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Editoriale

La complessa (e sconosciuta) interazione tra vitamina D e microbiota intestinale

Livelli circolanti di vitamina D_3 e rischio di epatopatia steatosica non alcolica: c'è un legame?

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EDITORIALE

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Cari Colleghi,

in questo numero, con il consueto contributo di esperti Autori, ci occupiamo di alcuni aspetti relativi al possibile ruolo della vitamina D in gastroenterologia.
Complessa e ancora in gran parte sconosciuta, quindi affascinante, è la possibile interazione tra vitamina D e microbiota intestinale, soprattutto in caso di alterazioni qualitative o quantitative di quest'ultimo.
Ricordando la fisiologia dell'assorbimento intestinale di vitamina D si comprende come qualunque alterazione anatomica o funzionale a carico del tubo digerente possa impattare sul microbiota e sullo status vitaminico D e d'altra parte, considerato il riconosciuto ruolo immunomodulante della vitamina D, come non si possa escludere che il ruolo riportato del microbiota nella patogenesi di molte malattie infiammatorie croniche intestinali (MICI) si realizzi, almeno in parte, attraverso un'alterata disponibilità locale della vitamina D.
Come vedremo molti studi hanno valutato gli effetti della vitamina D sul microbiota intestinale, in particolare, ma non solo, nel campo delle MICI, dove è stata descritta un'associazione tra deficit di vitamina D e attività di malattia, rischio di recidiva e fallimento delle terapie.
Ma sono prevedibili e descritti anche interessanti effetti del microbiota sulla vitamina D. Basti pensare alle possibili conseguenze sull'assorbimento della vitamina D delle modificazioni qualitative e/o quantitative del microbiota intestinale, secondarie ad esempio a ipochilia o ad alterazioni della motilità intestinale o alla somministrazione di probiotici.
La disbiosi intestinale appare coinvolta anche nella patogenesi della frequente steatosi non alcoolica (*non-alcoholic fatty liver disease*, NAFLD) e negli ultimi anni molti studi epidemiologici hanno documentato che i pazienti affetti da NAFLD hanno livelli circolanti di 25(OH)D significativamente ridotti rispetto alla popolazione di controllo.
Bassi livelli di vitamina D_3 si associano anche a una maggiore severità istologica della NAFLD. Benché i meccanismi eziopatogenetici che possono spiegare tale associazione non siano ancora chiariti, è stato ipotizzato che la vitamina D_3 possa espletare importanti effetti epatoprotettivi. In vitro è stato visto in particolare che la vitamina D è in grado di modulare positivamente il *signaling* insulinico (migliorando la resistenza insulinica anche a livello epatico) e di ridurre la proliferazione dei fibroblasti e la produzione di collageno.
Fino a oggi non sono tuttavia disponibili in letteratura né ampi studi prospettici di coorte né ampi trial clinici randomizzati che abbiano valutato la possibile associazione fra livelli circolanti di vitamina D_3 e il rischio di sviluppare o far progredire NAFLD, studi necessari per poter confermare la plausibilità biologica e il possibile ruolo causale della vitamina D_3 nello sviluppo e nella progressione del NAFLD.
Tuttavia vedrete che recentemente sono stati pubblicati i risultati di studi prospettici di coorte e di studi di randomizzazione mendeliana che suggeriscono che effettivamente il man-

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tenimento di sufficienti livelli di 25(OH)D possa rappresentare un approccio efficace alla prevenzione primaria e secondaria della NAFLD.
Vi segnalo inoltre che dovremmo cominciare a considerare la NAFLD tra le patologie che determinano osteoporosi secondaria. È quanto emerge da una metanalisi recentemente pubblicata su *Osteoporosis International* [1] che ha permesso di documentare una associazione significativa, soprattutto nei soggetti di sesso maschile, tra NAFLD e prevalenza e rischio di osteoporosi e fratture. Un altro motivo per pensare alla vitamina D in questi pazienti. Cosa ne pensate ?

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La complessa (e sconosciuta) interazione tra vitamina D e microbiota intestinale

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Riassunto

Nei Paesi industrializzati e in quelli in via di sviluppo stiamo assistendo a un critico aumento delle malattie infiammatorie croniche che hanno diversi organi come bersaglio e colpiscono soprattutto la popolazione in età produttiva. È implicito, quindi, che fattori ambientali quali la modalità del parto e dell'allattamento, l'alimentazione, l'inquinamento, gli additivi, i farmaci, il fumo, per citarne alcuni, giocano un ruolo importante sia nel determinismo che nel mantenimento del danno d'organo. La principale via attraverso cui tali fattori esplicano la loro azione è il microbiota intestinale, un complesso e cangiante ecosistema vivente che alberga nel tubo digerente e che svolge funzioni fondamentali per l'omeostasi non solo intestinale, ma dell'intero organismo umano. Parallelamente, negli ultimi anni, stanno emergendo gli effetti extra-scheletrici della vitamina D, soprattutto quelli coinvolti nel mantenimento della tolleranza immunologica e della barriera intestinale. Inoltre, gran parte della vitamina D circolante deriva dalla dieta e quindi deve essere assorbita a livello intestinale. Appare plausibile, pertanto, l'ipotesi di un'interazione tra vitamina D e microbiota intestinale, soprattutto in caso di alterazioni qualitative/quantitative di quest'ultimo, nonché dei possibili effetti della supplementazione di vitamina D sulla composizione del microbiota stesso. Questi i temi sviluppati nel presente articolo.

