Νεότερες απόψεις για το ρόλο του μικροβιώματος στα οστά

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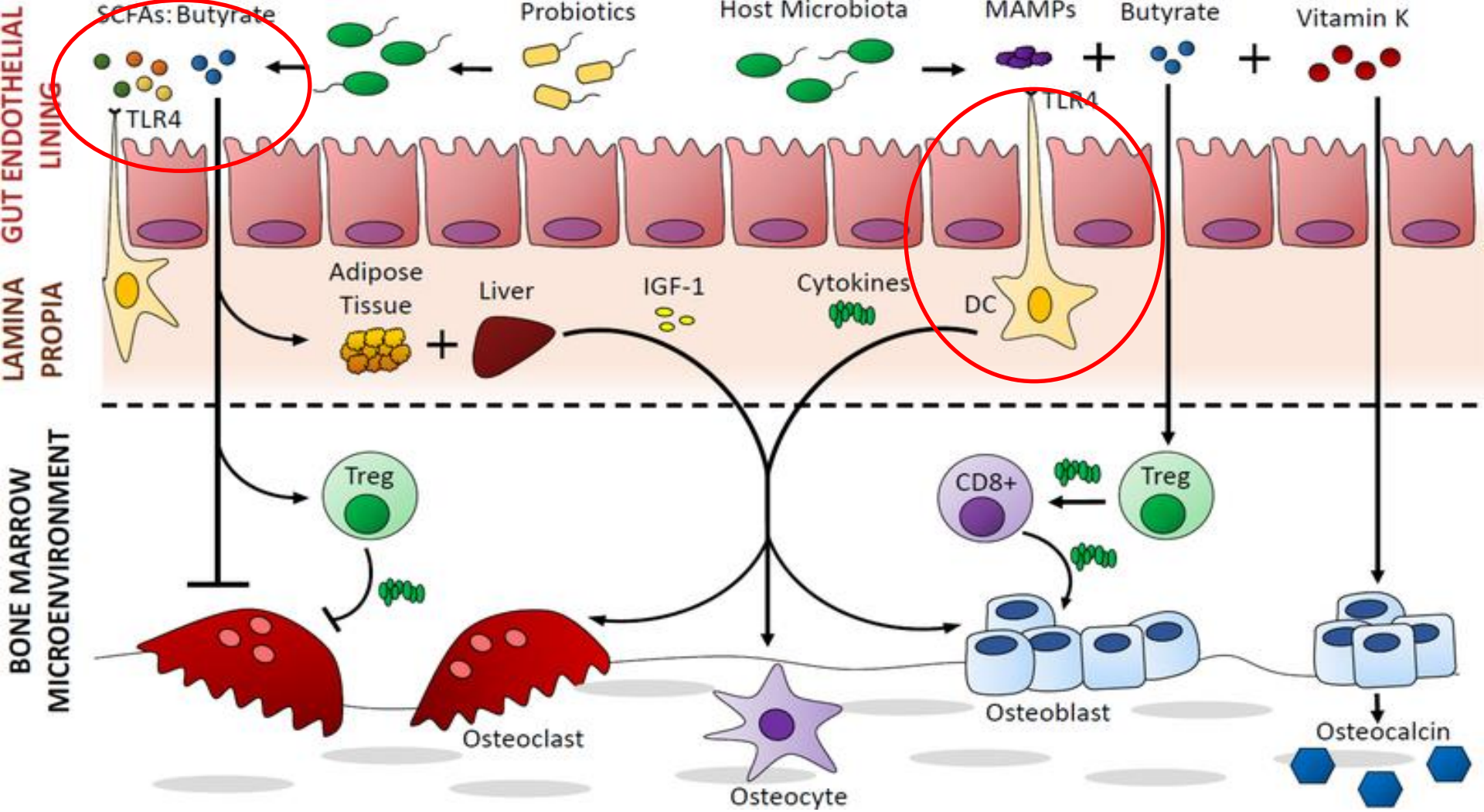
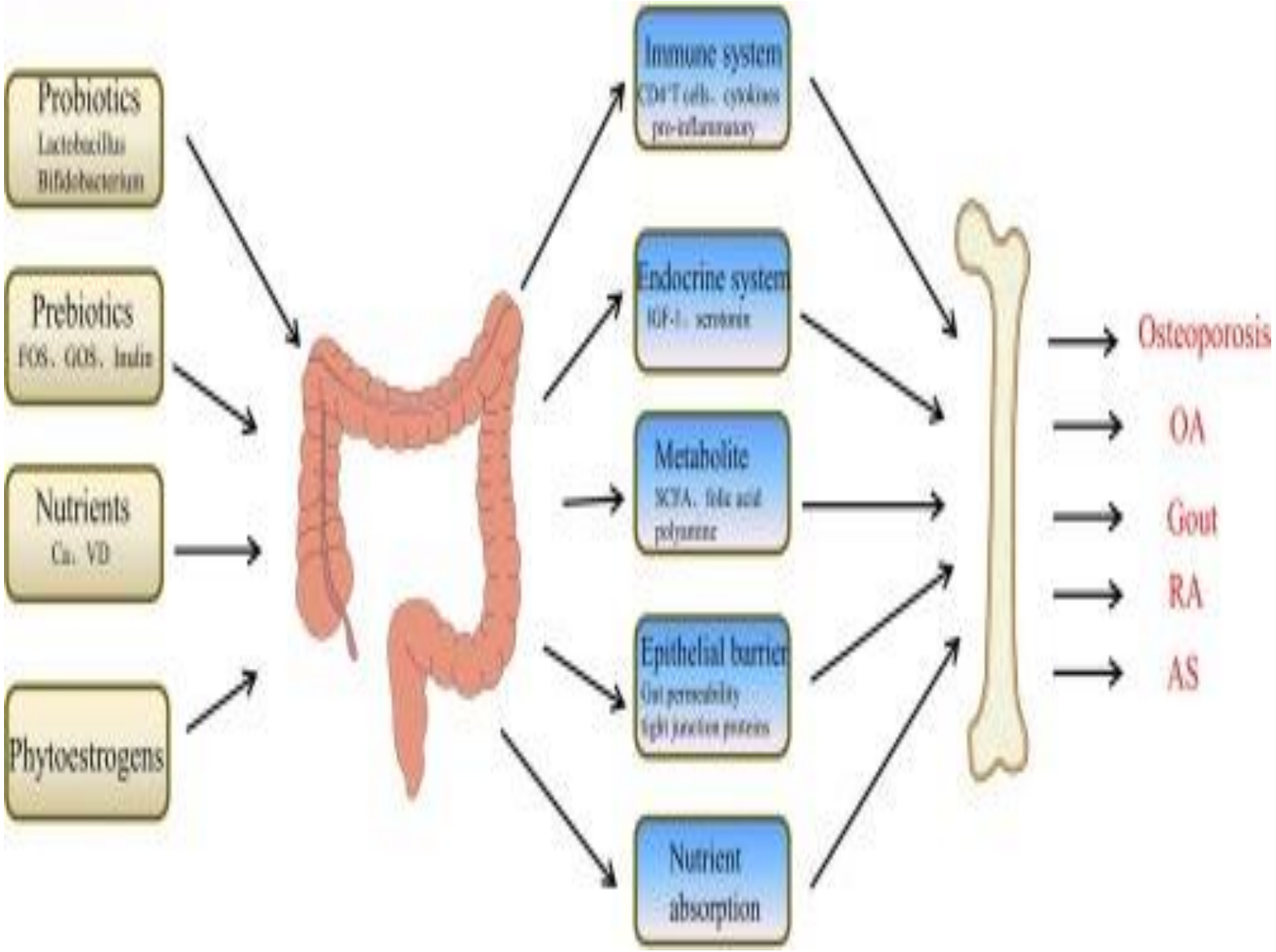
Ενδοκρινολόγος-Διαβητολόγος, Κλινική Αγίου Λουκά, Θεσαλονίκη



• Parathyroid hormone-dependent bone formation requires butyrate production by intestinal microbiota. J Clin Invest. 2020 Apr

• Involvement of the Gut Microbiota and Barrier Function in Glucocorticoid-Induced Osteoporosis. J Bone Miner Res. 2020 Apr


Probiotics
Lactobacillus
Bifidobacterium
Prebiotics
FOS, GOS, Inulin
Nutrients
Ca, VD
Phytoestrogens
Immune system
CD4+T cells, cytokines
pro-inflammatory
Endocrine system
IGF-1, serotonin
Metabolite
SCFA, folic acid
polyamine
Epithelial barrier
Gut permeability
tight junction proteins
Nutrient
absorption
Osteoporosis
OA
Gout
RA
AS

JCI The Journal of Clinical Investigation
Published by The American Society for Clinical Investigation | Founded 1908

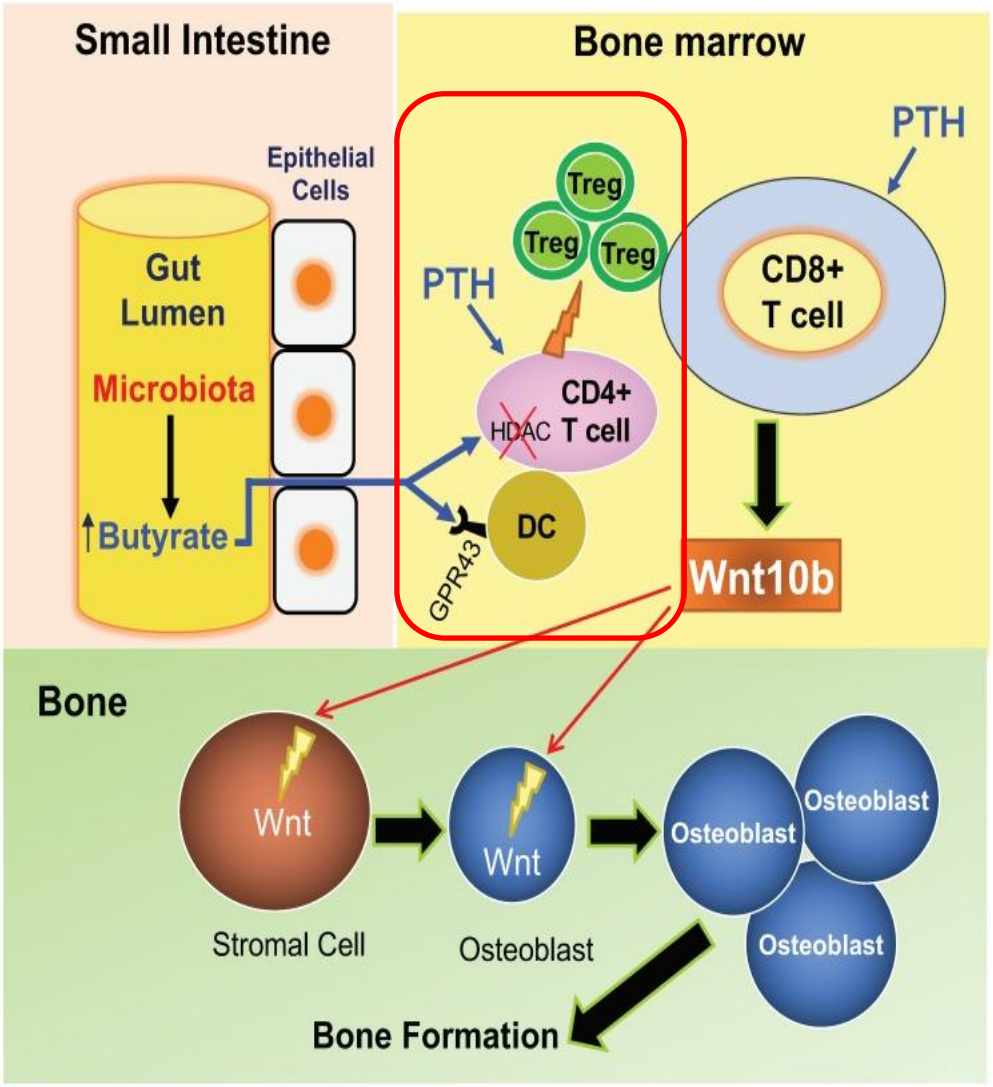
J Clin Invest. 2020 Apr 1; 130(4): 1767–1781.
Published online 2020 Mar 3. doi: 10.1172/JCI133473

PMCID: PMC7108906
PMID: 31917685

Parathyroid hormone–dependent bone formation requires butyrate production by intestinal microbiota

Jau-Yi Li,[1,2] Mingcan Yu,[1,2] Subhashis Pal,[1,2] Abdul Malik Tyagi,[1,2] Hamid Dar,[1,2] Jonathan Adams,[1,2] M. Neale Weitzmann,[1,2,3] Rheinallt M. Jones,[2,4,5] and Roberto Pacifici[1,2,5]

Background



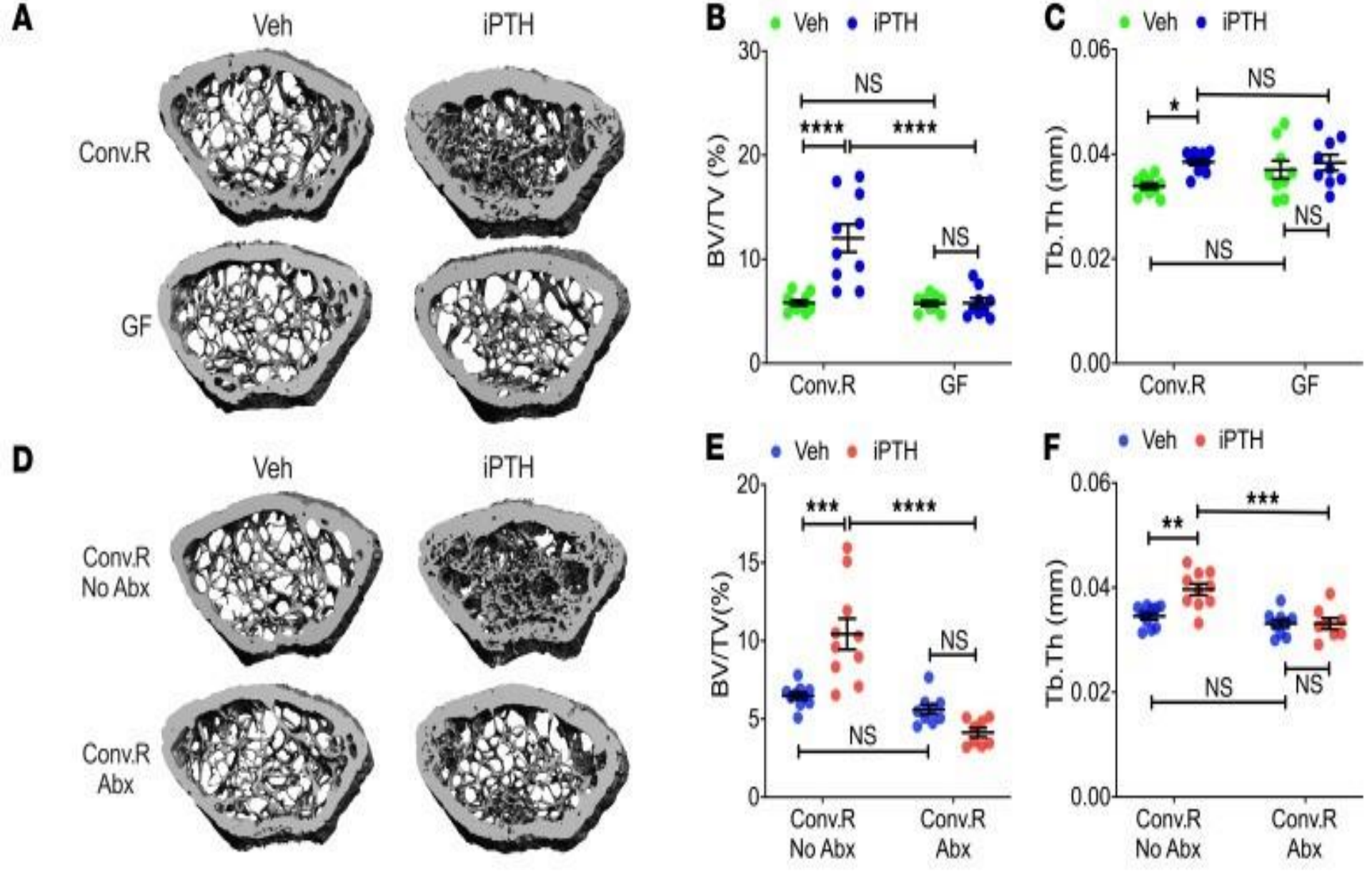
•PTH: κομβικός ρόλος στην οστική ομοιοστασία
• Δράση είτε αναβολική είτε καταβολική
• Δύο τουλάχιστον οδοί δράσης:
Wnt pathway αλλά και μέσω των Treg με….

