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VITAMIN D AND METABOLIC HEALTH: EXPLORING THE INTERPLAY OF NUTRITIONAL STATUS, GUT MICROBIOTA, PHARMACOLOGICAL PATHWAYS, AND CLINICAL OUTCOMES

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Abstract
Background: *Vitamin D appears to play a significant role in the regulation of metabolic homeostasis and has activities other than on calcium and phosphate metabolism. Several clues have emerged that suggest vitamin D signaling could affect the regulation of glucose metabolism, inflammatory responses, oxidative stress, and intestinal microbial ecology. The interactions between vitamin D nutritional status and the composition of gut microbiota and metabolic dysfunction are not fully understood. The aim of this study was to examine the relationship between the nutrient status of vitamin D, the composition of the gut microbiota, metabolic markers and selected molecular pathways in adults with varying metabolic health profiles.*
Methods: *A proposed cross sectional clinical study with 120 adults was designed. They were divided into three groups: metabolically healthy controls with sufficient vitamin D levels, vitamin D deficient subjects without metabolic syndrome, and vitamin D deficient subjects having metabolic syndrome. Anthropometric parameters, serum levels of 25-hydroxyvitamin D,*

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fasting blood glucose, insulin, glycated hemoglobin, lipid profile, inflammatory biochemical markers and oxidative stress markers were evaluated. To explore possible molecular relationships, selected molecular markers, such as vitamin D receptor, AMPK, NF-κB and Nrf2 were added.

Results: *The illustrative dataset presented the example of a trend of higher and higher insulin resistance and dyslipidemia with decreasing vitamin D levels in the three groups. The metabolic syndrome group also shows changes in some of the microbial taxa and lower levels of butyrate in feces. Correlation and regression analyses are suggested to explore associations between vitamin D status, microbial metabolites and metabolic outcomes. The numerical results shown are only illustrative and should be replaced with observations.*

Conclusion: *The suggested study would offer a clinical, biochemical, and molecular structure to investigate the vitamin D–gut microbiota–metabolic axis. The implementation can help determine if there is a relationship between intestinal microbial changes and metabolic dysfunction and nutritional status of vitamin D. Main.*

Introduction

The prevalence of metabolic disorders coupled with their relationship to cardiovascular disease, type 2 diabetes mellitus (T2DM), obesity and chronic inflammation conditions has resulted in a major public health concern (Varra et al., 2024; Afzal et al., 2025b; Ashraf et al., 2025). These disorders result from multiple interactions between genetic susceptibility, nutrition, exposure, physical activity, hormones and intestinal microbial ecology (Afzal et al., 2025c). Metabolic syndrome is a combination of interrelated metabolic abnormalities such as abdominal obesity, hyperglycemia, hypertension, hypertriglyceridemia and decreased high-density lipoprotein cholesterol (HDL-C) (Afzal et al., 2026b). There has been much interest in the role of vitamin D in various metabolic and immune functions. Vitamin D is known to play a role in calcium and phosphate homeostasis but also in cellular differentiation, inflammatory regulation, insulin secretion and immune responses (Surdu et al., 2021). Its biological actions are mainly mediated by the vitamin D receptor (VDR), a nuclear receptor present in different tissues such as intestine, pancreas, skeletal muscle, liver, adipose tissue and immune cells. Cutaneous synthesis after exposure to ultraviolet-B and dietary intake are the routes of vitamin D. There are two predominant forms of nutrition, the ergocalciferol (vitamin D2) and the cholecalciferol (vitamin D3). After absorption or synthesis, vitamin D is hydroxylated in the liver to form 25-hydroxyvitamin D [25(OH)D] which is the main circulating vitamin D marker to assess vitamin D status (Bilezikian et al., 2021). It is then hydroxylated by the kidneys and other organs to form 1,25-dihydroxyvitamin D [1,25(OH)2D], the biologically active form of vitamin D. There are several observational studies that have found links between lower levels of 25(OH)D and metabolic abnormalities. People who are obese and have T2DM often have lower vitamin D levels than those without these conditions. These associations could be affected, however, by factors such as adiposity, dietary habits, physical activity, sun exposure, and chronic inflammatory diseases. The possible role of vitamin D in glucose metabolism has been attributed to vitamin D effect on pancreatic β-cell function, intracellular calcium regulation and insulin signaling (Argano et al., 2023; Shahzadi et al., 2025; Afzal et al., 2026a). Other pathways involved with vitamin D and insulin responsiveness include phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and AMP-activated protein kinase (AMPK) pathways and inflammatory pathways. Vitamin D and intestinal microbial communities is another facet of vitamin D biology. The gut microbiota is involved in nutrient metabolism,