INTRODUZIONE

Quando si parla di vitamina D, o *calciferolo*, si fa riferimento a una famiglia di composti lipidici di derivazione steroidea indispensabili per l'organismo umano, i cui livelli sierici raccomandati sono > 30 ng/mL [1]. Il fabbisogno quotidiano viene soddisfatto sia attraverso l'esposizione solare sia attraverso la dieta. Nel primo caso, il 7-deidrocolesterolo presente nella cute viene trasformato in vitamina D_3 (*colecalciferolo*) attraverso una reazione fotochimica, nel secondo caso la vitamina viene assunta attraverso alimenti di derivazione sia animale (D_3, *colecaciferolo*) quali latte e derivati, sia vegetale (D_2, *ergocalciferolo*) quali funghi freschi o essiccati [1]. Queste ultime forme, strutturalmente differenti per le caratteristiche della catena laterale legata allo sterolo ma funzionalmente simili, necessitano di essere emulsionate e incorporate nelle micelle a opera della lecitina e dei sali biliari per poter essere assorbite a livello intestinale. Qui, attraversano la membrana apicale degli enterociti sia attraverso un meccanismo di diffusione passiva, sia attraverso specifici trasportatori proteici (Niemann-Pick C1-Like 1, recettore Scavenger di classe B tipo 1, CD36, trasportatore ATP-Binding Cassette A1) [2]. Una volta all'interno dell'enterocita la vitamina D viene incorporata nei chilomicroni che superando la membrana basale raggiungono la circolazione linfatica. La vitamina D, sia essa prodotta a livello cutaneo o assunta attraverso la dieta, è biologicamente inattiva ed è, pertanto, considerata un pro-ormone. Per divenire attiva, necessita di una doppia idrossilazione operata dapprima in posizione 25 a livello epatico

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dall'enzima vitamina D-25-idrossilasi e, successivamente, in posizione 1 a livello renale dall'enzima vitamina D-1α-idrossilasi, dando origine alla forma 1,25(OH)$_2$ D (*calcitriolo*) [1].
Da quanto premesso si evince come qualunque perturbazione a carico del tubo digerente, inteso non tanto anatomicamente come strato mucosale, quanto funzionalmente come barriera intestinale, microbiota e sali biliari, possa impattare sulla disponibilità di vitamina D. Inoltre, scarsamente conosciuto è l'effetto che la supplementazione orale di vitamina D ha sul microbiota intestinale. Obiettivo di questo articolo è, pertanto, quello di sintetizzare le attuali conoscenze sull'interazione tra vitamina D e microbiota intestinale dal momento che entrambi svolgono un ruolo preminente nella modulazione del sistema immunitario e nella patogenesi di molte malattie infiammatorie croniche.

IL MICROBIOTA INTESTINALE

Alla nascita, il corpo umano viene popolato da una moltitudine di microorganismi, comprendenti batteri, miceti, virus, fagi e archea, noti collettivamente con il termine di microbiota. Il tubo digerente, con la sua superficie di circa 400 m^2, ospita la più abbondante e diversificata comunità microbica del nostro organismo, comprendente più di 100 trilioni di microorganismi [3] codificanti più di 3 milioni di geni a loro volta responsabili della sintesi di migliaia di metaboliti [4]. Questo ecosistema è responsabile di funzioni fondamentali per l'omeostasi del nostro organismo quali la resistenza alla colonizzazione da parte di microorganismi patogeni, il mantenimento dell'integrità della barriera intestinale e del turnover epiteliale, la sintesi e l'assorbimento di nutrienti e metaboliti (vitamine, aminoacidi, lipidi, sali biliari, acidi grassi a catena corta), nonché lo sviluppo e la modulazione del sistema immunitario periferico e la regolazione dello stato nutrizionale [3]. Grazie alla possibilità di identificare le regioni iper-variabili della subunità 16S dell'rRNA batterico [5], la popolazione microbica meglio conosciuta è quella batterica che viene classificata in base al livello tassonomico in phyla, classi, ordini, famiglie, generi, specie [4]. Gli studi volti alla sua caratterizzazione qualitativa/quantitativa hanno messo in evidenza come la composizione si modifichi a seconda del tratto del tubo digerente (Fig. 1), essendo influenzata da

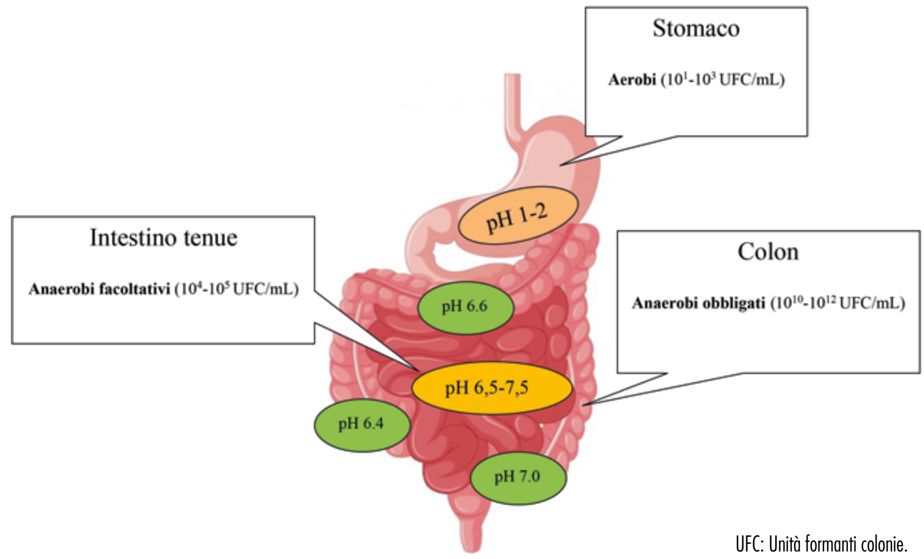


FIGURA 1.
La composizione qualitativa/quantitativa del microbiota intestinale cambia nei diversi tratti del tubo digerente in funzione di variabili quali il pH, la tensione parziale di ossigeno, la motilità intestinale. Alterazioni dei suddetti fattori conseguono a condizioni quali ipochilia gastrica, dismotilità intestinale, presenza di anse cieche e modifiche della valvola ileo-ciecale e favoriscono la sovraccrescita batterica del tenue per via discendente o ascendente.

