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RESEARCH ARTICLE

Lycopene inhibits lymphocyte proliferation through mechanisms dependent on early cell activation

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Scope: Epidemiological evidence suggests that lycopene is potentially cardio-protective. Recruitment and activation of T cells in the arterial wall is a critical process during atherogenesis, but the effects of lycopene on T-cell response remain to be elucidated. We aimed to determine whether lycopene could modulate T-cell function and activity.

Methods and results: Peripheral blood mononuclear cells from 16 healthy adults were cultured in the presence of lycopene-enriched liposomes (0–2.9 μg lycopene/mL) with or without mitogens. Cell cycle as well as the expression of CD69 (marker of early cell activation), CD25 (IL-2 receptor), and CD11a (late activation marker) were measured in T cells, T-helper cells, and T-cytotoxic cells by flow cytometry. IL-2 secretion and cell proliferation were determined by ELISA and [^{3}H]-thymidine incorporation, respectively. Lycopene significantly inhibited lymphocyte proliferation (up to 40%) in activated cells. Lycopene also significantly inhibited CD69 expression (by up to 12%) as well as IL-2 secretion (by up to 29%). However, CD25 and CD11a expression as well as the cell-cycle profile were unaffected by lycopene.

Conclusion: Lycopene influences lymphocyte proliferation through its effects on processes involved in early cellular activation, providing one possible mechanism to explain the beneficial effects of tomato-rich diets against cardiovascular disease.

Keywords:
Immune function / Lycopene / Lymphocytes / Proliferation

1 Introduction

Strong epidemiological evidence indicates that high consumption of fruits and vegetables reduces the risk of chronic disease such as cardiovascular disease (CVD) [1], but the actual components of these foods that confer the protective effect and the mechanisms by which they act have yet to be firmly identified. Increased consumption of tomato-rich diets has been reported as associated with a 30% reduction in the relative risk of CVD [2]. Such potential benefits to vascular health from a tomato-rich diet are often ascribed to high concentrations of lycopene in tomato products that can account for more than 80% of the dietary intake of this carotenoid [3].

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Abbreviations: **Con-A**, concanavalin A; **CVD**, cardiovascular disease; **ICAM-1**, intercellular adhesion molecule 1; **LFA-1**, leucocyte function antigen 1; **PBS**, phosphate buffer; **PBMCs**, peripheral blood mononuclear cells; **PHA**, phytohemagglutinin; **TCR**, T cell receptor

High lycopene levels in blood and adipose tissue correlate with a reduction in CVD incidence [4–7], and low concentrations are associated with early atherosclerosis [8]. Arterial intimal wall thickness is lower in subjects with higher adipose tissue lycopene concentrations, suggesting decreased risk of arterial occlusion [5, 9, 10]. Low plasma lycopene concentrations are also associated with elevated C-reactive protein concentrations, a risk marker for CVD [11, 12], and the CARDIA/YALTA study [13] found an inverse relationship between serum carotenoids, including lycopene, and markers of inflammation and vascular endothelial dysfunction. Corroborative evidence includes an inverse association between neopterin (a marker for cellular immune activation) and serum lycopene concentrations [14], suggesting that lower serum lycopene concentration may relate to higher grade chronic immune activation, a common feature in cardiovascular disorders. Furthermore, it has been suggested that lycopene could modulate the immune response by lowering inflammatory mediator production [15]. In vitro studies have shown that lycopene modulates the expression of adhesion molecules in human vascular endothelial cells [16], while in macrophages it increases the expression of LDL receptors involved in the regulation of cholesterol metabolism, inhibits

cholesterol synthesis, and increases LDL degradation [17]. More recently, lycopene was found to significantly decrease monocyte proliferation [18].

The effect of lycopene on lymphocytes, important immune cells actively involved in atherogenesis [19], has not yet been studied. The aim of the present study was to determine if lycopene could modulate T-lymphocyte activity, as measured by their ability to proliferate, differentiate, and express adhesion molecules after activation with a mitogen, and to determine whether these effects were dependent on modulation of early activation pathways.

2 Material and methods

2.1 Subjects

Ethical approval was granted by the North of Scotland research ethics committee (REC 07/S0801/32). Healthy males and females, 18–45-years old, were recruited from within the University of Aberdeen. Subjects with history of CVD, inflammatory and immune disorders, diabetes, or regularly taking any medication or dietary supplements known to affect inflammation or immune function were excluded. Smoking status was recorded.

