Reduced growth and integrin expression of prostate cells cultured with lycopene, vitamin E and fish oil *in vitro*

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Integrins are transmembrane proteins that facilitate the interaction of cells with the extracellular environment. They have also been implicated in cancer progression. The effects of nutrients thought to be involved in the prevention of prostate cancer on integrin expression have not been determined. Prostate cancer cell lines representing a range of malignancy from normal (RWPE-1) to highly invasive phenotypes (22Rv1 < LNCaP < PC-3) were cultured with or without lycopene (10 nM), vitamin E (5 μM) or fish oil (100 μM) for 48 h. Growth and integrin ($\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$) expression were assessed using Trypan Blue exclusion and monoclonal antibodies combined with flow cytometry. Vitamin E enhanced ($P<0.001$) whereas fish oil reduced the growth of all the cell lines tested ($P<0.001$). Lycopene had no effect on growth. All the malignant cell lines exhibited lower expression of $\alpha_2\beta_1$ with the addition of lycopene to culture media. Supplemental fish oil reduced $\alpha_2\beta_1$ in most invasive cell lines (LNCaP and PC-3). Each nutrient at physiological levels reduced integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in most invasive cell lines (PC-3). The results suggest that integrins may represent an additional target of bioactive nutrients and that the effects of nutrients may be dependent on the type of cell line used.

Antioxidants: *n*-3 Fatty acids: Metastasis: *In vitro* studies: LNCaP: PC-3: 22Rv1: RWPE-1

Epidemiological and clinical studies have identified a number of dietary compounds purported to influence the molecular events involved in the progression of prostate cancer. Carotenoids[1–3], lipid-soluble antioxidants[4,5], and fish oil-derived PUFA, EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3)[6,7], are of particular interest. Cell-culture studies have affirmed the anticancer properties of these nutrients; however, the relevance of these experimental data to human diseases is limited. One limitation is that concentrations of nutrients used in experimental studies are typically much higher than established human plasma levels. Furthermore, the majority of investigations utilising *in vitro* cell models supplement culture media with fetal bovine serum (FBS). Extrapolations from these studies to human disease are erroneous because an absence or imbalance of nutrients in FBS compared with human serum (HS) affects cell growth, development and function. Important differences in the composition of HS and FBS exist including the amounts of *n*-3 PUFA, the *n*-6:*n*-3 fatty acid ratio and the PUFA:SFA ratio[8]. FBS is also devoid of basal levels of testosterone, a regulator of the growth of many prostate cancer cell lines[9], as well as many other growth-regulating hormones found in HS derived from males. In the present study, initial work focused on establishing a range of prostate cancer cell lines in culture supplemented with HS and, subsequently, evaluating the effects of bioactive nutrients on cell growth and integrin expression.

The consumption of lycopene, abundant in tomato-based products, has long been associated with prostate health and a reduced risk of developing prostate cancer[1,10]. In addition to exhibiting potent antioxidant activity[11], lycopene has been shown to inhibit the proliferation of normal prostate epithelial cells[12], as well as several prostate cancer cell lines[13]. Accumulating experimental evidence also indicates that lycopene is capable of influencing other aspects of prostate cell functioning, including cell-cycle inhibition, increased cellular differentiation, inhibition of the insulin-like growth factor-1 signalling pathway and increased gap-junction communication[14–16], which would be expected to influence cell growth. The antioxidant activity of vitamin E has also been well documented[17], with specific isomers demonstrating an ability to inhibit the growth of prostate cancer cells[18,19]. However, aside from antioxidant properties, little is known about other potential mechanisms of action of vitamin E on prostate cancer progression[20,21]. Numerous epidemiological studies have indicated that high fish consumption may be an effective approach to reducing risk of several cancers, including prostate cancer[7,22]. These observations are supported by several experimental studies reporting inhibitory effects of EPA and/or DHA treatment on the growth of prostate cancer cells[23,24]. To date, most of the epidemiological and clinical prostate cancer research investigating specific dietary nutrient

intakes has focused on the reduction of disease risk. While further understanding of preventative mechanisms is essential, research into how nutrients affect malignant cell growth and disease progression following diagnosis is desperately needed.

