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ORIGINAL ARTICLE

Photobiomodulation of the microbiome: implications for metabolic and inflammatory diseases

Brian Bicknell[1,2] · Ann Liebert[1,3] · Daniel Johnstone[4] · Hosen Kiat[5,6]

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Abstract

The human microbiome is intimately associated with human health, with a role in obesity, metabolic diseases such as type 2 diabetes, and divergent diseases such as cardiovascular and neurodegenerative diseases. The microbiome can be changed by diet, probiotics, and faecal transplants, which has flow-on effects to health outcomes. Photobiomodulation has a therapeutic effect on inflammation and neurological disorders (amongst others) and has been reported to influence metabolic disorders and obesity. The aim of this study was to examine the possibility that PBM could influence the microbiome of mice. Mice had their abdomen irradiated with red (660 nm) or infrared (808 nm) low-level laser, either as single or multiple doses, over a 2-week period. Genomic DNA extracted from faecal pellets was pyrosequenced for the 16S rRNA gene. There was a significant ($p < 0.05$) difference in microbial diversity between PBM- and sham-treated mice. One genus of bacterium (*Allobaculum*) significantly increased ($p < 0.001$) after infrared (but not red light) PBM by day 14. Despite being a preliminary trial with small experimental numbers, we have demonstrated for the first time that PBM can alter microbiome diversity in healthy mice and increase numbers of *Allobaculum*, a bacterium associated with a healthy microbiome. This change is most probably a result of PBMt affecting the host, which in turn influenced the microbiome. If this is confirmed in humans, the possibility exists for PBMt to be used as an adjunct therapy in treatment of obesity and other lifestyle-related disorders, as well as cardiovascular and neurodegenerative diseases. The clinical implications of altering the microbiome using PBM warrants further investigation.

Keywords Photobiomodulation · Microbiome · *Allobaculum* · Infrared laser

Introduction

Over the past 15 years, it has become increasingly apparent that there is an intimate association between the microorganisms in the gut and human health. The gut microbiota contributes over 10×10^9 commensal and pathogenic microbes (more than the number of cells in our body) with over 1000 species-level taxonomic units. The genetic material that these microorganisms contribute is known as the microbiome and augments our own genetic make-up. The gut microbiome is sometimes considered as an additional organ, with the microbiome composition of lean and healthy humans (and model organisms) being quite different to that of obese humans and animals. Changes in the health status of humans and model organisms is accompanied by changes in the microbiome, including phylum-level fluctuations in the dominant microorganisms and reduced microbial and genetic diversity [1]. Changes in the gut microbiome are not only correlated with obesity, but also with a wide variety of metabolic and gastrointestinal disorders, such as inflammatory bowel disease, metabolic syndrome and type 2 diabetes, and cardiovascular disease and neurological disorders [2–4], encompassing the new field of neuromicrobiology [5]. Specific mechanisms to explain the correlation between gut microbiome and human health are in their infancy.

✉ Brian Bicknell
brian.bicknell@acu.edu.au

1 Australasian Research Institute, Wahroonga, Australia

2 Faculty of Health Sciences, Australian Catholic University, North Sydney, Australia

3 Department of Medicine, University of Sydney, Camperdown, Australia

4 Bosch Institute, University of Sydney, Camperdown, Australia

5 Faculty of Medicine and Health Sciences, Macquarie University, West Ryde, Australia

6 School of Medical Sciences, University of New South Wales, Kensington, Australia

There is communication between the gut microbiome and the host cells of the immune and neuroendocrine systems, with the importance of the gut-brain axis becoming ever more apparent. The microbiome appears to regulate host metabolism at a number of levels. The gut microbiota aids in digestion of food, increases kilojoule harvest, contributes to the body's intake of micronutrients, such as B vitamins (B_3, B_5, B_6, B_{12}), aids in mineral absorption, regulates glucose metabolism and lipid storage, and influences the epithelial integrity of the mucosa [2]. The more efficient energy production with a dysregulated microbiome may be one factor in obesity [6]. The microbiome also modulates the intestinal immune system and hence the nervous system. A major means of influence of the microbiome on the host appears to be the production of short-chain fatty acids (SCFA) such as acetate, propionate, formate, and butyrate, produced by microorganisms from the fermentation of complex (plant) polysaccharides that are not digestible with human or animal enzymes (so-called 'resistant polysaccharides'). SCFAs influence the body's energy balance and inflammatory response [2, 3]. Microbial signals are thought to regulate appetite, weight gain, insulin sensitivity, peripheral lipid storage, and liver and muscle energy balance.