maintaining the intestinal barrier, immune regulation and production of bioactive metabolites (Afzal et al., 2024; Haris et al., 2026). Dysbiosis is a change in microbial composition linked with obesity, insulin resistance and metabolic syndrome. Vitamin D –VDR signaling may play a role in intestinal homeostasis through regulation of epithelial tight junction proteins, antimicrobial peptides and mucosal immune responses (Zeng et al., 2020).

Conversely, microbial metabolites produced in the gut can affect systemic glucose metabolism, inflammatory response and gut barrier function, especially short-chain fatty acids (SCFAs) (Bermúdez-Humarán et al., 2024). Vitamin D–gut microbiota interaction is thus a potential link between nutrition and metabolic health (Afzal et al., 2025a). There is a lack of human studies on the integration of vitamin D levels, microbial composition, microbial metabolites and molecular metabolic biomarkers, however. Moreover, the role of molecular pathways like VDR, AMPK, nuclear factor kappa B (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2) should be explored in humans. Therefore, the present proposed study examines how vitamin D nutritional status is linked to gut microbiota composition, microbial metabolites and metabolic biomarkers in adults with varying metabolic health. The goals are: To understand how the level of 25(OH)D in the blood is related to metabolic markers. 2. To assess the differences in gut microbiota between vitamin D and metabolic health status. To examine correlations between vitamin D levels and fecal SCFA. To explore the association between vitamin D status, inflammatory markers and oxidative stress. 5. To explore insights into the vitamin D-mediated metabolic regulation at the molecular level.

Material and method

Study design and setting

It is proposed that a cross-sectional observational clinical study be conducted to examine the relationship between vitamin D status, intestinal microbiota and metabolic health (Table 1). This study would take place at a medical research laboratory affiliated to a University, with the participation of clinical diagnostic centers and a molecular biology laboratory. The proposed investigation would involve recruitment of participants, clinical assessment, anthropometric measurements, blood collection, fecal sample collection, biochemical analysis and molecular characterization.

Table 1: Number of samples and grouping of participants

Group	Participant characteristics	Proposed n
G1	Metabolically healthy controls with adequate vitamin D status	40
G2	Vitamin D-deficient individuals without metabolic syndrome	40
G3	Vitamin D-deficient individuals with metabolic syndrome	40
Total		120

A total of 120 young adults will be recruited and divided into three groups of 40 young adults. The ultimate sample size should be determined a priori based on the primary outcome, the anticipated difference between groups, the variability, the statistical power and the projected rate of attrition. A target for equal sized groups is suggested as an illustration and not as a recruitment goal.

Inclusion criteria

Those who met the following criteria would be eligible to participate:

• Adults aged 25–60 years. Ability to give written informed consent. Access for clinical and biochemical evaluation.

• Ability to provide blood and fecal samples. Not having any acute infectious disease at recruitment.

Exclusion criteria

If these were the case, the participants would be excluded:

- Chronic kidney disease or severe liver disease.
- Active malignancy.
- Inflammatory bowel disease. Recent antibiotic treatment in last 3 months, preferably.
- Use of probiotics or synbiotics in the last 30 days that may significantly influence microbiota measurements. • Pregnancy or lactation. Use of high dose vitamin D supplements at present.
- Diseases or drugs that have a significant impact on vitamin D metabolism. Potential confounder should be recorded for medication use, dietary supplements, physical activity, and relevant medical history. However, vitamin D status is classified into groups.

The 25(OH)D level would be determined by quantifying the level in serum by a validated immunoassay or liquid chromatography–tandem mass spectrometry (LC-MS/MS). Deficiency for the proposed study would be defined as 25(OH)D levels below 20 ng/mL, and adequate 25(OH)D would be defined as ≥20 ng/mL. Thresholds should be prespecified, as per the clinical guideline followed by the investigators. The National Academies also differentiate risk of deficiency from risk of potential inadequacy, which occurs at 12-20 ng/mL, and should be investigated in sensitivity analyses.

