Lutein exerts anti-inflammatory effects in patients with coronary artery disease.

Rosanna W S Chung, Per Leandersson, Anna K Lundberg and Lena Jonasson

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Abstract

Background and aims: Many coronary artery disease (CAD) patients exhibit chronic low-grade inflammation. Carotenoids are anti-oxidants with potential anti-inflammatory properties. Here, we first assessed relationships between interleukin(IL)-6 and individual carotenoids in plasma from CAD patients. Based on the results, we proceeded to assess anti-inflammatory effects of one carotenoid, lutein, in peripheral blood mononuclear cells (PBMCs) from CAD patients.

Methods: Lutein+zeaxanthin (isomers with lutein being dominant), β-cryptoxanthin, lycopene, α- and β-carotene and IL-6 were measured in plasma from 134 patients with stable angina (SA) and 59 patients with acute coronary syndrome. In 42 patients, plasma measurements were also performed 3 months after coronary intervention. PBMCs from SA patients were pre-treated with lutein (1, 5 and 25 µM) for 24h followed by 24h incubation +/- lipopolysaccharide (LPS). Cell pellets were collected for IL-6, IL-1β and TNF mRNA and intracellular lutein. Cytokine secretion was measured in cell media.

Results: Only lutein+zeaxanthin were inversely correlated to IL-6 in SA patients at baseline (r=-0.366, p<0.001) and follow-up (r=-0.546, p<0.001). *Ex vivo*, lutein was taken up by PBMCs from SA patients in a dose- and time-dependent manner. Pre-treatment with lutein dose-dependently lowered LPS-induced secretion of IL-6, IL-1β (p<0.01) and TNF (p<0.05), and also reduced IL-6, IL-1β and TNF mRNA expression (p<0.05).

Conclusions: Clinical findings highlighted the inverse association between lutein and IL-6 in CAD patients. Anti-inflammatory effects of lutein in PBMCs from CAD patients were consolidated in *ex vivo* experiments. Taken together, lutein has the potential to play a role in resolution of chronic inflammation in CAD patients.

Lutein exerts anti-inflammatory effects in patients with coronary artery disease

Rosanna W.S. Chung[1][*], Per Leanderson[2], Anna K. Lundberg [1], Lena Jonasson[1]

[1]Division of Cardiovascular Medicine, Department of Medical and Health Sciences, Linköping University, SE-581 85 Linköping, Sweden.

[2] Occupational and Environmental Medicine Center, Department of Clinical and Experimental Medicine, Linköping University, SE-581 83 Linköping, Sweden.

* _Corresponding author:_	Rosanna W.S. Chung
	Division of Cardiovascular Medicine
	Department of Medical and Health Sciences
	Faculty of Health Sciences
	Linköping University
	SE-581 85 Linköping
	Sweden
	Telephone: +46 10 10 37476
	Fax: +46 13 14 5949
	Email: Rosanna.chung@liu.se

Email addresses of co-authors:

Per Leanderson: per.leandersson@regionostergotland.se

Anna K. Lundberg: anna.lundberg@liu.se

Lena Jonasson: lena.jonasson@liu.se

Introduction

Inflammation has a prominent role in coronary artery disease (CAD) and other manifestations of atherosclerosis, from the development of plaques to plaque destabilization [1]. Elevated inflammatory biomarkers in the circulation, like C-reactive protein (CRP) and interleukin (IL)-6, have also been consistently shown to predict the risk of CAD [2, 3]. Over the last decades, significant improvements have been made in the medical and lifestyle management of CAD, all of which may lead to reductions in inflammatory activity. Despite this, low-grade systemic inflammation persists in many patients with CAD. There is growing evidence that anti-inflammatory diets, such as Mediterranean diet, may have beneficial effects [4, 5]. However, the specific mechanisms linking diet to inflammation remain to be elucidated.