The progression of prostate cancer primarily involves the formation of secondary metastatic lesions to bone[25,26], a process partially mediated by integrin cell adhesion proteins. Integrins are a family of heterodimeric transmembrane proteins composed of α- and β-subunits that provide the physical link between proteins of the extracellular matrix and cell cytoskeleton. Integrins modulate many important signalling pathways and cellular events, including cell growth, survival, invasion, migration and differentiation[27,28]. Differences in the expression levels of specific integrins in normal and malignant prostate cells have been reported[29]; however, molecular events responsible for these differences remain largely unclear. Moreover, bone matrix contains several components, including type-I collagen, vitronectin, fibronectin and osteopontin, which are ligands for specific integrins implicated in the metastatic progression of prostate cancer[30]. Interactions of integrin $\alpha_2\beta_1$ with type-I collagen have been implicated in the formation of bone metastasis[31]. The integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ mediate cell adhesion to a variety of extracellular matrix proteins, particularly vitronectin, and play a key role in tumour-mediated angiogenesis, as well as tumour cell migration, invasion and survival during the metastatic process[32,33]. Expression of the integrins $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ may facilitate migration and metastatic spread of cancer cells and correlate with increased tumour invasiveness[31,32], making integrins a potential biomarker for cancer progression[28] and enabling an assessment of the influence of bioactive nutrients on cancer growth and development.

For the present study, a range of cell lines representing the disease spectrum from normal prostate epithelial cells to highly invasive prostate cancer cells (RWPE-1 > 22Rv1 > LNCaP > PC-3) were utilised. It was hypothesised that the addition of physiologically relevant concentrations of the nutrients lycopene, vitamin E or fish oil to cell-culture media would reduce prostate cell growth as well as $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin expression in this series of prostate cell lines.

Materials and methods

Human serum

Ethical approval was provided by the Faculty of Agriculture Forestry and Home Economics Health Research Ethics Board at the University of Alberta (Edmonton, Canada) to obtain HS from healthy male subjects between the ages 18 and 35. The subjects had no history of cancer, autoimmune disease or other existing medical conditions. Subjects receiving steroidal medications, hormone therapies or having undergone surgery within the last 3 months were excluded. Blood samples (50 ml) collected from the male subjects were separated by centrifugation and the serum fractions from all the donors were pooled and stored at $-20°C$.

Cell culture

A range of cell lines representing the spectrum of human prostate cancer, from normal prostate epithelial cells to brain and bone metastases, were used in the experiments. RWPE-1, 22Rv1, LNCaP (androgen sensitive and prostate-specific antigen (PSA) positive), DU-145 and PC-3 (androgen insensitive and PSA negative) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in $75\,cm^2$ flasks (Fisher Scientific, Edmonton, AB, Canada) at $37°C$ in a humidified 5% CO_2 atmosphere. All the cell lines were maintained in culture media recommended by ATCC and contained 2 mM-L-glutamine supplemented with 1% (v/v) antibiotic/antimycotic solution (100 U/ml penicillin, $100\,\mu g/l$ streptomycin and 25 mg/l amphotericin B). All the culture media were further supplemented with either 10% (v/v) heat-inactivated FBS or 1% (v/v) HS. Serum concentrations were selected to standardise the total amount of fatty acids introduced into the cell cultures[8].

All experimental studies were performed on the cells after the fourth passage. The cell lines were maintained within two passages of each other. The cells were grown until they reached 90% confluence and subcultured routinely, with the culture medium being changed two to three times a week. After culturing the cell lines in either HS or FBS for 4 d, gross cell morphology was determined by light microscopy. The cells were then trypsinised and counted using the Trypan Blue exclusion method to determine cell viability. Integrin expression was measured using monoclonal antibodies ($\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$) combined with FACS flow cytometry analysis described later.