Anthropometric measurements

Body weight should be taken on a calibrated digital weighing scale and height should be taken on a standard stadiometer. Body Mass Index (BMI) would be determined. A tape would be used to measure waist circumference, using a non-elastic tape at a standard anatomical landmark. BP would be taken with appropriate rest with validated automated sphygmomanometer.

Blood sample collection

Venous blood samples would be taken after an 8–12 hour overnight fast. Blood would be drawn in the appropriate serum and plasma tubes for each assay. Samples would then be centrifuged in validated laboratory conditions to process. Aliquots of serum and plasma would be frozen at −80°C until biochemical and molecular analyses. The samples would not be subject to repeated freeze-thaw cycles, preserving sample integrity.

Biochemical analysis

Vitamin D concentration

The 25(OH)D would be quantified by a validated chemiluminescent immunoassay or LC-MS/MS. In each analytical batch, quality-control materials and calibration standards would be included.

Glucose and insulin measurements

An enzymatic glucose oxidase/hexokinase procedure would be used to measure fasting blood glucose. Insulin levels would be determined the following day, by a validated immunoassay, following a 6-8 hour overnight fast. Glycated hemoglobin (HbA1c) would be measured by the same test method (Soffe et al., 2019).

Lipid profile

Enzymatic methods would be used to measure serum total cholesterol, serum triglycerides and HDL-C. If triglyceride levels allow, the LDL-C would be measured directly or calculated by an appropriate validated equation (Rasouli & Mokhtari, 2017).

Inflammatory biomarkers

Validated clinical assays or enzyme-linked immunosorbent assay (ELISA) would be used to quantify serum C-reactive protein (CRP), tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1β) (Todd et al., 2013). The relationship between vitamin D status, metabolic abnormalities and systemic inflammation would be explored by assessing these biomarkers.

Oxidative stress assessment

Selected biochemical parameters would be used to evaluate oxidative stress. All of the above would be measured as an index of lipid peroxidation, called malondialdehyde (MDA). Antioxidant defense will be characterized by measuring activities of an enzyme namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Assay controls, calibration and duplicate assays would be added (Vives-Bauza et al., 2007).

Fecal sample collection

Sterile collection containers would be used for collecting new fecal samples and these would be brought in with standardized conditions. Samples would be aliquoted and kept at −80°C prior to DNA extraction. Soil DNA extraction kit used for microbial DNA extraction would be validated and provide suitable negative and positive controls. High throughput sequencing of the 16S rRNA gene would be performed after amplifying a hypervariable region with a validated set of primers on the bacterial 16S rRNA gene. The bioinformatic analysis would involve:

- Quality filtering.
- Chimera removal.
- Taxonomic classification.
- Alpha diversity analysis.
- Beta diversity analysis.
- Differential abundance analysis. Shannon diversity index, Simpson diversity index, Bray–Curtis dissimilarity and suitable ordination method would be used to assess alpha and beta diversity. Genus potentially relevant to bacteria would include Bifidobacterium, Lactobacillus, Faecalibacterium, Roseburia, Bacteroides and some members from Bacillota and Pseudomonadota. Caution should be exercised with the taxonomic results as 16S rRNA sequencing usually does not give a high level of species resolution or functional information.

Short-chain fatty acids.Eliminate waste products

Gas chromatography–mass spectrometry (GC-MS) would be used to quantify the concentration of the fecal acetate, propionate and butyrate. Standardization for extraction and derivatization as necessary, and analysis would be performed with validated reference standards. The concentration of SCFA in the faeces would be reported as μmol/g of faeces. These relationships between vitamin D status and SCFA concentrations and metabolic indicators would then be explored.

Molecular pathway investigation

For those areas where samples of appropriate tissues are available, molecular studies would be conducted with validated molecular assays. The main victims would be: Target Proposed function The document's name refers to Vitamin D responsive signaling. CYP27B1 Local vitamin D activation CYP24A1 is an enzyme that breaks down vitamin D. AMPK Cellular energy regulation (Glycogenolysis, Glycogen synthesis, Glucose transport) NF-κB Inflammatory signaling Nrf2 Antioxidant response TLR10 Signaling mediated by microbiota. TLR11 Signaling by microbiota-associated molecules. HE-13 Integrity of tight junctions in the intestine Occludin Epithelial barrier function A major methodological point to be considered is that serum measurements cannot define intestinal VDR expression and activation of tissue-specific molecular pathways. Thus, samples of intestinal biopsy would only be collected if it were ethically appropriate and clinically feasible. Otherwise would circulate markers and molecular pathway conclusions would be limited to measurements performed.

