Two common single nucleotide polymorphisms in the gene encoding β-carotene 15,15′-monoxygenase alter β-carotene metabolism in female volunteers

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ABSTRACT **The key enzyme responsible for β-carotene conversion into retinal is β-carotene 15,15′-monoxygenase (BCMO1). Since it has been reported that the conversion of β-carotene into vitamin A is highly variable in up to 45% of healthy individuals, we hypothesized that genetic polymorphisms in the *BCMO1* gene could contribute to the occurrence of the poor converter phenotype. Here we describe the screening of the total open reading frame of the *BCMO1* coding region that led to the identification of two common nonsynonymous single nucleotide polymorphisms (R267S: rs12934922; A379V: rs7501331) with variant allele frequencies of 42 and 24%, respectively. *In vitro* biochemical characterization of the recombinant 267S + 379V double mutant revealed a reduced catalytic activity of BCMO1 by 57% ($P<0.001$). Assessment of the responsiveness to a pharmacological dose of β-carotene in female volunteers confirmed that carriers of both the 379V and 267S + 379V variant alleles had a reduced ability to convert β-carotene, as indicated through reduced retinyl palmitate:β-carotene ratios in the triglyceride-rich lipoprotein fraction [−32% ($P=0.005$) and −69% ($P=0.001$), respectively] and increased fasting β-carotene concentrations [+160% ($P=0.025$) and +240% ($P=0.041$), respectively]. Our data show that there is genetic variability in β-carotene metabolism and may provide an explanation for the molecular basis of the poor converter phenotype within the population.—Leung, W. C., Hessel, S., Méplan, C., Flint, J., Oberhauser, V., Tourniaire, F., Hesketh, J. E., von Lintig, J., Lietz, G. Two common single nucleotide polymorphisms in the gene encoding β-carotene 15,15′-monoxygenase alter β-carotene metabolism in female volunteers. *FASEB J.* 23, 1041–1053 (2009)**

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VITAMIN A DEFICIENCY IS A SERIOUS public health problem that mostly affects pregnant or lactating women and preschool children, with an estimated 250 million at risk of developing vitamin A deficiency disorders (1). Deficiency in these vulnerable groups occurs largely due to increases in physiological requirements together with a low dietary intake of vitamin A (2). Vitamin A deficiency causes high mortality rates in Asia and Africa (3). However, low vitamin A intakes have also been described in Western societies; for example, 15% of young individuals aged 19–24 yr in the United Kingdom have a total vitamin A intake below the lower recommended nutrient intake level (4), and almost half of American postmenopausal women may experience frank or marginal vitamin A deficiency unless they supplement with vitamin A (5).

Provitamin A carotenoids such as β-carotene are a major dietary source of vitamin A. In Asia and Africa, ~80% of dietary vitamin A is derived from carotenoid-rich plants (6). To combat vitamin A deficiency, various efforts are being undertaken toward vitamin A intake, *e.g.*, by increasing the content and bioavailability of provitamin A sources (7). Due to the recognized adverse health effects of excess supplementation with preformed vitamin A, provitamin A supplementation has also been put forward as a more appropriate intervention to satisfy the increased demand of pregnant and lactating women in Western countries (8).

β-Carotene is the most abundant provitamin A carotenoid in the diet and, once absorbed, undergoes conversion into different metabolites. The enzyme responsible for β-carotene conversion into retinal is β-carotene 15,15′-monoxygenase (BCMO1), and ~95% of retinoids arising from β-carotene are produced by this pathway *in vivo* (9). Studies in a knockout mouse model for *BCMO1* have provided further evidence for the fundamental role of this enzyme in producing vitamin A from dietary β-carotene (10). BCMO1 is a soluble cytosolic enzyme that shows highest activity in intestinal

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mucosa, specifically in jejunal enterocytes (11). Elevated levels of *BCMO1* mRNA are also found in the liver, lung, and kidney, and lower levels can be found in the brain, prostate, ovary, colon, and skeletal muscle, which suggests the importance of local vitamin A synthesis (12). In animal models, evidence (13) has been provided that the intestinal expression of *BCMO1* is regulated by a negative feedback mechanism, since the vitamin A derivative retinoic acid down-regulates mRNA levels of *BCMO1* by up to 90%. This feedback regulation may involve the intestine-specific homeobox transcription factor Isx (14). In the liver and in peripheral tissues, additional factors influence mRNA expression of this gene, probably *via* the interaction with two regulatory elements (MEF2/PPARγ) in the *BCMO1* promoter region (15, 16).

The vitamin A activity of β-carotene, even when measured under controlled conditions, is highly variable and surprisingly low (17–21). In double-tracer studies, 27–45% of volunteers have been classified as poor converters (19–21). These individuals have a capacity to form only 9% vitamin A from β-carotene compared with those who are classified as normal converters (21). This large interindividual difference might be caused by reduced enzymatic activity as a consequence of genetic polymorphisms in the *BCMO1* gene. Indeed, it was recently shown (22) that the T170M missense mutation in the *BCMO1* gene causes a dramatic decrease in the enzyme activity *in vitro* and is associated with hypercarotenemia and hypovitaminosis A in a heterozygote carrier. However, given its very low frequency (22), this mutation cannot explain the high frequency of the low converter phenotype observed in humans.

The aims of the present study were to identify common single nucleotide polymorphisms (SNPs) in the human *BCMO1* gene and to determine their potential functionality by assessing their influence on BCMO1 enzyme activity *in vitro* and on β-carotene conversion efficiency in healthy human volunteers. Here we report on the identification of two nonsynonymous SNPs (R267S and A379V) in the human *BCMO1* gene with variant allele frequencies of 42 and 24%, respectively. Biochemical characterization of respective recombinant BCMO1 variants revealed an effect on enzyme activity for the double mutation (267S+379V). Responsiveness to a pharmacological dose of β-carotene in a human intervention study revealed that these SNPs were associated with significant alterations in β-carotene metabolism in female volunteers.

MATERIALS AND METHODS

Design of amplicons

The nucleotide sequence of *BCMO1* was obtained from Ensembl (Gene ID ENSG00000135697). The *BCMO1* gene (located on chromosome: 16; maps: 16q21-q23) contains 11 exons producing a 547-amino acid protein. Twelve amplicons were designed to span each of the 11 exons (**Table 1**). In addition, primers were designed to identify potential SNPs in the upstream regulatory region of *BCMO1* as defined by Gong *et al.* (16). Primer specificity was checked by blasting the primer sequence against the human genome using BLAST

TABLE 1. *BCMO1 amplicon primers and PCR annealing temperatures*

Amplicon	Primer	Sequence (5′ to 3′)	Annealing T_m (°C)	Amplicon length
1	Forward	CTCTTGGATTACGTCGGGCAACATAACAG	66.6	543
	Reverse	GAAGAAAGAGCCCAGTGTTTATCAGAATGCTC		
2	Forward	CAGTGCTAGCCAACTGCCCAACACATTATAC	66.8	537
	Reverse	CAAGTCAAGGAAAGCGGCTTCTAGAAGATAG		
3	Forward	GCCCTGTGTACTAAAGGAGCTTGTCCTAGTG	66.8	435
	Reverse	CTCTGGCAATCAGTGATCAAGAACTGTGTAG		
4	Forward	CTAAAGCCATCAAGGATAGACTTCTCTGG	64.5	425
	Reverse	GATACAGACACGAAGATAAAAACAACACAGTC		
5	Forward	CAAATCCCCATGTGAGGTTGTATAAGGTCAC	66.7	484
	Reverse	GTACCACACTGGATCTTGTGTCATCAACG		
6	Forward	GTCTGTCTACACGTTGCTGTTTAATGTAAAGG	65.5	392
	Reverse	GCACCCACTGGGCTAGAGTCCAG		
7	Forward	CAAACACTGTCCTCCTAGTGACTGCAAAGAC	68	585
	Reverse	CCACCTGGGCCGATATCTCTCTAGAAAG		
8	Forward	CTCAGACTGCATACGCAATTTGTCCTG	65.1	610
	Reverse	CTGTGAAAATCTGCCCCTTTCCTAAACTCAG		
9	Forward	CTTTGACCACCTTTGATCCAATGCAGG	65.1	570
	Reverse	CAGGGATTGGGAATCTCCCTGCTG		
10	Forward	GTCAAAAGTCTACATCCGATGGGCTTCATC	66.5	637
	Reverse	CTATCTGTGATCTACTTGGAGAGGCACCTG		
11A	Forward	CATCCCTCCGACTGAAGCCTGC	64.5	427
	Reverse	CTCTTGCCATGCCCTTGTCAAAGC		
11B	Forward	GTCAGAACTCCATGGATATGTTTCTTTGGATG	64.5	612
	Reverse	GGCTGTGAACCATCAAGCTCTGCAAC		
Promoter	Forward	GCTCAAGTTTCTCCTAATTCCCAG	66.0	540
	Reverse	CCTGCCAAATATTATATCCATTGCTC		