Carotenoids are lipid-soluble pigments found in a variety of fruits and vegetables. In several population-based studies, low plasma levels of carotenoids as well as low dietary intake of carotenoids have been associated with high cardiovascular disease risk [6, 7]. Moreover, inverse associations between circulating levels of carotenoids and inflammatory markers have been demonstrated in multiple studies [8-12] raising the hypothesis that carotenoids themselves exert anti-inflammatory effects. One might argue that individuals with low intake of carotenoid-rich food are more likely to have an overall unhealthy lifestyle since the association between carotenoids and inflammation has been shown to lose statistical significance after adjustment for potential confounders, such as overweight and smoking [13-17]. Moreover, majority of the mechanistic evidence were based on cell lines and animal models [7]. To the extent of our knowledge, it has not been investigated whether direct anti-inflammatory effects of carotenoids exist in human cells that are involved in the inflammatory response, such as peripheral blood mononuclear cells (PBMCs).

There are 6 major carotenoids in human plasma, lutein, zeaxanthine, β-cryptoxanthine, lycopene, α-carotene and β-carotene. Lutein and zeaxanthine are stereoisomers with identical chemical formulas. Lutein is the predominant one with ≈5-fold higher abundance in plasma [18, 19] and therefore the levels of lutein and zeaxanthine are commonly expressed as a combined figure. Although carotenoids share similar structural and chemical characteristics, there are evidence that they vary in biological effects. Previous studies that assess the effects of carotenoids on cardiovascular risk have focused mainly on β-carotene. Lately, lutein has emerged as a potentially stronger candidate for atheroprotection. A number of population-based studies comparing lutein with β-carotene have demonstrated that lutein, but not β-carotene, is

associated with early protection against atherosclerosis as assessed by carotid intima-media thickness (IMT) [20-23]. A recent meta-analysis showed a lower risk of CAD and stroke if the highest and lowest tertiles of lutein blood levels or intake were compared [24]. Also, lutein has been shown to protect against atherosclerosis in animal models [20, 25, 26] and to exert anti-inflammatory effects in various *in vitro* models [27, 28]. Moreover, recent studies in patients with early macular degeneration have shown that supplementation with lutein inhibits the systemic activation of the complement system [29, 30].

Comprehensive measurements of major carotenoids in plasma have been performed in population-based cohorts but are less common in patients with clinical manifestations of atherosclerotic disease. In a previous study, we found that CAD patients presenting with either acute coronary syndrome (ACS) or stable angina (SA) had significantly lower plasma levels of lutein+zeaxanthine compared with healthy controls, while levels of other major carotenoids, such as β-carotene and lycopene, did not differ between any subject groups [31]. In line with this, a larger case-control study on ACS reported that plasma levels of lutein were significantly lower in ACS patients compared with healthy controls while no significant differences were seen in β-carotene and lycopene [32].

In the present study, we first assessed the association between plasma levels of IL-6 and different carotenoids in patients with CAD. Using both a cross-sectional and longitudinal study design, we showed that IL-6 was inversely correlated with lutein+zeaxanthine but not with other major carotenoids in plasma at both baseline and follow-up. Since lutein is the dominant isomer, we proceeded to examine the anti-inflammatory effects of pure lutein on isolated PBMCs from patients with CAD and showed that lutein *i*) was stored in PBMCs and *ii*) suppressed their production of pro-inflammatory cytokines.

Materials and methods

Subjects

For plasma measurements of carotenoids and IL-6, 59 patients with recent onset of ACS (i.e. within 24 h after admission) and 134 patients with SA were consecutively recruited at the Department of Cardiology, University Hospital, Linköping, Sweden. Since IL-6 has a circadian rhythm, samples were always obtained in the morning. In order to avoid the inflammatory response to balloon injury and stent implantation, blood sampling was also performed prior to

coronary angiography. ACS patients were included if they had a diagnosis of unstable angina/non-ST elevation myocardial infarction, with the diagnosis based on typical ECG-changes (ST-T segment depression and/or T-wave inversion) and/or elevated troponins. SA patients were included if they had an angiographically verified diagnosis of CAD and exhibited angina class I-III in accordance with Canadian Cardiovascular Society Classification without any worsening of symptoms in 3 months. Patients were excluded if they were older than 75 years, had severe heart failure, immunological disorders, neoplastic disease, evidence of acute or recent (<2 months) infection, recent major trauma, surgery or revascularization procedure, treatment with immunosuppressive or anti-inflammatory agents (except low-dose aspirin). A longitudinal assessment was performed in 42 of the patients (14 ACS, 28 SA). In this subpopulation, blood samples were collected prior to coronary angiography and 3 months after coronary intervention. The reason that only 42 patients participated in the longitudinal study was mainly due to geographic or logistic factors. The study was conducted in accordance with the ethical guidelines of Declaration of Helsinki, and the research protocol was approved by the Ethical Review Board of Linköping University. Written informed consent was obtained from all study participants.

