Dear Editor,

Thank you for the feedback regarding our submission "Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data" (#2012:11:101:0:1:REVIEW).

Kindly find our responses to the reviewers' comments below. The reviewer comments are indicated by italics font. We hope that we have now adequately addressed all points raised up by the reviewers, and we are looking forward to hearing from You.

Kind regards,
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Editor's comments

You as authors might have two goals: (1) firstly to get this on record as normal EU process in a form that no one is ever expected to read but you can cite as being on record. Or (2) as experienced scientists you may wish to communicate to your colleagues interested in the topic just what they should be thinking and taking in to regard. Unless the manuscript is seriously revised (and of course with 8 authors and the EU practices, none of these has really thought this through adequately), I promise you this will fit into the first class and be ignored except by the authors. Of course, a modern editor is not usually so blunt – she or he merely shuffles papers or files. However, I hope to help PeerJ set a high standard, as I have in previous efforts with a hand-full of other journals. Therefore a 3rd set of comments are attached, dealing primarily with the figures and tables (of course, the data) and to a much less extent the writing. Good luck in your effort. It would be wonderful if all authors were required to be involved and learn and work together. It is our hope that after taking longer than wished-for in the initial review, the revised manuscript should be accepted for publications within a day or two of posting.

Thanks for the feedback. We have now carefully revised the manuscript, taking this and other comments into account. We do think that this paper has impact as we specified in our earlier letter – we also involved at least half of the authors in an endeavor to make this a worthwhile paper to read. This is not an EU project, fortunately…

Suggestions from the Editor:

1) Minor, but remove "lipidome" and "lipidomic" et alia. and other novel "omics" terms. A meta-genomics PhD student here suggested that the journal should charge 700€ for every "ome" or "omics" beyond the most-used 3-5. There are actually hundreds now and I recall when a new genomics centre was being started when I was at the University of Cape Town, in order to gain support for the Centre, it started with "nose-omics" for a mouse researcher in the Zoology Department) and "root-omics" for someone interested in gene expression in flooded maize fields. It is bad enough that we are probably stuck with metabolomics, but we do not need vitaminomics, sugaromics, aminoacidomics, et alia as well as lipomics. You have "mixOmics" from previous papers.

We consider lipidomics as an established term in the field. It is widely used in contemporary literature, including papers that have been published from the same cohort, i.e. Kekkonen et al. 2008a/b. Search patterns including "lipidomics" have stayed relatively constant since 2007 (Google Trends), and search in Google Scholar returns 19,900 hits (metabolomics: 26,600). However, we agree with your concerns and now have replaced "lipidome" and "lipidomic" with "lipid profiles" and "lipid profiling" to improve the manuscript. "mixOmics" is the name of a statistical software package but we do not use this term here either any more.

There are 3 Figures and 2 Tables, each of which could communicate its meaning much easier than now: Fig. 1 legend of course has a trivial capitalizing error that none of the 8 authors considers one's responsibility. There are three times for sampling and that information absolutely must be in the legend, as well as a simple statement of what "signal" is intended – as by telegraph? The sentence in the legend on "significance" should be moved from there to the text itself and the "bar" should be defined as for example +/- SD (N-x). The significant increase was only for the 2nd time data and not for the 1st or 3rd (earlier and later) time points, or else this reader does not understand the figure. Is the editor wrong or the authors just not careful?

We apologize for the sloppiness and thank for the feedback. We have now corrected the capitalization error and clarified the text as follows in Figure 1 caption: "Intervention effects on the abundance of *L. rhamnosus*. Mean abundance of *L. rhamnosus* among the study subjects before, during and after the probiotic intervention (the time points 1-3, respectively) quantified by the HITChip hybridization signal. The error bars denote the Gaussian 95% confidence limits based on standard deviation of the mean."

2) For Figure 2, "correlation heatmap" is neither science nor English. It does not made clearer by the first hit in google.com "Significance level added to matrix correlation heatmap using ggplot2 ".

