OKLAHOMA STATE UNIVERSITY

FINAL REPORT

Title of Study: Understanding how mango affects glucose homeostasis in type 2 diabetes

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INTRODUCTION

The prevalence of obesity and type 2 diabetes is increasing at alarming rates in the US and worldwide. The World Health Organization (WHO) estimates that in 2014, more than 1.9 billion adults were overweight and of these over 600 million adults are obese.[1] Diabetes on the other hand afflicts approximately 29.1 million people (9.3 % of the population) in the US alone in 2012.[2] Clearly, efforts should be directed to the understanding of obesity and its associated co-morbidities so effective prevention strategies can be implemented.

Many factors are linked to the development of both obesity and diabetes. Recent studies have shown a relationship between the composition of the intestinal microorganisms with obesity and diabetes.[3-5] The mammalian gut is colonized with diverse range of microorganisms and some bacterial species are linked to the development of obesity and can alter host metabolism.[3-5] For example, studies on mice models and in humans have provided evidence that increase in body weight was associated with less bacterial diversity and a larger proportion of *Firmicutes* and relatively less *Bacteroidetes.*[6-7] On the other hand, levels of *Bifidobacterium* significantly and positively correlated with improved glucose-tolerance and low-grade inflammation.[8] Some likely explanations by which bacterial population can mediate obesity and insulin resistance is by altering energy balance by affecting energy harvest, storage, and expenditure.[9]

Dietary manipulation can influence the composition of the intestinal microorganisms.[10-11] Fat intake and prebiotics have been shown to alter gut microflora.[10-11] High-fat diet feeding in rodents was demonstrated to change the gut microbiota in favor of an increase in the Gram negative to Gram positive bacterial ratio which can affect intestinal endotoxin levels and mucosal barrier function.[10] Prebiotics are well known to increase the population of *Bifidobacteria,* but are also implicated in the regulation of host energy homoeostasis by promoting the release of gut hormones and enhancing gut barrier integrity and/or the release of

bacterial-derived metabolites, all resulting to improve host health.[11] Additionally, metabolism of dietary phenolics by the gut microbiota can also modulate host health by altering the absorption, bioavailability, and biological activity of these bioactive compounds.[12] It can be inferred from these studies that dietary manipulations that shows positive effects in modulating body fat and blood glucose can partially be attributed to alterations in gut microbiota and/or increase microbial conversion of phenolic compounds to a more active metabolite.

Therefore, the *objective* of this proposed study is to investigate the effects of dietary supplementation of mango, some fruit rich in fiber, nutrients, and phytochemical, on gut-mediated immunity and microbiome and the corresponding changes in glucose homeostasis and body composition in a mouse model of diet-induced obesity. The *hypothesis* of the study is that diets containing mango, due to its fiber and phenolic compounds, will prevent the negative effects of a high fat diet by modulating the composition of the gut microbiota which then leads to positive effects on gut integrity, immune function, body composition and clinical parameters.

APPROACH

Sixty 6-wk old male C57BL/6 mice (Charles River Laboratory, Portage, MI) were acclimated for 1-wk and randomly assigned to one of four dietary treatment groups (n=15/group): control (AIN-93M; 10% fat kcal), high fat (HF; 60% fat kcal), and HF+1% or 10% mango (HF+1%M or HF+10%M, w/w) for 12 wk. Ripe mango (Tommy Atkins variety) was purchased from a local grocery store, peeled, and the pulp was freeze-dried, ground, analyzed for its nutrient composition and incorporated into the diet at 1% or 10% concentration by weight. The variety and doses of the mango used in this study were based on our earlier work.[14] All the HF diets were adjusted to have similar macronutrients, calcium, phosphorus and total fiber content. Mice were given access to food and deionized water *ad libitum*. Food intake was monitored three times each week and body weights were recorded weekly. Fecal samples were

collected at baseline and at the end of treatment for short chain fatty acid (SCFA) analysis. After 12 wk of treatment, mice were sacrificed and tissues were collected and various analyses were performed.

RESULTS

A. Manuscript and thesis

Ojo B, Davila El-Rassi G, Perkins-Veazie P, Clarke S, Smith BJ, Lucas EA. Mango supplementation prevents gut microbial dysbiosis and modulates short chain fatty acid production independent of body weight reduction in C57BL/6 mice fed a high fat diet. *Journal of Nutrition* (in press).

Ojo Babajide, MS student; Thesis Title: Effects of mango on gut microbial population and its impact on body composition and glucose homeostasis in mice fed high fat diet. Thesis Defense: July 2015.

B. Presentations at local and national meetings

Lucas EA. Functional food for heart health: focus on mango. *Oklahoma Baptist University, Science Club*, May 7, 2016.

