



Phytosterol and phytostanol-mediated epigenetic changes in cancer and other non-communicable diseases: a systematic review

Emtenan Jefrei, Mengfan Xu, J. Bernadette Moore and James L. Thorne*

School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

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Abstract

Phytosterols/phytosteranols are bioactive compounds found in vegetable oils, nuts and seeds and added to a range of commercial food products. Consumption of phytosterols/phytosteranols reduces levels of circulating LDL-cholesterol, a causative biomarker of CVD, and is linked to a reduced risk of some cancers. Individuals who consume phytosterols/phytosteranols in their diet may do so for many years as part of a non-pharmacological route to lower cholesterol or as part of a healthy diet. However, the impact of long term or high intakes of dietary phytosterols/phytosteranols has not been on whole-body epigenetic changes before. The aim of this systematic review was to identify all publications that have evaluated changes to epigenetic mechanisms (post-translation modification of histones, DNA methylation and miRNA expression) in response to phytosterols/phytosteranols. A systematic search was performed that returned 226 records, of which eleven were eligible for full-text analysis. Multiple phytosterols were found to inhibit expression of histone deacetylase (HDAC) enzymes and were also predicted to directly bind and impair HDAC activity. Phytosterols were found to inhibit the expression and activity of DNA methyltransferase enzyme 1 and reverse cancer-associated gene silencing. Finally, phytosterols have been shown to regulate over 200 miRNA, although only five of these were reported in multiple publications. Five tissue types (breast, prostate, macrophage, aortic epithelia and lung) were represented across the studies, and although phytosterols/phytosteranols alter the molecular mechanisms of epigenetic inheritance in these mammalian cells, studies exploring meiotic or transgenerational inheritance were not found.

Keywords: Phytosterols: Phytosteranols: Cancer: Epigenetics: Non-communicable disease: Histone deacetylase inhibitors: Gene regulation

Phytosterols/phytosteranols (PSS) are bioactive compounds found in vegetable oils, nuts seeds⁽¹⁾, which are also added to many commercial food products such as margarine and yoghurts⁽²⁾. There are a wide variety of PSS variants found in nature⁽³⁾, many of which are not yet fully characterised. Structural and functional differences between cholesterol and PSS derive from the presence of methyl or ethyl groups on the side chain, double bonds in the side chain at C22–C23, and in the case of phytosteranols, saturation of the B-ring (Fig. 1). Just as cholesterol is a major component of mammalian cell membranes, PSS are major components of plant cell membranes where they control fluidity and permeability⁽⁴⁾. In addition, PSS are precursors to steroid hormones such as brassinosteroids that control the life cycle physiological processes of the plants⁽⁵⁾, and production can change in response to stress, developmental stage and osmotic pressure. Despite a relatively low absorption rate (<5%) of dietary PSS^(6,7), compared with that of dietary cholesterol (30–80%) in humans⁽⁸⁾, PSS still reach micromolar concentrations

in human serum^(9,10) and exert physiological and systemic changes to the body. For example, consumption of 2–3 g/d of PSS reduces circulating levels of total cholesterol by 5–10%, LDL-cholesterol by 7.5–12%, and triacylglycerol by 0.1 mmol^(11–13). Therefore, for the treatment and management of hyperlipidaemia, PSS consumption is recommended by the National Lipid Association, the European Society of Cardiology and the European Atherosclerosis Society^(14,15). In addition, two meta-analyses have concluded that PSS consumption was linked to a reduced risk of developing some cancers in humans⁽¹⁶⁾ and reduced cancer burden in preclinical models⁽¹⁷⁾. More broadly, PSS have also been proposed to have anti-inflammatory, anti-microbial, blood pressure lowering, anti-diabetic and anti-obesity properties (reviewed in⁽¹⁸⁾).

The molecular mechanisms that convey inheritance of epigenetically regulated gene expression patterns include: post-translational modification of histones, DNA methylation and regulation of miRNA expression. Histone post-translational

Abbreviations: ATA, Antcin-A; CAMP, campesterol; DNMT, DNA methyltransferase; HDAC, histone deacetylase; miRNA, micro-RNA; PSS, phytosterols and phytosteranols; SITO, sitosterol; STIG, stigmasterol.

* **Corresponding author:** Dr J. L. Thorne, email medjlt@leeds.ac.uk



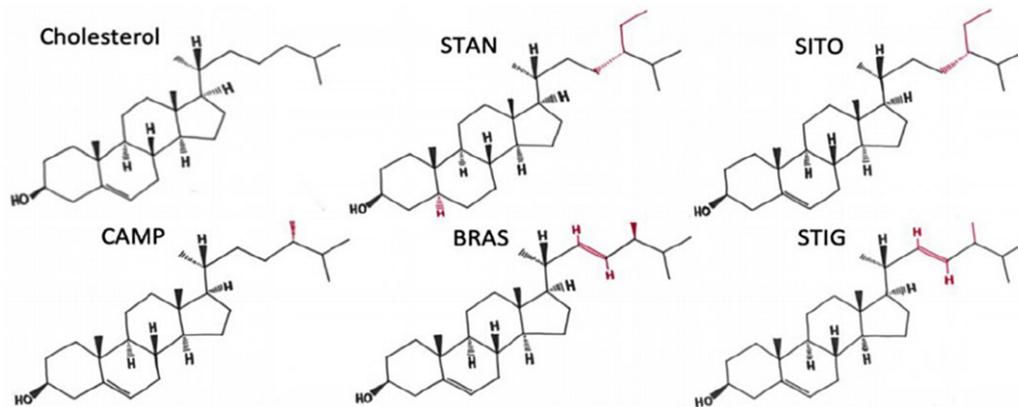


Fig. 1. The structures of cholesterol, sitostanol and phytosterols commonly consumed in a healthy human diet. STAN, sitostanol; SITO, β -sitosterol; CAMP, campesterol; STIG, stigmasterol. Regions of structural variability from CHOL are shown in red. Adapted from⁽³²⁾ and Licensed under CC BY 4.0.