Integrins

A range of prostate cell lines were assessed to determine $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ expression. Cells were plated onto ninety-six-well plates (5.0×10^4 cells/well) and coated with a buffer solution (PBS:4 % FBS (Gibco Invitrogen Corporation, Burlington, Ont., Canada)) for 20 min. The plates were centrifuged for 2 min and then the supernatant was removed from each well. The plates were lightly vortexed to displace the cells from the bottom of the wells. Antibodies recognising the integrins $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ ($10\,\mu g/ml$; Chemicon Int., Cedarlane Laboratories Ltd, Hornby, ON, Canada) were added to treatment wells with the buffer being added to control wells. The plates were then incubated in the dark at $4°C$ for 30 min, and the cells were rinsed with the buffer and vortexed three times. Fluorescein isothiocyanate ($20\,\mu g/ml$) was added to all the treatment wells and the buffer was added to the control wells. Plates were incubated in the dark at $4°C$ for 30 min, washed and vortexed three times. The cells were fixed in $300\,\mu l$ of PBS:0.5 % paraformaldehyde fixative solution. Finally, integrin expression of the prostate cell lines was assayed using flow cytometry using a FACS-Calibur instrument with CellQuest Software (BD Biosciences, Bedford, MA, USA). The cells were gated according to forward and side scatter characteristics and mean fluorescence of the gated cells was determined. Resulting percentages were corrected for background fluorescence (0–5 %) by incubating the cells with the appropriate isotype control (IgG_1).

Lycopene and vitamin E media preparation

Preliminary experiments were carried out to define the concentrations of nutrients to be used in these experiments

based on the physiological ranges reported in the literature at an amount that exhibited no toxicity (measured by lactate dehydrogenase assay, data not shown). Vitamin E ($\pm$ α-tocopherol), lycopene ($C_{40}H_{56}$) and Tween 40 were supplied by Sigma-Aldrich (Canada Ltd, Oakville, Ont., Canada). Stock solutions of vitamin E and lycopene were solubilised in Tween 40 (100 µl), diluted with complete culture media to a concentration of 5 mM, vortexed and filter sterilised using a 0·22 µm filter (Millipore Corporation, Bedford, MA, USA). The filtered stock solutions were serially diluted with additional complete culture media to a working concentration of 5 µM for vitamin E and 10 nM for lycopene, which are within human physiological ranges[34–38].

Fish oil media preparation

Preparation of fish-oil-supplemented media was adapted from a procedure used by Schley et al.[39]. A stock solution containing 10 mg/ml of fish oil was prepared in 95 % ethanol and stored at −80°C under $N_{2(g)}$. The stock solution was reconstituted in HS and then incubated in a water bath at 37°C for 1 h with vortexing every 10 min. The HS and fish oil mixture was diluted with culture media to a final working concentration of 100 µM and used immediately.

Nutrient treatment in cell culture

All the experiments using nutrient-supplemented media were carried out in cells cultured with 1 % HS. The cells were seeded onto six-well plates at a concentration of $1·0 \times 10^5$ cells/well containing 3 ml of complete culture media. Then the cells were allowed to adhere for 48 h at which time the media were decanted and replaced with 3 ml of nutrient-supplemented media containing either 10 nM-lycopene, 5 µM-vitamin E, 100 µM-fish oil, the respective control vehicle or complete culture media. The cells were detached from the respective wells after the 48 h nutrient incubation by the addition of 1 ml of 0·25 % trypsin−0·03 % EDTA. The wells were rinsed with appropriate media and the cells were counted using a haemacytometer and cell viability was evaluated via Trypan Blue exclusion. The expression levels of integrins, $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were determined for each prostate cell line using flow cytometry as described under the integrins section.