(http://www.ncbi.nlm.nih.gov/BLAST). DNA was extracted from buffy coat fractions as described previously (23). Samples were amplified using high-fidelity Expand *Taq* polymerase (Roche, East Sussex, UK) under the following conditions: 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at the optimized primer annealing temperature, 1 min elongation at 72°C, and a final cycle at 72°C for 1 min. polymerase chain reaction (PCR) fragments were then denatured at 95°C for 5 min and cooled to 40°C over 40 min using a Thermo hybrid Px2 cycler (Fisher Scientific, Loughborough, UK). Five-microliter aliquots of sample were analyzed on the Transgenomic WAVE denaturing HPLC (dHPLC) system (Transgenomic, Crewe, UK) using an acetonitrile gradient at several temperatures for optimal mismatch detection (Table 1). For each amplicon, 20 DNA samples were first analyzed by dHPLC to determine the presence of heterozygotes so that polymorphisms with a frequency of 5% or greater could be identified. Samples exhibiting heterozygosity were then sequenced (MWG-Biotech, London, UK) to confirm the presence of a SNP and to identify the polymorphism. The rs numbers were given to previously described SNPs, and where these were not available, Contig numbers were allocated (**Fig. 1**; **Table 2**).

Genotyping DNA samples for rs12934922 (R267S) and rs7501331 (A379V)

The 75 female and 56 male volunteers were recruited through poster- and web-based advertisements from Newcastle and North Tyneside, UK. Informed and written consent was obtained from all volunteers, and ethical permission was obtained from the Newcastle and North Tyneside Local Research Ethics Committee. DNA samples were genotyped for rs12934922 (R267S) and rs7501331 (A379V) using the Roche LightCycler 2.0 (Roche) and the Transgenomic WAVE dHPLC, respectively. Primers and probes used for genotyping are given in **Table 3**. Distinguishing between homozygote variant and homozygote wild-type genotypes using WAVE dHPLC requires that homozygote variant samples are mixed with known homozygote wild-type samples. Samples for the detection of A379V mutations were amplified with conditions set at 1 cycle at 94°C for 5 min, followed by 30 cycles for 30 s at 94°C, 30 s at 60.8°C, 1 min at 72°C for PCR product

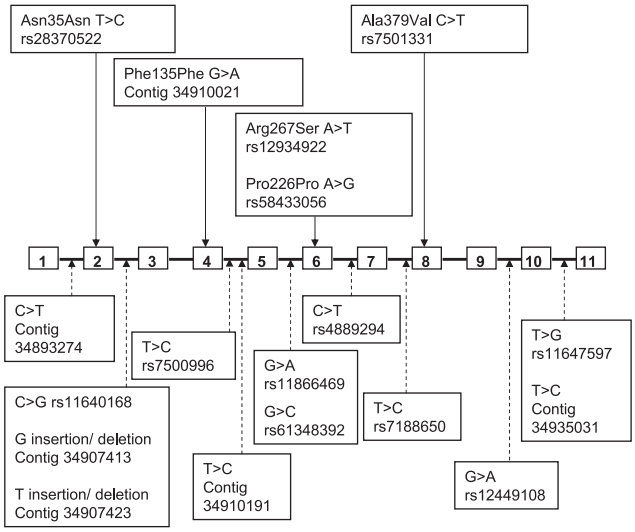


Figure 1. Location of identified SNPs within the *BCMO1* gene by dHPLC and sequencing. Exons are represented as numbered boxes and introns as straight lines.

elongation, and a final cycle at 72°C for 1 min. Optimal mismatch detection was at 58.5°C. The LightCycler PCR conditions were 1 cycle at 94°C for 2 min and 40 cycles of 94°C 15 s, and an optimal annealing temperature of 52°C for 1 min and 1 cycle at 72°C for 1 min. The melt curve analysis involved cooling the temperature to 35°C and slowly increasing (temperature elevation of 0.1°C/s) it to 80°C.

Gene cloning and protein expression

TC7 Caco-2 cells have been shown to contain intrinsic BCMO1 activity and *BCMO1* mRNA (24). To clone full-length human *BCMO1* cDNA, RNA was isolated from TC7 Caco-2 cells using TRIzol reagent (Invitrogen, Paisley, UK), and first-strand cDNA was synthesized using the Superscript II reverse transcriptase enzyme (Invitrogen). The resulting *BCMO1* cDNA was amplified by PCR using forward primer GCT GTT AAA ATC GAT CTC CCT CGG CAC CC and the reverse primer GGA GTT CTG ACA GCC GAG CTC CCC TC (MWG-Biotech) and Expand High Fidelity *Taq*DNA polymerase (Roche) using the following conditions: 1 cycle at 95°C for 5 min, 30 cycles at 95°C for 1 min, 70°C for 1 min 30 s, 72°C for 2 min, and 1 cycle at 72°C for 1 min. After the PCR product was purified with the Qiagen Qiaquick purification kit (Qiagen, West Sussex, UK), the *BCMO1* insert was cloned into the pCR Blunt vector using a Zero Blunt PCR cloning kit (Invitrogen). In a second ligation reaction, the *BCMO1* insert was extracted from the Zero Blunt vector using a gel extraction kit by Eppendorf (VWR, Lutterworth, UK) and cloned into the pTrcHis vector (Invitrogen) containing a C-terminal His_6 tag. The *Escherichia coli* strain TOP10 was transformed with the pTrcHis vector and plated onto ampicillin 100 μg/ml resistant Luria-Bertani agar media at 37°C. Plasmids were isolated and checked for the presence of the *BCMO1*-His insert by direct sequencing (MWG-Biotech). Expression of BCMO1-His protein in *E. coli* was confirmed by Western blot analysis as described below.

PCR-based site-directed mutagenesis

Site-directed mutagenesis was carried out using a PCR-based QuikChange kit (Stratagene, Amsterdam, The Netherlands) according to the manufacturer's instructions, using *BCMO1*-His insert as the template and the following primers synthesized by MWG-Biotech: forward primer for R267S: CAA CCG CAT ACA TCC GGA GAA TGA GCT GG; reverse primer for R267S: CCA GCT CAT TCT CCG GAT GTA TGC GGT TG; forward primer for A379V: CAC AAA TTT AAT CAA AGT GGC ATC TAC AAC AG; and reverse primer for A379V: CTG TTG TAG ATG CCA CTT TGA TTA AAT TTG TG. The following PCR conditions were used: 30 s at 95°C for 1 cycle; 30 s at 95°C, 1 min at 52°C, and 7 min at 68°C for 18 cycles; and 14 min at 68°C for 1 cycle. The validity of all point mutations and the integrity of the open reading frame were verified by sequencing (MWG-Biotech).