Dysfunction of the microbiome can lead to decreased mucosal integrity and the movement of bacteria and microbial products into the blood and liver, causing an activation of immune cells and contributing to the inflammatory response [3]. Potential products crossing into the blood include endotoxin, bacterial DNA, and inflammatory markers such as interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α). An altered microbiome can also affect lipid metabolism, glucose metabolism, protein turnover, and redox balance as well as increasing biomarkers such as HDL cholesterol, free fatty acids, fibroblast growth factor 21 (FGF-21), bilirubin, and lactate [7].

The microbiome can be altered by a number of factors. The most direct modifier of the microbiome is diet. Switching to a high fat diet, a high sugar diet, or a plant-based rather than meat-based diet can change the microbiome [3], as can kilojoule restriction, inclusion of fibre or resistant starch (prebiotics), or probiotics, such as fermented foods containing *Lactobacillus* and *Streptococcus* species. Bacteria that are known to be part of a healthy human microbiota, with increased abundance of the anti-inflammatory butyrate-producing bacteria genera *Blautia*, *Roseburia*, and *Coprococcus*, while putative pro-inflammatory bacteria present in a dysregulated microbiome include the genera *Akkermansia*, *Oscillospira*, and *Bacteroides*. Other bacteria identified as important in the microbiome include *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides uniformis*, and the genus *Prevotella* (associated with a high fibre diet) and the genus *Bacteroides* (associated with a long-term animal protein [8]. It is noteworthy that many of the bacteria suggested to be important in the functioning of a healthy microbiome are present in relatively low numbers [8]. Besides bacteria, other microorganisms present include methanogenic archaea, fungi, and bacteriophage. Mice are often used as model organisms in microbiome studies, but only 15% of their microbiota is also found in humans [9]. A healthy microbiome in mice include the genera *Allobaculum*, *Coprococcus*, *Eubacterium*, *Lactobacillus*, *Prevotella*, and *Turicibacter*, while those that are associated with a dysregulated microbiome include *Butyrivibrio*, *Oribacterium* and *Roseburia*, and *Bifidobacterium* [1, 10].

Photobiomodulation therapy (PBMt) has been used for almost 50 years to treat a range of diseases and conditions including wound healing, pain relief, arthritis, muscle and tendon repair, neurodegenerative diseases, and inflammation [11–13]. PBM is thought to act on cells by a number of mechanisms. One of the primary targets of PBM photons is believed to be the cytochrome C oxidase of the mitochondria, with the action of PBM being to increase ATP production and reactive oxygen species, which act on secondary signalling molecules to affect downstream pathways [14, 15]. In addition, it has been shown that PBM affects non-mitochondrial receptors, such as ion channels and NADP oxidase in cell membranes, as well as having a direct influence on the cellular cytoskeleton (reviewed by [16]).

PBM has been reported to reduce body fat, alter lipid metabolism and fat deposition; alter glucose metabolism and reduce the abdominal adipose tissue inflammation associated with metabolic syndrome and diabetes in mice [17, 18]; is used in body contouring [11]; and, in combination with exercise, has also been shown to differentiate fat deposits [19]. PBMt applied to remote tissues, including to the abdomen, can offer neuroprotection [20] and cardiac protection [21] and has delayed and abscopal effects on tissues and organisms [20, 22]. Thus, many of the conditions that PBMt has been shown to influence are also known to be influenced by the gut microbiome. PBMt is also known to have an inflammatory affect [23], a condition stimulated by a dysregulated microbiome [24]. The aim of the study was to determine if PBMt, delivered as low-level laser treatment, could alter the composition of the microbiome in mice.