Ojo B, Davila El-Rassi G, Perkins-Veazie P, Clarke S, Smith BJ, Lucas EA. Mango supplementation prevents gut microbial dysbiosis and modulates short chain fatty acid production independent of body weight reduction in C57BL/6 mice fed a high fat diet. Experimental Biology 2016 meeting, April 2016, San Diego, CA. *Babajide O chosen for the American Society of Nutrition's Young Minority Investigator Oral Competition*

Ojo B, Davila El-Rassi G, Perkins-Veazie P, Clarke S, Smith BJ, Lucas EA. Mango supplementation prevents gut microbial dysbiosis and modulates short chain fatty acid

production independent of body weight reduction in C57BL/6 mice fed a high fat diet. *Annual Oklahoma State University Research Week, Feb. 2016.*

Babajide O, Wu L, Gou X, Semkoff J, Janthachotikun SJ, Eldoumi H, Peterson S, Perkins-Veazie P, Lin D, Smith BJ, Lucas EA. Mango supplementation averts hepatic and cardiac mitochondrial dysfunction in mice fed a high-fat diet. Experimental Biology, March 2015; Boston, MA. *Chosen for the American Society of Nutrition's Emerging Leaders in Nutrition Science Poster Competition*

Babajide O, Wu L, Gou X, Semkoff J, Janthachotikun SJ, Eldoumi H, Peterson S, Perkins-Veazie P, Lin D, Smith BJ, Lucas EA. Mango supplementation averts hepatic and cardiac mitochondrial dysfunction in mice fed a high-fat diet. *OSU Research Symposium*, February 2015; Stillwater, OK.

C. Findings

Mango supplementation modulates gut microbiota in mice fed HF diet

We observed 36 significantly higher operational taxonomic unit (OTUs) in the control compared to the HF group ($P < 0.05$). Notably, one OTU belonging to the genus *Bifidobacterium* (species – *anomalis*) was 64-fold higher ($P = 0.04$) in the control compared to HF (**Figure 1A**). Additionally, nine OTUs belonging to the genus *Akkermansia* and *Ruminococcus* were at least 16-fold higher ($P < 0.05$) in the control group compared to HF (**Figure 1A**). Importantly, no OTU-level taxon belonging to *Akkermansia* or *Bifidobacteria* was significantly different ($P > 0.05$) in the control compared to HF+1%M or HF+10%M groups (**Figure 1D and 1E**).

We observed no significant changes ($P > 0.05$) in any of the OTUs belonging to presently classified bacteria genera in the HF group compared to HF+1%M (**Figure 1B**). However, in the HF+10%M group, we observed 26 significantly higher OTUs ($P < 0.05$) compared to HF (**Figure**

1C). One OTU belonging to the *Bifidobacteria* genus was 33- fold higher (P = 0.03) in the HF+10%M group compared to HF (**Figure 1C**). Similarly, one OTU of the *Akkermansia* genus was 109-fold higher (P = 0.01) in the HF+10%M group compared to HF, and this belonged to *Akkermansia muciniphila* (**Figure 1C**). Furthermore, one OTU belonging to the genus *Adlercreutzia* was 118-fold higher (P = 0.01) and four *Ruminococcus* OTUs were at least 8-fold higher (P<0.05) in the HF+10%M group compared to HF. On the other hand, two OTUs each belonging to the genus *Bacteroides* and *Parabacteroides* (phylum Bacteroidetes) were at least 8-fold lower (P < 0.05) in the HF+10%M compared to the HF group (**Figure 1C**). Despite these changes observed in OTU-level taxa, relative abundance of total bacterial genera in all the treatment groups did not reach statistical significance (P>0.05, **Figure 2**), suggesting that dietary treatments in this study selectively modulate the microbiome at the OTU level.

Mango supplementation did not reduce body and tissue weights, food intake and body composition in HF diet-fed mice

All HF-fed groups had approximately 25% higher caloric intake compared to control (P<0.0001) and the HF+1%M group had a 5% increase (P=0.046) in caloric intake than the HF group (**Table 1**). Body weights were similar prior to initiation of the dietary treatments. However, after 12 wk of treatment, there were significant differences in body weight with the HF+10%M group having a 10% and 7% higher body weight than the HF (P<0.001) and HF+1%M (P=0.006) groups, respectively. The increase in fat mass and % body fat due to HF diet was not prevented by the HF+1%M diet (P=0.61 and P=0.92 for fat mass and % body fat, respectively) and further increased (17%, P=0.004 and 7%, P=0.027 for fat mass and % body fat, respectively) by the HF+10%M. The mango fed groups had similar relative tissue weights to the HF group.

Mango supplementation had modest effect on glucose homeostasis in HF diet-fed mice

Similar to the results on body composition, both doses of mango had no effect on glucose homeostasis as shown by the glucose area under the curve from the glucose tolerance test (**Table 2**). However, plasma insulin was significantly increased by 119%, 59%, and 54% in the HF+10%M compared to control ($P<0.0001$), HF ($P=0.001$), and HF+1%M ($P=0.002$), respectively (**Table 2**). The HF+1%M group had statistically similar plasma glucagon-like peptide 1 (GLP1) to the control group (**Table 2**). Plasma gastric inhibitory peptide (GIP) concentrations were unaffected ($P=0.20$) by dietary treatments (**Table 2**).