Statistical analysis

Means and standard deviations were calculated for all integrins, and Student's unpaired t tests were conducted to determine the statistical significance ($P<0·05$) between control vehicles and nutrient treatments for each cell line.

Results

Cell growth in human v. fetal bovine serum

The fatty acid and hormone composition of FBS is markedly different from HS[8]. To determine the effect of these differences on prostate cell growth, RWPE-1, 22Rv1, LNCaP and PC-3 cell lines were cultured separately in either 10 % FBS or 1 % HS. Growth of the phenotypically normal RWPE-1

cell line was not significantly altered by culture in HS. However, all four malignant cell lines showed significant reductions in the growth of up to 50 % ($P<0·01$; Fig. 1) when cultured in HS compared with FBS. Photographs comparing the growth and morphology of PC-3 cells in 1 % HS and 10 % FBS are shown in Fig. 2 (a), 2 (b).

Cell growth in response to lycopene, vitamin E and fish oil treatments

The ability of physiological concentrations of lycopene, vitamin E and fish oil to affect cell growth in RWPE-1, 22RV1, LNCaP and PC-3 cell lines was examined. Trypan Blue exclusion demonstrated that cell viability following nutrient exposure was greater than 99 %, and the lactate dehydrogenase assay confirmed that nutrient delivery vehicles and individual nutrients were non-cytotoxic (data not shown). Culture media containing lycopene (10 nM) resulted in no reductions in cell growth in any prostate cell lines tested (Table 1). An increase ($P<0·0001$) in cell growth was observed in all the prostate cancer cell lines when vitamin E (5 µM) was added to the culture media; however, growth of the normal RWPE-1 cell line (Table 1) was not significantly affected by this nutrient. The addition of fish oil (100 µM) to the culture media produced a significant decrease ($P<0·004$) in the cell growth of all prostate cell lines (Table 1). No marked changes in cell morphology were observed via light microscopy in response to lycopene, vitamin E or fish oil supplementation of the culture media (data not shown).

Integrin expression in response to lycopene, vitamin E and fish oil treatments

Preliminary experiments measuring basal expression levels of integrins $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were conducted on the RWPE-1, 22Rv1, LNCaP, DU-145 and PC-3 cell lines (data not shown). Integrin $\alpha_2\beta_1$ was highly expressed on all the five prostate cell lines. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were expressed only by the more invasive DU-145 and PC-3 cell
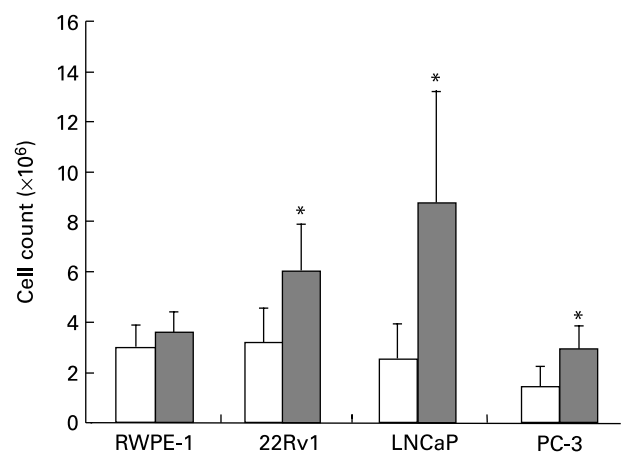