Methods

Animal treatment

All experiments were approved by the Animal Ethics Committee of the University of Sydney (approval number: 2017/1128), and all efforts were made to minimise animal suffering. Ten and a half-week-old Balb/c mice were held in five separate cages, with four mice per group. Treatment groups were: SHAM = sham treatment; S660 = single treatment with 660 nm laser; M660 = multiple (3 times per week)

treatments with 660-nm laser; S808 = single treatment with 808-nm laser; M808 = multiple (3 times per week) treatments with 808-nm laser. Laser treatments were delivered using low-level laser (Irradia MID-LITE models 8080 and 6565). The spot size was 0.8 cm^2 for both lasers, with a 40° divergence of the laser beam and a pulse frequency of 250 Hz. The 660-nm laser was set to 75 mW and produced an average output power of 75 mW, with a duty cycle of 25% and a power density of 93.75 mW/cm^2; the 808-nm laser was set to 80 mW and produced an average output power of 83 mW, with a duty cycle of 11.25% and a power density of 103.75 mW/cm^2. The laser lenses were gently pressed against the shaved skin of the abdomen of each mouse to give a total energy density (fluence) of 10 J/cm^2. Sham treatments were identical, but with the laser diodes were switched off. Faecal pellets were collected aseptically before any laser treatment (day 0) on day 7 and day 14. Faecal pellets were held at − 50 °C until DNA extraction.

Genomics

Genomic DNA was extracted using the Powerfecal DNA extraction kit (Qiagen®), following the manufacturer's instructions. The V4 hypervariable region of 16S rRNA was amplified to target bacteria and archaea using primers 514f (5′-GTGCAGAATTGCCCTATCC-3′) and 806r (5′-GACT ACHVGGGTATCTAATCC-3′). DNA was quantified using a Qubit® Fluorometer, and approximately 10 uL/μL of DNA was sent to the Australian Genomic Research Facility for pyrosequencing using the MiSeq platform (Illumina®). A total of 10,109,487 demultiplexed paired-end reads fastq sequences were imported into Qiime2-2017.10 [25] using Casava 1.8 paired-end demultiplexed fastq and following suggestions on the qiime2 website (https://docs.qiime2.org/2017.10/). Primers and barcodes were removed, and quality was trimmed to 250 bp, and chimeras were removed using DADA2, giving a total of 3,778,822 sequences. Two thousand nine hundred fourteen representative sequences (OTUs) were built into a phylogenetic tree. Taxonomy was assigned based on Greengenes (version 13_8) at 97% OUT, trained using a Naïve Bayes classifier. Alpha and beta diversity statistics were calculated using the q2-diversity plug-in at a rarefaction of 19,000 sequence sampling depth. Alpha diversity was calculated using both the Faith Phylogenetic Diversity and Evenness metrics. Beta diversity was calculated using PERMANOVA with both Bray-Curtis and unweighted unifrac statistics. The principle coordinate analysis generated was visualised with the Emperor plug-in. Differences in the abundances of OTUs (genus-level) between treatment groups was assessed using the q2-composition plug-in to calculate the ANCOM statistic [26]. Sequences of interest were subjected to a BLASTn search to assign taxonomy (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Results

One mouse in the M660 group died before the day 14 collection and was not included in the analysis. No methanogens or other archaea were detected in any microbiome sample. The microbiomes of both SHAM and treated mice were dominated by the Bacteroides and Firmicutes phyla (Fig. 1) and, in particular, by a genus of Bacteroidales S24-7 (16.4 to 71.7%) and by a genus of Clostridiales (3.3 to 63.3%) (Fig. 2). Two unrelated samples (SHAM mouse 3 and S660 mouse 1) showed a high percentage of *Mycobacterium* species in the day 7 sample but not the day 0 or day 14 samples.

Measures of α-diversity (evenness and faith) showed no significant differences in microbiome species richness between treatments. Measures of β-diversity (unweighted unifrac and Bray-Curtis) indicated a significant difference between untreated mice (SHAM plus day 0) and all treatment groups except S660 for Bray-Curtis ($p = 0.05$) (Fig. 3, Table 1).

Using the ANCOM statistic, two significantly ($p < 0.001$) changed microbiota genera were detected (Fig. 4): one being a *Cyanobacterium* species, which showed a 100% match to a chloroplast sequence from a grass (*Miscanthus* × *giganteus*) (Fig. 4a) and the other being an *Allobaculum* sequence, which showed a 100% match to *Allobaculum stercoricanis*. This genus was present as a minor percentage of the microbiome population (< 0.002%) in untreated mice (Fig. 4b) but increased to between 0.8 and 3.5% by day 14 in mice treated multiple times with infrared light.

Discussion

To our knowledge, this is the first report in the literature that uses pyrosequencing to demonstrate that PBM can alter the gut microbiota. A previous study used culture techniques [27] to show that a 635-nm laser reduced the numbers of pathogenic gut bacteria in rats.