2.2 Lycopene-enriched liposomes preparation

Freshly made liposomes were prepared by sonication using a mixture of egg yolk phosphatidylcholine (PC) (2 mg/mL) with various amounts of lycopene to obtain concentrations from 0 to 29 μg lycopene/mL. Lycopene and PC were dried under nitrogen then reconstituted with 9% sodium chloride (1 mL/2 mg PC). The solutions were then sonicated for 5 min following 30 s at 3 weeks, 4 min at 6 weeks, and a further 30 s at 3 weeks (Misonix Sonicator 3000, QSonica, CT). The mixture was then centrifuged for 10 min at 800 *g* before sterile filtration and use in cell culture. Liposome lycopene concentration was determined by HPLC after solvent extraction, based on a method previously published [20]. Samples were analyzed by HPLC (Waters 600E, Waters Ltd., UK) fitted with a Beckman ODS Ultrasphere 5-μm column (4.6 mm × 350 mm, Beckman Coulter, High Wycombe, UK) and a Phenomenex guard column (Macclesfield, UK). Samples were eluted isocratically (flow rate of 1.5 mL/min at 28°C) using 68.4% acetonitrile, 22.0% tetrahydrofuran, 6.8% methanol, and 2.8% ammonium acetate as mobile phase, and standard and lycopene peaks were detected at 450 nm. Trans-β-Apo-8′ carotenal was used as the internal standard. Analysis was carried out sing Waters™ Empower 2 software (Milford, MA).

2.3 Cell culture

Peripheral blood mononuclear cells (PBMCs) were isolated from fasted whole blood collected in lithium heparin tubes as previously described [21]. Cell number was determined by haemocytometry and adjusted to the required culture concentration using RPMI medium. PBMCs (1×10^6 cells/mL) were then cultured for 12–90 h at 37°C with 5% CO_2 in HEPES-buffered RPMI medium in the presence of 2.5% autologous serum, 0.2% antibiotics (10 units/mL penicillin and 10 μg/mL streptomycin), 2 mM glutamine, with or without concanavalin A (Con-A, 15 μg/mL), a nonspecific mitogen and anti-CD3 (0.5 μg/mL, specific for T-cell activation), and with or without lycopene-enriched liposomes (0–2.9 μg/mL lycopene). Nonactivated cells with lycopene-free liposomes were used as negative controls.

2.4 Cell proliferation

Lymphocyte proliferation was measured by the incorporation of tritiated thymidine. After 84-h incubation period, 1 mCi of tritiated thymidine (GE Healthcare, Buckinghamshire, UK) was added to each well and the cells were incubated for a further 6 h under the original incubation conditions. Cells were then harvested using a Harvester 96 Mach III M (Tomtec, Hamden, CT) onto a filter mat that was then left to dry overnight. Filter mats were placed in filter pockets and beta-scintillation fluid (Perkin Elmer, Waltham, MA) was added. The radioactivity of each well was counted using a Wallac1450 MicroBeta Trilux counter (GMI, Ramsey, MN) and measured as corrected counts per minute (CCPM).

2.5 Cell cycling

Propidium iodide (PI), Ribonuclease A (RNase A), Dulbecco's PBS, and sodium azide were all obtained from Sigma-Aldrich Co. (St. Louis). Fetal calf serum (FCS) was obtained from Biosera (East Sussex, UK) and ethanol from Joseph Mills (Liverpool, UK). Staining buffer was made up using Dulbecco's PBS containing 2% FCS and 0.01% sodium azide. RNase A was reconstituted to 1 mg/mL with Dulbecco's PBS and stored in aliquots at −20°C. PI was reconstituted at 1 mg/mL in distilled water with 0.1% sodium azide and stored at 4°C in the dark.

After 90-h culture, cells were harvested and the culture media supernatant removed and stored at −80°C until analysis. The cell pellet was washed twice with PBS and then fixed by the dropwise addition of 1 mL of ice-cold, 70% ethanol while vortexing. The sample was then incubated for 1 h at 4°C. Cells were then centrifuged (400 × *g*, 5 min, 4°C), the ethanol supernatant removed and the cells resuspended in 1 mL staining buffer. The RNA was then denatured by the addition of 100 μL of a 1 mg/mL RNAse A solution in Dulbecco's PBS and incubating for 30 min at 37°C. Cells were then stained with 1 mL of a 5 μg/mL PI solution in staining buffer and incubated for 30 min at room temperature.