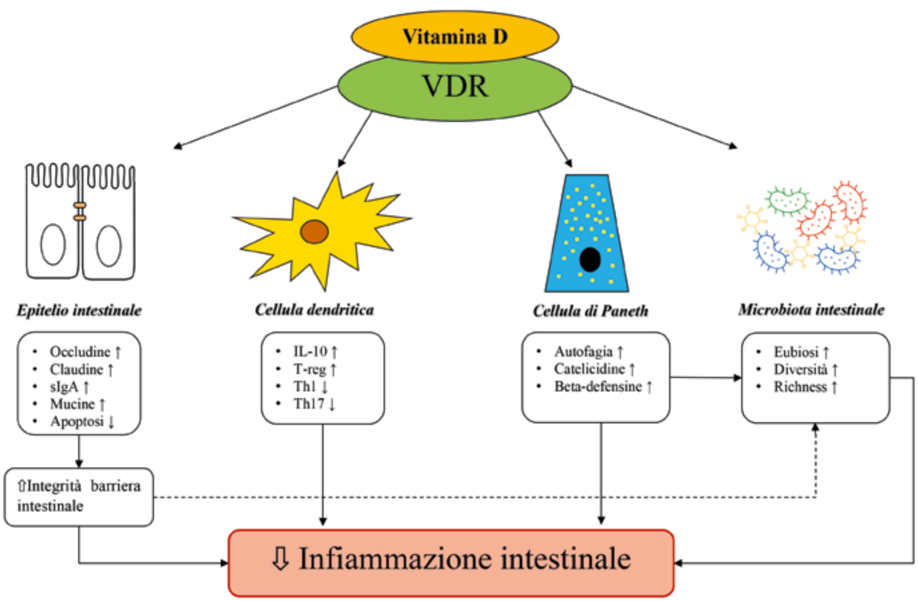
fattori intrinseci (ad esempio il pH, la tensione di ossigeno, la motilità intestinale) ed estrinseci (la modalità di nascita, il tipo di allattamento, la dieta, l'ambiente rurale o cittadino, il numero dei componenti il nucleo familiare, l'attività fisica, l'esposizione a xenobiotici) [3,4]. Benché ciascun individuo sviluppi un microbiota peculiare, numerosi studi hanno permesso di identificare il profilo di un microbiota "sano" (stato di eubiosi) caratterizzato da un'abbondanza relativa dei phyla *Bacteroidetes* (in particolare i generi *Bacteroides* e *Prevotella*) e *Firmicutes* (in particolare i generi *Lactobacillus, Clostridium, Enterococcus* e *Fecalibacterium*), ma, soprattutto, dotato di ricchezza (*richness*) e diversità (*diversity*) [3,4]. Di contro, tutte quelle condizioni caratterizzate da una perturbazione di questo stato prendono il nome di "disbiosi". Vista la grande capacità del microbiota intestinale di influenzare l'omeostasi dell'organismo umano, non sorprende che uno stato di disbiosi sia stato identificato in diverse condizioni patologiche sia intestinali, come la sindrome dell'intestino irritabile (IBS), le malattie infiammatorie croniche intestinali (MICI), la malattia celiaca, sia extra-intestinali, come l'obesità e la sindrome metabolica, le malattie reumatologiche, la psoriasi, la malattia di Alzheimer e di Parkinson, per citarne alcune [3,4]. A oggi, tuttavia, non è del tutto chiaro se le alterazioni del microbiota intestinale siano la causa e/o la conseguenza delle condizioni patologiche e, soprattutto, quali siano i meccanismi attraverso cui il microbiota influenzi il determinismo e il mantenimento dello stato patologico. A tal proposito, considerando il ruolo immunomodulante della vitamina D, non possiamo escludere che l'influenza del microbiota nella patogenesi di molte condizioni infiammatorie croniche avvenga, per lo meno in parte, attraverso un'alterata disponibilità della vitamina D stessa e/o che quest'ultima possa indurre modificazioni qualitative/quantitative della composizione del microbiota.

