Vitamin D and symptom severity in individuals with irritable bowel syndrome.

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Abstract

Vitamin D is typically associated with bone health, but there is increasing evidence that vitamin D deficiency plays a role in the risk and severity of several diseases including inflammatory bowel disease and cancer, and an association between low vitamin D status and irritable bowel syndrome (IBS) has been suggested. IBS is a chronic, relapsing functional disorder of the gut which has a considerable burden of cost to the NHS and to the individual living with this condition. The aetiology of IBS is unknown and the treatment that can be offered is not always effective. Treatment with vitamin D may be a relatively inexpensive and acceptable form of therapy. The aim of this thesis were to (i) to review the literature for evidence of a relationship between vitamin D and IBS, (ii) to investigate the efficacy of a sublingual/buccal vitamin D spray compared to vitamin D capsule, (iii) and to conduct a randomised control trial to investigate the possible effect of a 3000IU/ day vitamin D_3 sublingual spray on symptom severity and quality of life with individuals with IBS.

Methods:

The systematic literature search was completed using PRISMA guidelines to identify the current available research investigating an association between vitamin D and IBS. Three databases; Pubmed, Medline and Web of Science were used. A supplementary search was conducted using the same method to assess the literature published post 2018. In addition, we conducted an efficacy study comparing 2 vitamin D preparations: capsule and sublingual spray. This study recruited 75 healthy participants and randomly allocated 25 participants to one of the three treatments. Participants received; placebo capsule/active spray, active capsule/placebo spray or placebo capsule/placebo spray. Blood samples were collected at baseline, day 3, 7, 14, 21 and 28 using whole blood spot kits (Sandwell and Birmingham Hospitals) for the analysis of vitamin D status. The final study was a randomised, double blinded, placebo-controlled trial with 135 free living participants with a diagnosis of IBS to examine the effect of a 3000IU/day vitamin D_3 supplement for 12 weeks on symptom severity and quality of life in individuals with IBS. Fingerprick blood samples were collected and whole blood 25(OH)D was measured using liquid chromatography tandem mass spectrometry. Vitamin D status and quality of life was determined at baseline and exit. Quality of life was determined at baseline and exit using the IBS quality of life questionnaire and symptom severity was assessed fortnightly across the study using the IBS symptom severity score questionnaire (2). Habitual dietary intake of vitamin D was measured using the EPIC Food Frequency Questionnaire.

Results:

The systematic review yielded 7 studies; 3 intervention and 4 observation studies. The evidence from these studies suggest a beneficial effect of a vitamin D supplement on symptom severity and quality of life in people with IBS. The supplementary search generated a further 3 randomised controlled trials. These studies agree with the original review's findings that individuals with IBS may have improvement in their symptomology by supplementing with vitamin D. The efficacy study found a sublingual vitamin D spray to be as effective as a capsule at raising whole blood 25(OH)D concentrations. Baseline measurements of 25(OH)D concentrations showed a high prevalence ofvitamin D insufficiency (44.6%) among participants. The data also suggests that rates of change of vitamin D status in response to supplementation are higher in individuals with lower levels of 25(OH)D. The RCT showed there was a significant improvement in the vitamin D status of participants randomised to receive active vitamin D ($p=0.005$) after 12 weeks. No difference was seen in symptom severity and quality of life between arms at baseline and exit ($p=0.824$, $p=0.415$ respectively). There was no association between change in vitamin D status and change in symptom severity ($r= -0.071$, $p=0.434$), nor increase in vitamin D and change in quality of life ($r=-0.031$, $p=0.733$). This analysis found a weak but significant correlation between baseline serum concentrations of 25(OH)D and dietary intake of vitamin D ($p=0.046$, $r=0.17$).

Conclusions:

The results confirm that there is a prevalence of low 25(OH)D concentrations in individuals with IBS, and this warrants correction, if only for general health. The sublingual vitamin D spray proved to be an effective mode of delivery for raising 25(OH)D concentrations, which may be beneficial to those who have swallowing difficulties or malabsorption issues. We found no benefit of vitamin D supplementation on IBS symptom severity or quality of life. Low dietary intakes of vitamin D present in the general population

Acronyms

AEs - Adverse events

CRC - Colorectal cancer

CD - Crohn's disease

CVD - Cardiovascular disease

C-RP - C-reactive protein

DRIs – Dietary reference intakes

FGIDs - Functional gastrointestinal disorders

FDA - Food and Drug Administration

FODMAP - Fermentable oligosaccharides, disaccharides, monosaccharides and polyols

IBD - Inflammatory bowel disease

IBS - Irritable bowel syndrome

IBS-C - Constipation predominant irritable bowel syndrome

IBS-D - Diarrhoea predominant irritable bowel syndrome

IBS-M - Mixed (or alternating of both symptoms) irritable bowel syndrome

IBS-U - Undefined irritable bowel syndrome

IL-6 - Interleukin 6

LFD - Low FODMAP diet

NDNS – The National Diet and Nutrition Survey

PI-IBS - Post-infectious irritable bowel syndrome

RCT - Randomised controlled trial

SACN - Scientific advisory committee on nutrition

TNF-α -Tumor necrosis factor α

UC - Ulcerative colitis

UVB – Ultraviolet B

UVR – Ultraviolet radiation

VDBP – Vitamin D bindin protein

VDR – Vitamin D receptor

25(OH)D - 25 dihydroxyvitamin D

Declaration of Originality

"I, Claire Williams, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not been previously been presented for an award at this, or any other, university."

Claire E. T. Williams

Dissemination

Peer-reviewed publications

Williams, C., Williams, E., & Corfe, B. "Vitamin D Status in Irritable Bowel Syndrome and the Impact of Supplementation on Symptoms: What Do We Know and What Do We Need to Know?" European Journal of Clinical Nutrition 72.10 (2018): 1358-363. Web.

Williams, C., Williams, E., & Corfe, B. "Rate of Change of Circulating 25-hydroxyvitamin D following Sublingual and Capsular Vitamin D Preparations." European Journal of Clinical Nutrition 73.12 (2019): 1630-635. Web.

Williams, C., Williams, E., & Corfe, B. (2021). "Vitamin D supplementation in people with IBS has no effect on symptom severity and quality of life: results of a randomized controlled trial". European Journal of Nutrition, 61 (1). pp. 299-308.

Conference abstract publications

Williams, C., Williams, E., & Corfe, B. (2020). Vitamin D status and quality of life in people with Irritable Bowel Syndrome. Proceedings of the Nutrition Society, 79(OCE2), E653. doi:10.1017/S0029665120006023

Williams, C., Williams, E., & Corfe, B. (2020). Effect of vitamin D supplementation on irritable bowel syndrome symptom severity and quality of life. Proceedings of the Nutrition Society, 79(OCE1), E14. doi:10.1017/S0029665119001356

Conference presentations

Williams, C., Williams, E., & Corfe, B. "Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations." Junior Speaker at the Mini-Symposium on The Impact of Ethnicity on Risk of Diet-Related Diseases Rank Prize Funds Meeting, Lake District, February 2018.

Williams, C., Williams, E., & Corfe, B. "Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations." Poster presentation at the 21st Vitamin D Workshop, Barcelona, Spain, May 2018. (Appendix 1)

Williams, C., Williams, E., & Corfe, B. (2020). "Vitamin D status and quality of life in people with Irritable Bowel Syndrome." Poster presentation at the Nutrition Society Winter Meeting; Diet and Digestive Disease, December 2019. (Appendix 2)

Williams, C., Williams, E., & Corfe, B. (2020). "Effect of vitamin D supplementation on symptom severity and quality of life in people with Irritable Bowel Syndrome." Poster presentation at the 13th European Nutrition Conference, FENS; Malnutrition in an Obese World: European Perspectives, Dublin, October 2019. (Appendix 3)

Chapter One

Introduction

Vitamin D Biological function and metabolism

Vitamin D has a well-established role in bone health as it enhances the absorption of calcium, and deficiency can lead to rickets in children and osteoporosis/osteomalacia in adults (1). Vitamin D is recognised as a prohormone and is involved in the homeostasis of calcium and the parathyroid hormone (PTH) (2). Vitamin D has two main forms; D_3 (cholecalciferol) and D_2 (ergocalciferol), the difference between the two structures is in the side chain (see figure 1) (3). This difference however, does not affect either form in terms of function as a prohormone or its metabolism (4).

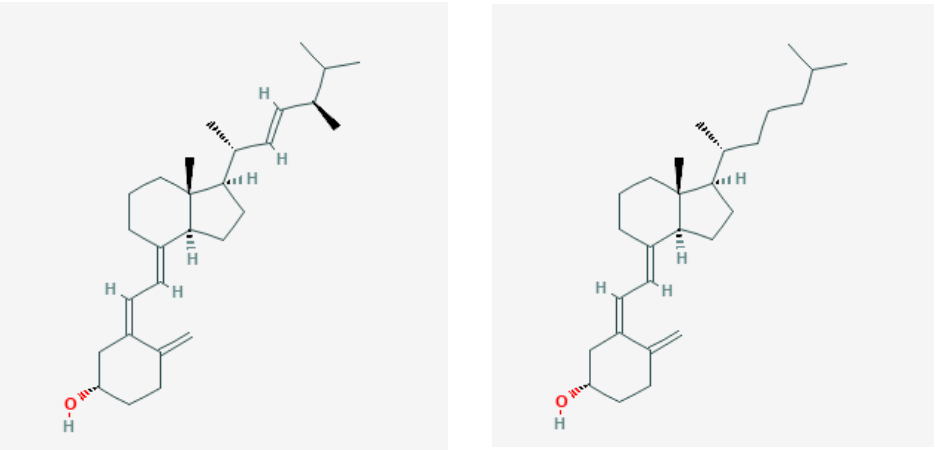
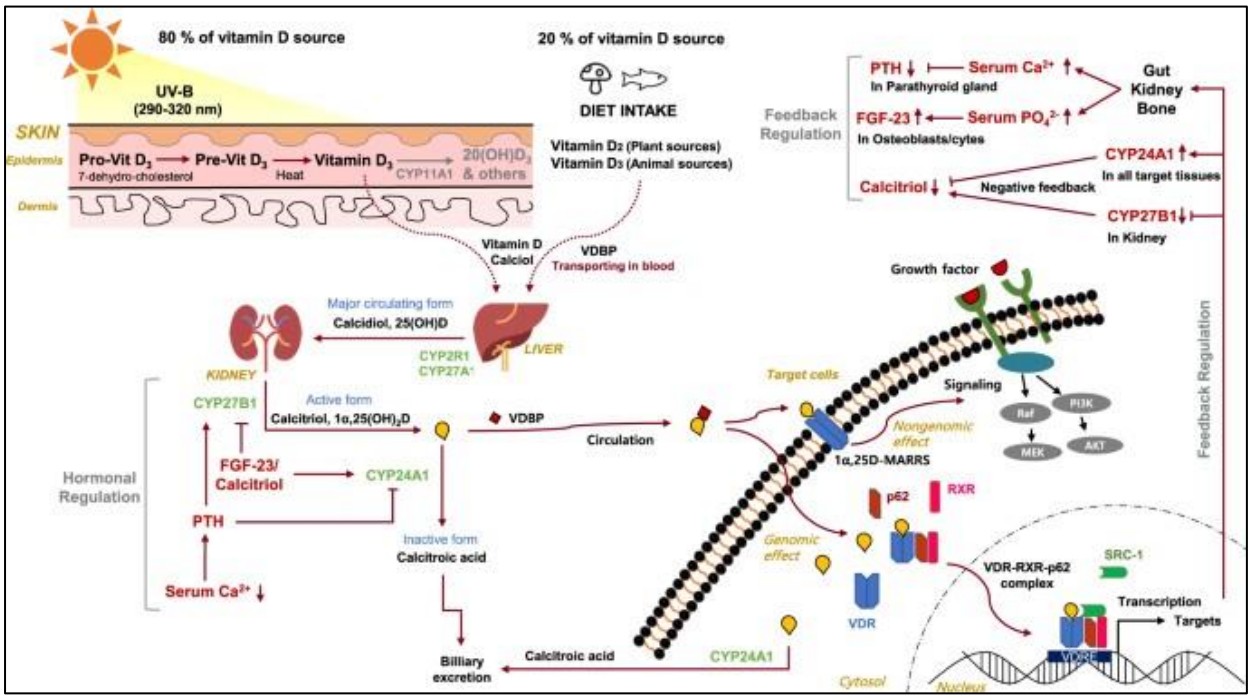


Figure 1: Structure of Vitamin D_2 and D_3, taken from PubChem, compounds (in public domain). (4)

Cholecalciferol is formed when the skin is exposed to sunlight or ultraviolet light is acquired by the irradiation of ergocalciferol in fungi and plants (5). Vitamin D_2 and D_3 are biologically inactive until hydroxylated enzymatically by the liver and the kidney (6). First, hydroxylation occurs in the liver where it is transported by vitamin D binding proteins (VDBP) and is converted to 25(OH)D, the precursor to calcitriol (7). VDBP is the main protein transport for all the metabolites of vitamin D (8). The enzymes P450 *CYP2RI, CYP27A1, CYP2R1, and CYP3A4* are involved in the initial hydroxylation process (9). 25(OH)D then enters the circulation and it is in this form that is typically used in the

clinical assessment of vitamin D status (10). Further activation occurs in the kidney utilising a key enzyme CYP27B1 to convert 25(OH)D to the hormonally active form 1, 25-dihydroxyvitamin D (calcitriol) (11). Calcitriol then enters circulation bound to VDBP, the same plasma carrier for vitamin D (8). This process of vitamin D activation is summarised in figure 2. Liver synthesis of calcitriol is controlled by two counteracting hormones; PTH and fibroblast-like growth factor-23 (FGF23) (12).



PTH aids in the uptake of calcitriol while FGF23 inhibits renal synthesis of calcitriol (13).

Figure 2: Overview of the vitamin D metabolic pathways showing the process involved in the activation of 25(OH)D from dietary/UVB sources and hormonal regulation of vitamin D metabolism (reprinted with permission) (14)

Sources of vitamin D and UK recommendations

Dietary sources

Dietary sources of vitamin D are available in both main forms; D_2 and D_3. Although in small quantities, vitamin D_3 is found in animal origin foods and D_2 is mainly found in fungi. Animal sources of vitamin D include red meat, oily fish (i.e. salmon, mackerel), and egg yolks (15). Oily fish is widely considered the best form of vitamin D_3 in the diet. For example, salmon (wild/raw) on average contains 8.6 µg/100g of vitamin D compared to eggs 3.2 µg/100g (16). As dietary intake of vitamin D is limited, it is difficult to achieve adequate vitamin D levels through food sources alone (17).

Dietary Recommendations

In the UK, dietary recommendations for nutrients (e.g. vitamins and minerals) are known as the RNI or reference nutrient intake (18). For vitamin D, the RNI was set for those deemed as being high risk of vitamin D deficiency (e.g. elderly or those with dark skin pigmentation) and assumed skin exposure in summer months would adequately provide the necessary vitamin D required for the rest of the population who are not sees at risk of deficiency (19). In 2016, The UK Scientific Advisory Committee of Nutrition (SACN) published their findings from an extensive review of the evidence and has recommended that all individuals over the age of four have a daily intake of 10µg/day of vitamin D (Table 1) (20). In the US, daily recommendations are slightly higher than the UK. The Institute of Medicine (IOM) recommends daily dietary intake of 15µg/day for vitamin D in people aged 1-70 and 20µg/day for those aged 71 and above (21). The National Diet and Nutrition survey (NDNS) conducts a continuous cross-sectional study which aims is to assess the nutritional status, nutrient intake and diet from a representative sample of the UK population each year (22). The NDNS has determined vitamin D dietary intake using 4-day diet diaries and vitamin D status using plasma concentrations of 25(OH)D. Dietary intake of vitamin D was less than the recommended 10µg/day for both children aged 4-18 years (mean= 3.3 µg/day) and adults aged 19-64 years (mean=5.4µg), with the exception of women aged 65-74 years (mean= 10.1µg/day) due to the use of supplements.

Table 1: Dietary recommendations from SACN and IOM (advisory committees)[1]

	SACN	IOM	
Age Groups	General Population (µg)	General Population (µg)	Populations at Risk (µg)
0-12 months	8.5-10	10	15-25
1-4 years	10	10	15-25
4-8 years	10	10	15-25
9-18 years	10	15	15-25
19-70 years	10	15	37.5-50
>70	10	20	37.5-50
Pregnant women 1-18 years	10	15	15-25
Pregnant women >18 years	10	15	37.5-50
Lactating women 14-18 years	10	15	15-25
Lactating women >18 years	10	15	37.5-50

[1]SACN: Scientific Advisory Committee on Nutrition (20); IOM: Institute of Medicine (23).

Cutaneous synthesis of vitamin D

The major source of vitamin D_3 for humans is skin exposure to sunlight (11). Cutaneous synthesis of vitamin D occurs through the action of UVB rays on 7-dehydrocholesterol found in the skin, which results in its conversion to 25(OH)D (24). Vitamin D is produced in the skin when exposed to specific wavelengths of ultraviolet radiation within the UVB range between 270-300nm (25). Factors that are dependent on the successful epidermis synthesis of vitamin D include season, time of day, latitude and skin pigmentation (26). Evidence from a prospective cohort study with an adult (20-60 years), white, UK population (n=125) showed that by September this population had not reached a serum 25(OH)D level that was sufficient enough to maintain adequate status throughout winter months (27). The authors define that optimum serum 25(OH)D levels for healthy, Caucasian adults aged 20-60 years should be 76nmol/L for women and 87.3nmol/L for men. This should be achieved by end of summer to provide sufficient levels of vitamin D for the winter months.

Other research reports that for Caucasian individuals living in the UK, 9-13 minutes of sun exposure daily at noon for the months of March through to September should be sufficient to maintain serum 25(OH)D concentrations of ≥25nmol/L for the winter months (28). It is important to note that populations living in the UK, with darker pigmented skin may need different guidance on adequate sunlight exposure. A single-centred, cross-sectional study with 124 participants examined the effect of skin colour on vitamin D status in individuals living in an urban setting (New York City) (29). Kaufman and colleagues state that there is an association between darker skin pigmentation and lower vitamin D status in this population. Recent research conducted in South Asian participants living in the UK, reported that individuals with brown skin needed sunlight exposure to be 2.5-3 times more than fairer skinned counterparts to achieve similar serum 25(OH)D concentrations over a 6 week period (24). Skin pigmentation will be discussed further in this chapter (vitamin D status).

Gastrointestinal absorption and transport of vitamin D

Vitamin D is fat soluble and is absorbed with other dietary fats in the upper part of the gastrointestinal tract (GI) (6). Original research by Hollander (1978), reported *in vitro* evidence that vitamin D can be absorbed though passive diffusion without the need for carrier mediated or active transport (7). Further research questioned this theory and has since shown that absorption of this fat-soluble vitamin may share common pathways with cholesterol (30). Reboul and colleagues (2011) used mouse models to show that along with simple diffusion, vitamin D is also absorbed,

partly, involving a cholesterol transporter (31). Studies continued to build on Reboul's research into the absorption mechanisms of vitamin D and observed similar mechanisms with cholesterol absorption. Studies have shown evidence that factors such long-chain fatty acids and phytosterols that inhibit the absorption of cholesterol also reduced the uptake of vitamin D (32, 33). Although gaps in the knowledge are present, a recent review concludes the absorption of vitamin D appears to be through passive diffusion with membrane transporters, particularly cholesterol transporters (34). The majority of the absorbed vitamin D is in the chylomicrons within the enterocyte which then enters the circulation and is quickly taken up by the liver (35, 36).

Vitamin D status

Definition and thresholds

Vitamin D status can be categorised as deficient, insufficient, and sufficient. The thresholds for each of these categories are defined in Table 2.

There is still no agreement over the specific thresholds of serum 25(OH)D concentrations that should be recognised as deficient, insufficient, and sufficient (37). SACN (UK) defines deficiency as less than 25nmol/L, while the IOM in the US has a slightly higher threshold of 25(OH)D concentrations of 30nmol/L which are considered deficient (20, 21). SACN did extensive research reviewing what is known about the different thresholds and the impact on musculoskeletal health, specifically reviewing literature on muscle strength and function, falls, rickets and osteomalacia (38). SACN found the data to be inconclusive and was unable to advise a specific serum 25(OH)D threshold which risk of osteomalacia increased (20). This resulted in their definition for deficiency to be 25(OH)D concentrations below 25nml/L as this was associated with increased risk of poor musculoskeletal health, however, this is not to be confused for use as a clinical diagnostic tool or threshold for disease (39). Symptoms of vitamin D deficiency may present as vague and non-specific such as fatigue or general aches in adults (40). Research shows that muscle weakness and pain is usual in the pelvis, thigh, foot and hip for adults with a vitamin D deficiency (41). In children, severe deficiency can present as seizures, bone deformation, swelling of the wrist and avoidance of weight bearing (42). Clinicians generally test for vitamin D deficiency if the patient is at high risk of vitamin D deficiency due to low sun exposure, disease (osteomalacia) or presents with symptoms (43). Individuals (e.g., the elderly and pregnant women) at high risk of vitamin D deficiency are outlined further in this chapter.

The IOM put together a committee of scientists and experts to review current research to set recommendations of dietary reference intakes (DRIs) for vitamin D and calcium for the benefit of musculoskeletal health (21). The synthesis of the research found serum concentrations of 30nmol/L or above were adequate for the protection of skeletal health, but no conclusive evidence was found to support recommendations for extra-skeletal outcomes (44). Although there remains debate on what levels of vitamin D should be considered for deficiency, insufficiency, and sufficiency, it appears that an agreement among researchers that having vitamin D levels below 25nmol/L will negatively affect skeletal health (45).

Although rare, toxicity of vitamin D occurs when blood concentrations of 25(OH)D reach >220 nmol/L (se

Table 2 table 2) as a result of extremely high intakes of vitamin D (supplement form) (46). Intoxication of vitamin D is facilitated through hypercalcaemia with symptoms including frequent urination, nausea, and weakness (47). Hypercalcaemia is the accumulation of calcium in soft tissues (48). Due to the lipophilic profile of vitamin D and its storage in human adipose, this mechanism can increase the time frame for toxic levels to remain longer after exposure has ended (49). Hypervitaminosis D with hypercalcaemia can be fatal as high concentrations of 25(OH)D induce renal failure and cardiac arrhythmias (50).

The means of how toxicity of vitamin D occurs remains unconfirmed, however, there are three theories used to explain the possible mechanisms behind vitamin D toxicity (50). These theories are all associated with increase in plasma concentrations of 25(OH)D that has contact with VDR in the nucleus of target cells which results in gene over-expression (51).

Table 2: Vitamin D thresholds from IOM and SACN

	IOM	SACN
Deficient	<30nmol/L	<25nmol/L
Insufficient	30-50nmol/L	n/a
Sufficient	>50nmol/L	>25nmol/L
Toxicity	>375 nmol/L	>375 nmol/L

Assessment

The assessment of vitamin D status is a changing landscape (52). Liquid chromatography mass spectrometry is the most common method used for testing 25(OH)D concentrations (53). There has been some debate regarding which metabolite should be tested to determine vitamin D status; 25(OH)D, 1,25 dihydroxyvitamin D or free (unbound) vitamin D (54). Concentrations of free 25(OH)D is measured directly using a centrifugal ultrafiltration, validated ELISA kit or can be calculated based on VDBP, albumin and total 25(OH)D serum levels (55). In a healthy population, free vitamin D status compared to total 25(OH)D is significantly correlated (56). However, some clinical conditions such as renal disease may affect albumin, VDBP, and an affinity for VDBP for 25(OH)D metabolites, therefore, affecting the amount of free vitamin D and the relationship between free and total 25(OH)D levels (56) .

Skin pigmentation

Vitamin D deficiency is now seen as a global public health issue (57) . This is especially true for those who are darker-skinned living in European countries of a northerly latitude (58). In the UK, 82% of the South Asian population have levels that are deficient during summer months which rises to 94% in the winter (59). Research focusing on ethnic minorities residing in a UK inner city in Birmingham found that Asian and Black Afro-Caribbean populations have higher prevalence of vitamin D deficiency rates (31% 21% respectively) compared to Caucasians (12%). Interestingly, it was noted that Asian women had a higher prevalence of deficiency compared to men (43% and 25% respectively) in this population (60).

Elderly

Research has shown a high prevalence of low vitamin D status in the elderly aged >65 years (61-63) . The term elderly is defined as having a chronological age of 65 years and above (64). This is relative to the economic status of the country and life expectancy of the population. This is reflected in the WHO definition of elderly in Africa as individuals of 50 years and older (65).

Many factors contribute to low vitamin D status in older persons, this includes compromised skin synthesis of UVB rays as a result of reduced sunlight exposure and reduced dietary intake. Sun exposure may be compromised due to poor mobility (house-bound or instutionalised) and an impaired ability to successfully synthesise UVB rays from the sun. (66). Malnutrition is common amongst the elderly, and this may also be a contributing factor for their lower vitamin D status (67). A German study recently reported the prevalence of vitamin D deficiency in hospitalised, frail elderly patients, which found sufficient serum 25(OH)D concentrations of >50nmol/L in 12.6% of the sample (n=167) (68). Similar findings were reported by Kweder et al (2018) who found that 43% of older adults aged 75+ had serum 25(OH)D concentrations less than 24 nmol/L in a population based study with 125 participants (69). This prevalence of low vitamin D status in older persons may increase the risk of poor musculoskeletal health, which is discussed further in this chapter.

Pregnancy and lactation

The prevalence of vitamin D deficiency during pregnancy has been reported to range from 18-84% across the globe, depending on which country the mother resides, sun exposure and dietary intake (70). A concern of low vitamin D concentrations in pregnancy is the effect it may have on the mother and unborn infant. Research has shown that having insufficient/deficient 25(OH)D concentrations during pregnancy increases the mother's risk of preeclampsia and gestational diabetes (71). Exclusively breastfed babies are at particular risk for low vitamin D levels as a result of low status in the mother (72). This was most apparent in unsupplemented babies with little or no sun exposure born to mothers with low vitamin D concentrations in pregnancy and lactation (73).

This low status appears to continue to be prevalent in young children (12-24 months)(74) and in adolescence (75). In support, an American cross-sectional study conducted with n=365 healthy children between 12 and 24 months found a relationship between the risk of vitamin D deficiency and breastfed infants that were unsupplemented and toddlers who had a higher BMI (74). A UK-based study was conducted using the NDNS survey data to determine the prevalence and predictors of vitamin D inadequacy in children aged 4-18 (n=1102) (75). This study reported that 35% of the sample (n=1102) had insufficient vitamin D concentrations (>50 nmol/L) and this was found to be associated with increasing age, specifically with adolescents aged 14-18 years compared to younger

children. Non-white skin was also seen to be at considerably higher risk of insufficiency in comparison to white skinned counterparts, as well as low levels of outdoor activity, increased screen time and BMI.

Approximately 20% of the infants required dietary intake, as recommended by the IOM is obtained from their mother's breast milk (76). There is a limited amount of research looking at vitamin D status in breastfeeding mothers, however, it is suggested by the literature that the mother's vitamin D status affects her offspring (77, 78). Research in Norway shows that immigrant mothers, specifically, Pakistani, Turkish and Somali and their infants had insufficient or low vitamin D levels, most notably in infants who were exclusively breastfed (79). North American research comparing Chinese and Mexican mothers to North American (Cincinnati) mothers found vitamin D deficiency (<50nmol/L) in 60% of the Mexican and 50% of Chinese mothers, compared to only 17% of deficiency in the Cincinnati mothers measured at four weeks postpartum (80). The research, albeit limited, shows that breastfeeding mothers appear to be at high risk of low vitamin D status and should be routinely assessed for serum 25(OH)D levels and supplemented where appropriate to benefit both mother and child.

Role in health

Musculoskeletal health

Vitamin D has a well-established role in bone health which plays an important role in bone mineralisation and aids calcium absorption (1). 25(OH)D is responsible for the maintenance of both serum calcium and phosphorus concentrations to promote mineralisation of the human skeleton and maintain vital cellular functions (81). Bone mineral density is positively correlated with increased serum 25(OHD) levels in all age groups (82). Vitamin D deficiency causes osteomalacia in adults and rickets in children (2).

It remains unclear whether vitamin D supplementation has a beneficial effect on muscle strength. A recent RCT showed no improvement in muscle strength, postural ability or physical performance in females aged 60-80 with a three-month vitamin D_3 supplement of 2800 IU/day compared to placebo (83) . In contrast, a systematic review and meta-analysis reviewed cross-sectional research to investigate whether there is a relationship between frailty in older adults with low serum 25(OH)D concentrations (84). Although limited to the cross-sectional data, the meta-analysis of 13 studies reported that lower 25(OH)D concentrations were significantly associated with an increase in frailty.

Low levels of vitamin D in older adults, especially those in residential care is seen as an important and ongoing public health issue (85).

In addition, a one year, population-based randomised controlled trial using 300 older women aged 70-90, with insufficient vitamin D status (<50nmol/L) at baseline, showed improvement in both muscle strength and function in the slowest and weakest participants of this cohort (86). These two studies are very similar in design using free-living, older female participants, with vitamin D insufficiency (<50nmol/L), but otherwise healthy and placebo controlled. However, the different results could be attributed to the much larger sample size (n=81 vs n=300) and longer trial duration (3 months vs 1 year) evidenced by Zhu et al. 2010. Low vitamin D levels and the risk of falls and fractures have also been researched (87, 88). In a recent (2018) systematic review with meta-analysis and trial sequential analyses reviewed 81 randomised controlled trials. This pooled analyses found that a vitamin D supplement ranging from 100IU/day-300,000 stat, had no effect on falls (n=37), hip fracture (n=20) or total fracture (n=36) (89). The evidence for a relationship between vitamin D and risk of falls and fractures appears to remain inconclusive.

Obesity

Low vitamin D status is associated with excess body mass and obesity (90). The mechanisms behind these low levels is unclear, however, many theories have arisen (91). Decrease in vitamin D levels in this population has been associated with raised plasma parathyroid hormone (92). This imbalance reduces the absorption of calcium and as a result can increase the risk of other conditions such as cardiovascular disease (93).

A systematic review and meta-analysis found a higher prevalence of vitamin D deficiency in participants that are obese compared to both normal and over-weight counter parts by 35% and 24% respectively (94). Further support for this in a more recent review found individuals who are obese to have on average 20% lower serum 25(OH)D levels than people with a normal weight (91). This was found to be consistent across ethnicity, age and geographical location. It is postulated that low dietary vitamin D intake, limited sun exposure, inflammation, and dilution and/or sequestration of vitamin D stored in the excess fat mass may account for the association between obesity and vitamin D. It has been argued whether vitamin D has been sequestered (95) into fat or simply diluted volumetrically (96, 97) without absolute conclusions. A small single centre study investigated the storage of vitamin D in adipose tissue in women with obesity undergoing bariatric surgery

compared women of normal BMI undergoing abdominal surgery not gynaecological reasons. They found similar vitamin D status in both groups and no difference in the distribution of vitamin D in adipose tissue (subcutaneous and omental) (95). It was also evidenced in both groups that adipose vitamin D concentration was directly related to serum vitamin D status. However the stores of vitamin D in the adipose tissue was higher in the women with obesity. The authors concluded that female participants who are obese store vitamin D in the excess adipose. This storage of vitamin D creates a need for further vitamin D to saturate this reservoir which may put individuals that are obese at risk of insufficient serum 25(OH)D concentrations. This suggests that storage of vitamin D is likely to be sequestered in those who are obese. The authors state this is the first study of its kind to investigate serum 25(OH)D concentrations in subcutaneous and omental adipose in individuals who are obese and non-obese. It is important to note that these results are not generalisable to all people with obesity since this was a convenience sample in a population undergoing surgery. It would be valuable to consider the adipose tissues stores of vitamin D in a population with obesity who were had low vitamin D status. The small sample of individuals who are obese (n=21) and non-obese (n=25) also make it difficult to draw definitive conclusions regarding how vitamin D is stored in individuals living with obesity.

A cross-sectional, population-based study investigated the relationship between participants that are obese and non-obese and serum 25(OH)D concentrations (97). Total sample size was 686; with 35 participants in the obese cohort and 651 in the non-obese cohort. This sample was derived from a previous study which recruited 1,179 postmenopausal women from a rural (east Nebraska, USA) community to investigate vitamin D and calcium over a 4 year period (98). All participants were unsupplemented and serum concentrations were seasonally adjusted. Regression analyses were performed using both hyperbolic and linear models. The authors found an inverse association between 25(OH)D concentrations and fat mass. The hyperbolic model proved to be most appropriate as it mathematically shows the association between volume and concentration. The research reports that these results offer a confirmation that serum 25(OH)D concentrations are stored volumetrically and not sequestered. However, the hyperbolic model when applied can only be approximated as this model relies on the data to be consistent. This is undermined by the inconsistent daily contribution of cutaneously sourced vitamin D. In addition, there is a limited sample size (n=35) and no deficient participants for the obese cohort study. This makes it a challenge to confidently confirm vitamin D is stored volumetrically.

