Healthy subjects differentially respond to dietary capsaicin correlating with the specific gut enterotypes

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Context: Previous population studies in evaluating the beneficial effects of capsaicin (CAP) have yielded inconclusive results, and the mechanisms responsible for possible benefit remain unclear.

Objective: The objective was to assess the impact of dietary CAP on metabolic and immune profiles and its potential associations with gut microbial patterns in healthy adults.

Design: In a 6-week controlled feeding trial, subjects were given the weight maintenance diet sequentially contained with 0, 5, 0 and 10 mg/d CAP from chili powder.

Setting and Participants: The study was conducted in 12 healthy subjects enrolled in Third Military Medical University in Chongqing.

Main Outcome Measures: At the end of each period, anthropometric and basal metabolism measures together with blood and fecal samples were collected. Plasma metabolic and inflammatory markers and gut microbial ecology of each subject were subsequently assessed.

Result: Dietary CAP increased the Firmicutes/Bacteroidetes ratio and *Faecalibacterium* abundance, accompanied with increased plasma levels of glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) and decreased plasma ghrelin level. Further enterotype analysis revealed that these subjects could be clustered into *Bacteroides* enterotype (E1) and *Prevotella* enterotype (E2), and the above beneficial effects were mainly obtained in E1 subjects. Moreover, E1 subjects had significantly higher fecal *Faecalibacterium* abundance and butyrate concentration after CAP interventions than those in E2 subjects.

Conclusion: Our study showed that gut enterotypes may influence the beneficial effects of dietary CAP, providing a new evidence for the personalized nutrition guidance of CAP intervention on health promotion linking with gut microbiota patterns.

Capsaicin (CAP) is the active and pungent ingredient of chili peppers, which are widely used in the cuisines around the world. Accumulating laboratory studies suggests that CAP has beneficial effects in controlling obesity, cardiovascular disease and cancer, which has lead to an increased focus on its pharmacological properties and clinical application (1, 2). However, previous studies have yielded inconsistent results when evaluating the above effects in human (3), and the underlying mechanisms are still unclear.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
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Received July 23, 2016. Accepted September 23, 2016.

Abbreviations:

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The gut microbiota is a complex microbial community and has attracted unprecedented attention because of its critical role in regulating host physiology and pathophysiology (4). Gut microbiota encodes enzymes that modulate the host energy metabolism, synthesis of short chain fatty acids (SCFAs) and systematic immune response (5). It also serves as a virtual endocrine organ which regulates the production of gastrointestinal (GI) hormones such as ghrelin, glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), playing important roles in energy homeostasis and glycolipid metabolism (6). In contrast, the imbalanced gut microbiota can produce toxins like lipopolysaccharide (LPS), which is closely associated with the development of obesity and related chronic disease (7). Therefore, it is of great significance to maintain its balance for health promotion.

It has been suggested that human gut microbiota can be divided into three enterotypes based on their enrichment in *Bacteroides, Prevotella* and *Ruminococcus*, which are not nation or continent specific (8). Although the stratification strategy of gut enterotype is still somewhat controversial (9), several lines of evidence have shown that the subjects in different enterotypes might have different metabolic profiles and gut microbiota responses under the same diet (8, 10–12). To clarify the gut microbial complexity and link the gut microbiota patterns with chronic disease control, more longitudinal studies are needed to further identify its biological and clinical significance. To approach this question, we were interested in whether the intervention effects of CAP will be correlated with the specific gut enterotypes.

Until now, only one study showed that CAP intervention enriched the abundances of gut *Prevotella*, *Lactobacillus* and *Akkermansia* in mice using qPCR approach (13), while the overall impact of CAP on gut microbial ecology is still poorly understood. In addition, no research has been conducted to evaluate its effects on human gut microbiota. This study was designed to evaluate the impact of dietary CAP on the metabolic and inflammatory profiles and gut microbial ecology of healthy subjects consuming a 6-week controlled diet, and further assess the association between CAP effects and gut enterotypes. We analyzed the anthropometric, metabolic and inflammatory markers periodically and simultaneously conducted the 16S rRNA gene sequencing, functional bacterial-metagenome prediction, fecal culture and SCFA analysis to characterize the dynamic changes of human gut microbiota.

Materials and Methods

Subject enrollment

Volunteers were initially recruited through leaflets distributed on-campus from July to August 2014 at the Third Military Medical University (TMMU), Chongqing. Subjects were enrolled if aged 18–40 years with a body mass index (BMI) of $<$ 28, and had received a complete physical examination within 3 months of enrollment. To be eligible for our study, all subjects were required to meet the following criteria: (i) free from any GI disease, endocrine disease, cardiovascular disease, cancer and other chronic diseases, (ii) had regular eating and lifestyle patterns, (iii) had a normal bowel frequency (minimum once every 2 days, maximum 3 times per day), (iv) had not taken antibiotics in the past 3 months, and (v) could follow the controlled feeding trial. Subjects with any incomplete information on the above items were excluded.