We have now clarified the description in Figure 2 caption as follows: "Correlations between intestinal genus-level phylogenetic groups and serum lipids. The correlations between the intestinal bacteria and serum lipids are indicated by colors (red: positive; blue: negative). The significant correlations ($q<0.05$) are indicated by '+'; only lipids and bacteria with at least one significant correlation are shown. Hierarchical clustering of the rows and columns highlights groups of significantly correlated bacteria and lipids. For abbreviations of lipid names, see the Methods section."

How does "q" less than 5% differ from the most usually used "p" less than 5% standard for weakly significant and for example on line 154, p. 7?

The q-value is a standard tool for multiple testing correction and for estimating the false discovery rate among the positive findings in high-throughput screening studies. We have now clarified the concept of q-values in the Methods section and have added the explicit statement that the q-value is used in the present paper exclusively in the high-throughput screening experiments where traditional multiple testing corrections are known to have severe shortcomings. Since q-value is a well-established statistical measure in this context we cite the original publication of Storey and Tibshirani (2003) for further details. The clarified text in the Methods section is as follows: "High-throughput screening studies involve considerable multiple testing and the traditional multiple testing correction approaches are prohibitively conservative in this context due to their emphasis on estimating the probability of a single false positive finding. Hence, we have used q-values (Storey and Tibshirani 2003) for multiple testing correction in the high-throughput screening tests that include parallel comparisons of large numbers of lipids and bacterial phylotypes."

And of course when the authors say in the legend of Figure 2 that "abbreviations of lipid names, see the Methods section", that is false, as line 141 on p. 7 says to look at the authors' 2008 paper for this list. Again, either the editor is making a major mistake or the 8 authors have failed to read their own manuscript. That long long list needs to be added to the Fig. 2 legend if any reader is expected to gain any understanding from it.

We agree fully and have now described the lipid names in more detail in Methods section and the Figure 2 legend. The Figure 2 legend now states: "Lipids have been named according to Lipid Maps (http://www.lipidmaps.org) with the following abbreviations: Cer: ceramide; ChoE: cholesteryl ester; lysoPC: lysophosphatidylcholine; PA: phosphatidic acid; PG: phosphatidylglycerol; PC: phosphatidylcholine; PS: phosphatidylserine; SM: sphingomyelin; TG: triglyceride. Where the fatty acid composition could not be determined, the total number of carbons and double bonds is indicated. The first number indicates the amount of carbon atoms in the fatty acid molecule, followed by the number of double bonds. For further details, see the Methods section." In the methods section we cite the original references.

3) For Fig. 3, "et rel" again is horrible narrow jargon and google.com shows it is used by microbiologists and USA government legal courts in different meanings. Which is intended here? Say it in words. Better of course is to use precise words and not confusing jargon.

We apologize for the confusion associated with this term, which indeed is horrible but we can not avoid it. It is consistently used in relation to the bacterial phylogeny discussed in this paper and unfortunately was not explained in the manuscript. We have now added the following sentence to Methods section to clarify the meaning: "Genus-level taxa with ≥90% sequence similarity in the 16S rRNA gene are referred to as *Species* and relatives, the latter being shortened in the text as "*et rel*." (Rajilić-Stojanović *et al.* 2009)."

"Cross plots" is again jargon rather than language. And any author with a sense of statistical meaning would be impressed with the "scatter" of data in Fig. 3A and B, and so "highly correlated" is a poor choice of words. There is a significant correlation and you can calculate an R value for this, but mostly there is wide scatter of the data. Of course we do not know what TG54:5 means. [TG is measured in our doctors' offices.]

We agree and have now clarified the caption of Figure 3 as follows: "Association between *Ruminococcus gnavus et rel.* and serum triglyceride (TG) lipids. The relative amounts of *R. gnavus et rel.* were quantified by the HITChip analysis and the triglyceride concentration was determined based on two independent techniques: **A** the triglyceride TG(54:5) (see Fig. 2 for explanation) by mass spectrometry (r=0.61); **B** triglyceride by an enzymatic assay (r=0.60)."