It remains consistent in the literature that people who are obese are at risk of low levels of vitamin D compared to normal or over-weight individuals (90) . This may or may not be due to volumetric dilution or sequestered in the excess fat mass. Little is known of the clinical impact of low serum vitamin D on the individuals living with obesity, however, BMI should be considered when evaluating serum 25(OH)D levels (99).

Obesity has also been associated with chronic low-grade inflammation. A cross sectional study found an inverse association with inflammation biomarkers C-reactive protein, Interleukin 6 (IL-6), tumor necrosis factor-α (TFN-α) and low vitamin D levels (100). C-RP is known for its lowering effect on fat-soluble vitamins, specifically vitamin D (101). One study suggests, for appropriate interpretations of plasma 25(OH)D, C-RP levels must be <10mg/mL (102).

The research shows a connection between obesity, inflammation and 25(OH)D levels. Inflammation, especially C-RP should be adjusted for when accounting for deficiency in people who are obese.

Type 2 Diabetes

In the UK, 1 in 40 people are diagnosed with type 2 diabetes (103). Type 2 diabetes is a major burden of cost to the NHS that can be largely preventable and manageable with a change in diet and lifestyle factors (104). Research has investigated the possible relationship between vitamin D deficiency and impaired insulin resistance, glucose intolerance, reduced insulin secretion and the metabolic syndrome (105). It is thought that vitamin D deficiency reduces the function of pancreatic β-cells which impacts on the secretion and resistance of insulin Mitri and colleagues (2011) found an inverse association between vitamin D status and high glucose levels that been reported from a number Fof other cross-sectional studies (106). A randomised controlled trial found a single bolus dose of vitamin D (400,000 IU), in 63 men and women with T2DM with vitamin D deficiency (concentrations ≤50 nmol/L) did not improve insulin secretion or sensitivity, over a 6-month period (107). A recent randomised, double-blind, placebo controlled clinical trial, with a large sample size (n=2423) compared a vitamin D 4000IU/day supplement to placebo over a 4-year duration in those who are considered to be at a high risk of developing T2DM (108). This RCT found no significant difference between placebo and treatment in reducing the risk of developing type 2 diabetes.

Another recent review concluded that vitamin D supplementation is not recommended to improve or prevent type 2 diabetes (109). In contrast, a review by Lips and colleagues (2019) did observe an

improvement with vitamin D supplementation in deficient participants on HbA1c, insulin resistance and insulin secretion in some clinical trials, however, effect size was small.

Grammatiki and colleagues also reviewed the available literature and concluded that current research is conflicted and fraught with limitations such as unpowered sample size and duration (110). In support of this, an expert panel has collaborated to review the evidence regarding vitamin D and disease. Their recommendation was that vitamin D supplementation would not prevent or treat T2DM, however, may benefit those early on in disease onset and have a deficient status (111). Indeed, Giovanni et al. (2016) state that it is unclear if supplementation is more effective when the decrease in β-cell and insulin function is in its early stages of damage. The possible relationship between vitamin D and type 2 diabetes remains disputed as observational studies (112) find positive associations and randomised control trial data draws no conclusions (109). The most recent (2020) evidence on the possible benefit of vitamin D supplementation on T2DM agrees that the large differences in the variables aforementioned makes evidence of a real effect challenging (113, 114).

Gastrointestinal

Vitamin D has been linked to several gastrointestinal disorders (115-117). Emerging research is providing evidence that suggests this prohormone may improve recurrence and survival rates in colorectal cancer and reduce symptom severity in inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (114, 118, 119). IBS and vitamin D in particular is a new and developing area of research. Vitamin D insufficiency (<50 nmol/L) has been observed in those with IBS, UC and CD (120-122). Evidence is limited in the area of IBS however, available literature shows low levels of vitamin D (115, 123, 124). Vitamin D deficiency has also been observed in IBD and colorectal cancer patients (125, 126). There is little understanding of whether low concentrations of 25 (OH)D is cause or effect in gastrointestinal disorders (127).

Colorectal cancer

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in women and the third most common cancer in men in Europe (128). In the UK, there are 42 000 new cases each year with an improved 5 year survival rate of 60% (129). Research has identified three patterns of incidence and mortality rates of global CRC (130) . Arnold et al. (2015) classify the patterns as; group one with

both incidence and mortality on the rise (e.g. Brazil, Croatia and Spain), group 2 have increase in incidence but a decrease in mortality (e.g. UK, Ireland and Sweden) and lastly, group 3 which has both a reduction in incidence and mortality (US, Japan and Austria). There are on average 100 new cases of CRC diagnosed every day in UK (131). Many CRC studies have reported low vitamin D levels and 2 recent meta-analyses shows strong evidence for an inverse association between serum concentrations of 25(OH)D and colorectal adenoma risk (132, 133). Evidence linking the effect of vitamin D supplementation and CRC prevention remains inconclusive. A large 7-year study with 36,282 post-menopausal women investigated the effect of vitamin D_3 (400IU) plus calcium (1000mg) daily supplement compared to placebo on the risk of developing CRC (134). No association between daily supplementation of vitamin D and calcium on the incidence of CRC was found. In contrast, a nested case-control study in western European populations (EPIC study) showed a strong inverse relationship between pre-diagnosis 25(OH)D levels and colorectal cancer risk using 1248 cases and 1248 age match controls (135). It remains unclear if vitamin D status has a causal link to the development or prevention of CRC. Fuchs et al. (2017), used predictive modelling for stage III colon cancer patients (n=1016). Predictive modelling uses validated regression models to forecast future outcomes. Specifically, this research assessed the effect of participants' vitamin D status after being diagnosed with CRC then created a model to predict levels of 25(OH)D using a score. Using Cox proportional hazards, the predicted plasma 25(OH)D scores were then investigated on cancer recurrence and mortality. It was determined that the higher predicted 25(OH)D levels post diagnosis are associated with lower cancer recurrence rates and increased survival (136).

In summary, the evidence suggests having higher concentrations of 25(OH)D may improve mortality and recurrence rates in those with colorectal cancer.

Inflammatory Bowel Disease

The two main types of inflammatory bowel disease (IBD) are Crohn's disease and ulcerative colitis. The aetiology of these conditions is unclear, however both of these chronic conditions cause inflammation to the gastrointestinal tract (137). Ulcerative colitis is primarily located in the large bowel, while CD has inflammation anywhere from mouth to anus, with the most common being the right colon and the terminal ileum (138, 139). In Europe, over 2 million people have been diagnosed with IBD which has an immense effect on health care and is considered a major public health concern (140). The prevalence of IBD has been increasing in western populations since 1990 at a substantial

rate; from 79.5/100 000 to 84.3/100 000 in 2017 (141). A recent prospective cohort study estimates prevalence for UC at 397 and for CD at 276 per 100 00 (142).

Numerous drug therapies for this condition are available, including vedolizumab and ustekinumab, however, approximately 30% of patients do not respond to this form of treatment (143). Patients may need to test many therapy options until the most effective option is discovered (144). Vitamin D deficiency has been associated with increased inflammation and disease activity in IBD (145). Research shows that vitamin D insufficiency is common in those with IBD (146, 147). In support of this, vitamin D levels were measured in participants with IBD and found 49.8% were vitamin D deficient (148). There are limited RCTs investigating the effect of vitamin D as a treatment. The most recent data from a pilot study supplemented patients with mild-moderate Crohn's disease up to 5000IU/day for 24 weeks (149). Their findings suggest achieving vitamin D levels to above 40ng/ml (100nmol/L), reduced Crohn's disease activity index (CDAI) scores from 230±74 at (baseline) to >150 for 67% (exit) ($p=<0.0001$) and improved quality of life scores from 156±24 (baseline) to180±26 (exit) ($p=0.0004$) in this small sample ($n=18$). An earlier study compared the effect of 1,25(OH)2 to 25(OH)D with CD participants to investigate effect on bone pathology and disease activity. This research presents beneficial effects on bone metabolism and disease activity in the short term (150). It is also suggested that vitamin D may play a role in reducing relapse frequency. A recent meta-analysis (151), provides evidence that all IBD relapse rates, including both UC and UD, may be controlled with improved 25(OH)D levels and recommends that vitamin D supplementation is included in the treatment of IBD.

Ananthakrishnan et al. (2012), conducted a prospective cohort study with over 70,000 females, aged 40-73 from the Nurses' Health Study from 1986-2008. Their evidence shows a decrease in incidence of CD in women with higher plasma levels of 25(OH)D (44).

The evidence presented offers a possible benefit of a vitamin D supplement to reduce relapse and disease activity in inflammatory bowel disease.

Vitamin D binding protein and the possible mechanism for GI disease

The possible mechanism for vitamin D in gastrointestinal disease is thought to be through the vitamin D receptor (VDR) and its mediated pathways within the colon (152). VDR, which is present in many tissues and almost all immune cells, has been investigated and found to have involvement in the possible development of inflammation in bowel disorders and colorectal cancers (153, 154).

VDR is strongly expressed in the colon and is a potential regulator of gene expression (118). The vitamin D receptor mediates the majority of recognised functions of 1,25-dihydroxyvitamin D_3 (153). In reference to IBD, VDR expression is significantly decreased in those with IBD (155). VDR may be a biomarker for these complex conditions and offer insight into those who may respond to vitamin D supplementation. Susceptibility genes have also been investigated in both IBD and IBS and may offer supplementary insight. Zucchelli and colleagues (2011) investigated single nucleotide polymorphisms (SNPs) that are commonly seen in Crohn's disease and reported the disease risk allele rs4263839 G in the *TNFSF15* gene (involved in inflammatory response) was also associated with IBS, suggesting that susceptibility to IBS is mediated via similar inflammatory pathways (156). This finding may inform future research to further investigate the means that the *TNFSF15* gene may offer to the pathogenesis of IBS.

Irritable Bowel Syndrome

Features

Irritable Bowel Syndrome (IBS) is a functional disorder of the gastrointestinal tract which is prevalent in approximately 5% to 20% of the global population (157-159). Functional gastrointestinal disorders (FGIDs) is a term for chronic or recurrent conditions of the gastrointestinal tract that are absent of any pathophysiology and that are diagnosed based on symptoms and include constipation, dyspepsia, oesophageal disorders and IBS (160).

In the UK IBS incurs a high cost to the NHS, in 2012-2013 it was estimated at over £11 000 000 (161). It is a relapsing condition that has been traditionally classified into 4 subtypes based on the patient's bowel habits; IBS-D (diarrhoea predominant), IBS-C (constipation predominant), IBS-M (mixed or alternating of both) and IBS-U (undefined) (162). Altered gut motility can be seen as the main pathophysiological component in IBS. Gut dysmotility can affect the rectum, colon, small intestine and the stomach in approximately 25-75% of IBS patients (163). Kanazawa and colleagues (2008) show that altered motility was associated with dissatisfaction in bowel habits and abdominal distention in their prospective study with 129 individuals with IBS (164). The cause of the syndrome appears to be multifactorial and may include social, biological and psychological influences (165).

Despite the high prevalence of this condition, the aetiology of IBS remains unclear (166). IBS can negatively impact a person's life and associations have been seen with depression, anxiety and post-

traumatic stress disorder (167). The effect of mental health on IBS could be explained by the gut-brain axis and visceral sensitivity. The gut-brain axis is the physiological connection between the GI tract and the central nervous system (168). As further discussed later in this chapter, visceral hypersensitivity in IBS participants have been identified through a lower pain tolerance to colonic or rectal distension compared to non-IBS counterparts (169).

Infectious enteritis is a possible risk factor for developing IBS. This is considered a subset of IBS known as post-infectious IBS (PI-IBS). This occurs after an acute episode of gastroenteritis in those who had no previous IBS symptoms or diagnosis (170). The associated risks for the development of PI-IBS may include; being younger in age, female, severity of the gastroenteritis event, and the presence of psychological distress before or during gastroenteritis (171).

IBS diagnosis

In 1978, the first effort to establish an objective criteria for the diagnosis of IBS, was known as the Manning criteria (172). Manning and colleagues found that IBS inpatients shared four common symptoms; abdominal distention, frequent and looser stools with the onset of pain, alleviation of pain upon defecation (173). In a similar study conducted in Germany, Kruis et al. (1984) reported three hallmark relapsing features reported by IBS participants; altered bowel function, bloating and pain (174) This led to a meeting in in Rome (1988) where experts in IBS gathered to create a standardised diagnostic tool for IBS (175).

The Rome criteria for the diagnosis of irritable bowel syndrome were first developed in 1992 to prevent patients from needing extensive and invasive investigations such as colonoscopy (176) and to be able to identify eligible patients for pharmaceutical intervention studies (175). IBS was and remains difficult to diagnose (177). The Rome criteria diagnostic tool has a set of standardised questions relating to abdominal pain, bloating, stool consistency/frequency and relief after defecation. These questions have altered as the criteria has been updated to II, III, and most recently in 2016 to Rome IV (178). There has been little validation for Rome I and II and none for the Rome III criteria until Ford and colleagues' evaluation in 2013, 5 years after its creation (179). There is debate around the efficacy of Rome IV. Research has shown that Rome IV vs Rome III significantly lowered the prevalence of IBS and may miss those with mild or less severe symptoms (180, 181).

Risk factors for IBS

Epidemiologic study of IBS is challenging due to symptom variability, a large proportion of those living with IBS not seeking medical attention and the lack of unbiased findings (182). Two known risk factors for developing IBS are being female and contracting a gastrointestinal infection (183, 184). Women have been shown to have a higher prevalence of IBS than males (185). Globally, IBS has an overall prevalence that is 67% higher in women than in men (186).

Research suggests a reason for this gender divide is that women are more likely to seek medical advice than men (159). If we compare IBS rates between men and women in Africa, South Asia and South America, they are similar in prevalence and in some instances, men have higher rates of the condition (187). As aforementioned post-infectious IBS (PI-IBS) is considered another way to subtype IBS. It is the onset of persistent abdominal symptoms post gastroenteritis infection (188). The estimated incidence rates for PI-IBS are as follows; epidemic infections (7-36%), traveller's diarrhoea (4-14%) and individual infections (4-36%)(189).

It may also be beneficial to consider certain psychological features (stress or anxiety) in this population (190). Anxiety, depression, and post-traumatic stress disorder are psychological traits that have been linked to IBS (191). One dominant theory is that IBS symptoms arise as a result of a disruption in neurotransmitter-related management of interaction between the brain and the enteric nervous system (the gut-brain axis) (192). This communication system combines GI and brain functions which include appetite, weight and gut motility (193).

IBS can also be associated with visceral hypersensitivity, which can be heightened following a meal (194). Visceral hypersensitivity is thought to be dependent on multiple factors and occur in the peripheral or central nervous system. It may also play a part in the cause of symptoms of IBS (195) . Visceral hypersensitivity is thought to be a distorted response to distension of the colon and a heightened reaction to pain (196). Research has shown that IBS participants have a lower pain threshold than the non-IBS counterparts (194, 197). A study using volunteers (n=136) with a diagnosis of IBS and fulfilling the Rome II criteria and healthy controls were recruited through physician referral and advertisement (164). This study aimed to establish; (i) pain sensitivity, phasic and tonic motility, (ii) if subtypes of IBS vary on these variables, (iii) does pain sensitivity correlate with tonic and phasic motility and (iv) if symptom severity is related to these 3 variables. Results found that regardless of subtype, participants with IBS had significantly lower pain thresholds than the controls. This lowered pain threshold was seen to contribute to symptom severity, with particular effect on frequency and intensity of abdominal pain.

The cause of visceral hypersensitivity is unknown but is recognised as a hallmark feature and is present in ~35% of individuals with IBS (198).

Management of IBS

Dietary interventions

Two thirds of patients with IBS believe their symptoms are caused by dietary triggers such as wheat or dairy (199). As a result of this perceived issue around certain food groups, diet has been a focus of research with individuals with IBS (200-202). The most widely used short term dietary intervention is the use of the low FODMAP (fermentable oligosaccharides, monosaccharides, disaccharides and polyols) diet (203). This diet restricts the consumption of fermentable carbohydrates for approximately 8 weeks (204). Examples of FODMAPs includes wheat, garlic, apples, onion, legumes and pulses (205). These short-chain carbohydrates are unsuccessfully digested in the small intestine, then fermented in the colon, thus yielding gas and bloating (206). An Australian study found lower overall improved symptoms in an IBS population compared to controls using a low FODMAP diet (LFD) versus a typical Australian diet (207). The low FODMAP diet is intended to identify certain foods that may cause or increase symptoms of IBS to assist the individual manage their condition and is not meant to be used as a long-term treatment or a cure (208).

In addition to the low FODMAP diet, the role of gluten is another focus of research. Current evidence, albeit limited, shows the removal of gluten may reduce symptom severity (209). To support this, research with a gluten-free diet led by a dietitian proved beneficial to diarrhoea predominant IBS, which reduced symptom severity significantly and in the participants who continued this gluten-free diet also had sustained reduction of symptom severity (210). It is argued that it may be other fructans present in the carbohydrates causing the disturbances and not the gluten protein (211).

A review of the literature presents good evidence for the use of LFD in the management of IBS in clinical practice (212). This review offers best practice with use of the LFD with dietary counselling by a specialist dietician. The review identified three significant and relevant stages; restriction, reintroduction and personalisation and offers evidenced based advice for each step. Whelan and colleagues (212) conclude that although there is inconclusive evidence for the long-term use of a

LFD, in the short term, the use of a LFD with dietary counselling delivered by a specialist dietician is an effective tool for the management of symptoms in functional gastrointestinal disorders.

Probiotics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"(213). Probiotics contain bacteria needed to maintain intestinal health, this includes; motility, visceral sensitivity and intestinal permeability (214). Information on probiotics is often sought out by patients with various clinical practitioners (215).
A recent systematic review and meta-analysis assessed 28 randomised controlled trials with the focus on safety and efficacy of probiotics as a treatment for IBS (216). This review found a positive effect on overall IBS symptoms and quality of life from studies that used a combination of probiotics but found nothing conclusive for single strains or species. Nor did they find an effect on individual symptoms such as bloating or satisfaction with bowel habit.
The probiotics most extensively used in human research are *Lactobacillus* and *Bifidobacterium* (29). It is postulated that changes in the gut microbiota may be a contributing factor to symptom severity in functional disorders of the gut which includes IBS (217). Indeed, a well-known theory on the normalising effect of probiotics is the possible increase of short chain fatty acids which in turn stabilises gut motility in those with constipation (218). Probiotics have been shown to have beneficial effects on pain, gas, and bloating in functional gastrointestinal disorders (FGIDs) (219). However, the amount of evidence provided in this area remains limited. A review of the research on probiotics, specifically to IBS, concluded probiotics elicited significant improvements in the overall and global symptoms of IBS, particularly using a combination of strains and L. *plantarum* DSM 9843 (220). This research also synthesised data that specifically looked at the probability of symptoms persisting for individuals with IBS in the treatment arm (probiotics) compared to a placebo. Twenty-three randomised controlled trials were analysed of which, twelve papers were selected for low risk of bias and presented dichotomous data. This review concluded that probiotics had a positive effect on bloating, flatulence, abdominal pain and global IBS scores. The RR was 0.79 (95% CI 0.70-0.89) for persisting symptoms of IBS with probiotics compared to placebo.

A recent review and meta-analysis (2017) assessing the effect of probiotics on constipation found that the specific strains *Lactobacillus* or *Bifidobacterium*, increased bowel movements by 0.8 /week in individuals with IBS-C and decreased intestinal transit time in those with IBS-D (221). The evidence shows promise for the use of probiotics in the treatment of IBS.

Prebiotics and symbiotics

There are limited data on the use of prebiotics and symbiotics for the treatment and improvement of symptoms for those living with IBS. Cappello et al. (2012) used a symbiotic mixture which included 9 different species of probiotics and one prebiotic in a powder form preparation compared to a placebo and examined its effect on quality of life, colonic transit time and overall symptoms (222). Sixty-four participants from an outpatient clinic were recruited for this study for four weeks after a two-week run in. The authors found a significant decrease in flatulence severity, and no improvement in bloating or global satisfaction of abdominal flatulence (222). This research was extended to a 6-month pilot study based on the previously published trial (223). The results were similar, with a change that more patients (n= 26) found relief of severe flatulence and for longer, however, with a much-reduced sample size.

As aforementioned, Ford and colleagues (2014) produced a systematic review and meta-analysis, covering latest research to explore the efficacy of prebiotics, probiotics and symbiotics in IBS and chronic idiopathic constipation (CIC). Although as stated above probiotics were found to be beneficial, there were too few intervention studies with prebiotics and/or symbiotics for conclusions to be drawn.

Pharmacological management

Before a pharmacological approach is taken, it is appropriate to first explore dietary changes mentioned above. Once these have been unsuccessful at relieving symptoms for the patient, then pharmacotherapy is offered (224).

Medications that are prescribed for constipation predominant IBS include; lubiprostone, linaclotide and plecanatide. These secretagogues offer an increase in fluid in the lumenal cavity and have been shown to be significantly more effective than placebo in RCTs. Although these treatments have been found to be successful, long-term safety has yet to be established (225).

Loperamide, eluxadoline, and alosetron are drug therapies offered to those with diarrhoea predominant IBS. Alosetron has shown benefit but is associated with severe adverse side effects such as faecal impaction (226). Loperamide is an over the counter anti-diarrhoeal used frequently

by those with IBS. However, toxicity or misuse of loperamide has shown to have serious cardiac effect (227). Eluxadoline a similar mu-opioid to loperamide have displayed adverse effects in this population, specifically, Lembo and colleagues found that in 4.5% of 1666 participants receiving eluxadoline reported AEs (228).

Selective serotonin re-uptake inhibitors (SSRI) are also used for the treatment of IBS. Traditionally, SSRIs are used to treat psychological conditions such as depression and anxiety; (229) comorbidities associated with IBS. A recent review (2019) by Ford et al., found SSRIs to most likely be effective in the treatment of IBS (230). This was an update on a previous review with similar results and only 5 more studies were included since 2011 (231).

A safe and effective treatment that can be administered long-term has yet to be discovered. Therefore, researchers and practitioners have explored various dietary interventions to improve gastrointestinal symptoms, which may offer less risk to pharmaceuticals.

Placebo Effect

The placebo effect has been defined as a positive response to an inert substance (232). It is argued, that it has broader meaning in that it is the improvements seen in individuals symptoms that are a result of participating in a therapeutic environment (233). The positive effect may be attributable to a change in behaviour or physiology in response to the participant's awareness of being studied (The Hawthorne effect) (234), this effect has been observed even when the research is double blind (235).

A Cochrane review of the literature found that of the 11 clinical conditions investigated, asthma, phobia pain and nausea showed significant placebo effect (236). In IBS specifically, elevated placebo response rates are approximately 40-70% (237). The reason for this high placebo rate is not completely clear, the endpoints that are measured are self-reported and herein lies a major contributing factor. Meta-analysis of the research suggests the more rigorous entry criteria and a higher number of treatment visits decreases the risk of placebo effect (237), while others evidence high variability in baseline scores (238, 239). Kaptchuk and colleagues (240) suggest the most robust predictor of placebo effect in IBS was placebo plus a sympathetic practitioner. In contrast Flik and colleagues (2017) reviewed the literature in psychological intervention studies and discovered similar placebo response rates to pharmacological intervention studies (241). This shows research

conducted in a supportive and sympathetic environment does not elicit more placebo response compared to traditional intervention studies.

A placebo run in phase for research with individuals with IBS has been suggested to reduce the placebo effect as it allows researchers to exclude placebo responders (242, 243). may contribute to However, the exclusion of placebo responders will contribute to the creation of heterogenic population samples which makes comparison of trials even more challenging (244).

Simplistic and subjective binary endpoints (whether the intervention worked or not) are used to assess positive and negative outcomes in IBS research (245). This may not be the most effective way to determine efficacy due to the complex and often variable symptoms. To reduce this effect in IBS research, the Food and Drug Administration (FDA) in the USA has modified the binary endpoints that have been typically used for global symptom relief of IBS to 2 endpoints directly relating to the primary motility issue. These include a more objective assessment focused on motility change and abdominal pain which is thought to minimise the placebo effect in clinical trials (246).

Vitamin D and IBS

Status of publications on vitamin D and IBS at the start of this study

The research available at the start of this study was limited to four observational studies (120, 247-249) and three randomised controlled trials (115, 250, 251). Chapter three is a published comprehensive systematic literature review of the current literature at the start of this PhD project in 2017. An updated review of the more recent publications (3 intervention studies) is presented in this chapter (114, 252, 253). Collectively, the studies in the systematic review and the supplementary research were mostly in agreement. Five out of the six intervention studies reported vitamin D levels to be insufficient among their participants (115, 250-253). Tazzyman (2015), albeit a formal pilot study was the only paper that did not find a positive effect from supplementing IBS participants (free-living) with vitamin D supplement compared to placebo (250). The subsequent intervention studies (114, 115, 251-253) suggests vitamin D supplementation improved both symptom severity and quality of life in clinical populations. We can determine from the intervention studies that a vitamin D supplement may be effective in participants from a clinical setting compared to a free-living population to reduce symptoms and improve quality of life. Although, since this benefit was seen in observational studies, these results are neither significant nor generalisable (120, 123, 247, 248). It highlights the need for vitamin D status to be assessed in IBS individuals and repletion is necessary

even if for general health reasons alone. The synthesis of this research is limited and warrants adequately powered trials with generalisable results.

Vitamin D deficiency prevalence in IBS and possible explanations

An association between low vitamin D status and irritable bowel syndrome (IBS) has been suggested, however, the published literature is limited and an established causal role in IBS has yet to be determined. Some of the research suggests that people with IBS have a common deficiency of vitamin D (120) which is thought to impact on both symptom severity and quality of life. The reason for this deficiency is yet to be determined as cause or effect of IBS. Low baseline serum concentrations have been recognised in both paediatric (249) and adult studies (120, 123). These studies found more than 50% (paediatrics) and 83% (adults) had baseline serum concentrations <50nmol/L. Deficiency in this population may be a result of their symptoms; people not comfortable leaving the house, food avoidance or possibly issues with malabsorption. Research has shown that malabsorption is present in other chronic gastrointestinal disease (i.e., ulcerative colitis) (254) however, this has not been a proven issue in FGIDs. It remains unclear whether the associated low vitamin D status in IBS is a malabsorption issue or due to lifestyle characteristics. Recent research with participants (IBS-D) has shown efficacious at improving IBS symptoms by using a high dose (50,000 IU/week) vitamin D supplement (255). As altered intestinal patterns are present in this population, particularly individuals with IBS-D along with possible lactose/dairy intolerance, this could suggest reduced absorption of fat-soluble vitamins such as vitamin D (34). This impaired intestinal absorption could be a contributing factor for the onset of deficiency in vitamin D.

Chapter 2

Aims and Objectives

The previous chapter has shown the various gaps in the research surrounding reliable and efficacious therapies for IBS. The limited research available at the start of this project suggested a potential benefit of vitamin D supplementation on IBS related symptoms and quality of life. Two intervention studies (115, 251) and one pilot study (250) reported positive results. Abbenezhad et al. (2016) (251) and Jallil et al. (2016) (115) both reported that high dose vitamin D (50,000IU/week) improved both symptom severity and quality of life in people with IBS recruited from a

gastrointestinal clinic. Tazzyman et al. (2015) (250), a pilot study which investigated the effect of vitamin D supplement, placebo or vitamin D and probiotics on symptom severity and quality of life in an IBS population. No association was found between treatment arms and improvement of quality of life or symptom severity. Although, it was noted that there was a negative correlation between serum 25(OH)D and quality of life. Tazzyman and colleagues also provided a power calculation of 74 per arm from which future studies should achieve.

This thesis intends to address some of the under-researched questions related to vitamin D supplementation and its possible effect on IBS symptoms and quality of life.

Thesis aim:

The main aim for this thesis is to investigate the relationship between vitamin D and irritable bowel syndrome and to address the question of whether vitamin D supplementation can ameliorate symptoms of IBS.

Hypothesis:

A vitamin D_3 supplement will improve symptom severity and quality of life in individuals diagnosed with irritable bowel syndrome.

Study Aims:

The aims of the thesis were:

1) To examine and synthesise all the available current literature on the relationship between vitamin D and irritable bowel syndrome.
2) To investigate the efficacy of a sublingual/buccal vitamin D spray compared to vitamin D capsule.
3) To conduct a RCT to investigate the possible effect of a 3000IU/ day vitamin D sublingual spray on symptom severity and quality of life with individuals with IBS.

Objectives

Study 1: *Vitamin D status in irritable bowel syndrome and the impact of supplementation on symptoms: what do we know and what do we need to know?*

Primary Aim: To systematically review the available literature with the focus of vitamin D and IBS.

Study 2: *Rate of change in circulating 25-hydroxyvitamin D following sublingual and capsular vitamin D preparations.*

Primary Aim: To measure and compare the rate of change in circulating 25(OH)D in response to a vitamin D supplement delivered in two preparations: a sublingual spray or a capsule. This study was an efficacy assessment of the proposed intervention preparation for the IBS trial (Chapter 4).

Study 3: *Effect of vitamin D supplementation on irritable bowel syndrome symptom severity and quality of life.*

Primary Aim: To undertake a randomised controlled trial of the effect of 3000IU vitamin D supplement on symptom severity and quality of life in individuals with irritable bowel syndrome from a free-living population.

Objectives: The broad objectives of the PhD were:

1. To conduct a systematic review of the literature exploring the relationship between vitamin D and IBS.
2. To conduct a randomised placebo-controlled efficacy trial of a vitamin D_3 supplement comparing two modes of delivery; capsule and sublingual spray.
3. To conduct a randomised placebo-control trial to investigate the effect of vitamin D supplementation on severity of symptoms in people with irritable bowel syndrome.

Chapter 3

General Methodology

This chapter is an overview of the methods used in this PhD. Each method is briefly reported in their respective chapters including the three research studies that have been peer-reviewed and published. The justification for each method is presented through an exploration of their appropriateness to the study conducted.

Vitamin D analysis

The assessment of vitamin D status of the participants of this research was fundamental to the interpretation of this thesis. For the purpose of this thesis, the total vitamin D status for each participant recruited was determined from whole blood samples collected using finger prick blood spot kits. For chapter 5 (the repletion study) the vitamin D status of participants was measured at days 0, 3, 7, 14, 21, and 42 during the study. For chapter 6 (the randomised control trial) the vitamin D status of participants was measured at baseline and trial exit in order to determine response to supplementation. The fingerprick blood spot kits were manufactured by Birmingham City Assays and supplied by the industry stakeholder BetterYou (Barnsley, UK). These kits are designed for the collection of dried blood spots on Whatman paper without the need for a phlebotomist. The kits provide participants with a lancet and detailed step-by-step written instructions, allowing an individual to collect their own finger-prick blood spot samples. For the purposes of the research each participant was given hands on guidance by the researcher to show how to perform the finger-prick blood spot test independently. For the repletion study the researcher performed only the first and final whole blood spot sample, leaving participants to complete the remaining 4 samples (chapter 5). For the intervention study the researcher completed the fingerprick blood spot sample at baseline and exit.

The samples were sent to City Assays, Department of Pathology, Birmingham Sandwell Hospitals NHS Trust, and analysed by liquid chromatography tandem mass spectrometry for total blood 25(OH)D (25(OH)D2 and 25(OH)D_3 (256). An example of the report with participants results can be seen in Appendix 12. This method was selected for its ease of use, quick turnaround of results (within 2-3 days), the reliability of service and precision of this technique.

The laboratory (Birmingham City Assays) are a member of DEQAS (vitamin D External Quality Assessment Scheme). The aim of this scheme is to ensure the validity and reliability of assays for 25 hydroxyvitamin D (25OHD) and 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) (DEQAS 2022). This shows a clear commitment to providing accurate results from this laboratory.

The fingerprick blood spot method is convenient for both participant and researcher as there is no need for a trained phlebotomist. In addition, capillary fingertip blood collection is seen as a non-invasive, quick technique which participants may feel more comfortable when compared to venepuncture.

It was important for this study to have a swift turnaround for results, especially as it was important to know baseline vitamin D status of participants prior to start of intervention in order to avoid toxicity. The results of vitamin D status were sent from the laboratory to the industry partner to ensure blinding. The staff member responsible for the results would flag any vitamin D levels to the researcher that were at a risk of toxicity (>220 nmol/L). If a participants' vitamin D status were to reach toxic levels the participant would be contacted and withdrawn from the study and advised to see their general practitioner. The studies conducted for this thesis did not have any toxic results.