«…Reports have highlighted the bone-regulating capacities of Tregs, describing mechanisms where Tregs blunt bone resorption, stimulate bone formation by promoting the differentiation of osteoblasts, and are pivotal for the stimulation of bone formation induced by nutritional supplementation with the probiotic *Lactobacillus rhamnosus* GG (LGG)»

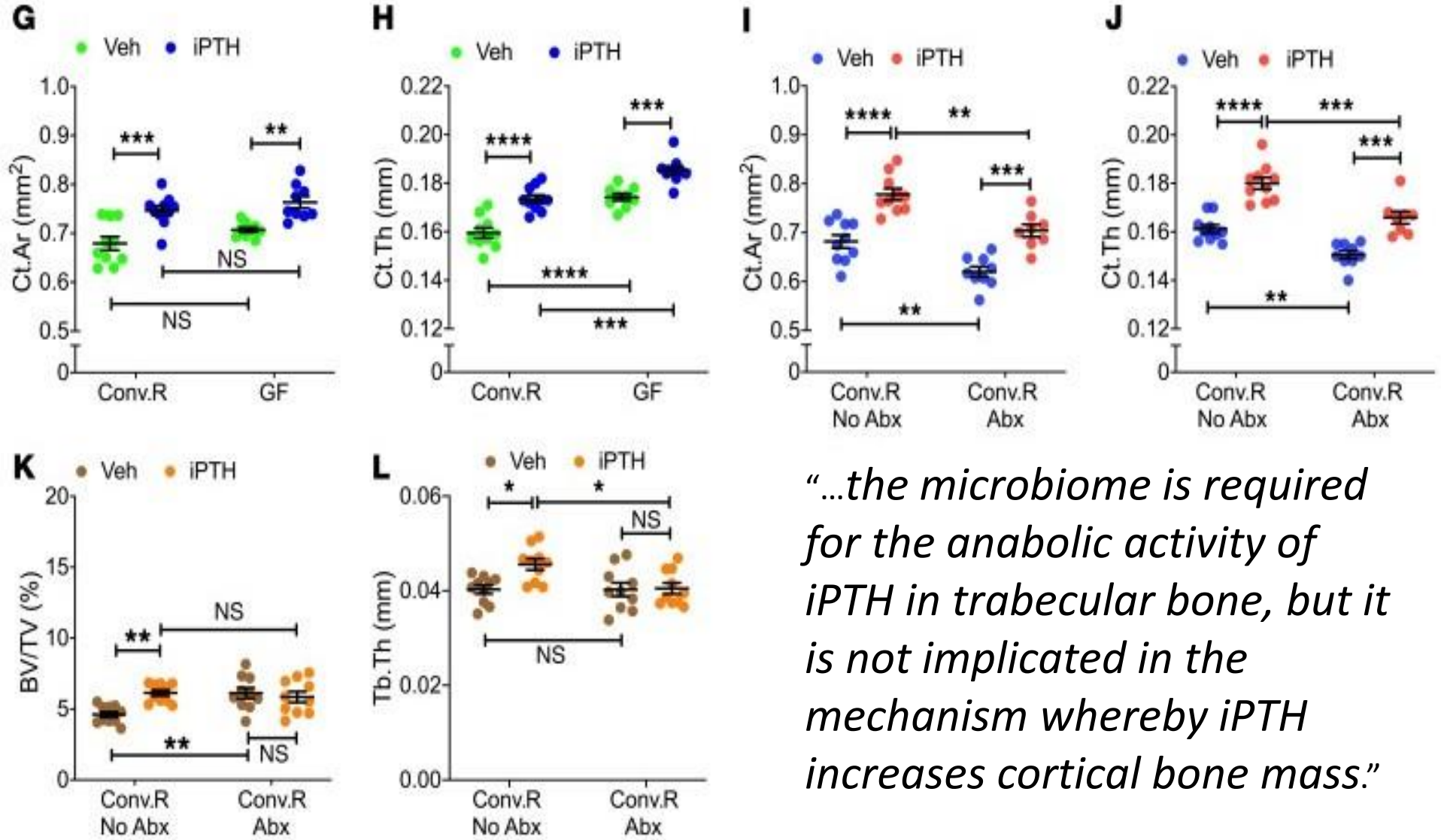
Ερώτημα μελέτης

- … We examined the role of the microbiota and of butyrate in the regulation of bone responses to iPTH in young female mice.
- Ποια η επίδραση σε germ-free mice and antibiotic-treated conventional mice;
- Και τι συμβαίνει στην iPTH και στον οστικό μεταβολισμό αν διορθωθεί η κατάσταση;

Αποτελέσματα (1)

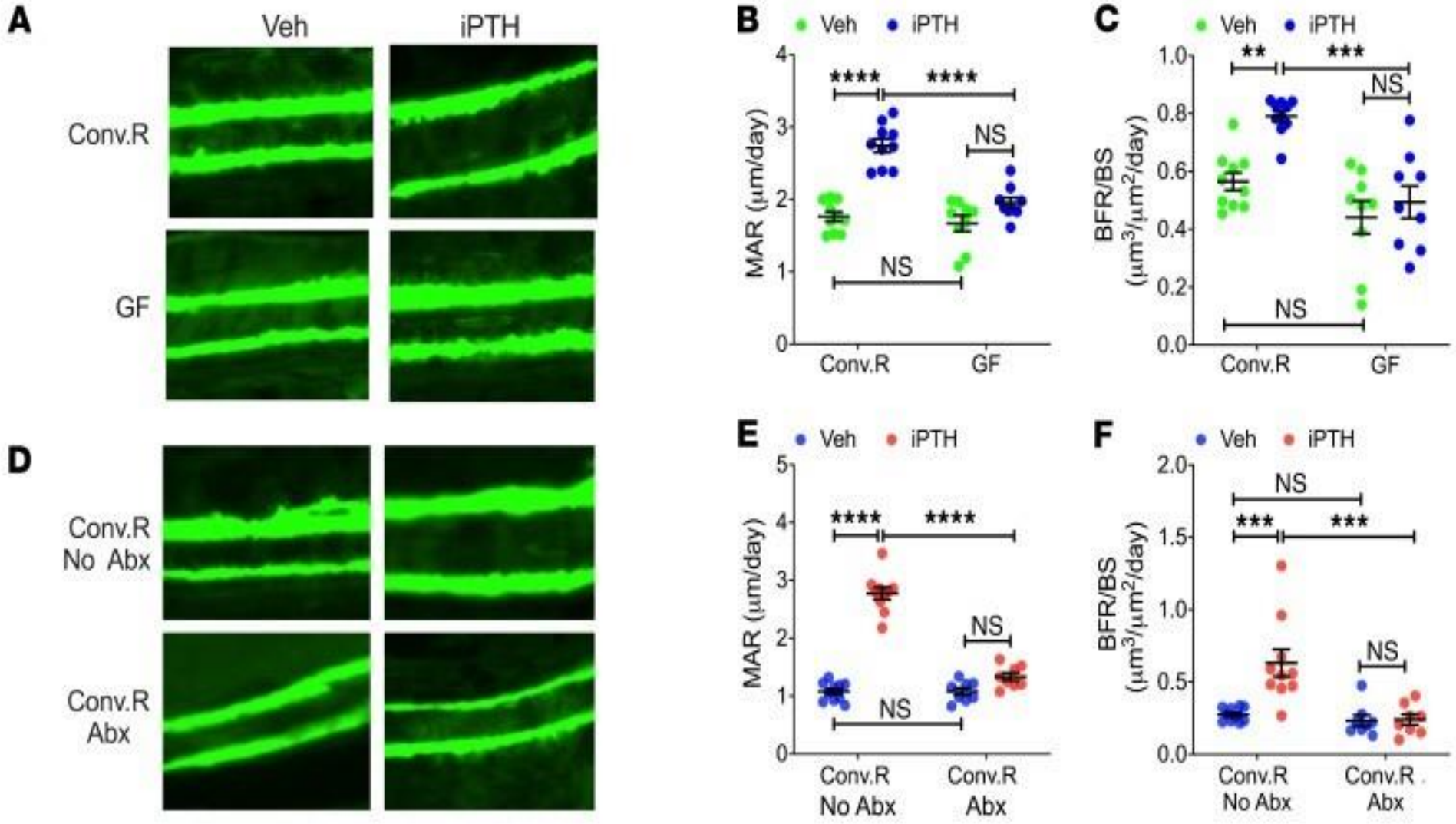


Αποτελέσματα (2)

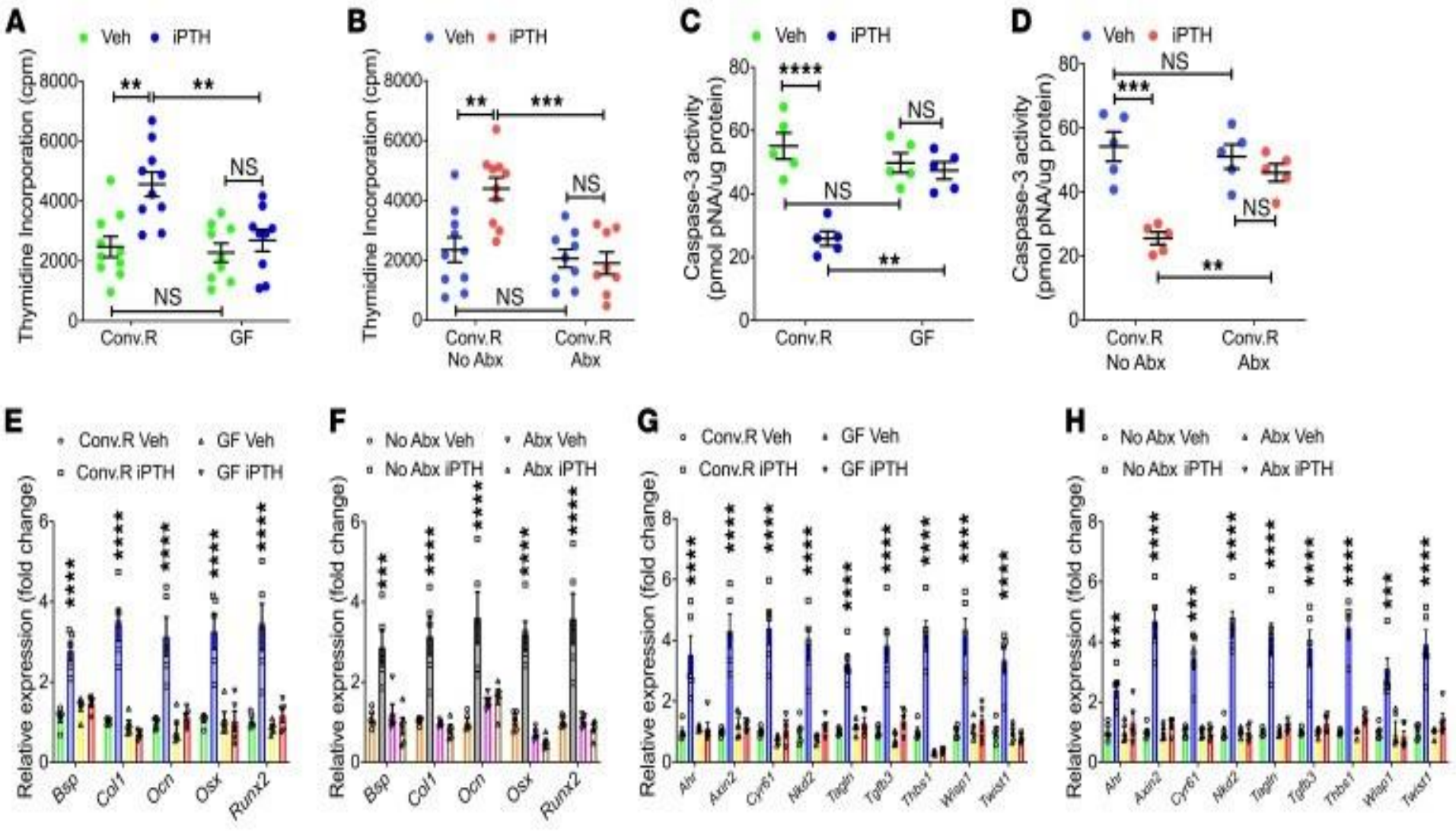


"…the microbiome is required for the anabolic activity of iPTH in trabecular bone, but it is not implicated in the mechanism whereby iPTH increases cortical bone mass."

Αποτελέσματα (3)



Αποτελέσματα (4)

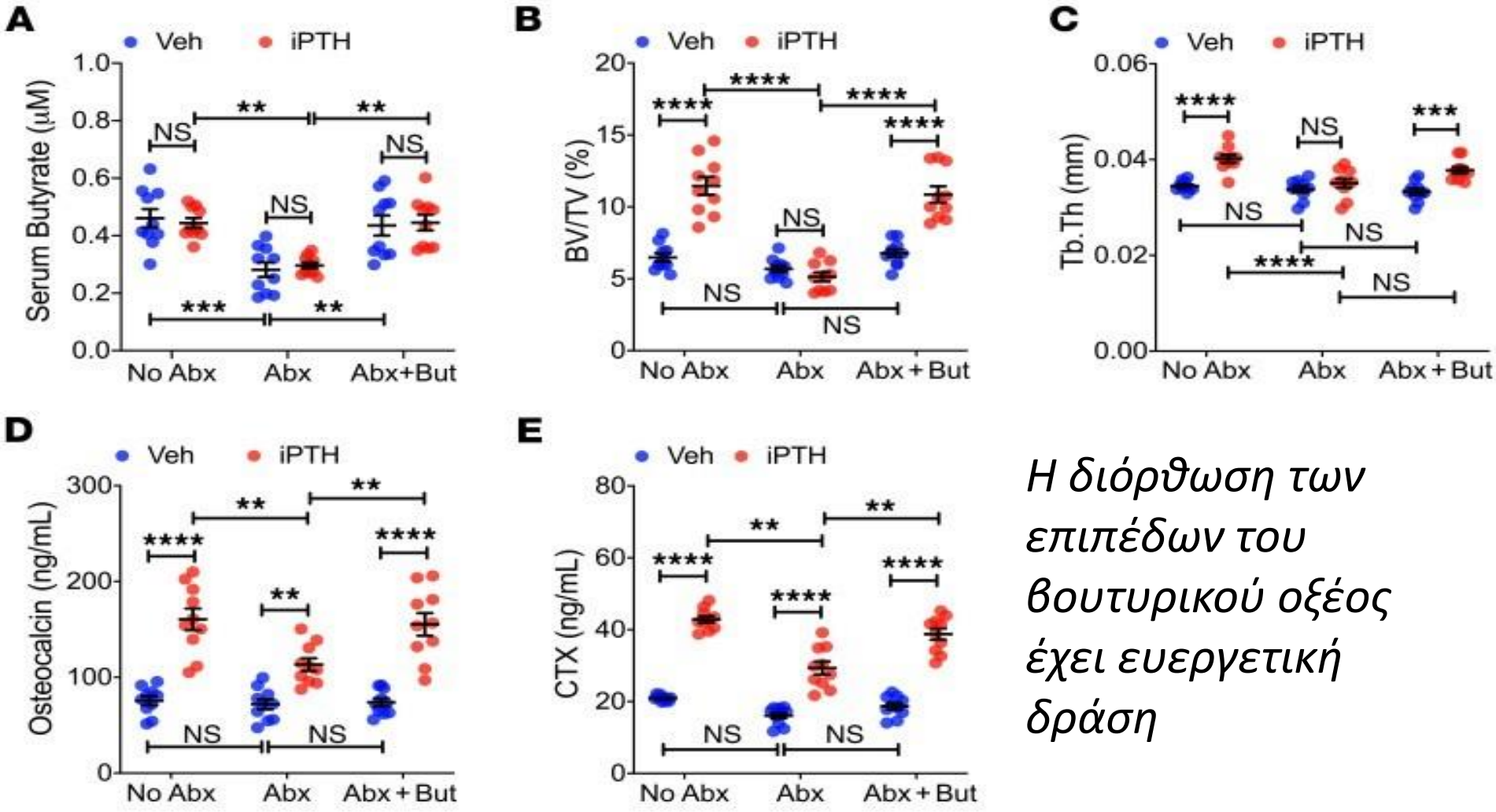


Ως τώρα...