Samples were stored at 4°C and out of the light until flow cytometry analysis using a FACS Calibur (Becton Dickinson, Oxford, UK). Samples were analyzed within 24 h of processing and analyzed at less than 400 events per second.

2.6 Cell surface marker expression and IL-2 determinations

CD3 FITC (mouse anti-human), CD4 FITC (mouse anti-human), CD8 FITC (mouse anti-human), and CD69 PE (mouse anti-human) were all purchased from Dako (Glostrup, Denmark). CD11a FITC (mouse anti-human) and CD25 PE (mouse anti-human) were purchased from Autogen Bioclear (Wiltshire, UK). Bovine serum albumin (BSA) and formaldehyde were obtained from Sigma-Aldrich Co. (St. Louis). CD3, CD4, CD8, and CD69 expression were measured after 12 h, and CD11a and CD25 after both 36 and 60 h. Expression was measured by flow cytometry (Becton Dickinson, Oxford, UK). IL-2 secreted into the cell medium after 18-h culture was determined by ELISA (Bender Medsystems, UK) according to the manufacturer's instructions.

2.7 Statistics

All data were normally distributed (Kolmogorov–Smirnov test). The effect of lycopene on all parameters measured was tested using one-factor ANOVA with lycopene as fixed factor. In order to adjust for each subject being used for each lycopene concentration, "subject" was entered as a "random factor" into the analysis. Significance was measured at $p < 0.05$. Differences between lycopene concentrations were analyzed using the Bonferroni post-hoc test.

3 Results

3.1 Effect of lycopene on T-lymphocyte proliferation

Lycopene significantly reduced T-lymphocyte proliferation in a dose-dependent manner in mitogen-stimulated PBMCs (Fig. 1). The inhibition was greater in cells activated by anti-CD3 than by Con-A, which was expected as both induce mitogenesis but via different pathways of activation. Anti-CD3 binds specifically to the T-cell receptor (TCR) to induce T-cell activation and proliferation, whereas Con-A binds to multiple receptors on the T-cell surface [22]. Con-A also activate monocytes via the Macrophage-1 antigen (Mac-1) on the monocyte surface [23], leading to further increase of lymphocyte activation [24].

In Con-A-activated cells, low lycopene concentrations (0.03, 0.11, and 0.31 μg/mL) significantly inhibited proliferation by nearly 20% and up to 30% at the highest lycopene concentrations tested (1.18 and 2.93 μg/mL) compared with the control ($p < 0.05$). A similar pattern was observed in anti-CD3-activated cells but with a greater magnitude of effect ($p < 0.05$), where proliferation decreased by 10% for the lycopene concentration of 0.03 μg/mL to reach a maximum inhibition of proliferation (50%) at the highest lycopene concentration (2.93 μg/mL).

3.2 Effect of lycopene on T-cell cycle

Increased cell death by apoptosis and/or necrosis, or inhibition of steps from the cell cycle inducing a decrease in cell mitosis by lycopene could be responsible for the reduction of cell proliferation observed. Lycopene had no significant effect on mitogen-activated T-cell cycle measured after 90-h incubation (Table 1). The proportion of apoptotic cells ($p = 0.797$, $p = 0.719$, and $p = 0.571$ for the control, Con-A and anti-CD3 activated cells, respectively), of cells in G_0/G_1 phase (control, $p = 0.735$; Con-A, $p = 0.606$; anti-CD3, $p = 0.156$), of S phase cells (control, $p = 0.848$; Con-A, $p = 0.116$; anti-CD3, $p = 0.354$) or the proportion of cells in G_2/M phase (control, $p = 0.376$; Con-A, $p = 0.213$; anti-CD3, $p = 0.367$) were not affected by lycopene. The proportion of apoptotic/necrotic cells was low (2%) for every concentration of lycopene tested, irrespective of the mitogen used for activation. The percentage of cells in both S and G_2/M phase was noticeably lower in the nonactivated cells (2 and 0%, respectively) than in Con-A-activated (8 and 10%) and anti-CD3-activated cells (4 and 5%). Consequently, the percentage of cells in the G_0/G_1 phase was higher for nonactivated cells (95%) than for either Con-A-activated (79%) or anti-CD3-activated (88%) cells.