Determination of enzymatic activity *in vitro*

E. coli XL1blue cells harboring *BCMO1*-His were cultured in Luria-Bertani broth at 37°C to midexponential phase (A_{600}=0.6), and protein expression was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside to 1 mM final concentration and incubation overnight at 25°C, 180 rpm. Cells were centrifuged at 6000 rpm for 10 min and resuspended in Tricine buffer (50 mM; with 100 mM NaCl), pH 7.5, containing 1 mM dithiothreitol and protease inhibitor (Complete Protease Inhibitor Cocktail Tablets EDTA-free; Roche). Cells were lysed using a French press (Thermo

TABLE 2. *Location, base change, and corresponding amino acid changes of mutations detected in the BCMO1 gene using dHPLC*

Amplicon	Contig position	Region	Heterozygosity in 20 samples	SNP allele	Amino acid change	Amino acid position
1			0	None		
2	34893274	Intron 1	9	C/T		
2	34893319	Exon 2	9	T/C	AAT/AAC (Asn/Asn)	35
2	34893577	Intron 2	9	C/G		
3	34907413	Intron 2	1	G insertion/deletion		
3	34907423	Intron 2	18	T insertion/deletion		
4	34910021	Exon 4	3	T/C	TTC/TTT(Phe/Phe)	135
4	34910101	Intron 4	1	T/C		
4	34910191	Intron 4	3	G/A		
5			0	None		
6	34915638	Intron 5	1	G/A		
6	34915659	Intron 5	7	G/C		
6	34915770	Exon 6	1	A/G	CCA/CCG (Pro/Pro)	226
6	34915893	Exon 6	8	A/T	AGA/AGT (Arg/Ser)	267
7	34917958	Intron 6	9	C/T		
8	34928580	Intron 7	6	T/C		
8	34928695	Exon 8	9	CT	GCA/GTA (Ala/Val)	379
9	34933637	Intron 9	6	G/A		
10	34935031	Intron 10	5	T/C		
10	34935497	Intron 10	2	T/G		
11A			0	None		
11B			0	None		
Promoter[a]			0	None		

[a]Heterozygosity determined in 80 samples.

Scientific, Pittsburgh, PA, USA), and lysates were centrifuged at 10,000 *g* for 5 min to remove insoluble protein and cell components. The supernatant fluid was kept on ice, and tests for enzymatic activity of different variants were then immediately performed. Protein concentrations were determined using the Roti-Nanoquant-solution (Roth, Karlsruhe, Germany). The supernatant of lysed *E. coli* cells and affinity purified His-tagged BCMO1 were subjected to SDS-PAGE. For Western blot analyses, anti-His-tag antibodies (Qiagen, Hilden, Germany) were used. Immunostaining was carried out with a dilution of 1:500 and the ECL system according to the manufacturer's protocol (Amersham Pharmacia Biotech). Quantification of bands was carried out by the Quantity-one (version 4.6) software (Bio-Rad, Richmond, CA, USA) in comparison with known amounts of affinity purified BCMO1.

Enzymatic reactions were performed in a volume of 100 μl in an assay buffer consisting of 50 mM Tricine-KOH (pH 7.5), 100 mM sodium chloride, 10 μM Fe_2SO_4, 1 mM dithiothreitol, protease inhibitor (Complete Protease Inhibitor Cocktail Tablets EDTA-free, Roche), catalase (1 mg/ml final conc.), and 3% (w/v) 1-*S*-octyl-β-D-thioglucopyranoside (OTG). For the production of the β-carotene micelles used for a 100 μl enzyme assay, 25 μl 12% OTG in ethanol was mixed with various concentrations of β-carotene in hexane (Wild, Heidelberg, Germany). The organic solvent was evaporated in a speedvac, and protein extract was added under vigorous vortexing for 30 s. The reaction mixture was incubated on an Eppendorf shaker (300 rpm, 28°C) and was stopped by addition of 200 μl 2 M hydroxylamine (pH 6.8) and 200 μl methanol. The β-carotene concentration in the assays varied between 2 and 16 μM, and the amount of BCMO1-His enzyme varied between 2.6 and 10.4 μg/reaction. Each parameter (protein amount, substrate concentration, and time linearity) was measured in triplicate. After indicated time points, samples were snap frozen at −80°C. Lipophilic compounds were extracted and subjected to HPLC analysis as described previously (25). All steps were carried out under a red dim safety light.

β-Carotene supplementation study

Since provitamin A supplementation has been proposed as an appropriate intervention to satisfy the increased demand for vitamin A in pregnant and lactating women in Western countries (8), we recruited young female volunteers up to the age of 30 yr with a maximum body mass index (BMI) of 30 for

TABLE 3. *Primers and probes used in the genotyping of samples for R267S (rs12934922) and A379V (rs7501331)*

SNP	Primer	Sequence	Length (nucleotides)	Annealing T_m (°C)
A379V	Forward	CACTAAAGCAAATGTTTGCTCTGG	24	65.8
	Reverse	CTGTGAAAATCTGCCCCTTTCCT	23	68.4
R267S	Forward	CTTTGGAGTCACCGAGAAC	19	55.3
	Reverse	GGTGAAGTTTAAGGCAAGG	19	54.6
R267S	Probe			
	Anchor	GGATATTCTCAAGATGGCAACCGC	19	
	Detector	CATCCGGAGAATGAGCTGG	24	

the β-carotene supplementation study. Out of the recruited 75 female participants, 28 volunteers were eligible and their age and BMI are given in **Table 4**. They were in good health, did not use medication suspected to interfere with absorption of fat soluble vitamins, and had not taken multivitamins A, C, E, or β-carotene supplements in the 3 months before the study. Exclusion criteria were high blood pressure, diabetes, liver disease, and heart attack in the past 2 yr; any abnormalities in fat metabolism; and being pregnant, lactating, or smoking. Informed and written consent were obtained from all volunteers, and ethical permission was obtained from the Newcastle and North Tyneside Local Research Ethics Committee. Volunteers fasted for 12 h before consuming a single oral dose of 120 mg β-carotene (10% CWS; DSM, Basel, Switzerland) together with a fat-rich meal. Fasting blood samples (20 ml) were collected on the morning of the experiment, and second blood sample (20 ml) was taken 3 h after consumption of the test meal. During the 3 h volunteers were only allowed to drink water. The test meal was designed to reflect the same nutrient content as described by Borel *et al.* (17).

Dietary assessment

The dietary intake of volunteers before the experiment was monitored by a 4 day (including 1 weekend day) estimated food diary followed by a brief interview to quantify the amount of food consumed using the Photographic Food portions atlas (Department of Nutrition and Dietetics, King's College London, London, UK). Daily nutrient intakes were calculated using the Windiets 2005 software program [Commercialisation, Research, and European Development Office (CREDO), The Robert Gordon University, Aberdeen, UK] (see Table 4).

Plasma and triglyceride-rich lipoprotein fraction preparation

Blood samples were collected in 10 ml EDTA Vacutainers (Southern Syringe Service Limited, Manchester, UK), placed in ice and covered to avoid exposure to light, and then centrifuged at 3000 rpm for 10 min at 4°C. Plasma was collected and stored at −80°C until analysis. The DNA containing buffy coat fraction was carefully removed to a sterile 1.5 ml tube and stored at −20°C for DNA extraction and genotyping. Triglyceride-rich lipoprotein (TRL) fractions were prepared by overlaying 1.6 ml of plasma with 0.3 ml of NaCl (Sigma, Dorset, UK) solution (density=1.006 g/ml) in polyallomer tubes and centrifuged at 100,000 rpm for 98 min at 4°C in an Optima Max ultracentrifuge (Beckman Coulter, High Wycombe, UK). Tubes were sliced, 0.3 ml of the upper layer was removed, and the lid was washed with 0.35 ml of NaCl solution to remove any residues. Samples were covered in foil to prevent oxidation of β-carotene by sunlight. TRL fractions were frozen at −80°C until they were extracted and analyzed by HPLC. After these protocols, the analyses of triglycerides, retinyl esters, and carotenes in the TRL fraction were shown to be unaffected by freezing (26).

HPLC analysis of plasma and TRL samples

The 100 μl of plasma or 600 μl of TRL was deproteinized with 1 ml of analytical grade ethanol (VWR) containing 10 μl of trans β-apo-8′-carotenal as the internal standard, extracted with 2 ml of analytical grade hexane containing 0.01% butylated hydroxytoluene and vortex mixed. After centrifugation at 3000 rpm and 4°C for 15 min, the hexane layer was removed and evaporated under a stream of nitrogen. Dried residues were redissolved in 200 μl of HPLC grade acetonitrile/chloroform/methanol (70/15/10 v/v/v; Fisher Scientific). Forty microliters per sample was injected for analysis of retinyl esters and carotenes. HPLC analysis was performed according to published methods (26).

TRL triglyceride analysis

Triglyceride analysis was performed on a Roche Cobas Mira that was first standardized with ABX Pentra Multical solution. Each reaction contained the following: 3 μl TRL, 10 μl water, and 290 μl Triglyceride CP ABX Pentra reagent; absorbance was measured at 500 nm.