Among the 48 subjects accessed for eligibility, 33 subjects were excluded because of their ineligibility (n = 29) or unwillingness to participate the study (n = 4). During the baseline intervention period, another 3 subjects were excluded because they could not follow the controlled feeding trial. Finally, 12 healthy subjects (6 females and 6 males with 27.8 ± 3.8 years old) met all the criteria and joined the study. The study protocol was registered at http://www.chictr.org/cn/ (ChiCTR-TTRCC-14 004 909) and approved by the Ethical Committee of TMMU. All participants provided informed consent.

Study design

All subjects were guided to consume the 6-week fully controlled diet and maintain their regular physical activities in this study. Dietary calorie levels were determined by the participants' initial basal energy expenditure (BEE) and physical activity levels as guided by the 2013 Chinese Dietary Reference Intakes (14). Body weight was measured every day and calorie intake was adjusted to maintain the body weight as suggested (15). Subsequently, food types and amounts for the weekly recipe were designed based on the 2007 Dietary Guidelines for Chinese Residents (16). During the study, all foods were served in cooked or ready-to-eat form at our study center, and the participants were required to eat the meals on-site. The uneaten foods were weighted for the daily dietary nutrient intake assessment.

The first week was the run-in period and contained no chili powder (defined as baseline period). After that, subjects underwent a two-week low CAP intervention period (5 mg/d) and two-week high CAP intervention period (10 mg/d), which was interspaced by one-week washout period. During the whole study, the diets among the four periods differed mainly in the CAP contents (see Supplementary Materials for more information). At the end of each period, anthropometric and basal metabolism measurements together with blood and fecal samples were collected from each participant, and appetite evaluation was conducted as suggested (17). The procedures for anthropometric and biochemical assessments together with SCFA measurement and microbial culturing are shown in Supplementary Materials. Meanwhile, a daily questionnaire was completed by each subject to monitor their symptoms of bowel movement, stool consistency, flatulence and other discomforts during the study.

Fecal DNA extraction and microbiota profiling

Fecal samples were collected within 1 hour of defecation and 12 hours of each blood sampling and stored at –80°C until DNA extraction with the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany). Bacterial 16S rRNA gene sequences were PCR amplified using primers binding to the V3-V4 regions, and the re-

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sulting amplicons were quantified and sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA) with paired-end 300-nucleotide reads. QIIME software (version 1.8.0) was used to filter and demultiplex the raw data (18), and the detailed information are provided in Supplemental Materials.

Microbial function prediction and enterotyping

Microbial function was predicted using PICRUSt (version 1.0.0) as suggested (19). The predicted genes and functions were aligned to KEGG database (version 66.1, May 1, 2013) and analyzed using QIIME and STAMP (P value < 0.05 and effect size > 1). To further cluster the gut enterotypes, we calculated the Jensen–Shannon (JS), Euclidean (EU), Bray-Curtis (BC), and weighted/unweighted UniFrac distances for the relative abundance at genus level using R (version 3.2.2) as suggested (8, 20). Besides, optimal number of clusters was determined by the Calinski–Harabasz pseudo F-statistic (CH) value and the Rousseeuw's Silhouette internal cluster quality index (SI) value as the relative and absolute measurement, respectively.

Statistical analyses

GraphPad Prism 6.01 (GraphPad Software Inc., CA, USA) was used for statistical analysis unless otherwise stated. The statistical analysis was conducted with ANOVA with Tukey's post hoc tests to compare the data acquired at different periods. If the data were skewed distributed, values were natural logarithm-transformed to normalize their distributions. ANCOVA adjusting for gender and baseline levels was used to compare the differences of change-from-baseline values between E1 and E2 subjects using SPSS 17.0 (SPSS Inc., Chicago, USA). Correlations between host biochemical measurements and bacterial genera were assessed by Pearson's correlation tests. QIIME, STAMP and R packages were used in the analysis of gut bacteria profiling as mentioned above. A P value < 0.05 was considered statistically significant.

Results

Effects of CAP interventions on physiological and biochemical markers

All subjects completed the study, and only one subject reported GI discomfort at the end of the high CAP period. As shown in Supplemental Table 1, the subjects' body weights remained stable over the study (average initial weight: 63.69 ± 14.86 kg), and their dietary nutrient intakes were comparable among the four periods after subtracting the uneaten foods from the weight of foods served, indicating good subject compliance with the study protocol.

Subsequent analysis showed that the subjects consistently exhibited significantly higher plasma levels of GLP-1 and GIP and lower plasma ghrelin level after both low and high CAP interventions, compared with the levels at baseline period (Figure 1, A-C). However, as shown in Supplemental Table 2, no significant difference was observed in BMI, waist-hip ratio (WHR), basal energy expenditure (BEE), body fat percentage and the area under curve (AUC) of appetite score in different periods. In addition, neither low nor high CAP intervention significantly changed the plasma levels of fasting blood glucose, insulin, glucagon, leptin and adiponectin in subjects. Similarly, the plasma levels of total cholesterol, triglycerides, HDL-C, LDL-C and proinflammatory cytokines (IL-6, TNF-α and LBP) did not differ during the study period.