3.3 Cell surface marker expression

Lycopene did not affect the proportion of $CD3^+$, $CD8^+$, or $CD4^+$ cells or the expression of these cell surface markers (data not shown). There was no effect of lycopene on the expression of the early activation marker CD69 in any of the nonactivated T-cell subsets ($CD3^+$, $p = 0.407$; $CD4^+$, $p = 0.952$; $CD8^+$, $p = 0.303$; data not shown). The effect of lycopene on CD69 expression in different T-cell subsets is shown in Table 2 (Con-A) and Table 3 (anti-CD3). In Con-A-activated cells, lycopene (1.18 μg/mL) significantly decreased CD69 expression in $CD3^+$ cells ($p = 0.007$) and in T-helper cells ($CD4^+$ cells) ($p = 0.029$). CD69 expression tended to decrease in $CD8^+$ cells but the effect was not significant ($p = 0.066$). A similar pattern was observed in anti-CD3-activated cells (Table 3) where lycopene (1.18 μg/mL) decreased CD69 expression in all of the T-cell subsets studied ($CD3^+$, $p = 0.001$; $CD4^+$, $p = 0.006$; $CD8^+$, $p = 0.002$) compared with the control.

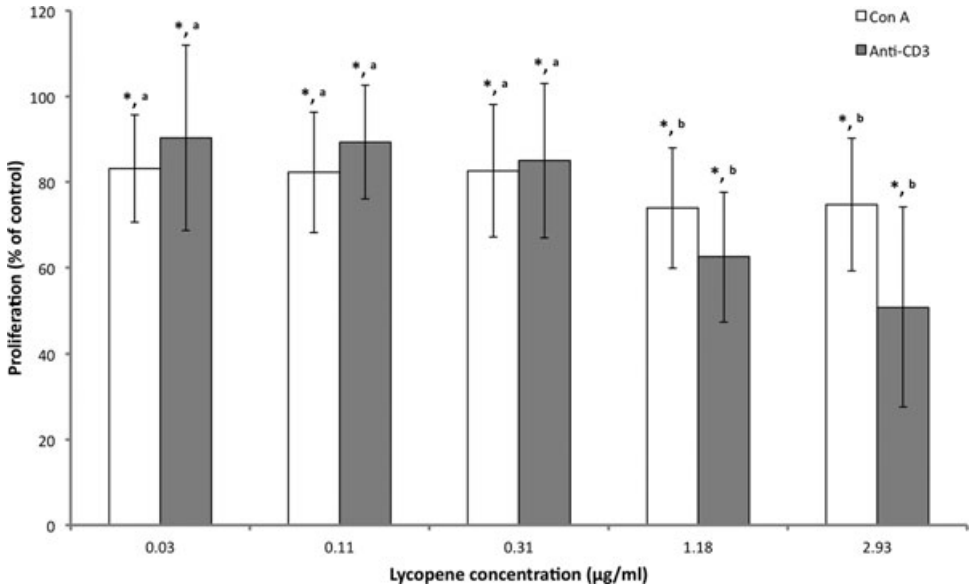

Figure 1. Effect of lycopene-enriched liposomes on lymphocyte proliferation. Proliferation was measured by tritiated thymidine incorporation after 90 h. Values presented are mean percentage (SD) of the control (0.0 μg lycopene/mL) of 16 independent experiments. Differences between lycopene concentrations were assessed by using one-factor ANOVA. *Significantly different from control (0.0 μg lycopene/mL), $p < 0.05$. Values with different superscript letters denote significant differences between lycopene concentrations ($n = 16$).

3.4 Effect of lycopene on IL-2 receptor (CD25) and CD11a expression and IL-2 production in mitogen-activated lymphocytes

As expected, the percentage of T-lymphocytess ($CD3^+$ cells) expressing the IL-2 receptor ($CD25^+$ cells) was very low (less than 5%) in the nonactivated cells, at both time points (data not shown). Over 60 and 27% of T-cells expressed IL-2 receptors after incubation with Con-A and anti-CD3, respectively, the percentage of positive cells being higher at 60 h than at 36 h (Table 4). There was no significant effect of lycopene on the percentage of T-lymphocytes expressing CD25 (Table 4). There was also no significant effect of lycopene on the level of CD25 expression in T cells, irrespective of the mitogen and lycopene concentrations used (36 h: Con-A, $p = 0.397$; anti-CD3, $p = 0.416$; 60 h: Con-A, $p = 0.871$; anti-CD3, $p = 0.075$). Due to practical problems, the analyses for the 36-h samples had to be carried out using another flow cytometer (LSR II, Becton Dickinson, Oxford, UK) resulting in higher fluorescence values compared with the 60h samples. Therefore both time points were not directly comparable.