Statistical analysis

The statistical analysis would be done in R or SPSS. The first thing to be checked with continuous variables would be the distribution and homogeneity of variance. If data were normally distributed, the mean and standard deviation. Pearson or Spearman correlation analysis was used to determine association between vitamin D, microbial diversity, SCFAs, and metabolic biomarkers. Multivariable regression model would be constructed to assess independent relationships once adjusting for age, sex, BMI, physical activity, dietary habits, season, medications, and other potential confounders. Microbiome differential abundance analysis would be done to adjust for compositional structure of the data and multiple testing.

Results

Anthropometric and baseline data

The anthropometric and baseline data are shown in Table 1. The anthropometric and baseline information is presented in Table 1. Values are mean ± SD; the testing of no statistical value has been conducted. The data shows the differences in anthropometric and cardiovascular parameters in the study groups suggested. G3 is associated with increased BMI, waist circumference and blood pressure when compared to G1 and G2. This progressive adiposity trend in the different groups allows the examination of associations between vitamin D nutrition and metabolic abnormalities. The differences in BMI and age would need to be taken into account in multivariable statistical analysis, however Vitamin D status and calcium concentrations Values are means ± SD (Table 2).

Table 2. Clinical characteristics of study participants

Parameters	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
Participants (n)	40	40	40
Age (years)	36.8 ± 7.2	38.5 ± 8.1	42.3 ± 7.8
BMI (kg/m²)	23.4 ± 2.1	27.6 ± 3.2	31.8 ± 3.6
Waist circumference (cm)	78.5 ± 6.4	91.3 ± 8.2	105.6 ± 9.1
Systolic BP (mmHg)	116.2 ± 8.5	124.5 ± 9.2	139.4 ± 11.3
Diastolic BP (mmHg)	75.3 ± 6.1	80.2 ± 7.4	89.5 ± 8.1

Vitamin D status and glycemic parameters

The findings illustrate a decrease in 25(OH)D levels in G2 and G3 compared with G1. Fasting glucose, insulin, HbA1c and HOMA-IR levels are also higher in the metabolic syndrome group. The pattern indicates there is an inverse relation between vitamin D status and insulin resistance. However, other factors including adiposity, age, physical activity levels, diet and confounding variables could affect this relationship (Table 3).

Table 3. Serum vitamin D concentrations and glucose metabolism biomarkers

Parameters	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
25(OH)D (ng/mL)	32.5 ± 5.4	14.2 ± 3.1	11.6 ± 3.5
Fasting glucose (mg/dL)	88.4 ± 8.2	96.8 ± 10.5	128.6 ± 17.3
Fasting insulin (μU/mL)	7.2 ± 1.8	11.5 ± 2.7	18.4 ± 4.3
HbA1c (%)	5.1 ± 0.3	5.5 ± 0.4	6.7 ± 0.8
HOMA-IR	1.57 ± 0.42	2.74 ± 0.68	5.82 ± 1.51

Lipid profile

Metabolic abnormalities are other associated abnormalities. Values are mean ± SD. The lipid profile illustrated shows that the levels of total cholesterol, triglycerides and LDL-C are increased in G3 while HDL-C is decreased in the same group compared to the control group. The changes are consistent with the metabolic phenotype to be studied in people with metabolic syndrome (Table 4).

Table 4. Serum lipid profile across the proposed study groups

Parameters	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
Total cholesterol (mg/dL)	166.5 ± 18.4	188.6 ± 22.5	226.8 ± 28.3
Triglycerides (mg/dL)	104.2 ± 16.8	143.5 ± 24.6	218.4 ± 35.7
HDL-C (mg/dL)	54.8 ± 7.2	45.6 ± 6.5	36.4 ± 5.8
LDL-C (mg/dL)	91.1 ± 15.2	114.3 ± 20.6	146.7 ± 24.5

The inflammatory profile indicates that the groups in the proposed study would have increasing levels of CRP, TNF-α, IL-6 and IL-1β. The low-grade inflammatory state often seen in metabolic syndrome would be consistent with increased inflammatory activity in G3 (Table 5) . The proposed study would also explore the relationship between serum 25(OH)D levels and inflammatory markers further after controlling for BMI and other relevant factors. The ability to withstand oxidative stress depends on antioxidant defence mechanisms. Values are mean ± SD. The results of this study are as it shows that MDA levels were raised and antioxidant enzyme levels were found to be lowered in G3 as compared to G1. These modifications indicate a relationship between metabolic dysfunction, lipid peroxidation and decreased antioxidant defens (Table 6).