For ex vivo experiments, blood was obtained from patients with SA with similar criteria as above.

Extraction of carotenoids

Carotenoids in heparinized plasma (150µL) and PBMCs in 150µL PBS were extracted in duplicates using Milli-Q water, 95% ethanol with 0.1% butylated hydroxytoluene and pure hexane in a ratio of 3:3:4 (v/v/v) in one round of extraction. In brief, water and ethanol were added to plasma or PBMCs suspended in PBS followed by vortex at full speed for a few seconds. Thereafter, hexane was added and samples were vortexed at 2400 rpm/min for 5 min. At this point, proteins and carotenoids were dissolved in the ethanol-water phase and the hexane phase, respectively. The hexane phase was isolated by centrifugation at 14000 x g for 2 min. After centrifugation, the upper phase (hexane phase) was transferred to a new tube and dried under nitrogen gas. The carotenoids were then reconstituted in 100µL mobile phase (80 % acetonitrile:20 % methanol) followed by 5 min sonication in a sonication water bath prior to high-performance liquid chromatography (HPLC) analysis.

Quantification of carotenoids

Carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene) levels in both plasma and PBMCs were determined by HPLC using a modified version as previously described [31]. As stereoisomers, lutein and zeaxanthin were measured together and presented as a combined value in plasma. The HPLC analyses were performed using a PU 980 HPLC Pump (Jasco Inc., Japan), C18-Chromolith® Performance RP-18 endcapped 100-4.6 HPLC column that has a 4.6 mm internal diameter packed with C18-Chromolith material and 2µm macropores (Merck KGaA, Germany) and Jasco MD-2010 Plus Multiwavelength Detector (Jasco Inc., Japan). The flow rate was set to 3 mL/min and 20 µL of samples were injected. Signals were recorded at 450 nm using Clarity version 2.6.5 (DataApex, Czech Republic). Carotenoid concentration were calculated from a standard curve generated from reference samples that were calibrated against an external standard (SRM 968E) from the National Institute of Standard and Technology. Inter-assay coefficient of variations were less than or equal to 5.

Cytokine measurements

Levels of IL-6, IL-1β and tumor necrosis factor (TNF) were analyzed by high-sensitivity human multiple Luminex assay (Bio-Techne, UK). The sensitivity limits of quantification for IL-6, IL-1β and TNF were 2.3-18880, 3.2-3261, and 5.8-95484 pg/mL, respectively. The interassay CVs for IL-6, IL-1β and TNF were 7 %, 6 % and 8 %, respectively.

Gene expression analysis

Total RNA was isolated from PBMCs using an RNeasy kit (Qiagen, Germany). Purity of RNA was tested in a 2100 Bioanalyzer (Agilent Technologies, USA). RNA (100 ng) was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, NM_001256799.2), IL-6 (NM_000600.4), TNF (NM_000594.3) and IL-1β (MN_000576.2) mRNA were amplified using TaqMan Gene Expression Assays in a 7500 Fast Real-Time PCR System (Applied Biosystems, USA). PCR conditions were as described by the manufacturer. The relative gene expression was calculated by normalizing cycle threshold (Ct) values for genes of interest with Ct values for GAPDH using the ΔΔCt method.