Much the same is for Fig. 4 with a lot of scatter more than a "positive association" being most visible and we do not know what ChoE920:5) is, although LDL is familiar.

We have now removed the word "positive" from the title. The revised caption for Figure 4 is as follows: "Association between *Collinsella* spp. and serum cholesterol. The relative amounts of *Collinsella spp.* were quantified by the HITChip analysis, and serum cholesterol levels were determined by two independent techniques: **A** Cholesterol ester ChoE(20:5) (see Fig. 2 for explanation) by mass spectrometry (r=0.59); **B** low-density lipoprotein (LDL) cholesterol by enzymatic assay (r=0.57)."

4) The problems continue with the Tables. "Pearson correlation" is unfamiliar to most journal readers, most microbiologists and most "omics" researchers. It is in fact a measure of the scatter r that we are not given for Figs. 3 and 4.For Table 1, please say what those times are, 1 hour, 1 week or 1 month? "Between subjects can be said once; it is not said 3x? And the4 correlations all look quite tight with small scatter.

Thanks for the suggestions. We have now added the scatter r in the captions in Figures 3-4, polished Table 1 and rephrased the caption for Table 1 accordingly: "Stability of microbiota and lipid profiles in the probiotic and placebo groups. We determined the similarity (expressed as Pearson's correlation) both within and between the time points (TP) for the microbiota and lipid profiles by the average scatter r of the profiles. Lipid data is available for the first two time points (TP1 and TP2, three weeks before the intervention and during the intervention, respectively), and not available (-) for the third time point (TP3) measured three weeks after the intervention.". To improve the readability we have also increased the size of the data points in the correlation plots (Figures 3-4).

4) Table 2 is again a Table that cannot be read by intended readers and has the sole purpose of allowing the authors to get something that can not be understood on record. Are these)probably) Pearson correlations again? -- we are not told. The values are a bit lower than in Table 1, and some correlations are negative.

We have now clarified the Table 2 caption as follows: "Associations between genus-level bacterial groups and enzymatically determined lipids. Associations between the relative amounts of genus-level bacterial groups as deterimend by the HITChip analysis and the serum lipid concentrations are quantified with a biweight midcorrelation. Only significant positive and negative correlations are shown ($q<0.05$; otherwise '-'). Abbreviations: Total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride (TG). Correlations between genus-level bacterial groups and mass spectrometry-determined lipids are provided in Supplementary Table S3."

Bacteriodes would be "genus level" and not two separate subgroups listed with species "et rel", which is not defined. Please try again. Of course, we all wish the authors would do their jobs carefully, so that outside peer reviewers can deal more with larger issues (a they have here with enthusiasm) and editors can not try to substitute for statisticians et rel. Good luck.

This is an inexact interpretation and we apologize for the confusion. We provide data on the genus-level groups, indicated here as for instance *Bacteroides plebeius et rel*. and *Bacteroides vulgatus et rel*. However, to support the reader in understanding their phylogenetic position, we also indicate the phylum level, which for these bacterial groups is that of the Bacteroidetes. Please note that also *Tannerela et rel.* are listed, which also belong to the phylum Bacteroidetes. Our reaction to comments #3 is addressing this and hopefully eliminates the confusion.

Reviewer 1 (Shira Doron)

Basic reporting *This is a well written study by experienced investigators in the field demonstrating several findings: First, the absence of an impact of LGG administration on the fecal microbiota, as measured by HITChip and quantitative PCR, with the exception of the Lactobacillus quantities. Second, the absence of an impact of LGG on serum lipid profiles. Third, the correlation between certain bacterial phylotypes and lipid classes. The negative findings support those of previously published studies and the positive findings are hypothesis generating and should lead to further research.*

Thanks for the encouraging comments.