The chloroplast sequence was presumably present as a component of the mouse feed, and the undigested chloroplasts may have been stimulated by the action of laser light on the light-sensitive chlorophyll molecules. *Allobaculum*, on the other hand, appears to be an important component of the mouse microbiome, being decreased in mice that are obese [10] or have lost their circadian rhythm [28] and being increased with exercise, fibre intake, weight loss [1, 10], and treatment with berberine and metformin [29]. *Allobaculum* is one of the genera associated with greater mucous layer strengthening and better mucosal integrity [10].

The response of *Allobaculum* to infrared light treatment might be due to the direct effect of light on this genus of bacteria, or it might be due to the response of the mouse to the light and subsequent communication to the microbiome.

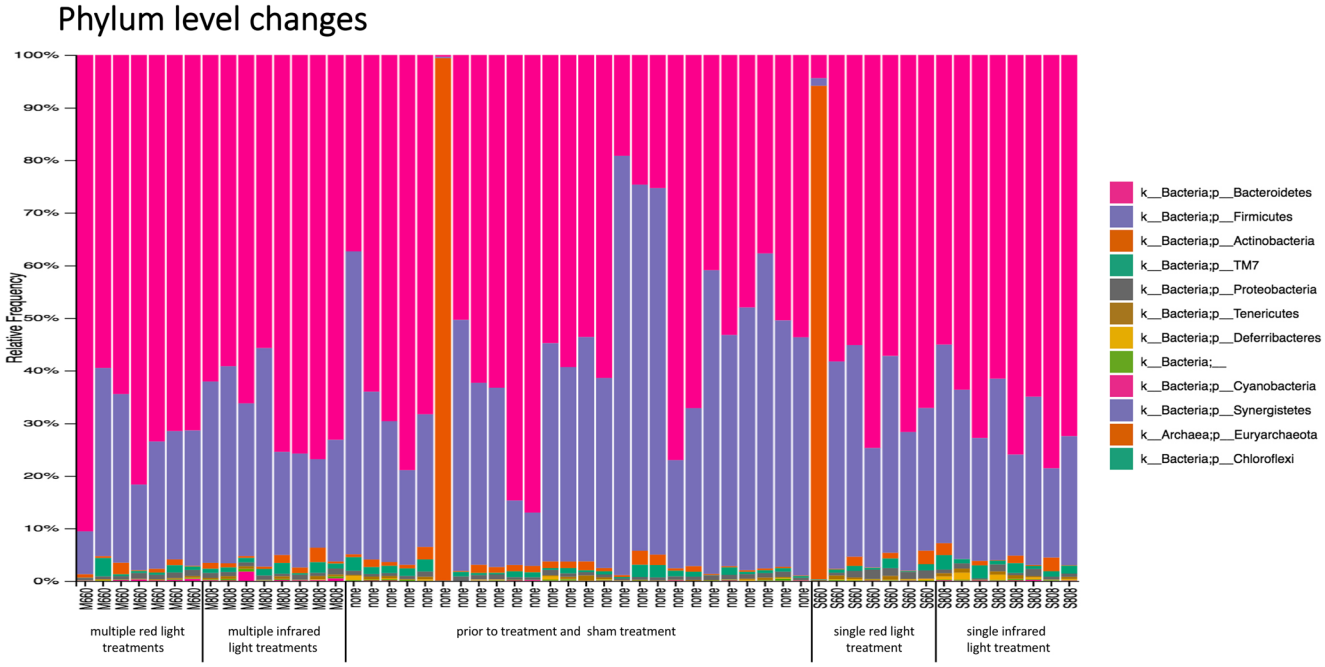

Fig. 1 Phylum-level differences between laser light-treated and laser light-untreated faecal samples. None no treatment or sham treatment, S single treatment, M multiple (3× per week) treatments

The delay in response to PBM (14 days) suggests the latter, given that rapid bacteria growth would be expected to produce a faster response. The increased effect of infrared compared to red light might be due to increased penetration or to different cellular targets of the different wavelengths [16]. At the power density used to treat the mice, it is unlikely that there is any significant heating effect [30, 31]. The possibility of a thermal effect that might have influenced the response of bacteria or cells cannot, however, be completely excluded [32].

