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Evaluation of the vitamin D response index in a Saudi cohort

Shareefa A. AlGhamdi [a,b,c,1,*], Ranjini Ghosh Dastidar [d,1], Maciej Rybiński [d], Hadeil M. Alsufiani [a,b,c], Sawsan O. Khoja [a,b], Nusaibah N. Enaibsi [a], Safa F. Saif [a], Carsten Carlberg [d,e,**]

[a] Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Vitamin D Pharmacogenomics Research Group, King Abdulaziz University, Jeddah, Saudi Arabia
[c] Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland
[e] Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio, Finland

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ABSTRACT

The concept of the vitamin D response index was developed based on vitamin D intervention studies conducted with Finnish cohorts. In this study, we challenged the concept by performing a single vitamin D_3 bolus (80,000 IU) intervention with a cohort of 100 native Saudis. The change of serum levels of the proinflammatory cytokines interleukin 6, interleukin 8 and tumor necrosis factor measured directly before intervention in comparison to samples taken one and thirty days after vitamin D_3 supplementation were used as biomarkers for distinguishing low, mid and high responders. Interestingly, we identified 39 % of the study participants as low responders. In contrast, when we used in a subset of 37 study participants whole blood expression changes of seven well-known vitamin D target genes one and thirty days after supplementation as alternative biomarkers, only 9 persons (24 %) were identified as low responders. In conclusion, in Saudi Arabia the rate of low vitamin D responders is equal or even higher than that in Finland. Therefore, similar to Nordic countries also in Saudi Arabia appropriate vitamin D_3 supplementation is essential, in order to fulfill the needs of low responders.

1. Introduction

Vitamin D_3 is an important signaling molecule, because its metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) acts a as high affinity ligand to the transcription factor vitamin D receptor (VDR) (Pike et al., 2017, Zmijewski and Carlberg., 2020. This makes vitamin D a direct regulator of gene expression (Carlberg 2022) and a molecule with a pleiotropic physiological profile (Dimitrov et al., 2021). The most important functions of vitamin D_3 are the regulation of calcium homeostasis (Fleet, 2022), which is essential for appropriate bone formation (van Driel and van Leeuwen, 2023), and the modulation of the immune system, such as boosting innate immunity and preventing overreactions of adaptive immunity (Bishop et al., 2020). Accordingly, insufficient circulating levels of 25-hydroxyvitamin D_3 ($25(OH)D_3$), which is the most stable vitamin D_3 metabolite and serves as a biomarker for the vitamin D status, are associated with rickets in children, osteomalacia in adults and osteoporosis in elderly (Uday and Hogler, 2017) as well as with inflammatory and autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis (Harrison et al., 2020).

When 7-dehydrocholesterol, a direct precursor of cholesterol, is exposed in human skin to the ultraviolet-B (UVB) component of sunlight, vitamin D_3 is synthesized in a non-enzymatic reaction (Wacker and Holick, 2013). However, changes in lifestyle like preferential indoor activities as well as living at higher latitudes with large seasonal variations in sun exposure, drastically reduced the endogenous vitamin D_3 production in nearly all human populations (Bendik et al., 2014, Spiro and Buttriss, 2014). This made vitamin D_3 a vitamin that needs to be taken up by diet or directly supplemented *via* pills. There is an ongoing discussion about the dose of daily vitamin D_3 supplementation as well as on the threshold levels distinguishing vitamin D sufficiency from insufficiency and deficiency (Pilz et al., 2019). In this study, we follow the well-accepted recommendation of the US Endocrine Society that a

* Corresponding author at: Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.
** Corresponding author at: Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland.
E-mail addresses: saaalghamdi1@kau.edu.sa (S.A. AlGhamdi), c.carlberg@pan.olsztyn.pl (C. Carlberg).
[1] Both authors contributed equally to the manuscript.

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25(OH)D_3 level above 75 nM (30 ng/ml) is considered as sufficient, while vitamin D deficiency is defined as a 25(OH)D_3 concentration below 30 nM (12 ng/ml) (Holick et al., 2011).