1. **iPTH treatment fails to improve trabecular bone structure in GF mice and conventional mice treated with antibiotics.**
2. **iPTH treatment fails to stimulate trabecular bone turnover in 12-week-old GF mice and conventional mice treated with antibiotics.**
3. **iPTH treatment fails to regulate SC proliferation and life span, T cell expression of Wnt10b, number of Tregs, and BM production of TGF-β and IGF-1.**

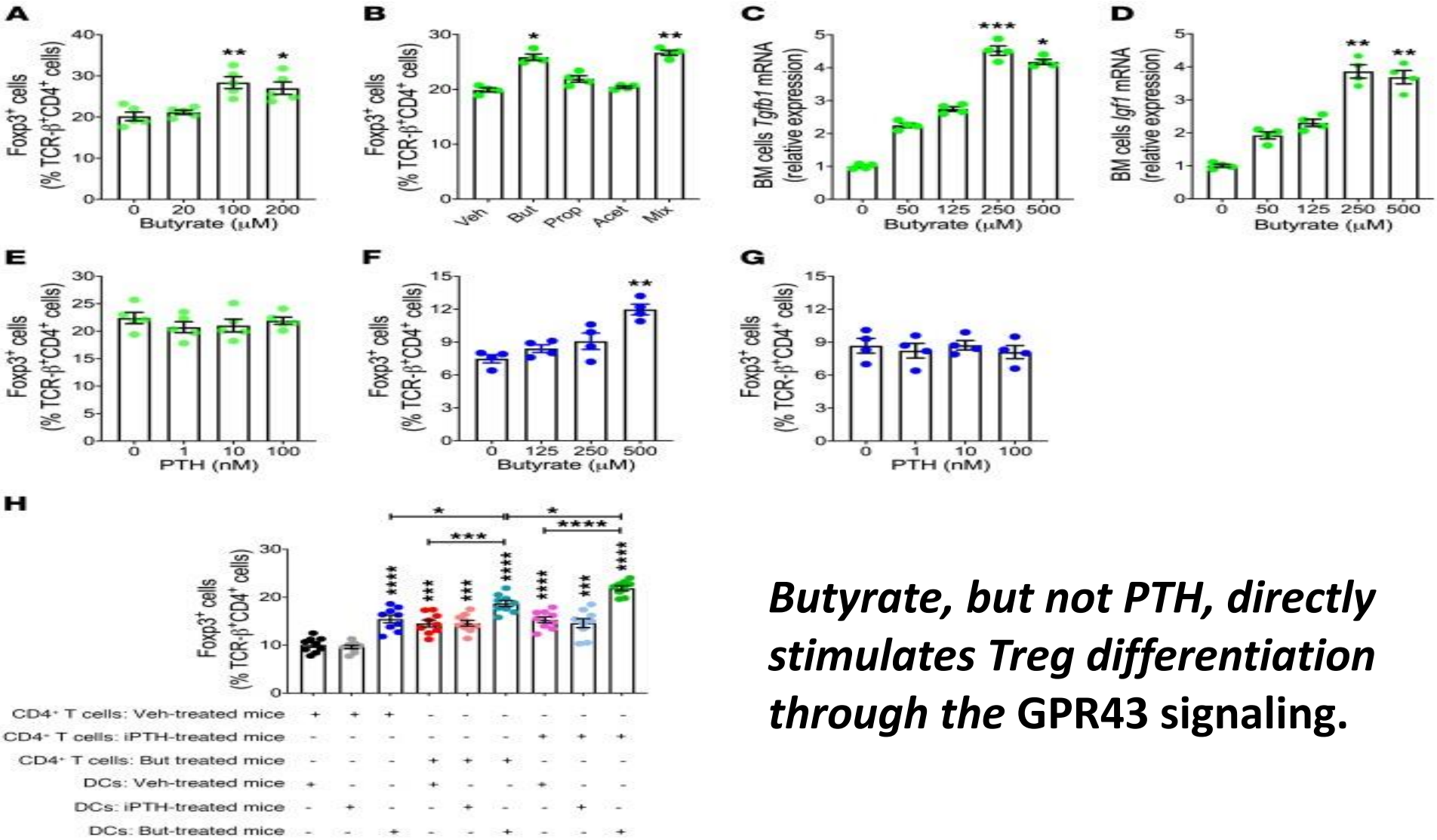
These findings demonstrate that iPTH regulates osteoblast proliferation, differentiation, and life span through a ***microbiome-dependent mechanism.***

Αποτελέσματα (5)



Η διόρθωση των επιπέδων του βουτυρικού οξέος έχει ευεργετική δράση

Αποτελέσματα (6)



***Butyrate, but not PTH, directly stimulates Treg differentiation through the* GPR43 signaling.**

Προοπτικές...

- An increase in the number of Tregs achievable by nutritional supplementation with butyrate may represent a novel therapeutic modality for osteoporosis or for potentiating the bone anabolic activity of PTH.
- Moreover, the use of butyrate to increase the number of Tregs may find wider applications, such as in transplant medicine or as a treatment for inflammatory and autoimmune conditions.

Journal of Bone and Mineral Research
JBMR
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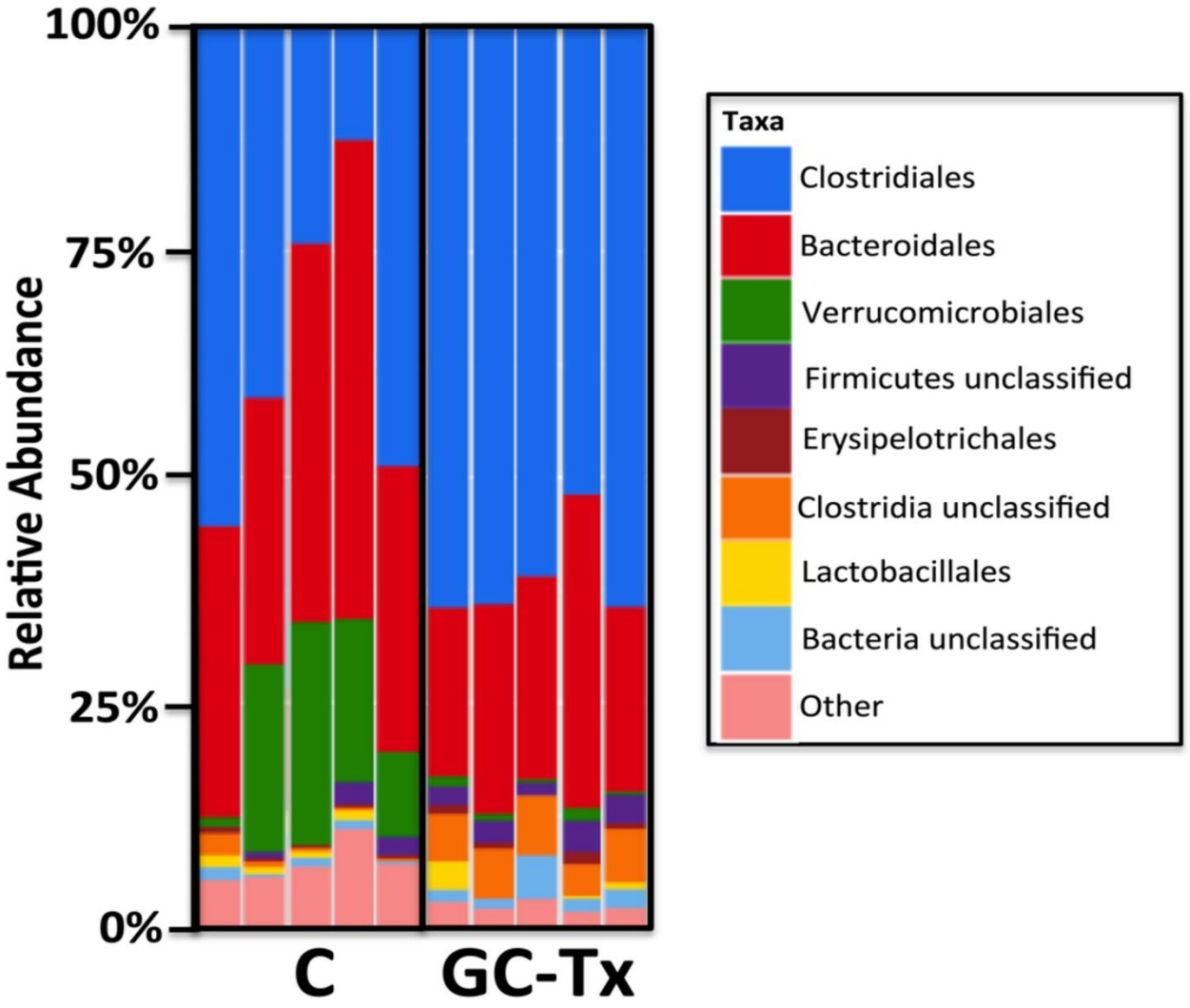
Original Article | Free Access

Involvement of the Gut Microbiota and Barrier Function in Glucocorticoid-Induced Osteoporosis

Jonathan D Schepper, Fraser Collins, Naiomy Deliz Rios-Arce, Ho Jun Kang, Laura Schaefer, Joseph D Gardinier, Ruma Raghuvanshi, Robert A Quinn, Robert Britton ... See all authors

First published: 30 December 2019 | https://doi.org/10.1002/jbmr.3947 | Citations: 33

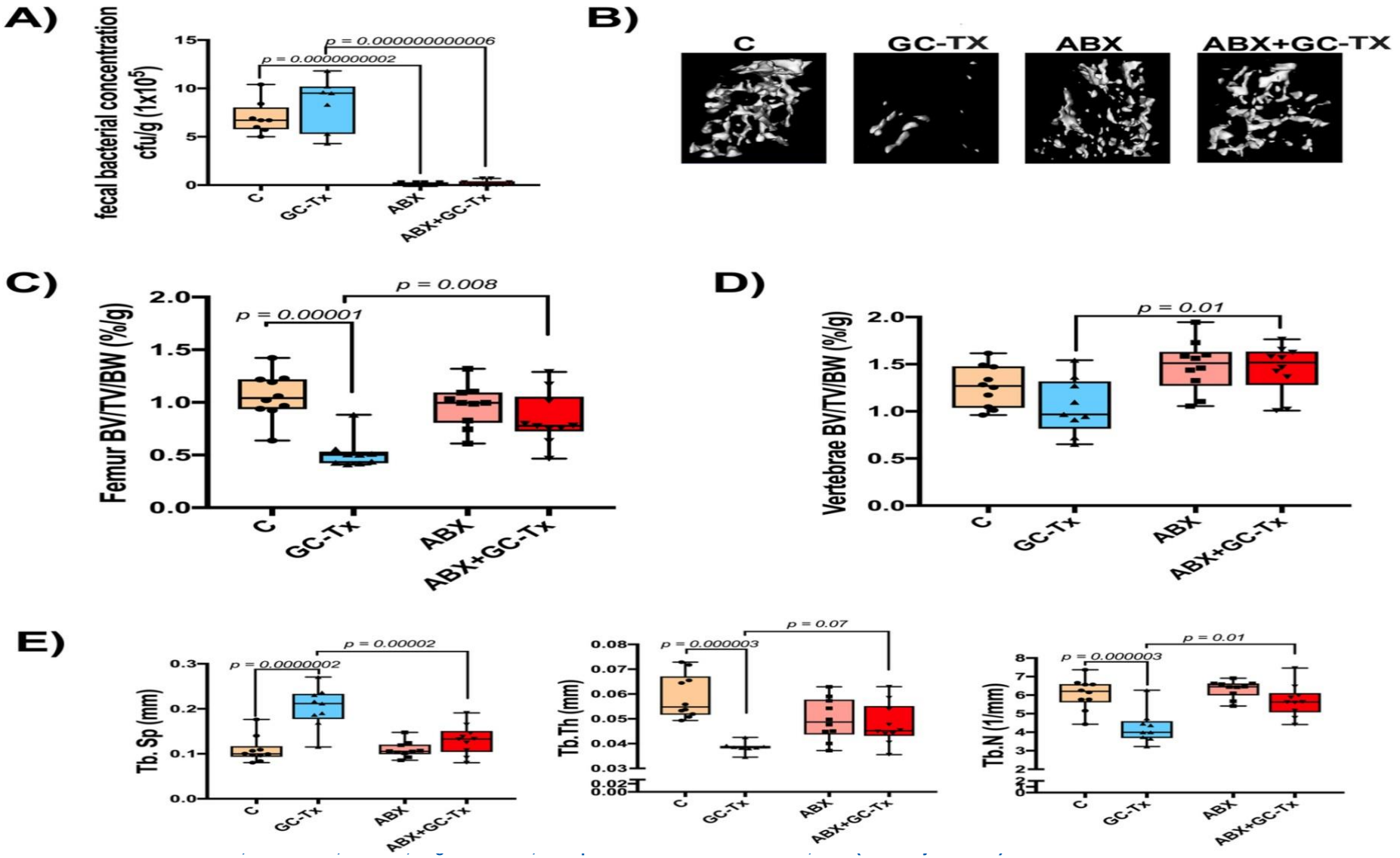
Glucocorticoid treatment causes dysbiosis



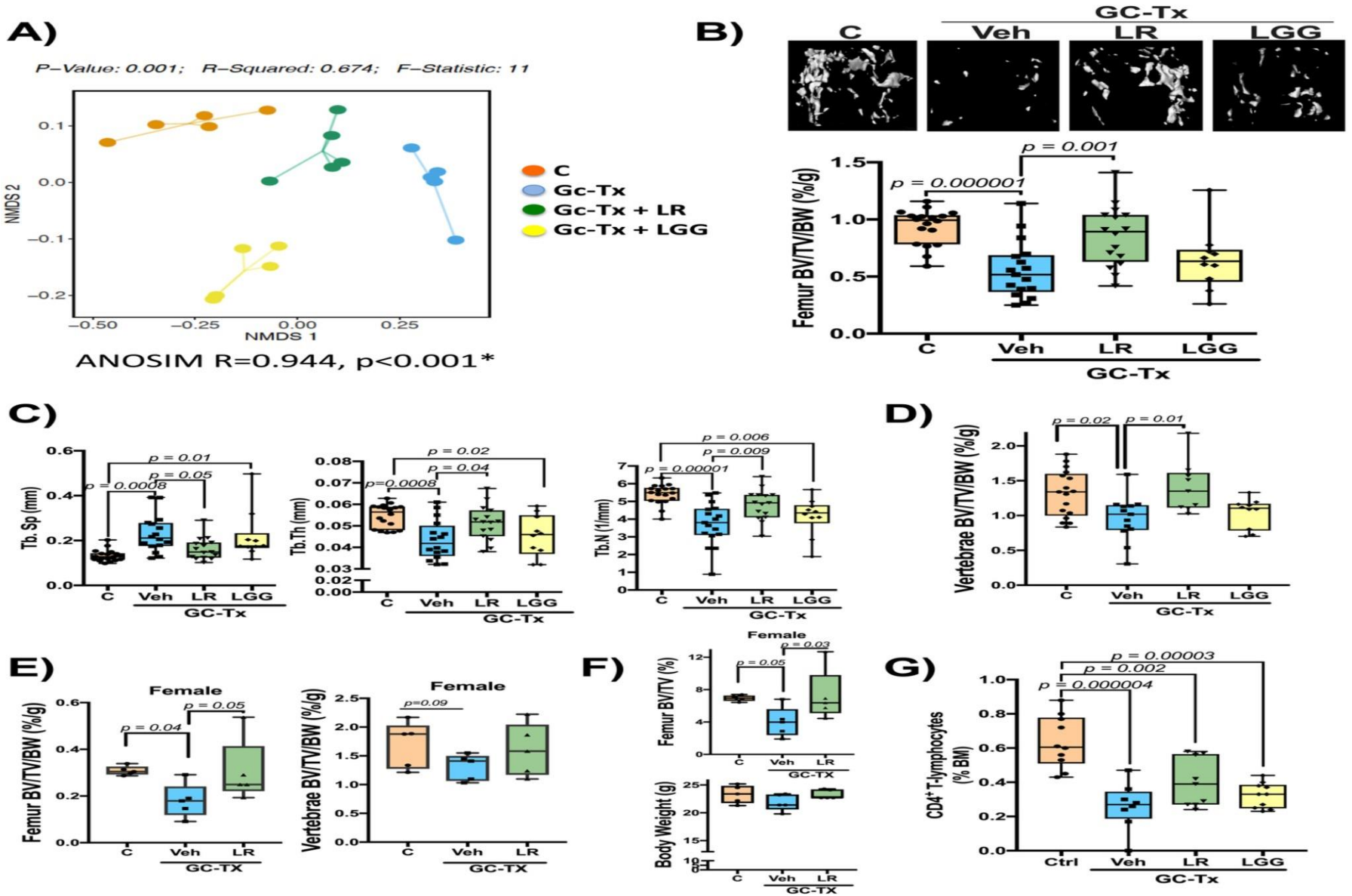
Glucocorticoid treatment causes ***dysbiosis***

J of Bone & Mineral Res, Volume: 35, Issue: 4, Pages: 801-820, First published: 30 December 2019, DOI: (10.1002/jbmr.3947)

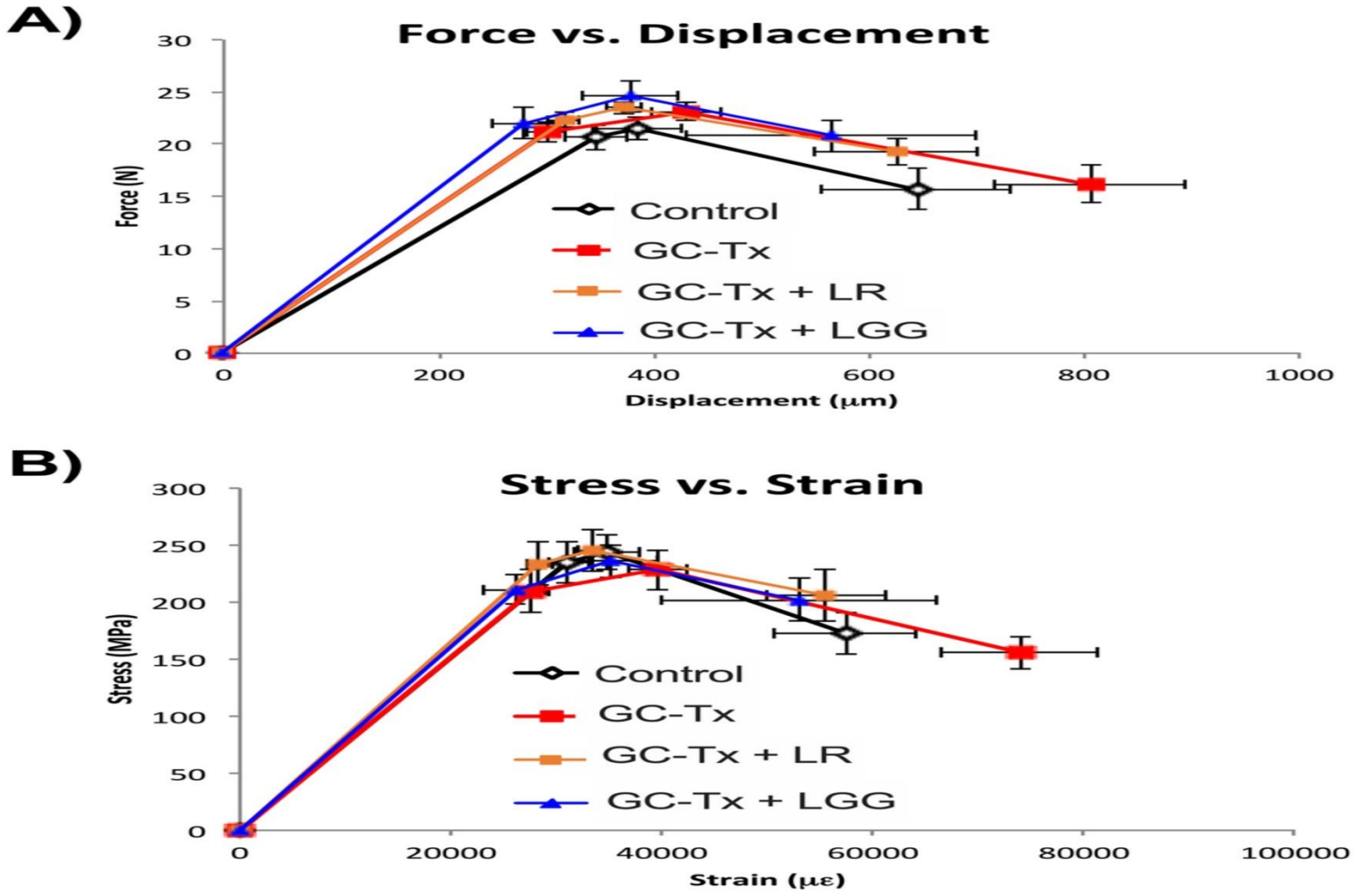
Depletion of the gut microbiota prevents glucocorticoid induced trabecular bone loss.