PBM is known to affect many cellular signalling processes within the cell, including a number of markers that are also influenced by the microbiome (e.g. FGF-21, transforming growth factor beta (TGF-β), cytokines, high-density lipoprotein (HDL), and cholesterol). The possibility that PBMt can alter the microbiome implies that PBMt could represent a further safe and non-invasive treatment option for a range of diseases associated with a dysregulated microbiome, such as metabolic, neurological, and cardiothoracic diseases. Despite the small numbers of mice used in this study, and the pitfalls of extrapolating mouse microbiome results to humans [9], the potential clinical implications of the results justify further investigation, including elucidation of the mechanism of PBM influence on the microbiome (via the host).

The gut microbiome modulates a surprising number of what have previously been considered common neurological conditions and behaviours in humans and model animals, including fear-related behaviour, risk-taking behaviour, social behaviours [33], and cognition [34], as well as mood and sleep. A dysregulated gut microbiome has also been implicated in a number of metabolic diseases, including gastrointestinal diseases such as ulcerative colitis, irritable bowel syndrome and Crohn's disease [35], metabolic syndrome, obesity, type 2 diabetes, cancer, cardiovascular disease, and neurological diseases. Obesity may be influenced by the increased energy harvest from polysaccharides and increased triglyceride deposition due to the changing microbiome. A healthy microbiome also inhibits fasting-induced adipose factor and monophosphate activated protein kinase, both of which increase triglyceride deposition [24]. Mice with a humanised microbiome show an increase in weight gain, most probably due to increased energy extraction from food, and increased lipid deposition [3] and transplantation of the microbiome transfers this trait. A Western diet results in changes in the microbiome of both humans [8] and mice [1]. There is now also substantial evidence that the microbiome is a risk factor and/or has an effect in the progression of a number of neurodegenerative diseases and neurological disorders [5, 36–38], including multiple sclerosis, Parkinson's disease, Alzheimer's disease (including the oral microbiome), Huntington's disease, autism spectrum disorder, schizophrenia, anxiety, and depression in humans and rodents. It has moreover become increasingly apparent that the microbiome is associated with chronic pain syndromes, including visceral pain [39], migraine (both gut and oral microbiomes) [40], and chronic prostatitis and pelvic pain (gut and urogenital microbiomes) [41]. Many of these conditions have been shown to be modulated to some extent by PBMt [13, 42, 43].

Recently there have been a number of reports of the use of PBMt to affect abdominal fat deposition, glucose metabolism,