EFFETTI DELLA VITAMINA D SUL MICROBIOTA

Una messe crescente di studi ha valutato gli effetti della vitamina D sul microbiota intestinale soprattutto nel campo delle MICI, condizioni multifattoriali in cui la disbiosi gioca un ruolo di primo piano nell'innesco e mantenimento delle lesioni [6]. Al contempo, il riscontro di bassi livelli di vitamina D in pazienti affetti da queste condizioni e, soprattutto, l'associazione dei livelli sierici con l'attività di malattia, il rischio di recidiva, la risposta e/o il fallimento alle terapie [7,8] ha determinato un crescente interesse sul possibile ruolo della vitamina D nella patogenesi delle MICI. Evidenze scientifiche, derivate principalmente da modelli sperimentali suggeriscono da una parte che modifiche epigenetiche indotte dall'infiammazione intestinale possano ridurre l'espressione del gene codificante il recettore nucleare della vitamina D (VDR) [9] e, dall'altra, che la via di segnale vitamina D/VDR possa regolare diversi fattori coinvolti nell'infiammazione intestinale [7,10]. In particolare, la vitamina D sembrerebbe influenzare l'integrità della barriera intestinale modulando l'espressione di componenti delle giunzioni strette e aderenti [7] e favorendo il rilascio di peptidi antimicrobici (catelicidine, beta-defensine) da parte delle cellule di Paneth [11] e di mucine, esercitando un effetto immunomodulante sia inibendo il rilascio di citochine pro-infiammatorie, sia favorendo il rilascio di quelle antinfiammatorie e l'induzione di linfociti T regolatori [7,10,11] (Fig. 2). Per quanto riguarda gli effetti sul microbiota intestinale, la supplementazione di vitamina D in una piccola coorte di soggetti affetti da colite ulcerosa ha determinato una riduzione del *Ruminococcus gnavus*, anche se non ha indotto modificazioni dell'α-diversità (indice della diversità batterica all'interno di un campione) [12], mentre in pazienti affetti da malattia di Crohn, ma non in soggetti di controllo, ha determinato un incremento relativo di batteri eubiotici quali, *Alistipes, Parabacteroides, Roseburia* e *Faecalibacterium* [13]. L'assenza di modificazioni del microbiota fecale a seguito di supplementazione di vitamina D in soggetti sani è stata confermata in uno studio [14], ma non in altri ove, per contro, si evidenziava un aumento degli indici di eubiosi quali l'α- e la β-diversità (indice della diversità batterica tra diversi campioni), un incremento del rapporto tra *Bacteroides* e *Firmicutes*, così come di ceppi ad azione protettiva come *Akkermansia muciniphila* [15,16]. Una possibile spiegazione a tale apparente discrepanza risiede nella capacità della vitamina D di influenzare soprattutto il microbiota adeso alla mucosa intestinale e, quindi, valutato attraverso il campionamento bioptico endoscopico, più che quello luminale valutato attraverso il campionamento fecale. In particolare, i maggiori effetti avverrebbero a livello del tratto intestinale superiore ove, dopo 8 settimane di supplementazione, sono stati riscontrati un decremento di patogeni opportunisti (come le specie *Pseudomonas, Escherichia, Shigella*) e un aumento della *richness* [17].

Tali evidenze hanno spinto a indagare su un potenziale ruolo della vitamina D anche nella IBS (*Inflammatory Bowel Disease* - sindrome dell'intestino irritabile). Quest'ultima è una condizione cronica che affligge almeno il 10% della popolazione mondiale, caratterizzata da sintomi quali dolore/discomfort addominale, meteorismo intestinale e alterazioni dell'alvo in senso prevalentemente stiptico e/o diarroico [18]. La sua eziopatogenesi vede coinvolti fattori che influenzano il funzionamento dell'asse cervello-intestino e che includono un'alterata permeabilità intestinale e una disbiosi [18,19]. Inoltre, è stata spesso evidenziata una carenza vitaminica in tale condizione, probabilmente legata a modifiche del regime alimentare, prima fra tutte l'esclusione di latte e latticini, nonché un miglioramento della qualità della vita e dei sintomi intestinali a seguito di opportuna supplementazione [19]. Tuttavia, la scarsità degli studi clinici, la diversità del loro disegno, l'eterogeneità dei soggetti arruolati, e la mancata considerazione di fattori confondenti quali, ad esempio l'esposizione solare e la dieta, non consentono di stabilire il ruolo giocato dalla vitamina D nell'IBS e, soprattutto, se questo è in parte mediato da alterazioni del microbiota intestinale.

La disbiosi intestinale appare coinvolta anche nella patogenesi della malattia da fegato grasso associata alla sindrome metabolica [20], la principale causa di epatopatia cronica nel mondo occidentale, nonché condizione associata a maggior rischio di deficit di vitamina D [21]. In particolare, la supplementazione di vitamina D in questa patologia sembrerebbe contrastare la fibrogenesi conseguente all'attivazione della via del fattore di crescita trasformante-β nelle cellule stellate [22], e migliorare alcuni parametri laboratoristici, quali il tasso delle transaminasi, dei trigliceridi, della glicemia a digiuno e della insulinemia [23]. Pur tuttavia, rimane ancora inesplorato il possibile effetto della supplementazione vitaminica sul microbiota intestinale in questo specifico setting clinico. Sempre nell'ambito della sindrome metabolica, studi *in vivo* sia su modelli animali che sull'uomo suggeriscono un ruolo del microbiota intestinale nella patogenesi dell'obesità. A tal proposito, lapalissiana è la dimostrazione che il trapianto di microbiota di soggetti obesi causa obesità nell'animale da esperimento [4]. Inoltre, un aumentato rapporto *Firmicutes/Bacteroidetes*, elevati livelli di *Ruminococcaceae* e *Lactobacillus* e ridotti di *Bacteroidaceae, Bacteroides* e *Bifidobacterium vulgatus* sem-



IL: Interleuchina; sIgA: Immunoglobuline A secretorie; T-reg: linfociti T regolatori; Th: linfociti T helper; VDR: Recettore vitamina D.

FIGURA 2.
Effetti della vitamina D sull'ecosistema intestinale: vedere testo.

brerebbero associati a obesità [4]. Di nota, l'evidenza che la supplementazione di vitamina D, ma non il placebo, ha determinato un aumento dei generi *Lachnospira* e *Coprococcus* (ritenuti associati a uno stato di salute) e una riduzione dei generi *Blautia* e *Ruminococcus* (relativamente abbondanti in condizioni infiammatorie e dismetaboliche), in una coorte di soggetti con sovrappeso corporeo e/o obesità (indice di massa corporea ≥ 25 kg/m^2) [24].