Preparation of lutein micellar cell medium solution

In order to prevent oxidation of lutein during incubation, lutein was incorporated into micelles before being supplemented to PBMCs. The micellar cell medium solution was prepared by dissolving 33 µM oleic acid, 16.7 µM L-α-phosphatidylcholine, 100 µM monoolein and 2 mM sodium taurocholate hydrate in complete cell medium via 15 min sonication in water bath followed by sterilization through a 0.22 µm filter [33]. The complete cell medium contained Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies, USA), 10 % fetal bovine serum (FBS), 100U/mL Penicillin-Streptomycin (Life Technologies, USA). All other chemicals were purchased from Sigma Aldrich (USA) unless specified otherwise. To make lutein micellar cell medium, 1µM, 5µM or 25µM lutein analytical standard (07168, Sigma Aldrich, USA) was dissolved with all of the micelle components mentioned above in complete cell medium via the same sonication and sterilization procedures. The lutein analytical standards had ≥ 97% purity, according to certificates of analysis provided by Sigma Aldrich.

To confirm the concentration of lutein used in the ex vivo PBMC treatment, a known amount of lutein analytical standard was first reconstituted in absolute ethanol. Several diluted lutein samples were then prepared via serial dilution and their absorbance at 445 nm were measured in a spectrophotometer. The actual concentrations of the original lutein analytical standard was then determined using molar extinction coefficient of lutein in absolute ethanol ($\varepsilon = 145 \times 10^3$).

Treatment of PBMCs with lutein in the presence or absence of LPS

PBMCs were isolated from venous blood of patients with SA using Ficoll-Paque Plus (GE Healthcare Life Science, UK) according to manufacturer's instructions. PBMCs were incubated in the micellar cell medium containing 1µM, 5µM or 25µM lutein for 24 hours under standard cell culturing conditions before incubation with (or without) additional 1 ng/mL lipopolysaccharide (LPS) for 24 hours. Countess II Automated Cell Counter (Life Technologies, USA) was used to obtain cell concentrations as per manufacturer's instructions. Seeding density was 1 000 000 cells/mL. At the end of the incubation, cell media and cell pellets were collected and stored in -80°C until analysis. CellTiter 96 aqueous one solution cell proliferation assay (MTS) (Promega, USA) was performed to assess viability of PBMCs, according to manufacturer's instruction.

Statistical analysis

IBM SPSS Statistics 23 was used for statistical analyses. For all clinical and laboratory characteristics, the significance of any difference between SA and ACS patients was analyzed using Mann-Whitney *U*-test. Bivariate correlations were analysed by Spearman´s rank correlation coefficient. A multiple linear regression analysis was performed to assess the independent contribution of different factors to lutein+zeaxanthin levels in plasma. Differences between related samples were analysed by Friedman´s or Wilcoxon Sign rank tests. Two-tailed p-values < 0.05 were considered significant.

Results

Characteristics of study population

The characteristics of patients with SA and ACS are listed in Table 1. There were no significant differences in age, gender, current smoking, waist circumference, hypertension or prior history of ACS between the two groups. Type 2 diabetes was more common in the ACS group. Also, the ACS patients had lower prevalence of statin use and higher levels of total cholesterol, LDL cholesterol and triglyceride levels compared with the SA patients. Moreover, the ACS group had significantly higher levels of plasma IL-6.

For ex vivo experiments, PBMCs were prepared from a total of 16 SA patients (25 % females, median age 65 years). All 16 patients were treated with high-dose statin, i.e. 80 mg atorvastatin.

Lutein+zeaxan8thin were inversely associated with IL-6 in plasma

The cross-sectional associations between plasma carotenoids levels and inflammatory status, as assessed by IL-6, are shown in Table 2. Lutein+zeaxanthin levels were inversely correlated to IL-6 in the whole cohort (r=-0.190, p<0.05) and in the SA group (r=-0.366, p<0.001), but not in the ACS group. Other carotenoids did not show any correlations to IL-6. In the SA group, lutein+zeaxanthin levels were inversely correlated to age (r=-0.262, p<0.01) and waist circumference (r=-0.208, p<0.05). In a multiple regression model, age, waist circumference and IL-6 levels were included as independent variables and lutein+zeaxanthin levels as dependent variable. Lutein+zeaxanthin levels remained significantly associated with IL-6 levels (adjusted r^2=0.064, p<0.05).