Fig. 1. Growth of RWPE-1, 22Rv1, LNCaP and PC-3 is greater when cells are cultured with fetal bovine serum (FBS; ▩) compared with human serum (HS; ▫). Viable cells were counted to determine cell population growth after 7 d in culture using Trypan Blue exclusion. Bars represent the means and standard errors ($n \geq 15$). * Statistical significance ($P<0·01$) between HS and FBS for the cell line as determined by Student's unpaired t test.
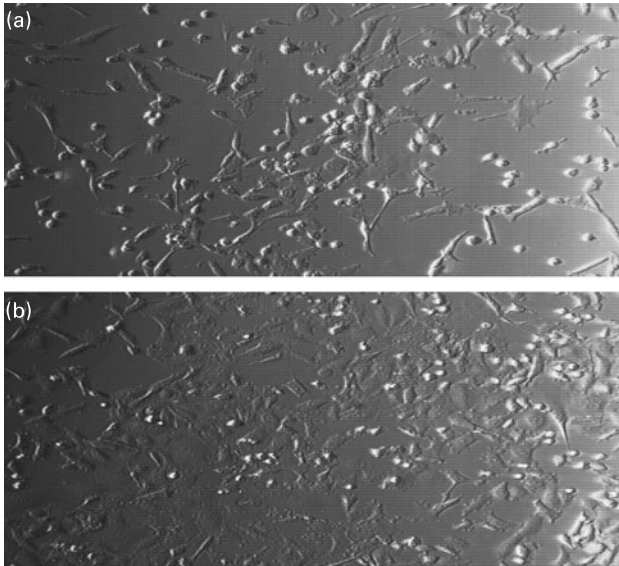
Fig. 2. Photograph of PC-3 in 1% human serum (HS) and 10% fetal bovine serum (FBS). Cells were visualised by light microscopy following 7 d in culture with either (a) FBS or (b) HS (seeded at 1.0×10^6 cells).

lines. These results are consistent with other reports, indicating that the changes in the expression of specific integrins may correlate with the progression to a more invasive phenotype[29,31]. In accordance with these data, additional experiments measured only integrins shown to be expressed on each individual cell line.

$\alpha_2\beta_1$ supplementation of the culture medium with 10 nM lycopene produced a significant increase ($P < 0.0001$) in integrin $\alpha_2\beta_1$ expression by RWPE-1 cells, and a corresponding significant reduction ($P < 0.04$) in the expression by 22Rv1, LNCaP and PC-3 cells in comparison with vehicle control (Table 2). Supplementation of culture medium with 5 μM-vitamin E resulted in a decrease ($P < 0.0001$) of integrin $\alpha_2\beta_1$ expression in 22Rv1 cells; however, the expression levels in RWPE-1, LNCaP and PC-3 cells were not significantly altered in comparison with vehicle control (Table 2). Supplementation of culture medium with 100 μM-fish oil increased ($P < 0.02$) $\alpha_2\beta_1$ expression in RWPE-1 and 22Rv1 cells, and decreased $\alpha_2\beta_1$ ($P < 0.01$)

expression in LNCaP and PC-3 cells in comparison with cells incubated with the control vehicle (Table 2).

$\alpha_v\beta_3$ and $\alpha_v\beta_5$ are up-regulated in more aggressive prostate cancer cell types and thus were expressed at the highest levels in the PC-3 cell line, in agreement with a previous report[40]. The addition of 10 nM-lycopene, 5 μM-vitamin E or 100 μM-fish oil to the culture medium resulted in a significantly lower ($P < 0.05$) expression of both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in the PC-3 cell line in comparison with the control vehicle (Figs. 3 and 4).

Discussion

Currently, *in vitro* cell culture is the principal model system employed for the studies of human prostate cancer. A range of cell lines spanning the spectrum of this disease, from phenotypically normal prostate epithelial cells to aggressive metastatic tumour cells, have been established. Nearly all studies culture these cells in media supplemented with FBS. The relevance of these results to man may be misleading, due in part, to the major differences in the fatty acid composition which exist between HS and FBS[8]. For example, the higher concentrations of *n*-6 fatty acids found in FBS have been repeatedly demonstrated to stimulate the growth of prostate cancer cells[41,42]. Testosterone, present in HS but lacking in FBS, is an integral regulator of prostate cell growth and survival in the *in vivo* microenvironment[9]. In the present study, significantly lower growth in all the malignant cell lines (22Rv1, LNCaP and PC-3) was observed when the cells were cultured in HS compared with FBS, whereas the growth of the phenotypically normal cell line RWPE-1 was not significantly affected by the culture in HS. Cell morphology of the prostate cell lines also differed between the two culture conditions, suggesting a reason for concern about the exclusive use of FBS as a media supplement.