modifications through their interactions with transcription factors provide a nuanced level of control over gene expression. Balance in the levels of histone acetylation is maintained by opposing activities of acetyl depositing enzymes (histone acetyltransferases) and the histone deacetylase (HDAC) enzymes that remove the acetyl group. The eighteen HDAC enzymes are grouped into four classes by sequence homology and play important roles in regulating gene expression⁽¹⁹⁾. Chemical inhibitors of HDAC (HDACi) are emerging as anti-cancer agents owing to their ability to induce cancer cell apoptosis⁽²⁰⁾, limit proliferation and modulate behaviour of immune system cells⁽²¹⁾. For example, the HDACi givinostat is in phase II clinical trials for the rare blood cancer polycythaemia vera⁽²²⁾ and a phase III trial for Duchenne muscular dystrophy (NCT02851797). DNA methylation, where a methyl group is added to the CpG Island in the promoter region by DNA methyltransferase (DNMT) enzymes, is essential for fundamental processes such as transcriptional regulation, chromatin structure, embryonic development, X chromosome inactivation and genomic imprinting⁽²³⁾. Lastly, expression of micro-RNA (miRNA) may also be conveyors of epigenetic information when inherited during cell division. Often referred to as oncomirs, miRNA are widely reported to be dysregulated in cancers of the breast^(24,25), blood⁽²⁶⁾ and thyroid⁽²⁷⁾. Interestingly, many miRNA are regulated by vitamins and other dietary components^(25,28,29).

The exact mechanisms through which PSS exert their effects remain unclear, but previous work has implicated induction of sterol metabolism and transport pathways^(30,31), interference with ligand dependent transcription factor activity^(32,33) and inhibition of oncogene signalling⁽¹⁷⁾. Given the change in steady-state levels of PSS in the body after long-term consumption, a key question is whether PSS, as for various other dietary compounds⁽³⁴⁾, may be dietary modifiers of epigenetic gene expression patterns. Therefore, the aims of this systematic review were to: (i) ascertain the depth of literature that has previously investigated links between PSS and epigenetic molecular mechanisms; (ii) understand the potential mechanisms through which PSS may influence epigenetic inheritance of gene expression patterns related to health and

disease and (iii) identify high confidence PSS-regulated epigenetic pathways that could be used as controls in future studies.

Methods

Search strategy

A systematic search of titles and abstracts was carried out up to and including February 2023 in three online databases: PubMed, Scopus and Web of Science, using pre-defined criteria informed by PROSPERO. Keywords encompassed PSS and epigenetic-related terms (Table 1). Records were collected and duplicates removed using Endnote X9-3-3, and titles/abstracts were screened using Rayyan software (<https://www.rayyan.ai/>).

Study selection

Titles and abstracts were screened for eligibility by two independent researchers (E. J, M. X) and a third researcher resolved any discordance. Inclusion criteria were (i) original papers; (ii) English, Chinese or Arabic languages; (iii) conducted on PSS or their derivatives and (iv) measured an epigenetic molecular mechanism. Epigenetic molecular mechanisms were defined as either: (i) changes to miRNA expression levels; (ii) changes to levels of histone post-translational modifications or expression of histone PTM enzymes or (iii) changes to CpG island methylation or expression of DNA methylation enzymes. We did not consider transience of founder event, or meiotic or mitotic heritability, as requisite factors for inclusion.

Data extraction

All data were extracted into Microsoft Excel. Data extracted included: PSS type, PSS delivery method, exposure time and concentration, epigenetic mechanism, molecular epigenetic factor such as regulated enzyme or miRNA, hypo/hyper-methylation of CpG promoters and cell/tissue type (Table 2).

Risk of bias assessment

The risk of bias analysis was performed using a designed multi-point questionnaire completed by two researchers working

Table 1. Search terms syntax for three databases

Database	Search Terms	Records
PubMed	(Phytosterol*[Title/Abstract] OR phytostanol*[Title/Abstract] OR Sitost*[Title/Abstract] OR Campe*[Title/Abstract] OR Stigmast*[Title/Abstract] OR Brassicasterol*[Title/Abstract] OR Avenast*[Title/Abstract] OR Spinast*[Title/Abstract] OR Ergost*[Title/Abstract] OR Daucost*[Title/Abstract] OR Peniocerol*[Title/Abstract] OR "Plant stanol*" [Title/Abstract] OR "Plant sterol*" [Title/Abstract]) AND (Epigenetics[MeSH Terms] OR epigenomics[MeSH Terms] OR Epigen* [Title/Abstract] OR "DNA methylation"[Title/Abstract] OR microRNA[Title/Abstract] OR miRNA[Title/Abstract] OR Histone[Title/Abstract] OR chromatin[Title/Abstract] OR "DNA methylation"[MeSH Terms]). Limit to: Journal Article and English Language.	88
Scopus	TITLE-ABS KEY ((phytosterol* OR phytostanol* OR sitost* OR campe* OR stigmast* OR brassicasterol* OR avenast* OR spinast* OR ergost* OR daucost* OR peniocerol* OR "Plant stanol*" OR "Plant sterol*") AND (epigen* OR "DNA methylation" OR microma OR mirna OR histone OR chromatin)) AND (LIMIT-TO(DOCTYPE, "ar")) AND (LIMIT TO (LANGUAGE, "English"))	187
Web of Science	TOPIC: ((phytosterol* OR phytostanol* OR sitost* OR campe* OR stigmast* OR brassicasterol* OR avenast* OR spinast* OR ergost* OR daucost* OR peniocerol* OR "Plant stanol*" OR "Plant sterol*") AND TOPIC: ((epigen* OR "DNA methylation" OR microma OR mirna OR histone OR chromatin)). Refined by: DOCUMENT TYPES: (ARTICLE) AND LANGUAGES: (ENGLISH)	134
The total records from all databases:		409
Records after removing duplicates:		229

independently; disagreements were resolved by discussion with a third member of the research team. A total of twenty-five questions were created to assess the experimental, statistical and reporting transparencies. Questions related to qPCR data were created from the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines⁽³⁵⁾; while those for immunoblotting data were created from the guidelines of the British Journal of Pharmacology⁽³⁶⁾.