Overall impact of dietary CAP on gut microbiota

To comprehensively monitor the dynamic changes of gut microbiota, we analyzed the fecal samples with bacterial 16S rRNA gene sequencing. In total, we characterized 1 006 958 high-quality sequences and the rarefaction diversity curves revealed that most of the diversity had already been captured (Supplemental Figure 1A). There was no significant difference of taxonomic alpha diversity (Chao1, Shannon and Simpson Index) during the study period (Supplemental Figure 1, B-D). Meanwhile, no significant variation among the study periods was found in relation to CAP intervention by either weighted or unweighted UniFrac PCoA analysis (Supplemental Figure 2, A and B), indicating the overall gut microbiota structure of subjects kept stable overtime and the response of dietary CAP interventions was highly correlated with the initial

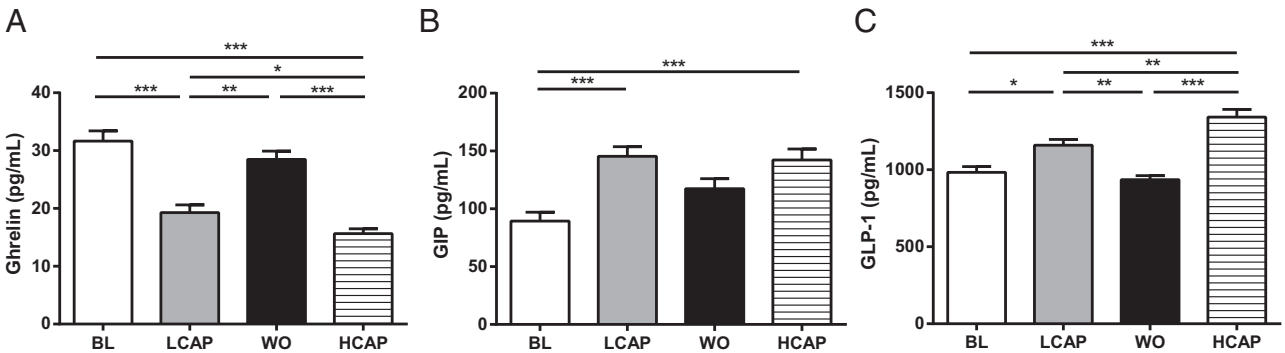

Figure 1. Effect of CAP intervention on the plasma levels of (**A**) ghrelin, (**B**) GIP and (**C**) GLP-1 in study subjects (n = 12). BL, baseline; LCAP, low CAP intervention; WO, washout; HCAP, high CAP intervention. Data are presented as means ± S.E.M.. *$P < .05$, ** $P < .01$, *** $P < .001$ using one-way ANOVA with Tukey's post hoc tests.

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state of the gut microbiota. In contrast, gut community was significantly different between men and women when using unweighted UniFrac PCoA analysis (Adonis test, $R(2) = 0.1$, $P < .001$), which suggested the potential role of gender on gut microbiota structure (Supplemental Figure 2, C and D).

Although the relative abundance of different phyla among subject samples changed over time and had apparent interindividual variability (Figure 2A), both low and high CAP interventions significantly increased the proportion of the phylum Firmicutes and high CAP also decreased the phylum Bacteroidetes (Figure 2B) In addition, low CAP intervention significantly reduced the abundance of family Lachnospiraceae and high CAP significantly enriched the abundances of family Ruminococcaceae and genus *Faecalibacterium* (Figure 2, C and D).

To further evaluate the changes of functional genes in gut microbial communities, we used PICRUSt to predict the relative abundance profiles of KEGG reference pathways. The average NSTI in our study was 0.095, suggesting that PICRUSt could be used to predict the metabolic potential of microbial communities. Despite the widespread differences in gut bacteria composition, KEGG pathways in the gut microbial community were comparatively stable among the subjects during CAP interventions (Supplemental Figure 3).

Enterotype stratification and functional characteristics

We next sought to cluster the gut microbial enterotypes simultaneously using the JS, EU, BC and weighted/unweighted UniFrac distance metrics. The highest CH value was obtained for all of the tested distance metrics when k = 2 (Supplemental Figure 4A). For two clusters, the SI