However, after 18-h incubation, lycopene significantly reduced the concentration of IL-2 recovered in the culture medium (Table 5). The reduction was lycopene dose dependent and reached a maximum of 11% in Con-A-activated cells and by 29% in cells activated by anti-CD3 for the highest lycopene concentration tested.

Table 6 shows the effects of different lycopene concentrations on the percentage of cells expressing CD11a as well as the level of CD11a expression, as measured by CD11a fluorescence, in PBMCs after 36- and 60-h incubation. CD11a was expressed in both nonactivated (not shown) and

Table 1. Effect of lycopene on lymphocyte cell cycle[a)]

		Lycopene concentration (μg/mL)			
Mitogen	Cycle phase	0.0	0.11	1.18	$p^{b)}$
0	Apoptotic/ necrotic	2.44 (1.08)	2.56 (1.16)	2.55 (1.22)	0.797
	G_0/G_1	95.74 (1.72)	95.49 (1.66)	95.52 (2.25)	0.735
	S	1.80 (0.97)	1.93 (1.18)	1.91 (1.47)	0.848
	G_2/M	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.376
Con-A	Apoptotic/ necrotic	2.39 (1.33)	2.41 (1.28)	2.32 (1.15)	0.719
	G_0/G_1	79.18 (4.09)	78.60 (4.36)	78.66 (4.41)	0.606
	S	8.15 (2.82)	8.60 (2.97)	7.86 (2.05)	0.116
	G_2/M	10.20 (2.89)	10.35 (3.10)	11.09 (3.68)	0.213
Anti-CD3	Apoptotic/ necrotic	2.63 (1.19)	2.42 (0.76)	2.59 (1.25)	0.571
	G_0/G_1	87.72 (5.13)	87.60 (6.49)	88.93 (4.80)	0.156
	S	3.87 (1.78)	4.32 (2.97)	3.39 (1.79)	0.354
	G_2/M	5.76 (2.98)	5.61 (3.35)	5.05 (2.39)	0.367

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 90 h. Data are mean% (SD) of total lymphocyte population ($n = 11$).
b) Differences between lycopene concentrations were assessed by using one-factor ANOVA.

Table 2. Effect of lycopene on CD69 expression in Con-A-activated T cells[a)]

		Lycopene concentration (μg/mL)			
		0.0	0.11	1.18	p[d)]
% CD69 positive cells[b)]	CD3[e)]	63.40[a] (9.60)	62.23[ab] (9.09)	60.19[b] (9.92)	0.020
	CD4	50.04 (11.20)	48.14 (8.09)	47.82 (8.47)	0.431
	CD8	34.73 (15.47)	38.68 (15.95)	34.91 (14.97)	0.176
CD69 MFI[c)]	CD3[e)]	609.19[a] (131.65)	608.27[a] (122.32)	577.17[b] (135.22)	0.007
	CD4[e)]	604.58[a] (137.85)	610.61[a] (131.11)	579.49[b] (149.00)	0.029
	CD8	527.33 (131.03)	519.70 (125.67)	477.05 (160.33)	0.066

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 14 h. Values are mean (SD) of 11 independent experiments.
b) Represents cells that are positive for both CD69 and T-cell subset markers after 14-h incubation.
c) MFI, mean fluorescence intensity representing the average geo-mean fluorescence of double-positive cells.
d) Differences between lycopene concentrations were assessed by using one-factor ANOVA.
e) Values with different superscript letters are significantly different (Bonferroni, $p < 0.05$).