Studies with Finnish cohorts being supplemented either daily with 1600 or 3200 IU vitamin D_3 (Carlberg et al., 2013) or once with a monthly dose of 80,000 IU suggested that individuals can be segregated into low, mid and high responders to vitamin D. This vitamin D response index concept (Carlberg and Haq, 2018) implies that there should be supplementation with personalized doses of vitamin D_3, because low responders need a higher daily vitamin D_3 dose than high responders. Determining the vitamin D response index requires at least two samples from the same individual and measurements of circulating vitamin D-triggered proteins, such as the cytokines IL6 (interleukin 6), IL8, TNF (tumor necrosis factor) and the peptide hormone PTH (parathyroid hormone) (Carlberg et al., 2013), or the expression of vitamin D target genes in immune cells, such as PBMCs (peripheral blood mononuclear cells). The molecular basis of the vitamin D response index is not well explored, but it is likely based both on genetic and epigenetic variations of individuals (Hanel and Carlberg, 2020). However, the vitamin D response index is independent from the vitamin D status, since persons with high 25(OH)D_3 serum levels can be low responders, while individuals with a low vitamin D status may be high responders (Carlberg and Haq, 2018).

Recently, we reported on single vitamin D_3 bolus (80,000 IU) supplementation of 50 males (AlGhamdi et al., 2022) and 50 females (Alsufiani et al., 2022) from Saudi Arabia. We found in both cohorts a significant reduction in the circulating levels of the proinflammatory cytokines IL6, IL8 and TNF. In this study, we performed a *meta*-analysis of the data from both cohorts and used them for determining the vitamin D response index. Furthermore, we used changes in the expression of vitamin D target genes in a subgroup of 37 study participants for the verification of response index determination.

2. Materials and methods

2.1. Study design and participants

Each of the 50 male and 50 female adults (18–53 years old) had been recruited from the staff of King Abdul Aziz University and King Fahad Medical Research Center. Details of the study have been described previously (AlGhamdi et al., 2022, Alsufiani et al., 2022). In brief, all participants were native Saudis, free of cancer, liver or kidney diseases and did not take vitamin D supplements within three months before the study. They orally received a single vitamin D_3 (80,000 IU) bolus and gave fasting blood samples directly before supplementation (d0) as well as one and thirty days after (d1 and d30). The study was approved by the ethical committee of the Faculty of Medicine, King Abdulaziz University (ref. no. 30–18) and all participants provided written informed consent. The individuals were considered healthy as per Saudi standards (set by the Saudi Central Board for Accreditation of Healthcare Institutions (CBAHI, https://portal.cbahi.gov.sa/english/cbahi-standards) and the Saudi Commission for Health Specialties (SCFHS, https://scfhs.org.sa/en)), which defines health as being free of serious ailments or lifelong medications.

2.2. Biomarker measurements

Quantitative measurements of serum levels of 25(OH)D_3 were done *via* the Architect 25-OH vitamin D assay kit (Abbott), which has a detection limit of 4 ng/mL, with an intra-assay coefficient of variation (CV) of approximately 5.0 % and an inter-assay CV of approximately 7.0 %. The cytokines IL6, IL8 and TNF were determined by fully automated ELISA (enzyme-linked immunosorbent assay, Bioassay Technology Laboratory). The detection limits for IL6, IL8, and TNF were 2 pg/ml, 1 pg/ml, and 2 pg/ml, respectively, with intra-assay CVs of less than 10 % and inter-assay CVs of less than 12 % as previously described (AlGhamdi et al., 2022, Alsufiani et al., 2022) (**Table S1**).

2.3. Gene expression measurements

RNA was extracted from fresh blood sample by using QIAamp RNA blood mini kit following the instructions of the manufacturer (Qiagen). RNA quantity was determined *via* a Nanodrop 2000 spectrophotometer and RNA integrity was assessed on an Agilent 2100 bioanalyzer. RNA with RIN > 8 was used for cDNA synthesis through the SuperScript™ IV VILO™ Master Mix (Invitrogen) according to manufacturer's protocol. For measuring the expression of the vitamin D target genes *ASAP2* (ArfGAP with SH3 domain, ankyrin repeat and PH domain 2), *CAMP* (cathelicidin antimicrobial peptide), *CD14* (CD14 molecule), *FBP1* (fructose-bisphosphatase 1), *ITGAM* (integrin subunit alpha M), *LRRC25* (leucine rich repeat containing 25) and *NINJ1* (ninjurin 1) in relation to *RPL13A* (ribosomal protein L13a) housekeeping gene, real-time PCR was performed using the EverGreen Universal qPCR Master Mix (Haven Scientific) and the primer oligonucleotides listed in Table 1. Triplicate reactions were done on an ABI7300 system applying the following protocol: 3 min at 95 °C followed by 40 cycles of 95 °C for 15 s and then 60 °C for 60 s (data collection). To eliminate inter-run variabilities, all assays for a given sample were run on the same plate. PCR product specificity was monitored using post-PCR melt curve analysis. Relative mRNA expression levels were determined using the formula $2^{-(\Delta Ct)}$, where ΔCt is Ct(target gene) – mean of Ct(reference gene).