Venous blood sampling by a trained phlebotomist has been the traditional approach to collect blood for vitamin D status analysis. However, dried capillary blood samples have been shown to perform to a high standard compared to venous blood for the measurement of serum 25(OH)D. McNally and colleagues compared measurements of 25(OH)D levels in both capillary and venous blood samples (257). Their results provided evidence that the use of finger lance technique for collecting blood samples for assessing vitamin D status is an accurate and reliable method. The blood spot finger-prick method used in two of the studies was most appropriate for time, efficiency, participant acceptability, and accuracy for this thesis.

The method of analysis of vitamin D status has been a topic of considerable debate over recent decades (52). This debate has included a consideration of which form of vitamin D should be considered as a biomarker of status and dispute over which laboratory method is most accurate (258). The debate over which form of vitamin D is the most appropriate biomarker continues, but the majority of the recent literature has recommended the use of 25-hydroxyvitamin D (25(OH)D) as the biomarker most reflective of status (259, 260). This is opposed to 1,25(OH)D2 since this has a short half-life, so difficult to measure and is less responsive to changes in intake (10, 261). These are important issues because deficiency and inadequacy of vitamin D has been increasingly recognised as risk factors for a number of diseases and international agreement over thresholds of deficiencies and standardised accurate methods are needed to allow comparison between datasets. This has led

to attempts to standardise the methodology. Two commonly used methods for assessment of 25(OH)D concentrations are: Chromatography (used in this thesis) and Immunoassays (IA) (262). There is strong evidence of the accuracy of the LC/MS/MS method (263). Research has compared to 2 liquid chromatography-tandem mass spectrometry, a radioimmunoassay (RIA), and 5 automated 25(OH)D immunoassays (264). To determine assay acceptability, the minimum requirements for mean bias and imprecision were calculated as ≤15.8% and ≤9.1% respectively. Additionally, the authors used the concordance correlation coefficient (CCC) defined as <0.9 as poor agreement and >0.99 as excellent. The results from this study found, the 5 automated immunoassays gave variable results with excessive bias scores and poor concordance with LC/MS/MS. The LC/MS/MS had excellent concordance of 0.99 and mean bias score of 2.8%. However, the RIA did show comparable results to the LC/MS/MS with a CCC of 0.97 and mean bias of 5.4%. It was also shown that all assays had adequate results for the imprecision score with the exemption of one of the automated immunoassays. It appears that immunoassays have over time improved efficacy but remain inferior to the LC/MS/MS method (265).

To ensure laboratories are producing high quality testing, the vitamin D Standardization Program (VDSP) organised an inter-laboratory (n=15) comparability study which assessed 16 assays (8 = IA, 8 = LC/MS/MS) against the VDSP specific evaluation criteria that included a CV of <10% and bias <5%. There were 7 countries involved in this study: Canada, United Kingdom, United States, Korea, Ireland, Australia and Germany. This study reported that 11 out of 13C/MS/MS assays met the standard benchmarks for measuring 25(OH)D concentrations compared to only 9 out of 18 of IAs tested. They concluded that the results from this comparability study should be used as a baseline that future studies could be benchmarked. There is also recent research exploring which vitamin D metabolite could be used to assess vitamin D status. The most abundant vitamin D metabolite available in the circulatory system is 25(OH)D_3, however, it has been argued that other active metabolites (e.g. 25(OH)D_3 -G) could prove beneficial when assessing status (266). It is important to note that 25(OH)D_3 is the inactive form of vitamin D and insights into the activated forms for the assessment of vitamin D status is an emerging area of research (see figure 3) (267). Recent research into different metabolites of vitamin D for assessing status; specifically conjugated (25OHD3-S and 25OHD-G) compared to unconjugated (25(OH)D_3 and 25(OH)D_2) forms found that approximately 50% of 25(OH)D_3 circulates in the conjugated (sulfate) form (268). Interestingly, the authors also evidence that the conjugated glucuronide (25OHD_3, 25OHD_2, and 24,25(OH)2D_3) forms are present in circulation, however in much smaller amounts than the sulfated forms. This supports the research that focussing on a single inactive metabolite may not be most accurate for the assessment of vitamin D status.

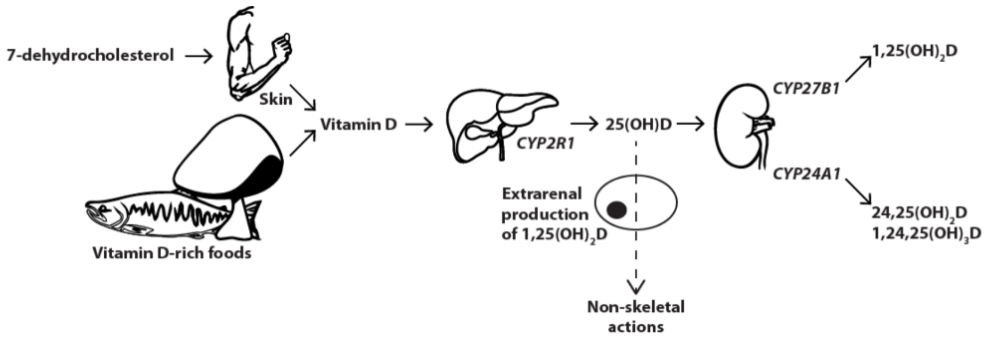


Figure 3: Metabolism of vitamin D from point of ingestion of vitamin-D rich foods or exposure from sunlight to active and inactive forms. (266)

The current study could have benefitted from using the active metabolites as a way of assessing vitamin D status in combination with the 25(OH)D_3 and would have contributed to this emerging research in supplementation studies.

There are notable variations in the laboratory assessments and in the types of vitamin D metabolites used to determine vitamin D status in the human body. It is crucial to get the correct method for assessing participant's/patient's vitamin D status. The metabolite and method selected will impact the interpretation of both observational and experimental research and will in turn contribute to evidence-based guidelines (269). Future studies should work towards an agreed 'gold standard' for which metabolite(s) and laboratory method when assessing the vitamin status of patients and participants in research.

Dietary analyses

Research into dietary intake involves the use of dietary assessment tools (DATs) such as food frequency questionnaires (FFQs), food records and 24-hour recall (270). Dietary assessment is challenging and often comes with limitations such as under/over-reporting, missing data, and recall bias (271). This is due to self-reported dietary intake being reliant on the memory of the participant, social desirability bias, and ability to estimate portion sizes (272). For the purpose of this thesis, a

DAT was needed to assess the dietary intake of vitamin D in participants from the previous year to determine whether this may reflect their baseline vitamin D status.

The Weighed food records method is commonly used for measuring dietary intake due to its known accuracy and detailed account of food and drink consumed (273). This accuracy is achieved when the leftover food waste is subtracted from the original full serving (274). Although accurate, this is a burdensome task for volunteers to weigh all food and drinks consumed pre and post meal and may lead to missing data or participant drop-out (275). This method of dietary assessment would not have been suitable for the study presented in chapter 6. Cost and time were limited as this research was a part of a PhD with a limited budget. The 24-hour recall is a relatively quick method with low participant burden lead by a trained professional (36). This tool is not limited to a specific type or groups of food, literacy is not needed by participants, and is sensitive to ethnic differences. However, validity is only achieved when multiple recalls are conducted, which increases both cost and time (276). The cost burden is gained through the administration of multiple recalls and subsequently, the analysis of the data collected (277). The 24-hour recall deemed not be an appropriate method to assess vitamin D intake in individuals with IBS. There is a limited amount of vitamin D rich foods available in the diet and this DAT could miss the variation in day-to-day intake of vitamin D by participants (278).

There is no agreed gold standard for the assessment of dietary intake, it is suggested that there is a need to combine methods as well as a comparison to biomarkers (279). For the purpose of the research presented in chapter 6, it was based on a formal pilot study, which used the EPIC FFQ to assess dietary intake of vitamin D in individuals with IBS.

The EPIC Food frequency questionnaire (FFQ) has been previously validated in a UK adult population. The EPIC-Norfolk study was part of the European Prospective Investigation of Cancer (EPIC). This multi-centre cohort study started recruitment between 1993-1997 and is one of the largest epidemiological studies of nutrition (280). There were 23 centres involved, which, EPIC-Norfolk was one. The aim of the EPIC study was to investigate the possible relationship between dietary intake and cancer incidence. This study used 4 different methods to assess dietary intake; FFQ, 7 day diet diary, 24-hour diet recall (paper version) and the diet web questionnaire. The validation of the dietary assessment was achieved using biological markers (e.g plasma vitamin C) and comparison between each assessment tool (281).

This questionnaire asks the participant to rate the frequency of the food consumed (list of 130 foods) using 9 categories, which starts with “never or less than once/month” to the most frequent “> 6 times/day” (282). The portions are specified using comprehensible sizes such as one slice of bread

or one orange or household units (spoon or cup) (283). To ensure accuracy, the participants were instructed how to fill in the FFQ and the researcher ensured each person was confident to complete this independently in their home setting. It is important that the FFQ that is selected is appropriate for the population size that you intend to measure and for which purpose. It is recommended that FFQs are used in research with larger sample size and validated for use in the same country or dietary habits (284). Additionally, Serra-Majem et al. (2009), reviewed FFQs from validation studies and 2 of the key components for quality included having a sample size of over 100 and compared intake results to a biomarker (285). The EPIC FFQ was appropriate in the present study as research was with a UK population and used for potentially 160 participants (n=135).

The FFQ EPIC Tool for Analysis (FETA) (https://www.epic-norfolk.org.uk/) was the tool to calculate nutrient and food group data from the entered food frequency questionnaires (FFQ). The data gathered from the FFQs are first inputted into an excel spreadsheet which is then uploaded into FETA software. FETA is a cross-platform, open-sourced tool that analyses the dietary data from the food frequency questionnaire. The 130 foods used in the FFQ are from McCance and Widdowson's Composition of food and the UK food composition database (286). For the research presented in chapter 6, n=115 FFQs were returned by participants and only these were included in the final analysis. No missing data was observed in the completed and returned questionnaires. The FETA software analysis gives 4 nutrient outputs. The first output is average intakes of daily food groups (14 basic) and nutrients (46) from all the consumed foods from the FFQ. This is in a format that is suitable to import into statistical software or spreadsheet for analysis.

A recent study have developed a rapid FFQ to estimate the dietary intake of vitamin D in healthy adult participants (n=50) in England (287). The FFQ was developed using 'The composition of Foods' by McCance and Widdowson, and 'Food Portion Sizes' from the Food Standards Agency. The food groups included are; pasta, breakfast cereals, milk and cream, egg and egg dishes, cheese, yoghurts, meat and meat products, dessert and sweet items, fish, drinks, sauces, butter and spreads, and supplements. To validate this FFQ, it was compared to a 4-day diet diary and plasma 25(OH)D concentrations. A Bland Altman plot was conducted to show the difference in vitamin D intakes between the 4-day diet diary and the rapid FFQ. Only one participant fell outside the 95 agreement, with the mean (SD) difference in reported vitamin D intake between the FFQ and 4-day diet diary was –1·8ug (SD 3·8). This research also reported a strong, significant correlation between the 4-day diet diary and the FFQ for evaluating dietary vitamin D intakes ($r= 0.609$, $p < 0.0001$. This research was conducted with university students, and ethnic minorities were not well represented, making results not representative of the general UK population. However, the results presented offer a promising FFQ to estimate dietary intake of vitamin D in future studies. This research was published

post recruitment of the present study and would have been a possible alternative FFQ to assess dietary intake of vitamin D in individuals with IBS. There appears to be a gap in the research for a FFQ specifically designed and validated to assess dietary intake of people living with IBS. There are limited studies assessing the dietary intake of individuals with IBS (288-291). The available research is based on short term dietary assessment tools such as food diaries or recalls and neglects to report long term habitual intakes.

The EPIC FFQ used in this thesis, was also used in research specifically evaluating dietary intake in individuals with IBS (292) and a formal pilot study this research was based on (250). In individuals, with IBS, dietary intake was investigated using the validated EPIC FFQ (292). Analysis of the FFQ data was compared to the Dietary Reference Values (food energy and nutrients) and intakes observed in the UK general population. The EPIC FFQ was successfully used in the previous research (250), suggesting that this is an acceptable tool to capture habitual dietary intake in people with IBS.

The methods discussed as alternatives to the FFQ are only a snapshot of a persons' diet. This thesis sought to examine the habitual dietary intake of a specific nutrient from the previous 12 months.

Questionnaires

To evaluate the possible effect of a vitamin D supplement (3000IU) on symptom severity and quality of life in volunteers with IBS, two IBS-specific questionnaires were used (293, 294). The IBS Quality of Life Questionnaire (IBS-QoL) is a recognised IBS-specific measure with recognised repeatability, and internal consistency (295). The IBS-Symptom Severity Score (IBS-SSS) questionnaire is extensively used to measure pain (abdominal) as it recognised as advantageous over standard pain measures and shown to be correlated to physical assessments (296). All the IBS research (RCTs) reviewed in chapter 4 have used these IBS specific questionnaires to measure improvement in the participants evidencing its widespread use (114, 115, 251, 297). The pilot study that the research in chapter 6 was based on used both the IBS-SSS and IBS-QoL, therefore these questionnaires were selected for use as they are widely used and validated tools for assessing pain severity and quality of life in an IBS population.

Alternatives to the IBS-SSS tool to measure pain severity in IBS are the Numeric Rating Scale (NRS) and the Functional Bowel Disorder Severity Index (FBDSI).

The NRS is an 11, 21 or 101 point scale which has endpoints of worst pain to no pain that can be adapted to the need of the researcher (298). Past research has shown the NRS to have poor reproducibility (299). However, when adapted to a 10-point scale for assessing pain severity in

individuals with IBS, the NRS scale proved to have exceptional validity and recommended for use in clinical trials with people with IBS (300).

The FBDSI is a simple scale developed by Drossman et al. 1995 with patients, to assess illness severity in individuals with functional bowel disorders for the use in intervention studies (301). The score is based on three variables current pain severity (VAS), clinical diagnosis of functional chronic abdominal pain and the number of visits in the past 6 months to their physician (300). The score rates the severity as mild (0-36), moderate (37-110) and severe (>110). This tool has been validated for its use in assessing illness severity in functional bowel disorders which includes IBS and its application for its use in screening participants for entry onto treatment studies (302). A recent systematic review concluded that NRS 10 point scale was valid and reliable in IBS research for abdominal pain, although the authors concluded the IBS-SSS to be the most appropriate tool to assess gastrointestinal severity in participants with IBS (303).

IBS is known to have a serious impact on the individuals' daily life which can include feelings isolation, anxiety and even suicide (304). Research suggests that people who live with IBS are at a higher risk of developing depression and have a lower quality of life compared to controls without IBS (305). Our study used the IBS-QoL to assess quality of life in those living with IBS. As aforementioned, this was to in keeping with the methodology used in the formal pilot study. Other health related measures available to assess quality of life that could be applied to an IBS population is the Functional Digestive Disorders Quality of Life Questionnaire (FDDQL) and the Irritable Bowel Syndrome Questionnaire (IBSQ).

Similar to the IBS-Qol, the FDDQL is a disease specific questionnaire created to measure health related quality of life in people living with functional disorders of the bowel. This self-reported questionnaire has 43 items related to 8 dimensions; sleep, daily activities, health perception, impact of stress, anxiety, diet, digestion discomfort and coping with disease (306). A specific calculation delivers a score for each of the 8 dimensions that can range from 0 (the worst) to 100 (the best) (307). The FDDQL, has yet to produce published evidence of validation for its use specifically for research with IBS to date (308).

Developed by Wong et al. (1998), the Irritable Bowel Syndrome Questionnaire (IBSQ) measures quality of life in participants with IBS (309). The items were produced from interviews with patients and clinicians, ensuring the main symptoms were determined and included (310). The final tool has 26 items in 4 domains (fatigue, activity limitations, emotional dysfunction, and bowel symptoms). A 7-point scale is used to score results, which a higher score indicates a better quality of life. This appears to be an appropriate way to measure quality of life in individuals with IBS, unfortunately this

method has not been adequately validated (311). At present, the IBS-QoL used in this thesis is the most extensively validated tool for assessing quality of life in individuals living with IBS (294, 312, 313).

Recruitment

Sample size and sampling technique are important considerations for research (314). A sample size should be established during the design phase of the research study (315) and for RCTs ab adequate sample size is needed to show a significant difference, if a difference is present (316). It is also important to have a sample size to ensure the research is able to provide clinically relevant results (317). The sample size of a study/research needs to be large enough to be statistically significant, however, too large and there may be unnecessary exposure to participants from potentially harmful interventions, not to mention ethical concerns (318). On the other hand, having a research study which is undersized can be a waste of valuable resources without usable results (319). The formal pilot from which chapter 6 was based on, produced a power calculation (sample size of >97) for future studies investigating the effect of vitamin D supplementation on an IBS population (250). The RCT reported in this thesis (Chapter 6) benefitted from a sample size of n=135.

Convenience sampling was used to recruit participants through the university email lists for both studies in chapter 5 and 6. The same volunteers in chapters 5 were recruited to participate in the qualitative phase (focus groups) of this study. Convenience sampling is considered a nonprobability and non-random sampling method (320). This is the most common sampling method that recruits individuals that can meet the inclusion criteria and consent to participate (321). A convenience sample could be expected be homogenous and consequently have fewer disparities in results compared to a random sample (322). Alongside an increase in bias from researchers, non-random sampling does not offer generalisable results (323). Although this method has its disadvantages, it is 16 times more likely than convenience sampling is used over probability samples (324). Convenience sampling offered this thesis an efficient, cost effective and simpler alternative to probability sampling.

Participants

Healthy adults over the age of 18 were recruited to take part in the in the efficacy study (chapter 5) and adults over the age of 18 with a self-reported diagnosis of IBS for the intervention study (chapter 6). Healthy adults were needed to compare the effect of two forms of 3000IU vitamin D supplements; capsule and oral spray on raising vitamin D concentrations. Low vitamin D status is prevalent worldwide (45) and this thesis explored vitamin D status in a healthy free living population while testing the efficacy of the two modes of delivery. Discovering whether the oral vitamin D spray is as effective as the capsule could be advantageous for those with swallowing or absorption difficulties (325). The industry partner (BetterYou) who supplied the oral spray were keen to explore whether their product could raise serum 25(OH)D concentrations to adequate levels. In both studies, volunteers were recruited in the winter months to ensure that their vitamin D levels were at the lowest due to limited skin synthesis of vitamin D and therefore at low risk of toxicity (326).

Low vitamin D status has been associated with individuals diagnosed with IBS (121). We recruited adults aged 18 and older with a clinical diagnosis of IBS including all the subtypes. It was important to include all subtypes of IBS to explore possible response to treatment between these groups. We included individuals diagnosed with IBS using Rome III – IV criteria. Clinical settings are regarded as the gold standard for research, although not representative of real world data as participants tend to be treatment compliant, excluded for other comorbidities, limiting to clinical practice (327). Free living settings are able to include those individuals that may be more generalisable and have the ability to capture data on those not familiar with clinical practice (328).

Current and available research exploring vitamin D supplementation and individuals with IBS (see chapter 4) recruit participants from gastrointestinal outpatient clinics. To recruit volunteers from a gastrointestinal clinic, the primary researcher must obtain NHS ethics approval is a lengthy process. The normal response time for opinion on an NHS ethics application is 60 days (hra.nhs.co.uk). This process could be made longer if amendments are required. PhD studentships are time sensitive and would not allow for prolonged waiting time before recruitment could begin. A decision was therefore taken to recruit via the general population rather than via the NHS.

This thesis was based on a formal pilot study whose participants were from a free-living community, and at this time, the only intervention research with this population (250).

Focus Groups

Focus Groups (2 sessions) were conducted with participants who had taken part in the efficacy study to understand the participant's experience of the two treatment preparations of vitamin D i.e. capsule and sublingual spray. This was firstly to ensure the acceptability of the sublingual spray and to determine a possible preference between the two modes of delivery. At exit interview the participants were asked whether they had a preference between the spray and capsule. This ensured that the preference data was captured in the event the focus groups were not adequately attended. The focus groups were recorded and transcribed. The main finding from both the interview question and focus group sessions was that there was an overall preference for the spray. This was disseminated to the industry partner. Focus groups are a suitable way to gain sufficient amount of in-depth detail in a relatively short time for a small cost (329). This type of technique aims to facilitate honest discussions in a natural way (330). Participants can feel the need to 'perform' and this may inhibit their true opinions being discussed freely (331). To encourage discussion the researcher's used photographs that represented different aspects of the study (i.e., spray bottle or tablets). Our focus groups did not discuss sensitive topics, and it was obvious in the group that the volunteers felt comfortable disagreeing with each other on the very practical discussion on whether the group preferred the oral spray or capsule. To ensure the focus groups were completed to a high standard, an experienced researcher with this method assisted in the running of the sessions. To support the findings of the focus groups, the participants were asked whether they had a preference of either of preparation or no preference. Quantitatively these responses were analysed to identify a preferred mode of supplementation if any. One to one interviews could have been conducted as an alternative to focus groups. Interviews for research is beneficial for when in depth detail information is required on a specific topic (330). Face to face interviews are considered a suitable method to gain insight into the volunteer's experience and views on a product or service and acceptability and delivery of the intervention study they participated in (332). In addition, interviews may be beneficial over focus groups as more rich detail answers may be given while not silenced by dominant speakers in a group setting (333). The practicality for this thesis to interview 75 participants would be time consuming for the analysis of the information recorded/transcribed and the time to conduct the interviews. The topic for discussion in the focus groups were not of a sensitive nature and volunteers were healthy adults

asked to discuss their experience of using both vitamin D supplement preparations. Focus group interviews was the most appropriate for extracting data on preference and experience of using two different methods of a vitamin D supplement.

Data analyses

Detailed account of the analytical tests performed in each study are available in their respective chapters. Once cleaned and locked, all data sheets were merged and imported into SPSS (SPSS Inc., USA, V.23). Baseline descriptives were explored and distribution of normality was determined. Not all data was distributed normally, and where needed non-parametric tests were performed and is stated in the publication/chapter. Tests specific to the research questions were performed including repeated measures ANOVA, independent and paired t-tests, and Pearson's correlation. All analyses were 2-tailed with a significance value of <0.05.

Other statistical software packages are available such as 'R', SAS and STATA. SPSS was the one package I (and my supervisors) was most familiar with and was able to attend a master's course for a semester to develop this skill and as such was the chosen platform for statistical analyses.

Ethical considerations

Ethical issues were considered and are an integral part of being a competent researcher. The main ethical considerations for this thesis included voluntary participation, informed consent, anonymity, confidentiality, potential for harm, and results communication. To ensure the main ethical considerations were considered, ethical approval (amendments where needed) was applied for and successfully approved through the University Research Ethics Committee (UREC) (reference 011865 and 016753). Additionally, it was mandatory for PhD researchers to successfully complete the module 'FCM 6100 research ethics and integrity'. This was completed in the first year of study aimed for post graduate researchers to be effective reflective practitioners. This was developed through group discussion of six case studies while considering the models of ethics and integrity. Students were encouraged to apply this learning to their own research and to discuss any challenges they may have faced. Good Clinical Practice (GCP) training was completed to ensure the research is conducted in this thesis was to an ethical and scientific standard. The participants' involved in the

research presented in this thesis were all over the age of 18 and fully aware of their right to withdraw from the study at any time before signing the consent form. Details of what was going to be involved by taking part in the research was explained in detail and documents with this information was given to participants to take home and review. Volunteers were informed that their identity would not be identifiable in documentation and numbers would be used in place of names to ensure confidentiality. The numbers were created by the researchers to substitute participants' names for identification codes which were only known to the researchers directly linked with the study. Encrypted hard drive was used to store electronic data and paper (hard) copies were stored in a locked cabinet. The key and password for the encrypted electronic data was the responsibility of the primary researcher. The two focus groups were recorded with the consent of the participants. The recordings were transcribed and saved to the encrypted device. Participant's safety was of importance and volunteers were informed of how to correctly consume the vitamin D (3000IU) oral spray/capsules and advised to contact the research team if any unwanted side effects occurred. There were two volunteers that experienced small bumps in the mouth as a result of the vitamin D oral spray. One participant appropriately stopped using the spray and the reaction stopped. The other participant was able to continue taking the spray with minimal discomfort. These adverse events are clearly stated in the publication in chapter 5.

Systematic review

Critical appraisal skills programme (CASP) and PRISMA guidance were used to ensure reproducibility and quality of the reviewed research (chapter 4). CASP is a validated tool to appraise the quality of conducted research including; randomise control trials, case studies, and cohort studies (334). Other validated tools are available such as the Effective Public Health Practice Project (EPHPP), Centre for Evidence Based Medicine (Oxford) Critical Appraisal Tools and Cochrane Assessing Risk of Bias in a Randomized Trial that could have been applied for the systematic review (335-337). The critical appraisal tools are designed for specific types of research and would not have been appropriate for this systematic review's focus of vitamin D and irritable bowel syndrome.

PRISMA guidance is a well-established and validated method for reporting and synthesising research in a transparent manner (338, 339). The Cochrane Handbook is another accepted method for systematic literature reviews, valued for its commitment to continuity (340). Either of these techniques are appropriate for systematically reviewing the literature. The PRISMA method was chosen as I was familiar with this tool and both of my supervisors promoted its use.

Study design

The first research study conducted compared two preparations for the efficacy of a vitamin D supplement at raising serum 25(OH)D concentrations (341). There is very limited data comparing a capsule to an oral spray with a placebo control. There were only 3 comparable studies available at the time of this research (274-276). Duration of study ranged in the research from 1-3 months, with our study time period of 6 weeks. This proved to be an adequate length of study and all participants were replete by day 21. This study was a part of a published systematic review scoring the quality of RCTs comparing the efficacy of a sublingual vitamin d spray compared to capsules (342). A total of 4 studies were included in the final review (326, 341, 343, 344). The efficacy study which is peer-reviewed and published (chapter 5) scored the highest for quality (341).

The 12-week RCT presented in chapter 6 was based on a formal pilot which therefore, used the same study duration. The research presented in chapter 4 reviews the duration of studies from the available literature investigating effect of vitamin D supplementation on symptoms/quality of life in those with IBS. This shows that study length among similar research ranged from 6-weeks to 6-months. As the total number of intervention studies is a very limited to 7, as previously mentioned, the 12-week duration was primarily chosen based on the formal pilot from which it was based.

The published version of this research has now been meta-analysed in 2 different reviews (345, 346). This is a strength of the RCT presented in this chapter. Abuelazm et al (2022), conducted a quality assessment of risk bias of the included research and reported that current study had a low risk of bias. The conclusions by both meta-analyses generally agree with the conclusions in this thesis; i) vitamin D supplementation is an additional therapeutic resource ii) effect of vitamin D supplementation on IBS symptom severity and quality of life remains unclear and iii) larger, first rate RCTs are needed to establish dosing regimen, effect of vitamin D supplementation in the long-term.

Chapter 4

Vitamin D status in irritable bowel syndrome and the impact of supplementation on symptoms: what do we know and what do we need to know?

This chapter is in the form of a paper which was published in the European Journal of Clinical Nutrition in 2018. This paper is a systematic review using PRISMA guidelines and shows the research available on vitamin D and IBS at the start of this study. This paper is presented in published format with permission from the publishers. The paper and supplementary data can be accessed here: https://doi.org/10.1038/s41430-017-0064-z

The rationale for conducting this review was to identify the literature available and to show, if any, the evidence for supplementation of vitamin D in individuals with IBS to relieve symptom severity and improve quality of life. The study presented in this chapter suggests that low vitamin D status appears to be common in IBS populations. Since the time of this publication, additional research has been published. A supplementary search to update what is further known on the subject was conducted. Using the same MESH terms "Irritable Bowel Syndrome" OR "IBS" AND "vitamin D" since 2018. the search was performed using the previously searched websites; Web of Science (core collection), Medline and Pubmed. This search yielded three intervention studies from 2018-2020. Two of these studies were based in Iran (114, 252) and one study in Egypt (253), with all studies using recruitment from a clinical setting and a randomised, placebo controlled trial design . Sample sizes were between 74 and 116 participants. Sikaroudi et al. (2018) and El Amrousy et al. (2020) had similar mean average baseline vitamin D concentrations of 43.6 nmol/L and 46.5 nmol/L respectively. These concentrations were similar for both treatment and placebo arm in both these studies. Conversely, Jalili et al. (2019) had sufficient vitamin D status across both arms 52.75 nmol/L. Jalili et al. (2019) offer no justification for this vitamin D status as criteria of inclusion.

Duration of study ranged from 6 weeks (252, 253) to 9 weeks (with a 3 month follow up) (252). The studies used a variety of endpoints (e.g. inflammatory biomarkers, HADs), however, IBS-SSS, and quality of life scores were present in all of the research. Each study reported significant results from the intervention group compared to placebo in these commonly assessed endpoints for IBS population (Table 3, Table 4). One study observed a significant improvement in IBS-SSS score ($p<0.001$) and quality of life ($p=0.007$) in the placebo arm (252). This placebo effect was also reported in the intervention study presented in chapter 6 of this thesis. No other research reviewed

in this chapter has reported this effect although very common within an IBS cohort (347). Indeed, a review of the placebo effect in an IBS population found that 30-40% of clinical trials reported a placebo effect with this participant group (75).

The data collected from the three papers was difficult to synthesise. Each study used various methods of reporting their findings. El Amrousy et al. (2018) neglected to state whether the data distribution (±) presented was standard deviation or standard error. While Jalili et al. (2019) used a combination of standard deviation at baseline then changed to standard error at exit. Lastly, a range rather than a mean or median was reported by the most recent study from Sikaroudi and colleagues making comparison with other trials challenging (252).

Assessment for serum vitamin D_3 was also varied across the studies. Sikaroudi used the LIAISON 25 (OH) vitamin D_3 assay and stated their classification of vitamin D3 status as deficient (<10ng/ml), insufficient (10-30 ng/ml), sufficient (30-100 ng/ml) and toxic (>100 ng/ml). This study took measurements at baseline and exit in IBS-D participants, which included the questionnaires (IBS-SSS, QoL, HADs and VSI) and blood samples 25(OH)D_3, 5-hydroxy tryptamine (5-HT), 5-hydroxy-indole acetic acid (5-HIAA) and 5-HT to 5-HIAA ratio. Jalili et al. (2019) used a similar design where all assessments were made at the start and finish of the trial. However, this study neglects to state which type of laboratory testing was used to determine vitamin D_3 concentrations and there is no definition for the classification of vitamin D status or justification for the required vitamin D status of <75 nmol/L. Jalili et al. (2019) does report using the IBS-SSS and IBS-QoL questionnaires along with data from dietary intake from 3-day dietary recalls.

El Amrousy et al. (2019) used a slightly different assessment schedule. This study measured IBS-SSS, IBS-QoL, and a total score of IBS-SSS (visual analogue scale) questionnaires at day 0, 3 months and exit (6 months). All laboratory assessments were taken at the start and end of the trial, similar to the other trials. Whole blood samples were obtained through venipuncture and vitamin D assay was completed using the vitamin D total assay kit. This study only recruited participants with vitamin D status of <50nmol/L; which they deem deficient based on the clinical practice guideline created by the Endocrine Society, hence no further classification was offered (348).

This data from the research shows a similar baseline mean IBS-SSS score (treatment + placebo) from each trial 240.5, 248.5, 235 respectively. This agrees with the RCTs reviewed in this chapter with mean baseline IBS-SSS scores of 245.8, 241.3 and 248.5. Across the three studies, similarities were observed in baseline QoL and IBS-SSS scores, study setting, design and at exit an improvement in IBS-SSS and QoL scores. Sample age was the one difference between trials. El Amrousy et al. (2018)

had adolescents for participants in comparison to adult populations in the other studies. All participants were recruited from hospital settings.

This supplementary search has provided a further three RCTs in this area of research which appears to agree with the previously reviewed literature that vitamin D supplementation improves symptom severity and quality of life. These IBS populations differ across the studies; in age, study duration, and type of sample used, making the results difficult to synthesise. Although, similar results were reported, these data cannot be simply applied to all individuals with IBS. Data collected from a clinical sample is not representative of the individuals living in a free-living population with IBS. Using a clinical sample reduces the ability to apply the results to the general population, while random samples will be able to deliver data that is more representative (349). It does provide a foundation from which well-defined research studies can stem from, recommending the need for further RCTs that are larger, adequately powered, with a control population, which will offer generalisable results that may confirm a justification for vitamin D as a potential treatment of IBS.

Table 3: IBS-SSS scores for baseline and exit.