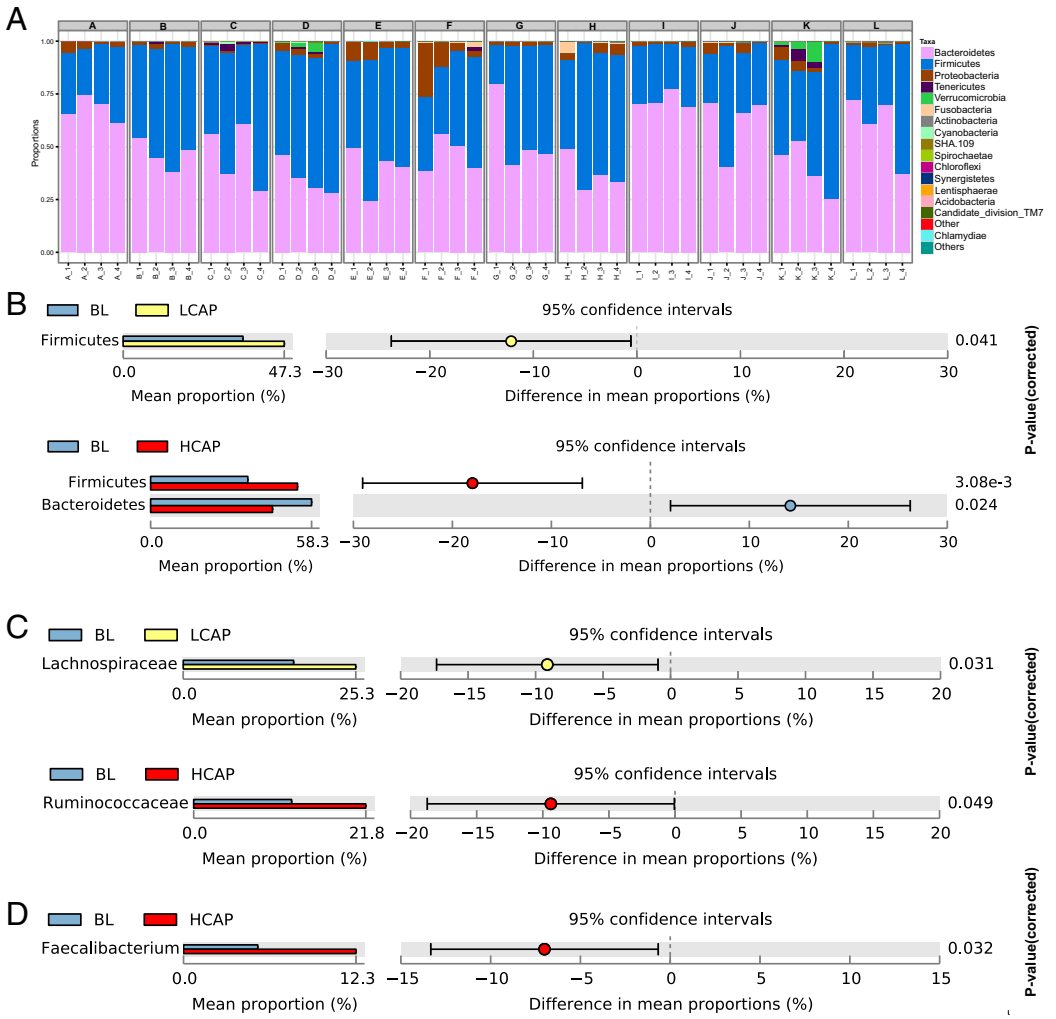

Figure 2. Effects of CAP intervention on gut microbial communities in study subjects (n = 12). (**A**) Changes of taxonomic composition of gut microbiota. A-L represents each subject and 1–4 represents the four periods in this study. Stacked bar charts show the individual variability of the relative abundances of major bacterial phylum during the study. (**B-D**) Plots show the significant differences in abundance of the gut microbial communities at (**B**) phylum, (**C**) family and (**D**) genus levels. Bar graphs on the left side display the mean proportion of each microbial taxa. Dot plots on the right side display the difference in mean proportions between baseline and low CAP or high CAP periods with associated *P* values. Error bars of dots represent the 95% CIs. BL, baseline; LCAP, low CAP intervention; WO, washout; HCAP, high CAP intervention.

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values were higher than 0.25 when using EU, JS and weighted Unifrac distance metrics (Supplemental Figure 4B). Therefore, we inferred that our subject microbiome data could be clustered into two enterotypes and applied JS distance for further analyses (Figure 3A), which were driven by levels of *Bacteroides* and *Prevotella* (Figure 3B). Besides, the ratio of *Prevotella* to *Bacteroides* presented a discrete distribution across these two enterotypes (Supplemental Figure 5). As shown in Figure 3B, subjects in *Bacteroides* Enterotype (E1, n = 6) also had significantly higher abundances of *Faecalibacterium, Phascolarctobacterium* and *Alistipes* bacteria when compared to the subjects in *Prevotella* Enterotype (E2, n = 6). Further evaluation on the enterotype stability showed that all E1 subjects and 4 of 6 E2 subjects maintained their baseline enterotypes during the whole study periods. Although the rest two E2 subjects were classified into E1 at the end of washout period and high CAP period respectively, their relative abundances of *Prevotella* were still much higher than those in E1 subjects. In addition, PICUSt analysis further demonstrated the significant differences of gut microbial functions between the subjects in E1 and E2 groups, reflecting the higher gene functions of glycolipid metabolism in E1 group and higher efficiency of nutrient digestion and absorption in E2 group (Figure 3C).