Results and discussion

After duplicates were removed, 229 titles and abstracts were included for abstract screening, which left sixteen records for full-text screening; of which eleven were eligible for data extraction (Table 2). The PRISMA flow diagram summary is shown in Fig. 2. The PSS reported were sitosterol (SITO) in five studies^(37–42), Antcin-A (ATA)^(43,44) and stigmasterol (STIG)^(45,46) in two studies each and ergosterol (ERGO)⁽⁴⁷⁾, and campesterol (CAMP)⁽⁴⁵⁾ in one each. Four studies completed experiments in breast cancer cell lines^(38,39,43,48), two in models of coronary obstructive pulmonary disease^(42,47), one in prostate cancer cell lines⁽⁴⁰⁾, one in neural stem cells⁽³⁷⁾, one in human aortic endothelial cells⁽⁴¹⁾, one in BALB/c-nude mice⁽³⁸⁾, one was in guinea pigs⁽⁴⁶⁾ and one in silico⁽⁴⁵⁾.

Phytosterols regulate expression and function of histone deacetylase enzymes

Five studies investigated the role of PSS on HDAC expression, activity or binding^(39,40,45–47). Two studies by Pradhan and colleagues in cancer cell lines provided *in vitro* evidence that PSS promote histone acetylation via HDAC inhibition. SITO inhibited the expression of HDAC1 and HDAC2 in MCF7 and MDA-MB-231 breast cancer cells⁽³⁹⁾ and in PC3 and DU145 prostate cancer cells⁽⁴⁰⁾. In a subsequent HDAC activity assay, HDAC activity was inhibited in a SITO dose-dependent manner that was separate from the effect of the PSS on HDAC expression⁽⁴⁰⁾. A relatively high concentration of 80 µM SITO

was used in this *in vitro* analysis; median serum concentration levels of SITO were previously reported to be 2148 ng/ml (5.2 µM) with a range of 308 ng/ml (0.74 µM) to 19 476 ng/ml (47 µM) in a study of >3000 people⁽⁹⁾. Ishola and Adewole⁽⁴⁹⁾ performed molecular docking experiments and predicted several phytosterols could physically interact with class I and class II HDAC leading to inhibition of activity⁽⁴⁵⁾. Forty-nine compounds isolated from *Morinda Lucida*, a plant found throughout central and southern Africa and used in traditional medicine for the treatment of cancer⁽⁵⁰⁾, were compared with the HDACi givinostat for their capacity to bind and inhibit HDAC⁽⁴⁵⁾. Of the forty-nine compounds tested, PSS were among the strongest HDAC-binding factors. Interestingly, CAMP and STIG were predicted to bind in the same HDAC pocket as givinostat and with higher binding affinity. An Absorption–Distribution–Metabolism–Excretion analysis using Swiss online Absorption–Distribution–Metabolism–Excretion web tool indicated that several PSS adhere to the Lipinski rules of five for drug development (Table 3), underlining their suitability for clinical translation⁽⁵¹⁾.

Contrary to publications above that found PSS inhibited HDAC expression and function were two publications exploring the effect of PSS in lung diseases that found PSS were associated with increased HDAC expression. In a model of steroid-resistant asthma, HDAC2 protein expression was measured in lung tissue of guinea pigs after the animals were given five daily intraperitoneal injections of 50 mg/kg or 100 mg/kg STIG⁽⁴⁶⁾. Steroid-resistant asthma animals had fivefold lower expression of HDAC2 compared with healthy controls and the addition of STIG (50 mg/kg or 100 mg/kg) ameliorated this loss of HDAC2 expression. In a study using a mouse coronary obstructive pulmonary disease model and mouse macrophage cell line (Raw264.7), exposure to cigarette smoke extract inhibited HDAC3 expression and induced expression of the histone acetylation coactivators P300 and PCAF. When increasing doses of ERGO (2.5, 5 and 10 mg/kg) were added to the cigarette smoke extract treatment, the cigarette smoke extract mediated loss of

Table 2. Summary of the data extracted from the included studies (n 11) illustrating the effects of PSS on the epigenetic mechanisms