	Vitamin D Group		**Placebo**		
AUTHOR	Baseline	Exit	Baseline	Exit	P
EL AMOURSY ET AL. (2018) (NOT STATED)	239.3±7.3	167.6±46.9	241.9±69.8	233±68.2	<0.001
JALILI ET AL. (2019) (MEAN±SD[£]/SE[$])	250.87±96.42	60.23±12.67*	245.78±112.82	35.55±13.54*	<0.05^
SIKAROUDI ET AL. (2020) (RANGE)	230 (190-290)[&]	100 (70-170)[&]	240 (200-300)[&]	190 (140-240)[&]	<0.001

*Used change from baseline data instead of full scores,^ ANCOVA adjusting the effects of age, IBS-QOL baseline score, and baseline value of each factor as covariates, [&]reported range of score, [£]standard deviation used at baseline, [$]standard error used at exit.

Table 4: Quality of life scores at baseline and exit

	Vitamin D Group		Placebo		
AUTHOR	Baseline	Exit	Baseline	Exit	**P**
EL AMOURSY ET AL. 2018 (NOT STATED)	59.2±14.7	75.2±9.2	58.8±15.2	60.8±13.8	<0.001
JALILI ET AL. 2019 (MEAN±SD/±SE)	57.35±27.96	48.13±4.24	46.70±31.37	35.18±4.24	0.039*
SIKAROUDI ET AL. 2020 (RANGE)	76 (65-109)	59 (50-89)	93 (68-120)	79 (61-99)	0.022

*ANCOVA used and adjusted the effects of age, IBS-QOL baseline score, and baseline value of each factor as covariates.

Statement of Authorship

I (CEW) conducted the literature searches, synthesised the literature and created the primary draft. My supervisors (BC and EAW) co-conceived the study. BC also carried out the literature searches, both BC and EAW reviewed and edited all drafts of the manuscript. The final version was agreed by CEW, EAW and BC.

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REVIEW ARTICLE

Vitamin D status in irritable bowel syndrome and the impact of supplementation on symptoms: what do we know and what do we need to know?

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Abstract

Background Low vitamin D status is associated with risk of colorectal cancer and has been implicated in inflammatory bowel disease. Irritable bowel syndrome (IBS) is a chronic, relapsing, functional bowel disorder. A nascent literature suggests a role for vitamin D in IBS, but this has not been collated or critiqued. To date, seven studies have been published: four observational studies and three randomised controlled trials (RCTs). All observational studies reported that a substantial proportion of the IBS population was vitamin D deficient. Two intervention studies reported improvement in IBS symptom severity scores and quality of life (QoL) with vitamin D supplementation.
There are limited data around the role of vitamin D in IBS.

Conclusions The available evidence suggests that low vitamin D status is common among the IBS population and merits assessment and rectification for general health reasons alone. An inverse correlation between serum vitamin D and IBS symptom severity is suggested and vitamin D interventions may benefit symptoms. However, the available RCTs do not provide strong, generalisable evidence; larger and adequately powered interventions are needed to establish a case for therapeutic application of vitamin D in IBS.

Introduction

The reported health benefits of vitamin D have recently extended from musculoskeletal health to focus on the potential relationships in systemic diseases, such as multiple sclerosis, colorectal cancer and inflammatory bowel disease (IBD) [1]. Vitamin D is a hormone that has two key roles within the body: (i) to aid the absorption of calcium and phosphate and (ii) control the secretion of parathyroid hormone [2]. The principal circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D; calcifediol; ChEBI:17933), which is used clinically to determine vitamin D status [3]. There is no universally agreed optimal level of vitamin D; however, the National Academy of Medicine (USA and Canada) has asserted that serum 25 (OH)D levels need to exceed 50 nmol/L (20 ng/mL) to be adequate to meet the needs of 97.5% of the population [4] and by extension levels <50 nmol/L (<20 ng/mL) are considered insufficient [5, 6]. Poor vitamin D status is of major public health concern with low vitamin D status affecting 8–24% of children and 20% adults in the UK [7]. Consequently, SACN guidelines recommend an intake of 10 µg/day for anyone aged 1 year and older [8]. Vitamin D has increasingly been implicated in the pathobiology of colorectal diseases. A meta-analysis and systematic review of observational studies in inflammatory bowel disease (IBD) suggested that patients were 64% more likely to be vitamin D deficient compared to controls without IBD ($p = 0.0001$) [9]. Similarly, a recent review and a meta-analysis of the potential relationship between vitamin D and colorectal cancer identified an association between vitamin D intake and colorectal cancer prevalence:

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a significant inverse association between dietary vitamin D intake, 25(OH)D status and colorectal cancer risk was reported [10, 11]. The potential for vitamin D as a secondary preventive of adenoma recurrence has also been investigated in several trials both alone and in combination with calcium [12].

Irritable bowel syndrome is one of the most common functional bowel disorders seen globally (10–20% of some populations [13] with significant healthcare cost [14]). The pathogenesis of the disease remains unclear and is categorised primarily by the symptoms experienced [15–17]. Symptoms of IBS include bloating, abdominal pain, diarrhoea and/or constipation; the ROME III criteria incorporate assessment of these symptoms to diagnose the condition [18]. There are three recognised sub-types of IBS: diarrhoea-predominant (Type D), constipation-predominant (Type C) and alternating diarrhoea and constipation (Type A) [19]. Other common features of this syndrome not covered in the diagnostic criteria are bloating, passing of mucus from the rectum, irregular stool habits and urgency of evacuation [20]. These symptoms have a serious impact on the person's every day quality of life and appear to have strong links to mental health issues such as anxiety and depression [21]. A number of reports linking vitamin D and IBS have received significant media attention; this review aims to collate and contextualise this research. The literature was searched systematically (see Supplementary Online Information Section I) to identify the full scope of publications in this area; seven reports were identified, comprising four observational studies and three randomised control trials (RCTs).

Summary of the literature to date

Observational studies

Four intervention trials were identified that assessed vitamin D status in IBS (see Table 1).

A case study reported that a high dose supplementation (50–75 µg per day throughout the year) of vitamin D significantly improved one woman's IBS symptoms [22], including a return to almost-normal bowel patterns and decreased anxiety and depression. This paper also systematically identified analysed social media (blogs by people with IBS), noting that 70% of 37 individuals' blogs reported that vitamin D supplementation resulted in an improvement of symptoms. This case resided in the UK (hence a Northerly latitude); however, blogs were from those living internationally and exact locations were not reported. Deficiency thresholds were not defined and serum 25(OH)D levels were not stated. Although in agreement with some intervention trials [23, 24], case studies are not generalisable or statistically significant.

A case control study reported vitamin D serum concentrations in patients with IBS attending a gastroenterology clinic in Saudi Arabia (International Medical Centre) [5]. Cases had a confirmed diagnosis of IBS using ROME III criteria and healthy controls were gender and age-matched staff members from the medical centre. This study defined deficient serum 25(OH)D concentrations as <50 nmol/L [23, 25]; mean serum 25(OH)D concentrations in patients with IBS was 21 ± 12 nmol/L, which was significantly different to 31 ± 16 nmol/L reported for the control group. It should be noted that this study only reported serum 25(OH)D concentrations retrospectively from medical records.

A second observational study in Saudi Arabia reported recruitment of subjects ($n = 498$) with both Crohn's disease (CD) and IBS and compared these to a control group of staff and students ($n = 442$) [26]. The study reported insufficiency of serum 25(OH)D concentrations in 67.3% of the patients; however, it is difficult to ascertain whether the insufficiency of vitamin D was a result of the IBS, CD, a combination of both or a common issue among this general population. This study neglected to define their threshold of 'vitamin D insufficiency'.

Both studies were conducted in Saudi Arabia known for its year-round sunshine which should have a positive effect on serum 25(OH)D levels. However, for religious reasons the population avoid direct exposure of their skin to sunlight and a recent systematic review [27] of 13 studies ($n =$ 24,399) found that 81% of different Saudi Arabian populations (e.g. pregnant/lactating women, children, adults) had serum concentration levels of 25(OH)D <20 ng/mL (<50 nmol/L).

In a US-based study (Atlanta, Georgia) medical records of 1000 IBS patients were reviewed [28]. The mean serum concentration of 25(OH)D of the population studied was 25.05 nmol/L. It was also reported that 72% of women and 3% of men with IBS had a serum concentration < 30 nmol/L. There were no controls used for comparison. Furthermore, this research is only available in abstract form and as such a full analysis is unavailable.

A retrospective case-controlled study [6] analysed the medical records of 55 children and adolescents aged 6–21 diagnosed with IBS living in Massachusetts, USA. This research shows that only 7% of the IBS cohort had sufficient vitamin D levels compared to 25% of body mass index-matched healthy controls attending a well-child clinic. This study suggested prevalent vitamin D insufficiency in both the IBS and control populations, albeit with a limited study design.

Table 1 Observational studies identified linking IBS symptoms and vitamin D status

Author, year	Relationship between IBS and vitamin D	n	Study design	Outcomes
Spricke et al. [22]	IBS symptoms improved following high doses of vitamin D	1	Single case study	Near normal bowel movements
			20,000–40,000 IU vitamin D_3 daily	Relapses only occur if supplementation is ceased, following three years of supplementation Two major themes identified; vitamin D is an effective management tool for IBS and people with IBS seem to be deficient in vitamin D
			Dosage varies according to season (20,000 IU in the summer and 3–4,000 IU in the winter)	5,000–10,000 IU doses were common among people with IBS
			Systematic review of commentary on 12 different online blogs/forums by 37 people with IBS	90% of individuals reported being diagnosed with deficient or low vitamin D levels
				70% of online commentary reported that their IBS condition improved with vitamin D_3 supplementation
Yazadi and Cirstea [28]; Abstract only	Prevalence of vitamin D deficiency in IBS patients	150	Screening patients medical records	The mean of vitamin D level was 25.05 nmol/L
				Caucasians had significantly higher level of vitamin D in comparison to African Americans (26.94 vs 20.40; $p = 0.008$)
				Seventy-two (72%) females and three (3%) males had serum vitamin D levels <30 nmol/L (African Americans)
Al-Ajlan [26]	Deficiency in IBS patients	492	Screening of patients with IBS for coeliac disease	67.3% of patients were vitamin D insufficient
Khayat and Attar [5]	Deficiency in IBS patients	150	Case control study compared IBS to healthy control	82% of IBS patients were diagnosed vitamin D deficient
				31% of controls were diagnosed vitamin D deficient
Nwosu et al. [6]	Vitamin D status in paediatric patients with IBS	170	Retrospective review of paediatric patients aged 6–21	>50% of IBS paediatric patients had serum 25(OH)D levels <50 nmol/L, $p = 0.021$
				90% of IBS paediatric patients had serum 25(OH)D levels <75 nmol/L, $p = 0.006$
				IBS subjects had a significantly lower mean plasma serum 25(OH)D compared to controls 53 ± 116 vs 65 ± 28 nmol/L, $p = 0.003$

Papers are in order of publication, showing populations used in the study

Intervention studies

Three intervention trials were identified that investigated the possible beneficial effect of vitamin D on IBS symptoms (see Table 2).

Tazzyman et al. (2015) conducted a 12-week randomised double-blind three-arm parallel pilot study in people with IBS which compared placebo to either vitamin D supplementation (75 µg/day) or combination of vitamin D (75 µg/day) plus probiotic (two strains of *Lactobacillus acidophilus* per capsule). The trial was conducted in the UK in January–April 2015. Analysis of baseline data illustrated that participants with low vitamin D (<50 nmol/L) had lower QoL (using the single question in the Total Symptom Severity IBS questionnaire [29] compared to their replete counterparts ($p = 0.034$)). Improvements were reported in all treatment arms, but no significant difference between the treatment arms was observed. The study provides valuable data on which to base power calculations for future RCTs.

A RCT conducted in Iran with 85 participants with IBS [23] found significant improvement of IBS symptoms ($p < 0.001$) and quality of life ($p < 0.001$) following very high dose (1250 µg fortnightly for 6 months) vitamin D_3 supplementation compared to a placebo over a period of 6 months. Separate tools measured symptom severity [29] and quality of life [30] at baseline and exit of the study.

A second Iranian study [24] used a 2 × 2 factorial design to conduct a blinded RCT with women aged 18–75 to investigate the effects of vitamin D, soy isoflavones or both on IBS symptoms and quality of life. One hundred participants were randomly assigned to one of four possible arms of the intervention; vitamin D and placebo (D + P), soy isoflavones and placebo (S + P), soy isoflavones and vitamin D (S + D) or both placebo vitamin D and placebo soy isoflavones ($p + P$). 50,000 IU (1250 µg) of vitamin D was administered fortnightly and 2 × 20 mg of soy isoflavones capsules daily. The length of study was a restrictive 6 weeks with a follow-up at 4 weeks post intervention. This study reported significant improvements in IBS symptom severity score and quality of life in participants randomised to either vitamin D isoflavones. Both S + P and the D + P groups significantly improved IBS total score ($p = 0.004$, $p = 0.015$, respectively). The combination effect of vitamin D and soy on IBS-TS was also significant ($p < 0.05$).

Both the Abbasnezhad and Jalili studies showed extraordinarily low standard deviations of IBS symptom severity scores (around 10% around the mean); our ongoing work suggests that the majority of such studies report the SD of symptom severity in the range of 20–70% of the mean (Corfe, unpublished). This suggests a significantly more homogeneous population than comparable publications, the reasons for this are unclear.

All three intervention studies reported low mean baseline vitamin D serum concentrations in the IBS populations studied, ranging from 14 to 21.23 ng/mL (35–53 nmol/L). Vitamin D deficiency is present in the general populations of both the UK and Iran [31, 32] populations and as such, no causal link with IBS can be inferred without control population data. Two [23, 25] out of the three studies showed an increase in the mean 25(OH)D levels from deficient (<20 ng/mL or <50 nmol/L) status to replete (>20 ng/mL or >50 nmol/L) in the active arm. Dosages of vitamin D supplement varied between the studies. The preparations were either in the form of one 50,000 IU (1250 ug) oral capsule fortnightly or a daily 3000 IU (75 ug) sublingual spray. Although optimal dosing strategy is not known, research suggests that both larger, less frequent doses and daily preparations are equal in effectiveness in their repletion of 25 (OH)D [33, 34]. Despite small losses to follow-up, final sample sizes from previous studies appear to be relatively similar.

Conclusions and directions

There is a nascent body of literature associating vitamin D status and the pathobiology and management of colorectal conditions including IBD and cancer. Four papers and one abstract report cross-sectional studies. A consistent limitation of these was that vitamin D status of the wider population is not reported. Cause and effect are difficult to determine as it might be argued that individuals with severe IBS may exhibit behaviour changes, for example elevated time indoors consequent to symptoms, that may impact on vitamin D status.

Two of three interventions studies report a positive benefit of vitamin D supplementation in people with IBS; however, the low variation in the study populations and unusual dosing regime in these two studies raises questions about the generalisability of the data. All three RCTs reported a relationship, either at baseline or in response to intervention, between vitamin D and QoL, a symptom domain of particular importance to the patient population.

Collectively the studies reviewed, although restricted, offer enough justification for further work in this subject area. In particular, future research may benefit from adequate powering (Tazzyman et al. suggest 74 subjects/arm), now that effect size data are in the public domain, to assure generalisability and conclusiveness. Future studies should include a broader spread of participant, or multiple studies should address the potential benefits in defined populations and limit claims to these populations.

Less equivocally, the body of evidence accrued across multiple populations already suggests that vitamin D status assessment should be incorporated as a routine assessment

Table 2 Summary of the effect of vitamin D supplementation on symptoms of IBS in intervention studies

Study, year	Relationship between IBS and vitamin D	n	Intervention	Outcome	Baseline mean serum 25(OH)D ng/mL (±SD)	End mean serum 25(OH)D ng/mL (±SD)	Length of study	TSS scores	QoL
Tazzyman et al. [23]	Improved quality of life vitamin D deficiency	51	300 IU vitamin D_3 plus probiotic (4 strains in total) vs 1000 IU vitamin D_3 vs placebo	81.8% of IBS-C, 70% of IBS-D and 81.6% of IBS-N with circulating levels of <20 ng/mL	Placebo 15 (±4.4) vitamin D 14 (±8.3) vitamin D + probiotic 16 (±6.0)	Placebo 25 (±4.0) vitamin D 37 (±12) vitamin D + probiotic 37 (±9)	12 weeks	244 (baseline VD); <65 (after 6 weeks) = 179	25(OH)D at baseline
				Increased vitamin D levels in all groups				237 (baseline D + P); ~62 = 195	
				Significant association between quality of life and vitamin D levels was observed.					
Abbasnezad et al. [22]	Gastrointestinal symptoms	85	50,000 IU fortnightly vs placebo	Significant improvement in IBS symptoms (overall GI symptoms, abdominal pain and distension, flatulence, vomiting) compared to placebo ($p \leq 0.05$)	Vitamin D 20 (±10) placebo 19 (±11)	Vitamin D 33 (±12) placebo 21 (±11)	6 months	253 (baseline) ~54 (after 6 months) = 197 ± 69	60; 51 (baseline) + 14 = 75
	Health-related quality of life			No improvement was seen in satisfaction with bowel habits					
				Significant improvement in IBS-QoL and IBSSS scores ($p \leq 0.001$)					
Jalili et al. [24]	Effect of soy isoflavones and vitamin D in management of vitamin D	100	4 arm study; placebo soy isoflavones and placebo vitamin D vs soy isoflavones and placebo vitamin D, vs vitamin D and placebo soy isoflavones vs soy isoflavones and vitamin D	IBS-TS score improved significantly in both S + P and D + P ($p = 0.004$, 0.015)	Soy + p 21 Soy + D 20; p + P 21 D + P 21	Not reported	6 weeks	S + p = 240, S + D = 241, p + P = 251, D + P = 251 (baseline)	S + p = 64, S + D = 58, p + P = 22, D + P = 20 (baseline)
				Interaction of effect of vitamin D and soy isoflavones were significant ($p \leq 0.05$)				S + P = 71, S + D = 72, p + P = 29, D + P = 69 (after 6 weeks)	S + p = 38, S + D = 31, p + P = 50, D + P = 40 (after 6 weeks)
				The main effect of vitamin d or the interaction effect of vitamin D and soy isoflavones on IBS-SSS were not statistically significant					
				S = P and D + P groups found a significant decrease on IBS-SSS scores ($p = 0.001$, 0.047)					

alongside IBS diagnosis in routine practice to identify individuals at risk and likely to benefit from vitamin D intervention for general health as much as for IBS symptoms.

Author contributions C.E.W. undertook the searches, collated literature and wrote the first draft. E.A.W. co-conceived the study, reviewed and edited all drafts. B.M.C. co-conceived the study, undertook the searches, collated the literature and edited all drafts. All authors agreed the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors authored two of the systematically reviewed papers. BetterYou markets vitamin D supplements.

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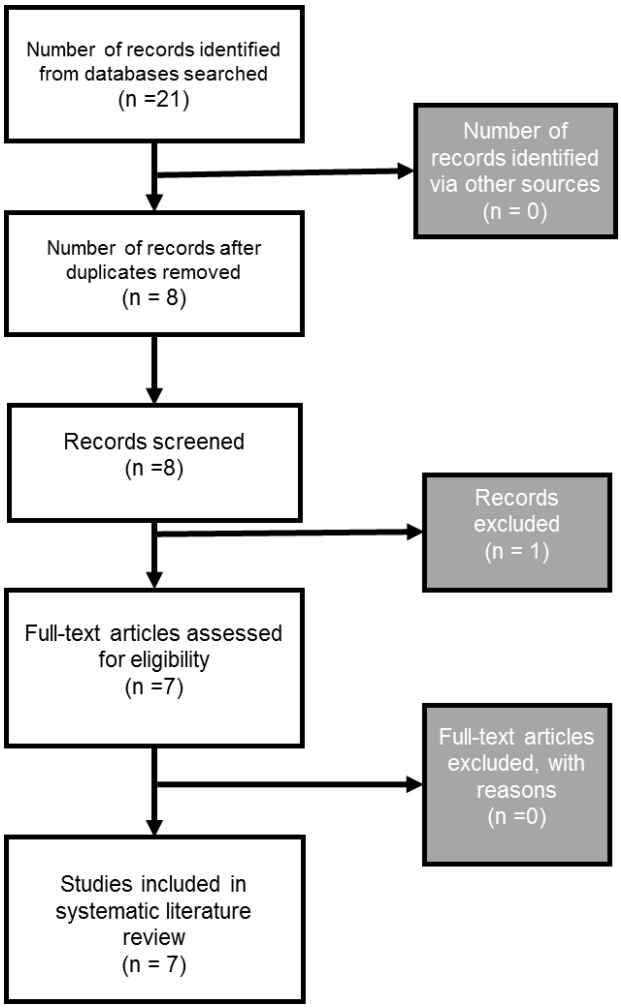
Supplementary Information

PRISMA workflow

This review was conducted in accordance to the PRISMA guidelines (338). The search terms used to identify relevant studies is review used the search terms “Irritable Bowel Syndrome” OR “IBS” AND “vitamin D” with no date limits. The databases searched were PubMed, Ovid and Web of Science (core collection) in August 2017. The clinicaltrials.gov was also searched for any current studies taking place whence further data could be extracted. Two reviewers (CEW and BMC) conducted the search independently and one reviewer (CEW) collated the data at The University of Sheffield. Studies included were of original data on the association of vitamin D and IBS and were limited to studies in humans. Only peer-reviewed studies were included in this review.



Chapter 5

Rate of change in circulating 25-hydroxyvitamin D following sublingual and capsular vitamin D preparations.

This chapter is in the form of a publication. This efficacy study was conducted in my first year of study and the results were published in the European Journal of Clinical Nutrition in 2019. The ethical approval, participant information sheet, and recruitment poster can be seen in Appendix 1-3. At the time of this publication, only two publications were available that had compared two different oral routes of delivering a vitamin D supplement (326, 343). With so few data available on the efficiency and acceptability of an oral spray to raise vitamin D levels, further research was warranted. The paper can be accessed here: https://doi.org/10.1038/s41430-019-0503-0 and has been presented in this chapter.

The rational for this research several fold. Firstly, the high prevalence of suboptimal levels of vitamin D in the general population merits resolving. This is of particular importance to people living at Northern latitudes and ethnic minorities (350). A recent randomised control trial (351) examined rates of deficiency and insufficiency across seven European countries; Germany, Spain, Greece, Poland, Netherlands, Ireland and UK in a sample size of 1075 participants. The rate of insufficiency (30-49.9 nmol/L) and deficiency (<30nmol/L) for the UK was 34% and 8.2% respectively (351). The UK was identified as having the highest prevalence of vitamin D insufficiency/deficiency within the European countries assessed, with the Netherlands having the lowest prevalence. It was further reported that females had a higher prevalence of deficiency/insufficiency of vitamin D compared to males (p=<0.001). Younger participants (18-35) compared to older participants (≥51 years) were also associated with a greater prevalence of vitamin D insufficiency and deficiency (p=0.003) (351). This shows the high prevalence of deficiency and insufficiency in the UK, especially for those who are between the age of 18-35 and female, which may need supplementation to correct. The current research in this chapter contributes to the current body of literature that an oral spray is an effective alternative to capsules. This may be advantageous for those with swallowing or malabsorption issues and have insufficient or deficient vitamin D status.

The UK SACN has recommended that a serum 25(OH)D concentration >25nmol/L is maintained throughout the year, leading to a RNI of 10µ/d from all sources, however this is difficult to achieve through diet alone and as a result supplementation of vitamin D is likely to be required (20). Despite

this, there is limited data comparing the different routes of vitamin D delivery, uptake rates and efficacy between preparations (326). Indeed, in total only four RCT studies have been published reporting the efficacy of a vitamin D sublingual spray (326, 341, 343, 344). Only one of these trials reported a superior absorption of a sublingual vitamin D oral spray compared to capsules in individuals with intestinal malabsorption syndrome and in healthy controls (343). This study was an open labelled, randomised, two periods, two-way cross over design. The authors compared absorption of vitamin D_3 in a soft gelatine capsule (1000IU/capsule) compared to a buccal spray (500IU/spray x2). The placebo was in the form of a soft gelatine capsule purchased over the counter. Thirty-eight participants aged 18-65 were randomised to receive the vitamin D_3 buccal spray, capsule or placebo for 30 days. The participants were based at 2 different hospitals in India; healthy controls at one and malabsorption patients at the other. Group allocation was the same for controls and malabsorption patients with n=7 (capsule), n=7 (spray) and n=6 (placebo) respectively. After a washout period of 30 days, those in the spray group moved to the capsule group (vice versa for capsule group) and placebo remained placebo. Two participants were lost due to compliance being less than 90% and were excluded from the final analysis. The study found a significant increase in serum 25(OH)D for both healthy and patient groups. The mean increase of 25(OH)D was 10.2nmol/L (capsules) and 20nmol/L (spray) for the healthy cohort. The patient group had a similar mean increase 10 nmol/L (capsules) 26.2 nmol/L (spray). The increase in serum 25(OH)D was noted to be higher in the individuals with malabsorption disease in comparison to the healthy group. The authors acknowledge the known inverse relationship between lower baseline 25(OH)D concentrations and uptake rate of vitamin D. This was also found in the study presented in this chapter.

It was concluded that the buccal vitamin D_3 buccal spray proved to be superior at raising 25(OH)D levels than the capsule in both the control group and individuals with intestinal malabsorption syndrome. In contrast, Todd et al. (2016), Penagini (2017) and Williams et al. (2019) found no difference in efficacy at raising vitamin D_3 levels between the capsule and oral spray preparations.

Since the time of the publication presented in this chapter, a further two studies (one systematic review and one RCT) have been published, both in 2020 (342, 352).

Zmitek et al. (2020) conducted a RCT with 105 participants aged 18-65 during wintertime in Slovenia with insufficient vitamin D levels (30-50nmol/L) (352). They investigated the efficiency of a vitamin D supplement in healthy adults and possible associations with physical activity, BMI and baseline status. Participants were randomised to one of four groups; vitamin D capsules, a vitamin D oil based oral spray, a vitamin D water-based spray or control group. The treatment arms received a

vitamin D_3 supplement of 1000IU/day for 2 months. This study reported a significant increase in serum 25(OH)D levels compared to the placebo group ($p<0.0001$ for each treatment vs control). There was no difference between modes of delivery (p not stated). It was also determined that the efficiency of the supplement was associated with a normal BMI <25 and those with prominent vitamin D insufficiency. This study cited our paper, which presented the details of my study design and concurred with our results that both modes of delivery were equally efficacious.

The systematic review had the focus of assessing recent RCTs to determine the efficacy of a buccal spray compared to other supplement formulations (342). Inclusion criteria were; human trials, any health status, any language and any age that evaluates the efficacy of a buccal vitamin D_3 spray to any other method of delivery. The included trials (n=4) were evaluated for their quality of study and risk of bias. The quality of the study was scored using the Jadad scale (353) and risk of bias using the Cochrane risk of bias (RoB 2.0) assessment tool (354). This review found it difficult to synthesise the results from the four trials. There was variability in the dose (800 -3000IU/day), duration of the study (30 days – 3 months), trial design (2 x cross over, 2 x parallel) and results. One out of the four reported the sublingual spray to be superior in raising 25(OH)D (343), while the remaining three found equal efficacy between methods of delivery.

My study scored highest for quality compared to the others; however, the study also scored high for risk of bias. It was also noted that the present study was the only one to report an adverse event and skin tone.

Criticisms of the study I conducted include; no flow diagram, not adhering to the original study duration, and the comparisons made between placebo and active groups were removed due to not meeting the authors “superiority” criteria for comparison.

The research discussed has created a body of evidence that shows a vitamin D_3 sublingual spray and capsule are equally efficacious at raising 25(OH)D levels. This finding was of importance for the proposed intervention study that is presented in chapter 5. It gives the study confidence that the sublingual spray is as effective as the capsule at raising 25(OH)D levels, but it is also an accepted mode of delivery for participants.

This study was conducted with an external industry partner BetterYou, which provided all the vitamin D supplements. In the exit interview, participants were asked if they preferred the tablet, oral spray or no preference. Two focus groups were conducted post-study with 12 participants in each session to determine if the oral spray was considered a good alternative to tablets and why, for the benefit of the industry partner. Focus groups were recorded with participants consent and

transcribed by administration staff in the department. The main themes that emerged were simply regarding preference between the two modes of delivery. As stated in the publication presented in this chapter, there was a preference for the oral spray compared to capsule. Reasons included; easy to take without water, nice taste, and travel friendly. We acknowledge that the oral spray is a convenient but expensive way to supplement individuals with vitamin D. Tablets purchased from a well-known pharmacy would cost £2.30 for 3-months' supply of 10ug vitamin D supplement. In contrast, the equivalent in spray format would cost over double at £6.95. As our findings show equal efficacy, we have not promoted this product as a superior alternative. This portion of the study was simply to explore these questions for the benefit of the industry stakeholder.

Statement of Authorship

I (CEW) and supervisors (BC/EAW) created the concept of the study. CEW conducted all aspects of the study, including; ethics approval, participant paper work, recruitment and participant interviews. CEW and VG (colleague), ran the focus groups post study. CEW collected the data and did the preliminary analysis, and interpretation. CEW wrote the primary draft of the manuscript. BC/EAW gave guidance through editing and reviewing all work produced by CEW. All authors agreed on the final manuscript for publication.

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BRIEF COMMUNICATION

Rate of change of circulating 25-hydroxyvitamin D following sublingual and capsular vitamin D preparations

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Abstract

Background Vitamin D is critical for skeletal health, and is increasingly associated with other pathologies encompassing gastrointestinal, immunological and psychological effects. A significant proportion of the population exhibits suboptimal levels of vitamin D, particularly in Northern latitudes in winter. Supplementation is advocated, but few data are available on achievable or typical rates of change. There has been considerable interest in the potential use of sublingual sprays for delivery of nutrient supplements, but data on efficacy remain sparse.

Methods A randomised, placebo-controlled, three-arm parallel design study was conducted in healthy volunteers (n = 75) to compare the rate of change of vitamin D status in response to vitamin D3 (3000 IU/day) supplementation in capsule and sublingual spray preparations over a 6-week period between January and April 2017. Blood 25(OH)D concentrations were measured after day 0, 3, 7, 14, 21 and 42 days of supplementation with 3000 IU per diem.

Results Baseline measurements show 25(OH)D deficiency (<30 nmol/l), insufficiency (31–46 nmol/l) and sufficiency (> 50 mmol/l) in 14.9, 44.6 and 40.5% of the participants, respectively. There was a significant elevation in blood concentrations of 25(OH)D in both of the treatment arms (capsule p = 0.003, spray p = 0.001) compared with control. The capsule and spray were equally efficacious. The rate of change ranged from 0.69 to 3.93 (capsule) and 0.64 to 3.34 (spray) nmol/L day with average change in blood 25(OH)D levels of 2 nmol/l/day. Rates followed a simple normal distribution in the study population (ks = 0.94 and 0.82 for capsule and spray, respectively). The data suggest that rates of change are higher in individuals with lower levels of 25(OH)D.

Conclusions A sublingual vitamin D spray is an effective mode of delivery for supplementation in a healthy population. The data provide reference values and ranges for the rate of change of 25(OH)D for nutrikinetic analyses.

Supplementary information The online version of this article (https://doi.org/10.1038/s41430-019-0503-0) contains supplementary material, which is available to authorized users.

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Introduction

Vitamin D is essential for the homoeostasis of calcium and phosphate, and well known for its role in the development and maintenance of bone health [1]. Once vitamin D has been ingested or synthesised via sunlight exposure, it requires activation in the liver to form 25-hydroxyvitamin D (25(OH)D) and in the kidney to form 1,25 dihydroxyvitamin D (1,25 $(OH)_2D$ [2]. 25(OH)D is the most abundant circulating form in the human body and is used to determine vitamin D status. 25(OH)D levels can be defined as; sufficient (≥50 nmol/L), insufficient (30 ≤ 5049 nmol/L) of deficient (<30 nmol/L) [3, 4]. There is limited research on rates of repletion; one paper reports amounts for maintenance of blood 25(OH)D at 50 nmol/L requires around 11 weeks of dosing at 1000 IU vitamin D per day [5]. Hypovitaminosis is evident worldwide, and is a major public

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health concern [6] leading to advocacy for supplementation in at-risk groups. Research has also shown African Americans may require a higher dose of vitamin D supplementation to reach optimal serum 25(OH)D concentrations compared with the Caucasian participants [7], perhaps as a result of lower baseline 25(OH)D levels in this population [8]. It is also known that serum 25(OH)D levels is inversely associated with body fat mass [9].

Supplementation has classically been with capsule preparations, but sublingual sprays are increasingly available. There are few data available on the relative efficacy of each type of preparation on rate of change in circulating levels. Dose response studies using capsular delivery of vitamin D supplementation [10–12] have shown evidence of efficiency in increasing serum 25(OH)D levels which plateau and begin to decrease.