Statistical analysis

Statistical analyses were performed by using the SPSS software package version 14.0 (SPSS, Chicago, IL, USA). Results are expressed as mean ± SE. Dietary macronutrients, dietary retinol, dietary β-carotene, plasma retinol, plasma β-carotene, TRL retinyl palmitate, TRL β-carotene, and TRL triglycerides were ln- transformed to improve normality. A χ^2 test was performed to determine whether genotype frequencies were in Hardy-Weinberg equilibrium. Haplotype analysis was performed using Haploview (27). Correlation coefficients and their probability levels were obtained from linear regression analyses. Stepwise multiple regression analysis was carried out to select significant covariates ($P<0.05$) among anthropometry factors, dietary intake, and related biological analytes. Then, multiple regression analysis was used to test relationships among retinol concentrations, carotenoid concentrations, and gene polymorphisms, accounting for significant

TABLE 4. *Characteristics and dietary habits of study volunteers (n=28)*

Variable	Geometric mean	Range	95% CI	CV (%)
Age (y)	20.3 ± 0.3[a]	19–26		7.5
Body mass index (kg/m^2)	22.1 ± 0.4[a]	18.3–26.4		8.6
Dietary intake				
Energy (MJ/day)	6.4		5.8–7.1	24.5
Carbohydrate (g/day)	193.9		175.6–214.1	24.8
Protein (g/day)	67.5		59.5–76.5	29.5
Fat (g/day)	47.4		40.2–55.8	40.4
Retinol (μg/day)	133		98–180	64.6
β-Carotene (μg/day)	1193		802–1774	95.4
Retinol equivalents (μg/day)	445		325–609	80.3

[a]Arithmetic mean ± SE.

covariates selected in the previous step. For this analysis, homozygote and heterozygote variants were combined and compared with wild-type carriers. When a polymorphism was significantly associated with plasma/TRL carotenoid and retinol concentrations, an independent sample *t* test or a one-way ANOVA was used. When the ANOVA was significant, a Tukey-Kramer test was used to detect which groups were statistically different from each other. A level of $P < 0.05$ was accepted as significant.

RESULTS

Novel SNP detection in *BCMO1* using dHPLC

To screen the *BCMO1* gene for novel polymorphisms, amplicons were designed to span the total open reading frame of the coding region, including exon-intron boundaries, to allow the detection of single base pair substitutions in splice junctions. Twelve different PCR products, ranging from 392 to 637 base pairs in length, were amplified from the *BCMO1* coding region. Twenty different DNA samples were screened for each of the 12 amplicons to detect potential SNPs with a frequency ≥5%. No heterozygosity was detected in amplicons 1, 5, 11A, and 11B (Table 2). The dHPLC mutation detection system revealed that the other eight amplicons contained genetic variations at varying frequencies. Sequencing of samples showing heterozygosity identified 18 different SNPs, of which 13 were located in exon-intron boundaries and 5 were within the coding region of *BCMO1* (Fig. 1; Table 2). Of the five SNPs located in the coding region, only two were nonsynonymous SNPs, with R267S (rs12934922) in exon 6 and A379V (rs7501331) in exon 8. The following rare SNPs in the coding region of *BCMO1* were not identified: T121T (rs35683292) in exon 4, T170M (Contig position 34912481) in exon 5, R228C (Contig position 34915774) in exon 6, S236Y (rs34746358) in exon 6, and K387K (rs7501162) in exon 8. Screening for SNPs in the promoter region of *BCMO1* failed to show heterozygosity in 80 samples, and the absence of SNPs in the promoter region was confirmed by sequencing of 25% of these samples (Table 2).

Frequency of A379V and R267S in UK Caucasians

Allele frequency for the A379V (rs7501331) SNP in exon 8 was determined by dHPLC in 131 samples (**Table 5**). The frequency of the wild-type C allele and variant T allele was 76 and 24%, respectively; 56% of the population was CC wild-type genotype, and 39% was heterozygote CT with the TT variant present in 5% of the population. Due to the complex elution profiles produced by the presence of up to 4 SNPs in amplicon 6, it was not possible to genotype R267S (rs12934922) using the dHPLC. A LightCycler assay was therefore developed to genotype R267S. The frequency of the wild-type A allele and variant T allele was 58 and 42%, respectively; 38% of the population was AA wild-type genotype, 40% was heterozygote AT, and 22% was TT homozygote (Table 5). Both the R267S (rs12934922) SNP and the A379V (rs7501331) SNP were found to be Hardy Weinberg equilibrium. Although R267S and A379V variants are not in linkage disequilibrium ($D'=0.409$, $r^2=0.038$, and LOD=1.26), 23.7% of volunteers in this study were found to carry at least one variant T allele from both SNPs (R267S+A379V; Table 5).

Biochemical characteristics of recombinant *BCMO1*

The full-length *BCMO1* cDNA was synthesized by reverse transcription PCR using mRNA isolated from the Caco-2 TC7 cells and cloned into Zero Blunt vector. Its sequence was in agreement with the published mRNA sequence of *BCMO1* (GenBank reference sequence NM_017429). The *BCMO1* insert was recloned into the pTrcHis vector containing a C-terminal His_6 tag. The *BCMO1* cDNA sequence inserted was 1727 nucleotides in length encoding a protein of 553 amino acids in length (547 amino acids excluding the hexahistidine tag) with a predicted molecular mass of 63.4 kDa (http://www.expasy.ch/tools/protparam.html). Three *BCMO1* variant plasmids were generated by site-directed mutagenesis containing one or both of the following SNPs, R267S (rs12934922) and A379V (rs7501331), and were confirmed by direct sequencing. Levels of BCMO1 variant expression from bacterial cell extracts were measured by Western blot analysis (**Fig. 4**). Comparable amounts of different BCMO1 variants were measured in all four cell lysates, thus excluding the possibility that polymorphism caused instability of proteins when expressed in *E. coli*. This approach allowed robust measurement and thus comparison of subtle differences of enzymatic properties of

TABLE 5. *BCMO1 R267S and A379V SNP frequencies (n=131)*

SNP	Genotype	*n*	%
R267S (rs12934922)	A/A	50	38.2
	A/T	52	39.7
	T/T	29	22.1
A379V (rs7501331)	C/C	74	56.5
	C/T	51	38.9
	T/T	6	4.6
R267S + A379V (combined)	AA/CC, AA/CT, AA/TT, AT/CC, TT/CC	100	76.3
	AT/CT, AT/TT, TT/CT	31	23.7

different protein variants in a single large experiment. In contrast, affinity-purified BCMO1 was sensitive to freezing and thawing and lost activity during storage at 4°C in a time-dependent manner (data not shown).

The activity of the recombinant BCMO1 variants was investigated by performing enzyme assays using the 10,000 *g* supernatant of the bacterial homogenate with β-carotene as the substrate. All four recombinant BCMO1 proteins were able to catalyze the cleavage of β-carotene *in vitro*, and the formation of retinal was linear up to 30 min when 10 μM β-carotene was used as a substrate with 10.4 μg of recombinant BCMO1 protein (**Fig. 2**). Since it is not uncommon for an enzyme to lose activity when it is diluted and since mutant enzymes are usually less stable than their natural counterparts, the enzyme inactivity was tested in all four recombinant BCMO1 proteins using the Selwyn test (28). Progress curves of product formation were compared at three different enzyme concentrations (2.6, 5.2, and 10.4 μg of recombinant BCMO1 protein; **Fig. 3**). Plots of synthesized product against time multiplied by the initial enzyme concentration were superimposable, indicating the absence of enzyme inactivation.

Biochemical analysis of recombinant enzymes revealed that the 267S + 379V double mutation significantly lowered the BCMO1 enzyme activity by 43%, with the specific activity of the 267S + 379V mutant being 1.0 nmol retinal/mg protein × min compared with 2.3 nmol retinal · mg protein^{-1} · min^{-1} for the wild-type (R267/A379) enzyme ($P<0.001$; **Fig. 4**). The Michaelis-Menten constant (K_m) was similar for both enzymes with 18.3 and 19.5 μmol for the wild-type enzyme and the 267S + 379V double mutant, respectively. Enzymatic analysis of the 379V mutation indicated a reduction in K_m by 33%; however, this was not statistically significant. Due to the effects of the 267S + 379V double mutation on enzyme activity, a β-carotene supplementation study was conducted to test whether the observed enzymatic changes would affect β-carotene conversion to retinyl palmitate in female volunteers.