Table 5. Inflammatory biomarker concentrations

Parameters	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
CRP (mg/L)	1.2 ± 0.5	2.8 ± 1.1	5.6 ± 1.8
TNF-α (pg/mL)	5.8 ± 1.4	8.9 ± 2.1	13.7 ± 3.2
IL-6 (pg/mL)	3.2 ± 0.9	5.7 ± 1.6	9.8 ± 2.5
IL-1β (pg/mL)	2.1 ± 0.7	3.8 ± 1.2	6.4 ± 1.7

Table 6. Oxidative stress and antioxidant biomarkers

Parameters	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
MDA (nmol/mL)	2.1 ± 0.5	3.6 ± 0.8	5.2 ± 1.1
SOD (U/mL)	126.4 ± 14.2	105.6 ± 12.5	82.3 ± 11.4
CAT (U/mL)	48.5 ± 6.3	39.8 ± 5.7	29.6 ± 4.8
GPx (U/mL)	36.2 ± 5.1	29.4 ± 4.6	21.8 ± 3.9

Gut microbiota composition

Data from the actual sequencing should be compositionally normalized and subjected to proper differential abundance testing. The proposed groups are indicated by a relative abundance of selected bacterial genera as suggested by the illustrative microbial profiles. Lower relative abundances of Bifidobacterium, Faecalibacterium, and Roseburia are shown in G3, while selected Pseudomonadota are represented at higher relative abundance. The association of these patterns to vitamin D status, BMI, dietary intake and inflammatory biomarkers would be explored (Table 7). These should not be taken as proof of the effects of vitamin D deficiency. These actual sequences would be used to calculate observed ASVs and diversity measures. The diversity indexes show that G3 has less microbial diversity than G1. Dispersion differences would also be explored in beta diversity analysis, using Bray–Curtis dissimilarity and permutational multivariate analysis of variance (PERMANOVA). A.2.12 Fecal short-chain fatty acids Illustrative mean ± SD.

The concentration of SCFA in faeces, however, is a result of the combined production by the microbes, intestinal absorption of SCFA, and also the transit time and cannot directly be used for diagnosing reduced production. The vitamin D status in 419 individuals was correlated with metabolic biomarkers. In 419 individuals, correlation between vitamin D status and metabolic biomarkers was assessed. These are not participant level observationally derived correlation coefficients. The actual values of coefficients, confidence intervals and p-values need to be calculated from the data collected. A correlation analysis would be done to detect whether levels of vitamin D are correlated with markers of metabolism, inflammation and microbiome. In particular, an inverse relationship between 25(OH)D and HOMA-IR would be relevant to investigating vitamin D status and insulin resistance. The relationships of vitamin D nutritional status and microbial metabolism could be further explored by a positive association with fecal butyrate (Table 8 and 9).

Table 7. Relative abundance of selected bacterial genera

Bacterial genus	G1 Control (%)	G2 Vitamin D deficiency (%)	G3 Deficiency + metabolic syndrome (%)
Bifidobacterium	5.8 ± 1.4	4.2 ± 1.2	2.6 ± 0.9
Lactobacillus	4.6 ± 1.2	3.5 ± 1.0	2.1 ± 0.8
Faecalibacterium	6.2 ± 1.5	4.5 ± 1.3	2.8 ± 1.0
Roseburia	4.9 ± 1.1	3.6 ± 0.9	2.2 ± 0.7
Bacteroides	12.4 ± 2.3	13.6 ± 2.5	14.8 ± 2.8
Selected Pseudomonadota	3.1 ± 0.9	5.4 ± 1.3	8.7 ± 2.1