In the pancreas, mango supplementation had no significant effect on the protein expression of the GLP1 receptor (GLP1R, $P=0.45$, **Figure 3A).** However, we observed a 33% increase ($P=0.04$) in GIP receptor (GIPR) in the HF+1%M group compared to HF (**Figure 3B**).

Mango supplementation increased both fecal and cecal SCFAs in HF diet-fed mice

SCFA analyses (**Table 3**) showed the impact of mango supplementation in modulating cecal and fecal SCFAs production. Compared to control, HF diet decreased cecal acetic (by 31%, $P=0.001$), propionic (by 32%, $P<0.0001$), isobutyric (by 35%, $P=0.008$), and isovaleric (by 26%, $P=0.001$) acids but not n-butyric ($P=0.25$) and n-valeric acids ($P=0.19$). Except for cecal propionic acid, the HF+10%M but not HF+1%M brought these cecal SCFA to the level of the control group. Similar results were seen with the fecal SCFAs. The HF+10%M further increased fecal acetic acid (27%, $P=0.036$), n-butyric (6-fold, $P<0.0001$), isovaleric (30%, $P=0.039$), and n-valeric acids (3-fold, $P<0.0001$) relative to the control group. Unlike the cecal SCFAs, HF+1%M significantly increased both fecal n-butyric ($P=0.037$) and n-valeric ($P=0.0006$) acids by at least 30% compared to the HF group.

Mango supplementation stimulated colonic Il10 gene expression independent of Gpr43

Mango supplementation had no significant impact on ileal and colonic mRNA expression of the SCFA receptor, *Gpr43* and the inflammatory peptide *Il1b and Il6* (**Figure 4A and 4B**). Colonic mRNA expression of *Il10* was significantly increased in the HF+1%M (by 70%, P=0.021) and HF+10%M (by 59%, P=0.048) compared to the HF group, an effect that was not observed in the ileum (**Figure 4A and 4B**).

Mango supplementation had modest effects on both plasma and liver lipids in high fat diet-fed mice

Plasma total- and non-HDL cholesterol was elevated due to HF feeding and mango had no reducing effect (**Table 4**). No significant change was observed with mango supplementation on plasma triglycerides (P=0.12) and plasma NEFAs (P=0.10) (**Table 4**). The HF and mango supplemented groups had 2-fold increase in liver triglyceride compared to the control (P<0.05). Finally, mango supplementation had no effect on plasma adipokines.

CONCLUSION

This study demonstrates that despite the inability of mango to prevent body weight gain, fat accumulation, glucose intolerance and dyslipidemia induced by a HF diet, it modulates gut bacteria in favor of the beneficial *Bifidobacteria* and *Akkermansia* and enhanced SCFA production. The results also show that mango supplementation in mice fed a HF diet improves insulin secretion possibly via the action of incretins and enhanced anti-inflammatory cytokine production in the gut. These results imply that mango supplementation in HF feeding may be useful in modulating some of the adverse effects that accompanies HF diet-induced obesity.

Table 1: Body and relative tissue weights, energy intake and body composition in C57BL/6 mice fed a control diet or a high fat (HF) diet containing 0%, 1% or 10% mango for 12 wk [1]

	Control	HF	HF+1%M	HF+10%M	P value
Energy intake *(kcal/d)*	11 ± 0.20^{c}	12 ± 0.20^{b}	13 ± 0.20^{a}	13 ± 0.20^{ab}	<0.0001
Body weights					
Initial *(g)*	21 ± 0.68	21 ± 0.61	21 ± 0.63	21 ± 0.60	0.99
Final *(g)*	31± 0.67^{c}	39 ± 1.2^{b}	40 ± 1.2^{b}	43 ± 1.2^{a}	<0.0001
Relative tissue weights *(mg/g body weight)*					
Liver	41 ± 1.0^{a}	35 ± 1.0^{b}	34 ± 1.3^{b}	36 ± 2.9^{b}	0.032
Cecal tissue	2.2 ± 0.090^{a}	1.7 ± 0.067^{b}	1.5 ± 0.046^{b}	1.6 ± 0.074^{b}	<0.0001
Abdominal fat	33 ± 2.6^{b}	62 ± 4.1^{a}	61 ± 2.5^{a}	62 ± 6.3^{a}	0.0008
Pancreas	5.0 ± 0.44^{a}	4.2 ± 0.26^{ab}	3.6 ± 0.31^{b}	3.4 ± 0.32^{b}	0.024
Thymus	1.8 ± 0.14	1.6 ± 0.076	1.6 ± 0.11	1.6 ± 0.032	0.64
Body composition					
Lean mass *(g)*	22 ± 0.36	24 ± 1.0	24 ± 0.70	24.7 ± 0.81	0.13
Fat mass *(g)*	9.9 ± 0.49^{c}	18 ± 0.44^{b}	18 ± 0.40^{b}	21 ± 0.48^{a}	<0.0001
% body fat	31 ± 0.88^{c}	43 ± 0.47^{b}	43 ± 0.38^{b}	46 ± 0.90^{a}	<0.0001