β-Carotene supplementation study

Twenty eight female volunteers (mean age=20 yr; mean BMI=22 kg/m^2; Table 4) took part in the study. The mean daily total energy intakes of 6.4 MJ were close to those reported in the National Diet and Nutrition Survery (29). Dietary intake of carbohydrates, protein, fat, and alcohol contributed 50.4, 18.6, 28.5, and 2.4% of total energy intake, respectively, which is typical of a Western diet. The mean daily total provitamin A carotene intake was 2.5 mg, with β-carotene being the main ingested provitamin A carotene (mean β-carotene concentration=1.2 mg/day; range=0.2–7.0 mg/day). Mean daily intake of preformed retinol was low (133 μg/day) and indicated that 64% of the subjects rely more than 70% on provitamin A sources to cover their vitamin A requirements. Interindividual variation in preformed retinol intake was high with a coefficient of variation (CV) of 65%, with the lowest intake at 15 μg/day and the highest retinol intake at 434 μg/day. Mean daily retinol equivalent (RE) intakes of 445 μg/day were below the recommended nutrient intake of 600 μg/day for women, but comparable with mean intakes of 467 μg/day reported by the National Diet and Nutrition Survery (4; Table 4). A total of 36% of women had RE intakes above the recommended nutrient intake of 600 μg/day, and 14% had intakes below the lower recommended nutrient intake of 250 μg/day. Dietary intake of total energy, carbohydrates, protein, fat, alcohol, provitamin A carotenes, and RE was not significantly different between carriers of the 379V and 267S + 379V variant alleles compared with wild-type R267/A379 alleles.

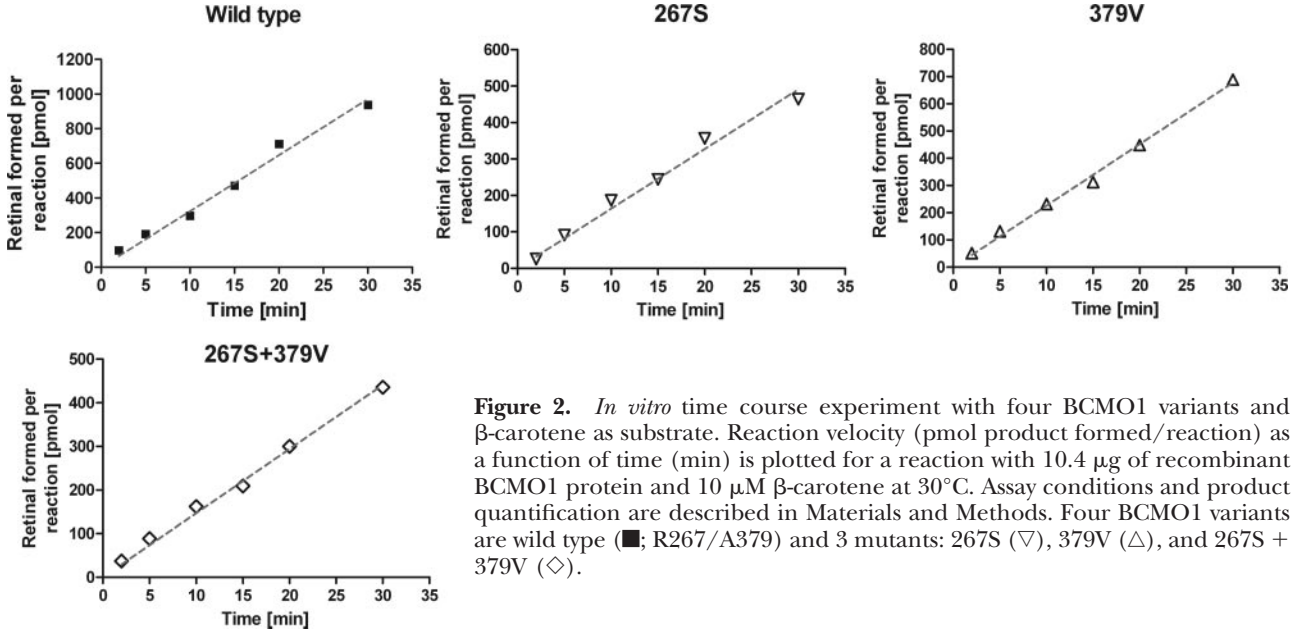


Figure 2. *In vitro* time course experiment with four BCMO1 variants and β-carotene as substrate. Reaction velocity (pmol product formed/reaction) as a function of time (min) is plotted for a reaction with 10.4 μg of recombinant BCMO1 protein and 10 μM β-carotene at 30°C. Assay conditions and product quantification are described in Materials and Methods. Four BCMO1 variants are wild type (■; R267/A379) and 3 mutants: 267S (▽), 379V (△), and 267S + 379V (◇).