Table 3. Effect of lycopene on CD69 expression in anti-CD3-activated T cells[a)]

		Lycopene concentration (μg/mL)			
		0.0	0.11	1.18	p[d)]
% CD69 positive cells[b)]	CD3	11.58 (14.71)	12.54 (13.96)	9.60 (12.63)	0.113
	CD4	36.51 (9.03)	35.31 (10.20)	33.51 (12.73)	0.088
	CD8[e)]	24.46[a] (11.19)	25.13[a] (11.91)	20.69[b] (10.57)	0.001
CD69 fluorescence intensity[c)]	CD3[e)]	626.25[a] (98.32)	341.83[a] (110.10)	298.84[b] (92.97)	0.001
	CD4[e)]	320.26[a] (152.99)	333.02[a] (150.82)	298.10[b] (137.73)	0.006
	CD8[e)]	303.76[a] (85.37)	308.63[a] (87.11)	274.45[b] (82.79)	0.002

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 14 h. Values are mean (SD) of 11 independent experiments
b) Represents cells that are positive for both CD69 and T-cell subset markers.
c) Represents the average geo-mean fluorescence of double-positive cells.
d) Differences between lycopene concentrations were assessed by using one-factor ANOVA.
e) Values with different superscript letters are significantly different (Bonferroni, $p < 0.05$).

activated cells. The percentage of positive cells was similar in the different mitogen groups and incubation times, with at least 90% of cells being CD11a positive irrespective of the mitogen or time. Lycopene did not affect the proportion of PBMCs expressing CD11a after 36 h irrespective of the mitogen used. After 60 h, however, lycopene slightly but significantly increased the proportion of $CD11a^+$ cells, compared with the control (0.0 μg lycopene per mL) in Con-A-activated cells ($p = 0.034$). However, this effect was not observed in anti-CD3-activated cells ($p = 0.767$). Furthermore, the level of expression of CD11a was not altered by lycopene.

4 Discussion

Lycopene suppressed proliferation in mitogen-stimulated cells, even at low concentrations, comparable with plasma lycopene concentrations found in populations with low intake of lycopene-rich foods such as tomato-based products [25]. The decrease in proliferation by lycopene suggests it may be effective in decreasing overall T-cell activation and therefore protective against atheromatous plaque development.

The lycopene concentrations used in this study were physiologically relevant. Maximum inhibitory effects (up to 50%) were observed for the highest lycopene concentrations tested, which are similar to lycopene concentrations found in plasma of subjects with daily consumption of lycopene around 30 mg [25]. Ex vivo measurements of Con-A- or phytohemagglutinin (PHA)-stimulated PBMCs proliferations were not affected by the consumption of tomato juice [26], lycopene supplementation [27], or tomato extract [28]. In line with these findings, both ex vivo PHA- [29] and Con-A-stimulated [30] PBMCs proliferations were significantly decreased after a high carotenoid diet. However, confounding factors such as other dietary components, age of the subjects, and related variability in absorption between individuals may account for the disparity of results observed. The studies by Corridan [26] and Watzl [27] were both carried out in the elderly who may have reduced capacity for intestinal absorption and transport of nutrients, such as carotenoids [31]. Others [29, 30, 32] involved periods of carotenoid depletion in the diet that may have already affected the immune response before increasing lycopene intake. However, proliferation and differentiation of a human monocyte/mactrophage cell line (U937) was significantly reduced by lycopene [18].

High numbers of T lymphocytes are usually found in atherosclerotic plaques [33]. During atherogenesis, T cells are recruited and then bound to the site of the damaged endothelium before transmigrating into the intima. The T cells are then activated through binding with antigen-loaded MHC molecules and costimulatory molecules, triggering a cascade of signaling events that result in an immune response including cell proliferation [34].

The mechanisms responsible could involve the modulation of the pathways involved in apoptosis and necrosis. Lycopene has previously been shown to affect caspase-3

Table 4. Effect of lycopene on mitogen-activated lymphocyte IL-2 receptor (CD25) expression[a)]

			Lycopene concentration (μg/mL)			
Mitogen			0.0	0.11	1.18	p[e)]
Con-A	36 h	% positive[b)]	60.35	59.79	58.54	0.450
		cells	(13.40)	(10.24)	(12.20)	
		MFI[c),d)]	32 608.52	28 511.54	30 071.27	0.397
			(45 326.15)	(38 947.98)	(41 007.22)	
	60 h	% positive	75.44	76.48	74.36	0.121
		cells	(8.66)	(10.32)	(9.35)	
		MFI	726.89	767.75	744.15	0.871
			(507.69)	(521.29)	(504.56)	
Anti-CD3	36 h	% positive	26.41	26.89	27.23	0.926
		cells	(16.57)	(15.68)	(19.51)	
		MFI[c),d)]	40 009.27	40 591.55	37 798.40	0.416
			(59 893.24)	(59 629.50)	(56 607.07)	
	60 h	% positive	32.04	32.89	32.54	0.963
		cells	(22.04)	(23.00)	(20.12)	
		MFI	509.45	503.52	501.16	0.075
			(375.85)	(364.00)	(363.19)	