2.4. Determination of the vitamin D response index

In order to determine the vitamin D response index, we scored all 100 study participants based on the downregulation of proinflammatory cytokines (IL6, IL8, and TNF) at days 1 (d1) and 30 (d30) after a single vitamin D_3 bolus (80,000 IU) supplementation at day 0 (d0). Absolute log fold changes (logFC) in cytokine serum levels between d1 and d0 and between d30 and d0 were compared to changes in 25(OH)D_3 serum concentrations. This scoring system was also applied to a subgroup of 37 individuals for the upregulation of vitamin D target genes (*ASAP2*, *CAMP*, *CD14*, *FBP1*, *ITGAM, LRRC25* and *NINJ1*).

For the visualization of the data, we plotted cytokine expression changes against 25(OH)D_3 serum levels and added a trend line to understand the pattern of responses. This line divided the participants into low responders (score 0), mid responders (score 1), and high responders (score 3) based on their relative positions to the line. Further, we utilized the *K-means* clustering algorithm to classify participants into low, mid and high responders based on their cumulative scores. We performed correlation tests to rule out confounding factors such as age, gender and BMI, confirming that the observed changes in cytokine serum levels and target gene expressions were primarily influenced by 25(OH)D_3 serum

Table 1
Oligonucleotide primers used for real-time PCR.

Gene name	Orientation	Oligonucleotide sequence	Amplicon size
ASAP2	Forward	TACGGATCTTCACACGATCAAAC	104
	Reversed	CCTCTTTCTGTTCAACCTGCAA	
CAMP	Forward	GATGCTAACCTCTACCGCCTC	61
	Reversed	CTGGGTCCCCATCCATCGT	
CD14	Forward	GCCGCTGTGTAGGAAAGAAG	85
	Reversed	CGCGCTCCATGGTCGATA	
FBP1	Forward	ACTCTGGTCTACGGAGGGAT	73
	Reversed	ACAGCAGTCTCAGCTTTCCAT	
ITGAM	Forward	GGTTCACCTCCTTCCAGGTT	81
	Reversed	ATGACATAAGGTCAAGGCTGTT	
LRRC25	Forward	CCCTCCACTCCCGACTATGA	83
	Reversed	GAAGGGTGAGCCCCTTGTTC	
NINJ1	Forward	GGCCTGGTGTTCATCATCGTG	106
	Reversed	CAGGGTCCTGGGTGTCCTACT	
RPL13A	Forward	GCTAAACAGGTACTGCTGGG	99
	Reversed	AGCCAGGTACTTCAACTTGTTTC	

levels. For seven participants with dissimilar scores between the two methods, we noted a trend of higher basal IL6 and TNF serum levels but no statistically significant differences were found.

3. Results

In this study, we took advantage of two vitamin D intervention studies that were performed with 50 males (AlGhamdi et al., 2022) and 50 females (Alsufiani et al., 2022) from Saudi Arabia using an identical protocol, which had been established with Finnish cohorts (Seuter et al., 2017). The protocol involves taking blood samples directly before (d0), one day after (d1) and 30 days after (d30) a single oral vitamin D_3 bolus (80,000 IU) supplementation.

The *meta*-analysis of the data of both studies showed an age range of the 100 participants from 18 to 53 and a body mass index (BMI) spanning from 14.9 to 39.1 (**Table S1**). The individuals were considered healthy, because they were not diagnosed of any major disease and did not take any medication. However, at baseline (d0) the serum levels of IL6 ranged from 179 to 1079 ng/l, that of IL8 from 67 to 1311 ng/l and that of TNF from 69 to 342 ng/l, *i.e.*, the study participants showed a large variation in basal chronic inflammation. Moreover, the basal vitamin D status ranged from 14.2 to 136.7 nM (5.7–54.7 ng/ml) 25 $(OH)D_3$, which diagnosed 45 % of the study participants vitamin D deficiency (serum 25$(OH)D_3$ levels less than 50 nM), for 49 % of them vitamin D insufficiency (serum 25$(OH)D_3$ levels between 50 to 75 nM) and only for 6 % of the persons vitamin D sufficiency (serum 25$(OH)D_3$ levels above 75 nM). At d1 only one person remained vitamin D deficient, 82 were still vitamin D insufficient but already 17 were vitamin D sufficient and at d30 1 % of the study participants were vitamin D deficient, 76 % were still vitamin D insufficient but 23 % became vitamin D sufficient. The average 25$(OH)D_3$ serum concentration raised from 39.1 nM (15.6 ng/ml at d0) to 61.0 nM (24.4 ng/ml at d1) and even 64 nM (25.6 ng/ml at d30), *i.e.*, by 56 and 64 %, respectively. In our previous studies (AlGhamdi et al., 2022, Alsufiani et al., 2022) we had reported that both in males as well as in female's vitamin D_3 bolus supplementation significantly reduced IL6, IL8 and TNF levels already after one day and even more after 30 days. As expected, the analysis of the samples of in total 100 study participants came to the same results (Fig. 1**A**).