[1]Values are mean ± SEM (n=15 mice/group). Within a row, labelled means without a common letter differ, $P < 0.05$. HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango

Table 2: Glucose parameters in C57BL/6 mice fed a control diet or a high fat (HF) diet containing 0%, 1% or 10% mango for 12 wk [1]

	Control	HF	HF+1%M	HF+10%M	P value
Fasting whole blood glucose *(mg/dL)*[2]	163 ± 5.44[c]	194 ± 12.6[bc]	233 ± 14.0[a]	207 ± 9.09[ab]	0.005
Glucose AUC *(g x min/dL)*[2]	40 ± 2.0[b]	58 ± 2.6[a]	60 ± 2.0[a]	62 ± 1.6[a]	<0.0001
Plasma insulin *(ng/mL)*[3]	0.64 ± 0.042[b]	0.88 ± 0.050[b]	0.91 ± 0.14[b]	1.4 ± 0.11[a]	0.0002
Plasma GLP1 *(pg/mL)*[3]	16 ± 0.92[c]	24 ± 2.8[ab]	19 ± 1.8[bc]	28 ± 2.4[a]	0.01
Plasma GIP *(pg/mL)*[3]	122 ± 4.20	171 ± 11.9	165 ± 16.7	161 ± 18.5	0.20

[1]Values are mean ± SEM. Within a row, labelled means without a common letter differ, $P < 0.05$. HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango.
[2]Fasting whole blood glucose and glucose area under the curve (AUC) were obtained following glucose tolerance tests after 11 wk of treatment (n=13 mice/group).
[3]Insulin, glucagon-like peptide 1 (GLP1) and gastric inhibitory peptide (GIP) were measured in the plasma at the end of the study (n=8 mice/group).

Table 3: Fecal and cecal short chain fatty acids (SCFAs) in C57BL/6 mice fed a control diet or a high fat (HF) diet containing 0%, 1% or 10% mango for 12 wk [1]

	Baseline	Control	HF	HF+1%M	HF+10%M	P value[2]
Fecal SCFAs *(umol/g)**						
Acetic acid	61 ± 8.5	37 ± 2.1^{*,b}	20 ± 4.1^{*,c}	23 ± 2.0^{*,c}	47 ± 3.1^{*,a}	<0.0001
Propionic acid	1.1 ± 0.10	0.86 ± 0.14	0.97 ± 0.21	1.2 ± 0.16	1.4 ± 0.021	0.13
Isobutyric acid	0.79 ± 0.10	0.14 ± 0.012*	0.13 ± 0.022*	0.16 ± 0.018*	0.20 ± 0.012*	0.053
n-Butyric acid	0.21 ± 0.0035	0.10 ± 0.017^{d}	0.24 ± 0.039^{c}	0.37 ± 0.037^{*,b}	0.56 ± 0.066^{*,a}	<0.0001
Isovaleric acid	0.92 ± 0.050	0.28 ± 0.020^{*,b}	0.22 ± 0.036^{*,b}	0.27 ± 0.021^{*,b}	0.40 ± 0.056^{*,a}	0.027
n-Valeric acid	0.16 ± 0.0071	0.15 ± 0.018^{d}	0.30 ± 0.018^{*,c}	0.42 ± 0.016^{*,b}	0.49 ± 0.025^{*,a}	<0.0001
Cecal SCFAs *(nmol/g)*						
Acetic acid	NA	8.4 ± 0.30^{a}	5.8 ± 0.67^{b}	5.5 ± 0.27^{b}	7.9 ± 0.018^{a}	0.0007
Propionic acid	NA	1.2 ± 0.0073^{a}	0.82 ± 0.029^{b}	0.74 ± 0.015^{b}	0.84 ± 0.0043^{b}	<0.0001
Isobutyric acid	NA	0.098 ± 0.0010^{a}	0.064 ± 0.0040^{b}	0.068 ± 0.0090^{b}	0.094 ± 0.0010^{a}	0.016
n-Butyric acid	NA	0.62 ± 0.12^{ab}	0.46 ± 0.0028^{b}	0.50 ± 0.080^{b}	0.83 ± 0.084^{a}	0.019
Isovaleric acid	NA	0.098 ± 0.0014^{a}	0.073 ± 0.0062^{b}	0.076 ± 0.0067^{b}	0.096 ± 0.0035^{a}	0.044
n-Valeric acid	NA	0.24 ± 0.026	0.20 ± 0.016	0.21 ± 0.015	0.23 ± 0.0094	0.48

[1]Values are mean ± SEM with n=15 mice/group or 11 mice/group for fecal and cecal SCFA, respectively. Within a row, labelled means without a common letter differ, $P < 0.05$. Caproic and heptanoic acids were below the limit of detection. HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango; NA, not assessed.

[2]P values are for comparison between treatment groups after 12 wk dietary treatment.

[*]Indicates significant difference ($P < 0.05$) from baseline value.