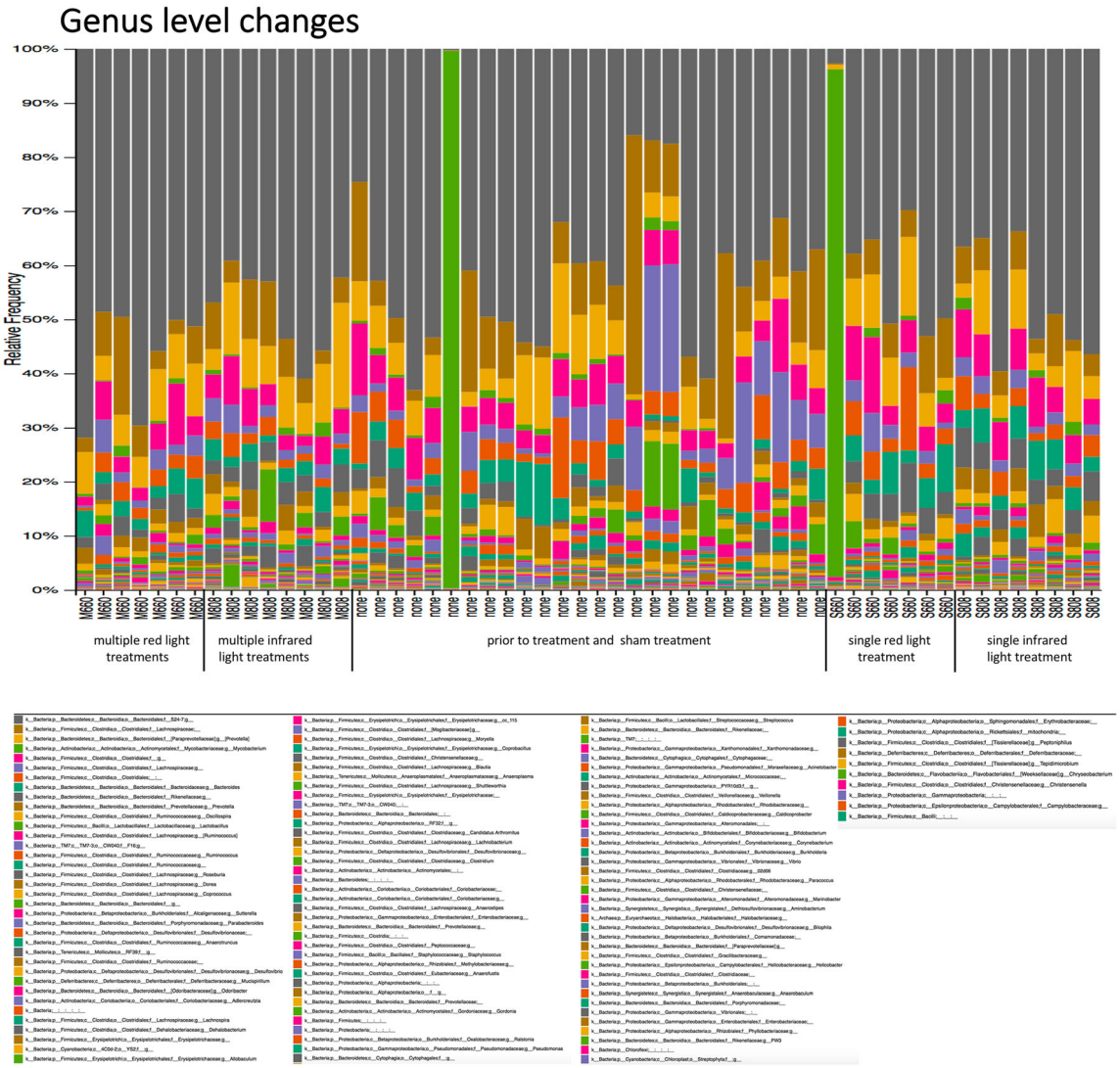

Fig. 2 Genus-level differences between laser light-treated and laser light-untreated faecal samples. None no treatment or sham treatment, S single treatment, M multiple (3× per week) treatments

and metabolic disease. PBMt combined with exercise has been shown to have a positive effect on obesity, insulin, fat deposits, and the inflammatory cytokines IL-6 and FGF-21, which can mediate differentiation of fat deposits (conversion of white fat to brown fat) [19] and to reduce cardiometabolic risk [44] in obese women. PBM has been also been shown to reduce body fat, alter glucose metabolism and insulin concentration, change lipid metabolism and fat deposition, reduce the abdominal adipose tissue inflammation [18], and effectively treat metabolic syndrome in rats [45] and mice [17]. This again demonstrates the overlapping effects on metabolic diseases of PBMt and the microbiome. Additionally, the microbiome has a strong impact on Parkinson's disease [46], as well as influences in other neurodegenerative diseases. The use of PBMt as a pre-conditioning therapy has been shown to be effective as a remote treatment against Parkinson's disease in mice [47–49], which, given the size of a mouse, would include irradiation of the abdomen. A recent study has demonstrated that targeting the gut with PBM/magnetic stimulation had a positive effect in the treatment of a mouse model of Alzheimer's disease [50], which the authors suggested could be due to a microbiome response to the treatment.

Changing the gut microbiome, by PBMt or by other means, has a direct consequence on the host, and the gut-brain axis is known to have effects on many disparate diseases. The gut-brain axis involves the sympathetic and parasympathetic nervous systems, the enteric nervous system (ENS), and the neuroimmune system. While the pathway of communication between microbiome and host is still not fully clear, a number of possibilities exist including bacterial secondary metabolites (such as SCFAs), metabolic precursors, immune signalling, vagus nerve signalling, hypothalamic–pituitary–adrenal (HPA) axis activation [34], and via the amygdala [33].