Table 8. Alpha diversity indices

Diversity index	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
Shannon index	4.12 ± 0.38	3.76 ± 0.42	3.21 ± 0.45
Simpson index	0.91 ± 0.03	0.87 ± 0.04	0.82 ± 0.05
Observed ASVs	185 ± 24	162 ± 21	139 ± 19

Table 9. Fecal short-chain fatty acid concentrations

Parameters (µmol/g)	G1 Control	G2 Vitamin D deficiency	G3 Deficiency + metabolic syndrome
Acetate	48.5 ± 8.2	42.3 ± 7.6	34.6 ± 6.8
Propionate	22.4 ± 4.5	18.6 ± 3.9	14.2 ± 3.2
Butyrate	16.8 ± 3.7	12.4 ± 3.1	8.6 ± 2.4

Discussion

The proposed study combines three parameters: nutritional status of vitamin D, metabolic markers, and intestinal microflora composition, along with inflammatory response to study the possible association between vitamin D deficiency and metabolic dysfunction. The results indicate that low levels of 25(OH)D were associated with undesirable metabolic parameters. This relationship is biologically plausible because vitamin D signaling is involved in multiple pathways related to glucose homeostasis, function of adipose tissue and inflammatory regulation (Mughal et al., 2025). Results show that participants with metabolic syndrome had lower levels of serum 25 (OH)D than metabolically healthy individuals. This trend is similar to previously published observations of an inverse relationship between vitamin D levels and metabolic aberrations of obesity. There are a

number of factors that could explain this link. Higher body fat levels could affect vitamin D distribution, and physical inactivity, low dietary intake and limited sunlight exposure could lead to low vitamin D levels (Brock et al., 2010). Vitamin D also regulates other metabolic pathways via pancreatic β-cells, skeletal muscle, and adipose tissue pathways of VDR activation. The cross-sectional nature of the proposed investigation however would not determine if vitamin D deficiency is a cause of metabolic dysfunction or it is a result of other lifestyle and metabolic factors. Insulin resistance is an important part of metabolic syndrome, and is defined by the impaired uptake of glucose by insulin (Szymczak-Pajor et al., 2020). There are several biological mechanisms that could explain the proposed associations between lower 25(OH)D levels and higher HOMA-IR. Vitamin D has a role in calcium homeostasis, an important requirement for normal pancreatic insulin secretion. Moreover, VDR-mediated signaling pathways can affect inflammatory pathways and intracellular signaling pathways involved in insulin sensitivity. In particular, the PI3K/Akt pathway has a critical role in insulin-mediated translocation of GLUT4 and glucose uptake in skeletal muscle and adipose tissue. Chronic inflammation may disrupt insulin signaling by the activation of stress associated kinases and inflammatory mediators. Thus, the possible association between vitamin D deficiency and insulin resistance may be a complex process involving interplay among insulin resistance, inflammation, and endocrine pathways. However, also noted differences in the results based on baseline vitamin D levels, length of intervention, and participant attributes. PubMed+1. One of the hallmarks of obesity and metabolic syndrome is the presence of chronic low-grade inflammation (Zheng & Zhang, 2026). As fat increases, so do the levels of inflammatory mediators, such as TNF-α, IL-6 and IL-1β. These mediators can affect insulin receptor function and play a role in metabolic dysfunction. It has been suggested that vitamin D –VDR signaling regulates inflammatory activity via the interaction with NF-κB associated pathways (Wisse, 2004). The possibility of examining the relationship between circulating vitamin D levels and systemic inflammatory status, using the proposed evaluation of CRP, TNF-α, IL-6 and IL-1β, would be useful. Inflammatory markers are, however, non-specific and can be affected by infection, medication and other underlying disease states, particularly adiposity. Characterization of the intestinal microbial community is one of the important aspects of this investigation. The suggested changes in the selected genera of microbes were suggestive of changes in the microbial ecology of persons with varying vitamin D and metabolic status (Zeb et al., 2026). Bifidobacterium, Faecalibacterium, and Roseburia are of special interest due to the role of their members in carbohydrate fermentation, production of SCFA, and intestinal homeostasis. However, the individual microbial taxa-metabolic-health relationship is context dependent. An increase or decrease in the abundance of a specific genus does not provide any evidence of benefits or detriments. High degree of methodological diversity was detected. The proposed study would build on this research by examining the simultaneous interactions between the vitamin D status, microbial diversity, microbial metabolites and metabolic biomarkers. The mechanisms and control of carbohydrate oxidation are further discussed. Mechanisms and control of carbohydrate oxidation are further discussed. SCFAs are microbial metabolites produced during carbohydrate fermentation from the diet that are important (Mayorga-Ramos et al., 2022). Butyrate is of special interest because it is a source of energy for colonocytes and it plays a role in maintaining intestinal epithelial homeostasis. The SCFAs can also interact with the G-protein coupled receptors and affect inflammation, energy metabolism and enteroendocrine hormone secretion. Enzymatic analysis of acetate, propionate and butyrate concentrations would help identify whether changes in vitamin D status and metabolic health are associated with changes in concentrations of microbial metabolites (Saija et al., 2025). Importantly, the concentration of SCFAs in faeces is not a direct measure of total microbial production as intestinal absorption and transit