This study aimed to measure and compare the rate of change of circulating vitamin D in response to capsular or sublingual delivery of a daily vitamin D supplement.

Methods

Study design

This was a 6-week double blind, placebo-controlled threearm parallel design study. The participants attended three visits at The Medical School of The University of Sheffield. The initial visit included anthropometrics, issue of first batch of blood test kits and completion of a first self-test blood sample. The second visit occurred ~2 weeks after the initial visit for issue of further test kits and to support participant retention in the trial. The final visit required participants to return their preparation bottles and answer five questions regarding the study.

Sample size and randomisation

There were no data upon which to base a power calculation. Seventy-five healthy male and female participants were recruited between January 2017 and February 2017, and were randomly assigned to one of three arms: (i) active capsules and placebo spray (n= 25); (ii) active spray and placebo capsules (n= 25); (iii) double placebo (n= 25). Participants were randomised according to a computer-generated random sequence using block randomisation with a block size of 9, with randomisation undertaken by an independent outside source. The allocation sequence was not available to any member of the team until databases had been completed and locked.

Participants

The University of Sheffield Research Ethics Committee granted ethical approval for this study (Ref: 011865). Participants were recruited via poster advertisements at the University of Sheffield and through a student volunteer email list. Inclusion criteria required participants to be fit and healthy, and aged between 18 and 50 years. Participants who reported any micronutrient supplement use (vitamin D, multivitamin, fish oils), recent or upcoming sunny holiday, pregnant or lactating, history of gastrointestinal disease, BMI > 30, diabetes, >50 years of age were excluded. A total of 124 potential participants were approached, of which 49 were excluded: 28 did not meet inclusion criteria and 21 had no further contact after initial consultation.

Participant measures

The concentration of 25(OH)D in the blood was assessed by blood sample using a finger-prick blood spot kits at 0, 3, 7, 14, 21 and 42 days of supplementation. Blood spots were analysed by liquid chromatography tandem mass spectrometry (Waters TQD and Acquity UPLC) for total blood 25(OH)D (25(OH)D_2 and 25(OH)D_3). LC-MS was undertaken by City Assays, Department of Pathology, Birmingham Sandwell Hospitals NHS Trust. Previous work has shown that this method is comparable with other commercial assays with intra and interassay coefficients of <10 and <11%, respectively [13–15]. Anthropometric measurements included: height, weight, BMI and body fat percentage. Body fat and weight were measured using Tanita BC-543 [16]. Skin tone was assessed by the researcher using 1 = Caucasian, 2 = Asian, 3 = Black.

Qualitative opinion of capsules and sprays were assessed via exit questionnaire. Participants were asked if they had a preference between preparations

> "Did you have a preference between the two preparations? If so which one?"

Answers were categorised as; "yes, the spray", "yes, the capsule" and "no preference".

Intervention

The vitamin D_3 and corresponding placebos were manufactured by Cultech Ltd., Port Talbot, UK and provided by BetterYou Ltd, Barnsley, UK. Preparations of vitamin D_3 and corresponding placebos were provided as 15 ml sprays and capsule. Each capsule and spray contained

3000 IU (75 µg) of vitamin D_3 per dose. The content of the spray and the capsule from the manufacturer was prepared to 97.5 µg/dose in order to maintain shelf life and to guarantee dose. Volunteers were instructed to ingest one capsule per day with water, and one spray orally per day for 6 weeks. Compliance was measured by weighing the spray bottles and counting the remaining capsules at the end of the study. In total, 86% and 96.4% of participants reached 100% compliance with the spray and capsules, respectively.

Table 1 Demographic characteristics and mean serum vitamin D at baseline and exit

	Capsules	Placebo	Spray	All	P-value
Participants, n	25	25	25	75	
Female, n	14	10	15	39	0.326
Mean age (± SD)	22.9 (±4.82)	22.4 (±2.72)	21.7 (±3.05)	22.4 (±3.65)	0.504
BMI (kg/m^2)	23.6 (±2.95)	22.7 (±2.72)	23.8 (±2.59)	23.4 (±2.77)	0.294
Body fat (%)	23.4 (±7.75)	19.1 (±5.91)	23.7 (±7.65)	22.1 (±737)	0.043
Height (m)	171.3 ±7.54)	173.5 (±10.20)	170.0 (±8.35)	171.6 (±8.77)	0.357
Weight (kg)	69.6 (±10.71)	68.6 (±12.77)	69.0 (±11.32)	69.1 (±11.48)	0.958
Skin tone	22/2/1	24/0/1	25/0/0	71/2/2	0.268
Mean serum 25(OH)D, nmol/L (baseline)	50.7 (±19.73)	45.6 (±21.30)	54.9 (±27.84)	50.5 (±23.24)	0.381
Mean serum 25(OH)D, nmol/L (exit)	91.35 (±19.78)	55.62 (±34.40)	95.78 (±28.03)	81.13 (±33.02)	0.001

The data are presented in means ± SD. Baseline characteristics are given along with exit serum 25(OH)D. Significant values are p > 0.005. A one-way ANOVA was used to compare means at baseline and exit for serum 25(OH)D

Adverse events

Two participants reported that small blisters formed on cheek and tongue after the study began. One participant stopped using the preparations for the duration of the study. The second participant continued to use the preparations throughout the intervention.

Statistical analyses

The data on vitamin D status were held by a third-party until all other data entry was complete, spreadsheets were then merged and analysis was undertaken at a group level with blinding to group identity. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, V.23; IBM Corp.). Percentage change in 25(OH)D from baseline was determined by analysis of variance (ANOVA) with Boneferroni correction. Pearson's correlations for rate of change in 25(OH)D per day was performed. Change in 25(OH)D over six time points were analysed by repeated measures ANOVA (there was a high failure rate in assessments of 25(OH)D at day 42, leading to the exclusion of this time point's data from the main analysis). Comparisons between percentage change in 25(OH)D from baseline in deplete and replete participants were assessed by Mann–Whitney U Test. Two-tailed tests were used in all analyses with the significance value of <0.05.

Results

Baseline demographics are shown in Table 1, and a CONSORT is supplied in online (Supplementary Fig. 1). The three arms were similar in numbers, age, BMI, body fat, height, weight, skin tone, sex and baseline blood 25(OH)D concentrations. Baseline blood 25(OH)D concentration showed 59% of participants had insufficient/deficient vitamin D status (<50 nmol/L).

Intention-to-treat analysis was used to evaluate the five time points up to day 21. Kolmogorov–Smirnov test (ks) indicates that the rate of change of 25(OH)D for both treatment arms follow a normal distribution (p = 0.200). Raw data are available online (Supplementary Table 1). Blood 25(OH)D concentration analysed across the timecourse in all three trial arms by ANOVA showed a significant improvement in 25(OH)D status in those receiving vitamin D compared with placebo. Post hoc analyses revealed significant differences between each of the active treatments and the placebo (capsules p = 0.003, spray p = 0.001), but no difference between the active preparations at any time point

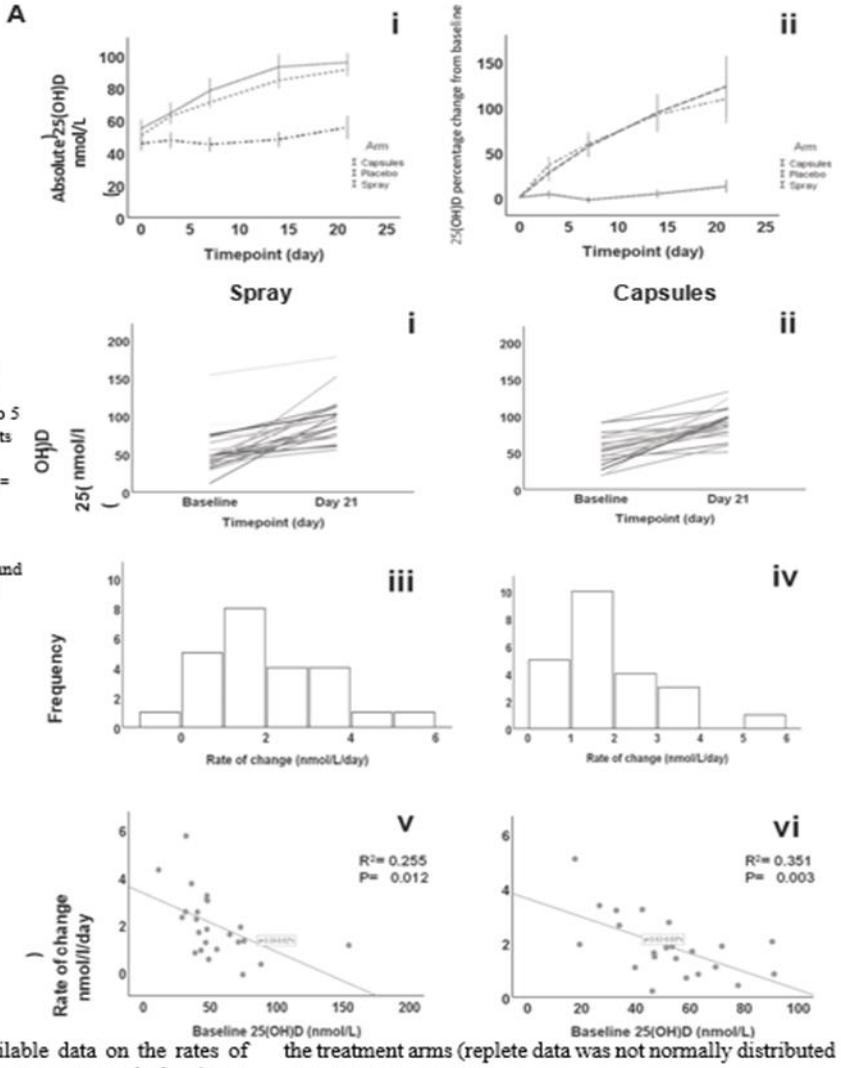

Fig. 1 Efficacy and rates of vitamin D uptake with differing delivery platforms. Panel a shows change in vitamin D circulating levels over time in each of the three study arms, presented as absolute levels (panel ai) or relative to baseline (panel aii). Panel b shows rates of uptake comparing spray (left column) with capsules (right column). Panels bi and bii show ladder plots for individuals in each arm of the trial plotting difference in vitamin D between day 0 and day 21 (the abscissa for uptake, based on panel a). Rates were derived as nmol/L/ day and binned into 5 nmol bins (panels biii and biv). KS tests showed the data were normally distributed (capsules p= 0.200, spray p= 0.200). Finally, the rates for each individual were correlated with the baseline serum concentration for that individual (panels bv and bvi). The r^2 and p-values for correlations are indicated

(Fig. 1a). As there are few available data on the rates of change of ingested vitamin D, we assessed the inter-individual and inter-preparation difference as change in whole blood nmol/L/day (Fig. 1bi, ii). Whilst there was a range of rates in each data set, assessment of the distribution of rate showed a monotonic normal distribution for both preparations with similar peak rates (Fig. 1biii, iv). Independent t test was performed, and found no significant difference between mean rates of change for capsule and spray. A Mann–Whitney U test was used to compare differences between deplete and replete participants within the treatment arms (replete data was not normally distributed with a KS score of $p = 0.001$). There was a significant difference ($p = 0.001$) in the percentage change of 25(OH)D between the replete and deplete from baseline to day 21.

In order to investigate a potential homoeostatic mechanism for 25(OH)D status, we investigated the relationship between 25(OH)D status and rate of change (Fig. 1bv, vi). We observed inverse relationships between baseline whole blood 25(OH)D and rates of change over 21

predisposing to development of excessive levels collectively identify a need for research on comparative efficacy of preparations and the saturability of uptake. This study used two commonly available vitamin D preparations: the widely used capsules and a more novel sublingual spray to investigate these factors.

Our findings show that a sublingual spray is equally effective at raising blood 25(OH)D concentrations with no significant difference between rate of change compared with capsules in this study population. The study participants reported a preference for the sublingual spray, and this study demonstrates that this delivery platform is of comparable efficacy. Sublingual sprays may be particularly advantageous in people with pre-existing malabsorption conditions or swallowing problems. Our analysis shows for the first time the likely rate of change in 25(OH)D and the range of these rates, albeit in a relatively small, healthy sample. The monotonicity of our rate distribution suggests a limited spread of rates with no suggestions of outliers or subpopulations; however, the relatively homogenous profile of the study population, whilst an advantage for this pilot exploration, is a limitation in terms of the prediction of rates in other groups (older adults, different ethnicities). A recent review [17] does offer suggested optimal supplementation rates to achieve adequate serum 25(OH)D levels (75 nmol/L) in regional, population and age-specific groups.

These data also suggest that baseline 25(OH)D status may influence the rate of change, as a correlation between baseline status and change exhibited a moderate inverse relationship, furthermore the circulating 25(OH)D concentrations started to level off towards the end of the intervention. This is in agreement with previous research by Lips et al., who reported that change in serum 25OHD in response to 6 months vitamin D supplementation was dependent on baseline vitamin D status, with the greatest change observed in people with the lowest baseline vitamin D [18]. Our research complements the previous work by undertaking an intervention over a shorter timeframe with sampling along the timecourse, demonstrating a baseline status-dependent response to the intervention and the possibility of a plateau effect. The mechanistic basis of this is unclear, and it is notable that both delivery platforms exhibit this effect, implying control in both enteric and transbuccal absorption. Future work may address the strength of this inferred relationship more thoroughly and identify implied control mechanisms. This study had no data from which a power calculation could be determined, however, the data presented herein may prove useful for the design of prospective intervention studies.

A limitation to this study is that we cannot show definitive absorption of the sublingual supplement. However, sublingual routes of drug delivery are established in pharmacokinetic studies [19, 20]. Recent research presented by Satia et al. found superior sublingual absorption compared with capsules in patients with malabsorption issues [21]. Participants were given clear guidelines on how to use the spray. Further studies should assess 25(OH)D and 1,25(OH)D levels in localised tissues with the use of labelled D3.

Conclusions

In summary, we have shown the capsule and sublingual spray are equally effective at delivery of a vitamin D supplement. There was an overwhelming preference (64%) for the spray over capsules for mode of supplement delivery. Rate of change, reported for the first time, exhibits a monotonic distribution in this population. This study saw a reduction in 25(OH)D levels as blood 25(OH)D concentrations increased over 21 days in both preparations. This suggests the oral spray has the same known mechanism as the capsule for slower conversions of vitamin D_3 when concentrations are higher [22]. These data illustrate the need for further studies to explore rate of change across mixed population groups, especially those identified as high risk.

Funding This work was jointly supported by BetterYou Ltd and The University of Sheffield.

Compliance with ethical standards

Conflict of interest BetterYou co-funded this PhD and provided the supplements and placebos. This sponsor was not involved in the study design, delivery or interpretation of the data, which was undertaken entirely by The University of Sheffield. The authors declare that they have no conflict of interest.

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Supplementary Information

Flow diagram of participant recruitment and allocation

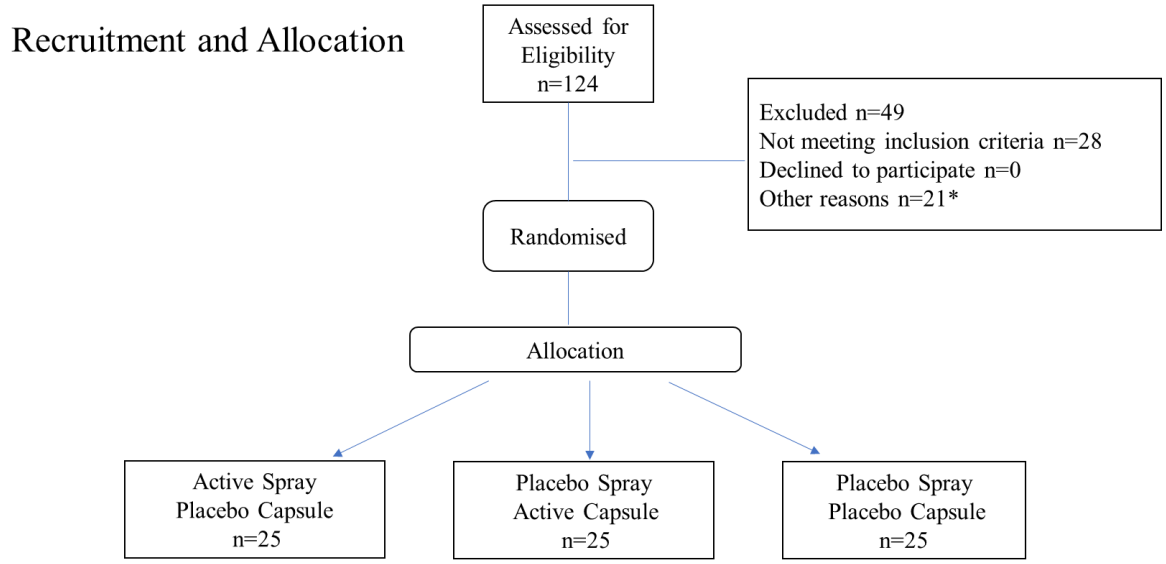

Figure 4: Flow diagram of study recruitment and allocation.

*These include; no further contact and missed/cancelled appointments.
Compliance for blood test kits at day 21; capsules and placebo 97.3%, and spray 98.6%.

Chapter 6

Effect of vitamin D supplementation on irritable bowel syndrome symptom severity and quality of life

This chapter is presented in manuscript form. As the primary author, I wrote this draft of the manuscript, contributed to design of the study, undertook recruitment and collection of the data and the data analysis. The ethical approval letter, participant information form, recruitment poster, and questionnaires can be seen in **Appendix 4-6.**

Another version of this manuscript has been written based on the one presented in this chapter and has been published in the European Journal of Nutrition. The publication has been attached to this chapter.

The published version of this research has now been meta-analysed in 2 different reviews (345, 346). This is a strength of the RCT presented in this chapter. The conclusions, pooled by both meta-analyses suggest; i) vitamin D supplementation is an additional therapeutic resource for bone health ii) effect of vitamin D supplementation on IBS symptom severity and quality of life remains unclear and iii) larger, first rate RCTs are needed to establish dosing regimen, effect of vitamin D supplementation in the long-term.

Effect of vitamin D supplementation on irritable bowel syndrome symptom severity and quality of life

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Short title: IBS and vitamin D supplementation

Keywords: vitamin D, Irritable Bowel Syndrome,

ABSTRACT

Background: Irritable Bowel Syndrome (IBS) is a common functional disorder of the gastrointestinal tract, affecting 17% overall of the UK population. The aetiology of this disorder is unknown, although it has been linked to environmental, psychological and social factors. Vitamin D deficiency and insufficiency is common within the IBS population, and vitamin D has been hypothesized as a potential remedy. We sought to test whether vitamin D supplementation improved symptoms or quality of life in IBS.

Methods: One hundred and thirty six volunteers were randomised to receive either a vitamin D (3000IU *p.d.*) or placebo oral spray in a 12-week double-blind, placebo-controlled, parallel design study. A reduction of ≥50 points on the symptom severity scoring system (IBS-SSS) was the primary endpoint of the study. Secondary endpoints included improvement of Quality of Life (QoL) and vitamin D status. QoL and serum vitamin D were determined at baseline and exit, symptom severity was assessed fortnightly across the study. Dietary intake of vitamin D was measured using the Food Frequency Questionnaire.

Results: One hundred and thirty-five participants (n=68, treatment, n=67, placebo) were included in the final analysis. Baseline demographics were similar between groups. After 12 weeks there was a significant improvement in the vitamin D status of participants randomised to receive the active vitamin D (p=0.005). Symptom severity was assessed across the study by trial arm: there was no difference between active and placebo (p=0.824). Quality of life showed no difference between baseline and exit for either trial arm (p=0.415). There was no association between increase in vitamin D and change in symptoms (r= -0.071, p=0.434), nor increase in vitamin D and change in quality of life (r=-0.031, p=0.733). There was a weak but significant correlation between baseline 25(OH)D concentrations and dietary intake of vitamin D (p=0.046, r=0.17).

Conclusions: Vitamin D insufficiency was prevalent in this sample confirming previous studies. Supplementation was efficacious. Patients with IBS should be tested for vitamin D status and, where appropriate, supplemented. In contrast to previous reports, this study shows no benefit of vitamin D supplementation on IBS symptomology. Dietary intake of vitamin Dare reflective of the general population and not clinically significant to individuals who have IBS.

Trial registration number: ISRCTN 13277340

What is already known about this subject?

- Irritable Bowel Syndrome (IBS) is a common functional disorder of the gastrointestinal tract, affecting 17% overall of the UK population.
- Vitamin D insufficiency is prevalent within this population.
- Vitamin D supplementation has been shown to have a positive associations with other gastrointestinal disease such as inflammatory bowel disease and colorectal cancer

What are the new findings?

- There is no relationship between vitamin D status and amelioration of symptoms or improvement of quality of life in this population.
- Vitamin D deficiency was prevalent at baseline in this population.

How might it impact on clinical practice in the foreseeable future?

- Patients with IBS should be tested for vitamin D status and, where appropriate, supplement for overall musculoskeletal health.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is a common functional disorder of the gastrointestinal tract, affecting 17% overall of the UK population (355). It is a chronic relapsing condition that can negatively impact on quality of life and in 2011-1012 had a burden of cost of over £11 000 000 to the NHS (356). Aetiology of this disorder is unknown, although research shows it may be related to environmental, psychological and social factors. IBS has been classified into 3 subtypes according to predominant symptoms; IBS-C (constipation predominant), IBS-D (diarrhoea predominant), and IBS-M (alternating between the two symptoms) (357). The heterogeneity of IBS symptoms makes diagnosis often difficult. Diagnosis is based on the Rome criteria, which assesses cumulative severity of symptoms such as bloating, abdominal pain, and bowel habit (358). Treatments are limited to the relief of the symptoms (359) and include anti-depressants, loperamide (diarrhoea) and laxatives.

Vitamin D has been traditionally associated to bone health (360), and intakes and repletion levels are judged for optimal bone health (361). Defined ranges are: Deplete (<30 nmol/L), Insufficient (30-50 nmol/L), replete (>50nmol/L), Toxic (>125nmol/L) (362). There is emerging evidence that vitamin D plays a role in non-skeletal conditions (363). Research has presented evidence of the potential beneficial effects of vitamin D supplementation on colorectal cancer (364) and cardiovascular disease (365), further showing an anti-inflammatory and immunomodulatory response.

The active form of vitamin D (calcitriol) which binds to and activates the vitamin D receptor (VDR) is highly expressed in intestinal epithelial cells (366) and may have an essential role in the maintenance and protection against inflammation of the mucosal barrier (367, 368). This suggests that vitamin D may play a role in symptom severity in individuals with IBS who are also vitamin D deficient and if inflammation is the cause. Indeed, this proposes that the lack of the biologically active prohormone ($1,25\ (OH)_2D_3$) in circulation may result in increased inflammation in the large intestine as seen in research associated with Inflammatory Bowel Disease (IBD) (122).

Vitamin D deficiency and insufficiency is common within the IBS population (120, 121), although it is unclear whether this reflects a cause or effect of the symptoms, or indeed whether the IBS population is different to the general population which is also widely insufficient at some times of year (369). A case study report suggested symptomatic relief with vitamin D supplementation (247). The benefit of vitamin D in IBS has been tested in three small trials. Three studies reported a symptomatic benefit, using a high dose (50,000IU) either weekly or fortnightly in clinically selected populations in Iran and Egypt (251, 370). A smaller study primarily focusing on molecular endpoints reported no benefit on symptoms (371). A pilot study using a recommendable dosing regime (250) found a potential benefit on quality of life, and was used to inform the power calculation for this

study. Herein we sought to test the potential benefit of a recommendable vitamin D dosing regimen on IBS symptoms using an adequately powered trial.

METHODS

Study design & measures

This was a 12-week double-blind, placebo-controlled, 2-arm parallel design study. The participants attended two visits at The Medical School of The University of Sheffield. 135 volunteers were randomised to receive either a vitamin D (3000IU) or placebo oral spray supplement each day for 12 weeks. The initial visit included anthropometrics, baseline fingerprick blood sample, and completion of 2 questionnaires (QoL, and IBS-SSS). Participants were given a further 5 IBS-SSS questionnaires to complete and post back fortnightly. The final visit occurred approximately 12 weeks after the initial visit. Participants gave an exit fingerprick blood sample to measure final 25(OH)D concentrations. A final IBS-SSS and IBS-QoL questionnaire was also completed at exit interview. Food frequency questionnaires were given in the initial interview with a prepaid envelope to post back upon completion. The data from the FFQ questionnaires were inputted and analysed by the FETA software which calculated dietary intake. In order to measure treatment compliance, participants were asked to return their used preparation bottles to be weighed and compared with a full unused bottle.

Participants

The University of Sheffield Research Ethics Committee granted ethical approval for this study (Ref: 016753). Recruitment occurred during two rounds of recruitment in the local area in winter (January-April) 2018 and 2019. Participants were recruited via poster advertisements at the University of Sheffield and through a student volunteer email list. All participants had a clinical diagnosis of IBS, met the Rome III or IV criteria, and obtained a severity score of 150 on SSS scale. According to Francis, Morris and Whorwell (1996), the developers of the IBS-SSS questionnaire define IBS severity as; <75 as control or in disease remission, 75-175 mild, 175 – 300 as moderate and >300 as severe (293). Participants were recruited from a free-living setting and as such may not have as severe symptoms as those from a clinical setting and therefore the cut off for >150 was used.

Participants who were pregnant or lactating, regular users of nutritional supplements, had a BMI >30, any history of gastrointestinal disorders (Crohn's Disease, Ulcerative Colitis, and diverticulitis) and diabetes mellitus were excluded. Participant enrolment and randomisation is shown in **Figure 4**.

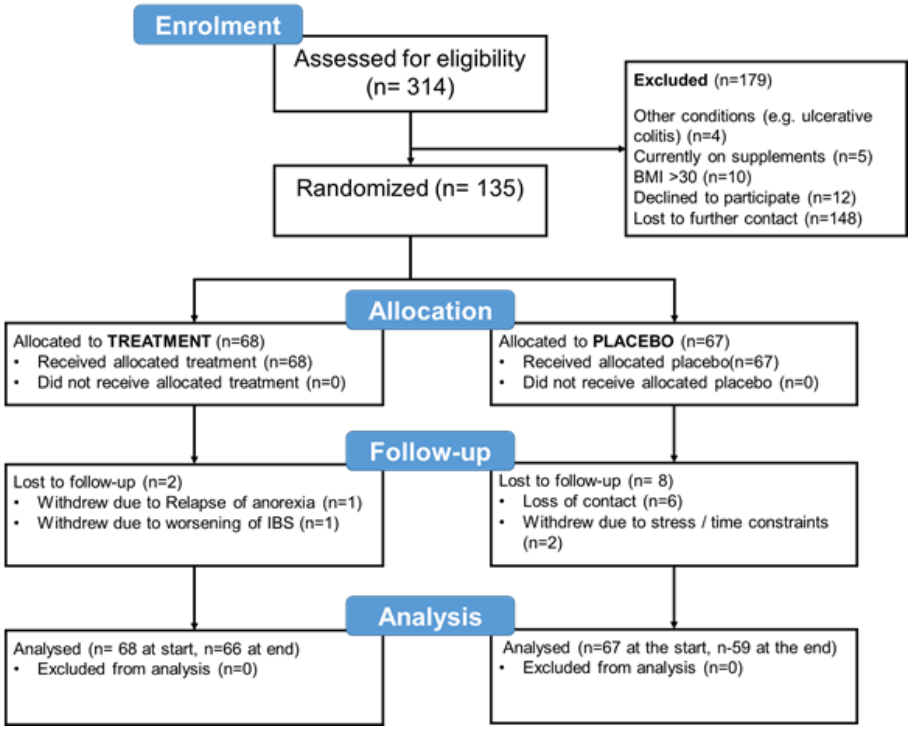

Figure 5: Flow diagram of participant enrolment

Participant measures

Participants had height and weight taken at baseline to determine BMI score. To measure serum 25(OH)D, participants provided a blood sample at baseline and exit. IBS symptom severity was assessed at baseline and exit, and bi-weekly during the study (293). A reduction of ≥50 points on the IBS-SSS scale was assessed as the primary endpoint. An IBS-specific quality of life questionnaire (313) was used at baseline and exit. The 34 questions assesses 8 subscales; dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual and relationships. Food frequency questionnaires were completed to evaluate dietary intake of vitamin D.

Sample size and randomisation

Based on power calculations determined by our previous pilot study (250) we aimed to recruit 160 participants. Randomisation was computer generated in blocks of 8 by a third party. Researchers and study participants were unaware of the allocation sequence until completion of databases.

Intervention

The vitamin D_3 oral spray and equivalent placebos were provided by BetterYou Ltd, Barnsley, UK. Volunteers received a vitamin D_3 oral spray or placebo equivalent oral spray for 12 weeks at a dose of 3000IU (75ug). These were provided as 15mL liquid, 100 dose spray bottle. Participants were asked to consume one oral spray daily for the duration of the study. Compliance was measured by weighing the spray bottles at the exit and compared to a full spray bottle. 75% compliance was achieved.

Biochemical assay

Serum 25(OH)D was analysed by liquid chromatography tandem mass spectrometry (Waters TQD and Acquity UPLC) for total blood 25(OH)D (25(OH)D_2 and 25(OH)D_3). LC-MS was undertaken by City Assays, Department of Pathology, Birmingham Sandwell Hospitals NHS Trust. Previous work has shown that this method is comparable to other commercial assays with intra and interassay coefficients of <10% and <11% respectively. (264, 372)

Statistical analysis

Baseline characteristics and exit serum levels are summarised and independent samples t-test and χ^2 were used to evaluate significant differences between the active treatment and placebo groups. The between group difference to assess treatment effect of this 12-week intervention, with at least a 50-point reduction on the IBS-SSS, from baseline to exit a χ^2 was calculated. Spearman's correlations were calculated for; (1) change in total symptom severity score and change in serum 25(OH)D, (2) change in quality of life score and change in serum 25(OH)D. Repeated measures ANOVA with Bonferroni correction was used to determine associations between change in each symptom severity score between groups across the 7 time points. Dietary intake of vitamin D was assessed using scatterplot with line of best fit. All analyses were two sided with a significance value set at <0.05 unless otherwise stated. Analysis was performed using SPSS V 25.0 (IBM, Armonk, New York, USA).

RESULTS

Recruitment and participant demographics

135 participants were enrolled to this randomised, double-blind, placebo-controlled intervention study during the winter months (January to April 2018/2019). Baseline characteristics and demographics of participants are similar in both groups (**table 6**). Randomisation, allocation and retention of participants are shown in **Figure 3**. 68 were allocated to the treatment group; 67 to placebo. Rate of withdrawal (placebo 12%, treatment 3%) was not different between groups (p=0.207). Reasons for discontinuation of the study included: worsening of current condition, voluntary withdrawal, and personal time constraints. Intention-to-treat analysis was used for this data.

Table 5: Baseline demographics of study participants.

	All	Placebo	Treatment	P
Participants n	135	67	68	
Females n (%)	106 (78.5%)	51 (76.1%)	55 (80.9%)	0.5 [a]
Age yr	30.01 (±10.46)	31.10 (±10.85)	28.94 (±10.03)	0.231 [b]
BMI kg/m2	23.37 (±2.88)	23.58 (±3.00)	23.15 (±2.76)	0.390 [b]
IBS-SSS	277.41 (±65.15)	273.22 (±69.01)	281.54 (±61.34)	0.460 [b]
IBS-QoL %	42.72 (±18.17)	43.35 (±19.24)	42.54 (19.45)	0.809 [b]
Blood 25(0H)D nmol/L (baseline)	49.23 (±27.38)	49.71 (±27.05)	48.75 (±27.91)	0.839 [b]
% with blood 25(OH)D <50mmol/l	60	61.2	58.8	0.779 [a]
% with blood 25(OH)D <25mmol/l	20.7	14.9	26.5	0.098 [a]
Dietary vitamin D intake µg/d (Baseline)	3.09 (2.379)	3.21 (2.383)	2.96 (2.389)	0.565 [b]

Data are numbers (%) and means (± SD) for the whole sample and by arm. No differences between arms and factors, a: analysed by chi-squared test and b: analysed by t-test.

Effect of vitamin D supplementation and status on IBS symptoms

Vitamin D deficiency/insufficiency was prevalent in participants at baseline with 60% of the cohort having serum 25(OH)D levels <50nmol/L. An increase in serum 25(OH)D from baseline to exit in the treatment arm compared to placebo was significant (p=0.005). Mean baseline serum 25(OH)D for the treatment arm was 48.75 (±27.91) nmol/L which increased to 94.29 (±33.70) nmol/L at exit. This demonstrates the vitamin D sublingual spray was effective at raising serum 25(OH)D levels.