Benefits of CAP intervention were increased in E1 subjects

We conducted further analyses to identify differences within and between groups of the above mentioned physiological and biochemical parameters during the study periods. Both low and high CAP could significantly increase the plasma levels of GLP-1 and GIP and decrease plasma ghrelin level in E1 subjects, while only high CAP intervention significantly influenced the ghrelin and GLP-1 levels in E2 subjects (Figure 4, A-C). We also found that the plasma LBP level in E1 subjects was significantly decreased after high CAP intervention (Figure 4D). In addition, ANCOVA analysis revealed that E1 subjects had significantly improved plasma GLP-1, GIP and LBP level than those in the E2 subjects after the CAP interventions (Figure 4, E-H).

We next compared the within-group and between-group differences in the gut microbiota response concerning the enterotypes during the study. Although there was no significant change of gut microbiota at family level when clustered into enterotypes, we found E1 subjects had borderline significant increases in Lachnospiraceae after low CAP intervention ($P = .098$) and Ruminococcaceae after high CAP intervention ($P = .055$). Besides, high CAP significantly increased the relative abundance of *Faecalibacterium* in E1 group (Baseline *v.s.* High CAP: 0.05 ±

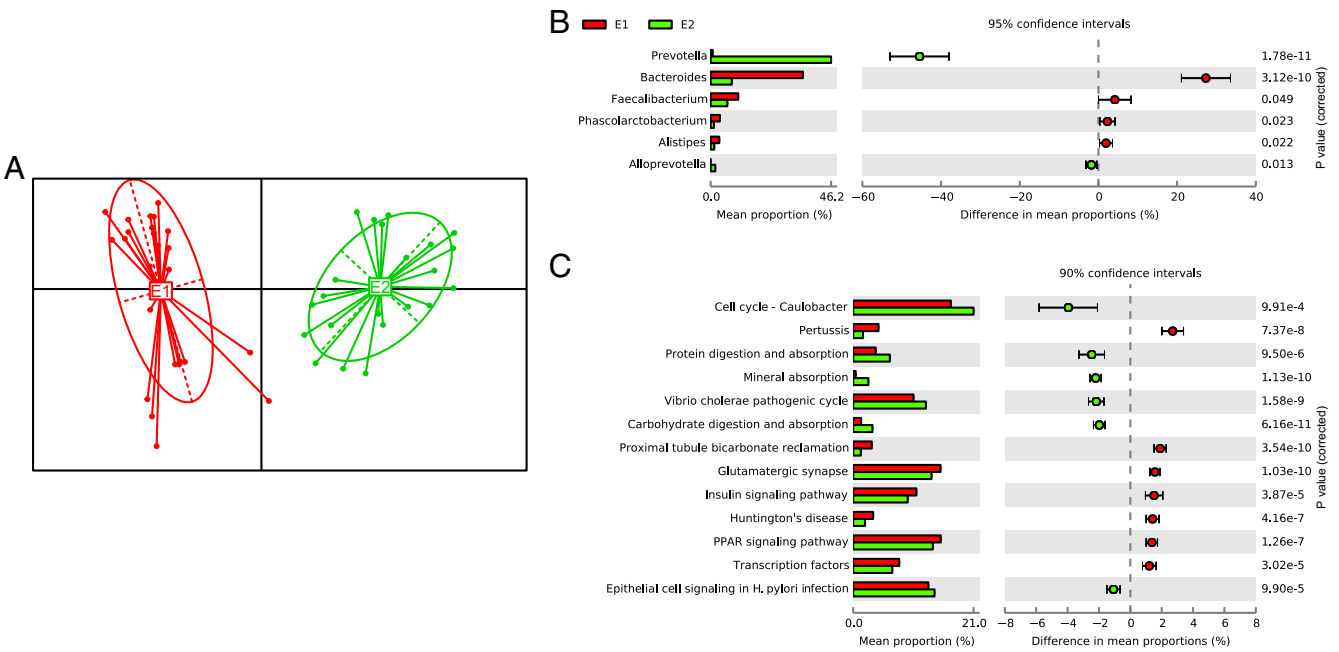

Figure 3. Enterotype stratification and functional diversity between the enterotypes. **A,** The first two principal coordinates of JS distances of the relative abundance profiles in genus level. Samples are colored by red for *Bacteroides* enterotype (E1) and *Prevotella* enterotype (E2). Comparisons of **(B)** the relative abundance of bacterial species between the subjects in E1 and E2 enterotypes at the genus level and **(c)** relative gene abundances in KEGG reference pathways. Bar graphs on the left side display the mean proportion of each microbial taxon. Dot plots on the right side display the difference in mean proportions between two enterotypes with associated *P* value. Error bars of dots represent the 95% CIs.

