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ORIGINAL ARTICLE

Diets naturally rich in polyphenols and/or long-chain n-3 polyunsaturated fatty acids differently affect microbiota composition in high-cardiometabolic-risk individuals

Claudia Vetrani[1,2] · Johanna Maukonen[3,4] · Lutgarda Bozzetto[1,2] · Giuseppe Della Pepa[1] · Marilena Vitale[1,2] · Giuseppina Costabile[1,2] · Gabriele Riccardi[1,2] · Angela Albarosa Rivellese[1,2] · Maria Saarela[3,5] · Giovanni Annuzzi[1]

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Abstract

Aims Gut microbiota significantly impacts human health and is influenced by dietary changes. We evaluated the effects of diets naturally rich in polyphenols (PP) and/or long-chain n-3 polyunsaturated fatty acids (LCn3) on microbiota composition in an ancillary analysis of a randomized controlled trial in individuals at high cardiometabolic risk.

Methods Seventy-eight individuals with high waist circumference and at least one additional component of the metabolic syndrome were randomized to an isoenergetic 8-week diet: (a) low LCn3 and PP; (b) high LCn3; (c) high PP; or (d) high LCn3 and PP. Microbiota analysis was performed on feces collected before and after the intervention. DGGE analysis of the predominant bacteria, *Eubacterium rectale* and *Blautia coccoides* group (*Lachnospiraceae*, EREC), *Clostridium leptum* (*Ruminococcaceae*, CLEPT), *Bacteroides spp.*, *Bifidobacteria,* and *Lactobacillus* group was performed. A quantitative real-time PCR was performed for the same group, additionally including *Atopobium* cluster (*Coriobatteriaceae*). Before and after the intervention, participants underwent a 75 g OGTT and a high-fat test meal to evaluate glucose and lipid response.

Results Adherence to the four diets was optimal. PP significantly increased microbial diversity ($p=0.006$) and CLEPT ($p=0.015$), while it reduced EREC ($p=0.044$). LCn3 significantly increased the numbers of *Bifidobacteria* ($p=0.041$). Changes in CLEPT numbers correlated with changes in early insulin secretion ($r=0.263$, $p=0.030$). Changes in *Atopobium* numbers correlated with postprandial triglycerides in plasma ($r=0.266$, $p=0.026$) and large VLDL ($r=0.313$, $p=0.009$), and cholesterol in large VLDL ($r=0.319$, $p=0.008$).

Conclusions Diets naturally rich in PP or LCn3 influenced gut microbiota composition in individuals at high cardiometabolic risk. These modifications were associated with changes in glucose/lipid metabolism.

Keywords Diet · Polyphenols · Long-chain n-3 polyunsaturated fatty acids · Microbiota diversity · Gut microbiota · Glucose tolerance · Lipid response

Claudia Vetrani and Johanna Maukonen have contributed equally to this work.

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✉ Angela Albarosa Rivellese
rivelles@unina.it

[1] Department of Clinical Medicine and Surgery, University of Naples "Federico II", 5, Sergio Pansini, 80131 Naples, Italy

[2] Task Force on Microbiome Studies, University of Naples "Federico II", Naples, Italy

[3] VTT Technical Research Centre of Finland, Espoo, Finland

[4] Present Address: DuPont Nutrition and Health, Kantvik, Finland

[5] Present Address: South Australian Research and Development Institute, Urrbrae, Australia

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Abbreviations

LCn3 Long-chain n-3 polyunsaturated fatty acid
PP Polyphenols

Introduction

Human gut microbiota is known to significantly impact health status of the host. Indeed, harmful alterations in the microbiota composition, known as dysbiosis, contribute to the pathogenesis of intestinal disorders as well as extra-intestinal disorders, including metabolic diseases [1].

Cross-sectional studies have shown that the composition of the gut microbiota is altered in individuals with prediabetes or type 2 diabetes (T2D) compared with healthy controls [2–4].

In addition, the association between microbiota composition, obesity, and insulin resistance has been widely studied [5–7]. However, to date, no consistent footprint of the microbial communities has been identified able to determine these adverse health conditions.

Internal and external factors are known to influence microbiota composition. Recently, genetics has shown to exert a minor role in defining microbiota composition, whereas it is more influenced by environmental factors [8]. Antibiotic therapies or fecal transplantations have dramatic but temporary effects on the host microbiota [9], while dietary changes represent a more feasible tool to induce long-lasting modifications in the gut microbiota.

The effects of dietary changes on gut microbiota are still not well known, although modifications in microbiota composition have been observed after controlled changes of the habitual diet—e.g., weight-loss diet, Mediterranean diet, high-protein diet, high-fat diet, and probiotics and prebiotics [10–13]. In addition, specific dietary compounds may have selective effects on the microbiota. Among these, polyphenols and long-chain n-3 polyunsaturated fatty acids have raised interest since it has been hypothesized that their beneficial metabolic effects could be mediated, at least in part, by changes in the microbiota.