Symptom severity was assessed biweekly (total of 7) by trial arm. There was no difference in mean symptom severity between active and placebo groups (p=0.824) over the 7 time points shown in **Figure 4.** **Figure 5** demonstrates total symptom severity score as a percent from baseline across the duration of the study. As presented, no significant difference was discovered between arms (p=0.872). The ANOVA analyses evaluating each symptom at all the time points (**Figure 4**) showed

no difference between active and placebo. **Figure 12** illustrates no association between increase in vitamin D and change in symptoms **Figure 5** (r= -0.071, p=0.434). Treatment group was not different to placebo when each symptom was assessed. Thus, demonstrating vitamin D supplementation does not relieve any symptoms.

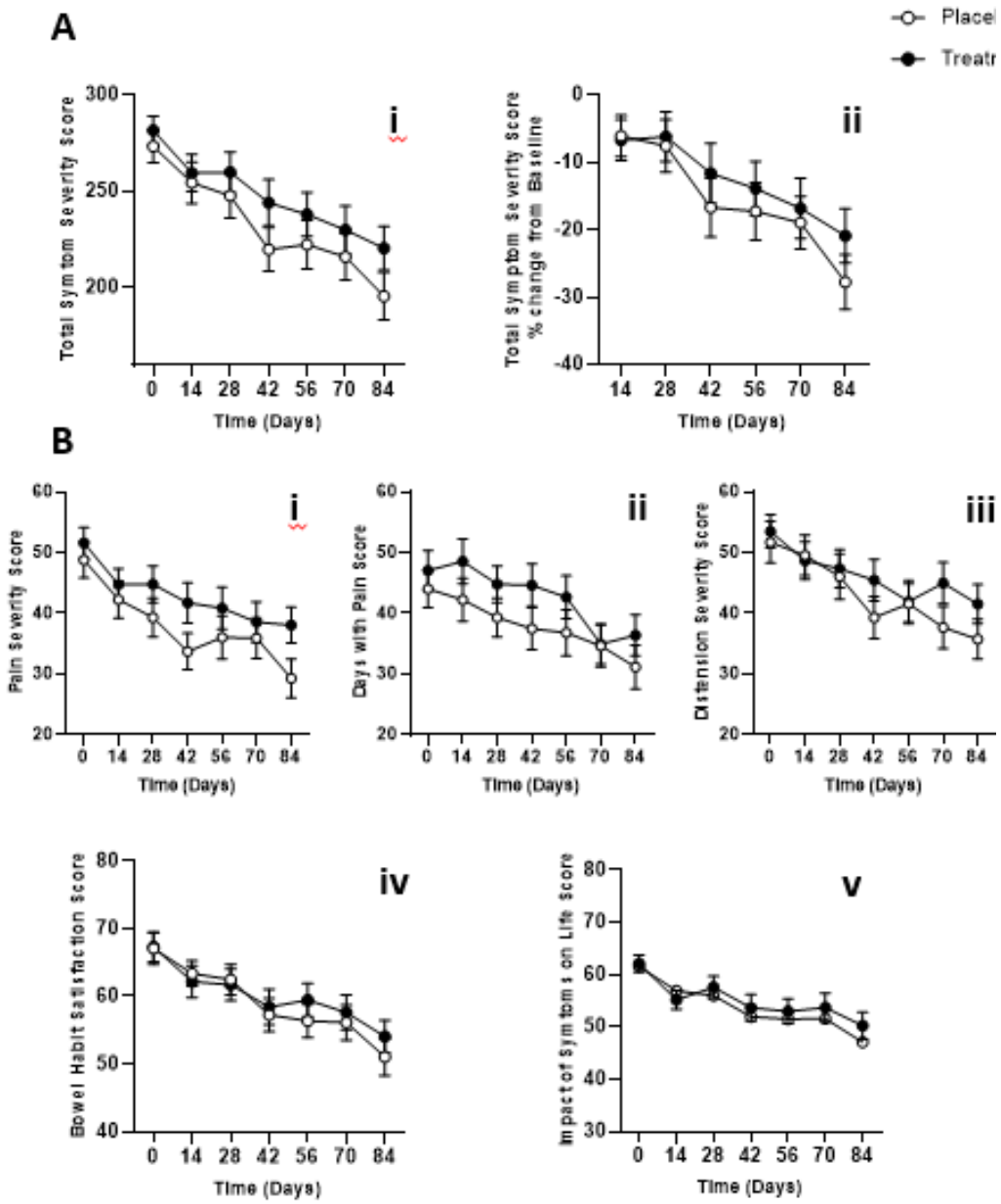

Figure 6: Effect of Vitamin D supplementation on IBS symptoms

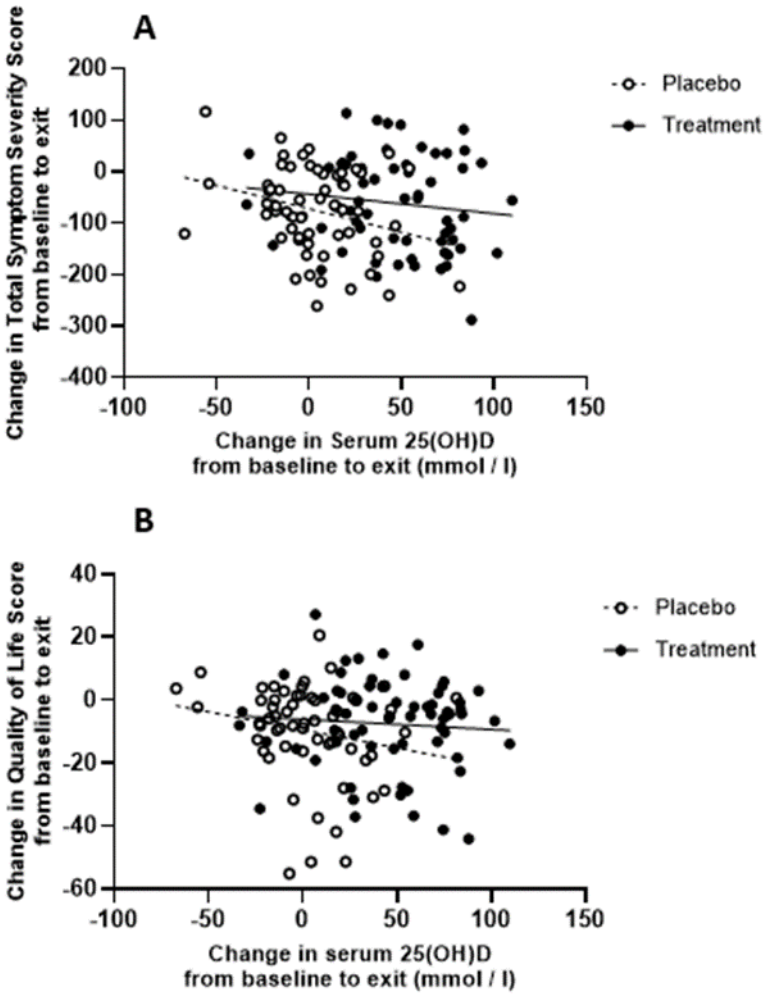

Figure 7: Effect of change in vitamin D status on IBS symptoms and quality of life

The effect of change in circulating levels of vitamin D was assessed for both outcome measures (TSS and QoL). Panel A shows correlation between change in circulating vitamin D from start to end of the trial against change in IBS symptoms. Panel B shows correlation between change in circulating vitamin D from start to end of trial against change in quality of life. There was no relationship between either endpoint and the vitamin D status change.

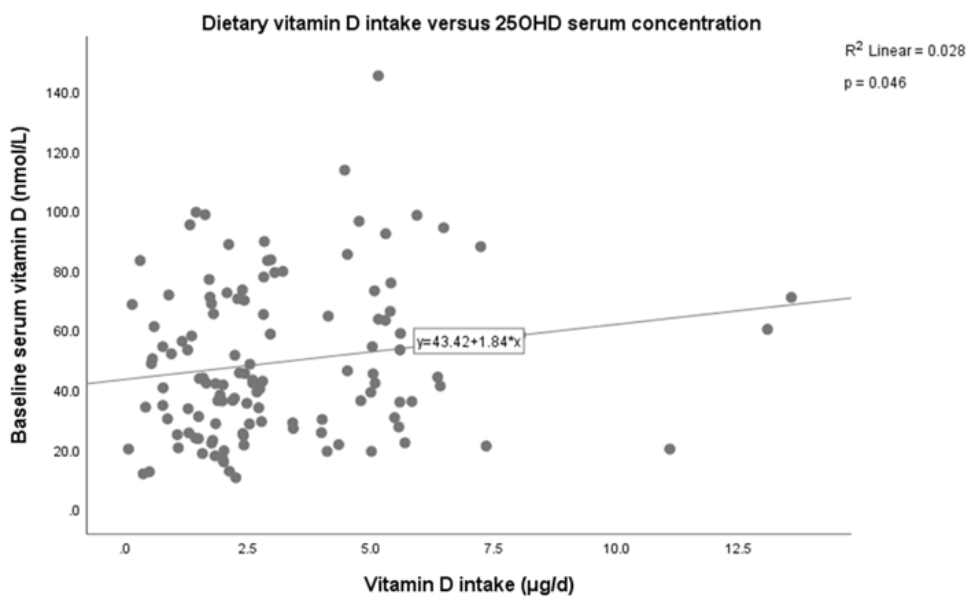

Figure 8: Scatterplot with line of best fit between baseline serum 25(OH)D concentrations and dietary intake of vitamin D.

Effect of vitamin D supplementation and status on quality of life

We assessed whether a 3000IU/day vitamin D supplement could improve quality of life using an IBS-specific QoL questionnaire (312) shows no correlation between the change in QoL at baseline and exit compared to vitamin D status from baseline to exit (r= -0.031, p=0.73). QoL showed no difference between baseline and exit for either trial arm (p=0.415) (**Figure 11**).

Dietary intake of Vitamin D

Dietary intake of vitamin D was analysed using food frequency questionnaires to determine a possible correlation between baseline 25(OH)D concentrations and dietary intake (373). A total of 115 returned questionnaires, 114 were used for the analysis, as 1 participant did not have a specific subtype. We assessed dietary vitamin D intake and serum 25OHD concentration using a scatterplot

with a line of best fit, establishing an incline that mirrored a dose-response relationship with gradual increases of 1.84nmol/L (0.74ng/ml) per g of dietary vitamin D. This analysis found a significant but weak correlation (p=0.046, r=0.17) (see figure 7).

Further exploratory analysis

Exploratory analysis was conducted to evaluate whether there were changes in response by IBS subtype to vitamin D. There was no change in symptom severity (SSS: p = 0.719, 0.962, 0.697 constipation, diarrhoea and mixed symptoms, respectively, Repeated measures ANOVA) or change in Quality of life (QoL p = 0.316. 0.946, 0.090 constipation, diarrhoea and mixed symptoms, respectively, Mann–Whitney U test) in response to vitamin D supplementation within any of the IBS subtype groups.

Adverse Events

One participant reported worsening of their current condition and withdrew from the study.

DISCUSSION

Our main finding is that providing a 3000IU/day vitamin D supplement for 12 weeks to participants with IBS did not reduce symptom severity nor improve quality of life. We found no relationship between vitamin D status and individual symptoms at any time point for the duration of the study.

Our findings are in contrast to the published trials that have shown a benefit of supplementation of vitamin D on IBS symptoms. Collectively, these studies were conducted in the Middle East and recruited from clinical populations from endoscopy and gastroenterology outpatient clinics (114, 115, 251-253).

Jalili and colleagues (115) used 50,000IU capsule biweekly with 25 participants per arm and report a significant decrease in symptom severity (P<0.05). Abbasnezhad et al. (2016) (251) conducted a RCT with 45 participants per arm with IBS. This study also found a significant improvement of IBS symptoms (P< 0.001) and quality of life (P<0.001) following a 50,000IU/biweekly vitamin D_3 (capsule) supplement compared to a placebo over a period of 6 months. Another study with a significant response to vitamin D supplementation was reported in research with a paediatric cohort. El Amrousy et al. recruited 56 paediatric outpatients per arm for a duration of 21 weeks. A significant improvement was shown for total score (*P*<0.02), IBS-QoL (P<0.001) and IBS-SSS (*P* <0.001). Jalili et al. conducted a second intervention study with a high bolus dose of 50,000IU/week, with 58 patients each arm for 6 weeks that again found a significant improvement in symptom severity and quality of life (P< 0.05) (114). Recently, Sikaroudi et al. randomised patients with IBS-D to receive Mebeverine 135mg (twice a day) and either 50,000IU/week (n=39) vitamin D supplement or placebo (n=35) for 9 weeks. The results from this study reported significant improvement in the vitamin D group for symptoms severity and quality of life (<0.001 and <0.049 respectively). However, the authors do not offer any commentary on the possible impact or effect of the Mebeverine on symptom severity or quality of life.

This study required a baseline symptom severity score of <150. Although considered within the mild range by its creators (293), studies that have used IBS clinical populations have no inclusion criteria surrounding a cut off for symptom severity from which to compare to the present study. It is evidenced in the research that reducing the severity score by 50 points is seen as a clinically significant improvement which is the main endpoint this study used (312). The mean symptom severity score in presented in

Table 5, show the mean baseline IBS-SSS was 277.41 (±65.15) considerably higher than the cut off of <150.

Compared to our study, this small collection of research from the Middle East used high doses of vitamin D (e.g. 50,000/week) which would not be advisable for individuals with IBS living in the general population. These studies have also stated high levels of compliance, very good retention of participants, however, no reporting of a placebo effect. Although, the current study had a low drop-out rate and sufficient compliance (75%) we did observe a strong placebo effect which appears to be common in research with this IBS (75, 237, 239). It is difficult to generalise the results from these clinical setting studies to the wider IBS population that are free living. Further real world studies are needed to determine the effect of vitamin D supplementation in individuals with IBS.

We explored dietary intake of vitamin D to establish whether a correlation between dietary vitamin D and baseline serum 25(OH)D concentrations. Using data from self-reported food frequency questionnaires (FFQ), the data from the FFQs was analysed by the FETA software to provide the nutrient and food intake of the participants (281). Food frequency questionnaires are a validated tool for the assessment dietary intake (284) . Research has shown this method of dietary analysis is competent at comparing against a biomarker (373). This study found a weak but significant correlation ($r=0.17$, $p=0.046$) between baseline vitamin D status and dietary intake. These findings are reflective of the general population and therefore, not clinically significant to individuals who have IBS. Research shows in a healthy UK population, individuals aged between 20-40 years have a daily intake of 3.6 µg of vitamin D similar to the individuals in this study who had an intake of 3.1 µg/day (374). The availability of vitamin D in the diet is negligible and therefore low dietary intake is common in UK adults with or without IBS (22, 375, 376).

A strength of the present study is the use of a safe and conservative dose of vitamin D. We used a commercially available vitamin D (3000IU/day) sublingual oral spray to the treatment arm of this study. This is 1000IU under the upper tolerable limit (362). Recruitment was in winter months to ensure low vitamin D status to minimise any possible risk of toxicity. We also used a length of study to reduce placebo effect (377). Lastly, this study was based on a formal pilot study using similar endpoints and type of population. The mean intake of participants was 3.1µg compared to the SACN recommended 10µg (20).

The main limitations of the current study include the heterogeneity of the sample and a large placebo response. However, these shortcomings are present in research with IBS populations. In our

study, QoL scores ranged from 8.82-97 (baseline) and 5.15-88.97 (exit) and for IBS-SSS total score ranged from 155-420 (baseline) and 0-460 (exit) showing a highly varied response rate. Future research should focus on reducing the placebo response, which may benefit the results from larger sized studies. Self-reported improvement from high dose vitamin D_3 supplementation has been evidenced through a systematic analysis from self-reported experiences on social media sites (247). This data has identified a subset of individuals living with IBS that have responded to supplementation, albeit high doses that range from 5000–10 000 IU/day. Further RCTS using high daily dose vitamin D_3 supplementation in this population are needed.

We asked for a diagnosis of IBS and as such, all Rome and any other criteria was included. This would also have contributed to the population sample being more diverse in their symptom severity and quality of life scores. It may have been beneficial to have had a specific subtype of IBS to allow for improved homogeneity.

In conclusion, this is the largest vitamin D and IBS intervention trial to date. We have found no association between vitamin D supplementation and reduction in symptom severity or improvement in quality life in free-living individuals with IBS. Patients with IBS should routinely have serum 25(OH)D levels checked if only for musculoskeletal benefits.

ACKNOWLEDGEMENTS

FINANCIAL SUPPORT

This work was jointly supported by BetterYou Ltd and The University of Sheffield.

CONFLICT OF INTEREST

BetterYou co-funded this PhD and provided the supplements and placebos. This sponsor was not involved in the study design, delivery or interpretation of the data, which was undertaken entirely by The University of Sheffield.

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ORIGINAL CONTRIBUTION

Vitamin D supplementation in people with IBS has no effect on symptom severity and quality of life: results of a randomised controlled trial

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Abstract

Purpose Several small trials suggest a benefit of vitamin D supplementation in irritable bowel syndrome (IBS). The generalisability of these reports is limited by their design and scale. This study aimed to assess whether vitamin D supplementation improved IBS symptoms in a UK community setting.

Methods This was a randomised, double-blind, placebo-controlled study. Participants were recruited from the community in winter months between December 2017 and March 2019. 135 participants received either vitamin D (3,000 IU *p.d.*) or placebo for 12 weeks. The primary outcome measure was change in IBS symptom severity; secondary outcomes included change in IBS-related quality of life.

Results The participants were analysed on an intent-to-treat basis. 60% of participants were vitamin D deficient or insufficient at baseline. Although vitamin D levels increased in the intervention arm relative to placebo (45.1 ± 32.88 nmol/L *vs* 3.1 ± 26.15 nmol/L; $p < 0.001$). There was no difference in the change of IBS symptom severity between the active and placebo trial arms (− 62.5 ± 91.57 *vs* − 75.2 ± 84.35, $p = 0.426$) over time. Similarly there was no difference between trial arms in the change in quality of life (− 7.7 ± 25.36 *vs* − 11.31 ± 25.02, $p = 0.427$).

Conclusions There is no case for advocating use of vitamin D in the management of IBS symptoms. The prevalence of vitamin D insufficiency suggests routine screening and supplementation should be implemented in this population for general health reasons.

This trial was retrospectively registered with ISRCTN (ISRCTN13277340) on 24th April 2018 after recruiting had been initiated.

Keywords Irritable bowel syndrome · Vitamin D · Vitamin D deficiency · Symptom management · Quality of life

Introduction

Irritable Bowel Syndrome (IBS) is a highly prevalent functional bowel disorder, with estimates of numbers affected in westernised populations ranging widely, but often in the region of 10–15%[1], although this estimate has been revised to under 5% [2] with the introduction of revised ROME IV criteria for assessment [3]. It is characterised by chronically relapsing perturbed bowel habit, associated pain and sensitivity, and dissatisfaction with bowel movements[4]. Symptoms may be severe and significantly impact both social function and work, with predicted cost to the NHS in excess of £11 M *p.a*[5] and estimates of direct healthcare costs from £45-200 M in the UK [6], indirect costs are likely to be higher when the impacts of the condition on work are considered [*ibid.*]. The aetiology of IBS is not well-understood:

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infection, stress, dietary factors, impaired gut-brain signalling are all implicated, but none conclusively[7]. As a result, treatment is limited to symptom management. Pharmaceutical approaches include anti-spasmodic and anti-depressive drugs. Whole dietary approaches to symptom management include low-FODMAP diets and other exclusion-led approaches[8]. There is also interest in supplementation strategies, including probiotics, prebiotics[9] and recently glutamine supplementation[10]. What is unequivocal is that in all trials and approaches there is a heterogeneity of response (Williams & Corfe; manuscript in preparation); for patients, trial and error lead to restrictive behaviours in a form of personalised dietary management[11] although nutritional intake seems generally adequate[12]. The nature and impact of symptoms, coupled to lack of a clear treatment path, have associated impacts on mental health and well-being[13].

Vitamin D is a prohormone produced by epidermal photoconversion of 7-hydroxycholesterol to vitamin D_3, followed by sequential hepatic, then renal, dihydroxylation to yield 25(OH) vitamin D then 1,25(OH) vitamin D[14]. The monohydroxylated form has a longer half-life and is usually used as a status marker. Low sunlight exposure through latitude, reduced mobility, or for cultural reasons is a risk factor for low vitamin D status[15]. Vitamin D is also obtained through diet and through supplementation. Low vitamin D status is a risk factor for poor bone health, with guidance on intake informed by reduced risk of fracture[16]. Nonetheless vitamin D is also implicated in non-skeletal pathologies[17]. From a gastroenterological perspective, the vitamin D receptor is strongly expressed in the colon[18]. Low vitamin D is a potential risk factor for colorectal carcinogenesis[19] and inflammatory bowel disease[20]. However, causal relationships between observed low vitamin D status in inflammatory conditions may be confounded by potential sequestration of the vitamin driven by inflammatory pathways[21].

Exploration of links between vitamin D status and IBS has arisen due to links between vitamin D and other colorectal pathobiologies. An untargeted analysis of mRNA from patients with IBS compared with controls suggested altered expression of serotonin update and metabolism pathways[22]. The same study showed reduced levels of TPH1 expression in IBS associated with vitamin D status, and went on to show with in vitro models that vitamin D treatment restored expression of EphA3 and CYP24A1 (vitamin D 24-hydroxylase) [22]. A case study[23] systematically collated patient reports of self-administration and suggested a potential benefit of vitamin D supplementation. Our review of vitamin D trials in management of IBS symptoms[24] noted that studies consistently reported prevalent vitamin D deficiency in participants with IBS, although there is inconsistency as to whether this is greater than in the general population (*ibid.*). Five RCTs have tested the effect of vitamin D in the management of IBS symptoms[25–29], with all reporting significant positive outcomes. However, four of these trials used bolus dosing (50,000 IU), one [27] (and potentially two—the dosing regime is ambiguous in [26]) with an effective dose above safe upper limit. Two trials used 6-week interventions[26, 27], which can obscure effect size relative to placebo in IBS studies[30]. All these studies were conducted in patients recruited from clinics and had small sample sizes relative to our pilot-study derived calculation of numbers needed for a powered trial of vitamin D intervention with IBS SSS as the outcome[31]. In view of this emerging literature and the potential benefit of vitamin D on IBS, coupled with the ease and relative safety of delivery we identified the need to assess the potential benefit of moderate dose vitamin D supplementation in the UK IBS population. Here we report on a double blind, placebo-controlled, adequately powered trial to investigate the effect of 12 week, moderate dose vitamin D supplement on symptoms of IBS. We hypothesised that vitamin D supplementation would reduce IBS symptom severity. This study was designed to test the hypothesis, and used a previous pilot study to inform the design [31].

Materials and methods

Study design

This was a randomised, double-blinded, placebo-controlled, two-arm parallel trial of 12-week duration. The study design and planned endpoints were registered at http://www.isrctn.com (ISRCTN13277340) seven weeks after recruitment had been initiated, but 11 months before trial closure or analysis. Ethical approval was granted by The University of Sheffield Medical School Research Ethics Committee (Ref: 11,865) and the trial was conducted in accordance with the Declaration of Helsinki. A sample size calculation (reported in our pilot study [31]) suggested, that 74 participants per arm were needed to achieve 80% power with 0.05 α–error (based on a reduction in total symptom severity score at exit of a mean of − 16 in the placebo arm, a mean of − 54 in the vitamin D intervention arm and a SD of 82). To achieve this target and allow for 10% withdrawals, a recruitment target of 160 participants was set.

Participants and recruitment

Participants were recruited through online mailshots to volunteer lists through the University of Sheffield, via the IBS Network (The UK National charity for IBS) and through poster and postcard advertising in the local areas. Respondents were assessed according to trial criteria. The *Inclusion criteria* were: a previous clinical diagnosis of IBS by ROME

criteria (as participation was open to individuals with long-standing IBS, potentially predating ROME IV or III, and as this was a community-based trial, of a potentially over-the-counter remedy, researchers required confirmation from participants of a previous clinical diagnosis, coupled to a total symptom severity score of 150 or over, rather than a clinical diagnosis using ROMEIV), age ≥ 18 years. *Exclusion criteria* were: regular use of nutritional supplements; pregnant or lactating; BMI > 30 kg/m^2; BMI < 18 kg/m^2; any history of other gastrointestinal disorders (e.g. inflammatory bowel diseases, diverticulitis, cancer); diabetes, recent or planned vacation. Due to circannual variation in vitamin D status[15] recruitment was undertaken seasonally in October–March 2017–18 and October–March 2018–19.

Respondents to advertisements were pre-screened against inclusion and exclusion criteria by telephone, provided with study information and subsequently invited to attend the Clinical Research Facility at the Royal Hallamshire Hospital, Sheffield for a study orientation and consent interview. At interview, potential participants' inclusion/exclusion criteria were cross-checked, consent taken, BMI was measured, and the dosing and symptom reporting protocols were explained. Fortnightly symptom questionnaires (see below) were returned by post. Quality of life measures and blood spots for circulating 25(OH) vitamin D were taken at entry and exit interview.

Participants were provided with a sublingual flavoured liquid spray for delivery of 3,000 IU vitamin D3 *per diem*, and were instructed how to use the spray format. This trial is designed to support the option of self-administration / over the counter supplementation as an option for people with IBS. Dose was therefore selected to be (i) below the safe maximum daily dose [32]; (ii) effective at increasing circulating vitamin D in deplete subjects within the intervention period [33]. Placebo was an identically presented spray with vector and flavouring only. The vitamin D spray and identically packaged placebo were provided by BetterYou Ltd (Barnsley, UK). Randomisation was computer generated in blocks of eight using sealedenvelope.com by a third party (G. Weatherhead, BetterYou Ltd). Additional detail on the blinding process is in the online supplement (for additional detail see supplementary online material).

Endpoints

Biometric data included age, sex, height (SECA 213 Height Measure), body weight (Tanita BC-543), circulating levels of vitamin D, severity of IBS and IBS-related Quality of Life. Participants' circulating vitamin D was measured as 25(OH) vitamin D_2 and 25(OH) vitamin D_3 in a dry bloodspot using blood collected from a finger-prick blood sample at baseline and after 3 months on the intervention. The 25(OH)D assay was conducted by a clinical service provider (Black Country Pathology Services, Sandwell and West Birmingham NHS Trust) using a validated LC–MS-MS assay as previously described [33]. IBS symptoms were assessed every two weeks throughout the trial using a widely applied IBS symptom severity questionnaire [34]. The questionnaire scores both severity and duration of abdominal pain (Pain severity; days with pain), abdominal distension (Distension severity), satisfaction with bowel habits (bowel habit Satisfaction) and global well-being (Impact of symptoms on life). Scores for composite individual factors (each with an arbitrary score of 100) were combined to give the total Symptom Severity Score (SSS) which has a maximum value of 500. Participants were reminded to complete questionnaires and to continue to take vitamin D via fortnightly text messaging throughout the duration of the study. Quality of Life was assessed at baseline and exit using an IBS-specific QoL instrument [35]. Participants who completed the study received a £50 voucher to thank them for their time and effort.

Data management and statistics

Consented participants were allocated consecutive trial numbers. The researcher (CEW) managed and inputted each participant's biometric data, symptom severity scores and QoL data into a spreadsheet in SPSS v25.0 (IBM, Armonk, New York, USA). The standard duration of the intervention was 84 days.

Participants were advised to continue supplementation between day 84 and the exit meeting. "Days on trial" represents time from commencement to exit blood sampling, or to the day of the last recorded symptom questionnaire in the case of withdrawal.

Data on serum 25(OH)D were returned to a third party (Mr G Weatherhead, BetterYou Ltd) who was blinded to all other participant data. Only on completion of the trial and data entry were spreadsheets merged. Analyses was undertaken by the research team whilst blinded to the identities of the trial arms. Analysis was performed on an intention to treat basis. Data missing for patients at the end of the trial period due to drop-out (see CONSORT diagram, Fig. 1) were not imputed. Statistical analyses were performed using SPSS V 25.0. Baseline demographic data were tested for normality and differences tested by t-test except where indicated; the primary endpoint (Symptom Severity Score) and contributing variables were analysed using repeated measures ANOVA. Non-normally distributed data are presented as medians with interquartile ranges and analysed by Mann–Whiney *U* test.

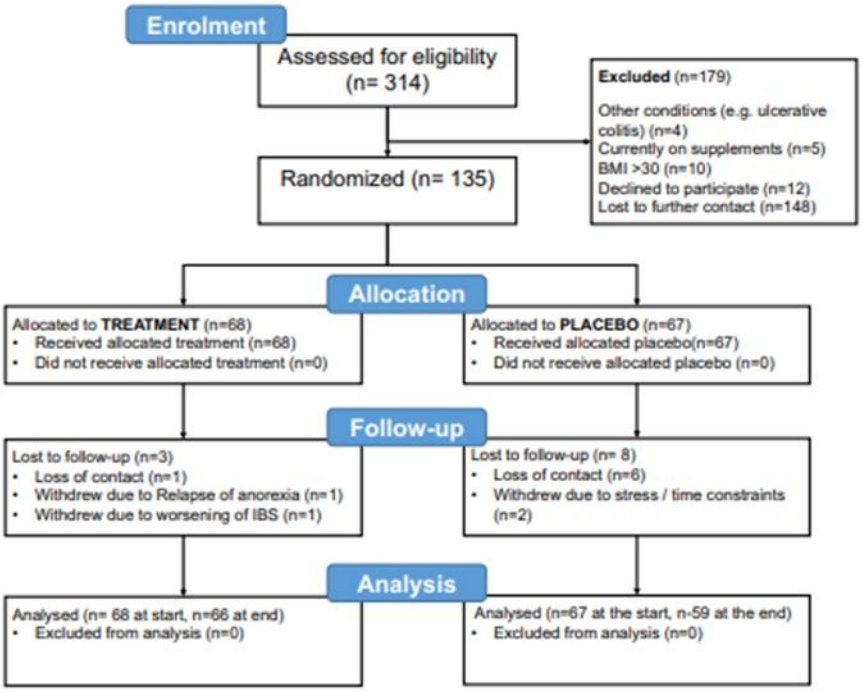

Fig. 1 Consort diagram summarising participant recruitment and retention in this trial. Of 314 expressions of interest, 19 candidates did not meet the inclusion criteria, 10 declined further involvement and 148 did not follow-up on initial contact. 135 participants were entered into the trial; 92% were retained until scheduled exit, two were unable to meet the time commitment for involvement, one was for unrelated health reasons, one due to increased symptoms (not overtly framed as an adverse event by the participant) and seven lost contact

Results

Recruitment and patient demographics

Participants were recruited to this trial across two successive winters (2017–2018 and 2018–2019). In total, 135 participants were recruited from an initial 314 responses to trial publicity, with 179 either excluded or lost to contact prior to consent (see Fig. 1 for the CONSORT workflow). In total 80 participants were recruited in the 2017–2018 round and 55 in the 2018–2019 round. Sixty-eight participants were entered into the treatment arm and 67 received placebo; 92.5% of participants completed the trial, reasons for withdrawal are indicated where known. Only one participant (in the treatment arm) withdrew reporting worsened symptoms. Demographic data for the whole group and comparison of trial arms are shown in Table 1.

There were no differences between trial arms at baseline in proportion of females, mean IBS severity, mean IBS-related quality of life or serum 25(0H)D. In common with previous studies we found a high proportion of participants with IBS were below recommended vitamin D adequacy levels: 20.7% were deficient (<25 nmol/l) and 60% were insufficient (<50 nmol/l). Dietary intake of vitamin D was assessed at baseline, intake was 3.1 ± 2.38 µg/day in the study sample and there was no difference in intake between arms (Table 1).