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 36 and 60 h. Values are mean (SD) of 11 independent experiments.
b) Represents cells that are positive for both CD25 and T-cell subset markers.
c) MFI, mean fluorescence intensity representing the average geo-mean fluorescence of double-positive cells.
d) Due to practical problems, the analyses for the 36-h samples had to be carried out using another flow cytometer (LSR II, Becton Dickinson, Oxford, UK) resulting in higher fluorescence values compared with the 60-h samples.
e) Differences between lycopene concentrations were assessed by using one-factor ANOVA.

levels, an enzyme required in the intrinsic apoptotic pathway [35]. However, apoptosis and necrosis were not affected by lycopene in our study. The signaling cascade process following T-cell activation involves sequential phosphorylation reactions by protein kinases that ultimately regulate the processes of gene transcription. Some evidence suggests that lycopene may affect such signaling pathways, e.g. mitogen-activated protein kinase (MAPK) [35], or possibly modulate redox-sensitive transcription factors, thereby affecting gene transcription of proteins required to initiate proliferation through the cell cycle [36]. However, the cell cycle remained unaffected by lycopene. Cell-cycling phases were measured after 90 h, identical to the time at which proliferation was measured. Therefore, longer incubation time might be required to observe any effects of lycopene on the cell cycle.

CD69 represents a marker of early T-cell activation and acts as a costimulatory molecule that increases T-cell responses following the TCR-ligand interaction [37]. Lycopene did not significantly affect the proportion of cells expressing CD69. However, its expression was significantly reduced in both Con-A- and anti-CD3-stimulated T-lymphocytes, indicating that lycopene reduces T-cell proliferation through mechanisms involving early activation pathways. After T-cell activation, both IL-2 and the IL-2 receptor (CD25) are rapidly upregulated and therefore represent two additional early activation markers [38]. In line with the decrease in proliferation observed, IL-2 production was also significantly reduced by lycopene in both Con-A- and anti-CD3-stimulated cells. IL-2 is one of the first cytokines to be released by T cells after activation, and drives the G_1-S phase progression and T-cell clonal expansion after binding to the IL-2 receptor (IL-2R) on other T cells, leading to a further stimulation of T-cells proliferation at the site of inflammation [39]. A previous study reported a decrease in IL-2 due to lycopene in LPS-stimulated PBMCs [40]. However, ex-vivo studies measuring IL-2 production in stimulated PBMCs after a period of tomato or lycopene nutritional intervention, showed no effect of the dietary interventions [27–29, 33]. During an immune challenge, IL-2 is upregulated along with IL-2R expression but our

Table 5. Effect of lycopene on PBMC IL-2 production (pg/mL)[a)]

	Lycopene concentration (μg/mL)			
Mitogen	0.0	0.11	1.18	p[b)]
0	1.00	2.64	1.55	0.517
	(2.05)	(6.28)	(2.70)	
Con-A[c)]	3251.73[a]	3071.00[b]	2885.90[c]	0.001
	(992.22)	(1011.42)	(1153.23)	
Anti-CD3[c)]	527.56[a]	472.60[a]	375.22[b]	0.006
	(595.38)	(466.68)	(472.55)	

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 18 h. Values are mean (SD) of 11 independent experiments.
b) Differences between lycopene concentrations were assessed by using one-factor ANOVA.
c) Values with different superscript letters are significantly different (Bonferroni, $p < 0.05$).

results showed no effect of lycopene on IL-2R. This implies that the upregulation of IL-2 and IL-2R are separately regulated. This is supported by the results of a previous study [41] who found that blocking the IL-2R in stimulated PBMCs did not reduce IL-2 expression. Furthermore, blocking the binding of IL-2 to IL-2R in PBMCs does not affect CD69 expression, suggesting that both pathways of activation are independent [41]. However, T-regulatory cells expressing $CD25^+$ were already present in our samples prior to activation. It is therefore possible that lycopene could modulate the expression of CD25 in some T-cell populations while increasing the proportion of T-regulatory cells or vice versa, resulting overall in no obvious or detectable change. However, this remains to be determined. The potency of lycopene to inhibit proliferation, CD69 expression, and IL-2 secretion to a greater extent in anti-CD3-activated cells compared with Con-A-activated cells suggests that lycopene might be more effective in suppressing TCR-dependent activation processes. A reduction of IL-2 secretion in atherosclerosis would potentially reduce the chronic inflammation occurring in the lesion. It would decrease the proliferation and activation of T cells that would have knock on effects on other immune cells also involved in plaque formation [19].