Evidence from randomized clinical trial is controversial, and even less information is available on the interplay between changes in microbiota and metabolic effects [14, 15]. In addition, to the best of our knowledge, no interventions combining polyphenols and long-chain n-3 polyunsaturated fatty acids have been carried out.

Therefore, this study aimed to evaluate whether dietary polyphenol, long-chain n-3 polyunsaturated fatty acids, or their combination could influence microbiota composition in individuals at high cardiometabolic risk participating in a medium-term nutritional trial. In addition, we explored the association between the dietary induced changes in microbiota and fasting and post-challenge glucose and lipid metabolism.

Materials and methods

Experimental design

The study design has been described in detail previously [16] and was registered at clinicaltrial.gov (NCT01154478). Briefly, 78 high-risk individuals (33 M/45 F), with high waist circumference (above 102 cm for men and 88 cm for women), and at least one more feature of the metabolic syndrome according to NCEP-ATPIII criteria [17] completed this nutritional trial. The participants were randomly assigned to one of four dietary interventions for the duration of 8 weeks. The four diets differed only for long-chain n-3 polyunsaturated fatty acids (LCn3) and/or polyphenols (PP): (a) low LCn3&PP, diet low in LCn3 (1.5 g/day) and PP (365 mg/day); (b) high LCn3, diet high in LCn3 (4 g/day) and low in PP (363 mg/day); (c) high PP, diet high in PP (2903 mg/day) and low in LCn3 (1.4 g/day); and (d) high LCn3&PP, diet high in PP (2861 mg/day) and LCn3 (4 g/day).

The difference in LCn3 and/or polyphenols amount was obtained through the selection of natural foods and beverages. In particular, the main dietary sources of LCn3 were salmon, dentex, and anchovies. Polyphenols were provided by decaffeinated green tea and coffee, dark chocolate, blueberry jam, extra-virgin olive oil, and polyphenol-rich vegetables (rocket salad, fennels, onions).

At baseline and after the 8-week intervention, all participants underwent a 75-g oral glucose tolerance test (OGTT) to evaluate indices of glucose tolerance and insulin secretion [18]. Two days after the OGTT, participants underwent a high-fat meal challenge having the same foodstuff characterizing their assigned diet to evaluate postprandial lipids response. In particular, meals were prepared with rice, butter, parmesan cheese, *bresaola* (cured beef meat), and white bread, with intakes of olive oil, salmon, and decaffeinated green tea differing in order to obtain a similar composition as the assigned diet [18]. In addition, before and after the 8-week intervention, fecal samples were collected at home in a plastic container, stored overnight at − 20 °C, and thereafter transferred freezed to the laboratory to be stored at − 80 °C. The samples were shipped frozen to VTT where they were stored at − 80 °C until the analysis.

Analysis of fecal microbiota

DNA was extracted as described by Maukonen et al. [19] from 0.2 g of sample. Partial 16S rRNA gene was amplified for the analysis of predominant bacteria, *Eubacterium rectale—Blautia coccoides* (EREC) (clostridial cluster XIV) [20], *Clostridium leptum* (CLEPT) (clostridial cluster IV)

[19], *Bacteroides* spp., *Bifidobacteria* and *Lactobacillus* group (comprised of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*) as previously described [19, 21–23]. Denaturing gradient gel electrophoresis (DGGE) analyses of the predominant bacteria EREC, CLEPT, *Bacteroides* spp., *Lactobacillus* group, and *Bifidobacteria* were performed as previously described to study the diversity of bacterial populations [22, 23]. In addition, a quantitative real-time PCR of the predominant bacteria EREC, CLEPT, *Bacteroides* spp., *Lactobacillus* group, *Bifidobacteria,* and *Atopobium* cluster (comprised of genera such as *Atopobium*, *Collinsella,* and *Eggerthella*) were performed as previously described to assess the numbers of bacteria [23, 24].

Biochemical measurements

Large VLDL (Svedberg flotation unit, Sf 60–400) was isolated from plasma by discontinuous density gradient ultracentrifugation [25]. Cholesterol, triglyceride, and glucose concentrations were assayed by enzymatic methods (Roche Molecular Biochemicals, Mannheim, Germany) on ABX Pentra 400 (HORIBA Medical, Montpellier, France).

Insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA; DIAsource ImmunoAssays S.A., Nivelles, Belgium) on Triturus Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain).