When plasma carotenoids and IL-6 were reassessed in 42 patients 3 months after a percutaneous coronary intervention procedure, the bivariate correlation analysis still showed a significant inverse correlation between lutein+zeaxanthin and IL-6 levels, $r=-0.546$, $p<0.001$.

Lutein was taken up by PBMCs from SA patients in a dose- and time-dependent manner

PBMCs from SA patients were treated with unloaded micelles or micelles loaded with lutein at different concentrations (1, 5 or 25 µM) in a standard cell culture setting. As shown in Figure 1A, the intracellular concentrations of lutein increased dose-dependently during a 24-hour incubation. Over 48 hours, the uptake of 25 µM lutein by PBMCs also occurred in a time-dependent manner (Figure 1B).

Lutein suppressed cytokine production by PBMCs from SA patients

To investigate the effect of lutein on LPS-stimulated cytokine production, PBMCs from SA patients were incubated with unloaded micelles or micelles loaded with lutein at different concentrations (1, 5 or 25 µM) for a total of 48 hours. As shown in Figure 2, the concentrations of IL-6, IL-1β and TNF in cell supernatants were dose-dependently reduced by lutein. We also investigated whether lutein affected gene expression of cytokines in PBMCs from SA patients and found that 25 µM lutein suppressed LPS-induced mRNA expression of IL-6, IL1-β and TNF (Figure 3). The cell viability, as assessed by the MTS assay, was not affected by the lutein treatment, nor by the LPS treatment (data not shown).

Discussion

In a clinical investigation of CAD patients, we found that lutein+zeaxanthine was the only type of carotenoid in plasma that correlated inversely with IL-6 at baseline (prior to coronary angiography) and at 3 months follow-up. We therefore proceeded to investigate the direct-acting effects of pure lutein on PBMCs from CAD patients and found that lutein suppressed both production and release of IL-6 and related cytokines.

Circulating levels of total and individual carotenoids, including lutein+zeaxanthine, have previously been shown to correlate inversely with slightly elevated markers of inflammation, i.e. low-grade systemic inflammation, in large population-based studies [8-11]. Fewer studies have examined the association between carotenoids and inflammatory markers in patients with chronic diseases. Also, most studies have focused on measuring CRP instead of IL-6. To our

knowledge, there is only one study of elderly women showing that those with lowest carotenoid levels were more likely to have increasing IL-6 levels over a 2-year period [34]. Our rationale behind choosing IL-6 was that CRP is not considered the ideal marker of inflammation in individuals on statin treatment. Statins significantly reduce CRP levels in plasma, an effect that may be partly explained by prevention of IL-6-induced CRP expression in hepatocytes [35, 36]. On the other hand, statins has not been shown to affect IL-6 levels in plasma [37-39] and may therefore be more useful for assessing inflammation in patients with CAD, at least under metabolic stable conditions. We did not see any relationship between lutein+zeaxanthine and IL-6 in patients with ACS, a condition associated with an acute-phase inflammatory response. Instead, the relationship between lutein+zeaxanthine and IL-6 was confined to the SA patient group, illustrating a potential role of lutein in low-grade chronic inflammatory conditions. The correlation also remained significant after adjustment for smoking and waist circumference. This is in line with a large population-based study of more than 7000 US inhabitants showing that lutein+zeaxanthine were inversely associated with CRP levels after adjustment for traditional cardiovascular risk factors [9].

The ex vivo results in the present study confirmed that lutein was capable of exerting anti-inflammatory effects in human PBMCs. The administration of lutein to PBMCs, isolated from SA patients, dose-dependently led to intracellular accumulation of lutein and suppression of LPS-induced IL-1β, TNF and IL-6 at both mRNA and protein levels. Others have examined anti-inflammatory effects of lutein in various cell lines, such as mouse macrophages and human colorectal adenocarcinoma cells. Pretreatment with lutein at doses between 10 and 20 μM has been shown to prevent leukocyte adhesion and expression of inflammatory genes, e.g. TNF and IL-6, by inhibiting NF-κB signaling [40-42]. Also, parenteral administration of lutein in various rodent models of endotoxin-induced inflammatory disease has suppressed NF-κB activation and inflammatory gene expression in various cells and tissues, including iris-ciliary body and peritoneal macrophages [40, 42]. Besides PBMCs, there are several potential sources of IL-6 in human plasma, including fibroblasts, endothelial cells, smooth muscle cells and adipocytes. Thus, it is reasonable to assume that lutein can affect circulating levels of IL-6 by suppressing cytokine production in other cell types besides PBMCs.