Epigenetic mechanism	References	PSS	Tissue and cell type	Exposure: Concentration/ Time	Significantly regulated by PSS		Methods used	Risk of bias	
					Up	Down			
Histone post-translational modification	Pradhan <i>et al.</i> 2019a	β -SITO	BCa: MCF7, 231	40, 60 μ M 24 h		mRNA HDAC1 mRNA HDAC2 Protein HDAC1 Protein HDAC2	qPCR WB	Low	
	Pradhan <i>et al.</i> 2019b	β -SITO	PCa: PC-3, DU-145	80 μ M 24 h		Protein HDAC1 Protein HDAC2 Activity HDAC1 Activity HDAC2	WB HDAC Activity Assay	Low	
	Hohoyai <i>et al.</i> 2022	STIG	Guinea pigs (steroid resistant asthma)	50, 100 mg/kg 6 days	Protein HDAC2		ELISA	Low	
	Sun <i>et al.</i> 2019	ERGO		RAW264.7 cells	5, 10, 20 μ M/L 24 h	mRNA HDAC3 mRNA CBP mRNA PCAF Protein HDAC3 Protein CBP Protein PCAF		qPCR WB	Low
				Sprague-Dawley rats (COPD)	2.5, 5, 10 mg/kg				
Ishola <i>et al.</i> 2019	CAMP STIG	N/A	N/A	N/A	Binding of PSS to: HDAC3, HDAC7 HDAC8		Molecular docking	Low	
DNA methylation	Pradhan <i>et al.</i> 2019a	β -SITO	BCa: MCF7, 231	40, 60 μ M 24 h		mRNA DNMT1 Protein DNMT1	qPCR WB	Low	
	Pradhan <i>et al.</i> 2019b	β -SITO	PCa: PC-3, DU-145	80 μ M 24 h		Activity DNMT1 Protein DNMT1	DNMT Activity Assay WB	Low	
miRNA expression	Jiang <i>et al.</i> 2013	β -SITO	NSC	40 μ M 72 h		miR-129-5p miR-301a-3p miR-322-5p	MicroRNA Array qPCR validation	Low	
	Xu <i>et al.</i> 2018	β -SDG	BCa: MCF7, 231	32 μ M 24 h	miR-10a-5p		qPCR	Low	
			BALB/c-nude mice	30, 60, 120 mg/kg					
	Kumar <i>et al.</i> 2019a	ATA	BCa: MCF7, 231	10 μ M 24 h	miR-200c		qPCR	Low	
	Kumar <i>et al.</i> 2019b	ATA	BCa: MCF7, 231	10 μ M 24, 72 h	miR-708		qPCR	Low	
	Jiang <i>et al.</i> 2020	β -SITO	HAEC	4.8 μ M 24 h	miR-126-3p miR-216a-3p miR-490-5p miR-511-5p miR-548j-3p miR-2682-5p miR-3175 miR-4746-5p	miR-29b-2-5p miR-34a-3p miR-154-5p miR-339-5p miR-374c-3p miR-374b-5p miR-424-5p miR-664b-3p miR-708-5p	qPCR validation of sequencing	Low	
	Shen <i>et al.</i> 2021	γ -SITO	BEC: BEAS-2B, H292	60 nM 24 h	miR-361-5p	miR-196-5p	qPCR	Low	

PSS, phytosterols/phytosterols; 231, MDA-MB-231; ATA, Antcin-A; BCa, breast cancer; BEC, bronchial epithelial cells; β -SITO, β -sitosterol; β -SDG, β -SITO-D-glucoside; CAMP, campesterol; COPD, chronic obstructive pulmonary disease; ERGO, ergosterol; HAEC, human aortic endothelial cells; ELISA, enzyme-linked immunosorbent assay; N/A, not applicable; NSC, neural stem cells; PCa, prostate cancer; qPCR, quantitative polymerase chain reaction; SITO, sitosterol; STIG, stigmaterol; γ -SITO, γ -sitosterol; WB, western blot.

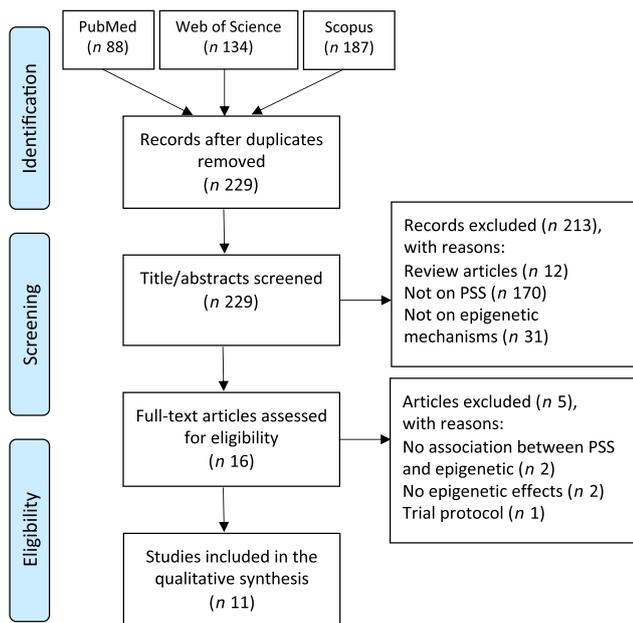


Fig. 2. PRISMA flow diagram showing numbers of records. The numbers of the included and excluded studies in each step; identifications from the three databases, titles/abstracts, full-text screening and the final numbers that are eligible for this systematic review.

Table 3. The oral drug likeness of SITO, CAMP, STIG and ATA by Lipinski's rule of five⁽⁵¹⁾

Lipinski rules of drug development	CAMP	STIG	SITO	ATA
≤ 5 hydrogen bond donors	1	1	1	1
≤ 10 hydrogen bond acceptors	1	1	1	4
Molecular mass < 500 Daltons	400.68	412.69	414.71	454.64
MLOGP < 4.15	6.54	6.62	6.73	4.51
Lipinski Violations	1	1	1	1
Drug Likeness	Yes	Yes	Yes	Yes

SITO, sitosterol; CAMP, campesterol; STIG, stigmasterol; ATA, Antcin-A; MLOGP, Moriguchi octanol–water partition coefficient.

HDAC3 was ameliorated⁽⁴⁷⁾. Neither the data presented by Hohoayi *et al.*, regarding STIG mediated increases in HDAC2 expression, nor by Sun *et al.*, regarding ERGO induction of HDAC3, included analysis of the effect of the PSS on HDAC in control animals, nor did they directly assess HDAC activity. Given that both disease models are associated with very high free-radical environments, HDAC degradation may be inhibited by PSS via protection from oxidative damage and adduct formation from the high levels of free radical characteristic of both these disease models; indeed, this was proposed by Hohoayi and colleagues as a reason why HDAC2 levels remained high.

The experimental observations showing PSS has HDAC modulatory properties may help explain why PSS are so potent at lowering LDL-cholesterol^(12,13), yet have not been conclusively linked to a reduction in CVD risk^(10,52–55). Whether HDACi are beneficial in terms of CVD prevention is context and end point dependent. In their 2016 review, Yoon and Eom described evidence regarding the use of pharmacological HDACi to reduce risk of several CVD outcomes, such as myocardial infarction and hypertension. However, HDACi is was also linked to worse

vascular calcification and indeed, and thrombus was a side effect of significant clinical importance in patients taking vorinostat⁽⁵⁶⁾. The molecular mechanisms of action of how PSS inhibit HDAC function in the context of CVD therefore require further elucidation. This would also shed light on the role of PSS in other diseases where HDAC activity is important, such as cancer.