Statistical analyses

All data are expressed as mean ± SEM unless otherwise stated. The effects of LCn3, polyphenols, and the interaction between LCn3 and polyphenols on microbiota composition (expressed as absolute changes: 8 weeks *minus* baseline) were evaluated by two-factor ANOVA. The associations between microbiota composition and the main metabolic outcomes observed in the trial were explored by Pearson's correlation analyses. A p value < 0.05 was considered significant. Statistical analysis was performed according to standard methods using the Statistical Package for Social Sciences software 25.0 (SPSS/PC; SPSS, Chicago, IL, USA).

Results

Subjects and compliance to dietary intervention

Baseline characteristics of the participants that could affect microbiota composition were not different between the groups observed in Table 1. Dietary compliance was optimal in all experimental groups, as shown by (1) the evaluation of the 7-day food records filled in during the study [16], (2) the detection of plasma long-chain PUFA-containing triglycerides after the high-LCn3 diets [26], and (3) the assessment of the phenolic metabolites profile in the 24-h urine collection [27].

Changes in the gut microbiota

An increase in the diversity of fecal predominant bacteria (evaluated as number of bands in DGGE) was observed after the polyphenol-rich diets, whereas the diversity decreased after low-LCn3&PP and High-LCn3 diets ($p = 0.006$ for polyphenol effect, Fig. 1). No changes in the diversity of the other groups (EREC, CLEPT, *Bacteroides*, *Bifidobacteria, Lactobacilli*) were detected (data not shown).

Polyphenols significantly reduced the numbers of EREC (as determined by qPCR) while increasing the numbers of CLEPT ($p = 0.044$ and $p = 0.015$, respectively, Fig. 2).

Table 1 Baseline characteristics of the participants in the dietary intervention trial

	Low LCn3&PP	High LCn3	High PP	High LCn3&PP	p value (ANOVA)
Gender (M/F)	8/12	8/11	9/11	8/11	
Age (years)	54 ± 9	56 ± 8	53 ± 9	55 ± 9	0.645
Body mass index (kg/m^2)	33 ± 3	32 ± 4	32 ± 3	30 ± 3	0.126
Fasting plasma glucose (mg/dl)	104 ± 12	104 ± 12	100 ± 9	103 ± 11	0.498
Fasting plasma insulin (µU/ml)	17 ± 5	20 ± 7	21 ± 6	17 ± 6	0.220
HOMA	4.45 ± 5	5.24 ± 7	5.09 ± 6	4.50 ± 6	0.351
Fasting total cholesterol (mg/dl)	194 ± 38	191 ± 26	194 ± 34	193 ± 27	0.992
Fasting HDL-cholesterol (mg/dl)	43 ± 10	41 ± 11	43 ± 9	44 ± 14	0.855
Fasting triglyceride (mg/dl)	120 ± 47	138 ± 68	120 ± 60	125 ± 78	0.787
Systolic blood pressure (mmHg)	120 ± 7	121 ± 12	126 ± 16	119 ± 9	0.231
Diastolic blood pressure (mmHg)	76 ± 8	74 ± 7	76 ± 9	73 ± 8	0.663

Data are M ± SD

HOMA homeostasis model assessment, *LCn3* long-chain n-3 polyunsaturated fatty acid, *PP* polyphenols

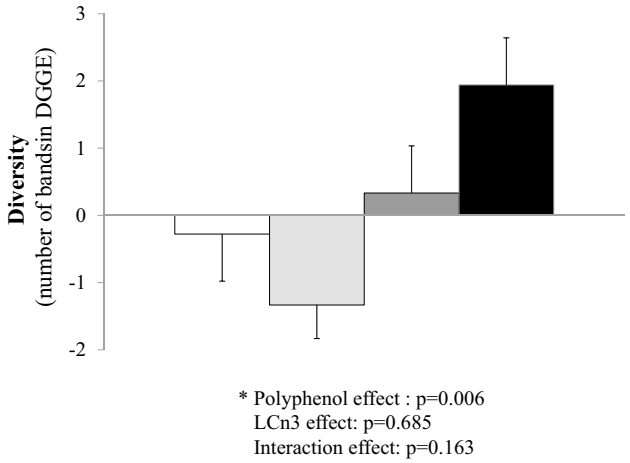

Fig. 1 Changes in fecal bacterial diversity (number of bands) between baseline and after the dietary intervention in the four experimental groups. *White bar*: low LCn3&PP, diet low in LCn3 and PP; *light gray bar*: high LCn3, diet rich in LCn3 and low in PP; *dark gray bar*: high PP, diet rich in PP and low in LCn3; *black bar*: high LCn3&PP, diet rich in PP and LCn3. *LCn3* long-chain n-3 polyunsaturated fatty acid, *PP* polyphenols. Mean ± SEM. * two-factor ANOVA

A "bifidogenic effect" was detected for LCn3 at the end of the intervention ($p = 0.041$ for LCn3 effect, Fig. 2). No changes were observed for *Lactobacillus* group and *Atopobium* cluster numbers (Fig. 2).