Probiotic Lactobacillus reuteri 6475 supplementation prevents glucocorticoid-induced bone loss.

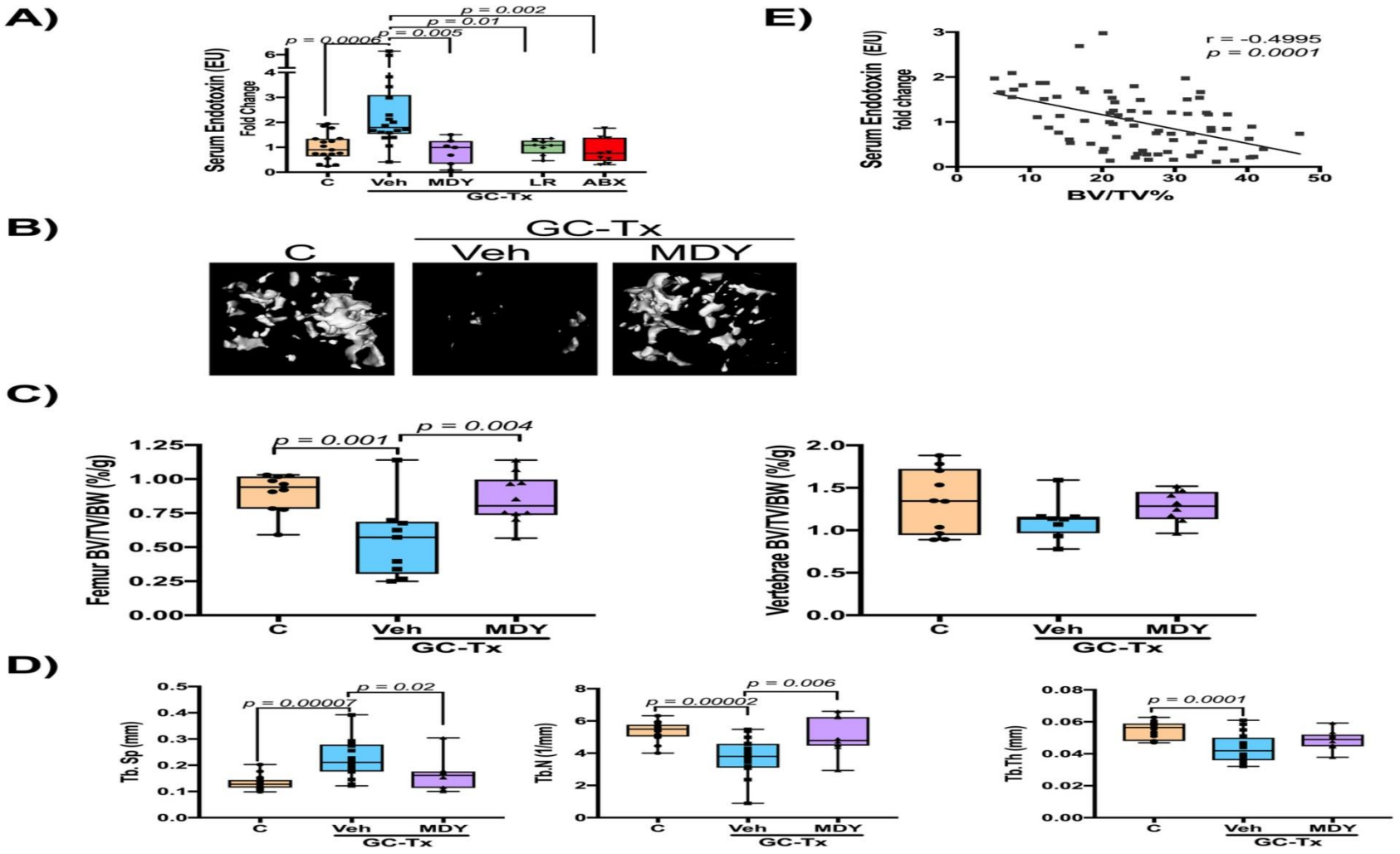


Probiotic treatment did not affect GC-Tx induced changes in cortical bone mechanical strength properties



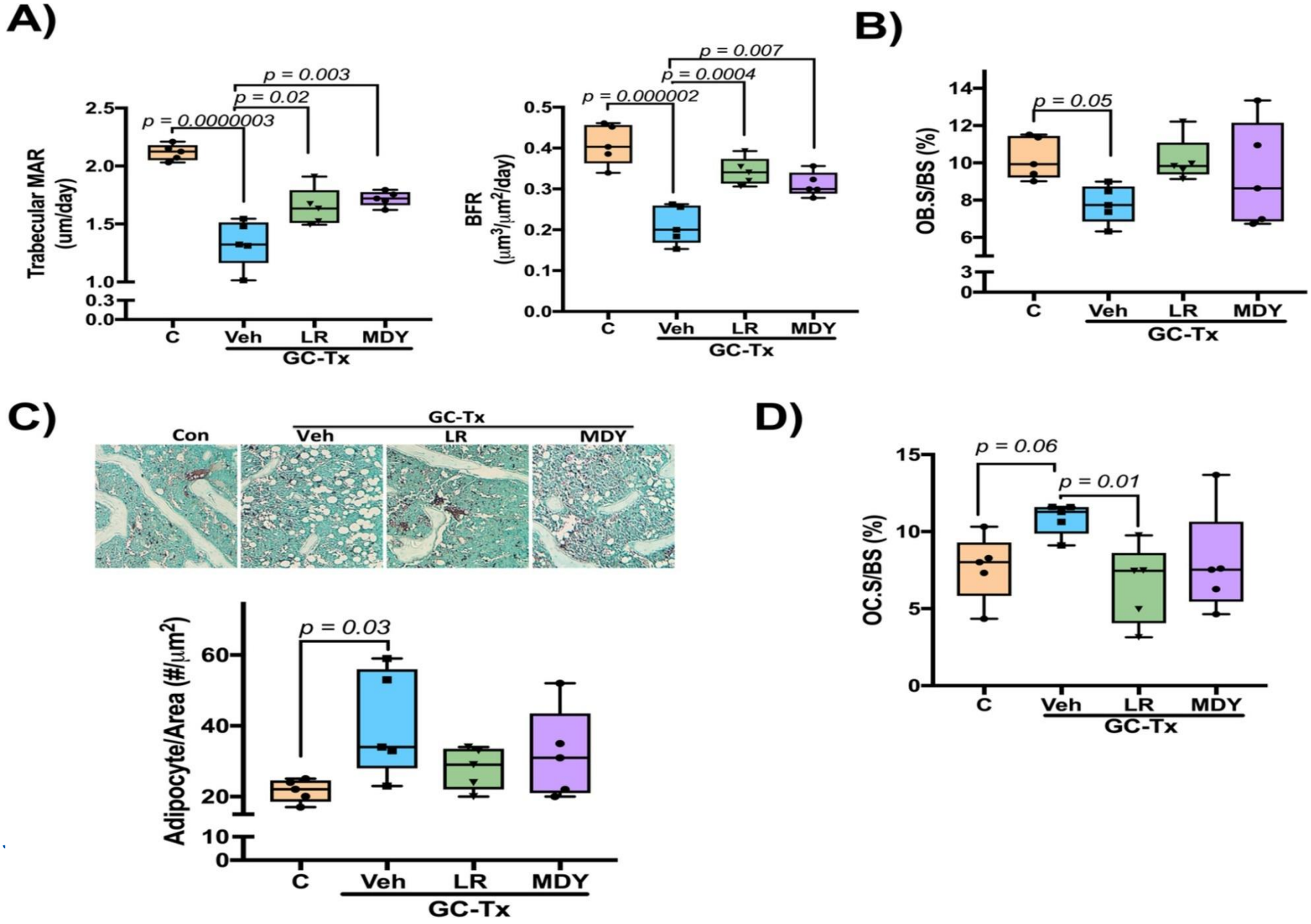
J of Bone & Mineral Res, Volume: 35, Issue: 4, Pages: 801-820, First published: 30 December 2019, DOI: (10.1002/jbmr.3947)

Barrier dysfunction mediates glucocorticoid-induced bone loss.



J of Bone & Mineral Res, Volume: 35, Issue: 4, Pages: 801-820, First published: 30 December 2019, DOI: (10.1002/jbmr.3947)

Prevention of GIO by LR and MDY is the result of retaining anabolic bone activity and reducing catabolic activity under GC treatment conditions.



Συμπεράσματα…

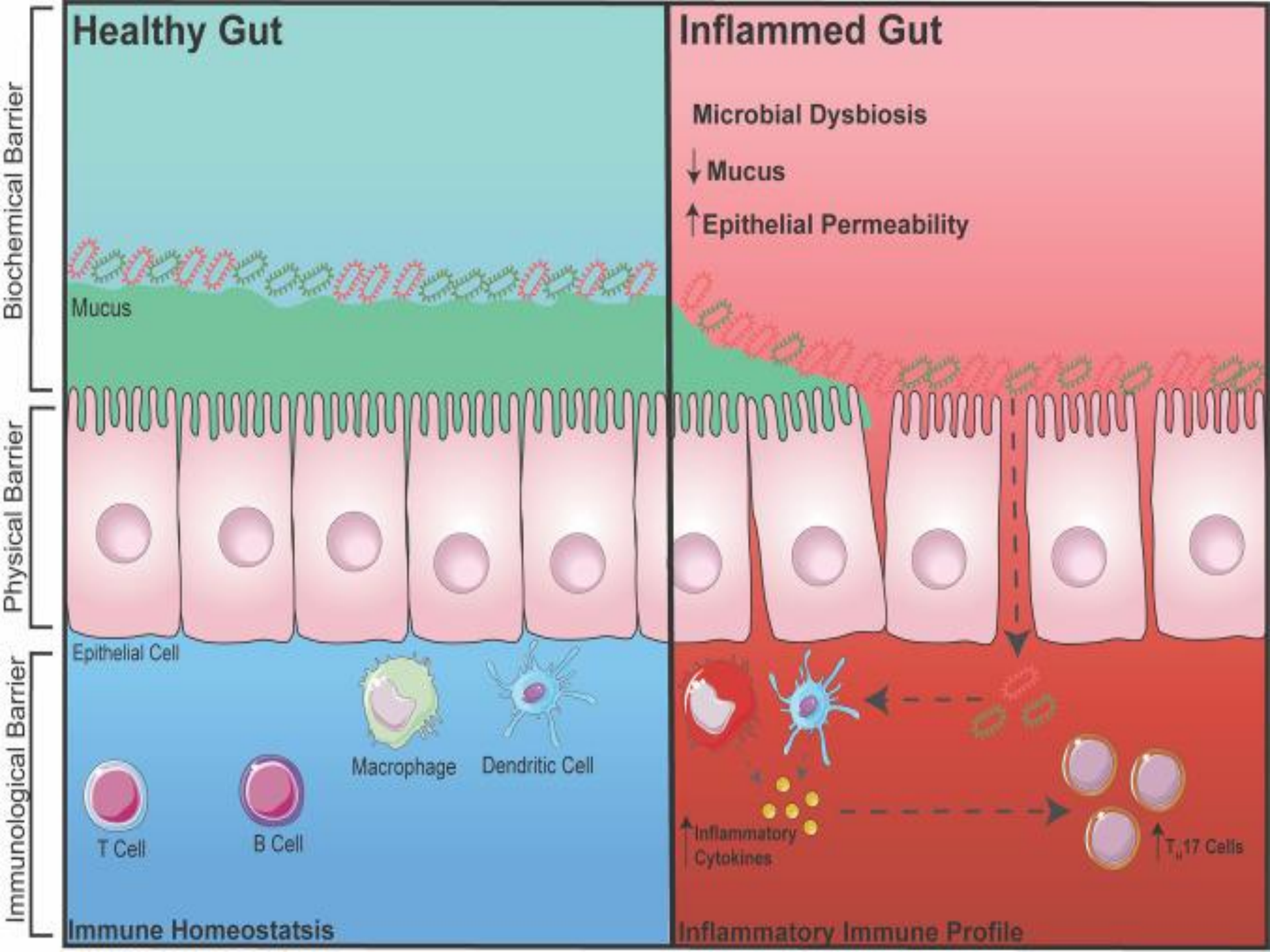
• Significant evidence for the role of the microbiota in mediating GIO

• Overall increase in the relative abundance of Clostridiales and a decrease in Bacteroidales and Verruomicrobiales groups.

•LR was able to prevent trabecular bone loss

• Alterations in the gut microbiota that reduce beneficial bacteria and increase unhealthy bacteria can promote intestinal permeability and increase serum endotoxin

• Enhancing barrier function with MDY prevented trabecular bone loss


Biochemical Barrier
Physical Barrier
Immunological Barrier
Healthy Gut
Mucus
Epithelial Cell
Macrophage
Dendritic Cell
T Cell
B Cell
Immune Homeostatsis
Inflammed Gut
Microbial Dysbiosis
Mucus
Epithelial Permeability
Inflammatory Cytokines
T_H17 Cells
Inflammatory Immune Profile

JBMR PLUS Open Access ASBMR

JBMR Plus. 2021 Mar; 5(3): e10452. PMCID: PMC7990138

Published online 2021 Jan 19. doi: 10.1002/jbm4.10452 PMID: 33778322

The Gut Microbiome Is Altered in Postmenopausal Women With Osteoporosis and Osteopenia

Elizabeth A Rettedal,[1] Bolaji L Ilesanmi-Oyelere,[1,2,3] Nicole C Roy,[2,4,5,6] Jane Coad,[7] and Marlena C Kruger[2,3,5]

Υπόθεση και σκοπός

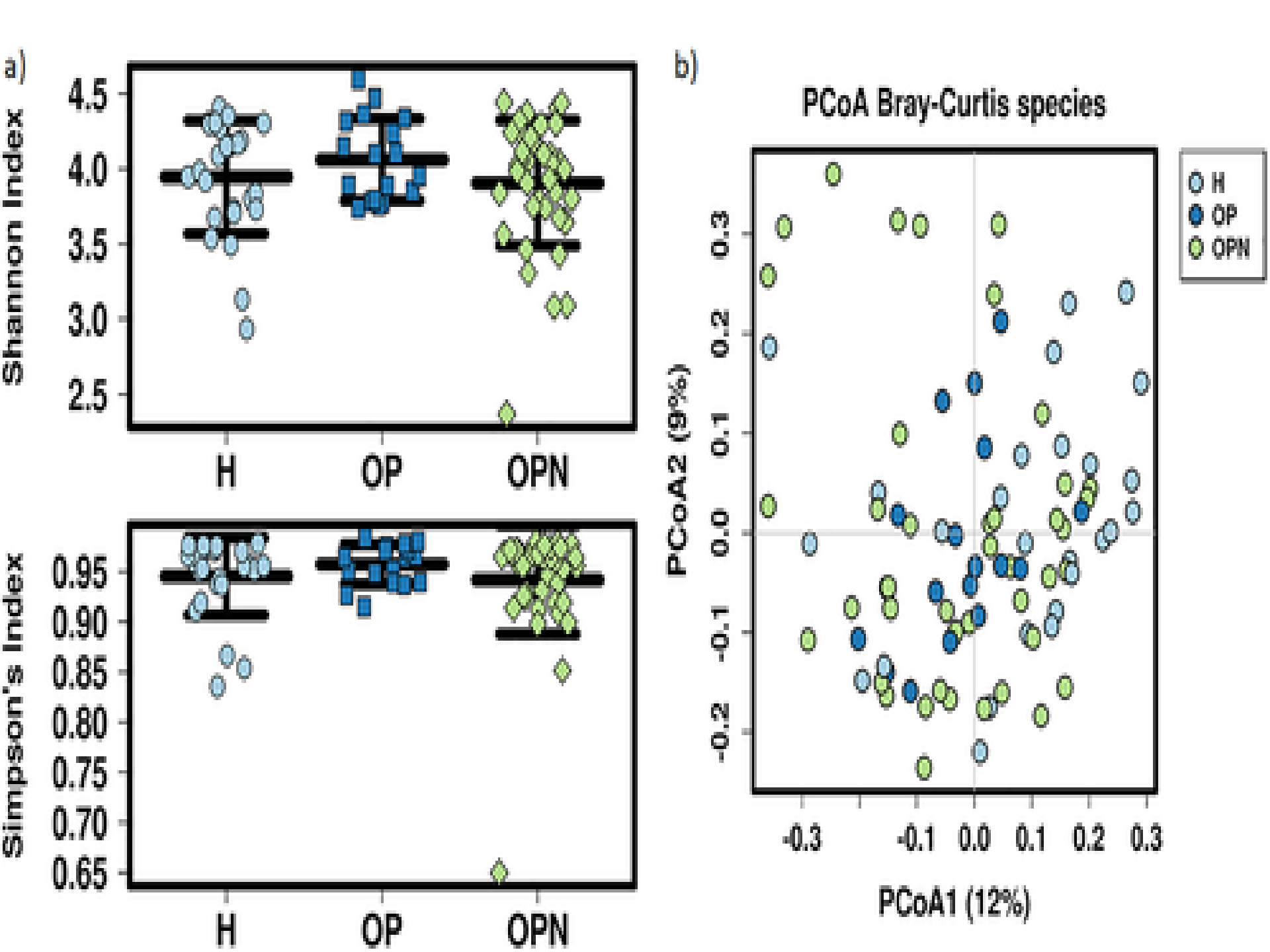
To characterize the diversity, composition, and functional gene potential of the gut microbiota of healthy, osteopenic, and osteoporotic women.