As prostate cancer cells become more invasive, they acquire phenotypic changes that alter their affinity for the components of the extracellular matrix, as well as other cellular components[29]. In the present study, we focused on several members of the integrin family of transmembrane proteins, $\alpha_2\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, demonstrated to have different levels of expression in more aggressive *v.* less invasive cells[31,43].

Table 1. Growth of cell lines with the addition of physiological levels of lycopene, vitamin E and fish oil (10 nM, 5 μM and 100 μM, respectively) was measured using Trypan Blue exclusion after 4 d in culture†

(Mean values and standard deviations for $n \geq 9$ for each nutrient)

	Number of cells ($\times 10^4$)											
	Lycopene				Vitamin E				Fish oil			
	Control		Nutrient		Control		Nutrient		Control		Nutrient	
Cell line	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nutrient treatment												
RWPE-1	13	11	12	9	25	5	29	10	39	21	21*	13
22Rv1	14	8	11	5	6	4	15*	6	25	6	7*	3
LNCaP	9	5	9	5	8	5	19*	5	27	6	7*	3
PC-3	5	3	7	3	3	3	11*	6	2	1	1*	1

* $P < 0.0001$.
† Lycopene had no effect on the growth of any of the cell lines, whereas fish oil reduced the growth of all the cell lines. Vitamin E reduced growth of the malignant cell lines.

Table 2. Expression of the $\alpha_2\beta_1$ integrin in cell lines with the addition of lycopene, vitamin E and fish oil (10 nM, 5 μM and 100 μM, respectively) shows nutrient-specific effects depending on the stage of the cancer each cell line represents†

(Mean values with their standard deviations for n 5–21 for each nutrient)

	Relative % of cells expressing $\alpha_2\beta_1$ integrin											
	Lycopene				Vitamin E				Fish oil			
	Control		Nutrient		Control		Nutrient		Control		Nutrient	
Cell line	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nutrient treatment												
RWPE-1	78	6	86**	1	78	6	75	2	72	7	79*	4
22Rv1	67	2	13**	7	67	2	61**	3	58	4	63**	4
LNCaP	54	3	34**	4	54	3	54	8	56	3	54*	3
PC-3	81	11	73*	2	81	11	78	2	75	3	65**	7

* $P < 0.05$.
** $P < 0.0001$.
† In the phenotypically normal cells, $\alpha_2\beta_1$ was higher in the nutrient-treated cells. In most advanced cell types (PC-3), fish oil and lycopene reduced the expression of $\alpha_2\beta_1$.

In addition to facilitating the migration of prostate cancer cells out of the primary site, the integrins also mediate adhesion to several bone matrix components including type-I collagen, fibronectin, laminin and osteopontin[44]. As a majority of prostate cancer metastases occur in bone, inhibiting the ability of tumour cells to adhere to bone matrix components represents an important therapeutic target[30]. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have been shown to be highly expressed by the tumour cells capable of forming bone metastases[29,45]. In agreement with previous reports[31], we demonstrated that the androgen-responsive and PSA-producing (and less invasive) cell lines 22Rv1 and LNCaP exhibit lower basal expression of integrin $\alpha_2\beta_1$ in comparison with the cell lines derived from metastasis. Furthermore, in the present study, integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were expressed in only 1 % of the less invasive cell lines, RWPE-1, 22Rv1 and LNCaP cells, whereas PC-3 cells exhibited higher expression levels of 10 and 35 %, respectively. Collectively, these results denote a shift in integrin expression and subsequently in the potential for prostate cancer cells to adhere to extracellular and bone matrix components, especially type-I collagen, during disease progression. These

observations suggest that integrins may represent important markers of the metastatic capacity of prostate cancer.