Phytosterols decrease expression of DNA methyltransferase enzymes and reduce global DNA methylation

Two studies investigated whether PSS exposure led to changes in DNA methylation by affecting DNMT expression^(39,40). In the first, H₂O₂ was used to induce expression of DNMT1 in MCF7 and MDA-MB-231 breast cancer cells⁽³⁹⁾. However, in the presence of 40 μM or 60 μM SITO, DNMT1 induction was prevented and both mRNA and protein levels of DNMT were reduced by PSS in both cell lines. Moreover, H₂O₂ down-regulated the expression of anti-epithelial to mesenchymal transition factor N-cadherin/CDH1, while lupeol and SITO reversed this pro-metastatic repression of CDH1 at mRNA and protein levels. In prostate cancer cell models, DNMT1 was also reduced following exposure of cell lines (PC-3 and DU-145) to methanolic extract of *Paederia foetida* leaves, lupeol or SITO, for 24 h or 72 h. The data show SITO inhibited DNMT1 expression and activity, decreased the expression of anti-apoptotic protein Bcl2 and upregulated the expression of pro-apoptotic protein Bax; and, again as for breast cancer cells, CDH1 expression was increased⁽⁴⁰⁾.

Given that DNMT play essential roles in activating or silencing oncogenes and tumour suppressor genes, it is interesting to note that PSS appear to have therapeutic actions in regulating these enzymes, in cell lines at least. Further molecular analyses are required to provide a mechanistic understanding of how PSS are able to regulate activity of DNMT enzymes. Specifically, the use of coarse grained and atomistic computational simulations, which have been applied previously to determine lipid–protein interactions (reviewed in⁽²⁹⁾), molecular docking simulations (such as those in⁽⁴⁵⁾ or molecular binding analyses) would determine if physical interactions occur. Further work in pre-clinical models and ultimately dietary interventions in cancer patients will be critical to understanding if PSS-mediated changes to DNA methylation patterns can translate into clinically meaningful effects.

Phytosterols regulate miRNA expression

The link between miRNA and PSS exposure was investigated in six records^(37,38,41–43,48), of which two were screening studies^(37,41). The screening studies evaluated SITO in the context of rat neuronal stem cells⁽³⁷⁾, and human aortic endothelial cells⁽⁴¹⁾. In neuronal stem cells, 40 μM SITO upregulated 30, and downregulated 84, miRNA⁽³⁷⁾. While in the HAEC cells, 4.8 μM SITO upregulated 579 and down-regulated 112 miRNA, albeit this was in comparison to an ox-LDL treated group⁽⁴¹⁾. Interestingly, despite being performed in different cell types from different species, and with a ten-fold difference in concentration of SITO utilised, four miRNA

were significantly altered by SITO in both studies (miR-17-5p, miR-20a-5p, miR-210-3p and miR-532-3p). Although these were not in the top regulated lists in either study and thus were not validated by PCR. In NSC cells, several SITO-regulated miRNA were found to activate the IGF1 signalling pathway, and treatment was associated with increased cellular proliferation⁽⁴¹⁾. Similarly in HAEC, signalling pathway analysis indicated that SITO treatment enriched for AKT signalling, forkhead box O transcription factors and cell cycle signalling⁽³⁷⁾. Contrary to this, the bronchial epithelial airway cell line model of chronic obstructive pulmonary disease (BEAS-2B) responded to very low concentrations of SITO (60 nM for 24 h) by induction of miR-196-5p and suppression miR-361-5p, leading to inhibition of mTOR signalling and reduced proliferation⁽⁴²⁾.

In cancer, like in the pulmonary model, PSS did not stimulate proliferative or cell survival signalling, but instead tended to suppress these pathways. In BCa cell lines MCF7 and MDA-MB-231 cells, 32 μ M SITO-induced apoptosis via induction of the tumour suppressor miR-10a-5p that in turn limited the activity of PI3K-AKT signalling⁽³⁸⁾. Also, in MCF7 and MDA-MB-231 cells, 10 μ M ATA upregulated miR-200c⁽⁴³⁾ and miR-708⁽⁴⁴⁾. Of note is that miR-708 was significantly downregulated by SITO in HAEC cells⁽⁴¹⁾. ATA inhibited multiple components of the epithelial to mesenchymal transition pathway, a critical step in cancer metastasis. ATA induced miR-200c expression in a p53-dependent manner, which led to repression of transcription factor ZEB1, and molecular effectors of epithelial to mesenchymal transition such as E-cadherin and vimentin⁽⁴³⁾. ATA suppressed the pro-inflammatory NF κ B pathway via miR-708-dependent inhibition of IKK β ⁽⁴⁴⁾.

These data support the hypothesis that PSS regulate important cell fate decisions such as proliferation and differentiation. Importantly, cell fate decisions can be driven in either direction, in cancer PSS are anti-proliferative, anti-metastatic and pro-apoptotic, but in the non-cancer models of NSC and HAEC, PSS-enhanced proliferation and in some cases via upregulation of the same pathways that are downregulated by PSS in cancer. This context dependence indicates the molecular effectors of PSS control over cell fate are yet to be fully elucidated.