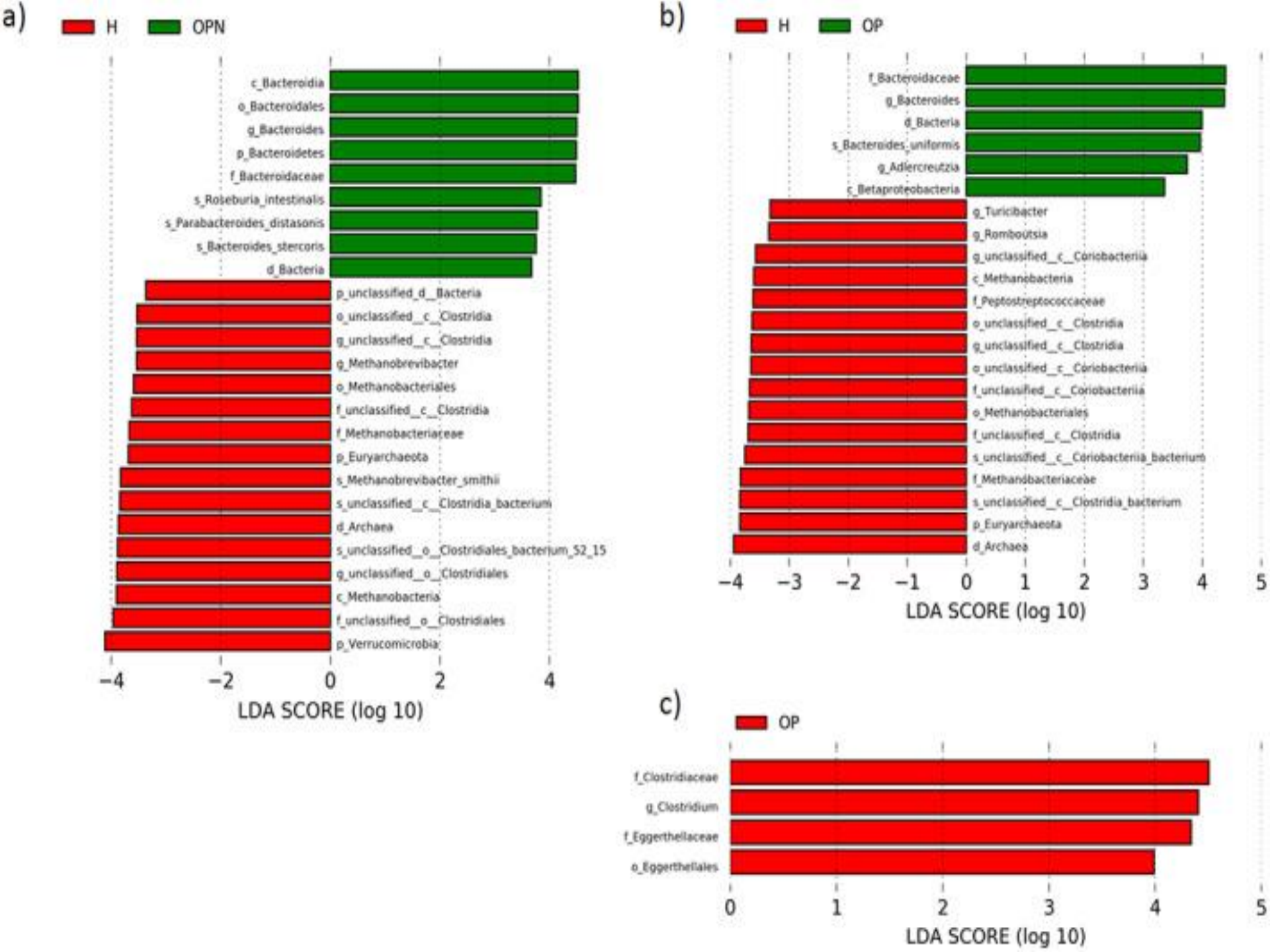
Μέθοδος

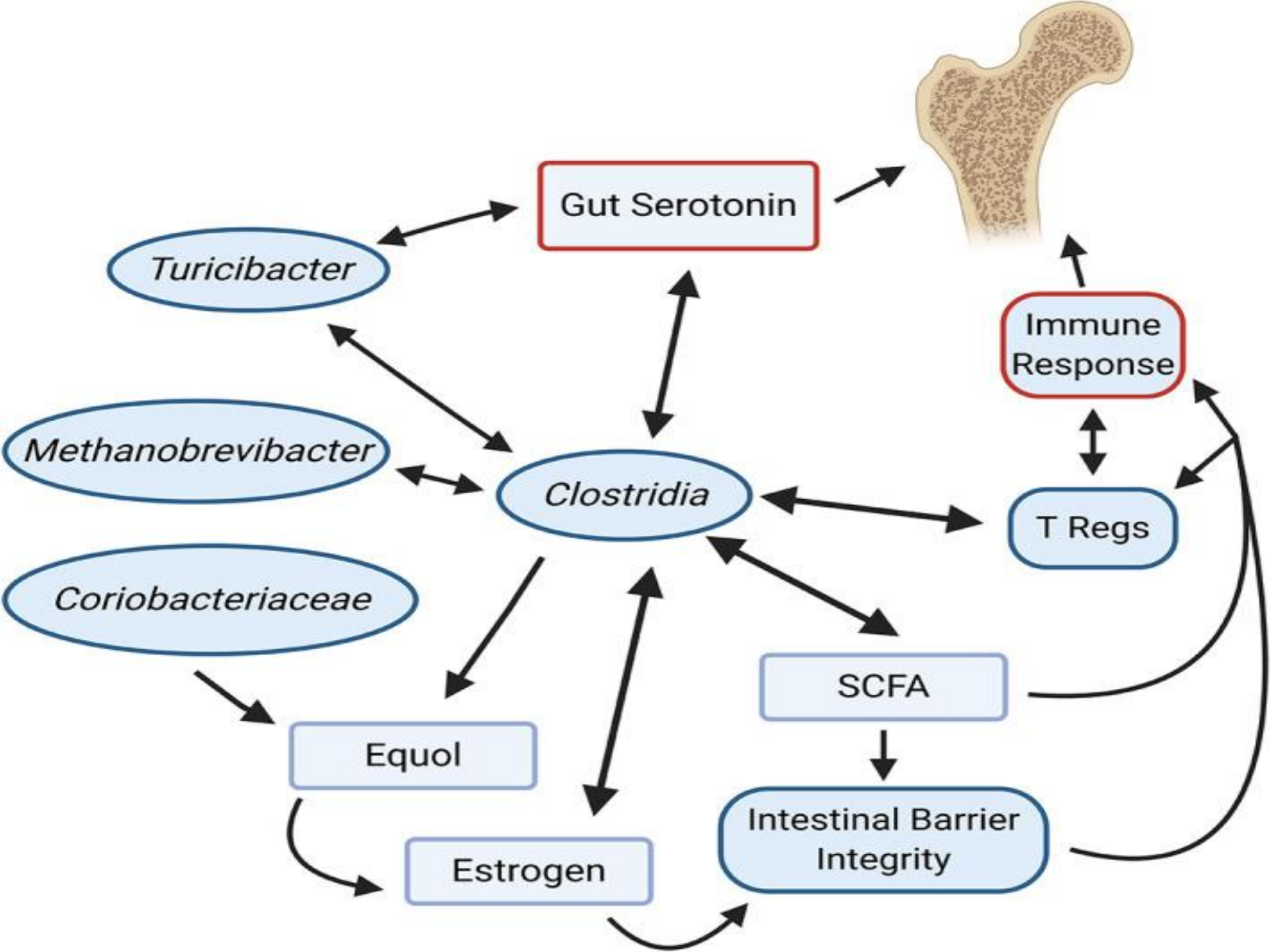
•Body composition, bone density, and fecal metagenomes were analyzed in 86 postmenopausal women.

•The women were classified as healthy, osteopenic, or osteoporotic based on *T*-scores.

•The taxonomic and functional gene compositions of the microbiome were analyzed using shotgun metagenomic sequencing.


a)
Shannon Index
4.5
4.0
3.5
3.0
2.5
H
OP
OPN
Simpson's Index
0.95
0.90
0.85
0.80
0.75
0.70
0.65
H
OP
OPN
b)
PCoA Bray-Curtis species
H
OP
OPN
PCoA2 (9%)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.1
0.0
0.1
0.2
0.3
PCoA1 (12%)


a)
H
OPN
c_Bacteroidia
o_Bacteroidales
g_Bacteroides
p_Bacteroidetes
f_Bacteroidaceae
s_Roseburia_intestinalis
s_Parabacteroides_distasonis
s_Bacteroides_stercoris
d_Bacteria
p_unclassified_d__Bacteria
o_unclassified__c__Clostridia
g_unclassified__c__Clostridia
g_Methanobrevibacter
o_Methanobacteriales
f_unclassified__c__Clostridia
f_Methanobacteriaceae
p_Euryarchaeota
s_Methanobrevibacter_smithii
s_unclassified__c__Clostridia_bacterium
d_Archaea
s_unclassified__o__Clostridiales_bacterium_52_15
g_unclassified__o__Clostridiales
c_Methanobacteria
f_unclassified__o__Clostridiales
p_Verrucomicrobia
−4
−2
0
2
4
LDA SCORE (log 10)
b)
H
OP
f_Bacteroidaceae
g_Bacteroides
d_Bacteria
s_Bacteroides_uniformis
g_Adlercreutzia
c_Betaproteobacteria
g_Turicibacter
g_Romboutsia
g_unclassified__c__Coriobacteriia
c_Methanobacteria
f_Peptostreptococcaceae
o_unclassified__c__Clostridia
g_unclassified__c__Clostridia
o_unclassified__c__Coriobacteriia
f_unclassified__c__Coriobacteriia
o_Methanobacteriales
f_unclassified__c__Clostridia
s_unclassified__c__Coriobacteriia_bacterium
f_Methanobacteriaceae
s_unclassified__c__Clostridia_bacterium
p_Euryarchaeota
d_Archaea
−4
−3
−2
−1
0
1
2
3
4
5
LDA SCORE (log 10)
c)
OP
f_Clostridiaceae
g_Clostridium
f_Eggerthellaceae
o_Eggerthellales
0
1
2
3
4
5
LDA SCORE (log 10)


Gut Serotonin
Turicibacter
Methanobrevibacter
Clostridia
Coriobacteriaceae
Immune Response
T Regs
SCFA
Equol
Estrogen
Intestinal Barrier Integrity

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Osteoporos Int. 2021; 32(1): 145–156. PMCID: PMC7755620
Published online 2020 Nov 25. doi: 10.1007/s00198-020-05728-y PMID: 33241467

Association between gut microbiota, bone metabolism, and fracture risk in postmenopausal Japanese women

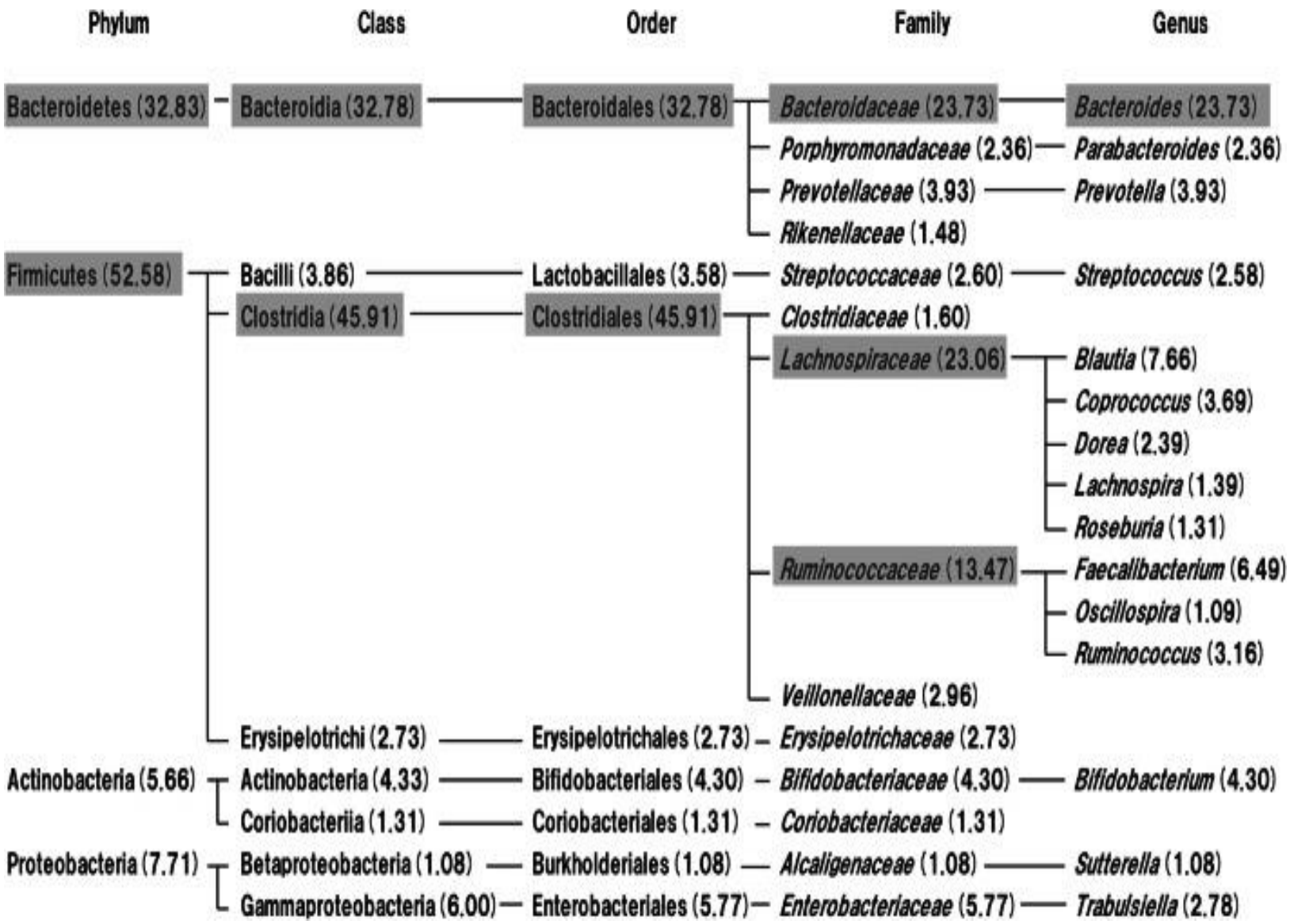
D. Ozaki,[1] R. Kubota,[1] T. Maeno,[2] M. Abdelhakim,[3] and N. Hitosugi[4]