Diversity and numbers of bacteria were not affected by the interaction between polyphenol and LCn3.

Bivariate association

In the four groups combined, the absolute changes of the CLEPT were correlated with the changes in the early insulin secretion during OGTT (0–30 min; $r = 0.263$, $p = 0.030$) (Fig. 3).

A strong correlation was found between the changes in *Atopobium* cluster and postprandial triglycerides in plasma ($r = 0.266$, $p = 0.026$) and large VLDL ($r = 0.313$, $p = 0.009$), and cholesterol in the large VLDL ($r = 0.319$, $p = 0.008$) (Fig. 3).

Discussion

Diet is a main determinant of gut microbiota composition. Despite the relevance of the interplay between diet and microbiota in terms of human health, there are little data about how specific dietary changes interact with microbial communities and the role of such interactions on cardiometabolic risk factors. In this study, we focused on two dietary components: polyphenols and long-chain n-3 polyunsaturated fatty acids that have been hypothesized to exert their metabolic effects through changes in microbiota composition [14, 15]. Moreover, to the best of our knowledge, this is the first study aimed to evaluate the effect of the combination of these two dietary components on microbiota composition.

The main finding of this study is that a polyphenol-rich diet increases the diversity of fecal predominant bacteria (evaluated as number of bands in DGGE). Previous studies have shown that plant-based diets are associated with an increased bacterial diversity compared to Western-type diet [28–30]. At the same time, mounting evidence suggests that maintaining or increasing bacterial diversity is one of the key activities of plant-based diets for the prevention of cardiometabolic diseases [31–33]. Previous studies described dietary patterns that result from multiple dietary changes, and therefore, it is difficult to assess the effect of individual dietary components on the microbiota and health outcomes. Although the evidence for major dietary changes in the prevention of cardiometabolic diseases is outstanding, it does not provide clear information on how and how much each dietary component may contribute to the beneficial effect. Polyphenols are widely represented in plant-derived foods suggesting that the metabolic benefits associated with plant-based diets may be due, at least in part, to their polyphenol content. In fact, the polyphenol-rich diet used in our intervention showed multiple favorable effects related to glucose/lipid metabolism [16, 18, 34, 35]. The present study shows that a polyphenol-rich diet affects microbial composition independently of the other dietary components. Three metagenomic studies [32, 33, 36] have previously shown that increased microbial diversity was associated with enhanced metabolic health. In this light, our data provide additional information on the mechanisms behind the beneficial effect of dietary polyphenols on cardiometabolic diseases.

As for the effects on specific bacterial species, in the present study polyphenols reduced *Eubacterium rectale—Blautia coccoides* (clostridial cluster XIV) numbers, while they increased *Clostridium leptum* numbers (clostridial cluster IV). This finding is in line with previous studies demonstrating a selective antimicrobial effect of phenolic compounds [37, 38]. The clinical implications of this effect related to phenolic compounds are unknown. However, it supports the potential to develop tailored dietary changes to obtain specific modifications in the microbiota. This approach represents a tool that can be part of advanced "precision nutrition" strategies for preventing and managing cardiometabolic diseases [39, 40].

Interestingly, we detected a clear bifidogenic effect of LCn3. This finding has been reported in two previous studies with LCn3 supplementation [41, 42]. However, it is noteworthy that in our trial the increase in the numbers of *Bifidobacteria* was obtained through the consumption of fatty fish three times/week, thus representing a feasible approach easily transferable to real-life setting.