Anche l'invecchiamento rappresenta una condizione frequentemente associata sia a deficit di vitamina D [25] che a variazioni nella composizione del microbiota intestinale [4]. Uno studio *cross-sectional*, multicentrico, in cui sono stati arruolati 567 anziani americani non residenti in comunità protette, ha evidenziato come più alte concentrazioni sieriche di *calcitriolo* fossero associate a maggiori α- e β-diversità [26]. Inoltre, i livelli sierici di *calcitriolo* risultavano correlare positivamente con microrganismi appartenenti soprattutto al phylum *Firmicutes* [26], produttori di butirrato, un acido grasso a catena corta con noti effetti benefici sull'omeostasi intestinale [3, 4].

Infine, in un elegante studio randomizzato, doppio cieco condotto su 41 soggetti affetti da fibrosi cistica, una malattia cronica geneticamente determinata che causa ispessimento delle secrezioni soprattutto a livello polmonare, cutaneo e dell'apparato digerente (in particolare il pancreas) a causa della perdita di funzione della proteina CFTR (*Regolatore della Conduttanza Transmembrana della Fibrosi Cistica*), un canale ionico che regola il flusso di ioni cloruri, e quindi di acqua, a livello delle cellule epiteliali, è risultata evidente una abbondanza relativa della classe *Bacteroida* nei soggetti con normali livelli di vitamina D, laddove in coloro con bassi livelli di vitamina D si osservava una abbondanza relativa di *Gammaproteobacteria*, classe di batteri gram-negativi comprendenti patogeni quali *Yersinia Pestis, Vibrio Cholera, E. coli* e *Pseudomonas aeruginosa*, quest'ultimo spesso responsabile di infezioni polmonari in tali pazienti [27]. Inoltre, la successiva supplementazione di vitamina D con normalizzazione dei livelli sierici determinava un aumento relativo del genere *Lactococcus* e una riduzione del genere *Veillonella* (famiglia *Erysipelotrichaceae*), il cui riscontro nei campioni di lavaggio broncoalveolare è stato associato a uno stato infiammatorio subclinico [27].

EFFETTI DEL MICROBIOTA SULLA VITAMINA D

Consideriamo ora le conseguenze che modificazioni quali-quantitative del microbiota intestinale o la somministrazione di probiotici possono avere sull'assorbimento e quindi sulla disponibilità della vitamina D. Un chiaro esempio è fornito dalla contaminazione batterica dell'intestino tenue in cui, a causa della presenza di uno o più fattori predisponenti quali l'ipochilia gastrica (da terapia cronica con prazoli, da gastrite atrofica, da interventi chirurgici), le alterazioni della motilità (soprattutto in corso di diabete, malattie autoimmuni sistemiche come la sclerodermia, diverticolosi, stenosi, by-pass intestinali) e della valvola ileo-ciecale (da malattia di Crohn o interventi chirurgici), si verifica una sovraccrescita/contaminazione della flora intestinale per via discendente o ascendente, rispettivamente [28]. Soprattutto in quest'ultimo caso, la contaminazione del tenue da parte di batteri anaerobi e Gram- determina la scomposizione delle micelle con malassorbimento delle vitamine liposolubili, compresa la vitamina D. Ne consegue un quadro clinico caratterizzato non solo da meteorismo intestinale e diarrea ma, a lungo andare, anche da osteoporosi, e anemia/neuropatie periferiche da deficit di vitamina B_{12} [28]. La scomposizione delle micelle, inoltre, altera il circolo enteroepatico dei sali biliari che, giungendo nel colon in maggiori quantità, determinano l'insorgenza di diarrea definita "coleretica" e legata all'aumentata produzione intracellulare di AMP e GMP ciclici che, a loro volta, aumentano la secrezione attiva di acqua ed elettroliti. Infine, la flora microbica può anche danneggiare la porzione apicale degli enterociti con perdita/riduzione del patrimonio enzimatico, compresa la lattasi, e conseguente sviluppo di sintomi da intolleranza al lattosio.

Per quanto riguarda la possibilità che probiotici (definiti dall'Organizzazione Mondiale della Sanità come organismi vivi in grado di esercitare effetti benefici) possano influenzare il tasso di vitamina D circolante, riportiamo le poche evidenze presenti in letteratura. In un'analisi post-hoc di uno studio clinico randomizzato controllato, la somministrazione del probiotico Lactobacillus reuteri NCIMB 30242 BSH-attivo (esprimente l'enzima idrolasi dei sali biliari [BSH] responsabile della loro deconiugazione [29], con conseguente ridotta capacità di formare micelle) per 9 settimane a soggetti dislipidemici ha determinato un incremento statisticamente significativo dei livelli di vitamina D circolante rispetto a coloro che avevano assunto placebo; di nota il fatto che l'effetto era selettivo sulla vitamina D ma non sulle altre vitamine liposolubili [30]. Inoltre, in studi clinici condotti su pazienti sottoposti a interventi di chirurgia bariatrica (bypass gastrico con singola anastomosi o con ricostruzione con ansa Roux-en-Y) la somministrazione di probiotici (tra cui l'associazione di *Lactobacillus acidophilus* NCFM e *Bifidobacterium lactis* Bi-07) fino a 3 mesi dopo l'intervento, determinava un incremento dei livelli sierici della vitamina D [31,32]. Tra le possibili spiegazioni, gli autori ipotizzano un aumento dell'assorbimento della vitamina D grazie all'acidificazione del contenuto intestinale secondaria alla sintesi di acido lattico, la stimolazione dell'enzima vitamina D-25-idrossilasi e/o un aumento della sintesi del 7-deidrocolesterolo [30].