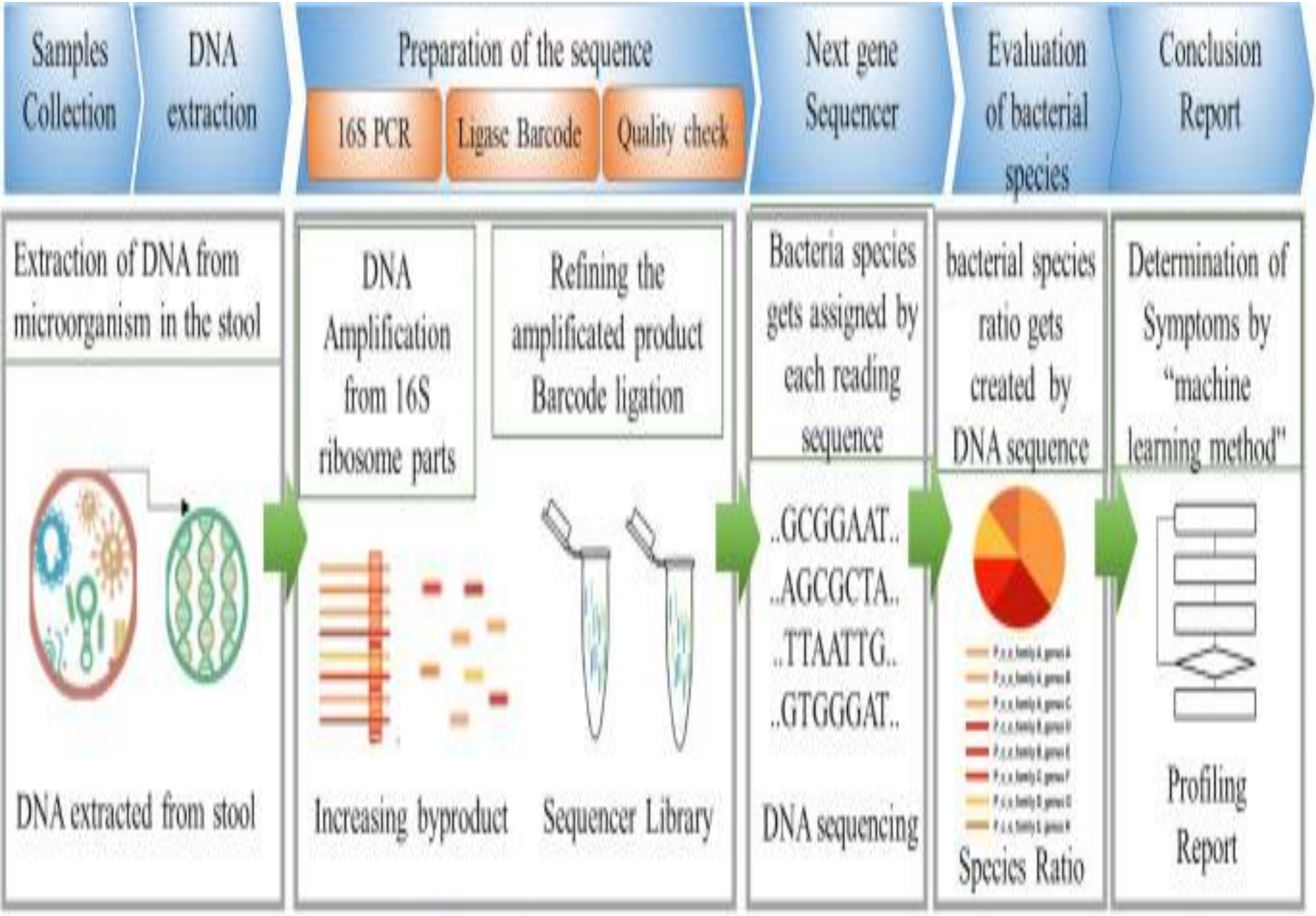
Υπόθεση και σκοπός

To investigate the relationship between gut microbiota composition and osteoporosis/fracture risk
Japanese postmenopausal women

Μέθοδος

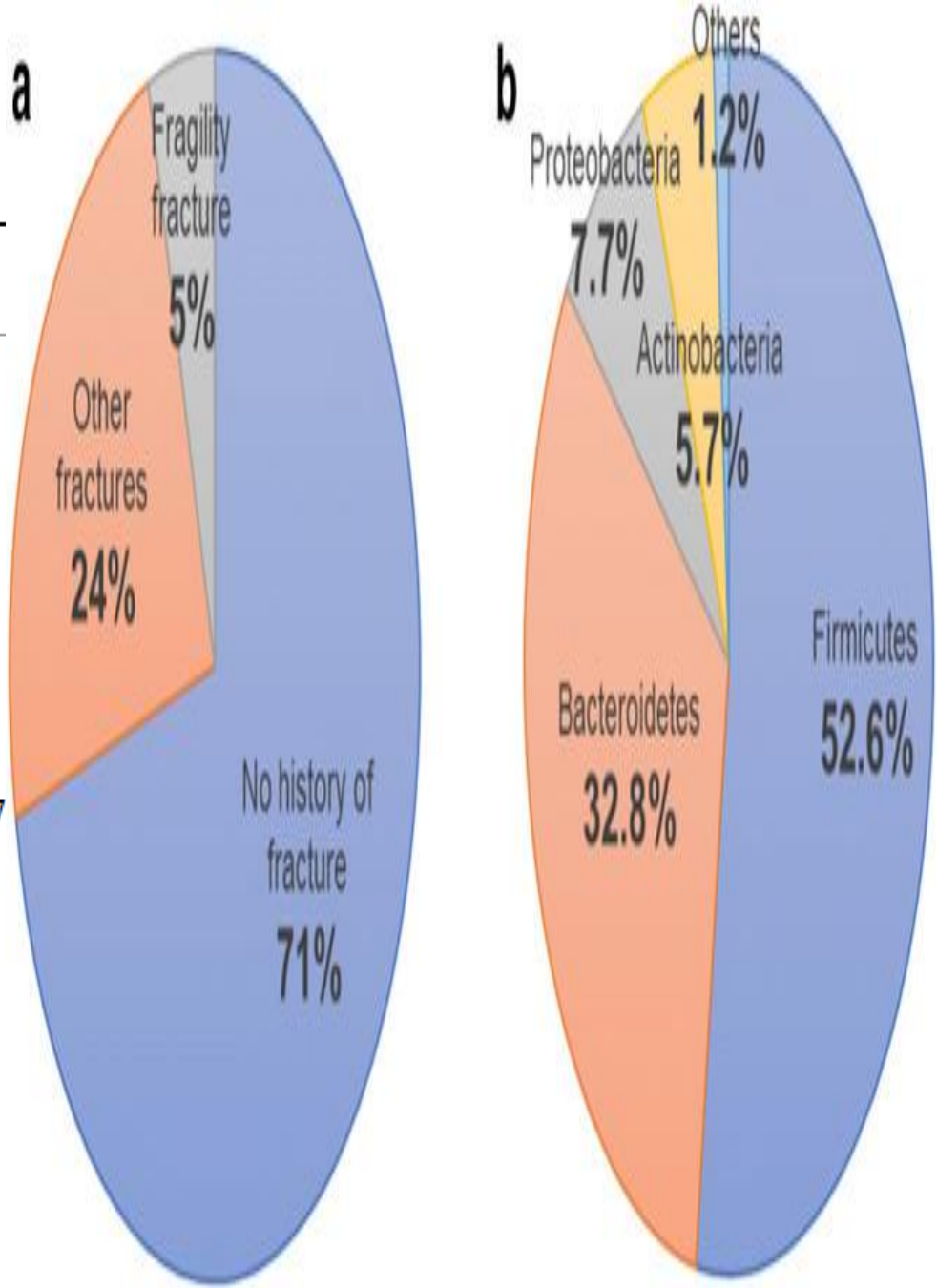
- 16S rRNA gene sequencing, FRAX, bone mineral density, biochemical bone parameters (vitamin K fraction and tartrate-resistant acid phosphatase 5b; TRACP-5b), and a self-administered questionnaire.
- Fracture incidence and relative risk were investigated for each bacterium.

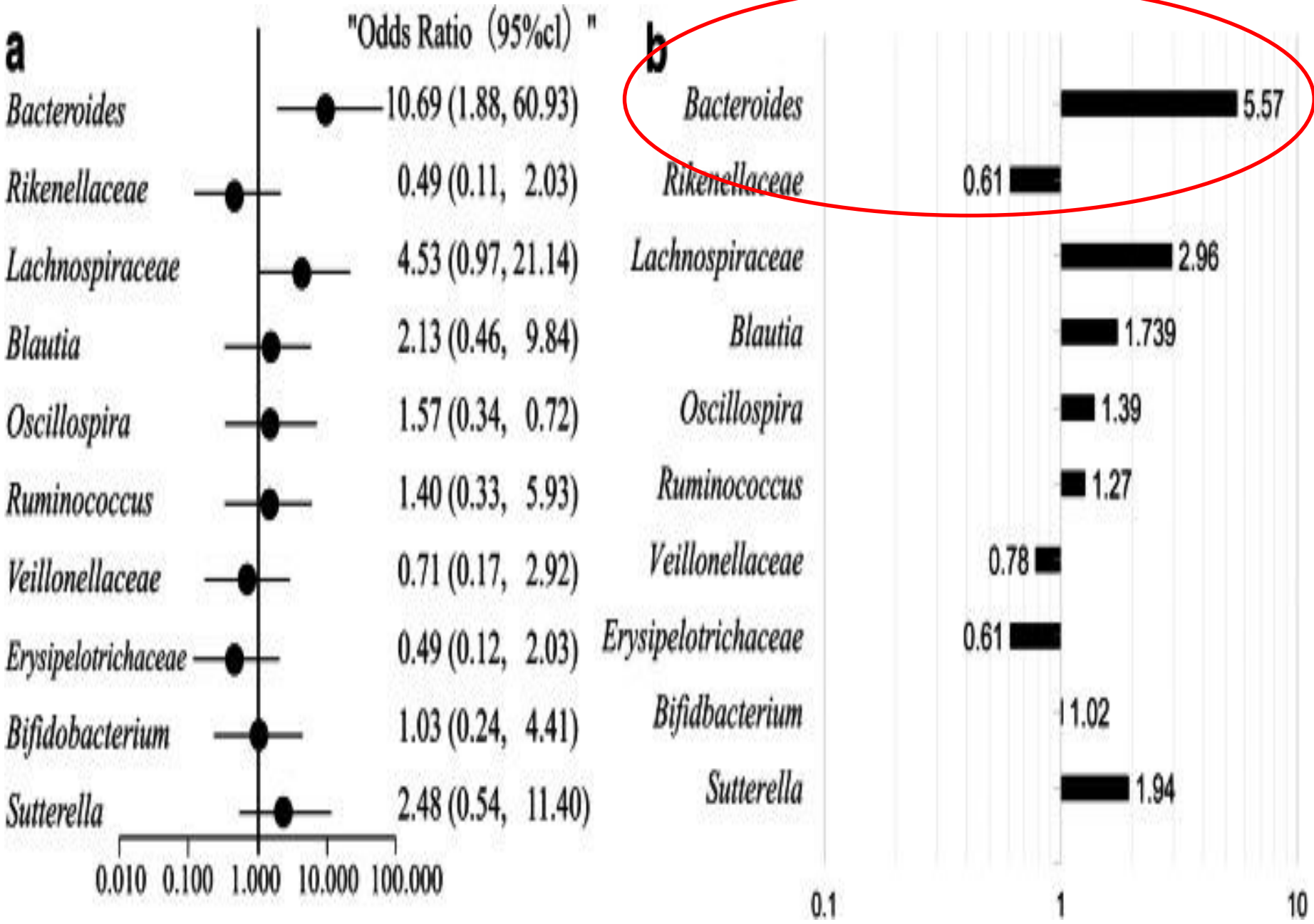

Phylum
Class
Order
Family
Genus
Bacteroidetes (32.83)
Bacteroidia (32.78)
Bacteroidales (32.78)
Bacteroidaceae (23.73)
Bacteroides (23.73)
Porphyromonadaceae (2.36)
Parabacteroides (2.36)
Prevotellaceae (3.93)
Prevotella (3.93)
Rikenellaceae (1.48)
Firmicutes (52.58)
Bacilli (3.86)
Lactobacillales (3.58)
Streptococcaceae (2.60)
Streptococcus (2.58)
Clostridia (45.91)
Clostridiales (45.91)
Clostridiaceae (1.60)
Lachnospiraceae (23.06)
Blautia (7.66)
Coprococcus (3.69)
Dorea (2.39)
Lachnospira (1.39)
Roseburia (1.31)
Ruminococcaceae (13.47)
Faecalibacterium (6.49)
Oscillospira (1.09)
Ruminococcus (3.16)
Veillonellaceae (2.96)
Erysipelotrichi (2.73)
Erysipelotrichales (2.73)
Erysipelotrichaceae (2.73)
Actinobacteria (5.66)
Actinobacteria (4.33)
Bifidobacteriales (4.30)
Bifidobacteriaceae (4.30)
Bifidobacterium (4.30)
Coriobacteriia (1.31)
Coriobacteriales (1.31)
Coriobacteriaceae (1.31)
Proteobacteria (7.71)
Betaproteobacteria (1.08)
Burkholderiales (1.08)
Alcaligenaceae (1.08)
Sutterella (1.08)
Gammaproteobacteria (6.00)
Enterobacteriales (5.77)
Enterobacteriaceae (5.77)
Trabulsiella (2.78)


Samples Collection
DNA extraction
Preparation of the sequence
16S PCR
Ligase Barcode
Quality check
Next gene Sequencer
Evaluation of bacterial species
Conclusion Report
Extraction of DNA from microorganism in the stool
DNA Amplification from 16S ribosome parts
Refining the amplificated product Barcode ligation
Bacteria species gets assigned by each reading sequence
bacterial species ratio gets created by DNA sequence
Determination of Symptoms by "machine learning method"
..GCGGAAT..
..AGCGCTA..
..TTAATTG..
..GTGGGAT..
DNA extracted from stool
Increasing byproduct
Sequencer Library
DNA sequencing
Species Ratio
Profiling Report

Characteristics of the participants

	Average value
Age	62.87 ± 6.22
Menopausal age	51.79 ± 4.65
BMD (YAM: %)	87.05 ± 11.78
ucOC (ng/ml)	4.38 ± 2.16
TRACP (mU/dL)	371.45 ± 126.77
Vitamin D (pg/mL)	12.59 ± 4.59
Vitamin K1 (ng/mL)	1.08 ± 0.75
VitaminK2 (ng/mL)	0.08 ± 0.08
FRAX (%)	8.38 ± 3.94




a
"Odds Ratio (95%cl) "
Bacteroides
10.69 (1.88, 60.93)
Rikenellaceae
0.49 (0.11, 2.03)
Lachnospiraceae
4.53 (0.97, 21.14)
Blautia
2.13 (0.46, 9.84)
Oscillospira
1.57 (0.34, 0.72)
Ruminococcus
1.40 (0.33, 5.93)
Veillonellaceae
0.71 (0.17, 2.92)
Erysipelotrichaceae
0.49 (0.12, 2.03)
Bifidobacterium
1.03 (0.24, 4.41)
Sutterella
2.48 (0.54, 11.40)
0.010 0.100 1.000 10.000 100.000
b
Bacteroides
5.57
Rikenellaceae
0.61
Lachnospiraceae
2.96
Blautia
1.739
Oscillospira
1.39
Ruminococcus
1.27
Veillonellaceae
0.78
Erysipelotrichaceae
0.61
Bifidbacterium
1.02
Sutterella
1.94
0.1
1
10

Συμπεράσματα

The genus *Bacteroides* was predominant in the high vitamin K2 group (29.73% vs 21.58%, *P* = 0.022). Fracture incidence was significantly higher in the low *Bacteroides* group, with a 5.6-times higher risk ratio of fracture history.

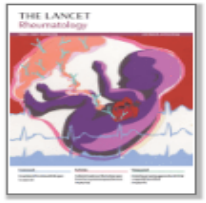
The family *Rikenellaceae* was more abundant in the low BMD group and more abundant in the high TRACP-5b group (2.15% vs 0.82%, *P* = 0.004; 2.38% vs 1.12%, *P* = 0.013, respectively).