The main focus of this study was to determine the vitamin D response index of the study participants on the level of vitamin D-triggered changes of cytokine levels. For each individual calculations was made with respect to the respective basal levels, *i.e.,* without vitamin D_3 supplementation. We used the approach to display the changes in cytokine expression (in absolute log scales) between d1 and d0 (Fig. 1**B**) as well as between d30 and d0 (Fig. 1**C**) in relation to the respective changes in 25$(OH)D_3$ serum levels. A linear regression line through the zero point subdivided the graphs into low responders (red, score 0), mid responders (yellow, score 1) and high responders (green, score 3) for the respective parameter. In this way, each of 100 study participants got six scores, the sum of which is proportionally to the vitamin D response index. The index numbers varied from 0 to 16 and allowed the display of all study participants ascendingly (Fig. 2). *K-means* clustering was applied on this dataset and segregated the study participants into 39 low responders (red), 39 mid responders (yellow) and 22 high responders (green).

In order to challenge the scoring results based on cytokine level changes, we used expression changes of the vitamin D target genes *ASAP2*, *CAMP*, *CD14*, *FBP1*, *ITGAM*, *LRRC25* and *NINJ1* in whole blood cells as alternative biomarkers for the responsiveness to vitamin D_3

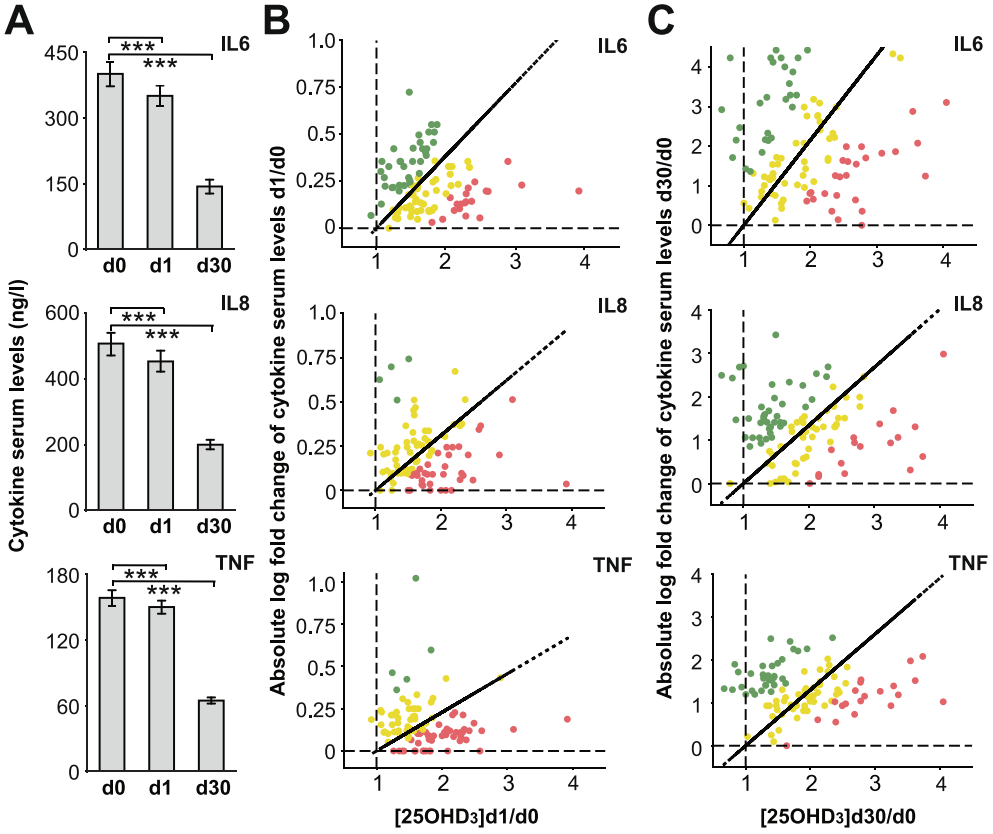

Fig. 1. Change of serum levels of the cytokines IL6, IL8 and TNF of the cohort of 100 participants in response to vitamin D_3 supplementation (**A**). Scoring of the study participants based on individual changes of the cytokines serum levels at d1 (**B**) and d30 (**C**) in relation to d0. Red (low responder): score 0, yellow (mid responder): score 1, green (high responder): score 3. ***, $p < 0.001$ (Student's *t*-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