Population-based studies have consistently shown that higher levels of lutein are associated with decreased carotid IMT, raising the hypothesis that lutein plays a role in early protection against atherosclerosis [20-23]. This has been confirmed in animal models where lutein

supplementation prevents the development of atherosclerotic lesions [20, 25, 26]. Atheroprotective effects have been attributed to both antioxidant and anti-inflammatory actions. The ability of lutein to prevent cell lipid oxidation and oxidative cell damage is well documented [7]. Dwyer et al [20] reported that lutein supplementation inhibited the inflammatory response of monocytes to oxidized LDL in atherosclerosis-prone mice. In a guinea pig model, a lutein-rich diet prevented atherosclerosis by lowering concentrations of oxidized LDL as well as decreasing malondialdehyde and cytokine production in aortic tissue [25]. In the present study, lutein was shown to suppress LPS-induced inflammatory gene expression in PBMCs, thus highlighting its anti-inflammatory properties.

It is worthwhile to note that we used PBMCs from patients with manifest CAD in the ex vivo experiments. This may be considered a strength since, in a secondary prevention perspective, it is important to verify that lutein is able to exert anti-inflammatory effects in PBMCs from patients who are on optimal medical therapy, including high-dose statin.

Some limitations of our study deserve consideration. The sample size was small and did not allow us to evaluate the impact of potential confounders like diabetes, obesity and health behaviors. The lack of food frequency questionnaires providing data on carotenoid intake may also be considered a limitation. However, the methodological problems associated with measurements of dietary intake are well-known. Also, in a previous study [14], no correlations between intake of carotenoid-rich fruits and vegetables and inflammatory markers were seen in a population-based cohort. Another limitation of the present study is the lack of a healthy control population. However, if a difference in correlates or effects of lutein had been observed between medicated patients and unmedicated healthy controls, it would have been difficult to interpret whether it represented the effect of CAD itself or the effect of medication. Furthermore, we only measured one of the cytokines (IL-6) in plasma since TNF and IL-1β cannot be measured reliably in clinical samples due to their pulsatile secretion pattern and short half-lives.

In conclusion, our data indicate that lutein by itself has the potential to exert significant anti-inflammatory effects in patients with CAD, thus revealing one mechanism by which lutein can contribute to atheroprotection. Future dietary intervention studies are warranted to confirm whether increasing the consumption of lutein has beneficial effects on clinical outcomes in patients with CAD.

Conflict of interest

There is no conflict of interest to be declared by any of the authors with respect to this manuscript.

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Author contributions

R.W.S. designed and performed the experiments and analyzed the data. R.W.S. and L.J. interpreted the data and wrote the manuscript. A.L. and P.L. provided scientific expertise and reviewed the manuscript.

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Figure legends

Figure 1. Intracellular lutein concentrations increased in a dose- and time-dependent manner when peripheral blood mononuclear cells from patients with stable angina were treated with lutein *ex vivo*. (A) Intracellular lutein concentrations after treatment with cell medium containing unloaded micelles (0 µM) or micelles loaded with different concentrations of lutein (1 µM, 5 µM or 25 µM) for 24 hours (n = 6-16). *** *p* for trend<0.001. (B) Intracellular lutein concentrations after treatment with cell medium containing unloaded micelles (filled circles) or micelles loaded with 25 µM lutein (filled squares) from baseline to 48 hours (n = 6). ** *p* for trend<0.01. Differences between related samples over time were analyzed by Friedman´s test. Points and error bars represent mean ± SEM.