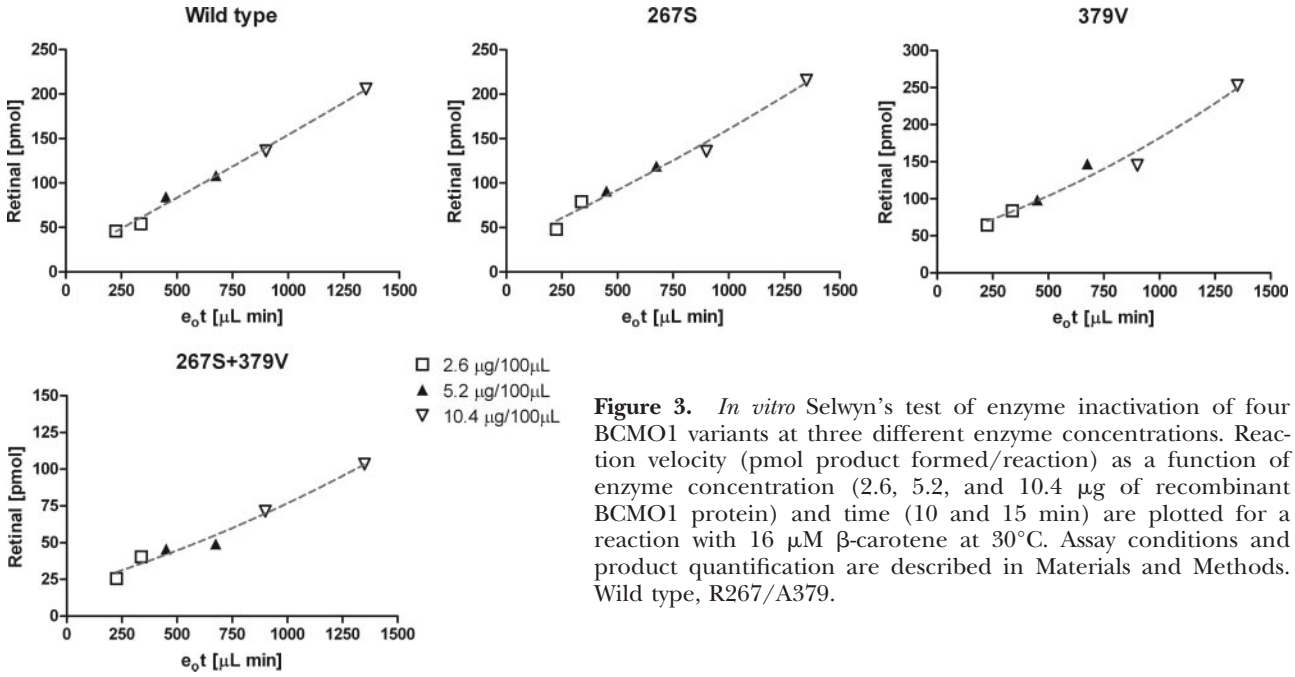


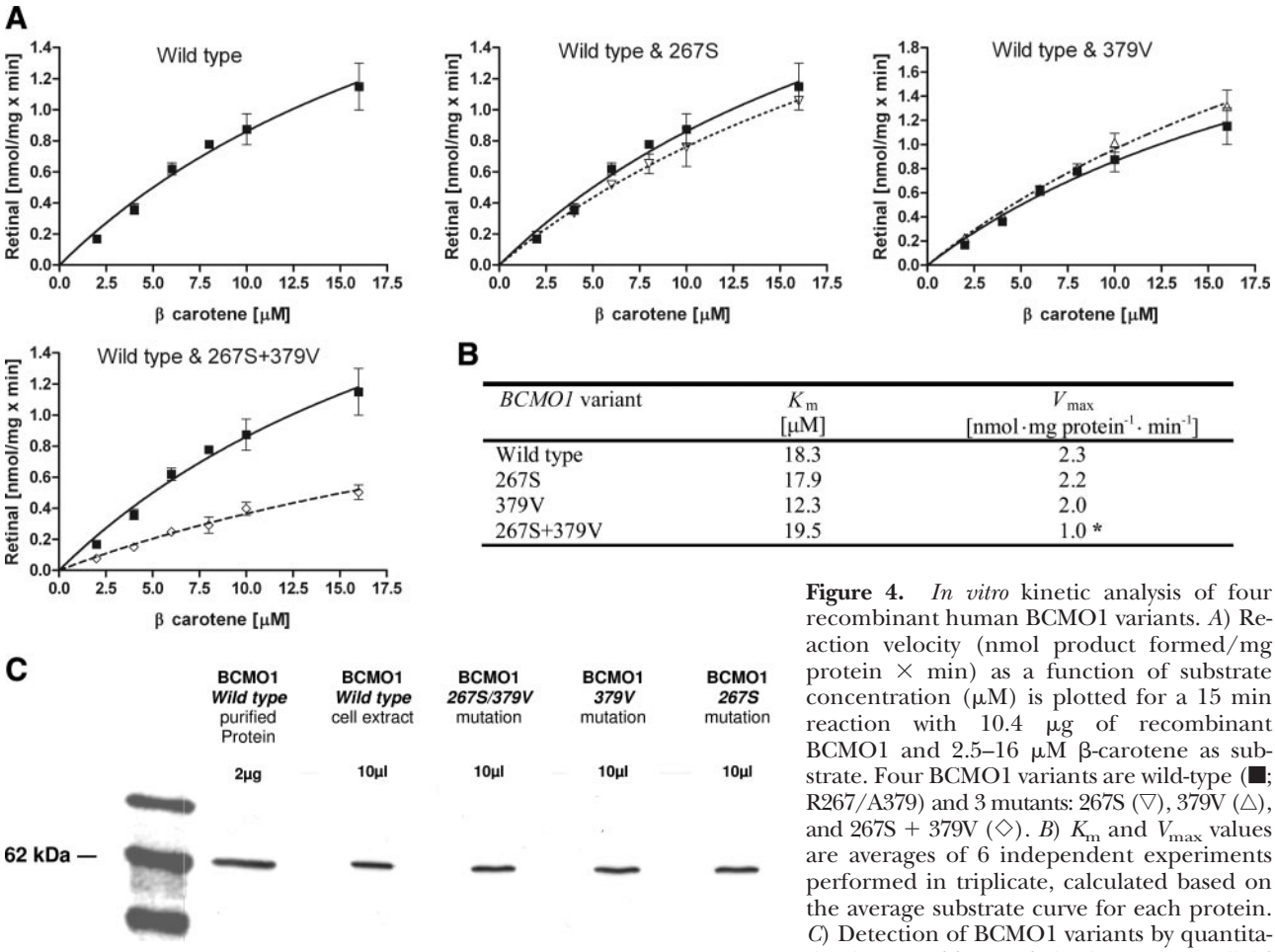
Figure 3. *In vitro* Selwyn's test of enzyme inactivation of four BCMO1 variants at three different enzyme concentrations. Reaction velocity (pmol product formed/reaction) as a function of enzyme concentration (2.6, 5.2, and 10.4 μg of recombinant BCMO1 protein) and time (10 and 15 min) are plotted for a reaction with 16 μM β-carotene at 30°C. Assay conditions and product quantification are described in Materials and Methods. Wild type, R267/A379.

Fasting mean plasma concentrations for β-carotene and retinol were 361.9 and 1516.7 nM, respectively (**Table 6**). Fasting plasma β-carotene concentrations showed a high interindividual variability with a CV of 74%, with the lowest and highest plasma concentration of 112.4 and 1448.7 nM, respectively. Fasting plasma retinol distribution was less variable with a CV of 28%, and the lowest plasma concentration was 963.8 nM, indicating that all volunteers had adequate serum vitamin A concentrations. Age, BMI, plasma triglyceride concentrations, and dietary vitamin A and β-carotene intake were not related to fasting plasma β-carotene and retinol concentrations. However, a positive significant correlation between fasting plasma β-carotene concentrations and both the 379V variant allele (r=0.42; P = 0.013) and the combined 267S + 379V variant alleles (r=0.39; P=0.021) was found. After adjustment for age, BMI, plasma triglyceride concentrations, dietary vitamin A and β-carotene intake, multiple linear regression analysis showed that carriers of the 379V and 267S + 379V variant alleles had significantly higher fasting plasma β-carotene concentrations of 586.2 nM (r^2=0.178; P=0.025) and 697.4 nM (r^2=0.151; P=0.041), respectively, compared with 276.4 nM in the wild-type variant (**Fig. 5*B***). Volunteers carrying the wild-type R267/A379 alleles also showed a lower interindividual variation in fasting plasma β-carotene concentrations with a CV of 28%.

To test if the 379V and 267S + 379V variant alleles could influence the ability of an individual to convert β-carotene into retinol, retinyl palmitate, β-carotene, and triglyceride concentrations were measured in the TRL fraction 3 h after a single 120 mg dose of β-carotene in a fat-rich meal. All 28 volunteers showed a β-carotene and retinyl palmitate response measured in the TRL fraction (Table 6). The retinyl palmitate:β-carotene ratio was used to determine β-carotene conversion efficiency in each subject. Interindividual variability was high, with a nearly 8-fold difference between the lowest (0.14) and highest value (1.06) and a CV of 60%. Negative significant correlations between the TRL retinyl palmitate:β-carotene ratio and fasting plasma β-carotene (r=−0.44; P=0.010), TRL triglyceride (r=−0.44; P=0.010), the 379V variant allele (r=−0.49; P=0.004), and the combined 267S + 379V variant alleles (r=−0.56; P=0.001) were observed, whereas age, BMI, and dietary vitamin A and β-carotene intake were not related to the TRL retinyl palmitate:β-carotene ratio. Multiple linear regression analysis showed that after adjustment for fasting plasma β-carotene and TRL triglyceride concentrations, carriers of the 379V and 267S + 379V variant alleles had significantly lower TRL retinyl palmitate:β-carotene ratios of 0.305 (r^2=0.520; P=0.005) and 0.156 (r^2=0.572; P=0.001), respectively, compared with 0.585 in the wild-type R267/A379 allele carriers (Fig. 5*A*). Volunteers carrying the wild-type R267/A379 alleles also showed a lower interindividual variation in the TRL retinyl palmitate:β-carotene ratio with a CV of 46%. Plasma triglyceride and TRL triglyceride concentrations were not significantly different between carriers of the 379V and 267S + 379V variant alleles compared with wild-type R267/A379 alleles (Fig. 5), indicating that subjects absorbed triglyceride in a manner that was independent of these SNPs.