CD11a is part of the receptor leucocyte function antigen 1 (LFA-1), which is expressed on T cells, B cells, macrophages, and neutrophils, and binds to the intercellular adhesion molecule 1 (ICAM-1), expressed on the endothelium, T cells, and other leukocytes. A reduction in LFA-1 expression decreases the potential for T cells to bind to the endothelium and consequently transmigrate into the subintima, a critical step in atherosclerotic plaque progression [42]. The proportion of $CD11a^+$ cells was slightly reduced only in Con-A-activated cell, not in anti-CD3-activated cell treated with 0.11 μg/mL lycopene. However, such a small effect (less than 1%), even if statistically significant, might result from normal fluctuation in LFA-1 expression independent of lycopene treatment and therefore not physiologically relevant. This is further supported by the lack of change in LFA-1 expression in cells stimulated with anti-CD3. Anti-CD3 would only activate T cells while Con-A would also activate other PBMCs leading to the modulation of CD11a expression. Interestingly, a previous ex vivo study also found no effect of lycopene supplementation for 26 days on CD11a expression in isolated monocytes [43].

Overall, our results indicate that low and physiological concentrations of lycopene can inhibit early T-cell activation through the modulation of cell proliferation, CD69 expression, and IL-2 secretion. The mechanism involved remains to be elucidated but could involve the direct [44] or indirect activation via oxidized products of lycopene [45] of the electrophile response element/antioxidant response element (EpRE/ARE). Dietary lycopene is rapidly metabolized into polar metabolites [46]. It is possible that these hydrophilic-oxidized products of lycopene generated during lycopene cell metabolism could be responsible for the modulation of lymphocyte activity. Such a reduction in T-cell activation during atherosclerosis would reduce the inflammatory responses involved in atherosclerotic plaque formation and development,

Table 6. Effect of lycopene on mitogen-activated lymphocyte expression of CD11a[a)]

			Lycopene concentration (μg/mL)			
Mitogen			0.0	0.11	1.18	p[d)]
Con-A	36 h	% CD11a	92.12	92.84	92.24	0.166
		Positive cells[b)]	(4.45)	(4.65)	(4.28)	
		MFI[b),c)]	1595.42	1622.44	1491.54	0.173
			(2147.99)	(2209.22)	(2022.51)	
	60 h	% CD11a	94.61[a]	95.36[b]	94.40[a]	0.034*
		Positive cells[e)]	(2.96)	(2.57)	(3.03)	
		MFI	56.26	57.48	57.86	0.881
			(34.20)	(32.95)	(32.68)	
Anti-CD3	36 h	% CD11a	96.64	96.50	96.56	0.947
		Positive cells	(2.28)	(2.49)	(3.28)	
		MFI[b),c)]	2706.54	2765.58	2892.04	0.179
			(3655.90)	(3757.96)	(3934.43)	
	60 h	% CD11a	96.54	96.67	96.50	0.767
		Positive cells	(1.37)	(1.60)	(2.09)	
		MFI	70.73	71.31	72.44	0.296
			(49.92)	(46.43)	(44.20)	

a) PMNCs were incubated with or without mitogen and lycopene-enriched liposomes for 36 and 60 rs. Values are mean (SD) of 11 independent experiments.
b) MFI, mean fluorescence intensity representing the average geo-mean fluorescence of $CD11a^+$ cells.
c) Due to practical problems, the analyses for the 36-h samples had to be carried out using another flow cytometer (LSR II, Becton Dickinson, Oxford, UK) resulting in higher fluorescence values compared with the 60-h samples.
d) Differences between lycopene concentrations were assessed by using one-factor ANOVA.
e) Values with different superscript letters are significantly different (Bonferroni, $p < 0.05$).

and could explain the potential cardioprotective effects of lycopene suggested by observational studies.

F. T. and H. W. conceived and designed the study. Under the supervision of F. T. and H. W., L. M. M. recruited volunteers, assigned volunteers to treatment groups and carried out the experiments. L. M. M. and F. T. did the statistical analyses. F. T. drafted the report. The work was supported by the Rural and Environment Science and Analytical Services of the Scottish Government.

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