Table 4: Plasma and liver lipids, and plasma adipokines in C57BL/6 mice fed a control diet or a high fat (HF) diet containing 0%, 1% or 10% mango for 12 wk [1]

Lipid	Control	HF	HF+1%M	HF+10%M	P-value
Plasma					
Total cholesterol *(mg/dL)*	109 ± 6.39^{c}	163 ± 1.90^{ab}	155 ± 4.59^{b}	180 ± 8.14^{a}	<0.0001
Triglycerides *(mg/dL)*	41 ± 1.6	52 ± 7.4	45. ± 4.3	36 ± 1.4	0.12
HDL cholesterol *(mg/dL)*	61 ± 4.8^{b}	81 ± 1.1^{a}	81 ± 1.8^{a}	82 ± 1.7^{a}	0.0005
Non-HDL *(mg/dL)*	48 ± 2.9^{c}	82 ± 1.9^{b}	74 ± 3.8^{b}	98 ± 4.9^{a}	<0.0001
NEFA *(mEq/L)*	0.84 ± 0.045	0.81 ± 0.055	0.81 ± 0.034	0.69 ± 0.023	0.10
Leptin *(ng/mL)*	3.2± 0.66^{b}	12 ± 0.75^{a}	8.6 ± 1.6^{a}	12 ± 2.0^{a}	0.002
PAI1 *(ng/mL)*	0.19 ± 0.018^{b}	0.30 ± 0.029^{a}	0.35 ± 0.050^{a}	0.32 ± 0.040^{a}	0.041
Resistin *(ng/mL)*	31 ± 3.9^{b}	76 ± 7.6^{a}	60 ± 8.1^{ab}	65 ± 16^{a}	0.004
Liver *(mg/g tissue)*					
Total lipids	108 ± 8.01	150 ± 12.3	144 ± 10.2	169 ± 26.1	0.10
Cholesterol	3.7 ± 0.049	3.6 ± 0.18	4.0 ± 0.21	3.3 ± 0.22	0.29
Triglycerides	24 ± 5.9^{b}	61 ± 9.4^{a}	58 ± 4.6^{a}	66 ± 11^{a}	0.012

[1]Values are mean ± SEM with n=15 mice/group or 8 mice/group for plasma and liver lipids, respectively. Within a row, labelled means without a common letter differ, $P < 0.05$. HDL, high density lipoprotein; HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango; NEFA, non-esterified fatty acids; PAI1, plasminogen activator inhibitor1.

Figure 1: Bacterial genera in C57BL/6 mice fed (A) a high fat (HF) diet compared to those fed a control diet (B) HF compared to HF + 1%M, (C) HF compared to HF + 10%M, (D) control diet compared to HF+ 1%M, and (E) control diet compared to HF+ 10%M for 12 wk