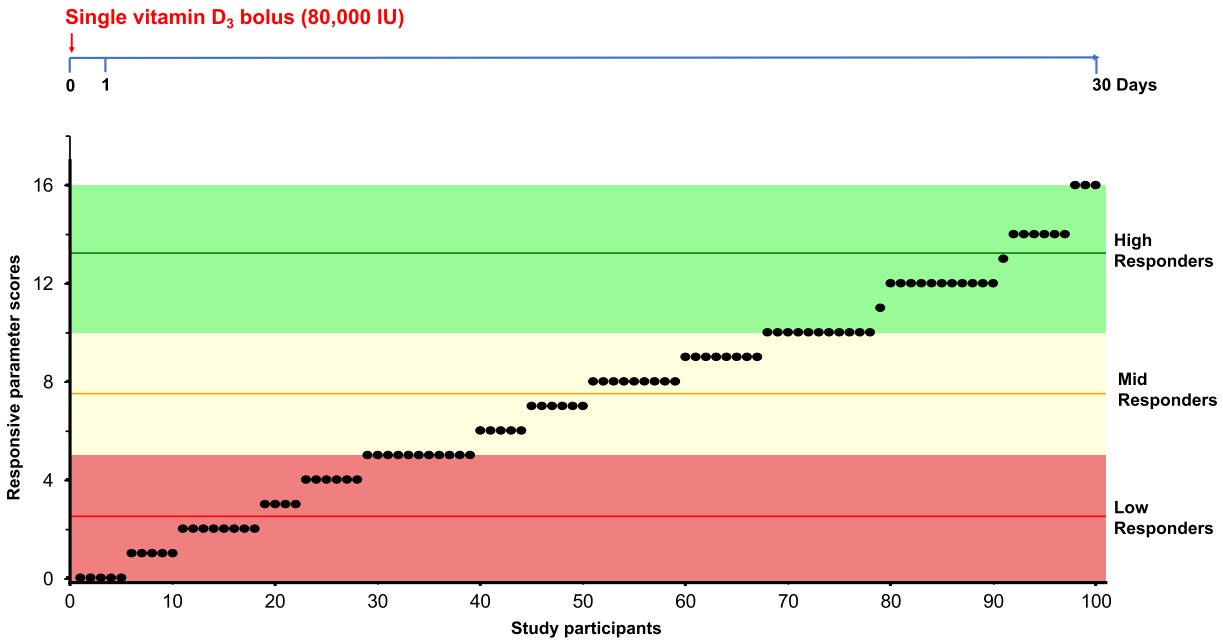

Fig. 2. Ranking of study participants. The *K-means* clustering algorithm was used to segregate the 100 study participants based on the sum of their scores in the responsiveness of cytokines at d1 and d30 (Fig. 1) into low (red), mid (yellow) and high (green) vitamin D responders. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

supplementation. All seven genes are well-known vitamin D target genes as shown by previous vitamin D intervention studies. The vitamin D target gene expression evaluation was done with a subgroup of 37 study participants (13 low, 16 mid and 8 high responders based on cytokine level changes) and resulted in 24 % low responders, 49 % mid responders and 27 % high responders (Fig. 3). Thus, this approach indicates for the chosen subgroup a lower percentage of low responders but more mid responders and high responders than the classification *via* cytokine serum levels.

The direct comparison of both methods indicated for 30 of 37 study participants (81 % of all) a similar scoring and classification. However, for 7 persons the classification based on target gene expression changes in whole blood differed more than 33 % from that calculated from changes in cytokine serum levels. In order to identify the reason for the difference in the classification, we compared the group of 30 persons with a similar score to the group of 7 individuals with a dissimilar score for their BMI and basal serum levels of IL6, IL8 and TNF (Fig. 4). For IL6 and TNF serum levels there is a trend that the medians of the values are higher for the persons with a dissimilar profile than for those with a similar profile, but no statistically significant difference was observed. To rule out other factors for the observed changes in serum levels of cytokines as well as the target gene expressions, we carried out significance tests for other variables like BMI, gender and age, but only 25(OH)D_3 serum levels showed to cause these changes.