Epidemiological studies have associated lycopene, vitamin E and *n*-3 PUFA with reduced prostate cancer risk[1,5,7], although the mechanisms mediating these effects are not fully understood. Human plasma lycopene concentrations are approximately 0·5 μM[46], but range from 0·01 to 1·8 μM depending on the region of study[34–38,47,48]. Kim *et al.*[49] reported a dose-dependent effect of lycopene on the growth of LNCaP cells with complete inhibition of growth occurring at 100 μM lycopene. Tang *et al.*[50] showed that the growth of DU-145, PC-3 and LNCaP cells was inhibited 50 % by lycopene concentrations of 26·6, 40·3 and 168·5 μM, respectively. However, these lycopene concentrations are far in excess of what is physiologically achievable in man. Ivanov *et al.*[15] reported the growth of LNCaP and PC-3 cells to be significantly inhibited by physiologically relevant lycopene concentrations of 0·5 μM or more. By contrast, the physiologically attainable lycopene concentration used in the present study did not alter the growth of any prostate cancer cell line, a result consistent with the recent reports in DU-145, PC-3[51]
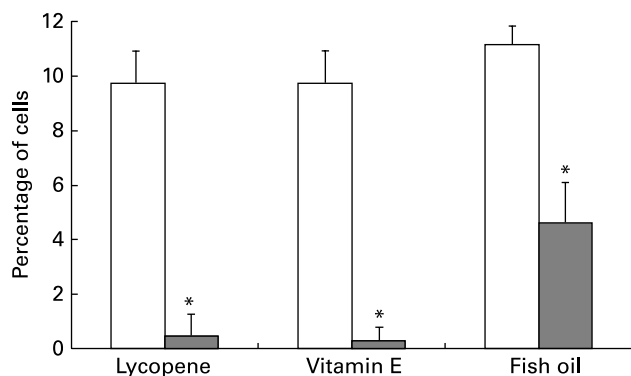

Fig. 3. PC-3 expressed less $\alpha_v\beta_3$ integrin when cells were cultured with lycopene, vitamin E or fish oil. The cells were cultured in media containing physiological levels of either lycopene, vitamin E or fish oil (10 nM, 5 μM and 100 μM, respectively) for 2 d. The cells were collected and incubated with integrin anitbodies and analysed using flow cytometry. Bars represent the means and standard errors (n 5–11 per treatment). (■), Nutrient treatment; (□), vehicle control. * Statistical significance ($P < 0.001$) between nutrient treatment and vehicle control as determined by Student's unpaired *t* test.
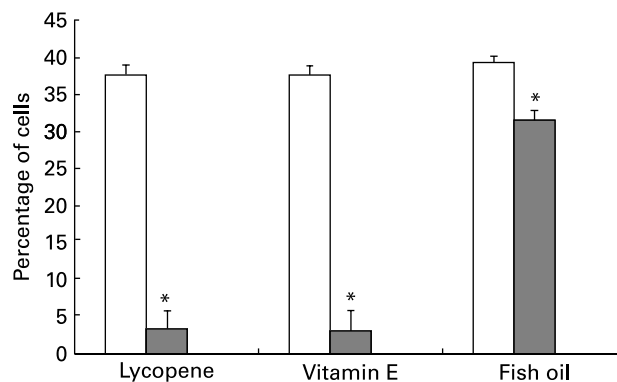

Fig. 4. PC-3 expressed less $\alpha_v\beta_5$ integrin when cells were cultured with lycopene, vitamin E or fish oil. Expression of $\alpha_v\beta_5$ was reduced when PC-3 cells were cultured with media containing physiological levels of lycopene, vitamin E or fish oil (10 nM, 5 μM and 100 μM, respectively) for 2 d. The cells were collected and incubated with integrin anitbodies and analysed using flow cytometry. Bars represent the means and standard errors (n 11). (■), Nutrient treatment; (□), vehicle control. * Statistical significance ($P < 0.05$) between nutrient treatment and vehicle control.