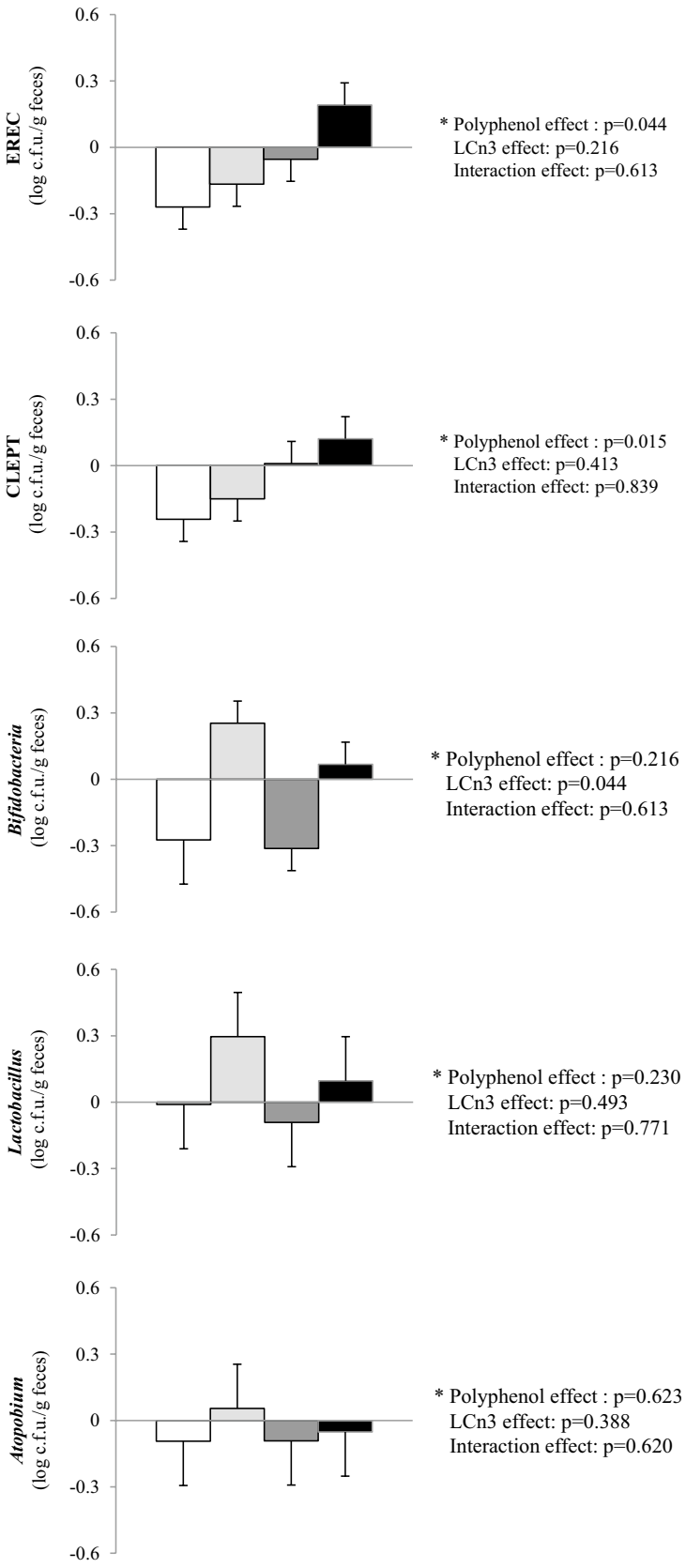

Fig. 2 Absolute changes of bacterial species between baseline and after the dietary intervention in the four experimental groups. *White bar*: low LCn3&PP, diet low in LCn3 and PP; *light gray bar*: high LCn3, diet rich in LCn3 and low in PP; *dark gray bar*: high PP, diet rich in PP and low in LCn3; *black bar*: high LCn3&PP, diet rich in PP and LCn3. *LCn3* long-chain n-3 polyunsaturated fatty acid, *PP* polyphenols. Mean ± SEM. * two-factor ANOVA