Degna di nota, infine, è la possibile interazione funzionale tra microbiota residente e/o probiotici e il VDR. In modelli animali di colite, infatti, l'azione antinfiammatoria del butirrato potrebbe essere connessa all'aumentata espressione genica del VDR indotta dallo stesso composto [7,33].

EFFETTI DEL MICROBIOTA SULL'ASSORBIMENTO DI LATTOSIO

Il lattosio, il principale zucchero presente nel latte dei mammiferi, è un disaccaride composto da glucosio e galattosio, il cui assorbimento intestinale dipende dalla idrolisi operata dall'enzima lattasi (*beta-galattosidasi*) presente sull'orletto a spazzola degli enterociti. La carenza congenita (condizione molto rara tipica del bambino) o acquisita (condizione molto frequente tipica dell'adulto) di tale enzima determina la mancata digestione del lattosio che, permanendo nel lume intestinale, viene catabolizzato da parte della flora residente con produzione di piccole molecole osmoticamente attive (acidi grassi a catena corta) e gas (anidride carbonica, metano e idrogeno), con conseguente sviluppo dei sintomi da intolleranza quali diarrea, meteorismo intestinale e crampi addominali [34]. In particolare, la carenza acquisita può essere primaria, quindi legata a un deficit geneticamente determinato che colpisce circa due terzi della popolazione adulta caucasica, o secondaria a enteropatie quali la malattia celiaca, la malattia di Crohn, da farmaci, da radiazioni, per citare le più frequenti, ma anche alla contaminazione batterica del tenue a causa della già citata capacità dei batteri di danneggiare l'orletto a spazzola degli enterociti ove risiede l'attività disaccaridasica. È noto che l'insorgenza dei disturbi legati all'ingestione di latte e derivati induce il soggetto a evitarli, con importanti ripercussioni nutrizionali, metaboliche e immunologiche dovute soprattutto alla conseguente carenza di vitamina D [34]. Alcune di queste ripercussioni sono secondarie a modificazioni quali-quantitative del microbiota intestinale che verrebbe a essere depauperato della quota di Lattobacilli con conseguente perdita del loro importante ruolo immunomodulante. Di nota anche il fatto che lo sviluppo dei sintomi da intolleranza al lattosio dipenda non solo dalla quantità di alimento ingerita, ma anche dalla capacità del microbiota dell'individuo di scindere il disaccaride [34,35]. A oggi, i ceppi con attività lattasica riconosciuta comprendono *Bacteroides/Prevotella*, *Bifidobacterium*, *Eubacterium rectale/Clostriudium coccoides* [36]. Pertanto, una possibile strategia terapeutica è rappresentata dal ricorso a probiotici, come dimostrato in alcuni studi in cui l'assunzione di *Lactobacillus casei Shirota* e di *Bifidobacterium breve Yakult* per 4 settimane [37] o di *Lactobacillus reuteri* per 10 giorni [38] ha determinato una riduzione dei sintomi da intolleranza e dei valori del breath test al lattosio. Degna di nota, infine, è l'evidenza che l'assunzione di latte in soggetti con deficit di lattasi sia associata a un aumento dei livelli di acido indoleproprionico, un metabolita del triptofano prodotto dal microbiota intestinale, in particolare dal *Bifidobacterium*, e inversamente associato al rischio di sviluppare diabete mellito tipo 2 [39].

CONCLUSIONI

Gli studi riportano che la vitamina D potrebbe avere un ruolo fisiologico nella modulazione del microbiota intestinale, e che alcuni suoi effetti immunoregolatori sono legati proprio all'interazione con quest'ultimo. Tuttavia, la complessità dell'universo rappresentato dal microbiota intestinale e gli attuali limitati mezzi a nostra disposizione per esplorarlo rendono difficile comprendere a pieno il suo possibile ruolo nei vari scenari clinici e, di conseguenza, le eventuali strategie preventivo-terapeutiche. Si raccomanda, pertanto, di supplementare i soggetti con carenza di vitamina D o a rischio di carenza, poiché lo stato carenziale prolungato nel tempo, potrebbe comportare un'alterazione non solo dell'omeostasi osteo-metabolica, ma anche nutrizionale e immunologica.

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Livelli circolanti di vitamina D_3 e rischio di epatopatia steatosica non alcolica: c'è un legame?

VITAMIN D
UpDates
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La carenza di vitamina D_3 è stata associata alla coesistenza di molteplici patologie croniche non scheletriche (tra cui obesità, diabete tipo 2, malattia cardiovascolare, alcune neoplasie ed epatopatia steatosica non alcolica), suggerendo la possibilità che tale vitamina possa svolgere molteplici e benefici effetti pleiotropici a livello extra-scheletrico, grazie alla distribuzione ubiquitaria del suo specifico recettore [1-5].

Tra queste patologie croniche extra-scheletriche, che sono associate a ridotti livelli circolanti di vitamina D_3, l'epatopatia steatosica non alcolica (nota con l'acronimo inglese di NAFLD, *non-alcoholic fatty liver disease*) ha rappresentato un importante *focus* della ricerca scientifica, specialmente negli ultimi 6-7 anni [6].

La NAFLD comprende un ampio spettro di patologie epatiche che sono caratterizzate dall'accumulo di trigliceridi nel fegato in soggetti con anamnesi negativa per un eccessivo consumo alcolico. Lo spettro di tali patologie include la steatosi semplice, la steatoepatite non alcolica (NASH), che può progredire a cirrosi e talora a epatocarcinoma [7]. La NAFLD rappresenta attualmente l'epatopatia cronica di più comune riscontro nei paesi occidentali e si stima che sia presente in circa il 25-30% della popolazione generale adulta [8,9]. La sua prevalenza globale è in progressivo aumento in molte parti del mondo, analogamente a quanto si osserva per la prevalenza di obesità e diabete tipo 2, che sono due patologie metaboliche con le quali la NAFLD è strettamente associata e di cui ne rappresentano i più importanti fattori di rischio [9,10].