Figure 2. Lutein treatment of LPS-stimulated peripheral blood mononuclear cells (PBMCs) decreased the release of cytokines into cell supernatants in a dose-dependent manner. PBMCs from patients with stable angina (n=8-14) were treated with unloaded micelles (0 µM) or micelles loaded with different concentrations of lutein (1 µM, 5 µM or 25 µM) for 24 hours prior to addition of 1ng/mL LPS for a further 24 hours. The percentage changes of supernatant concentrations of IL-6, IL-1β and TNF compared to cells treated with 0 µM Lutein are shown in (A), (B) and (C), respectively. Bars represent the median percentage decrease of each cytokine with different doses of lutein in relation to unloaded micelles. Error bars represent interquartile range. * *p*<0.05 *vs* 0 µM lutein; ** *p*<0.01 *vs* 0 µM lutein (2-tailed Wilcoxon Signed Ranks Test).

Figure 3. Lutein treatment of LPS-stimulated peripheral blood mononuclear cells (PBMCs) reduced the gene expression of cytokines. PBMCs from patients with stable angina (n = 5) were treated with unloaded micelles (0 µM) or micelles loaded with 25 µM lutein for 24 hours before incubation with 1 ng/mL LPS for a further 4 hours. Figures represent the relative mRNA levels of *IL-6* (A), *IL-1β* (B) and *TNF* (C) in each of the 5 experiments. * *p*<0.05 *vs* 0 µM lutein (2-tailed Wilcoxon Signed Ranks Test).

Table 1. Baseline characteristics of SA and ACS patients. Data are presented as n (%) or median (inter-quartile range).

	SA	ACS	p-values
	n=134	n=59	
Age, years	66 (60-72)	68 (61-74)	0.187
Female, n (%)	27 (20)	16 (27)	0.285
Current smokers, n (%)	18 (13.4)	10 (17)	0.061
Waist circumference (cm)	100 (94-108)	98 (93-108)	0.831
Hypertension, n (%)	72 (54)	29 (49)	0.558
Type 2 diabetes, n (%)	21 (16)	20 (34)	0.004
Prior history of ACS, n (%)	21 (16)	10 (18)	0.765
Statin treatment, n (%)	104 (78)	17 (29)	0.000
IL-6 (pg/mL)	2.21 (1.45-3.03)	5.01 (2.68-9.36)	0.000
Total cholesterol, mM	3.90 (3.40-4.65)	5.10 (4.20-6.10)	0.000
LDL cholesterol, mM	2.10 (1.70-2.50)	3.20 (1.98-3.85)	0.000
HDL cholesterol, mM	1.20 (0.97-1.40)	1.20 (1.00-1.50)	0.370
Triglycerides, mM	1.20 (0.93-1.70)	1.40 (1.08-2.13)	0.028
Total carotenoids, µM	1.43 (0.96-2.02)	1.57 (1.08-2.46)	0.311
Lutein+zeaxanthin, µM	0.27 (0.19-0.34)	0.30 (0.19-0.42)	0.083
β-cryptoxanthin, µM	0.09 (0.05-0.20)	0.10 (0.05-0.19)	0.569
Lycopene, µM	0.41 (0.25-0.65)	0.37 (0.26-0.58)	0.685
α-carotene, µM	0.14 (0.078-0.25)	0.18 (0.11-0.34)	0.112
β-carotene, µM	0.33 (0.20-0.58)	0.35 (0.21-0.68)	0.732

SA, Stable angina; ACS, Acute coronary syndrome; IL-6, Interleukin-6; LDL, Low density lipoprotein; HDL, High Density Lipoprotein.

Table 2. Cross-sectional associations between total or single carotenoids and IL-6 in plasma in SA patients, ACS patients and the whole cohort (SA + ACS). Values are given as Spearman´s rank correlation coefficients.

	SA	ACS	Whole cohort
Total carotenoids	-0.203*	-0.126	-0.125
Lutein+zeaxanthin	-0.366***	-0.267	-0.190*
β-cryptoxanthin	0.017	-0.080	0.022
lycopene	-0.170	-0.058	-0.157
α-carotene	-0.184	0.023	-0.053
β-carotene	-0.092	-0.036	-0.051

SA, Stable angina; ACS, Acute coronary syndrome.

* p<0.05; *** p<0.001

Figure 1

Figure 2

Figure 3