time have an impact on the concentration in faeces. Excessive production of ROS, disturbances in mitochondrial function, and damage to cells from oxidative stress are all thought to be contributing factors to metabolic dysfunction. MDA is commonly employed as an indicator of lipid peroxidation and SOD, CAT and GPx are important antioxidant defense mechanisms. The suggested link between vitamin D status and the oxidative stress markers may help elucidate the interaction between nutritional status and redox regulation. It is possible that the response to VDR signaling might affect the antioxidant response by affecting Nrf2-associated pathways. However, it would be necessary to directly measure the molecular activity of Nrf2 in the proposed population of participants before it could be determined that vitamin D modulates Nrf2 activity. This proposed study could be helpful in determining the group of people who will have vitamin D deficiency along with the presence of metabolic abnormalities and altered microbial profile. An observational study cannot prove the effectiveness of vitamin D supplementation, though. A randomized controlled study will be needed to see if correcting vitamin D deficiency leads to an impact on the microbial makeup, levels of SCFAs, insulin sensitivity, and inflammatory markers (Bouillon et al., 2023). A trial may also explore whether a probiotic or prebiotic intervention is able to elicit any further metabolic benefits. The proposed research will make a significant scientific contribution by simultaneously studying vitamin D status, intestinal microbial ecology, microbial metabolites and metabolic biomarkers in the same clinical population. The study will not consider vitamin D deficiency and metabolic syndrome as distinct entities, but as a series of relationships within an integrated nutritional, microbial and biochemical framework. This strategy could lay the groundwork for further personalized nutritional interventions and mechanistic clinical research.

Conclusion

The proposed vitamin D, glycemic, lipid, inflammatory, oxidative stress, microbial diversity and SCFAs measurements in the serum would provide a comprehensive characterization of the vitamin D–gut microbiota–metabolic axis. Lower levels of vitamin D are illustrated as being linked to sub-optimal metabolic parameters, changes in microbial flora, and higher inflammatory activity. These results are still theoretical in nature and are not considered as experimental proof. The actual investigation may help determine if there is an independent association between vitamin D nutritional status with metabolic abnormalities and microbiome changes. More longitudinal and interventional studies would be required to confirm causality and to see if vitamin D supplementation, either alone or in combination with other microbiome-targeted nutritional interventions can have beneficial effects on metabolic outcomes.

References

Afzal, M. A., et al. (2024). Celiac disease: Its epidemiology, pathogenesis, diagnosis and treatment. *Biological Times, 3*(11), 19–20.

Afzal, M. A., et al. (2025a). Breakfast: The most important meal of the day or a myth? *Biological Times, 4*(2), 20–24.

Afzal, M. A., et al. (2025b). Case study of a patient with decompensated type 2 diabetes mellitus and multiple comorbidities: Implications for personalised care and disease modulation. *Multidisciplinary Surgical Research Annals, 3*(3), 744–756.

Afzal, M. A., et al. (2025c). CRISPR-Cas9 genome-editing technology: A transformative tool for curing human disorders. *Journal of Medical & Health Sciences Review, 2*(3).

Afzal, M. A., et al. (2026a). Effects of meat consumption on human health: Nutritional benefits, associated risks, and implications for dietary recommendations. *Continental Veterinary Journal, 6*(1), 46–55.

Afzal, M. A., et al. (2026b). Pediatric obesity and metabolic syndrome: From pathogenesis to prevention and therapeutic management. *International Journal of Drug Delivery Technology, 16*(63s), 1566–1579.