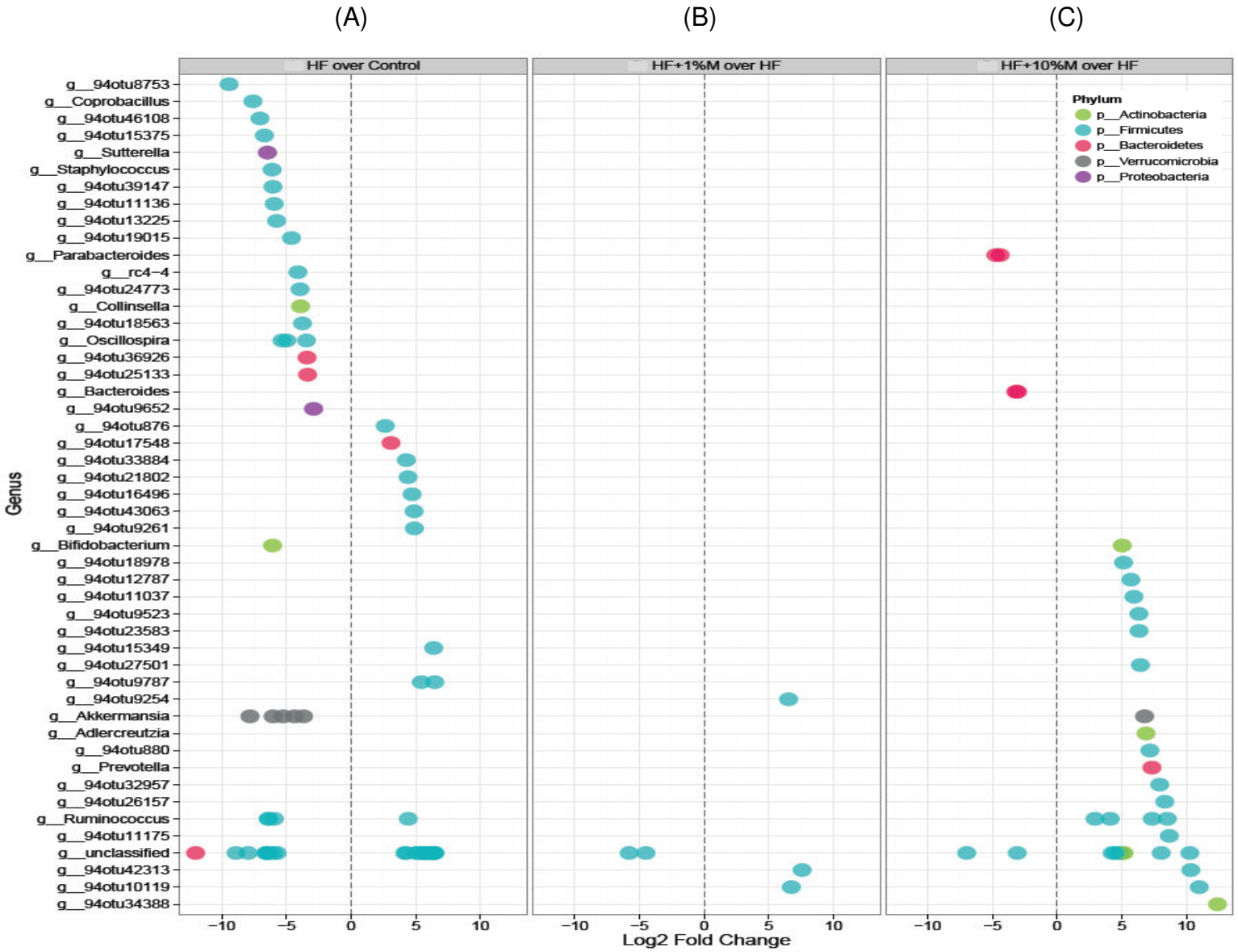
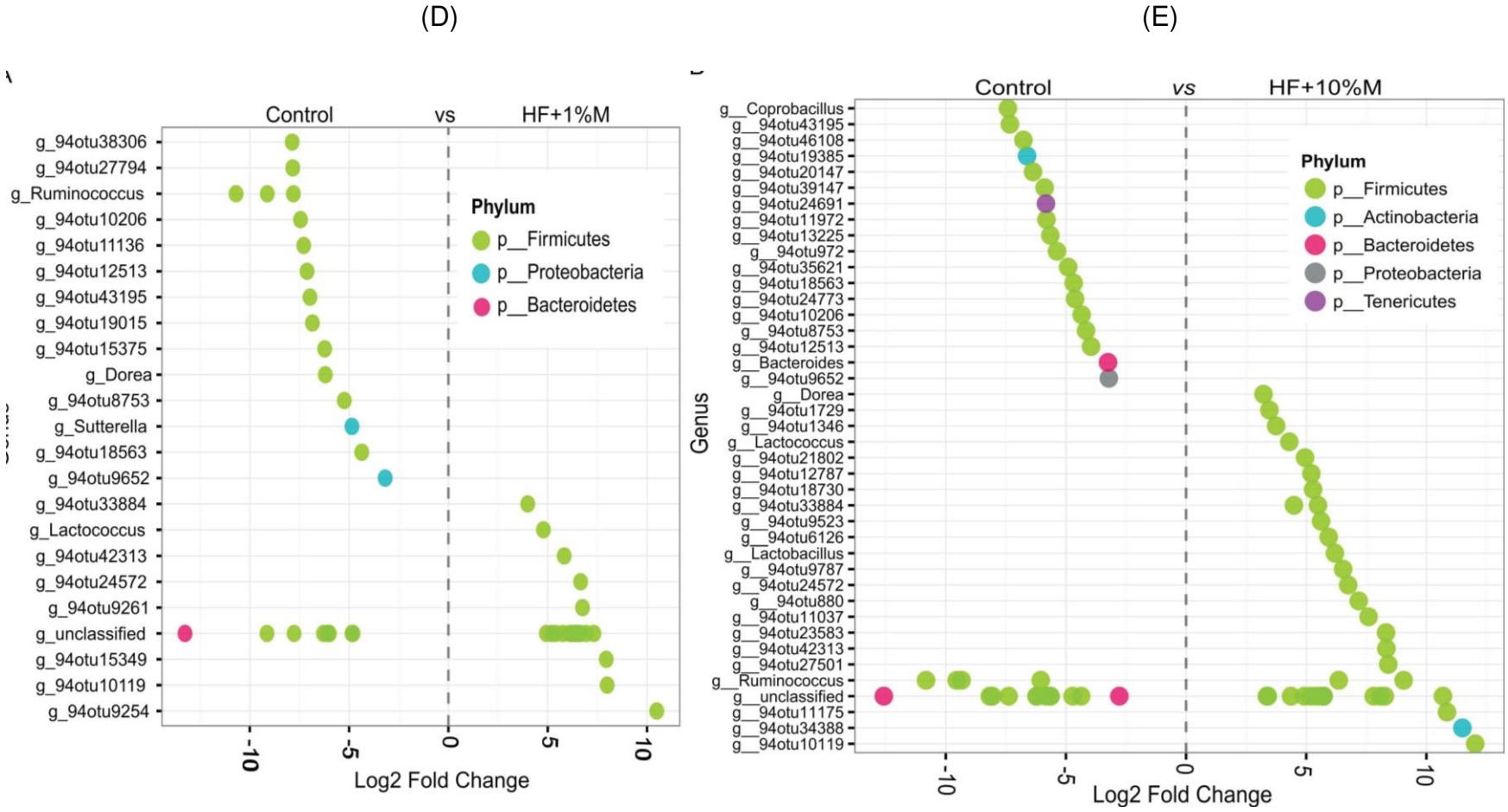
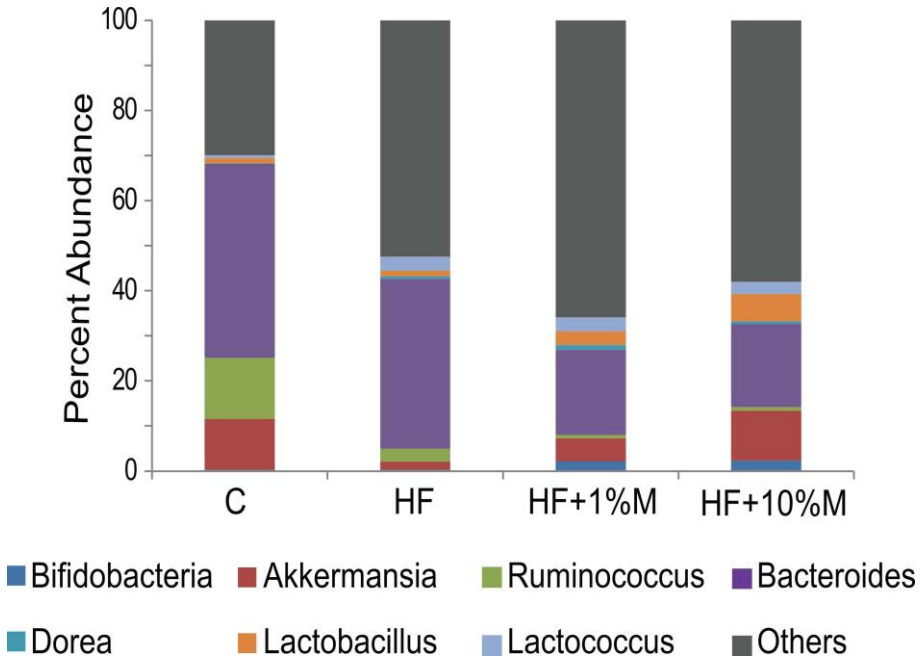