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0.02 *v.s.* 0.18 ± 0.03, $P < .05$; Figure 5A), and the increase in E1 group was significantly higher than that in E2 group (0.13 ± 0.03 *v.s.* 0.07 ± 0.02, $P < .05$; Figure 5B). Further correlation analysis showed that the fecal *Faecalibacterium* abundance in E1 group was significantly positively correlated with plasma GIP level ($r = 0.44$, $P < .05$), and negatively correlated with plasma ghrelin and LBP levels ($r = -0.42$, $P < .05$, and $r = -0.52$, $P < .05$, respectively; Figure 5, C-E). Similarly, high CAP significantly increased the fecal butyrate concentration in E1 group (Supplemental Figure 6, A and B), which was significantly correlated with plasma ghrelin and GLP-1 levels ($r = -0.56$, $P < .01$, and $r = 0.49$, $P < .05$, respectively; Supplemental Figure 6, C and D). In addition, both low and high CAP inter-

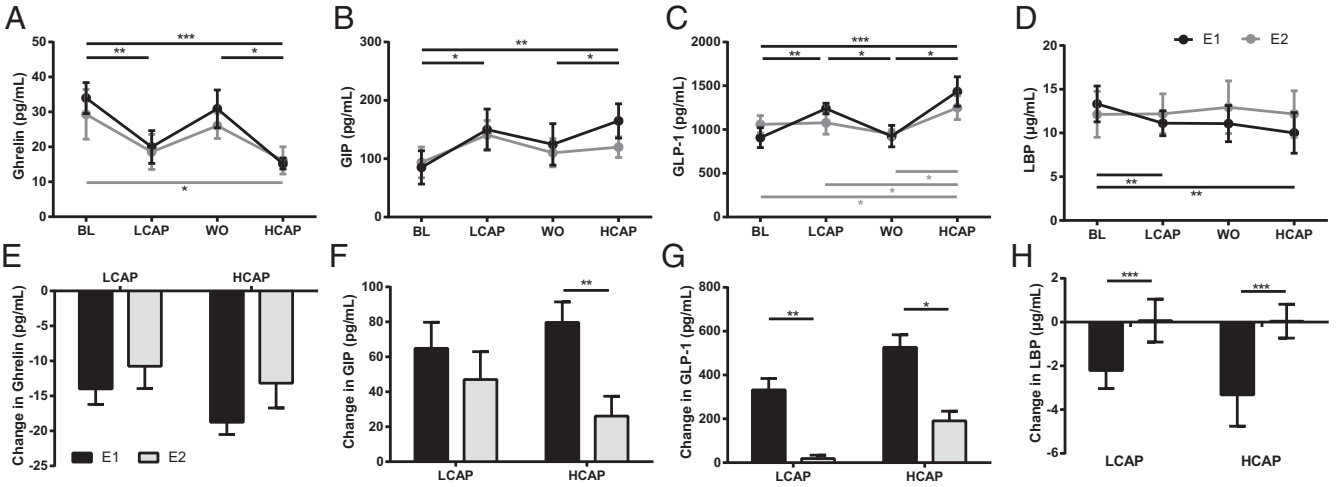

Figure 4. Comparison of changes in plasma GI hormones and LBP levels within and between the enterotypes. (**A-D**) One-way ANOVA with Tukey's post hoc tests was used to compare the within-group changes in (**A**) ghrelin, (**B**) GIP, (**C**) GLP-1 and (**D**) LBP during the study period. (**E-H**) ANCOVA adjusting for gender and baseline values was used to compare the intergroup changes between the E1 and E2 subjects in (**E**) ghrelin, (**F**) GIP, (**G**) GLP-1 and (**H**) LBP. E1 group, black bars and lines; E2 group, gray bars and lines. Data are presented as means ± S.E.M., $*P < .05$, $**P < .01$, $***P < .001$.