4. Discussion

This report combined the data of two recently published intervention studies having 50 native males (AlGhamdi et al., 2022) and 50 females (Alsufiani et al., 2022) from Saudi Arabia as participants. The in total 100 individuals were considered representative for the population in Saudi Arabia, since they were not diagnosed of any major disease. However, at baseline 45 % of the study participants were vitamin D deficient and only 6 % were vitamin D sufficient. Although this is not a good sign for the overall vitamin D health in Saudi Arabia, it provides optimal start conditions for vitamin D_3 intervention studies (Bouillon et al., 2022). The single oral vitamin D_3 bolus supplementation increased the average 25(OH)D_3 serum concentration already after one day by more than 20 nM (8 ng/ml). This increased vitamin D status was maintained 30 days later. The latter is not very surprising, since the bolus (80,000 IU) represents a monthly dose (Mazess et al., 2021). Thus, vitamin D_3 bolus supplementations are effective for rapidly elevating the vitamin D status and confirmed comparable previous studies (Witham et al., 2013). However, in clinical routine a vitamin D bolus should be

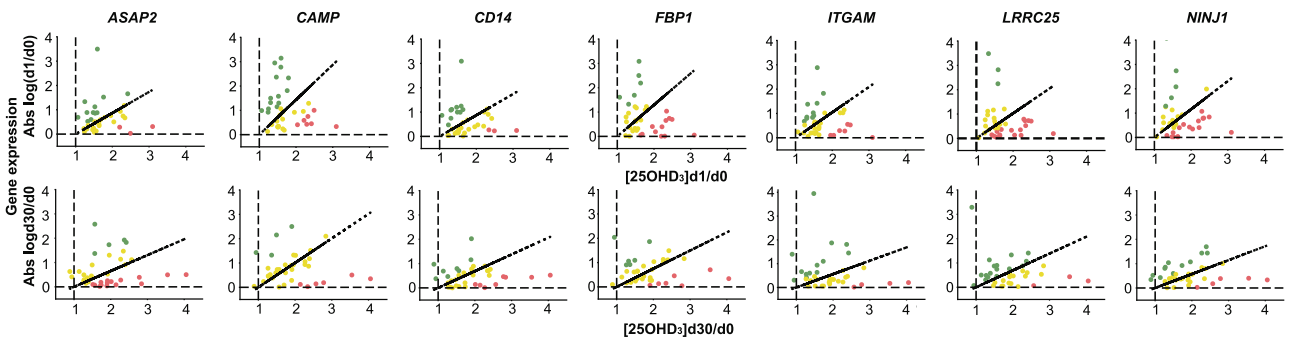

Fig. 3. Scoring of 37 study participants based on changes of the expression of the indicated upregulated vitamin D target genes. Red (low responder): score 0, yellow (mid responder): score 1, green (high responder): score 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