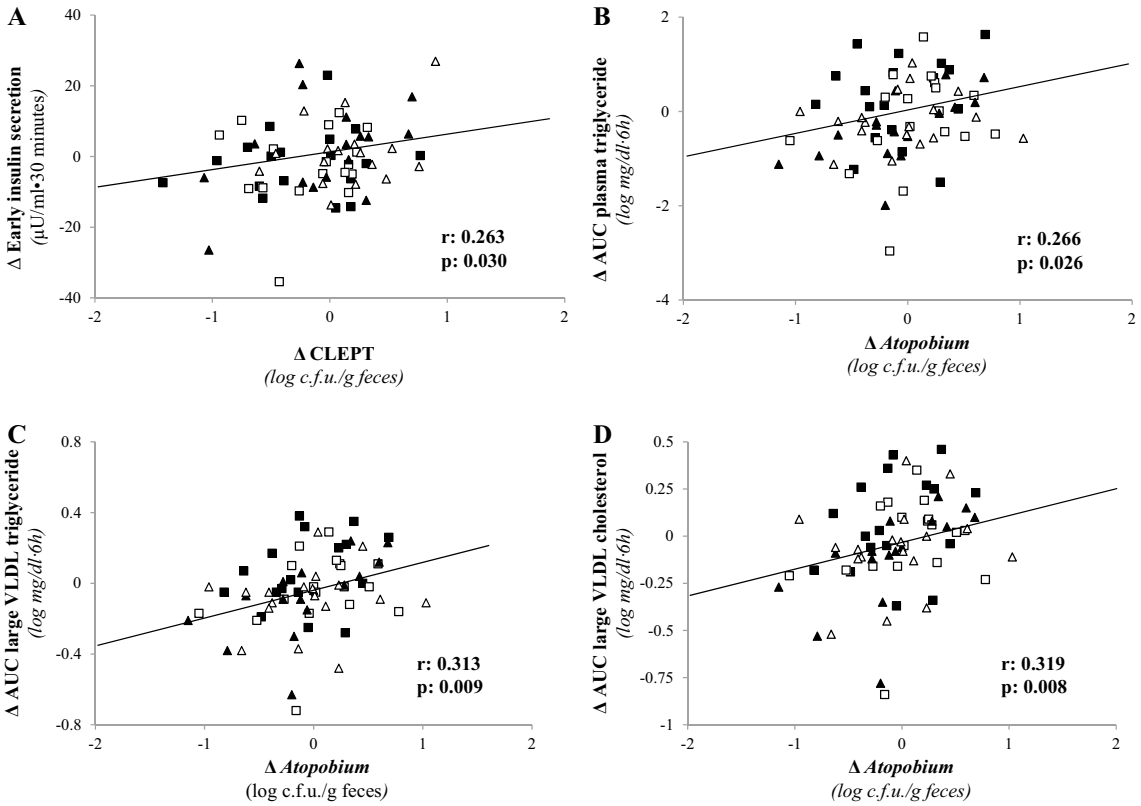

Fig. 3 Pearson correlation between the absolute changes between baseline and after the dietary intervention of **a** *Clostridium leptum* (clostridial cluster IV; CLEPT) and early insulin secretion, **b** *Atopobium* group and postprandial triglycerides in plasma and **c** large VLDL, and **d** postprandial cholesterol in large VLDL. *Black square*: diet low in LCn3 and PP; *white square*: diet rich in LCn3 and low in PP; *black triangle*: diet rich in PP and low in LCn3; *white triangle*: diet rich in PP and LCn3. *AUC* total area under the curve calculated by trapezoidal method, *LCn3* long-chain n-3 polyunsaturated fatty acid, *PP* polyphenols

As for the relation between changes in microbial species and host metabolism, the increase in the *Clostridium leptum* numbers was directly correlated with the early insulin secretion, an index of good glucose tolerance. *Clostridium leptum* group contains numerous butyrate-producing species, and butyrate has shown to influence glucose homeostasis, likely improving insulin secretion [43, 44]. Unfortunately, we did not perform any measurement of fecal or plasma butyrate to test this hypothesis.

In our trial, *Atopobium* group numbers were not influenced significantly by polyphenols or LCn3 or their combination. However, it highly correlated with postprandial plasma lipids, in particular in the large VLDL fractions. In a previous study carried out in healthy subjects [45], an association between *Collinsella* spp., the predominant member of *Atopobium* group, and fasting plasma lipid concentrations was observed. Intriguingly, a recent cross-sectional study [46] found an increased bacterial count of *Atopobium* group in patients with ischemic stroke compared to age- and sex-matched controls. Therefore, it could be hypothesized that the link between increased count of *Atopobium* numbers and cardiometabolic disease may be explained by a worse lipid profile.

A limitation of our study was that we only amplified certain bacterial species; therefore, it remains unknown whether small changes in not-so-abundant species might have affected clinical outcomes. In addition, we did not perform any measurement of fecal metabolome to assess microbial metabolites (i.e., short-chain fatty acids or phenolic compounds) that could represent the connection between diet, microbiota, and the metabolism of glucose and lipids [47, 48].

In conclusion, the present study demonstrates that diets naturally rich in polyphenols or long-chain n-3 polyunsaturated fatty acids can modulate gut microbiota and these changes are related to an improvement in the cardiometabolic risk profile.

These findings provide new information for future investigations on the interplay between dietary changes and microbiota composition that may help to develop tailored nutritional approach for preventing cardiometabolic disease in humans.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard This study was approved by the Ethics Committee of the Federico II University of Naples.

Human and animal rights disclosure All human rights were observed in keeping with the Declaration of Helsinki 2008 (ICH GCP) and with the ethical standards of the responsible committee on human experimentation (Ethics Committee "Federico II University"). There are no animal rights issues as this is a clinical study.

Informed consent Written informed consent was obtained from all participants being included in the study.

