0.55	0.26, 1.18	1.09	0.64-1.88	0.88	0.55, 1.41
Q4	0.93	0.56, 1.52	0.77	0.36, 1.67	1.19	0.57, 2.48	0.47	0.21, 1.05	0.57	0.33, 1.00	0.95	0.74, 1.23
P for trend	=0.79		=0.72		= 0.37		= 0.04		= 0.13		= 0.53	
Lycopene												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref

Q2	0.98	0.54, 1.78	1.00	0.73-1.38	1.01	0.47, 2.19	0.93	0.59, 1.44	0.76	0.47, 1.24	1.32	0.96, 1.80
Q3	0.72	0.44, 1.17	0.97	0.63, 1.48	0.76	0.48, 1.22	0.64	0.37, 1.09	0.88	0.54-1.42	1.09	0.80, 1.50
Q4	0.76	0.48, 1.21	0.83	0.53, 1.29	0.75	0.47, 1.19	0.64	0.37, 1.11	1.13	0.86, 1.48	0.98	0.62, 1.57
P for trend	=0.14		= 0.29		=0.07		= 0.06		= 0.20		= 0.54	
Total serum carotenoids												
Q1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Q2	0.63	0.47, 0.85	0.66	0.45, 0.98	1.03	0.66, 1.61	0.63	0.33, 1.18	0.61	0.26, 1.43	0.62	0.35, 1.12
Q3	0.87	0.51, 1.50	0.86	0.66, 1.12	0.88	0.68, 1.12	0.68	0.32, 1.41	0.98	0.73, 1.32	0.57	0.34, 0.94
Q4	0.43	0.23, 0.79	0.60	0.35, 1.02	0.57	0.34, 0.96	0.41	0.20, 1.85	0.56	0.26, 1.22	0.47	0.27, 0.81
P for trend	=0.04		=0.10		= 0.03		= 0.05		= 0.14		= 0.008	

[1]OR = Odds Ratio

Logistic regression adjusted for Adjusted for age (25-34, 35-44, 45-54, 55-64, 65-74, 75+); sex (male, female); education (post-graduate qualification, trade-certificate and bachelor degree, secondary school); alcohol intake (>60drinks per month, <60 drinks per month, none); smoking (current, former, never); physical activity (sedentary, insufficiently active, sufficiently active); vitamin use (yes, no) and BMI (obese, overweight, normal).

Table 5. Relation between serum carotenoids and metabolic syndrome by smoking status

	Current smokers (n=183)		P value	Former smokers (n=391)		P value	Never smokers (n=817)		
	Metabolic syndrome absent	Metabolic syndrome present		Metabolic syndrome absent	Metabolic syndrome present		Metabolic syndrome absent	Metabolic syndrome present	P value
	Mean (95% CI)	Mean (95% CI)		Mean (95% CI)	Mean (95% CI)		Mean (95% CI)	Mean (95% CI)	
Alpha carotene	0.08 (0.07-0.09)	0.07 (0.03-0.14)	0.59	0.14 (0.10-0.21)	0.11 (0.07-0.17)	0.03	0.15 (0.11-0.20)	0.11 (0.08-0.16)	0.04
Beta-carotene	0.42 (0.36-0.50)	0.38 (0.19-0.76)	0.72	0.63 (0.47-0.84)	0.44 (0.29-68)	0.02	0.63 (0.51-0.78)	0.49 (0.37-0.64)	0.01
Beta-cryptoxanthin	0.12 (0.10-0.15)	0.11 (0.09-0.15)	0.27	0.22 (0.19-0.25)	0.20 (0.15-0.28)	0.57	0.24 (0.20-0.28)	0.22 (0.18-0.27)	0.40
Lutein/zeaxanthin	0.33 (0.28-0.40)	0.32 (0.29-0.36)	0.80	0.40 (0.34-0.48)	0.41 (0.34-0.48)	0.87	0.43 (0.35-0.53)	0.41 (0.35-0.48)	0.65
Lycopene	0.37 (0.27-0.50)	0.39 (0.28-0.53)	0.80	0.48 (0.40-0.58)	0.37 (0.29-0.48)	0.06	0.43 (0.39-0.48)	0.43 (0.39-0.49)	0.97
Total serum carotenoid	1.47 (1.29-1.67)	1.43 (1.02-2.00)	0.87	2.11 (1.79-2.50)	1.80 (1.45-2.24)	0.10	2.13 (1.85-2.47)	1.92 (1.77-2.10)	0.08

Multivariable linear regression adjusting for age (25-34, 35-44, 45-54, 55-64, 65-74, 75+); sex (male, female); education (post-graduate qualification, trade-certificate and bachelor degree, secondary school); alcohol intake (>60drinks per month, <60 drinks per month, none); physical activity (sedentary, insufficiently active, sufficiently active); vitamin use (yes, no) and BMI (obese, overweight, normal).