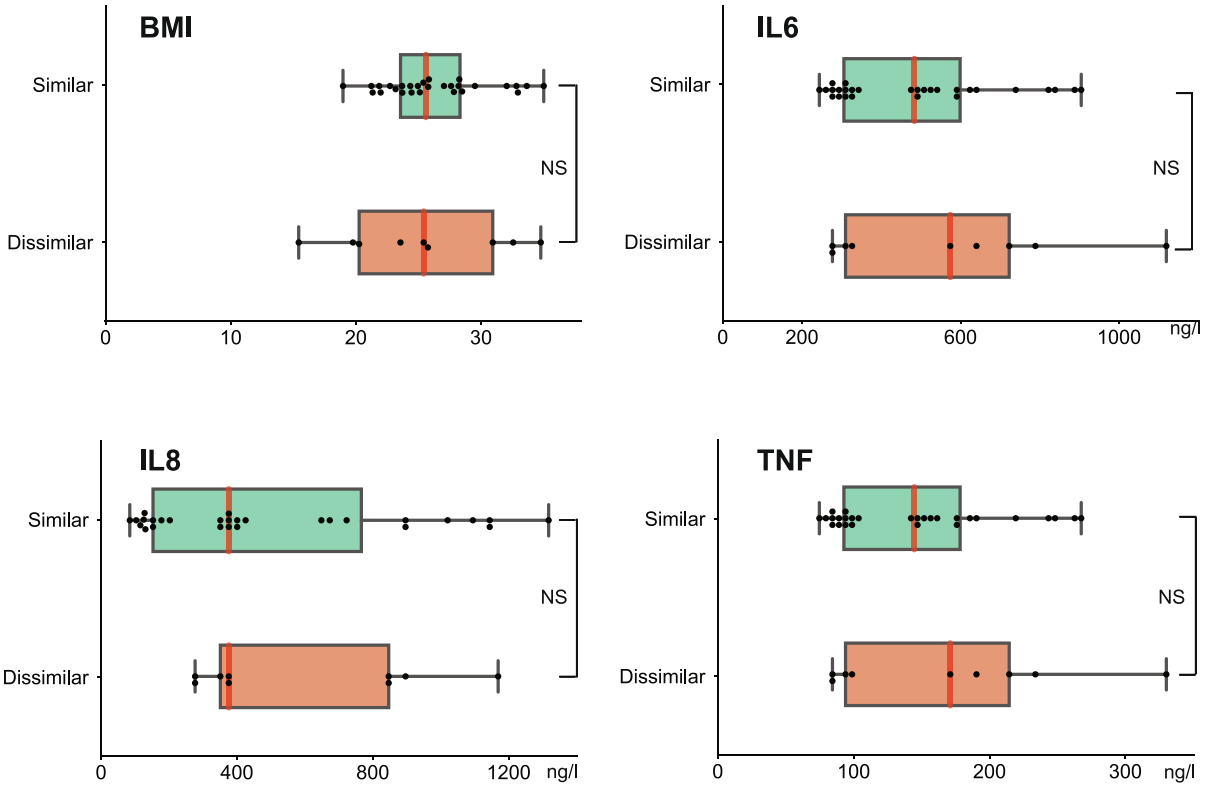

Fig. 4. Comparing similar with dissimilar study participants. Box plots were used to compare the 30 study participants with a similar score in both response index evaluations (green) with 7 persons showing a dissimilar result (red) concerning their BMI and basal serum levels of the cytokines IL6, IL8 and TNF. The red line indicates the median. NS, non-significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

applied only in cases of severe vitamin D deficiency to individuals who do not comply daily supplementation routines (Hill et al., 2022).

The main aim of this study was to use the vitamin D_3 bolus treatment, in order to follow the protocol of the Finnish vitamin D intervention studies VitDbol (NCT02063334) and VitDHiD (NCT 03537027) (Hanel et al., 2020) for a fast determination of the vitamin D response index. In principle, the response index can also be evaluated after a longer-term daily vitamin D_3 supplementation, such as demonstrated by the VitD-met study (NCT01479933) (Carlberg et al., 2013), but there should be a few months between both measurements, *i.e.*, the investigations take longer. With the combined data of both studies from Saudi Arabia we first applied the classical approach (Carlberg, 2016) and used the decrease in the serum levels of the proinflammatory cytokines IL6, IL8 and TNF one and thirty days after vitamin D_3 bolus supplementation as a biomarker for the effects of vitamin D. The combined score of the six single response evaluations allowed to segregate the 100 individuals into 39 % low responders, 39 % mid responders and 22 % high responders. The most critical subpopulation is that of the low responders and based on the vitamin D-triggered decrease in proinflammatory cytokines their proportion in this Saudi cohort is clearly higher than the rate in Finnish cohorts (25 %) (Carlberg et al., 2013, Seuter et al., 2017).

The measurement of the expression changes of vitamin D target genes like in this or previous studies is an alternative approach for determining the vitamin D response index. This study is the first one directly comparing both methods and demonstrated that for the majority of the tested persons the result is similar. This is an interesting result, since the populations in Saudi Arabia and Finland are genetically rather different and live in a distinct environment (Investigators et al., 2021). Therefore, this study provides evidence that the concept of the vitamin D response index may be extrapolated to all other populations.

Nevertheless, there is some discrepancy in the determination of vitamin D response index, which may result from higher rate of chronic inflammation detected by elevated basal levels of proinflammatory cytokines, such as IL6 and TNF. Although the study participants did not have any major diseases, they differed significantly in their BMI, which ranged from 14.9 (underweight) to 39.1 (severe obesity). Accordingly, the basal serum levels of IL6, which may be majorly derived from adipocytes and associated macrophages (Han et al., 2020), also showed a large variation. This suggests that for overweight and obese persons the determination of the vitamin D response index *via* the downregulation of proinflammatory cytokines can lead to inaccurate results. Since previous assessment of vitamin D response indices had been performed with normal weight persons (BMI ranging from 20 to 25) (Carlberg et al., 2013, Seuter et al., 2017), this aspect before had not been an issue. Thus, vitamin D response index evaluations of overweight and/or diseased persons should be determined on the basis of gene expression rather than *via* changes of cytokine levels.