Figure 1 (Continuation)



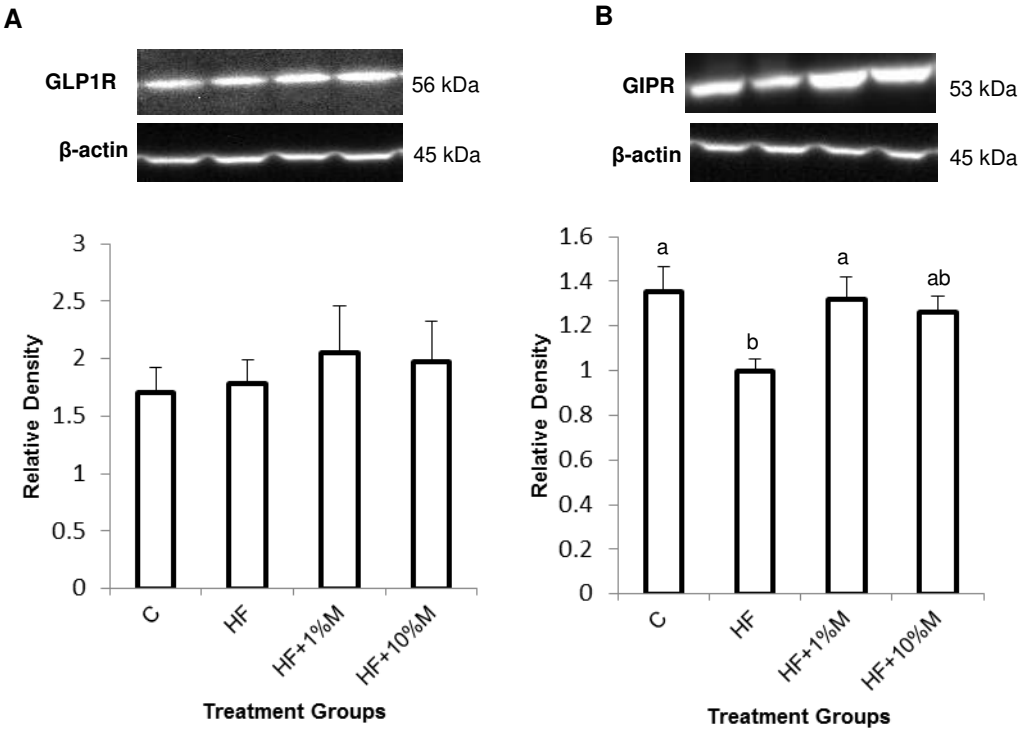
DNA isolated from cecal samples was subjected to 16S rDNA sequencing (n = 4 mice/group). Genus level changes due to dietary treatment are presented (log 2-fold changes) and points represent OTUs belonging to that genus. OTUs were considered significant if their FDR-corrected *P* value < 0.05 and the absolute value of their log 2-fold change was ≥ 1. Only statistically significant OTUs are presented. The numbered genera represent OTUs unclassified beyond the family level. FDR, false discovery rate; HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango; OTU, operational taxonomic uni

Figure 2: Relative abundance of cecal bacterial genera in C57BL/6 mice fed a control diet compared to those fed a HF diet containing 0%, 1% or 10% mango for 12 wk.