Effect of vitamin D supplementation on vitamin D status and IBS Symptoms

The intervention was effective at elevating total 25(OH) D levels, increasing circulating vitamin D in the intervention arm at 12 weeks relative to control (94.29 ± 33.70 *vs* 53.59 ± 23.21, $p < 0.0001$, *t* test) and relative to baseline (94.29 ± 33.70 vs. 48.75 ± 27.91, $p < 0.001$, t test). Exploratory analyses showed that the increase in circulating vitamin D in response to vitamin D intervention was greater in

Table 1 Participant demographics at baseline

	All	Placebo	Treatment	*p*
Participants *n*	135	67	68	
Females *n* (%)	106 (78.5%)	51 (76.1%)	55 (80.9%)	0.5[a]
Age year	30.01 (±10.46)	31.10 (±10.85)	28.94 (±10.03)	0.231[b]
BMI kg/m^2	23.37 (±2.88)	23.58 (±3.00)	23.15 (±2.76)	0.390[b]
IBS-SSS	277.41 (±65.15)	273.22 (±69.01)	281.54 (±61.34)	0.460[b]
IBS-QoL %	42.72 (±18.17)	43.35 (±19.24)	42.54 (19.45)	0.809[b]
Blood 25(0H)D nmol/l (baseline)	49.23 (±27.38)	49.71 (±27.05)	48.75 (±27.91)	0.839[b]
% with blood 25(OH)D < 50 mmol/l	60	61.2	58.8	0.779[a]
% with blood 25(OH)D < 25 mmol/l	20.7	14.9	26.5	0.098[a]
Dietary vitamin D intake µg/day (baseline)	3.09 (2.379)	3.21 (2.383)	2.96 (2.389)	0.565[b]

Data are summarised for the whole sample and by trial arm, where appropriate means (±SD) are listed, for days on trial medians (IQR) are shown. There were no between arm differences between any factor

[a] χ^2 test

[b] *t* test

Table 2 Outcome measures

Outcome	Placebo	Treatment	*p*
Adverse events	2	2	
Days on Trial (IQR)	83 (15)	85 (11)	0.240[a]
IBS-SSS (Baseline)	273.22 (±69.01)	281.54 (±61.34)	0.460
IBS-SSS (Exit)	195.37 (±97.27)	220.32 (±93.72	0.147
IBS-QoL % (Baseline)	43.64 (±18.33)	41.81 (±18.09)	0.560
IBS-QoL % (Exit)	33.12 (±17.95)	34.24 (±17.56)	0.726
Blood 25(0H)D nmol/l (baseline)	49.71 (±27.05)	48.75 (±27.91)	0.839[b]
Blood 25(0H)D nmol/l (exit)	53.59 (±23.21)	94.29 (±33.70)	<0.0001[b]

Data are comparisons by trial arm; where appropriate the means (±SD) are listed, for Days on Trial medians (IQR) are shown. There were no between arm differences for the primary outcome measure (IBS-SSS) or QoL. There was a significant difference between trial arms in circulating vitamin D at trial exit ($p < 0.0001$

[a] Mann–Whitney *U* test

[b] *t* test

participants with insufficient vitamin D status (> 50 nmol/l) at baseline versus their replete counterparts (increasing by 56.1 ± 27.48 nmol vs 30.0 ± 34.1 nmol, p = 0.001) and also greater for those participants who were deficient (> 25 nmol/l) at baseline (increasing by 60.1 ± 31.02 nmol vs 40.1 ± 32.26 nmol, $p = 0.034$) (Table 2).

The primary outcome measure was IBS-SSS. To assess the effect of vitamin D on IBS symptoms, the symptom severity was assessed every 2 weeks across the course of participation. Analysis of total symptom severity over time by trial arm is shown in Fig. 2Ai. Both groups reported significant improvement in their IBS symptoms, but there was no difference between vitamin D and placebo treatment arms ($p = 0.824$, ANOVA). The data were also considered as change from baseline (Fig. 2Aii) and again no difference was identified between the trial arms ($p = 0.872$, ANOVA). The IBS-SSS was compared at the 12 week point (see Table 1). At this timepoint, there was no difference between trial arms in total symptom severity (Vit D = 220.3(± 93.73), vs Placebo = 194.2 (± 97.67) $p = 0.147$). When individual symptom scores were assessed (Severity of pain, days with pain, distention, satisfaction with bowel habit, and impact of symptoms on life) there were no differences between trial arms across the course of the study for any individual symptom (data for all timepoints are provided in the Supplementary material). No differences in response to the intervention were identified according to IBS subtype (data not shown).

Response to intervention may be dichotomised; a reduction in symptom severity of more than 50 points is invoked as clinically effective/ beneficial [34]. When proportions of participants exhibiting > 50point were compared for treatment vs. placebo (Table 3), there was no difference in response rate between arms.

Finally, we hypothesised that the extent of improvement in circulating vitamin D level might lead to improvement

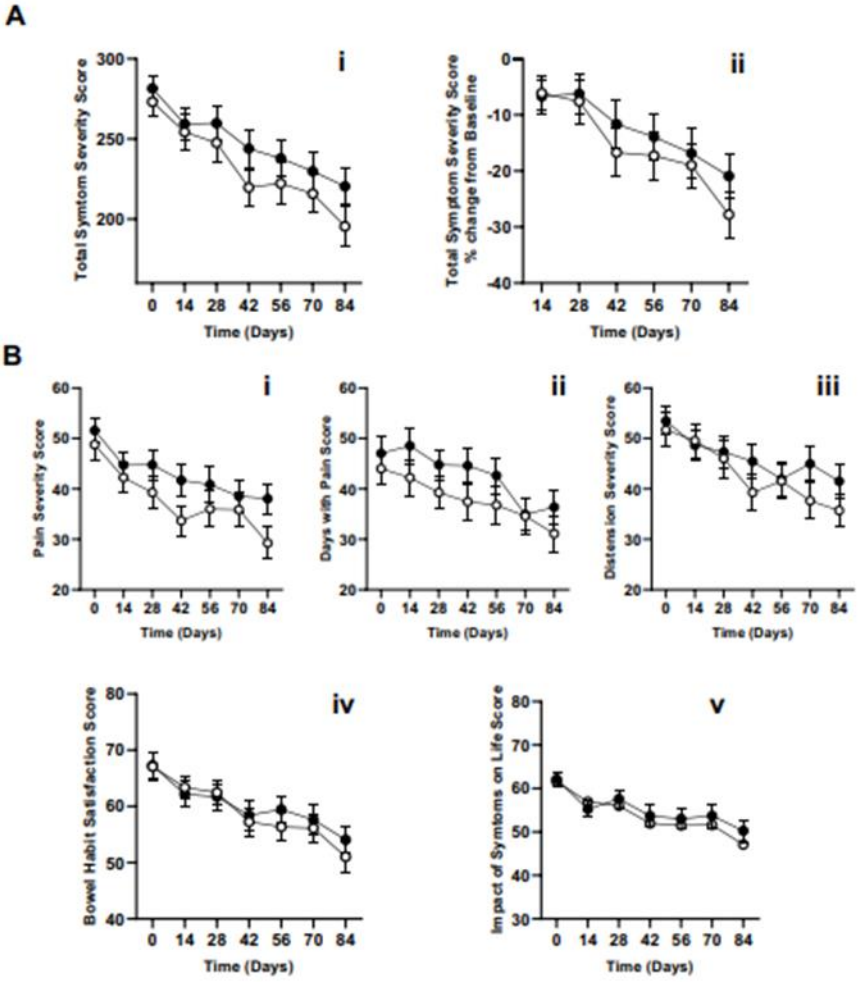

Fig. 2 Effect of vitamin D supplementation on IBS symptoms. Participants were assessed every 2 weeks on their symptoms. In all plots, placebo arm is the open circle and active arm is the solid circle; plots show mean ± SEM at each timepoint. **A** Shows change in total symptoms across the course of the trial, Panel Ai shows actual symptom severity, Panel Aii shows change from baseline. **B** Shows each symptom score plotted in the same way. I-iv are, respectively, pain severity, days with pain, distention severity, satisfaction with bowel habit and affected life

in symptoms and tested this by correlating change in vitamin D with change in symptoms. There was no apparent relationship between change in serum 25(OH)D and change in total symptom severity (Fig. 3i; $r = -0.071$, $p = 0.434$, Spearman's rank correlation coefficient).

Effect of vitamin D status on quality of life in IBS

Several studies have used an IBS-specific QoL instrument [36] and reported a benefit of vitamin D intervention. The instrument was applied at baseline and at exit from the intervention. Whilst there was an improvement in QoL in

Table 3 Comparison of response rate between trial arms

	Frequency (%)	*p*
All Participants		
Placebo	38/60 (63.3%)	
Treatment	37/65 (56.9%)	0.465
Vitamin D insufficient/deficient participants (25(OH) D < 50 nmol/L)		
Placebo	22/36 (61.1%)	
Treatment	20/37 (54.1%)	0.542
Vitamin D deficient participants ((25 (OHD) < 25 nmol/L)		
Placebo	5/8 (62.5%)	
Treatment	8/15 (53.3%)	0.673

Response is defined as > 50 point reduction in TSS score at trial exit. There were no differences in the proportions of participants responding to the intervention by trial arm in the whole study, or in either lower vitamin D status category (inadequate and deficient, deficient) (χ^2 test)

each arm of the trial ($p < 0.001$ for each arm, Mann–Whitney), there was no difference between the change in QoL score from baseline to exit between trial arms ($p = 0.525$, Mann–Whitney). We investigated whether improvement in circulating vitamin D level might improve QoL; no relationship was found between change in serum 25(OH)D and change in QoL (Fig. 3ii; $r = -0.031$, $p = 0.73$, Spearman's rank correlation coefficient).

Exploratory and signal-seeking analyses

Trials in IBS often either select or subdivide participants according to IBS subtype (constipation, diarrhoea or alternating symptoms). A signal seeking analysis was undertaken to assess whether there were differences in response to vitamin D by IBS Subtype. There was no difference in symptom severity (SSS: $p = 0.719$, 0.962, 0.697 constipation, diarrhoea and alternating symptoms, respectively, Repeated measures ANOVA) or change in Quality of life (QoL $p = 0.316$. 0.946, 0.090 constipation, diarrhoea and alternating symptoms, respectively, Mann–Whitney *U* test) in response to vitamin D within any of the IBS subtype groups.

The response according to IBS severity was investigated. Participants were categorised by IBS severity[34] (75–174—Mild; 175–299—Moderate; > 300—Severe) and response to the intervention was analysed. There were no differences in symptom severity ($p = 0.25$, 0.518, 0.554 mild, moderate and severe, respectively, repeated measures ANOVA) or Quality of life ($p = 0.262$. 0.275, 0.900 mild, moderate and severe, respectively, Mann–Whitney *U*) in response to intervention when analysed according to IBS symptom severity at baseline.

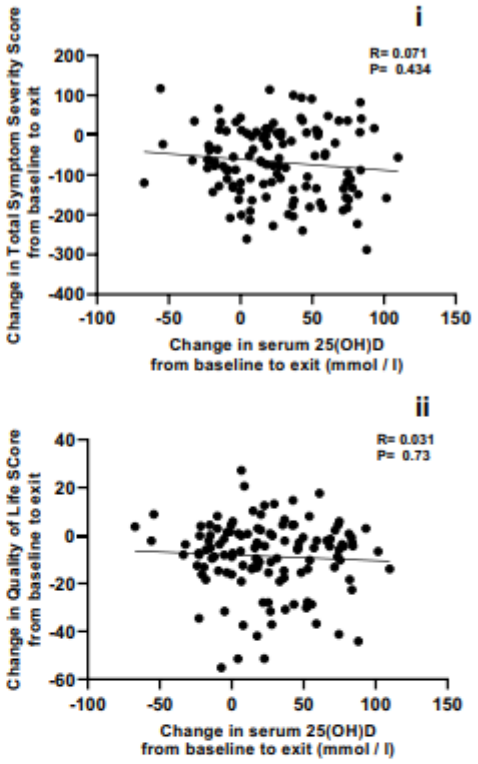

Fig. 3 Effect of change in vitamin D status on IBS symptoms and quality of life. The effect of change in circulating levels of vitamin D was assessed for both outcome measures (TSS and QoL). **a** shows correlation between change in circulating vitamin D from start to end of the trial against change in IBS symptoms. **b** shows correlation between change in circulating vitamin D from start to end of the trial against change in Quality of Life. There was no relationship between either endpoint and the vitamin D status change (Spearman's rank correlation coefficients shown)

Discussion

This study sought to investigate the potential of vitamin D supplementation as a management strategy for IBS, the design was community-based, seeking to be applicable to the general IBS population in addition to clinical settings. This study found no benefit of vitamin D supplementation on either symptoms of IBS or on QoL measures using standardised assessments. In addition, we found no relationship between change in vitamin D and change in symptomology.

The study has several hallmark features: it was based on a formal pilot study using the same intervention, endpoints

and population for the full trial; it is the largest trial of vitamin D in people with IBS; it used a moderate and safe dose of vitamin D; the duration of intervention was determined to minimise placebo effect [30]. Due to circannual variation in vitamin D status, we undertook recruitment during the winter to potentiate the maximum increase in circulating vitamin D at the annual low, concomitantly minimising risk of reaching toxic levels of the vitamin. Limitations of our trial include the potential heterogeneity of the sample (although this was deliberately a real-world study). We may have achieved more sample homogeneity and reinforced IBS diagnosis through reassessing participants with the ROMEIV criteria at screening. This sample would be more homogenous, although not necessarily more responsive. A general risk in nutrient supplement trials is that patients may self-supplement, obscuring effects; this was minimised by analysing outcomes against change in circulating vitamin D as well as by trial arm. We did not meet our target sample size, based on the power calculation. The implementation of GDPR regulations in 2018 led to a substantial impact on our recruitment rate in the second winter (80 vs target of 80 in first season; 55 versus target of 80 in second season). The absence of any signal of an effect suggests that failure to recruit did not affect interpretation of the outcome. Finally, despite our design, the placebo effect remained large.

Our findings are in contrast to a cluster of recent trials reporting a benefit of vitamin D supplementation on symptoms of IBS[25–28]. Abbasnezhad et al.[25] based in Iran recruited 45 outpatients / arm to a 50,000 IU fortnightly dose for 21 weeks and reported a significant reduction in symptoms ($p < 0.001$) of over 70 TSS points on average. Jalili et al.[26] had only 25 patients/arm recruited from an endoscopy clinic in Iran to 50,000 IU "biweekly"[1] dose for 6 weeks, again reporting a significant ($p < 0.05$) response. El Amrousy et al. [28] had a larger sample size (56/arm) recruited from paediatric outpatients in Egypt, undertook a power calculation based on a vitamin D intervention in IBS,[2] and used a longer intervention (21 weeks), again finding a significant ($p < 0.001$) benefit of supplementation. Jalili et al. [27] (2019) again recruiting in Iranian endoscopy clinics and using a dose (50,000 IU p.w.) considerably in excess of what would be regarded as safe, for 6 weeks with 58 patients per arm, again found a significant ($p < 0.05$) benefit of vitamin D. Most recently Sikaroudi et al. [29] recruited 88 patients from a gastroenterology clinic, dosing with 50,000 IU p.w. for 9 weeks, and reported a significant improvement in IBS-SSS. A further publication from the same group appears to be a restatement of these outcomes[37]. We note that these trials have several consistent features that limit their generalizability—all are based on clinically recruited groups in the Middle East; three used an intermittent bolus dose (50,000 IU), with one study using an extremely high effective dose of 7,142 IU *p.d*. Nonetheless, all four studies reported high compliance, low rates of drop out and high levels of significance notwithstanding sample sizes (25–58/arm) which our power calculation suggests were small. Despite the success of these trials their features suggest caution is needed about generalisability of their findings to the wider IBS population; in particular a bolus dose of 50,000 IU would not be a recommendable approach for general symptom management in IBS.

A recurrent feature of IBS trials is the heterogeneity of response, which may in part reflect the ill-defined nature of the syndrome. A meta-analysis of coefficients of variation (CV) in the IBS symptom tool used in this study reveals an average CV of 25% (SD = 8%) (Williams & Corfe, manuscript in preparation). It may be the case that there are subsets of the IBS population who do benefit from vitamin D supplementation[23]. Predicting responders, in terms of IBS symptoms, merits further research as vitamin D supplementation is a viable long-term management option. Our work shows that neither vitamin D status nor repletion is a predictor of a therapeutic response to vitamin D supplementation (in contrast, for example, to IBD[38]). Exploratory analyses of larger datasets would be needed to identify such potential predictors.

Critically, this study is in line with others in identifying vitamin D deficiency as widespread in IBS. There is recent, increasing recognition that IBS associates with increased risk of fracture[39] and of osteoporosis[40]. A causal inference is not yet possible, but poor vitamin D status in IBS may contribute to the observed association of these conditions. This suggests that, notwithstanding any benefit of vitamin D on IBS symptomology, IBS patients should be screened for vitamin D status and supplemented appropriately for general health reasons.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00394-021-02633-w.

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Author contributions CEW undertook the research including participant recruitment and day-to-day trial management, collation and

[1] It is unclear whether "biweekly" meant twice weekly or fortnightly in the context of this paper.

[2] Although the calculation appears to be based on work published after the group started recruiting.

analysis of data, reviewed and approved the manuscript. EAW co-designed and co-conceived this study, supervised and undertook data analysis, reviewed and approved the manuscript. BMC conceived, co-designed and co-supervised this study and wrote the manuscript.

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Availability of data and material Anonymised spreadsheets will be made available on reasonable request to the corresponding author.

Declarations

Conflict of interest BetterYou Ltd are a supplier of vitamin D supplements. CEW's PhD was part-funded by BetterYou Ltd. EAW and BMC were holders of the grant from BetterYou.

Ethics approval Ethical approval was Granted by The University of Sheffield Medical School Research Ethics Committee (Ref: 11,865) and the trial was conducted in accordance with the Declaration of Helsinki.

Consent to participate All participants provided informed, signed consent in accordance with the ethics approval.

Consent for publication All authors reviewed and approved the final submitted version of the paper.

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Supplementary information

Supplementary information #1 shows the randomisation strategy and supplementary information #2 presents the data table for IBS-SSS outcomes.

Supplementary Information for:

Vitamin D supplementation in people with IBS has no effect on symptom severity and quality of life: results of a randomized controlled trial

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Supplementary Information #1

The randomisation schedule was generated independently by G. Weatherhead (BetterYou Ltd) using sealedenvelope.com and a block size of eight. Identically presented boxes containing coded bottles and corresponding coded vitamin D bloodspot tests were provided to the research team by the supplier. The research team (CEW, EAW, BMC) was blinded to the content of each bottle.

In addition, the vitamin D assay results were returned and collated by a third party (G. Weatherhead, BetterYou) who was blinded to the IBS-SSS scores, whilst the researcher (CEW) entered the IBS-SSS data into a parallel sheet, blinded to the vitamin D baseline status or change. As the trial took place over a long period, across two successive winters, this protocol ensured that no interim unblinding or analyses were undertaken until all the data entry was completed.

Supplementary Information #2 – Data Table for IBS-SSS outcomes

		Time (days)						
		0	14	28	42	56	70	84
Pain severity† mean (SD)	Vit D	51.6 (21.20)	44.7 (21.63)	44.8 (24.64)	41.7 (27.35)	40.9 (28.10)	38.6 (26.43)	38.1 (23.91)
	Placebo	48.8 (24.59)	42.3 (25.23)	39.29 (26.16)	33.7 (24.05)	36.0 (28.44)	35.9 (25.74)	29.2 (24.82)
Days with pain† mean (SD)	Vit D	47.1 (27.58)	48.6 (30.07)	48.3 (28.57)	44.6 (28.53)	42.7 (28.52)	34.9 (26.13)	36.4 (27.35)
	Placebo	44.0 (25.47)	42.2 (28.75)	43.7 (29.73)	37.4 (28.01)	36.8 (30.19)	34.6 (27.15)	31.1 (27.93)
Distension severity† mean (SD)	Vit D	53.4 (22.69)	48.6 (25.38)	47.3 (26.07)	45.5 (28.25)	42.0 (27.78)	45.0 (26.72)	41.5 (26.31)
	Placebo	51.7 (28.46)	49.6 (26.99)	46.1 (30.66)	39.27 (28.1)	41.5 (26.27)	37.7 (26.77)	35.7 (25.28)
Bowel Satisfaction† mean (SD)	Vit D	67.3 (18.37)	62.2 (19.41)	61.7 (19.23)	58.4 (21.54)	59.4 (19.99)	57.6 (20.36)	54.1 (19.43)
	Placebo	67.1 (19.60)	63.4 (15.49)	62.5 (18.51)	57.3 (19.86)	56.4 (19.98)	56.1 (19.94)	51.0 (21.45)
Impact on Life† mean (SD)	Vit D	62.1 (13.64)	55.2 (14.94)	57.6 (17.57)	53.7 (20.97)	53.0 (19.71)	53.7 (21.60)	50.3 (20.8)
	Placebo	61.6 (14.93)	57.0 9 (17.15)	56.0 (20.39)	52.0 (19.71)	51.5 (20.83)	51.6 (21.67)	47.1 (22.96)
Total-SSS mean (SD)	Vit D	281.54 (61.33)	259.3 (79.26)	259.8 (86.15)	243.9 (99.17)	237.9 (92.06)	229.79 (98.40)	220.3 (93.73)
	Placebo	273.2 (69.01)	254.4 (87.48)	247.5 (93.80)	219.6 (90.78)	222.2 (100.86)	215.9 (92.95)	194.2 (97.67)

Table S1 Data detail for response to treatment
The table shows mean and standard deviations for each composite IBS symptom and for the total Symptom Severity Score at each timepoint in the study for each arm of the study, supporting the data presented graphically in Fig 2. Individual scores have a maximum score of 100; Total SSS _: Total symptom severity has a maximum possible score of 500.

Chapter 7

Discussion

This thesis has presented evidence from 4 studies that were conducted during this PhD. The over-arching aim was to investigate the relationship between vitamin D and irritable bowel syndrome and the possible impact vitamin D may have on symptom severity and quality of life. Each chapter is presented in either manuscript or publication format and each has its own discussion, specific to the research conducted. This discussion chapter will summarise the objectives achieved, results from the previous chapters, while addressing whether this agrees or conflicts with current evidence. It will discuss limitations/strengths of the findings and the contribution this makes to the wider IBS research community.

Summary of findings

1**.** The systematic literature review (Chapter 4) yielded a total of 7 studies (115, 120, 247, 248, 250, 251, 378) has shown the limited research available on the subject of vitamin D and IBS. Four observation and three intervention studies were identified using PRISMA guidelines. All four observational studies reported vitamin D deficiency was evident in a high proportion of the IBS population. Improvements in IBS symptom severity scores and QoL were reported in two of the intervention studies (115, 251) (Chapter 4). In the supplementary search post publication, all 3 intervention studies observed an inverse relationship between vitamin D levels and symptom severity/QoL (114, 252, 379).

2. A randomised control trial (Chapter 5) was conducted in healthy volunteers to compare rate of uptake of vitamin D using an oral spray versus a capsule. Vitamin D status was deplete or insufficient at baseline in 44.6% of the volunteers. There was a significant increase in serum vitamin D (capsules 50.7±19.73 to 91.35±19.78nmol/L p=0.003; spray 54.9±27.84 to 95.78±28.03 p= 0.001) in both treatment arms compared to the control over a 6 week period. The capsule and the spray were equally effective at raising vitamin D levels to sufficiency. The data suggest that those with a lower vitamin D serum had a higher repletion rate. The majority (71%) of participants preferred the oral spray to the capsule for vitamin D supplementation.

3. A randomised, double-blinded placebo-controlled, 2 arm trial (Chapter 5) was completed with 135 free living participants with IBS. The data concluded that a vitamin D 3000IU/day supplement for 12 weeks did not improve symptom severity or quality of life in this population. The intervention was

successful at raising vitamin D levels in the treatment arm from 48.75 (±27.91) nmol/L to 94.29 (±33.70) nmol/L at exit compared to placebo (p=0.05).

Detailed summary of studies conducted

In the first year of this project, a systematic review was conducted using PRISMA guidelines, presented in chapter 2. The review found a consistent observation of an insufficiency/deficiency of serum vitamin D levels in the IBS population and suggests amelioration of symptoms after supplementation with vitamin D. Systematically reviewing the literature identified 7 papers, 3 of which (at the start of this project) were intervention studies (115, 250, 251) . All three studies agreed there was a high level of deficiency/insufficiency in this IBS population. Two (115, 251) out of three studies found vitamin D supplementation to have a significant benefit on symptom severity and quality of life. It would have benefitted the review to have contacted the authors to better understand their decision to present the data in standard deviation, standard error, or a range of scores. In hindsight, the standard error presented in Jalili et al. (2016) could have been converted to a standard deviation to offer comparable data. This was unfortunately not identified at the time. Future research conducted will include this best practice when completing systematic reviews.

The efficacy study established that the sublingual vitamin D spray was as effective as a capsule for raising serum vitamin D levels in healthy populations. This offered confidence for its use in the RCT with IBS participants and an opportunity to assess and compare the sublingual spray's ability to raise serum 25(OH)D in a different sample. We established that all participants were replete by day 21 with a 3000IU daily supplement of vitamin D. The time taken to repletion informed the design of the subsequent RCT. Participants reported in focus groups a higher preference for the sublingual spray over the capsule, finding it easy to use and pleasant tasting. This provided assurance for the sublingual spray's acceptability for participants recruited to the RCT.

Chapter 6 presents the randomised control trial investigating the effect of vitamin D supplementation on symptom severity and quality of life in a free living IBS population. It is the largest study to date with a sample size of n=135 based on a formal pilot study drawn from the same population type and similar endpoints. We hypothesised that a 3000IU/day vitamin D supplement would ameliorate symptom severity and improve quality of life in people with IBS.

Our main finding was that providing a 3000IU/day vitamin D supplement for 12 weeks to participants with IBS did not reduce symptom severity nor improve quality of life. In agreement with current research we also found a high proportion of our sample to be low in vitamin D, which may just be a reflection of the deficiency seen in the wider population (45). A higher number of responders (reduction of ≤50 points on the TSS score) were found in the placebo arm compared to the treatment (63.3% versus 56.9% respectively). This confirms the current evidence of a high placebo effect in this population, this is discussed further in this chapter (377).

Contrast in findings

This next section will discuss the possible factors that may result in different outcomes in research conducted with IBS populations and vitamin D supplementation. In contrast to our main finding, previous research by several authors has reported (114, 115, 251, 252) a highly significant a reduction in IBS symptom severity with vitamin D supplementation. Our study (Chapter 6) used 3000IU/day sublingual spray within a free living population, while Jalili et al. used 3571IU/day (2016) or 7142IU/day (2019) with a clinical population (114, 115). Similarly, Abbasnezhad and colleagues (2016) conducted a RCT with 85 participants with IBS and found significant improvement of IBS symptoms ($p < 0.001$) and quality of life ($p < 0.001$) following a 3571IU/day vitamin D capsule (251). A recent study conducted in Iran, (2020) with 88 participants, from a clinically recruited sample, used a weekly bolus dose of 50,000IU in adults with IBS-D for 9 weeks. This study found a significant improvement in both IBS-SSS and QoL scores ($p=<0.001, 0.049$) in the treatment arm compared to the placebo for 9 weeks (252). Alongside vitamin D all participants received 135mg (twice daily) of Mebeverine which is known to relieve symptoms of IBS (380). It may be this anti-spasmodic that improved the symptom severity and quality of life in volunteers, however, no discussion is provided.

One could postulate that it may be the clinical population have a higher symptom severity score at baseline than free living participants. However, our study shows (Chapter 6) the mean baseline IBS-SSS score (277.29) to be higher than the scores from a clinical population which ranged from 235-248. It may be that having a clinical sample reduces symptom severity as this group is already under the care of a specialist medical team compared to our random sample that may have not received any medical intervention thus far, and there may have been confounding factors that may have influenced the response to intervention.

Baseline serum 25(OH)D concentrations could also be a differing factor. Again, this is similar across all the studies presented with a baseline mean 25(OH)D concentrations from 46.40nmol/L-

52.67nmol/L. As aforementioned, the studies conducted in the Middle East used bolus doses of vitamin D 50,000IU per week/ biweekly. It could be that this higher dosage in one bolus treatment is more effective in relieving symptom severity in individuals with IBS compared with a daily regimen.

The combination of a high rate of fluctuation in symptom severity and a tendency for individuals to volunteer when symptoms are more severe, it is inevitable that their symptoms will improve (381).

It is known that intervention research with IBS populations encounter high placebo rates (237). Areas of focus include practitioner-patient relationship, number of visits, entry criterion, and duration of study (377). Research has shown that personality of the practitioner leading the research may positively influence placebo rates in the IBS population (246). A practitioner that has a positive relationship with the participant appears to raise placebo responses, which is independent of the treatment being used (75). By reducing the amount of contact time and lowering participant expectations is thought to counteract this placebo effect (246) . A meta-analysis suggests strict entry conditions such as the inclusion of the Rome criteria reduces placebo rates on average by 8.9% (237). A clinical diagnosis of IBS was part of the inclusion criteria of the present study and therefore all known Rome criteria was included and possibly unknown diagnostic measures. This may have contributed to the heterogeneity of the sample. The research studies which recruited from gastroenterology clinics used specifically the Rome III criteria for their inclusion criteria (114, 115, 251, 297), apart from the most recent study which used Rome IV (252) and this may be why they were able to observe significant results.

Our research, although conflicting with current literature, evaluates the efficacy of a widely available, over the counter vitamin D supplement in free living individuals with IBS. The reason for the lack of response to vitamin D in our population is uncertain, and this prompted an investigation of the variability between trials using the IBS-SSS questionnaire (chapter 7).

The IBS-SSS questionnaire is common in all the reported studies and has been used regularly for over 20 years in IBS research to evaluate symptom severity (293). The data collected from these IBS specific questionnaires can vary in scores from 0-500. Due to the symptoms of IBS being heterogeneous in nature, and as such, this is reflected in the highly varied scores in the IBS-SSS questionnaire in trials with this population.

Strengths and limitations

The systematic review (Chapter 4) highlighted the novelty of the topic and an opportunity to explore the possible relationship between vitamin D and IBS. This was also the first systematic review to

investigate and synthesise the evidence surrounding vitamin D supplementation within populations of people with IBS.

The intervention trial presented in Chapter 6, was based on a formal pilot study drawn from the same population type and similar endpoints (250). Guided by the pilot study it was the aim to recruit from a free living population with IBS and not from a clinical cohort.

Only safe doses were used that are available to members of the public and are able to purchase over the counter. The duration of study, is in line with advice to be over 8 weeks in length to reduce placebo effect (377). We sought to recruit participants in the winter months when 25(OH)D concentrations are at their lowest, for both the efficacy and intervention study. This was a way to minimise any risk of toxicity to the participants, maximise the potential benefit of receiving the intervention to a population that may need it the most and to ease interpretation of the data.

The studies presented in this thesis were conducted in a robust manner, however limitations are still present. The efficacy study was not based on a power calculation, however, it may provide a foundation from which future studies may be based. Another limitation of the efficacy study is the inability to be definitive in the absorption of the supplement in capsule form or sublingually, however pharmacokinetic studies have confirmed both these oral routes (382-384).

The limitations in the intervention study include; the large placebo effect, heterogeneity of the sample, and a possibility of participants self-supplementing.

Future work

This thesis has shown the rate of change in serum 25(OH)D following 6 weeks supplementation with vitamin D_3 (3000IU/day) in a healthy cohort. This thesis has contributed to the understanding of the rate of change of serum 25(OH)D in a healthy and primarily Caucasian cohort. Future work should explore the rate of change in other population groups, particularly those at high risk of deficiency, such as the elderly or those with darker pigmentation (66, 385). There is evidence showing further work is necessary with South Asian and Black African and Caribbean to determine guidelines for adequate intake of vitamin D for immigrants living in the UK (17). The evidence presented in chapter 5, may be used as a comparative data set to measure and compare the rate of change in other population such as individuals from BAME communities.

It has been evidenced by Ford and colleagues that having rigid entry criteria (latest Rome criteria/specific subtype) appears to lower placebo effect creating a more homogeneous population sample (377).

As aforementioned, this thesis required participants to have a clinical diagnosis which could include any of the Rome criteria or any other IBS diagnostic tool. This may have created a more heterogeneous sample and therefore reduced the likelihood of observing an effect.

Because this thesis found no significant difference between the treatment and the placebo arms, it may be worth future research focussing on subtypes of IBS that may benefit from vitamin D supplementation. To improve this work I would suggest utilising one single Rome criteria preferable the most recent which would contribute to the homogeneity of the sample (386). The use of the volunteer email and advertising with IBS charity would still be used as an effective recruitment tool, it would be beneficial to access IBS groups within other cities around the UK to increase participant numbers and diversity. It would be advantageous to stratify according to vitamin D status and subtype to compare the effect of a vitamin D supplement between arms only if a larger sample size could be achieved. I would also use a run in phase to exclude volunteers who are showing a placebo response which is recommended in studies using participants with IBS to lessen placebo response (387).

This may identify one or more subtype of IBS that may have improved symptomology and quality of life with repletion. Lastly, recruitment from a gastroenterology clinics would confirm diagnosis of IBS.