Risk of bias and study limitations

The risk of bias score was generated by assessing experimental, statistical and reporting transparency for each included study. Questions regarding experimental protocols were created according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines⁽³⁵⁾ and for reporting guidelines for immunoblotting data⁽³⁶⁾. See the table in Supplementary (Table S1). Generally, all the studies received a minimal risk of bias rating (Fig. 3). In terms of statistical and experimental transparency, most studies had minimal risk of bias. Other aspects of reporting transparency assessed as low across the studies were: a lack of information on reagent catalogue numbers, reverse transcription and qPCR experimental variables, a lack of size marker reporting in immunoblot images and a lack of reporting across all studies about how PSS were stored and delivered to cell lines (e.g. use of carriers). Apart from these gaps,

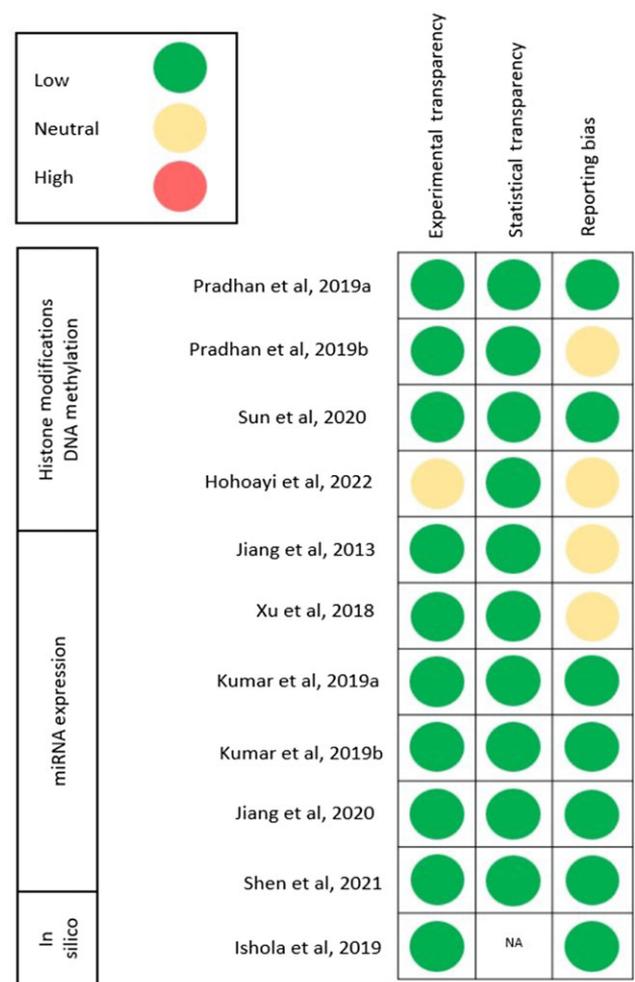


Fig. 3. Risk of bias score. The risk of bias assessment for the included studies using a designed multi-point questionnaire. The score presented as green indicated the low risk of bias, red indicated the high risk of bias and yellow indicated the neutral risk of bias.

other criteria were generally sufficiently reported so risk of bias for the included studies was considered low.

Perhaps the major limitation of this systematic review was the paucity of studies returned during the search phase. The corollary of which is that we have been unable to adopt previous examples of good practice when reviewing miRNA pathways; namely, requiring a miRNA to be both experimentally independently validated (e.g. QPCR, genetic or other functional confirmation of high-throughput expression data) and reported as differentially regulated in two or more publications to warrant inclusion⁽⁵⁷⁾.

Conclusion

There is international consensus that the high consumption of seeds, nuts, legumes, fruits and vegetables reduces the risk of cardiometabolic disease and cancer. However, further studies characterising the bioactive compounds and mechanisms of action conferring health benefits are required. PSS enriched in plant-based diets lower pathogenic blood lipids^(12,14,58), are

linked to reduced cancer burden and risk^(16,17) and a range of other diseases. Our systematic review has collated important knowledge regarding how PSS may act as epigenetic modifiers with the following notable observations.

PSS could be amongst the most potent naturally occurring HDACi reported to date⁽⁴⁵⁾. This potency, coupled with PSS's suitability as orally administered agents as suggested by their high scores in the Lipinski test, indicates that further examination exploring their use as HDACi in clinic is warranted (Table 3)⁽⁵¹⁾. Nonetheless, clarity regarding their role in preventing and exacerbating different CVD clinical endpoints is required. As described recently, PSS are very unlikely to exacerbate CVD risk⁽⁶⁰⁾, but gaps remain in understanding the mechanism of action of how they reduce LDL-cholesterol and global histone acetylation analyses may shed light on this phenomenon. PSS inhibit expression and activity of DNMT that methylate CpG islands, leading to re-expression of genes silenced in disease states^(39,40). PSS regulate perhaps hundreds of miRNA, but validation of individual miRNA in multiple studies was only possible for five miRNA in total. More screens in more cell and tissue types are required to understand the true scale of miRNA regulation by PSS across tissues.

These epigenetic gene expression regulatory mechanisms impinge on several diseases. In cancer, PSS induce miRNA that lower expression of factors such as ZEB1 that promote the epithelial to mesenchymal transition, a critical step in cancer metastasis⁽⁴³⁾ and suppress miRNA allowing increase in expression of factors that maintain an epithelial phenotype, such as CDH1⁽⁴³⁾. PSS also inhibit components of inflammatory pathways, including NFκB and mTOR signalling, in models of coronary obstructive pulmonary disease⁽⁴²⁾ indicating a potential dietary route to ameliorating these conditions. Investigations into the epigenetic effects of PSS on many cell types, including those of the immune system, are lacking from the literature thus far. Given the emerging understanding that PSS may ameliorate or even prevent a range of diseases, evaluating the role of PSS in epigenetic reprogramming is of increasing clinical and public health interest.

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J. L. T. and E. J. contributed to the review conception and design. E. J. screened records, extracted and analysed data, created figures and tables and contributed to an initial manuscript draft. M. X. screened records and extracted data. J. L. T. and J. B. M. supervised the project. J. L. T. wrote the final manuscript. All authors critically reviewed the manuscript for intellectual content and approved final version. Fig. 1 is Adapted Material from⁽³²⁾ and shared under CC BY 4.0 License. The graphical abstract was created with BioRender.com under license by E. J.

All authors declare that they have no competing interests.

Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114523002532>

References

- Phillips KM, Ruggio DM & Ashraf-Khorassani M (2005) Phytosterol composition of nuts and seeds commonly consumed in the United States. *J Agric Food Chem* **53**, 9436–9445.
- St-Onge MP & Jones PJ (2003) Phytosterols and human lipid metabolism: efficacy, safety, and novel foods. *Lipids* **38**, 367–375.
- Nes WD (2011) Biosynthesis of cholesterol and other sterols. *Chem Rev* **111**, 6423–6451.
- Ratnayake WM, L'Abbe MR, Mueller R, *et al.* (2000) Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. *J Nutr* **130**, 1166–1178.
- Fujioka S & Yokota T (2003) Biosynthesis and metabolism of brassinosteroids. *Annu Rev Plant Biol* **54**, 137–164.
- Jimenez-Escrig A, Santos-Hidalgo AB & Saura-Calixto F (2006) Common sources and estimated intake of plant sterols in the Spanish diet. *J Agric Food Chem* **54**, 3462–3471.
- Ostlund RE Jr, McGill JB, Zeng CM, *et al.* (2002) Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. *Am J Physiol Endocrinol Metab* **282**, E911–E916.
- Bosner MS, Lange LG, Stenson WF, *et al.* (1999) Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J Lipid Res* **40**, 302–308.
- Stiles AR, Kozlitina J, Thompson BM, *et al.* (2014) Genetic, anatomic, and clinical determinants of human serum sterol and vitamin D levels. *Proc Natl Acad Sci U S A* **111**, E4006–E4014.
- Genser B, Silbernagel G, De Backer G, *et al.* (2012) Plant sterols and cardiovascular disease: a systematic review and meta-analysis. *Eur Heart J* **33**, 444–451.
- Wu T, Fu J, Yang Y, *et al.* (2009) The effects of phytosterols/stanols on blood lipid profiles: a systematic review with meta-analysis. *Asia Pac J Clin Nutr* **18**, 179–186.
- Ras RT, Geleijnse JM & Trautwein EA (2014) LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomised controlled studies. *Br J Nutr* **112**, 214–219.
- Musa-Veloso K, Poon TH, Elliot JA, *et al.* (2011) A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: results of a meta-analysis of randomized, placebo-controlled trials. *Prostaglandins Leukot Essent Fatty Acids* **85**, 9–28.
- Jacobson TA, Ito MK, Maki KC, *et al.* (2014) National lipid association recommendations for patient-centered management of dyslipidemia: part 1 – executive summary. *J Clin Lipidol* **8**, 473–488.
- Catapano AL, Graham I, De Backer G, *et al.* (2016) 2016 ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis* **253**, 281–344.
- Jiang L, Zhao X, Xu J, *et al.* (2019) The protective effect of dietary phytosterols on cancer risk: a systematic meta-analysis. *J Oncol* **2019**, 11.
- Cioccoloni G, Soteriou C, Websdale A, *et al.* (2022) Phytosterols and phytostanols and the hallmarks of cancer in model