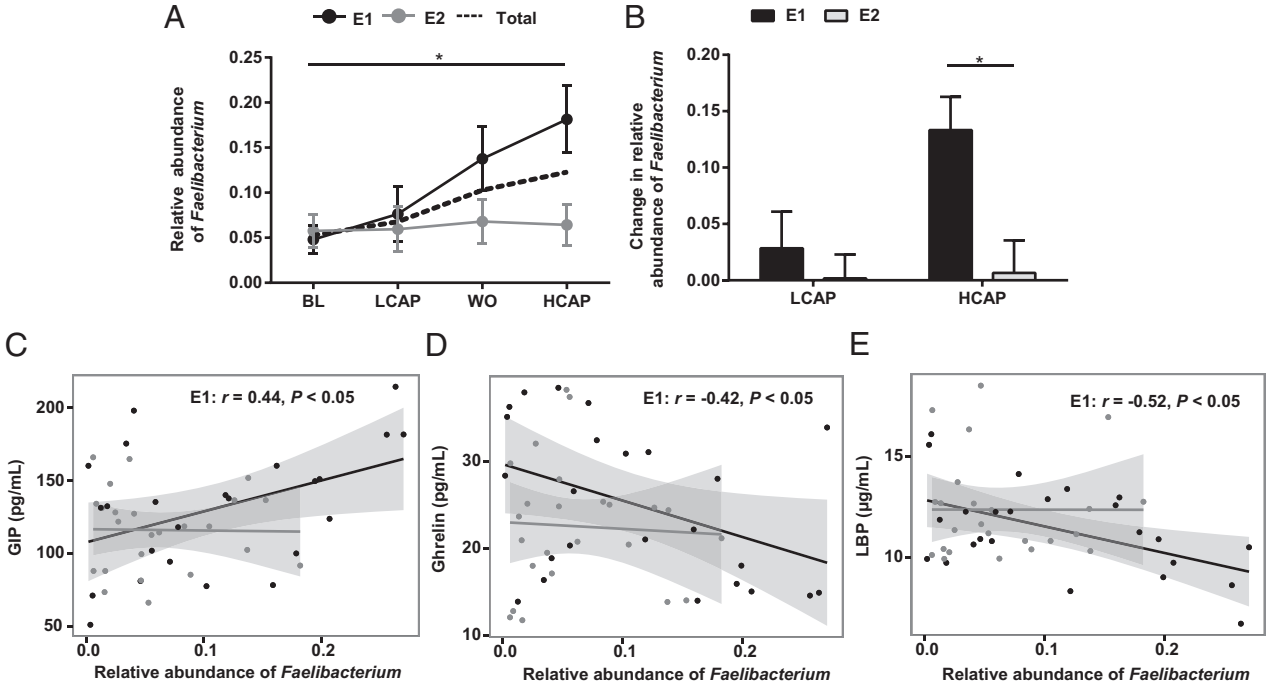

Figure 5. *Faecalibacterium* abundance changes and their associations with plasma biochemical parameters linking with enterotypes. **A,** One-way ANOVA with Tukey's post hoc test was used to measure the within-group change. **B,** ANCOVA adjusting for gender and baseline values was used to compare the intergroup changes between the E1 and E2 subjects. (**c-e**) Plots show the relationship between *Faecalibacterium* abundance and serum ghrelin, GIP and LBP, represented as a smoothing spline with a 95% confidence interval (CI) (shaded region). E2 group, gray bars and lines. Addition dashed lines were presented to show the respective changes in total subjects. Data are presented as means ± S.E.M., $*P < .05$, $**P < .01$, $***P < .001$. Pearson's correlation (r) and the corresponding P-values are presented when the statistic is significant.

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ventions dramatically decreased the abundances of Gram-negative bacteria in E1 subjects, which were also significantly lower than those in E2 subjects (Supplemental Figure 7, A and B).

Discussion

In this controlled feeding trial, we found that the short-term dietary intervention of CAP increased the Firmicutes/Bacteroidetes ratio and *Faecalibacterium* abundance that coincided with the increase of plasma levels of GLP-1 and GIP and the decrease of plasma ghrelin level in healthy Chinese adults. Strikingly, further gut enterotype stratification analysis revealed that the above benefits were mainly obtained in E1 subjects, who also had significantly lower plasma LBP and fecal gram-negative bacteria levels and higher fecal butyrate concentration when compared to the E2 subjects.

Previous laboratory studies have demonstrated that CAP is not only a hot topic in chili pepper but also a promising pharmacological molecule on regulating lipid metabolism, glycemic control, GI function and immune response (2, 21, 22). Comparatively, the studies conducted to evaluate these effects on human are few and their results are inconsistent (23). In this study, we did not find any significant changes in appetite, glycolipid metabolism and inflammatory markers in subjects after either low or high CAP intervention. These results were consistent with some (24, 25) but not all (26, 27) reported studies, possibly due to the strong ability to maintain homeostasis in healthy people, the less sufficient dose and duration of CAP interventions, and the comparable dietary energy and macronutrient intakes during the whole study with controlled diet. However, in line with the previous result, our study showed that both low and high CAP interventions significantly improved the plasma levels of ghrelin, GIP and GLP-1, which are comprehensively involved in energy homeostasis and metabolic regulation (6).

Up to now, no studies have evaluated the impact of dietary CAP on human gut microbiota. In this study, we found that although the overall gut microbiota structure remained stable during the study, the relative abundance of microbial taxa varied over time, consistently with the previous reports (28, 29). Specifically, both low and high CAP interventions increased the Firmicutes/Bacteroidetes ratio, which was also observed in the populations with long-term consumptions of whole grain, dietary fibers and vegetables (30, 31), and the healthy subjects after the short-term whole grain intervention (32). In addition, we found that the dietary CAP increased the abundances of family Lachnospiraceae and Ruminococcaceae and genus *Faecalibacterium*, indicating the enrichment of butyrate-producing members in gut microbiota (33). Collectively, these results showed that dietary CAP intervention have substantial effects on the composition and function of gut microbiota in healthy population.