and LNCaP cells[52]. In addition to the growth-inhibitory effects, lycopene also influences other aspects of prostate cell functioning, including cell-cycle inhibition, increased cellular differentiation, inhibition of the IGF-1 signalling pathway and increased gap-junction communication, as reviewed by Wertz et al. [14]. Much like gap junctions, the integrin $\alpha_2\beta_1$ is involved in the regulation of cell-to-cell signalling for adhesion and migration[53]. In the present study, the addition of lycopene to media reduced $\alpha_2\beta_1$ in the malignant 22Rv1, LNCaP and PC-3 cell lines and, likewise, decreased $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in the PC-3 cell line. Therefore, lycopene may impact the progression of prostate cancer by altering invasive and migratory potential, as suggested by integrin expression.

Reports published to date on the effects of vitamin E and prostate cancer are conflicting[4]. Richards et al. [54] reported LNCaP cell growth to be reduced at concentrations of 0·2 and 2·0 μM. By contrast, Jiang et al. [18] reported that α-tocopherol failed to inhibit the growth of PC-3 and LNCaP cells at a concentration of 11·6 mM. The physiological concentration of vitamin E in HS ranges from 1·16 to 116 μM[55]. The present study suggests that vitamin E within this range enhances cell growth in all malignant cell lines, but not the phenotypically normal RWPE-1 cell line. Although preliminary, this nutrient may be important for the prevention of prostate cancer, but less effective once tumours are established. Recent data derived from an erythroleukaemia cell line demonstrated the ability of α-tocopherol to reduce the expression of several integrins in this cell line[56]. Expressions of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were significantly reduced by vitamin E treatment of the PC-3 cell line in the present study, suggesting that these cells may be less able to adhere to and migrate on the extracellular matrix components[57].

n-3 PUFA found in fish oils have documented the effects on several stages of prostate carcinogenesis[23,41]. The present study demonstrated a significant reduction in the growth of all prostate cell lines in response to treatment with fish oil at human physiological levels. We based this concentration on plasma fatty acid data from healthy human subjects who were not supplementing with fish oil[58], as well as the levels found in infants who were fed human milk[59,60]. In vitro treatment with n-3 PUFA at similar concentrations resulted in growth reductions in a breast cancer cell line[61]. Demonstration that fish oil treatment significantly reduced the expression of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in the PC-3 cell line is suggestive of decreased ability for this cell line to form metastatic lesions to bone[57]. Additionally, when introduced into cell-culture medium, n-3 fatty acids are incorporated into plasma membrane phospholipids of normal prostate cells, as well as prostate cancer cells, altering structural and chemical properties[6]. The observed growth inhibition and alteration in the integrin expression observed in the present study may be due to the changes in plasma membrane structure caused by long-chain PUFA incorporation.

In conclusion, differences in cell growth, morphology and integrin expression were evident when the cells were grown in HS compared with FBS. Physiologically relevant concentrations of lycopene had no significant effect on the growth of normal and malignant prostate cells. Vitamin E had no apparent growth-inhibitory effect on phenotypically normal prostate cells, but rather enhanced the growth of prostate cancer cell lines. Fish oil inhibited the growth of all prostate cell lines tested, further supporting its beneficial role in cancer prevention and as an adjuvant nutrient. The ability of lycopene, vitamin E and fish oil to decrease the expression levels of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in most invasive prostate cancer cell lines represents a crucial finding. Integrins are mediators of the metastatic progression of prostate cancer, particularly to the bone microenvironment and represent an important therapeutic opportunity. Further work is required to determine functional changes associated with the observed differences in integrin expression when the cells are cultured with lycopene, vitamin E or fish oil.

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