- organisms: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr* **62**, 1145–1165.
18. Nattagh-Eshstivani E, Barghchi H, Pahlavani N, *et al.* (2022) Biological and pharmacological effects and nutritional impact of phytosterols: a comprehensive review. *Phytother Res* **36**, 299–322.
 19. Ropero S & Esteller M (2007) The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* **1**, 19–25.
 20. Bao L, Diao H, Dong N, *et al.* (2016) Histone deacetylase inhibitor induces cell apoptosis and cycle arrest in lung cancer cells via mitochondrial injury and p53 up-acetylation. *Cell Biol Toxicol* **32**, 469–482.
 21. Kim HJ & Bae SC (2011) Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *Am J Transl Res* **3**, 166–179.
 22. Rambaldi A, Iurlo A, Vannucchi AM, *et al.* (2021) Long-term safety and efficacy of givinostat in polycythemia vera: 4-year mean follow up of three phase 1/2 studies and a compassionate use program. *Blood Cancer J* **11**, 53.
 23. Meng H, Cao Y, Qin J, *et al.* (2015) DNA methylation, its mediators and genome integrity. *Int J Biol Sci* **11**, 604–617.
 24. Verghese ET, Drury R, Green CA, *et al.* (2013) MiR-26b is down-regulated in carcinoma-associated fibroblasts from ER-positive breast cancers leading to enhanced cell migration and invasion. *J Pathol* **231**, 388–399.
 25. Thome JL, Battaglia S, Baxter DE, *et al.* (2018) MiR-19b non-canonical binding is directed by HuR and confers chemosensitivity through regulation of P-glycoprotein in breast cancer. *Biochim Biophys Acta Gene Regul Mech* **1861**, 996–1006.
 26. Calin GA, Ferracin M, Cimmino A, *et al.* (2005) A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* **353**, 1793–1801.
 27. He H, Jazdzewski K, Li W, *et al.* (2005) The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci USA* **102**, 19075–19801.
 28. Thome JL, Maguire O, Doig CL, *et al.* (2011) Epigenetic control of a VDR-governed feed-forward loop that regulates p21(waf1/cip1) expression and function in non-malignant prostate cells. *Nucleic Acids Res* **39**, 2045–2056.
 29. Zhang Z, Moon R, Thome JL, *et al.* (2021) NAFLD and vitamin D: evidence for intersection of microRNA-regulated pathways. *Nutr Res Rev* **36**, 120–139.
 30. De Smet E, Mensink RP & Plat J (2012) Effects of plant sterols and stanols on intestinal cholesterol metabolism: suggested mechanisms from past to present. *Mol Nutr Food Res* **56**, 1058–1072.
 31. Nakano T, Inoue I & Murakoshi T (2019) A newly integrated model for intestinal cholesterol absorption and efflux reappraises how plant sterol intake reduces circulating cholesterol levels. *Nutrients* **11**, 310.
 32. Hutchinson SA, Lianto P, Moore JB, *et al.* (2019) Phytosterols inhibit side-chain oxysterol mediated activation of LXR in breast cancer cells. *Int J Mol Sci* **20**, 3241.
 33. Plat J, Nichols JA & Mensink RP (2005) Plant sterols and stanols: effects on mixed micellar composition and LXR (target gene) activation. *J Lipid Res* **46**, 2468–2476.
 34. Tiffon C (2018) The impact of nutrition and environmental epigenetics on human health and disease. *Int J Mol Sci* **19**, 3425.
 35. Bustin SA, Benes V, Garson JA, *et al.* (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**, 611–622.
 36. Alexander SPH, Roberts RE, Broughton BRS, *et al.* (2018) Goals and practicalities of immunoblotting and immunohistochemistry: a guide for submission to the British Journal of Pharmacology. *Br J Pharmacol* **175**, 407–411.
 37. Jiang LH, Yang NY, Yuan XL, *et al.* (2013) Microarray analysis of mRNA and microRNA Expression profile reveals the role of β -sitosterol-D-glucoside in the proliferation of neural stem cell. *Evid Based Complement Alternat Med* **2013**, 360302.
 38. Xu H, Li Y, Han B, *et al.* (2018) Anti-breast-cancer activity exerted by β -Sitosterol-D-glucoside from sweet potato via upregulation of microRNA-10a and via the PI3K-Akt signaling pathway. *J Agric Food Chem* **66**, 9704–9718.
 39. Pradhan N, Parbin S, Kar S, *et al.* (2019) Epigenetic silencing of genes enhanced by collective role of reactive oxygen species and MAPK signaling downstream ERK/Smad axis: ectopic application of hydrogen peroxide repress CDH1 gene by enhanced DNA methyltransferase activity in human breast cancer. *Biochim Biophys Acta Mol Basis Dis* **1865**, 1651–1665.
 40. Pradhan N, Parbin S, Kausar C, *et al.* (2019) Paederia foetida induces anticancer activity by modulating chromatin modification enzymes and altering pro-inflammatory cytokine gene expression in human prostate cancer cells. *Food Chem Toxicol* **130**, 161–173.
 41. Jiang YH, Li X, Niu W, *et al.* (2020) β -Sitosterol regulated microRNAs in endothelial cells against an oxidized low-density lipoprotein. *Food Funct* **11**, 1881–1890.
 42. Shen HF, Liu Y, Qu PP, *et al.* (2021) MiR-361-5p/abca1 and MiR-196-5p/arhgef12 axis involved in γ -sitosterol inducing dual anti-proliferative effects on bronchial epithelial cells of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* **16**, 2741–2753.
 43. Kumar KJS, Vani MG, Hsieh HW, *et al.* (2019) Antcin-A modulates epithelial-to-mesenchymal transition and inhibits migratory and invasive potentials of human breast cancer cells via p53-mediated mir-200c activation. *Planta Med* **85**, 755–765.
 44. Kumar KJS, Vani MG, Hsieh HW, *et al.* (2019) MicroRNA-708 activation by glucocorticoid receptor agonists regulate breast cancer tumorigenesis and metastasis via downregulation of NF- κ B signaling. *Carcinogenesis* **40**, 335–348.
 45. Ishola AA & Adewole KE (2019) Phytosterols and triterpenes from *Morinda lucida* Benth exhibit binding tendency against class I HDAC and HDAC7 isoforms. *Mol Biol Rep* **46**, 2307–2325.
 46. Hohoayi A, Antwi AO, Amoah V, *et al.* (2022) Coadministration of stigmaterol and dexamethasone (STIG+DEX) modulates steroid-resistant asthma. *Mediators Inflamm* **2022**, 2222270.
 47. Sun X, Liu Y, Feng X, *et al.* (2020) The key role of macrophage depolarization in the treatment of COPD with ergosterol both *in vitro* and *in vivo*. *Int Immunopharmacol* **79**, 106086.
 48. Senthil Kumar KJ, Gokila Vani M, Hsieh HW, *et al.* (2019) MicroRNA-708 activation by glucocorticoid receptor agonists regulate breast cancer tumorigenesis and metastasis via down-regulation of NF- κ B signaling. *Carcinogenesis* **40**, 335–348.
 49. Sowemimo AA, Fakoya FA, Awopetu I, *et al.* (2007) Toxicity and mutagenic activity of some selected Nigerian plants. *J Ethnopharmacol* **113**, 427–432.
 50. Daina A, Michielin O & Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* **7**, 42717.
 51. Ras RT, van der Schouw YT, Trautwein EA, *et al.* (2015) Intake of phytosterols from natural sources and risk of cardiovascular disease in the European prospective investigation into cancer and nutrition-the Netherlands (EPIC-NL) population. *Eur J Prev Cardiol* **22**, 1067–1075.
 52. Zhao Y, Zhuang Z, Li Y, *et al.* (2023) Blood phytosterols in relation to cardiovascular diseases and mediating effects of blood lipids and hematological traits: a Mendelian randomization analysis. *Metabolism* **146**, 155611.





53. Klingberg S, Ellegard L, Johansson I, *et al.* (2013) Dietary intake of naturally occurring plant sterols is related to a lower risk of a first myocardial infarction in men but not in women in northern Sweden. *J Nutr* **143**, 1630–1635.
54. Witkowska AM, Waskiewicz A, Zujko ME, *et al.* (2022) Dietary plant sterols and phytosterol-enriched margarines and their relationship with cardiovascular disease among polish men and women: the WOBASZ II cross-sectional study. *Nutrients* **14**, 2665.
55. Yoon S & Eom GH (2016) HDAC and HDAC inhibitor: from cancer to cardiovascular diseases. *Cbonnam Med J* **52**, 1–11.
56. Soteriou C, Kalli AC, Connell SD, *et al.* (2021) Advances in understanding and in multi-disciplinary methodology used to assess lipid regulation of signalling cascades from the cancer cell plasma membrane. *Prog Lipid Res* **81**, 101080.
57. Katan MB, Grundy SM, Jones P, *et al.* (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc* **78**, 965–978.
58. Windler E, Beil FU, Berthold HK, *et al.* (2023) Phytosterols and cardiovascular risk evaluated against the background of phytosterolemia cases – a German expert panel statement. *Nutrients* **15**, 828.