DISCUSSION

The extent to which β-carotene is converted to vitamin A is highly variable between well-nourished healthy individuals (17–20). This variable response to β-carotene has led to the characterization of the poor converter phenotype in 27–45% of volunteers in double-



B

BCMO1 variant	K_m [μM]	V_{max} [nmol·mg protein^{-1}· min^{-1}]
Wild type	18.3	2.3
267S	17.9	2.2
379V	12.3	2.0
267S+379V	19.5	1.0 *

Figure 4. *In vitro* kinetic analysis of four recombinant human BCMO1 variants. *A*) Reaction velocity (nmol product formed/mg protein × min) as a function of substrate concentration (μM) is plotted for a 15 min reaction with 10.4 μg of recombinant BCMO1 and 2.5–16 μM β-carotene as substrate. Four BCMO1 variants are wild-type (■; R267/A379) and 3 mutants: 267S (▽), 379V (△), and 267S + 379V (◇). *B*) K_m and V_{max} values are averages of 6 independent experiments performed in triplicate, calculated based on the average substrate curve for each protein. *C*) Detection of BCMO1 variants by quantitative immunoblot analysis. Supernatant fluid from the cell lysate (used for enzymatic activity tests) was subjected to SDS-PAGE, and proteins were electrotransferred to membranes. BCMO1 variants were then detected by anti-His antibodies and ECL system, and were quantified using affinity purified wild-type BCMO1 protein. *$P < 0.001$ *vs.* wild type; independent sample *t* test.

tracer studies (19–21). The frequency of a rare missense mutation in the *BCMO1* gene that caused a dramatic decrease in the enzyme activity (22) is so low that this SNP cannot explain the high occurrence of the poor converter phenotype. The present report shows for the first time that two common nonsynonymous SNPs that exist in the *BCMO1* gene occur at frequencies similar to those of the poor converter trait observed in human intervention studies and that they lead to impaired function of the BCMO1 enzyme *in vitro* and *in vivo*. The 267S + 379V double mutation indicated a reduced catalytic activity of BCMO1 *in vitro* by 57%. The *in vivo* results from this intervention trial are consistent with the biochemical characterization of the 267S + 379V double mutant and indicate that female volunteers carrying the combined 267S + 379V variant alleles show a 69% lower ability to convert β-carotene into retinyl esters. Although *in vitro* results did not indicate that the 379V mutant would affect the catalytic activity of BCMO1, female volunteers carrying the 379V variant allele showed a reduced ability to convert β-carotene by 32%. As expected, this pheno-

TABLE 6. *Fasting plasma and TRL triglyceride, β-carotene, retinol, and retinyl palmitate concentrations in all volunteers (n=28)*

Variable	Geometric mean	95% CI	CV (%)
Fasting plasma retinol (nM)	1516.7	1362.6–1688.2	28.3
Fasting plasma β-carotene (nM)	361.9	288.8–453.4	73.6
TRL triglyceride (μM)	1272.1	1033.1–1566.5	54.7
TRL retinyl palmitate (nM)	170.2	128.3–225.8	79.4
TRL retinyl palmitate/triglyceride (nmol/μmol)	0.13	0.09–0.20	95.2
TRL β-carotene (nM)	445.6	361.6–549.0	52.8
TRL β-carotene/triglyceride (nmol/μmol)	0.35	0.27–0.46	66.1
TRL retinyl palmitate/β-carotene	0.38	0.30–0.49	60.4

type was accompanied by higher fasting β-carotene concentration, with 1.6 and 2.4 times higher β-carotene concentrations in female 379V and 267S + 379V variant allele carriers, respectively, compared with wild-type R267/A379 allele carriers.

In this first systematic evaluation of novel and existing SNPs in *BCMO1* occurring at frequencies over 5%, dHPLC identified a total of 18 gene variations, the majority being single base pair substitutions at splice junctions. Rare SNPs occurring at frequencies <5% would not necessarily have been detected by our approach. Although five gene variations were identified in the coding region, only two of these SNPs caused alterations to the amino acid sequence. Variant allele frequencies of R267S and A379V were 42 and 24% in the current study, respectively. Similar frequencies in a Caucasian population were observed in the International HapMap Project (http://www.hapmap.org/), with 48 and 26% for the R267S and A379V variant alleles, respectively. The combined 267S + 379V variant alleles had a frequency of 24 and 36% in Caucasians in the current study and the HapMap database, respectively (Table 5; **Fig. 6**). The Hapmap database shows both the 379V and 267S variant alleles to be present in Han Chinese and Japanese populations but indicates large differences in frequencies between ethnic groups. The 379V variant allele was found at a frequency of 31 and 24% in the Han Chinese and Japanese population, whereas the combined 267S + 379V variant alleles were observed in 9 and 2% of the Han Chinese and Japanese population, respectively (Fig. 6). It is important to note that the 379V variant allele was not found in the Yoruba population in Nigeria (http://www.hapmap.org/). To evaluate whether a mutation in the promoter region of the *BCMO1* gene could have influenced the β-carotene conversion to retinal, the MEF2 and the PPARγ/RXR transcription binding sites as characterized by Gong *et al.* (16) were screened for novel SNPs.

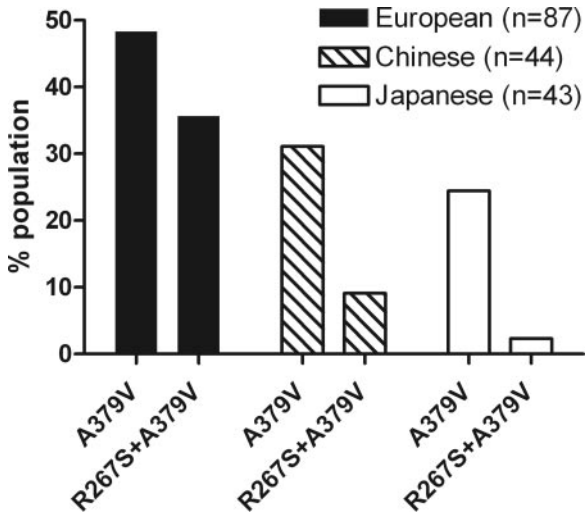


Figure 6. Allele frequencies of A379V and R267S+A379V variants in 3 different ethnic groups according to Hapmap (www.hapmap.org). European, Utah residents with Northern and Western European ancestry; A379V, at least one T allele; R267S+A379V, at least one T allele in both R267S and A379V.

The absence of SNPs within these two transcription binding sites in 80 samples together with the observation that neither the HapMap (http://www.hapmap.org/), Ensembl (http://www.ensembl.org), nor NCBI (http://www.ncbi.nlm.nih.gov/SNP/) SNP databases recorded any SNPs within the *BCMO1* promoter region confirmed that no SNPs are present at frequencies ≥2%. It is therefore unlikely that the poor converter phenotype could be caused by genetic variances in the promoter region of the *BCMO1* gene.

Both wild-type and 267S + 379V recombinant BCMO1 proteins expressed in *E. coli* produced all-trans-retinal as the main product of β-carotene cleavage. Both the K_m (18.3 μM) and V_{max} (2.3 nmol retinal