Conclusions and contributions to research

- This thesis has contributed to the growing body of evidence that there is a high prevalence of vitamin D deficiency/insufficiency present in individuals with IBS.
- Our efficacy study offers a data set for rate of change in a healthy population from which other populations can be compared, such as the elderly or individuals who are obese.
- The efficacy study has also contributed to existing work that shows baseline 25(OH)D concentrations influence uptake of vitamin D (388).
- This thesis has also added to the knowledge vitamin D supplementation in free living individuals with IBS. This was achieved by conducting the largest trial to date with n=135 participants

Concluding remarks

This thesis as a whole has contributed to current research that IBS is an ongoing public health issue that needs an effective and evidence-based intervention to alleviate symptoms and improve quality of life. The impact of an effective treatment means a reduced burden of cost to the NHS but most importantly improved quality of life for the individual. Although we did not find a significant relationship between vitamin D supplementation and decreased symptom severity, these findings are useful for future research.

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Appendix 1: Poster from Barcelona conference

The University Of Sheffield.

Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations

BetterYou™ just as nature intended

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BACKGROUND

- Vitamin D is critical for skeletal health and is increasingly associated with other pathologies encompassing gastrointestinal, immunological, psychological effects.
- A significant proportion of the population exhibit suboptimal levels of vitamin D, particularly in Northern latitudes in winter.
- Supplementation is advocated, but few data are available on relative efficacy of uptake rates, or platform of delivery.

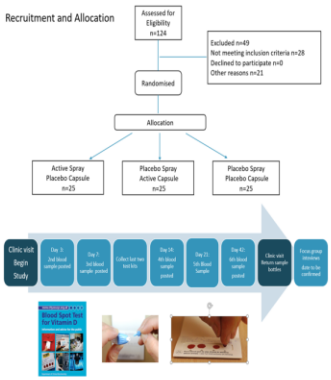
Hypothesis/Aim

Hypothesis: It was hypothesised that there was no difference in rate of uptake of vitamin D delivered using sublingual spray compared to a capsule.

Aim: To conduct a trial to investigate the efficacy of a vitamin D sublingual spray compared to a capsule.

METHODS

A double blind, randomised, placebo-controlled, 3-arm parallel design study was conducted in healthy volunteers (n=75) to compare uptake rates of vitamin D supplementation in capsule and sublingual spray preparations over a six week period between February and April 2017. Serum 25(OH)D concentrations were measured on day 0, 3, 7, 14, and 21 days of supplementation with 3000IU *per diem*.



RESULTS

1. No significant difference in efficacy of repletion between platforms

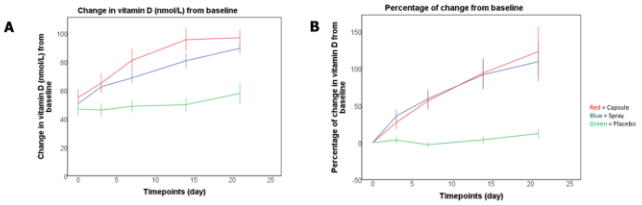


Figure 1. Absolute serum 25(OH)D over 6 time points (A). Percentage change in 25(OH)D from baseline (B).

2. Baseline vitamin D status may influence uptake rate

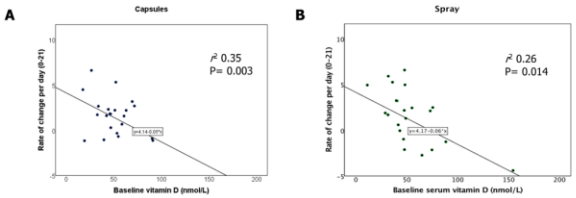


Figure 2. Significant negative correlation between baseline; day 21 vitamin D and rate of change in vitamin D in capsule (A) and spray (B).

SUMMARY OF RESULTS

- Both capsule and spray resulted in significant improvement in vitamin D status within three days.
- Serum vitamin D levels analysed across the time course in all three trial arms by ANOVA showed a significant difference (capsules p= 0.003, spray p= 0.001) compared to placebo (*Figure 1*).
- *Post hoc* analyses revealed significant differences between each active and placebo, but no difference between the active preparations at any time point.
- Independent t-test found no significant difference between mean uptake rates for capsule and spray (data not shown).

  These data suggest equal efficacy between methods of delivery.
- We found a negative relationship between serum 25(OH)D status and uptake rate of vitamin D.

  These data suggest a homeostatic regulation of uptake.

CONCLUSIONS

1. A sublingual vitamin D spray is an effective mode of delivery for supplementation in a healthy population.
2. Achievable rates of vitamin D increment are approximately 2 nmol/ml/day at the dose of 3000IU (75ug)/day.

FUTURE WORK

To conduct further studies using this as a reference data set to examine other populations for possible differences in uptake rates such as ethnic minorities and the elderly.

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Appendix 2: Poster presentation at the Nutrition Society Winter

Vitamin D status and quality of life in people with Irritable Bowel Syndrome.

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Background

Irritable Bowel Syndrome (IBS) is a chronic and debilitating functional disorder of the gastrointestinal tract with serious and detrimental impacts on quality of life[1].

Its aetiology is largely unknown, and the identification of effective management strategies remains far from complete. Research suggests that people with IBS have a high prevalence of vitamin D deficiency, which may impact on symptom severity and quality of life[2].

Aim

To investigate the relationship between serum 25(OH)D and quality of life and total symptom severity in people with IBS.

Methods

- 135 people with IBS were recruited from the Sheffield and South Yorkshire region to a vitamin D intervention (January-April 2018 and 2019).
- Baseline data was examined to explore associations between vitamin D status, Quality of Life (IBS-QoL) and IBS symptom severity score (IBS-TSS).
- Finger prick blood samples were collected for the measurement of vitamin D status[3].
- Questionnaires were completed to assess quality of life[4] and symptom severity[5].
- Associations between the data were examined using Spearman's correlation analysis using SPSS (Version 25).

Conclusions

1. Contrary to expectations no relationship was found between vitamin D status and either quality of life or symptom severity.
1. Analysis of a RCT of vitamin D in this population is underway and will determine if vitamin D supplementation improves either quality of life or symptom severity.
- People with IBS may benefit from vitamin D supplementation during winter months given high prevalence of vitamin D insufficiency, for general musculoskeletal health.

Results

1. Baseline participant characteristics

Participants n	135
Female n (%)	106 (78.52%)
Height (m)	1.70 (±0.080)
Weight (kg)	67.20 (9.95)
Age (years)	30.01(±10.46)
BMI kg/m^2	23.37 (±2.88)
IBS-QoL	42.95 (±19.28)
IBS-SSS	277.29 (±64.03)
Serum 25(OH)D nmol/L	49.23 (±27.38)
***Deficient** <30 nmol/L n (%)	39 (28.9%)
Insufficient >30-<50 nmol/L n (%)	42 (31.1%)
Adequate >50 nmol/L n (%)	54 (40.0%)

*Institute of Medicine (2011)[6]

2. No relationship between serum 25(OH)D and quality of life or symptom severity

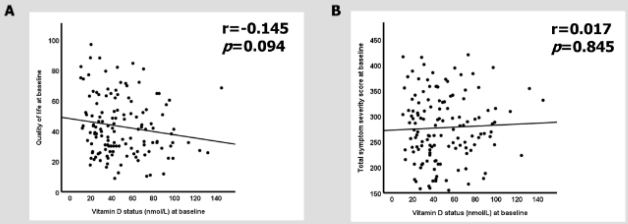

Figure 2. No correlation between baseline serum 25(OH)D status and quality of life (A) or total symptom severity (B).

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For further details please contact cewilliams1@sheffield.ac.uk

Appendix 3: Poster presentation for FENS conference

The University Of Sheffield.

Effect of vitamin D supplementation on symptom severity and quality of life in people with Irritable Bowel Syndrome

BetterYou™ just as nature intended

Claire E. Williams[1], Elizabeth A. Williams[2] & Bernard M. Corfe[1]

1. Molecular Gastroenterology Research Group, Academic Unit of Surgical Oncology, Department of Oncology & Metabolism, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX
2. Department of Oncology & Metabolism, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX

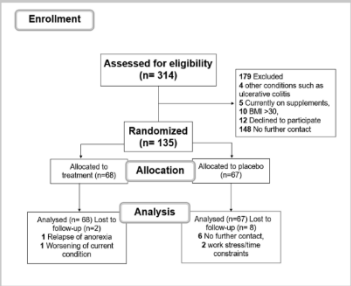
Background

- Irritable Bowel Syndrome (IBS) is a common functional disorder of the gastrointestinal tract, affecting 17% overall of the UK population[1].
- The aetiology of this disorder is unknown, although it has been linked to environmental, psychological and social factors[2].
- Vitamin D deficiency and insufficiency is common within the IBS population[3], and vitamin D has been hypothesized as a potential remedy.
- We sought to test whether vitamin D supplementation improved symptoms or quality of life in people with IBS.

Aim

To investigate the effect of a 3000IU vitamin D supplement on symptom severity and quality of life in people with IBS.

Methods



- Data was examined to explore associations between vitamin D status, Quality of Life (IBS-QoL) and IBS symptom severity score (IBS-TSS).
- Finger prick blood samples were collected for the measurement of vitamin D status[4].
- Questionnaires were completed to assess quality of life[5] and symptom severity[6].
- Spearman's rho correlation, chi square, and t-test were used to analyse results (SPSS Version 25).

Results

1. Baseline participant characteristics.

Participants n		135
Female n (%)		106 (78.52%)
Height (m)		1.70 (±0.080)
Weight (kg)		67.20 (9.95)
Age (years)		30.01(±10.46)
BMI kg/m^2		23.37 (±2.88)
IBS-QoL		42.95 (±19.28)
IBS-SSS		277.29 (±64.03)
Serum 25(OH)D nmol/L		49.23 (±27.38)
*Deficient	<30 nmol/L n (%)	39 (28.9%)
Insufficient	>30-<50 nmol/L n (%)	42 (31.1%)
Adequate	>50 nmol/L n (%)	54 (40.0%)

*Institute of Medicine (2011)[7]

2. Percentage of participants with a >50 point reduction in TSS at exit.

	Frequency (%)	P value
Placebo	38/60 (63.33%)	
Treatment	37/65 (56.92%)	0.465

3. Intervention increased vitamin D status, no difference in in quality of life or symptom severity.

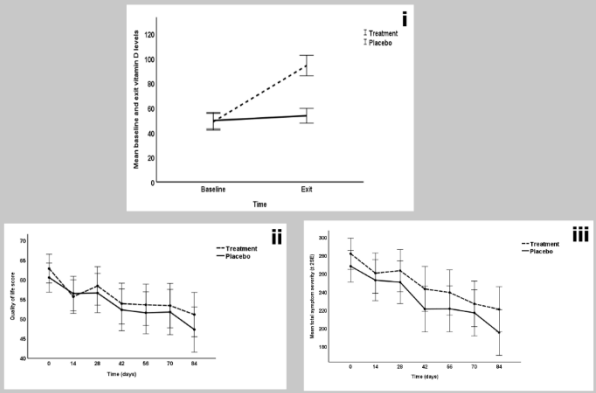


Figure 2. Significant improvement of vitamin D status (i); no difference between arm for quality of life (ii) or symptom severity score (iii).

Conclusions

1. There was a significant improvement of vitamin D status in the intervention arm (*p=0.005).*
2. Contrary to expectations, vitamin D supplementation did not improve either quality of life or total symptom severity.
3. There was no relationship to vitamin D status and total symptom severity or quality of life.
4. Future work may benefit from a longer study duration to reduce the strong placebo effect in this population.
5. People with IBS may benefit from vitamin D supplementation during winter months given high prevalence of vitamin D insufficiency, for general musculoskeletal health.

For further details please contact cewilliams1@sheffield.ac.uk

References

1. Ford, A. C., & Vandvik, P. O. (2010). Irritable bowel syndrome. *BMJ clinical evidence, 2010*, 0410.
2. Tazzyman S, Richards N, Trueman AR, Evans AL, *et al.* Vitamin D associates with improved quality of life in participants with irritable bowel syndrome: outcomes from a pilot trial. *BMJ Open Gastroenterol*; 2015: 2(1): e000052.
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4. Volmer, Dietrich A. ; Mendes, Luana R. B. C. ; Stokes, Caroline S.. Analysis of vitamin D metabolic markers by mass spectrometry: current techniques, limitations of the "gold standard" method, and anticipated future directions. *Mass Spectrum Rev.* 2015; 34(1): 2-23.
5. Andrae DA, Patrick DL, Drossman DA, Covington PS. Evaluation of the Irritable Bowel Syndrome Quality of Life (IBS-QOL) questionnaire in diarrheal-predominant irritable bowel syndrome patients. *Health Qual Life Outcomes.* 2013;11(1):1–12.
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7. Ross, A. Catharine, Joann E Manson, Steven A Abrams *et al.* "The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know." *J Clin Endocrinol* Metab. 2011; 96(1): 53-58.

Appendix 4: Ethical approval for efficacy study

Downloaded: 20/07/2021
Approved: 16/12/2016
Claire Williams
Registration number: 160216727
Oncology
Programme: PhD
Dear Claire

PROJECT TITLE: Spray versus capsule for effective delivery of vitamin D in a healthy population.

APPLICATION: Reference Number 011865

On behalf of the University ethics reviewers who reviewed your project, I am pleased to inform you that on 16/12/2016 the above-named project was approved on ethics grounds, on the basis that you will adhere to the following documentation that you submitted for ethics review:

University research ethics application form 011865 (form submission date: 09/12/2016); (expected project end date: 30/12/2017).

Participant information sheet 1025627 version 2 (09/12/2016). Participant consent form 1025628 version 3 (22/12/2016).

The following optional amendments were suggested:

Thank you for making the changes requested and suggested at the last review. This has been amended effectively. Only one minor point remains on this review, which is to modify the consent form: please state "I wish to opt in TO the focus group" on the amended consent form (presently reads "I wish to opt in OF..."). I do not need to review this again, but suggest you make this change for clarity to the participants.

If during the course of the project you need to deviate significantly from the above-approved documentation please inform me since written approval will be required.

Your responsibilities in delivering this research project are set out at the end of this letter.

Yours sincerely

Paula Blackwell

Ethics Administrator Medical School

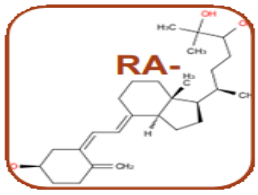
Please note the following responsibilities of the researcher in delivering the research project:
The project must abide by the University's Research Ethics Policy:
https://www.sheffield.ac.uk/rs/ethicsandintegrity/ethicspolicy/approval-procedure The project must abide by the University's Good Research & Innovation Practices Policy:
https://www.sheffield.ac.uk/polopoly_fs/1.671066!/file/GRIPPolicy.pdf
The researcher must inform their supervisor (in the case of a student) or Ethics Administrator (in the case of a member of staff) of any significant changes to the project or the approved documentation.
The researcher must comply with the requirements of the law and relevant guidelines relating to security and confidentiality of personal data.

The researcher is responsible for effectively managing the data collected both during and after the end of the project in line with best practice, and any relevant legislative, regulatory or contractual requirements.

Appendix 5: Recruitment poster for efficacy study

Version 1: 6/12/16

Vitamin D: Spray or Capsule?



You are invited to take part in the RADaR study: Rate of vitamin D uptake and repletion.

Uptake rates of vitamin D using an oral spray compared to a capsule. Researchers at the University of Sheffield are looking to recruit 100 participants aged 18-50 to take part in a 6-week study.

During the 6-weeks, participants will be asked to either take a vitamin D supplement or a placebo. They will also be asked to provide fingerprick blood samples using at home blood spot kits on 5 occasions. Upon completion of the study you will receive a £50 amazon voucher to thank you for your involvement.

For more information about the RADaR study and how to participate please contact one of the research team:

Tel:07594930676
Email: radar@sheffield.ac.uk

BetterYou™ just as nature intended

The University Of Sheffield.

RADaR study
Tel: 07594930676
Email: radar@sheffield.ac.uk

Appendix 6: Participant information sheet for efficacy study

Participant Information Sheet

Is there a difference between spray and capsule for effective delivery of vitamin D in a healthy population? (RaDaR)

You are being invited to take part in the RaDaR (Rate of vitamin D uptake and Repletion) study. Before deciding whether to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please feel free to ask the researchers any questions if there is anything that is unclear or if you would like any more information. Thank you for taking the time to read this.

What is the project's purpose?

This study aims to explore whether dietary supplements of vitamin D are best delivered as an oral spray or a capsule. Low vitamin D levels are a growing concern in the general population in the UK. It is estimated that 10 million people in England have low vitamin D levels. Vitamin D plays an important role in bone health and is now becoming a focus for other health concerns such as irritable bowel syndrome, asthma and multiple sclerosis. We wish to investigate the best method of delivering a vitamin D supplement by comparing an oral spray of vitamin D with the equivalent dose delivered via an oral capsule.

Why have I been chosen?

This trial is open to anyone aged 18-50 who is healthy and is currently not taking multivitamins or vitamin D supplements.

Do I have to take part?

No. Taking part in this trial is entirely voluntary and if you decide not to enter the trial there will be no penalty or loss to you. Similarly, if you wish to leave the trial at any stage you may do so without giving reason. If you do decide to leave the trial for any reason, please notify the researchers and we will arrange for any leftover supplements and paperwork to be returned to us. If you decide to withdraw from the study, then unless you explicitly ask for your data to be destroyed then the information already collected may still be used.

What will happen to me if I take part?

Prior to taking part in the project you will have the opportunity to discuss the study with a researcher. If you do decide to take part, you will be asked to sign a consent form, which you will be given a copy of. Once you have been enrolled in the trial, you will be asked to complete a fingerprick blood test (performed by yourself) which you will be shown how to do this by one of the researchers. We will also measure your weight and height and ask a few questions about your lifestyle.
You will be randomly allocated into either the vitamin D supplement capsule group, vitamin D spray group or the placebo group. All participants in every group will receive an oral spray and capsules to be taken every day for the duration of the trial (6 weeks). Only one of the treatments (either spray or capsule) will have the active vitamin D, the other will be a placebo and in some cases both will be a placebo.
You will then be asked to complete the at home blood spot kits at day 3 of the first week, then every week for the remainder of the study (total of 8 samples). The kits have everything you need to complete your sample including a postage paid envelope for you to send for your blood to be analysed. You will have an opportunity to practice with a researcher to ensure that you are happy and confident completing the samples at home.

At the end of the 6-week period, you will return to see one of the researchers, and hand in all remaining sprays and capsule bottles.

Both of the meetings, at the beginning and end of the 6-week period, will be held at the Medical School located at the Royal Hallamshire Hospital. A timetable is attached to this leaflet.

At the end of the trial you will be invited to participate in a focus group to discuss your experiences of the trial and your opinion on preference to taking a daily capsule or an oral spray. This will take place in a conference room in the University's Medical School and will involve 1 or 2 University researchers and up to seven other participants. This part of the study is optional and you may participate in the intervention trial without contributing to the focus group.

Members of the focus groups will be encouraged to keep everything that is discussed confidential but this is something that we cannot guarantee. If your contribution is used in University research it will be anonymised and identifying details, such as your job and age will be changed to protect your anonymity. Audio recordings will be used at these focus group sessions, though these will be transcribed and then destroyed so as not to be identifiable. Direct quotations may be used from the transcript.

What are the possible disadvantages and risks of taking part?
The main disadvantage to you is that you will need to spend some time in the initial appointment getting familiar with the at home blood spot kits. You will also need to remember to take the capsules and oral spray you have been given every day for 6-weeks.
You may experience soreness or slight bruising at the site of the fingerpick. You will be shown how to reduce this where possible.

The supplements you will be taken are safe and are available over the counter at most pharmacies and the dosage does not exceed safe levels. There should be no significant adverse effects from the supplements. We will ask you to refrain from taking any other vitamin D supplements during the study.

What are the possible benefits of taking part?

At the end of the study we will tell you what your vitamin D level was at baseline and how much it improved as a consequence of being on the study. If you are allocated to a vitamin D treatment, then we would expect to see an improvement in your vitamin D levels over the course of the trial. Improved vitamin D levels are associated with the maintenance of healthy bones and teeth and may have other health benefits. If you are allocated to the placebo arm we don't expect to see a significant change in your vitamin D levels, but at the end of the study all participants will receive vitamin D supplements to enable them to supplement with vitamin D should they wish.

What if something goes wrong?

If you find that you are having difficulties in completing the trial, for whatever reason, please contact one of the researchers with any issues that you may have (contact details below). If you wish to make a complaint about the conduct of the research or the research team, you can contact the research supervisor, Dr Bernard Corfe at b.m.corfe@sheffield.ac.uk.

If you feel your complaint has not been handled to your satisfaction, you can use the University complaints procedure and contact Professor Tim Skerry, Head of Oncology & Metabolism , either by post:, Department of Oncology & Metabolism, Medical School, Beech Hill Road, Sheffield S10 2RX; or email t.skerry@sheffield.ac.uk

Will my taking part in this project be kept confidential?

Any information collected from you throughout the project will remain completely confidential. When you sign the consent form to agree to participate in the project, you will be given a unique ID number which will be used for all information about you within the project. The only link between your name and your ID number will be on the original consent form. This will be kept in a locked filing cabinet, in a locked office, at all times.

You will not be able to be identified in any reports or publications that result from this project.

What type of information will be sought from me and why is this information relevant to the research project?

First of all, we will collect personal details from you such as you name, gender, date of birth and contact details. We will also collect some information on your age, height and weight. Your blood test will tell us how much vitamin D is circulating in your blood.

What will happen to the results of the research project?

The results of the research will be published in scientific journals, presented at relevant conferences and will contribute to the PhD thesis of Claire Williams.

It is important to remember that you will not be identifiable in any of the published research.

Who is organising and funding the research?

The project is supervised by academic members of staff in the Department of Oncology & Metabolism of the University of Sheffield. It is funded by a company named BetterYou who will be providing the oral sprays and capsules.

Who has ethically reviewed the project?

This project has been ethically reviewed and favourably approved by the University of Sheffield, Medical School Research Ethics Committee (Ref: 011865).

Please note that if you decide to take part, you will be compensated £50 upon completion of the project for your time and effort and a £5 Amazon voucher upon completion of the focus group interview.

Contact for further information

Please contact either of the researchers Claire Williams or Bernard Corfe.
Email: radar@sheffield.ac.uk
Telephone: 07594930676
Participants will be given a copy of this information sheet to keep for the duration of the project, as well as a copy of their signed consent form.

Thank you for reading this information sheet

Appendix 7: Ethical Approval for IBS intervention study

Downloaded: 07/01/2019
Approved: 19/12/2017

Claire Williams
Registration number: 160216727
Oncology
Programme: PhD

Dear Claire

PROJECT TITLE: Vitamin D and IBS
APPLICATION: Reference Number 016753

On behalf of the University ethics reviewers who reviewed your project, I am pleased to inform you that on 19/12/2017 the above-named project was **approved** on ethics grounds, on the basis that you will adhere to the following documentation that you submitted for ethics review:

- University research ethics application form 016753 (dated 04/12/2017).
- Participant information sheet 1037862 version 2 (19/12/2017).
- Participant consent form 1037863 version 1 (04/12/2017).

The following optional amendments were suggested:

The participant information sheet contains a lot of minor typos, such as additional words that mean sentences don't quite make sense or lack of spaces between full stops and the next sentence, as well as things like 'Taken' where it should be 'taking'. Please read through carefully and amend. In the methodology section you mention that that the blood samples will be posted by the participants, but elsewhere in the documentation you mention that the samples will be taken at the Clinical research centre and therefore presumably will be posted by the research team. Please clarify that information throughout.

If during the course of the project you need to deviate significantly from the above-approved documentation please inform me since written approval will be required.

Yours sincerely

Laura Williams
Ethics Administrator
Medical School

Appendix 8: Participant information sheet for the IBS intervention study

Participant Information Sheet

To assess whether an increase of vitamin D in subjects with irritable bowel syndrome improves symptoms (DIBS)

You are being invited to take part in the DIBS (vitamin D and IBS) study. Before deciding whether to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please feel free to ask the researchers any questions if there is anything that is unclear or if you would like any more information. Thank you for taking the time to read this.

What is the project's purpose?

This study aims to explore whether a dietary supplement of vitamin D reduces symptoms of Irritable Bowel Syndrome (IBS) in people with low levels of vitamin D. Low vitamin D levels are a growing concern in the general population in the UK. It is estimated that 10 million people in England have low vitamin D levels. Vitamin D plays an important role in bone health and is now becoming a focus for other health concerns such as irritable bowel syndrome, asthma and multiple sclerosis. We wish to investigate whether a vitamin D supplement can improve symptoms in people with IBS and low levels of vitamin D.

Why have I been chosen?

This trial is open to anyone aged 18-65 who has a clinical diagnosis of IBS and is currently not taking multivitamins or vitamin D supplements and have had no previous.

Do I have to take part?

No. Taking part in this trial is entirely voluntary and if you decide not to enter the trial there will be no penalty or loss to you. Similarly, if you wish to leave the trial at any stage you may do so without giving reason. If you do decide to leave the trial for any reason, please notify the researchers and we will arrange for any leftover supplements and paperwork to be returned to us. If you decide to withdraw from the study, then unless you explicitly ask for your data to be destroyed then the information already collected may still be used.

What will happen to me if I take part?

Prior to taking part in the project you will have the opportunity to discuss the study with a researcher. If you do decide to take part, you will be asked to sign a consent form; you will be given a copy of. Once you have been enrolled in the trial, you will be asked to complete a fingerprick blood test (performed by yourself) which you will be shown how to do this by one of the researchers. We will also measure your weight and height and ask a few questions about your lifestyle. By giving us your personal information we may be able to see similarities to participants in same age group, gender or BMI category. You will also be asked to fill in 3 questionnaires asking you about your food, symptom severity and quality of life
You will be randomly allocated into either the vitamin D supplement or the placebo group. All participants in every group will receive an oral spray to be taken every day for the duration of the trial (12 weeks). Only one of the treatments will have the active vitamin D, the other will be a placebo.
You will then be asked to complete 2 questionnaires fortnightly for the duration of the trial.
At the end of the 12-week period, you will return to see one of the researchers, and hand in the spray bottle and complete your final fingerprick blood test and questionnaires.

Both of the meetings, at the beginning and end of the 12-week period, will be held at the Clinical Research Facility (CRF) at the Royal Hallamshire Hospital. A timetable is attached to this leaflet.

What are the possible disadvantages and risks of taking part?

The main disadvantage to you is that you will need to spend some time in the initial appointment filling in the questionnaires and a fingerprick blood test. You will also need to remember to take the oral spray you have been given every day for 12-weeks.
You may experience soreness or slight bruising at the site of the fingerpick. You will be shown how to reduce this where possible.
Appendix
The supplements you will take are safe and are available over the counter at most pharmacies and the dosage does not exceed safe levels. If you receive the active vitamin D supplement, it is above the recommended 400IU/day at 3000IU/day. The recommendation of 400 IU/day is set at a population level and is safe from the age of four. 3000IU/day is below the upper tolerable level that is set at 4000IU/day. There should be no significant adverse effects from the supplements. We will ask you to refrain from taking any other vitamin D supplements during the study.

What are the possible benefits of taking part?

At the end of the study, we will tell you what your vitamin D level was at baseline and how much it improved as a consequence of being on the study. If you are allocated to a vitamin D treatment, then we would expect to see an improvement in your vitamin D levels over the course of the trial. Improved vitamin D levels are associated with the maintenance of healthy bones and teeth and may have other health benefits such as reduced symptom severity and improved quality of life. If you are allocated to the placebo arm we do not expect to see a significant change in your vitamin D levels, but at the end of the study all participants will receive vitamin D supplements to enable them to supplement with vitamin D should they wish.

What if something goes wrong?If you find that you are having difficulties in completing the trial, for whatever reason, please contact one of the researchers with any issues that you may have (contact

details below). If you wish to make a complaint about the conduct of the research or the research team, you can contact the research supervisor, Dr Bernard Corfe at b.m.corfe@sheffield.ac.uk.
If you feel your complaint has not been handled to your satisfaction, you can use the University complaints procedure and contact Professor Allan Pacey, Head of Oncology & Metabolism , either by post:, Department of Oncology & Metabolism, Medical School, Beech Hill Road, Sheffield S10 2RX; or email a.pacey@sheffield.ac.uk

Will my taking part in this project be kept confidential?
Any information collected from you throughout the project will remain completely confidential. Only the University of Sheffield and BetterYou will have access to your data. Your data will not be shared with anyone else. When you sign the consent form to agree to participate in the project, you will be given a unique ID number, which will be used for all information about you within the project. The only link between your name and your ID number will be on the original consent form. This will be kept in a locked filing cabinet, in a locked office, at all times.
You will not be able to be identified in any reports or publications that result from this project.

What type of information will be sought from me and why is this information relevant to the research project?
First of all, we will collect personal details from you such as you name, gender, date of birth and contact details. We will also collect some information on your age, height and weight. Your blood test will tell us how much vitamin D is circulating in your blood. Your questionnaires will tell us how much vitamin D you may get from your diet, how severe your symptoms are and your overall quality of life.

What will happen to the results of the research project?
The results of the research will be published in scientific journals, presented at relevant conferences and will contribute to the PhD thesis of Claire Williams.
It is important to remember that you will not be identifiable in any of the published research.

Who is organising and funding the research?
The project is supervised by academic members of staff in the Department of Oncology & Metabolism of the University of Sheffield. It is funded by a company named BetterYou who will be providing the oral sprays and capsules.

Who has ethically reviewed the project?
This project has been ethically reviewed and favourably approved by the University of Sheffield, Medical School Research Ethics Committee (Ref: XXXX).
Please note that if you decide to take part, you will be compensated £50 amazon voucher upon completion of the project for your time and effort.

Contact for further information
Please contact either of the researchers Claire Williams or Bernard Corfe.
Email: DIBS@sheffield.ac.uk
Telephone: XXXX
Participants will be given a copy of this information sheet to keep for the duration of the project, as well as a copy of their signed consent form.

Thank you for reading this information sheet

Appendix 9: Food frequency questionnaire

Web Link for the FFQ Questionnaire

https://www.epic-norfolk.org.uk/for-researchers/ffq/

Appendix 10: Recruitment poster for IBS intervention study

Appendix 11 : IBS-SSS questionnaire

Subject initials ☐☐☐

Study ID ☐☐☐☐☐☐☐

Study week ☐☐

-IBS QUESTIONNAIRE

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS symptoms. Please try and answer the questions based on how you currently feel

i.e. over the **7 days**. All information will be kept in strict confidence.

1. Some questions will require you to write in an appropriate response.
2. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example: How severe was your pain?

Please place your cross (**X**) anywhere on the line between 0-100% in order to in order to indicate as accurately as possible the severity of your symptoms. This example shows a severity of approximately 90%.

0% ◆——————————————————X——————◆ 100%

No pain Not very (severe) Quite severe Severe Very severe

1a) Have you suffered from abdominal (tummy) pain at any point in the past 7 days?

Yes ☐ **No** ☐

b) If **yes**, how severe is your abdominal (tummy) pain?

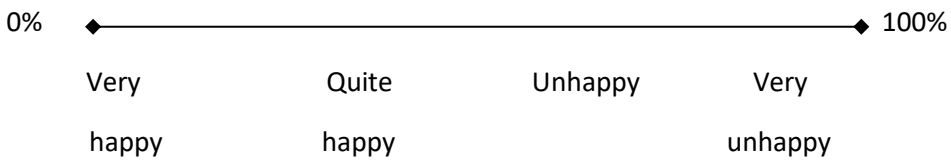
0% ◆——————————————————————————◆ 100%

No pain Not very (severe) Quite severe Severe Very severe

c) Please enter the number of days that you have experienced pain in the last 7 days. For example, if you enter 4 it means that you had pain 4 out of 7 days. If you had pain every day, enter 7.

Number of days with pain _____

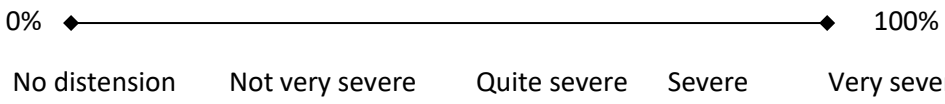
c) How satisfied are you with your bowel habits?



2a) Do you currently suffer from abdominal distension* (bloating, swollen or tight tummy).
(*women, please ignore distension related to your periods)

Yes No

b) If yes, how severe is your abdominal distension/ tightness?



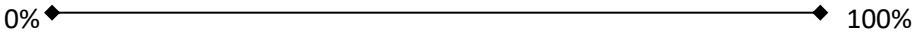
3.a) What is the most number of times you have opened your bowels per day?

Number of times per day

b) What is the least number of times that you opened your bowels?

Number of times per day

4. Please indicate how much your Irritable Bowel Syndrome has affected or interfered with your life in general.



Not at all Not much Quite a lot Completely

5. What year were you diagnosed with IBS?

6. Have you had any illness lately requiring antibiotics?

Yes ☐ **No** ☐

If yes, please give details