Περνώντας από την εμβρυική στην παιδική ηλικία…

ELSEVIER

The Lancet Rheumatology

Volume 1, Issue 3, November 2019, Pages e154-e162

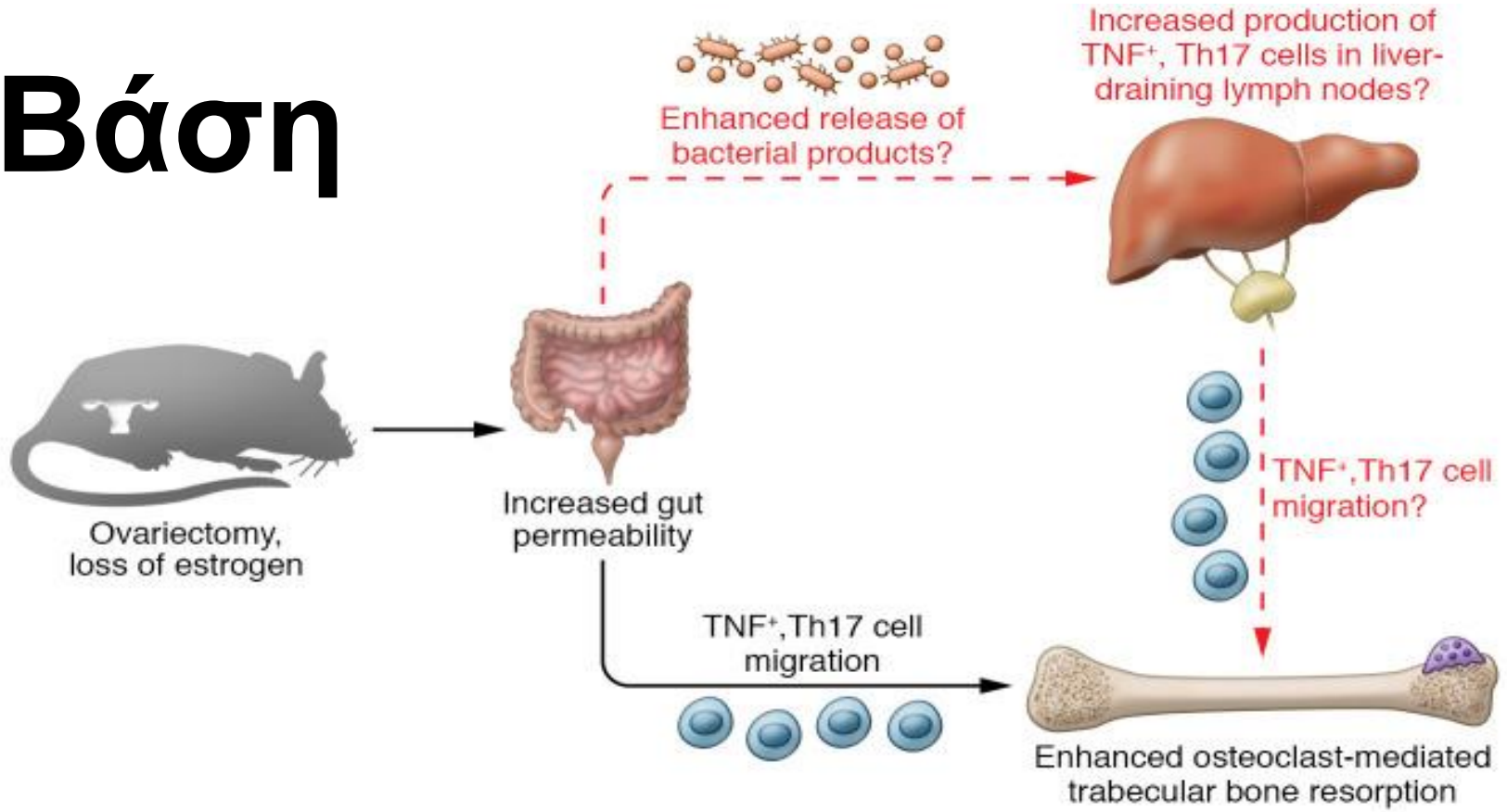


Articles

Probiotic treatment using a mix of three *Lactobacillus* strains for lumbar spine bone loss in postmenopausal women: a randomised, double-blind, placebo-controlled, multicentre trial

Per-Anders Jansson MD [a], Dan Curiac MD [b], Irini Lazou Ahrén PhD [c], Fredrik Hansson PhD [d], Titti Martinsson Niskanen PhD [c], Klara Sjögren PhD [e], Claes Ohlsson MD [e]

Βάση



Convincing evidence from ovariectomized mice, where supplementation with ***L. reuteri*** ATCC PTA 6475 protected against bone resorption and loss associated with oestrogen deficiency

Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. Britton RA et al, J Cell Physiol. 2014 Nov

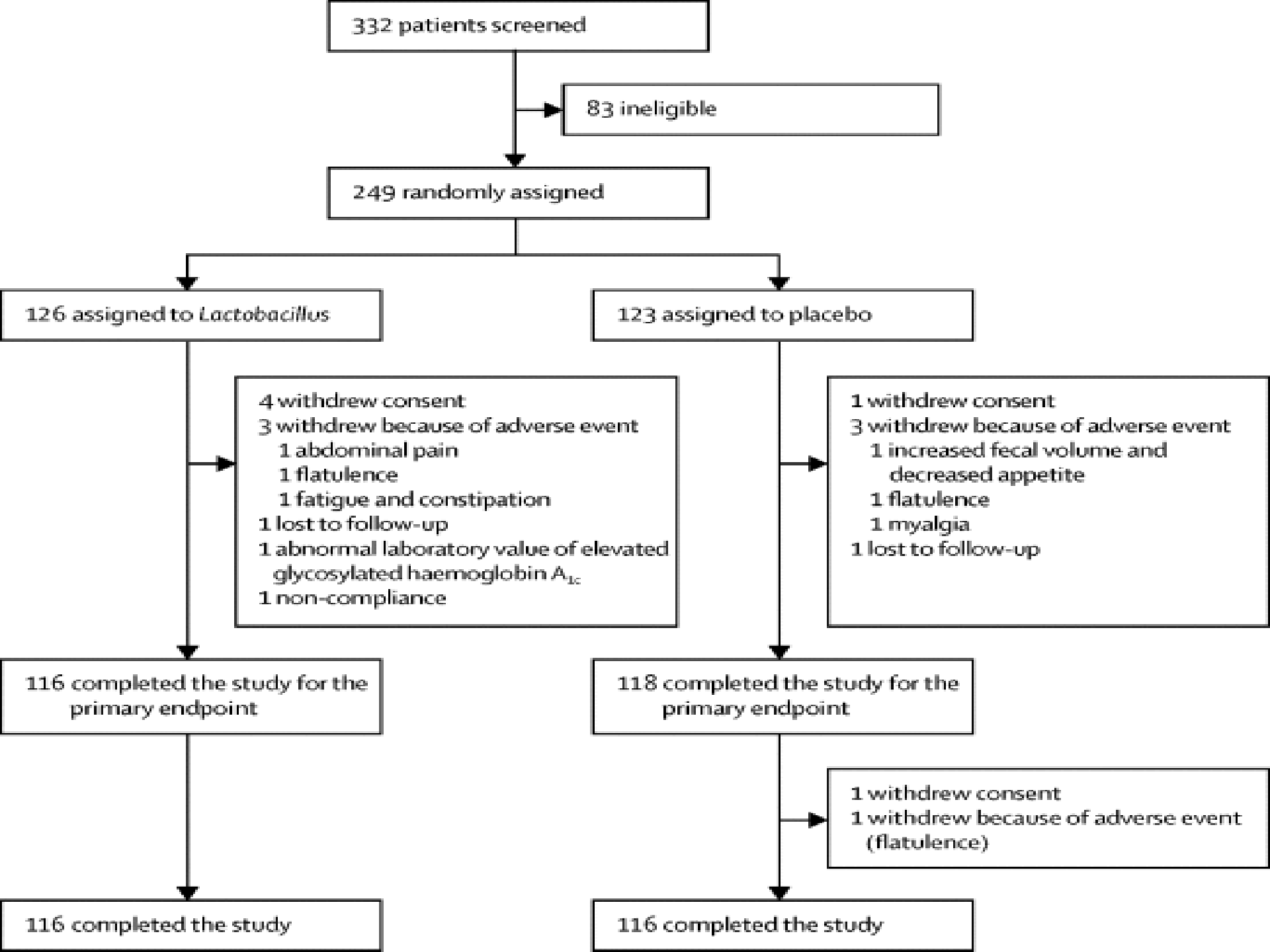
Probiotics protect mice from ovariectomy-induced cortical bone loss. Ohlsson C et al, PLoS One. 2014

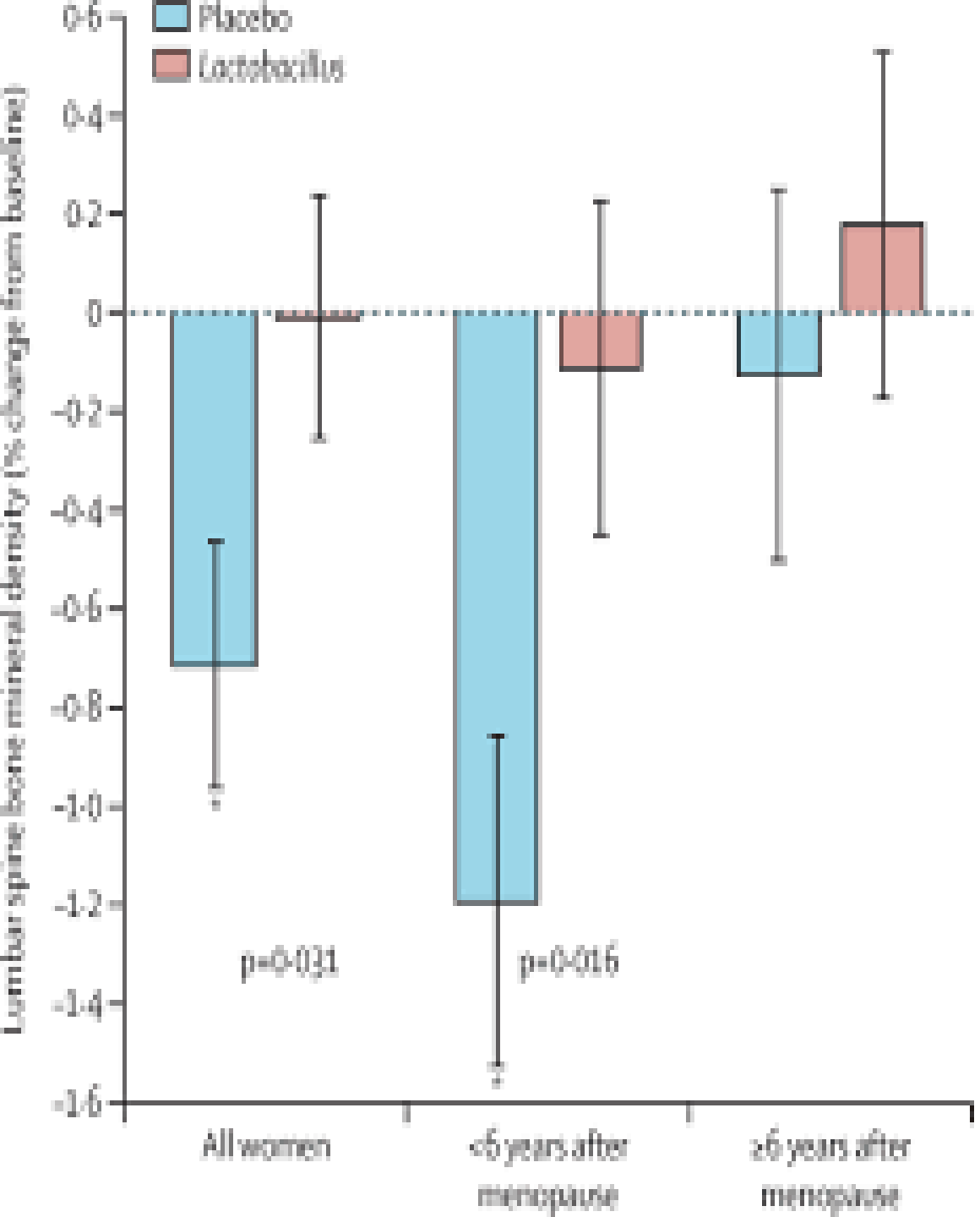
Σκοπός

To determine if treatment with a combination of three bacterial strains protects against the rapid spine bone loss occurring in healthy early postmenopausal women.

Μέθοδος

- Randomised, double-blind, placebo-controlled, multicentre trial was done at four study centres in Sweden.
- 232 μετεμμηνοπαυσιακές γυναίκες
- *L. reuteri* ATCC PTA 6475 $\times 10^{10}$ colony-forming units (CFU) per day vs placebo
- 1 έτος


332 patients screened
83 ineligible
249 randomly assigned
126 assigned to Lactobacillus
123 assigned to placebo
4 withdrew consent
3 withdrew because of adverse event
1 abdominal pain
1 flatulence
1 fatigue and constipation
1 lost to follow-up
1 abnormal laboratory value of elevated glycosylated haemoglobin A_{1c}
1 non-compliance
1 withdrew consent
3 withdrew because of adverse event
1 increased fecal volume and decreased appetite
1 flatulence
1 myalgia
1 lost to follow-up
116 completed the study for the primary endpoint
118 completed the study for the primary endpoint
1 withdrew consent
1 withdrew because of adverse event (flatulence)
116 completed the study
116 completed the study



• *Lactobacillus* treatment reduced the LS-BMD loss compared with placebo (mean difference 0·71%, 95% CI 0·06 to 1·35).

• The LS-BMD loss was significant in the placebo group (–0·72%, −1·22 to −0·22), whereas no bone loss was observed in the *Lactobacillus*-treated group (–0·01%, −0·50 to 0·48).

• The adverse events were similar between the two groups.

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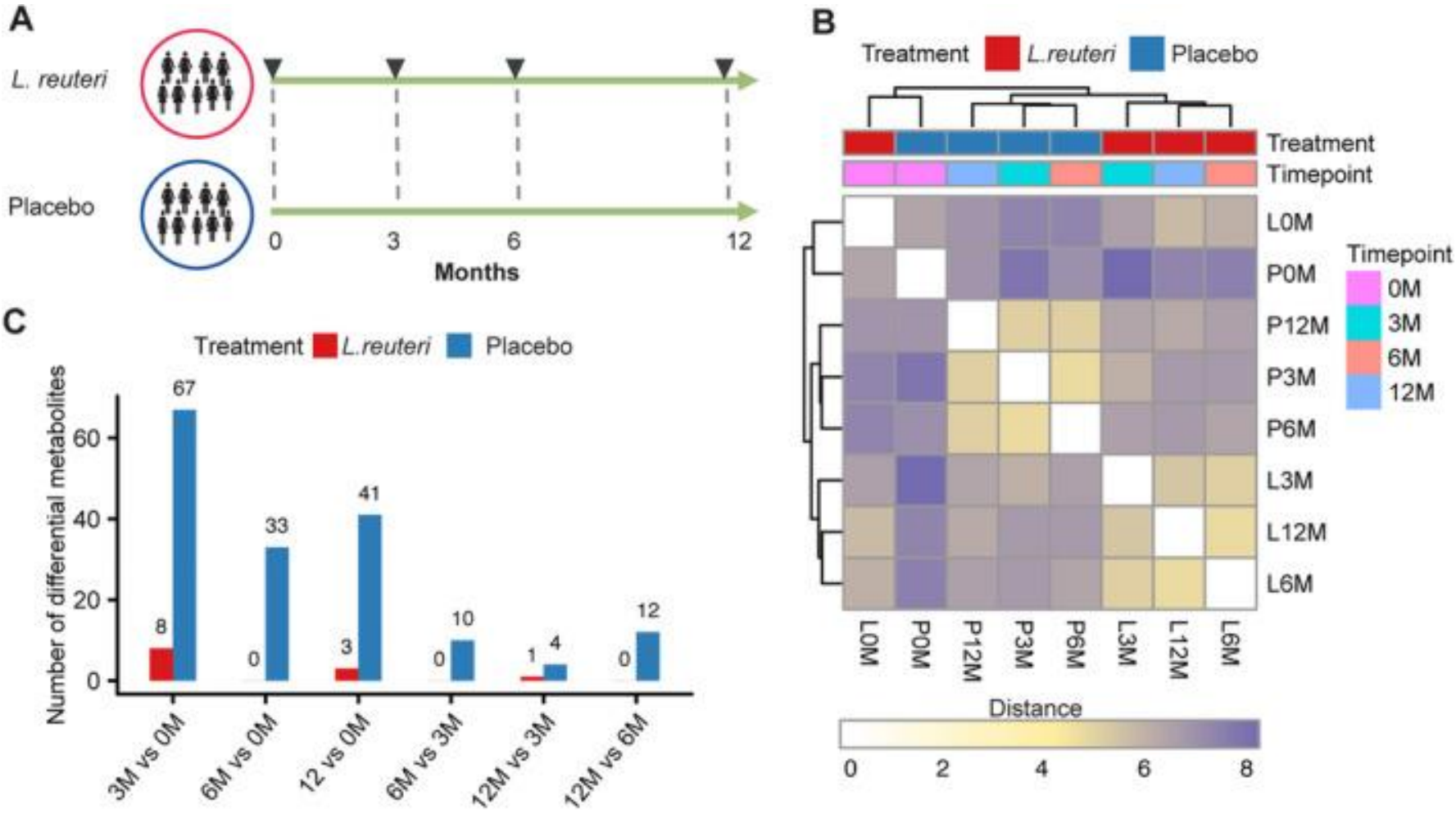
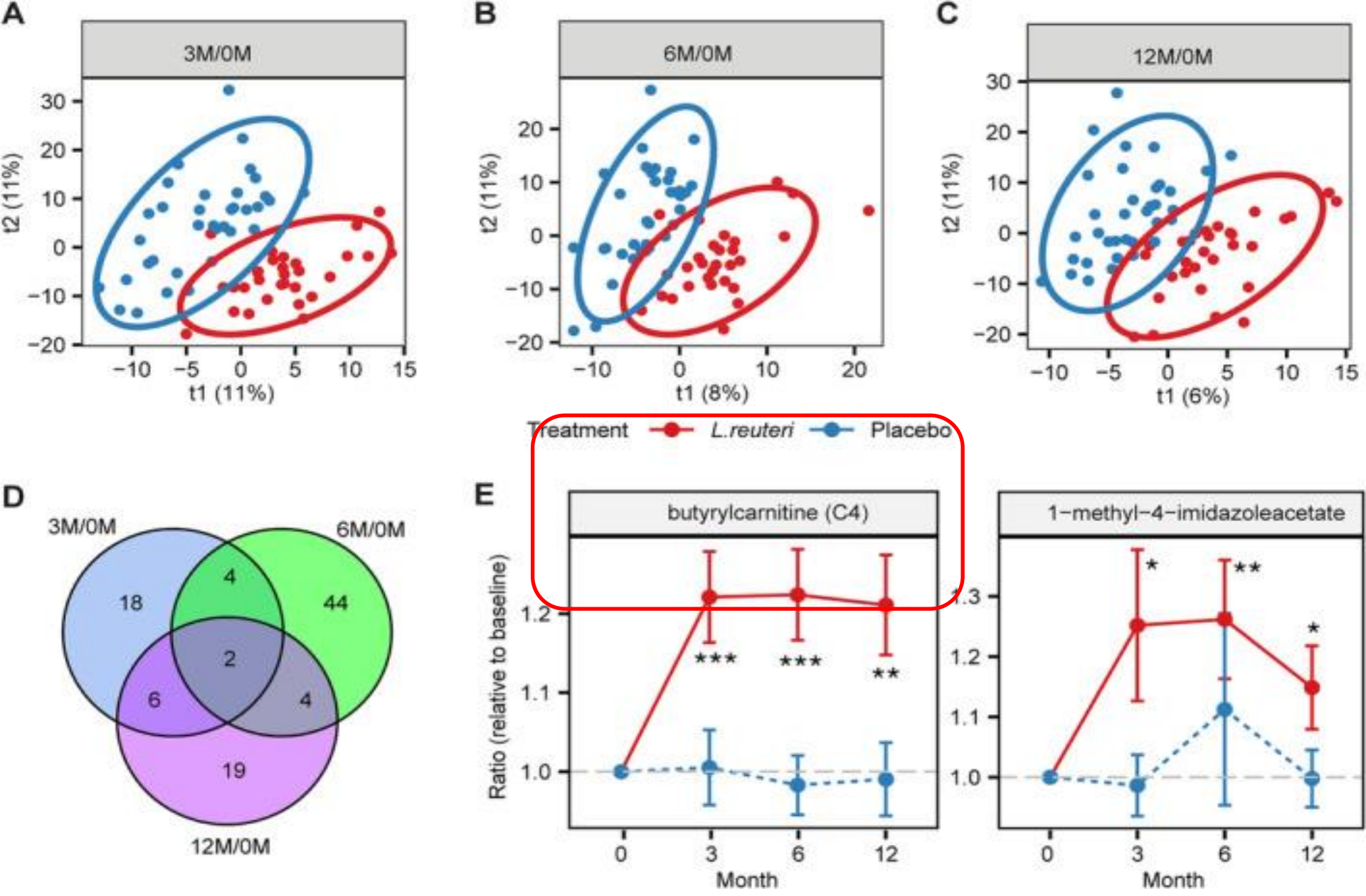
PMCID: PMC8046097
PMID: 33869994