La storia naturale e la progressione della NAFLD verso i suoi stadi istologici più avanzati è spiegata da diversi fattori di rischio (genetici, epigenetici, ambientali e clinici), che rendono conto dell'ampio spettro di fenotipi patologici che ne derivano e per i quali possono essere necessari diversi approcci terapeutici individuali [7]. Questo aspetto differenzia la NAFLD da altre epatopatie croniche, dove l'agente eziologico è ben definito (come per esempio l'epatopatia da abuso alcolico e le epatiti virali), o per le quali esiste un trattamento farmacologico specifico (come nel caso delle epatiti virali e autoimmuni). La sempre più crescente importanza della NAFLD dal punto di vista clinico e socio-sanitario deriva anche dal fatto che tale patologia non si associa solo a un aumentato rischio di progressione verso la NASH e la cirrosi, ma si associa anche frequentemente a un aumentato rischio di sviluppare importanti complicanze extra-epatiche, tra cui la malattia cardiovascolare (che rappresenta la principale causa di morte di questa popolazione di pazienti), la malattia renale cronica, il diabete tipo 2 e lo sviluppo di alcuni tipi di neoplasie (specialmente a livello del colon-retto, pancreas e mammella) [7,11]. Per tali motivi, è particolarmente importante dal punto di vista clinico conoscere la storia naturale della NAFLD e identificare tutti i principali fattori di rischio che possano modificarne la sua progressione verso le complicanze epatiche ed extra-epatiche.

Come accennato in precedenza, negli ultimi 6-7 anni, molti studi epidemiologici hanno documentato che i pazienti con NAFLD hanno livelli circolanti di 25-idrossi-vitamina D_3 significativamente ridotti rispetto alla popolazione di controllo senza steatosi epatica e che bassi livelli di vitamina D_3 si associano anche a una maggiore severità istologica della NAFLD, indipendentemente dalla coesistenza di obesità, diabete e altre alterazioni tipiche della sindrome metabolica [6,12]. Benché i meccanismi eziopatogenetici che possono spiegare tale associazione non sono ancora del tutto chiari, è stato ipotizzato che la vitamina D_3 possa espletare importanti effetti epatoprotettivi. Tra questi, è stato riportato, sia in studi sperimentali che in studi *in vitro*, che la vitamina D_3

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è in grado di modulare positivamente il signaling insulinico (migliorando la resistenza insulinica a livello muscolare ed epatico), di ridurre la produzione di molteplici fattori protrombotici, procoagulanti e pro-ossidanti, di espletare un effetto immunomodulatorio oltre che di ridurre la proliferazione dei fibroblasti e la produzione di collageno [6,12].

Fino a oggi, non sono tuttavia disponibili in letteratura né ampi studi prospettici di coorte né ampi trial clinici randomizzati, che abbiano valutato la possibile associazione fra livelli circolanti di vitamina D_3 e il rischio di sviluppare NAFLD, o che abbiano esaminato se una supplementazione protratta di vitamina D_3 sia in grado di ridurre il rischio di sviluppo e/o progressione della NAFLD/NASH (documentata mediante biopsia epatica, che rappresenta il "gold standard" per la diagnosi e la stadiazione di tale patologia epatica). Tali dati sono clinicamente importanti per poter confermare la plausibilità biologica e il possibile ruolo causale della vitamina D_3 nello sviluppo e nella progressione del NAFLD.

A uno di questi specifici aspetti ha cercato di dare una risposta il recente studio osservazionale che è stato condotto da Kim e colleghi su una ampia coorte di soggetti coreani [13]. In questo interessante studio denominato "Kangbuk Samsung Health Study", gli autori si sono posti due obiettivi principali e cioè quello di: (1) valutare se i livelli circolanti di 25-idrossi-vitamina D_3 al baseline erano in grado di predire il rischio di sviluppare nuove forme incidenti di NAFLD e/o la probabilità di risoluzione di forme note di NAFLD al follow-up; (2) esaminare l'associazione delle variazioni temporali dei livelli di 25-idrossi-vitamina D_3, misurati al baseline e follow-up, con la probabilità di sviluppo e/o risoluzione della NAFLD [13].

Per il primo obiettivo dello studio (valutazione dell'associazione tra livelli basali di vitamina D_3 e incidenza di NAFLD), gli autori hanno arruolato durante una campagna di screening una coorte di oltre 139.000 soggetti adulti (~44% maschi, età media 36,8 anni, BMI medio 22 kg/m^2), che non avevano abuso alcoolico e che erano privi di NAFLD e di altre epatopatie note al baseline [13]. Tali soggetti non sono stati selezionati sulla base del loro stato vitaminico al baseline; ~77% del campione aveva valori di 25-idrossi-vitamina D_3 < 20 ng/mL al baseline. In tutti i partecipanti, sono stati misurati i livelli circolanti di 25-idrossi-vitamina D_3 ed è stata eseguita una ecografia epatica sia al baseline che al follow-up. In questa prima parte dello studio, come riportato in Tabella I, gli autori hanno chiaramente documentato che esisteva una associazione inversa fra livelli circolanti di 25-idrossi-vitamina D_3 e rischio di sviluppare NAFLD (all'ecografia epatica), durante il periodo di follow-up dello studio (mediana di circa 4 anni). Tale rischio era indipendente da molteplici fattori di rischio noti e altri possibili confondenti [13].