References

1. Carding S, Verbeke K, Vipond DT et al (2015) Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 26:26191. https://doi.org/10.3402/mehd.v26.26191
2. Karlsson FH, Tremaroli V, Nookaew I et al (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498:99–103. https://doi.org/10.1038/nature12198
3. Larsen N, Vogensen FK, van den Berg FW et al (2015) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS ONE 5:e9085. https://doi.org/10.1371/journal.pone.0009085
4. Qin J, Li Y, Cai Z et al (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490:55–60. https://doi.org/10.1038/nature11450
5. Bäckhed F, Manchester JK, Semenkovich CF et al (2007) Mechanisms underlying the resistance to diet induced obesity in germ-free mice. Proc Natl Acad Sci USA 104:979–984. https://doi.org/10.1073/pnas.0605374104
6. Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489:242–249. https://doi.org/10.1038/nature11552
7. Zhang X, Shen D, Fang Z et al (2013) Human gut microbiota changes reveal the progression of glucose intolerance. PLoS ONE 8:e71108. https://doi.org/10.1371/journal.pone.0071108
8. Rothschild D, Weissbrod O, Barkan E et al (2018) Environment dominates over host genetics in shaping human gut microbiota. Nature 555:210–215. https://doi.org/10.1038/nature25973
9. Lynch SV, Pedersen O (2016) The human intestinal microbiome in health and disease. N Engl J Med 375:2369–2379. https://doi.org/10.1056/NEJMra1600266
10. Lopez-Legarrea P, Fuller NR, Zulet MA et al (2014) The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. Asia Pac J Clin Nutr 23:360–368. https://doi.org/10.6133/apjcn.2014.23.3.16
11. Holscher HD (2017) Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes 8:172–184. https://doi.org/10.1080/19490976.2017.1290756
12. So D, Whelan K, Rossi M et al (2018) Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr 107:965–983. https://doi.org/10.1093/ajcn/nqy041
13. Seganfredo FB, Blume CA, Moehlecke M et al (2017) Weight-loss interventions and gut microbiota changes in overweight and obese patients: a systematic review. Obes Rev 18:832–851. https://doi.org/10.1111/obr.12541
14. Tomás-Barberán FA, Selma MV, Espín JC (2016) Interactions of gut microbiota with dietary polyphenols and consequences to human health. Curr Opin Clin Nutr Metab Care 19:471–476. https://doi.org/10.1097/MCO.0000000000000314
15. Costantini L, Molinari R, Farinon B et al (2017) Impact of omega-3 fatty acids on the gut microbiota. Int J Mol Sci 18(12):2645. https://doi.org/10.3390/ijms18122645
16. Annuzzi G, Bozzetto L, Costabile G et al (2014) Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. Am J Clin Nutr 99:463–471. https://doi.org/10.3945/ajcn.113.073445
17. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2002) Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 106:3143–3421
18. Bozzetto L, Annuzzi G, Pacini G et al (2015) Polyphenol-rich diets improve glucose metabolism in people at high cardiometabolic risk: a controlled randomised intervention trial. Diabetologia 58:1551–1560. https://doi.org/10.1007/s00125-015-3592-x
19. Maukonen J, Mättö J, Satokari R et al (2006) PCR DGGE and RT-PCR DGGE show diversity and short-term temporal stability in the Clostridium coccoides-Eubacterium rectale group in the human intestinal microbiota. FEMS Microbiol Ecol 58:517–528. https://doi.org/10.1111/j.1574-6941.2006.00179.x
20. Collins MD, Lawson PA, Willems A et al (1994) The phylogeny of the genus clostridium: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 44:812–826. https://doi.org/10.1099/00207713-44-4-812
21. Mättö J, Maunuksela L, Kajander K et al (2005) Composition and temporal stability of gastrointestinal microbiota in irritable bowel syndrome–a longitudinal study in IBS and control subjects. FEMS Immunol Med Microbiol 43:213–222. https://doi.org/10.1016/j.femsim.2004.08.009
22. Maukonen J, Mättö J, Suihko ML et al (2008) Intra-individual diversity and similarity of salivary and faecal microbiota. J Med Microbiol 57:1560–1568. https://doi.org/10.1099/jmm.0.47352-0
23. Maukonen J, Simoes C, Saarela M (2012) The currently used commercial DNA-extraction methods give different results of clostridial and actinobacterial populations derived from human fecal samples. FEMS Microbiol Ecol 79:697–708. https://doi.org/10.1111/j.1574-6941.2011.01257.x
24. Simões CD, Maukonen J, Kaprio J et al (2013) Habitual dietary intake is associated with stool microbiota composition in monozygotic twins. J Nutr 143:417–423. https://doi.org/10.3945/jn.112.166322
25. Rivellese AA, De Natale C, Di Marino L et al (2004) Exogenous and endogenous postprandial lipid abnormalities in type 2 diabetic patients with optimal blood glucose control and optimal fasting triglyceride levels. Exogenous and endogenous postprandial lipid abnormalities in type 2 diabetic patients with optimal blood glucose control and optimal fasting triglyceride levels. J