Metabolic Alterations in Older Women With Low Bone Mineral Density Supplemented With *Lactobacillus reuteri*

Peishun Li, [1] Daniel Sundh, [2] Boyang Ji, [1] Dimitra Lappa, [1] Lingqun Ye, [1] Jens Nielsen, [1,3,4] and Mattias Lorentzon [2,5,6]

Metabolomic-based analysis of serum samples from participants in both treatment groups using liquid chromatography—tandem mass spectrometry to identify possible mechanisms for these effects.


A
L. reuteri
Placebo
0
3
6
12
Months
B
Treatment
L.reuteri
Placebo
Treatment
Timepoint
L0M
P0M
P12M
P3M
P6M
L3M
L12M
L6M
Timepoint
0M
3M
6M
12M
Distance
0
2
4
6
8
C
Treatment
L.reuteri
Placebo
Number of differential metabolites
0
20
40
60
67
8
33
0
41
3
10
0
4
1
12
0
3M vs 0M
6M vs 0M
12 vs 0M
6M vs 3M
12M vs 3M
12M vs 6M


A
3M/0M
t2 (11%)
t1 (11%)
B
6M/0M
t2 (11%)
t1 (8%)
C
12M/0M
t2 (11%)
t1 (6%)
Treatment
L.reuteri
Placebo
D
3M/0M
6M/0M
12M/0M
18
4
44
2
6
4
19
E
butyrylcarnitine (C4)
1−methyl−4−imidazoleacetate
Ratio (relative to baseline)
Month
***
***
**
*
**
*

Ωστόσο…

97 metabolites involved in multiple processes, including amino acid, peptide, and lipid metabolism which showed trends for differences between the treatment groups, but none remained significant after correction for multiple testing

Πού βρισκόμαστε;

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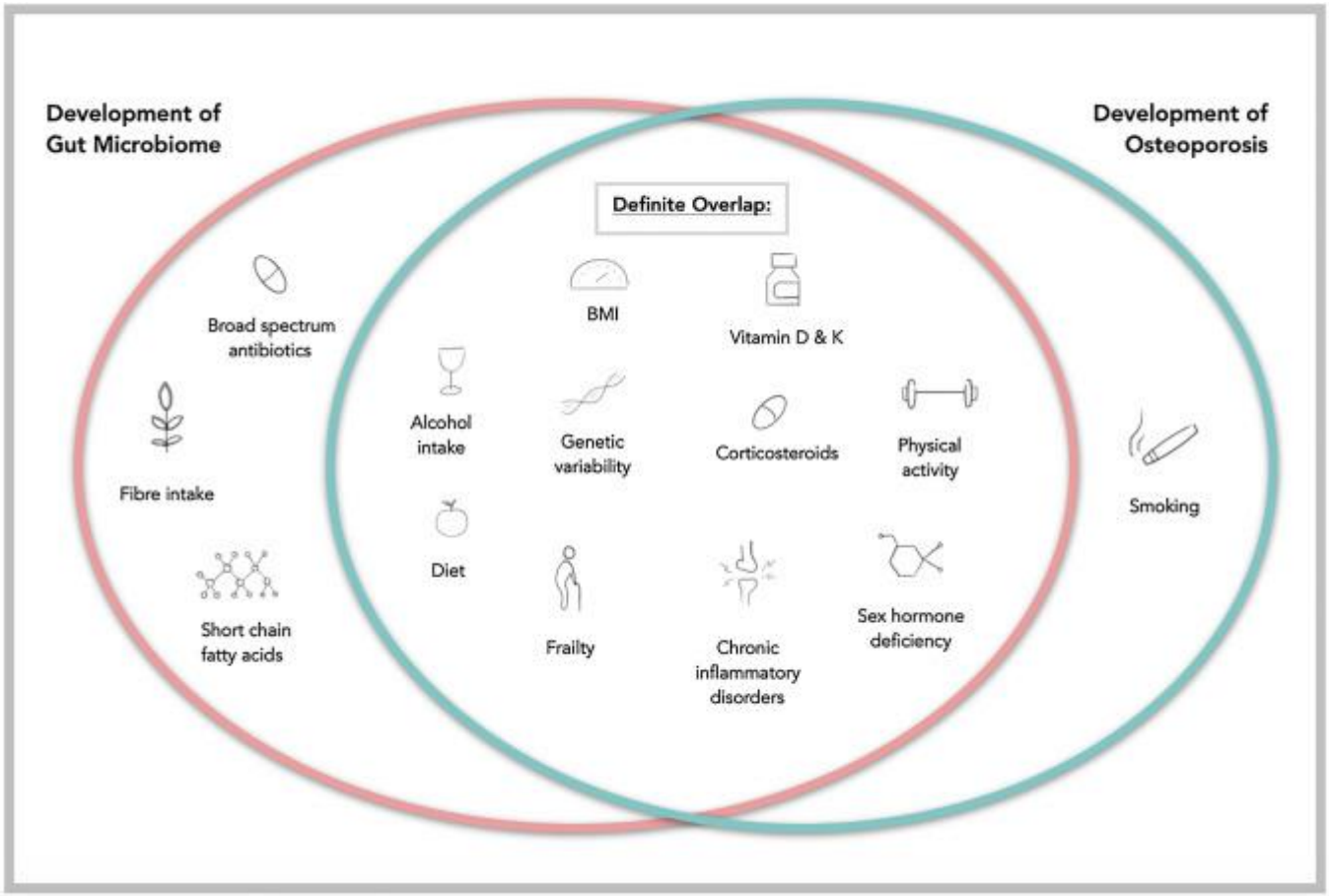
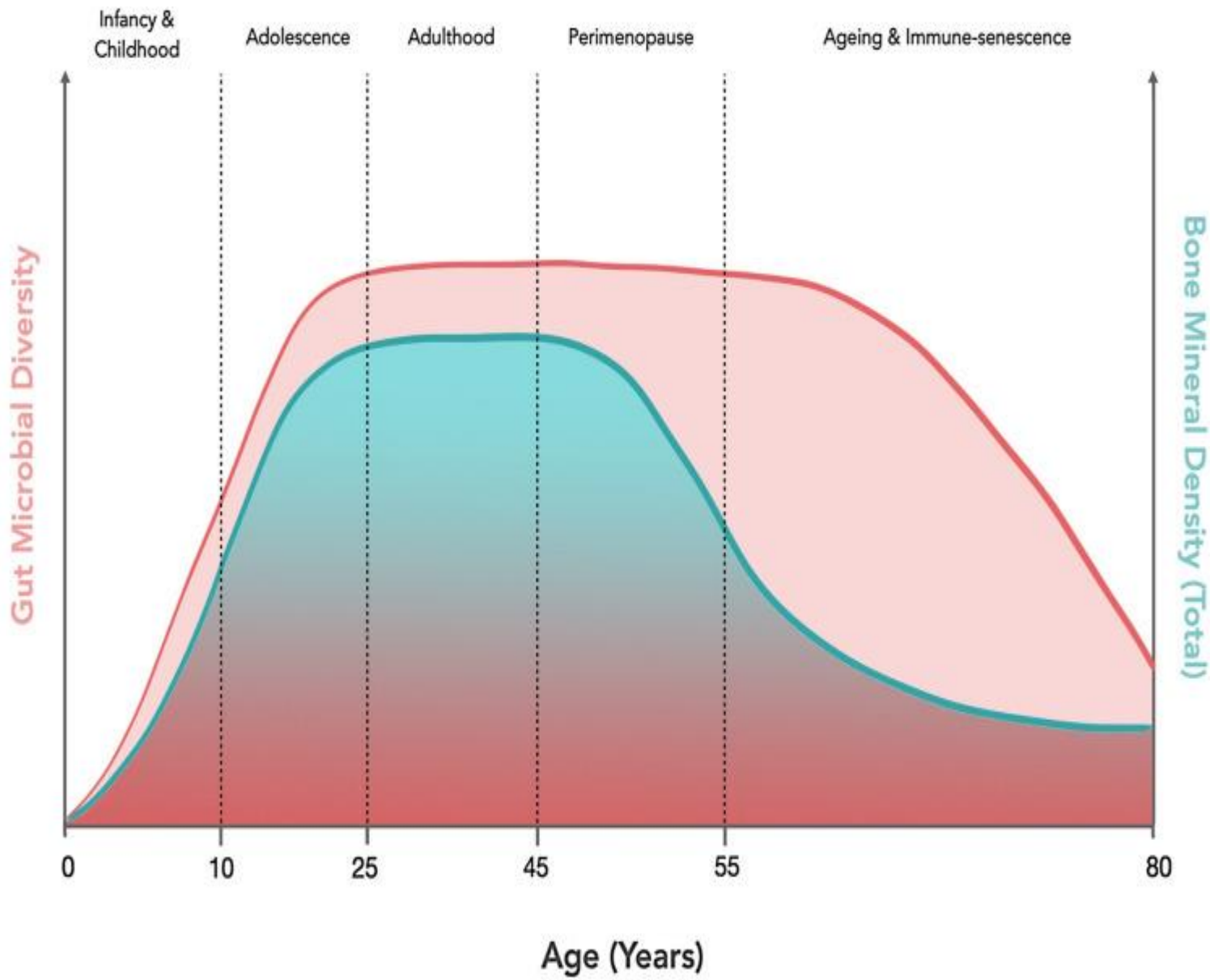
Calcif Tissue Int. 2022; 110(3): 273–284. PMCID: PMC8860778
Published online 2021 Dec 6. doi: 10.1007/s00223-021-00924-2 PMID: 34870723

Role of the Microbiome in Regulating Bone Metabolism and Susceptibility to Osteoporosis

Owen Cronin,[1,2] Susan A. Lanham-New,[3] Bernard M. Corfe,[4] Celia L. Gregson,[5,6] Andrea L. Darling,[3] Kourosh R. Ahmadi,[3] Philippa S. Gibson,[7] Jon H. Tobias,[5,6] Kate A. Ward,[8,9] Maria H. Traka,[10] Megan Rossi,[7] Claire Williams,[11] Nicholas C. Harvey,[8,9] Cyrus Cooper,[8,9,12] Kevin Whelan,[7] André G. Uitterlinden,[13] Paul W. O'Toole,[14] Claes Ohlsson,[15] Juliet E. Compston,[16] and Stuart H. Ralston[1,2]


Development of Gut Microbiome
Development of Osteoporosis
Definite Overlap:
Broad spectrum antibiotics
Fibre intake
Short chain fatty acids
BMI
Vitamin D & K
Alcohol intake
Genetic variability
Corticosteroids
Physical activity
Diet
Frailty
Chronic inflammatory disorders
Sex hormone deficiency
Smoking


Infancy & Childhood
Adolescence
Adulthood
Perimenopause
Ageing & Immune-senescence
Gut Microbial Diversity
Bone Mineral Density (Total)
0
10
25
45
55
80
Age (Years)

Οι έως τώρα «κατακτήσεις»…

Α. Μελέτες σε ζώα

1. Germ-Free (GF) Murine Models

Το εντερικό μικροβίωμα παίζει ρόλο στον οστικό μεταβολισμό
short chain fatty acids (SCFA) – butyrate

2. Antibiotic-Intervention Studies

Μικτά αποτελέσματα – ανάλογα με τη διάρκεια χορήγησης των αντιβιοτικών
Μηχανισμοί: Αλλαγή στα επίπεδα κλωστηριδίων και στα επίπεδα βιταμίνης Κ2

3. *Models of Postmenopausal Osteoporosis*

Οι έως τώρα «κατακτήσεις»…

Β. Μελέτες σε ανθρώπους

1. Human Observational Studies

2. Intervention Studies
Lactobacillus reuteri

3. Vitamin D and microbiome

	Question of interest	Possible mechanism of investigation
1	Are nutrition and lifestyle effects on the gut microbiota in early life (birth to adolescence) related to peak bone mass (PBM) attainment?	Longitudinal cohort study
2	Are nutritional and lifestyle effects on the gut microbiota in early life (birth to adolescence) related to risk of osteoporosis in adulthood?	Longitudinal cohort study
3	Are the effects of early-life antibiotic exposure (birth to adolescence) on the gut microbiota related to PBM accrual and/or bone mineral density in later life?	Longitudinal cohort study
4	What are the effects of childhood illness on the development of a healthy gut microbiota and how does this affect PBM attainment and risk of osteoporosis in adulthood?	Case control studies, longitudinal cohort study
5	Can the negative impact of inflammatory diseases in childhood such as IBD, asthma, JIA on adult bone health be attenuated by manipulating the gut microbiota through diet, and pro- or prebiotic use?	Targeted exploratory and intervention studies in patient populations
6	Will recent changes in dietary habits such as vegan diets and gluten free diets in non coeliac individuals in younger generations affect future BMD and does this operate through alteration of the gut microbiota?	Case–control studies, Longitudinal cohort study
7	Can BMD loss be attenuated by the use of targeted dietary modification or supplementation with pre- or probiotics during the peri-menopausal time-span?	Clinical trials
8	Can a patient's gut microbiota influence the individualized response to medications such as bisphosphonates, PTH, calcium and vitamin D supplements?	Prospective observational study (1–5 year duration)
9	Is the increased osteoporosis risk evident in underweight individuals mediated by the gut microbiota?	Longitudinal cohort study
10	How does the gut microbiota affect the availability and absorption of calcium, vitamin D and other mineral nutrients such as magnesium from the gut lumen?	Experimental animal and human studies
11	Can a specific or complex dietary modification such as an increased fibre, protein intake, Mediterranean or DASH diet, prebiotic or probiotic-use improve bone mineral density and at what stage in the lifecourse is the greatest benefit seen?	Clinical trials with long-term follow-up
12	Can short chain fatty acid supplementation (directly or via increased non-starch polysaccharide intake) improve BMD and if so, at what stage of the life-cycle?	Clinical trials
13	Is the reduction in BMD evident in the frail and elderly directly related to the concurrent decrease in gut microbial diversity that occurs in later life?	Cross-sectional, case–control studies, longitudinal cohort study

Σας ευχαριστώ!