Per esaminare l'associazione tra livelli circolanti di 25-idrossi-vitamina D_3 al baseline e la probabilità di risoluzione di forme note di NAFLD durante il follow-up, è stato arruolato invece un campione di circa 48.700 soggetti affetti da NAFLD al baseline. In questo caso, come riassunto in Tabella II, gli autori hanno documentato una associazione positiva e di tipo dose-effetto fra i livelli circolanti di 25-idrossi-vitamina D_3 al baseline e la probabilità di osservare la risoluzione della NAFLD al follow-up [13].

Per il secondo obiettivo dello studio (esaminare l'associazione delle variazioni temporali dei livelli di vitamina D_3 con la probabilità di sviluppo e/o risoluzione della NAFLD), questi stessi autori hanno documentato che un incremento nei livelli circolanti di 25-idrossi-vitamina D_3, passando cioè da valori insufficienti al baseline (< 20 ng/mL) a uno stato vitaminico adeguato (≥ 20 ng/mL) al follow-up (mediana di 1,8 anni), si associava a un rischio significativamente ridotto di sviluppare NAFLD [Hazard Ratio (HR) aggiustato 0,87, 95% IC (intervallo di confidenza) 0,82-0,91] nei soggetti che non avevano NAFLD al baseline; al contrario, valori persistentemente adeguati di 25-idrossi-vitamina D_3, sia al baseline che al follow-up (≥ 20 ng/mL), si associavano a una maggiore probabilità di risoluzione della NAFLD (HR aggiustato 1,10, 95% IC 1,02-1,20) nei soggetti che avevano NAFLD al baseline [13].

I risultati di questo ampio studio prospettico di coorte suggeriscono pertanto che il mantenimento di sufficienti livelli di 25-idrossi-vitamina D_3 possono rappresentare un efficace approccio per la prevenzione primaria e secondaria della NAFLD [13]. Tuttavia, il disegno osservazionale di questo studio non permette di trarre alcuna inferenza causale riguardo all'associazione osservata fra livelli di 25-idrossi-vitamina D_3 e rischio di NAFLD, ma è comunque in linea anche con le recenti osservazioni di uno studio di randomizzazione mendeliana, condotto su 3 popolazioni europee, che ha riportato una significativa e inversa associazione fra livelli geneticamente predetti di 25-idrossi-vitamina D_3 e rischio di NAFLD [14]. Altre importanti limitazioni di questo studio pubblicato da Kim e colleghi [13] sono che la diagnosi di NAFLD è stata formulata mediante ecografia epatica, che può non essere sufficientemente accurata per evidenziare la presenza di steatosi epatica di grado lieve e che la popolazione in studio era costituita esclusi-

TABELLA I.
Rischio di sviluppare NAFLD (all'ecografia epatica) al follow-up in base ai livelli circolanti di 25-idrossi-vitamina D_3 in soggetti senza NAFLD al baseline (n = 139.599) (da Kim et al., 2022, [13], mod.).

Baseline 25(OH)D, ng/mL	Persone-anni	Casi incidenti NAFLD (n)	HR (95% IC) aggiustati per sesso ed età	HR (95% IC) aggiustati per molteplici fattori confondenti
< 10	114.688	4.310	1,00 (reference)	1,00 (reference)
10-19	343.137	16.487	0,95 (95% IC 0,92-0,99)	0,89 (95% IC 0,86-0,92)
20-29	102.627	5.740	0,91 (95% IC 0,88-0,95)	0,81 (95% IC 0,78-0,85)
≥ 30	20.569	994	0,76 (95% IC 0,71-0,82)	0,72 (95% IC 0,67-0,77)
P–value per trend			< 0,001	< 0,001

TABELLA II.
Risoluzione della NAFLD (all'ecografia epatica) al follow-up in base ai livelli circolanti di 25-idrossi-vitamina D_3 in soggetti con NAFLD al baseline (n = 48.702) (da Kim et al., 2022, mod.) [13].

Baseline 25(OH)D, ng/mL	Persone-anni	Casi con risoluzione di NAFLD (n)	HR (95% IC) aggiustati per sesso ed età	HR (95% IC) aggiustati per molteplici fattori confondenti
< 10	25.318	1.819	1,00 (referenza)	1,00 (referenza)
10-19	118.651	8.202	1,12 (95% IC 1,06-1,18)	1,09 (95% IC 1,03-1,15)
20-29	41.262	2.929	1,17 (95% IC 1,10-1.24)	1,13 (95% IC 1,06-1,21)
≥ 30	6.140	499	1,23 (95% IC 1,12-1,36)	1,21 (95% IC 1,09-1,35)
P-value per trend			< 0,001	<0,001

vamente da soggetti coreani (limitando così la estendibilità di tali osservazioni a popolazioni di diversa etnia).
Ciò detto, i risultati di questo ampio studio longitudinale [13] sottolineano in maniera sempre più evidente la necessità di condurre nel prossimo futuro degli ampi trial clinici randomizzati con una adeguata durata di trattamento per valutare i possibili benefici della supplementazione con vitamina D_3 sul rischio di insorgenza e progressione della NAFLD/NASH (e dove tali outcome epatici siano esaminati mediante biopsia). È inoltre necessario che in tali trial clinici venga tenuto in considerazione anche lo stato vitaminico D dei partecipanti arruolati, dato che è ragionevole ritenere che l'eventuale beneficio della supplementazione con vitamina D_3 ad alte dosi sulla NAFLD/NASH possa essere maggiore nei pazienti con carenza di vitamina D rispetto a quelli che hanno valori di vitamina D_3 circolante nella norma [15].

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