In a Finnish cohort of healthy, normal weight persons the rate of low vitamin D responders was determined as 25 % (Seuter et al., 2017). In contrast, 50 % of the participants of the Saudi cohort were overweight or obese and, based on cytokine levels, their rate of low responders was 39 %. This higher rate of low responders may be related to the fact that in the past in Saudi Arabia there was no evolutionary pressure leading to their outnumbering, since the traditional lifestyle provided enough sun exposure for endogenous vitamin D_3 production. However, it is also possible that in northern Europe with long dark winters, like in Finland, there was evolutionary selection of high vitamin D responders, for which a rather low vitamin D status is still sufficient for the activation of vitamin D target genes. Thus, the change of lifestyle in Saudi Arabia over the last two generations made the higher rate of low responders a challenge that needs to be overcome by more intensive vitamin D_3 supplementation (Al-Daghri et al., 2019, Amer et al., 2022, Sabico et al., 2023). In this context also the significant rise in average BMI (Althumiri et al., 2021) needs to be considered, *i.e.*, vitamin D_3 supplementation should be adjusted by weight.

Determining the vitamin D response index of a person is far more

effort than measuring the vitamin D status, since for the latter only a single measurement of the 25(OH)D_3 serum level is needed, while for the response index two samples and a number of parameters, such as 25(OH)D_3, cytokines and/or gene expression have to be assessed (Carlberg, 2016). Therefore, it is unlikely that response index measurements will enter routine clinical diagnosis. However, more important is the public awareness of a vulnerable subpopulation of low responders, which need a higher dose of daily vitamin D_3 supplementation than indicated by national guidelines. In most countries the recommendations for daily vitamin D_3 supplementation are in the order of 10–20 μg (400–800 IU) (Spiro and Buttriss, 2014). This may be sufficient for high vitamin D responders but is insufficient for low responders. Since this study confirmed that low vitamin D responders are found not only in Finland and that their percentage may be even higher in countries closer to the equator, but the recommended doses of national guidelines should also be reconsidered. A supplementation with 1 μg vitamin D_3 (40 IU)/kg body weight may serve as a rule that can be easily memorized.

Progress in technology makes measuring gene expression on a transcriptome-wide level a standard for all types of future intervention studies. Moreover, in the context of many nutritional intervention studies 25(OH)D_3 serum level are determined, even if the primary focus of the trials may not be vitamin D. In this way, data are collected that allow the assessment of the vitamin D response index of the study participants. This will facilitate the segregation of the study participants in low, mid and high responders, which could lead to more specific presentations the trial results. The underlying hypothesis of this suggestion is that due to the pleiotropic actions of vitamin D, a low vitamin D response index may affect the results of a larger number of nutritional intervention studies.

This study has a few limitations. Although the cohort size is with 100 individual larger than that of comparable studies from Finland, which had 35–71 participants, an even larger number of studied persons would have been desirable, in order to obtain more accurate numbers on the percentage of low vitamin D responders. Moreover, the underlying studies (AlGhamdi et al., 2022, Alsufiani et al., 2022) are not classical controlled trials. The studies had a focus on adults in an age range of 18–53, but it would be interesting to obtain data also from children as well as from elderly. Finally, the availability of transcriptome-wide data would have further increased the accuracy of the vitamin D response index determination.

5. Conclusion

The concept of the vitamin D response index could be confirmed by a Saudi cohort. In Saudi Arabia the rate of low vitamin D responders is at least as high as in Finland but probably even 14 % larger when determined based on cytokine level changes. This suggests that under the present modern lifestyle, which also in sunny Saudi Arabia is characterized by low UV-B exposure, there should be daily vitamin D_3 supplementation in doses between 2000 and 4000 IU (depending on body weight), which are sufficient even for low vitamin D responders.

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Institutional review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethical committee of the Faculty of Medicine, King Abdulaziz University (ref. no.30–18).

Informed consent Statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Shareefa A. AlGhamdi: Writing – review & editing, Supervision, Methodology, Conceptualization. **Ranjini Ghosh Dastidar:** Software, Formal analysis. **Maciej Rybiński:** Visualization, Formal analysis. **Hadeil M. Alsufiani:** Writing – review & editing, Methodology, Conceptualization. **Sawsan O. Khoja:** Conceptualization. **Nusaibah N. Enaibsi:** Methodology, Data curation. **Safa F. Saif:** Methodology, Data curation. **Carsten Carlberg:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2024.102137.

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