It has been suggested that the three enterotypes (*Bacteroides, Prevotella*, and *Ruminococcus*) of human gut microbiota might respond differently to the same diet, which provides a new approach to explore the gut microbiota complexity and the interactions among diet, gut microbial ecology and human health (8). However, only *Bacteroides* and *Prevotella* enterotypes were observed in our study. This result was consistent with a few published studies (34–36), possibly due to the small sample size or the different habitual dietary intakes. Although a recent study showed that the gut microbiota is represented as a smooth gradient (20), we found a discrete distribution of *Prevotella/Bacteroides* ratio across the two enterotypes. To evaluate the applicability of enterotype stratification, we further assessed the enterotype stability of the subjects during the study. Only 2 samples scored in E2 subsequently switched into E1 at the end of washout period and high CAP period respectively, yet their *Prevotella* abundances were still significantly higher than those in E1 subjects. These results indicated that the enterotypes remained generally stable at least for 6 weeks, which is in line with the previous studies (11, 35, 37).

Owing to the fact that only few studies concerning gut enterotypes have been conducted, the biological and clinical significance of enterotypes remains unclear. Nonetheless, a study showed that E2 individuals had significantly higher plasma concentrations of trimethylamine-N oxide (TMAO), a gut microbiota-generated proatherogenic metabolite, than those in E1 individuals, suggesting the potential role of enterotype in affecting host metabolism (10). Another study used New Nordic Diet (contains more fruits, vegetables and whole grain with less added sugar and saturated fat) with 6-month intervention period found that the subjects with high *Prevotella/Bacteroides* ratio had significantly higher total plasma cholesterol levels than those in low ratio group (11). Similarly, we found that dietary CAP had better improving effects on the plasma ghrelin, GIP and GLP-1 concentrations in E1 subjects although both E1 and E2 subjects responded to the CAP interventions. Furthermore, high CAP interventions significantly increased the concentrations of fecal butyrate only in E1 group, which plays important roles in protecting mucosal barrier function and stimulating the secretions of GI hormones (38).

When clustered into enterotypes, we also found that high CAP intervention significantly enriched the abundance of *Faecalibacterium* (belonging to the family *Ru-*

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minococcaceae) in E1 group. Meanwhile, E1 subjects had significantly lower plasma LBP level and fecal Gram-negative bacteria abundance after both low and high CAP interventions when compared to the E2 subjects, which indicated the reduced risk of local and systemic inflammation (39). In addition, all significant correlations between host biochemical markers and gut microbiota were only found in E1 group. Taken together, our data complemented and strengthened evidence that the individuals in different enterotypes might have different metabolic and gut microbiota responses under the same diet (8).

To our knowledge, this is the first study to systematically evaluate the impact of CAP on human gut microbiota linking with the metabolic and inflammation profiles. However, some limitations should be addressed when interpreting the findings of this study. Firstly, the influences of dietary CAP on metabolic and inflammatory profiles and gut microbiota might be affected by the relative small sample size and short intervention period. To minimize this influence, we provided the 6-week fully controlled and weight maintenance diet and all subjects were enrolled from the same university who had the comparable lifestyles and considerable compliance. Secondly, metagenomic analysis was not applied to comprehensively evaluate the dynamic changes of gut microbiota structure and functions. Alternatively, we investigated these characteristics using 16S rRNA gene sequencing, product determinations and microbial function prediction. Thirdly, although our study discovered the large effect of gender on gut microbiota structure, we found no significant correlation of gender with enterotype, or with the changes of physiological and biochemical markers (data not shown). Owing to the potential influence of sex hormone fluctuation on GI hormones like GLP-1 and ghrelin (40), further studies should take the menstrual status into consideration when evaluating the regulating effects on GI hormones in premenopausal women. In addition, the association among the effects of dietary CAP, gut enterotypes and gut microbiota response was only evaluated in healthy subjects in this study. To validate and expand this significance, we have initiated the parallel clinical trials including subjects with obesity and type 2 diabetes mellitus. Meanwhile, more studies with larger sample size, longer period, wider age range and different chronic diseases are warranted.

In conclusion, our study suggests the possible link between CAP beneficial effects and host gut enterotypes, which may partly explain the inconsistent results of previous trials evaluating the effects of CAP on human health. Also, we provide a new evidence for the potential feasibility of gut microbiota stratification for the personalized nutrition guidance of dietary capsaicin intervention.

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Acknowledgments

This research was supported by Project 81 472 973, Project 81 470 562 and Project 81 673 157 of the National Natural Science Foundation of China (NSFC). We thank Dr. Kanakaraju Kaliannan at Massachusetts General Hospital in Boston for proofreading of our manuscript.

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This work was supported by This research was supported by Project 81 472 973, Project 81 470 562 and Project 81 673 157 of the National Natural Science Foundation of China (NSFC).

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Disclosures Statement: The authors have declared that no conflict of interest exists.

Conflict of interest: The authors declare no competing financial interests.

Data Availability: The sequence information has been deposited in the GenBank Sequence Read Archive with accession number SRP063480.

Author Contributions: The authors' responsibilities were as follows: CK, BW, and M-T M designed the experiments; CK, YZ, XZ, XW, KL, MC, SH, LH, JW, J-D Z and Q-Y Z collected samples and performed the experiments; CK, YZ and HC analyzed the data; CK, BW and M-T M prepared the manuscript and had primary responsibility for final content; and all authors read and approved the final manuscript. None of the authors reported a conflict of interest.

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