Experimental design *Here I have only one suggestion, and that is to clarify whether subjects were instructed not to consume store-bought probiotics during the study period, whether subjects were questioned on compliance with the intervention and with abstinence from store-bought probiotics, and to clarify not just the average excretion of LGG in the placebo group, but the range, so that the reader can ascertain whether the treatment and placebo groups were indeed distinct in their exposure to probiotics.*

Thanks for the suggestion. To answer these questions, we have now clarified the compliance and exposure to probiotics in more detail in the Methods section as follows: "No other probiotic-containing products were allowed three weeks prior or during the intervention; a list of fermented foods and commercial probiotic-containing products was given to the subjects. The subjects were not questioned about their abstinence from probiotic products, but they filled a study diary recording the daily intake of the study product." In the beginning of the results part, both average and standard deviation of the excretion of LGG is given for both treatment groups.

Validity of the findings *Because most of the latest studies in this field are using pyrosequencing technology, some discussion of how these results might be expected to differ or be the same if that technology had been used of HITChip instead would be very helpful.*

Thanks for the suggestion. We have now added the following sentences in Discussion: "While good correspondence between the HITChip and pyrosequencing studies have been previously demonstrated, the main advantage of the HITChip microarray compared to the pyrosequencing studies is that the microarray technology provides very standardized and cost-efficient tools for deep and reproducible analysis of intestinal microbiota, including phylotypes that are only present in low concentrations (Claesson et al., 2009; Salonen et al. 2012). We expect that our major findings, the associations between specific lipid species and bacteria related to *Collinsella* or *R. gnavus c*ould have been detected also in a standard pyrosequencing study due to the relatively high abundance of these organisms. However, the associations involving less abundant taxa (Bacilli, Proteobacteria, *Actinomycetaeae*) would likely have been missed with conventional sequencing depth."

Reviewer 2

Basic reporting *The authors present a two compelling study that demonstrates, firstly, that there was no impact of a probiotic containing "L. rhamnosus GG" on the diversity and composition of the native gut community or the blood chemistry of 11 individuals versus 14 individuals given a placebo; secondly, the authors go onto to explore potential correlations between the microbial taxa detected in the study and blood serum lipids. Overall the manuscript is well written and presents an interesting study. It fulfills all the requirements of the journal.*

Thanks for the encouraging comments.

Experimental design *I have one small criticism. The correlations in the second part of the paper, while based on 22 observations, are still only correlations between 2 time points. I know very well that you can generate such correlations and they do indeed turn out to be meaningful. However, in this current manuscript, as the authors themselves say, these correlations are deemed to be hypothesis generating.*

With this in mind, the authors need to statement to the methods (statistics section) and a paragraph to the discussion, which explicitly deals with the limitations of the experimental design for exploring such correlations. Even with the statistical tests applied, the correlation across 2 time points could be indicative of many other variables – the authors go so far as to suggest diet – but this is the tip of the iceberg. To get proper statistical power you would need to explore a more resolved longitudinal analysis. The authors absolutely need to expand on the limitations of their study. Especially, also toning down the rhetoric in the abstract, the final sentence of which is simply untrue "Our results suggest that several members of the Firmicutes, Actinobacteria and Proteobacteria are involved in the metabolism of dietary and endogenous lipids"

Thank you for the suggestion. This is a very relevant point and the revised sentence in the Abstract states now: "Our results suggest that several members of the Firmicutes, Actinobacteria and Proteobacteria may be involved in the metabolism of dietary and endogenous lipids."

We also have added the following two paragraphs to Discussion:

"It is important to note, however, that the present analysis is based on a Finnish cohort and data from only two time points, and does not consider causal relationships between these variables. Therefore it cannot be excluded that some of the observed correlations may be associated with the diet or other confounding variables that simultaneously affect both lipid and microbiota profiles.";

And later: "Confirmation of the findings in an independent cohort and a more thorough longitudinal analysis will be necessary to assess the effect of external variables on the microbiota-lipid correlations and their potential causal associations."

Validity of the findings *See experimental design, the findings are valid if you accept that the experimental design is a little bit strained.*

Agreed. We believe that the above statements of the limitations of the experimental design now sufficiently cover this issue.

Minor comment. *Ln 35 – 'affecting' not 'affect'.*

Thanks, fixed.