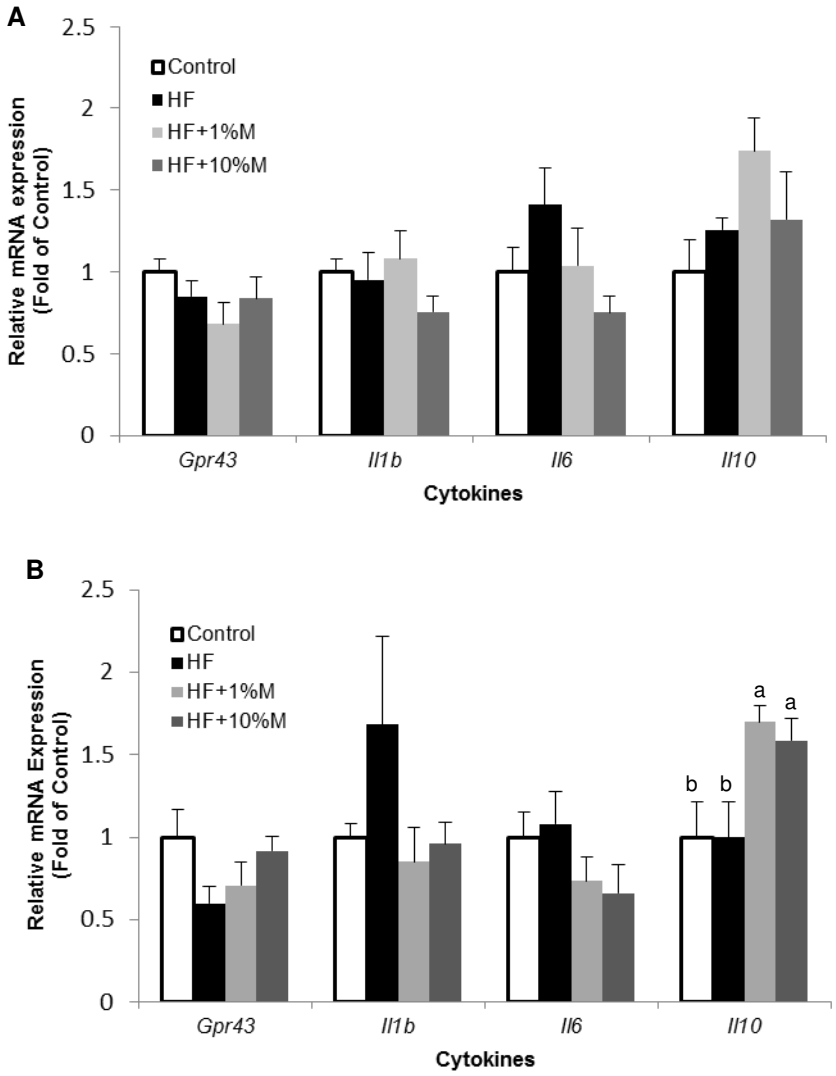
n = 4 mice/group. HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango

Figure 3: Protein expression of pancreatic incretin receptors in C57BL/6 mice fed a control diet or a high fat (HF) supplemented with 0%, 1% or 10% mango for 12 wk.



Pancreatic GLP1R (A) and GIPR (B) protein expression were determined via immunoblot analysis (normalized to β–actin) after 12 wk of dietary treatment. Data are mean ± SEM (n = 5 mice/group). Labelled means without a common letter differ, $P < 0.05$. GIPR, gastric inhibitory peptide receptor; GLP1R, glucagon-like peptide 1 receptor; HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+1% mango.

Figure 4: Gene expression of inflammatory molecules (*Il1*β*, Il6 and Il10*) and the SCFA receptor (*GPR43*) in C57BL/6 mice fed a control diet or a high fat (HF) diet supplemented with 0%, 1% or 10% mango for 12 wk.



mRNA expression of these genes were quantified via qRT-PCR in the ileum (A) and colonic lamina propria (B) after 12 wk of dietary treatment. Results are presented as relative mRNA expression (fold of control) with the qRT-PCR reactions normalized to cyclophilin. Data are mean ± SEM (n = 6 mice/group). Labelled means without a common letter differ, $P < 0.05$. GPR43, G-protein coupled receptor 43; HF, high fat; HF+1%M, high fat+1% mango; HF+10%M, high fat+10% mango; *Il1*β, interleukin 1 beta; *Il6*, interleukin 6; *Il10*, interleukin 10; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

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