Clin Endocrinol Metab 89:2153–2159. https://doi.org/10.1210/jc.2003-031764
26. Bondia-Pons I, Pöhö P, Bozzetto L et al (2014) Isoenergetic diets differing in their n-3 fatty acid and polyphenol content reflect different plasma and HDL-fraction lipidomic profiles in subjects at high cardiovascular risk. Mol Nutr Food Res 58:1873–1882. https://doi.org/10.1002/mnfr.201400155
27. Vetrani C, Rivellese AA, Annuzzi G et al (2014) Phenolic metabolites as compliance biomarker for polyphenol intake in a randomized controlled human intervention. Food Res Int 63:233–238. https://doi.org/10.1016/j.foodres.2014.01.018
28. Graf D, Di Cagno R, Fåk F et al (2015) Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis 26:26164. https://doi.org/10.3402/mehd.v26.26164
29. Garcia-Mantrana I, Selma-Royo M, Alcantara C et al (2018) Shifts on Gut Microbiota Associated to Mediterranean Diet Adherence and Specific Dietary Intakes on General Adult Population. Front Microbiol 9:890. https://doi.org/10.3389/fmicb.2018.00890
30. Segata N (2005) Gut microbiome: westernization and the disappearance of intestinal diversity. Curr Biol 25:R611–R613. https://doi.org/10.1016/j.cub.2015.05.040
31. Chierico F, Del Vernocchi P, Dallapiccola B et al (2014) Mediterranean diet and health: food effects on Gut microbiota and disease control. Int J Mol Sci 15:11678–11699. https://doi.org/10.3390/ijms150711678
32. Le Chatelier E, Nielsen T, Qin J et al (2013) Richness of human gut microbiome correlates with metabolic markers. Nature 500:541–546. https://doi.org/10.1038/nature12506
33. Cotillard A, Kennedy SP, Kong LC et al (2013) Dietary intervention impact on gut microbial gene richness. Nature 500:585–588. https://doi.org/10.1038/nature12480
34. Della Pepa G, Vetrani C, Vitale M et al (2019) Effects of a diet naturally rich in polyphenols on lipid composition of postprandial lipoproteins in high cardiometabolic risk individuals: an ancillary analysis of a randomized controlled trial. Eur J Clin Nutr. https://doi.org/10.1038/s41430-019-0459-0
35. Vetrani C, Vitale M, Bozzetto L et al (2018) Association between different dietary polyphenol subclasses and the improvement in cardiometabolic risk factors: evidence from a randomized controlled clinical trial. Acta Diabetol 55:149–153. https://doi.org/10.1007/s00592-017-1075-x
36. Turnbaugh PJ, Hamady M, Yatsunenko T et al (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484. https://doi.org/10.1038/nature07540
37. Marín L, Miguélez EM, Villar CJ et al (2015) Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int 2015:905215. https://doi.org/10.1155/2015/905215
38. Most J, Penders J, Lucchesi M et al (2017) Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women. Eur J Clin Nutr 71:1040–1045. https://doi.org/10.1038/ejcn.2017.89
39. de Toro-Martín J, Arsenault BJ, Després JP et al (2017) Precision nutrition: a review of personalized nutritional approaches for the prevention and management of metabolic syndrome. Nutrients 9(8):pii: E913. https://doi.org/10.3390/nu9080913
40. Wang DD, Hu FB (2018) Precision nutrition for prevention and management of type 2 diabetes. Lancet Diabetes Endocrinol 6:416–426. https://doi.org/10.1016/S2213-8587(18)30037-8
41. Watson H, Mitra S, Croden FC et al (2018) A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. Gut 67:1974–1983. https://doi.org/10.1136/gutjnl-2017-314968
42. Noriega BS, Sanchez-Gonzalez MA, Salyakina D et al (2016) Understanding the impact of omega-3 rich diet on the gut microbiota. Case Rep Med 2016:3089303. https://doi.org/10.1155/2016/3089303
43. Everard A, Cani PD (2014) Gut microbiota and GLP-1. Rev Endocr Metab Disord 15:189–196. https://doi.org/10.1007/s11154-014-9288-6
44. Sanna S, van Zuydam NR, Mahajan A et al (2019) Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet 51:600–605. https://doi.org/10.1038/s41588-019-0350-x
45. Lahti L, Salonen A, Kekkonen RA et al (2013) Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data. PeerJ. 1:e32. https://doi.org/10.7717/peerj.32
46. Yamashiro K, Tanaka R, Urabe T et al (2017) Gut dysbiosis is associated with metabolism and systemic inflammation in patients with ischemic stroke. PLoS ONE 12:e0171521. https://doi.org/10.1371/journal.pone.0171521
47. Federici M (2019) Our second genome and the impact on metabolic disorders: why gut microbiome is an important player in diabetes and associated abnormalities. Acta Diabetol 56:491–492. https://doi.org/10.1007/s00592-019-01315-8
48. Abdul Rahim MBH, Chilloux J, Martinez-Gili L et al (2019) Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. Acta Diabetol 56:493–500. https://doi.org/10.1007/s00592-019-01312-x

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