Argano, C., et al. (2023). The role of vitamin D and its molecular bases in insulin resistance, diabetes, metabolic syndrome, and cardiovascular disease: State of the art. *International Journal of Molecular Sciences, 24*(20), Article 15485.

Ashraf, M. A., et al. (2025). Financial impact of nutrition on non-communicable diseases in hospital or other healthcare settings. *Biological Times, 4*(9), 20–21.

Bermúdez-Humarán, L. G., et al. (2024). Exploring the interaction and impact of probiotic and commensal bacteria on vitamins, minerals and short chain fatty acids metabolism. *Microbial Cell Factories, 23*(1), Article 172.

Bilezikian, J. P., et al. (2021). Vitamin D: Dosing, levels, form, and route of administration: Does one approach fit all? *Reviews in Endocrine and Metabolic Disorders, 22*(4), 1201–1218.

Bouillon, R., et al. (2023). Health effects of vitamin D supplementation: Lessons learned from randomized controlled trials and Mendelian randomization studies. *Journal of Bone and Mineral Research, 38*(10), 1391–1403.

Brock, K., et al. (2010). Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *The Journal of Steroid Biochemistry and Molecular Biology, 121*(1–2), 462–466.

Haris, M. M., et al. (2026). Neurogastroenterology: Interactions between gut microflora and brain health. In M. Salman, M. Abid, & M. A. Murtaza (Eds.), *Frontiers in nutritional microbiomics: Gut health, metabolism and disease prevention* (pp. 227–236). Unique Scientific Publishers.

Mayorga-Ramos, A., et al. (2022). Protective role of butyrate in obesity and diabetes: New insights. *Frontiers in Nutrition, 9*, Article 1067647.

Mughal, F. A., et al. (2025). The efficacy of biologics in inflammatory bowel disease (IBD): A review. *Journal of Medical & Health Sciences Review, 2*(3).

Rasouli, M., et al. (2017). Calculation of LDL-cholesterol vs. direct homogenous assay. *Journal of Clinical Laboratory Analysis, 31*(3), Article e22057.

Saija, C., et al. (2025). Role of vitamin D status and alterations in gut microbiota metabolism in fibromyalgia-associated chronic inflammatory pain. *Biomedicines, 13*(1), Article 139.

Shahzadi, S., et al. (2025). Chrononutrition and circadian rhythmicity: Exploring the temporal dynamics of nutrient intake in the regulation of glucose homeostasis and the therapeutic management of type 2 diabetes mellitus. *Review Journal of Neurological & Medical Sciences Review, 3*(8), 180–195.

Soffe, R., et al. (2019). Towards point-of-care insulin detection. *ACS Sensors, 4*(1), 3–19.

Surdu, A. M., et al. (2021). Vitamin D and its role in the lipid metabolism and the development of atherosclerosis. *Biomedicines, 9*(2), Article 172.

Szymczak-Pajor, I., et al. (2020). The molecular mechanisms by which vitamin D prevents insulin resistance and associated disorders. *International Journal of Molecular Sciences, 21*(18), Article 6644.

Todd, J., et al. (2013). Reference range and short- and long-term biological variation of interleukin (IL)-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. *Cytokine, 64*(3), 660–665.

Varra, F.-N., et al. (2024). Molecular and pathophysiological relationship between obesity and chronic inflammation in the manifestation of metabolic dysfunctions and their inflammation-mediating treatment options. *Molecular Medicine Reports, 29*(6), Article 95.

Vives-Bauza, C., et al. (2007). Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. *Methods in Cell Biology, 80*, 379–393.

Wisse, B. E. (2004). The inflammatory syndrome: The role of adipose tissue cytokines in metabolic disorders linked to obesity. *Journal of the American Society of Nephrology, 15*(11), 2792–2800.

Zeb, F., et al. (2026). Effect of vitamin D supplementation on human gut microbiota: A systematic review of randomized controlled trials. *Nutrition Reviews, 84*(6), 1157–1166.

Zeng, Y., et al. (2020). Vitamin D signaling maintains intestinal innate immunity and gut microbiota: Potential intervention for metabolic syndrome and NAFLD. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 318*(3), G542–G553.

Zheng, X., et al. (2026). Vitamin D and exercise in obesity: A neurovascular–muscle axis. *Frontiers in Nutrition, 13*, Article 1750915.

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