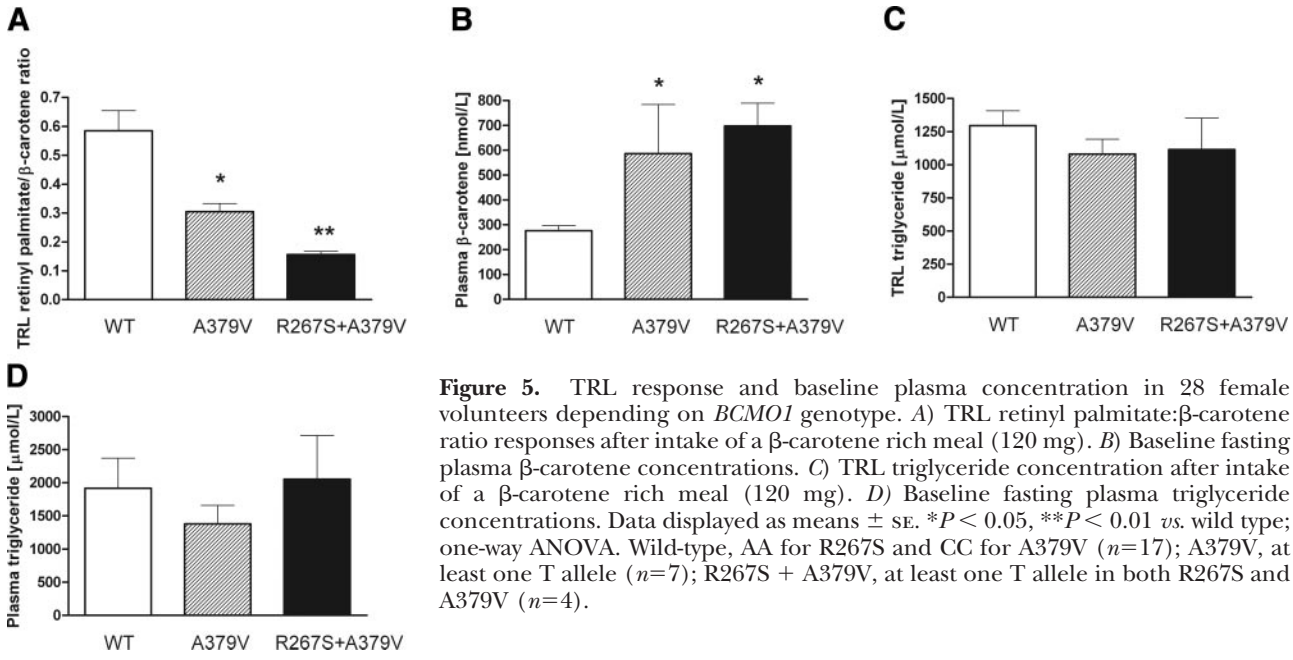


Figure 5. TRL response and baseline plasma concentration in 28 female volunteers depending on *BCMO1* genotype. *A*) TRL retinyl palmitate:β-carotene ratio responses after intake of a β-carotene rich meal (120 mg). *B*) Baseline fasting plasma β-carotene concentrations. *C*) TRL triglyceride concentration after intake of a β-carotene rich meal (120 mg). *D)* Baseline fasting plasma triglyceride concentrations. Data displayed as means ± SE. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* wild type; one-way ANOVA. Wild-type, AA for R267S and CC for A379V (n=17); A379V, at least one T allele (n=7); R267S + A379V, at least one T allele in both R267S and A379V (n=4).

formed·mg protein^{-1}·min^{-1}) of the wild-type BCMO1 protein were within the range previously reported for human recombinant BCMO1 protein (22, 30, 31). The biochemical analysis of the BCMO1 variants revealed that only the 267S + 379V double mutant showed a significant decrease in specific activity compared with the wild-type enzyme due to changes in V_{max}. The K_m value for β-carotene of the 267S + 379V double mutant was not significantly different from the wild-type enzyme, suggesting that this double mutation does not influence substrate binding but rather the turnover rate (K_{cat}), which was reduced from 0.14 min^{-1} for the wild-type enzyme to 0.06 min^{-1} for the 267S + 379V double mutant. The crystal structure of cyanobacterial apocarotenoid 15,15′-oxygenase PCC 6803 (ACO) has recently been successfully used to elucidate the effects of mutations located in the vicinity of conserved histidines and acidic residues within the BCMO1 enzyme (22, 32), but the observed 267S + 379V double mutation is not close to any conserved histidine or acidic residues. Furthermore, ACO and BCMO1 only have 24% sequence identity (33), making structure predictions outside conserved areas of the protein unreliable. Thus, at present, the reason for the lower specific activity of the 267S + 379V double mutant in BCMO1 is unknown. However, the recent discovery of an increased catalytic activity of the BCMO1 T381L mutation (31) indicates that the region in direct vicinity of A379V is critical for enzyme catalysis. Thus, our *in vitro* results provided evidence that carriers of the 267S + 379V variant alleles may generate lower amounts of retinal from dietary β-carotene sources in comparison with subjects without these polymorphisms.

Indeed, data from our human intervention study showed that female volunteers carrying either the 379V or combined 267S + 379V variant alleles have a reduced ability to convert dietary β-carotene into vitamin A, as indicated by reduced TRL retinyl palmitate:β-carotene ratios combined with increased fasting plasma β-carotene concentrations. After adjustment of covariates (dietary vitamin A and β-carotene intake, plasma β-carotene, and TRL triglyceride), carriers of the combined 267S + 379V variant alleles showed a reduced β-carotene conversion efficiency by 69%, and an increase in fasting plasma β-carotene concentrations by 240%. This finding corresponded well to the *in vitro* results for which a 57% reduction in specific BCMO1 activity of the 267S + 379V mutant was observed. It is important to note that the 267S + 379V variant alleles were only found in the heterozygous form. Since the 267S and 379V variant alleles are not in linkage disequilibrium, it is unlikely that both SNPs always occur on the same chromosome. However, the discovery of the heterozygous T170M mutation indicated that haploinsufficiency of the BCMO1 enzyme may cause symptoms of hypercarotenemia and hypovitaminosis A (22). Although we did not observe any significant effects of the 379V mutant on enzymatic parameters *in vitro*, our results from the β-carotene supplementation study indicated that carriers of the 379V variant allele have a 32% reduced β-carotene conversion efficiency and an increase in fasting plasma β-carotene concentrations by 160%. This discrepancy between *in vitro* and *in vivo* data may be caused by the fact that the recombinant BCMO1 enzyme exists as a 230-kDa tetramer in solution in the presence of the detergent OTG, but as a monomeric enzyme in the absence of detergent (22). Other additional factors affecting the enzymatic activity of BCMO1 that cannot be properly mimicked in the test tube may include protein stability, post-translational modifications, and interactions with downstream components in the retinol synthesis pathway.

For the evaluation of the poor converter phenotype, we adopted the study design from Borel *et al.* (17) for several reasons: first, the measurement of β-carotene and retinyl esters in the TRL fraction after a dose of β-carotene with a vitamin A free meal is an appropriate method to evaluate intestinal absorption and cleavage of β-carotene in humans (26). Second, by giving pharmacological doses of β-carotene, all subjects in the current study and in the study from Borel *et al.* (17) responded with elevated TRL β-carotene concentrations, suggesting that there are apparently no nonresponders to pharmacological doses of β-carotene in the healthy population. Third, although the TRL retinyl palmitate:β-carotene ratio displays a negative slope when physiological concentrations of β-carotene are consumed (26, 34), the ratio is constant during the postprandial period when pharmacological doses of β-carotene are given and therefore allows the determination of β-carotene conversion 3 h after the test meal (17).

Baseline retinol and β-carotene concentrations in the current study were similar to previous studies (17, 20, 35) with values ranging from 1.3 to 1.7 μM and 234 to 450 nM, respectively. In agreement with Borel *et al.* (17), interindividual β-carotene, retinyl palmitate, and triglyceride responses in the TRL fraction were highly variable. However, all subjects in the present study had measurable concentrations of β-carotene and retinyl palmitate in the TRL fraction, in contrast to Borel *et al.* (17) who identified 12% of subjects with no detectable retinyl palmitate after supplementation. The present study also observed higher absorption and conversion of β-carotene, since both TRL β-carotene and retinyl palmitate concentrations were higher in the current study in comparison with Borel *et al.* (17). High TRL β-carotene concentrations consequently reduced the relative conversion efficiency in the current study since β-carotene conversion to vitamin A decreases as the β-carotene dose increases (17, 26, 34, 35). This is confirmed by the observation that retinyl palmitate:β-carotene ratios of 2.68 (26) and 2.39–2.73 (34) were observed in volunteers who consumed physiological concentrations of β-carotene, compared with retinyl palmitate:β-carotene ratios of 1.0 (17) and 0.54 (current study) when pharmacological concentrations of β-carotene were administered. Both the current study and that of Borel *et al.* (17) gave 120 mg of β-carotene in a meal with a similar nutrient content. However,

β-carotene was administered in a water soluble beadlet form in the current study but as an oil suspension in the earlier work (17). Thus, the present data suggest that absorption as well as conversion might be higher when β-carotene is given in the form of water soluble beadlets.

In conclusion, the present study shows that two common nonsynonymous SNPs exist in the human *BCMO1* gene that occur at high frequencies and that alter β-carotene metabolism. Female volunteers carrying either the 379V or the combined 267S + 379V variant alleles showed a reduced ability to convert β-carotene and had higher fasting β-carotene concentrations. Thus, our study provides a putative explanation for the poor responder phenotype in β-carotene metabolism. The recent discovery that β-carotene plasma concentrations are also reduced in carriers of the lipoprotein lipase X447 allele (36) and are higher in women homozygous for the T allele of the hepatic lipase C-480T variant (37) demonstrates that β-carotene status is influenced by multiple SNPs and genes, which warrants further study. This genetic variability should be taken into account in future recommendations for vitamin A supplementation. Based on our data, populations with a high frequency of the 379V variant allele may benefit from supplementation with preformed vitamin A rather than increased intake of plant provitamin A sources to combat vitamin A deficiency. FJ

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