The most direct route from gut ENS to the brain is via the vagus nerve, which is both afferent and efferent and has a central function in the transmission of signals between the

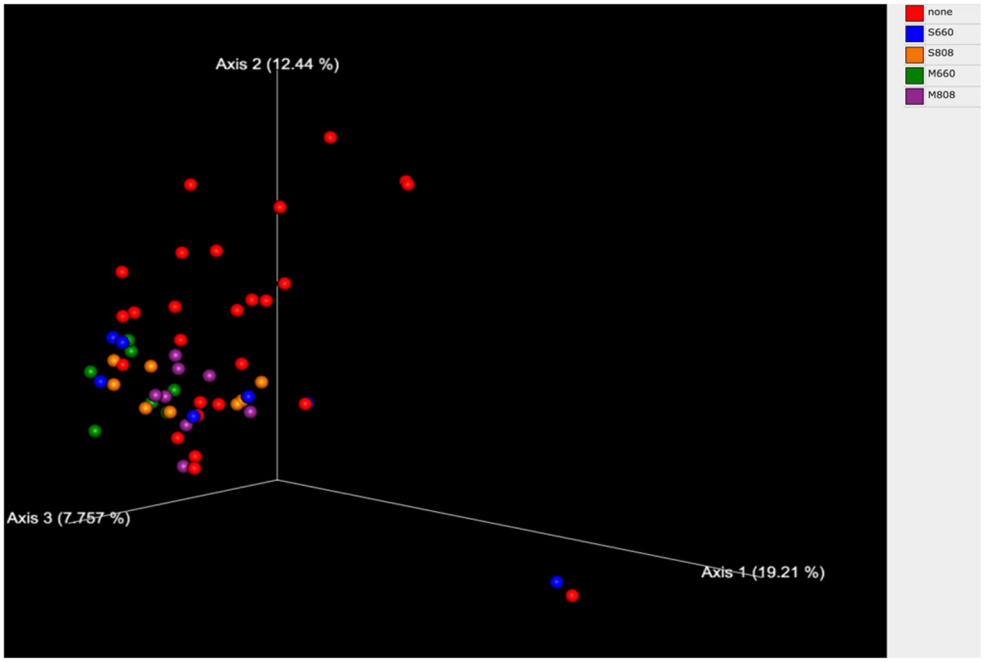

Fig. 3 Principle component analysis plot of the Bray-Curtis statistic, comparing treatment groups

gut and the brain. The microbiota in the gut may directly stimulate the ENS to signal the brain via the vagus nerve.

Bacteria in the gut are known to produce neurotransmitters identical to human molecules, which can cross the intestinal mucosa, such as serotonin (by *Candida*, *Streptococcus*, and *Enterococcus*), gamma-aminobutyric acid (GABA) (by *Lactobacillus* and *Bifidobacterium*), dopamine (by *Bacillus*), acetylcholine (by *Lactobacillus*), and norepinephrine (by *Bacillus* and *Saccharomyces*) [51]. Other metabolites include SCFAs, tryptophan precursors, and catecholamines, which can have both local and remote targets, epithelial cells, the ENS, and/or the vagus nerve. It appears that many circulating metabolites originate from the gut microbiome [52].

The microbiota produces metabolites that may be beneficial (such as SCFAs) or deleterious (such as lactic acid and ammonia) to host cells. SCFAs help maintain epithelial integrity and produce a strong mucous layer in the gut. Butyric and propionic acids inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and may improve insulin sensitivity. SCFAs also have longer range signalling effects, activating G-protein-coupled receptors and inhibiting histone deacetylases, which regulate epigenetic expression.

The leaking of microbial metabolites, products, or entire microbes from the intestinal lumen into the tissues will set up immunological reactions. While low levels of these may be the norm, high levels of endotoxin (bacterial outer membrane lipopolysaccharides) may initiate a stronger inflammatory processes associated with type 2 diabetes and obesity [24]. A dysregulated microbiome increases gut permeability, leading to an inflammatory response and further reducing the integrity of the gut barrier, allowing the increased movement of microorganisms from the gut and leading to further (systemic) inflammation [53]. Chronic inflammation, which changes homeostasis, appears to be the basis of not only

Table 1 Pairwise Permanova analysis of β-diversity between faecal samples of untreated and laser light-treated mice. *None* no treatment or sham treatment, *S* single treatment, *M* multiple (3× per week) treatments

Treatment	Treatment	Bray-Curtis *p* value	Unweighted unifrac *p* value
None	S660	0.109	0.155
	M660	0.012	0.054
	S808	0.010	0.007
	M808	0.006	0.003
S660	M660	0.002	0.028
	S808	0.015	0.040
	M808	0.002	0.003
M660	S808	0.001	0.001
	M808	0.004	0.003
S808	M808	0.001	0.008

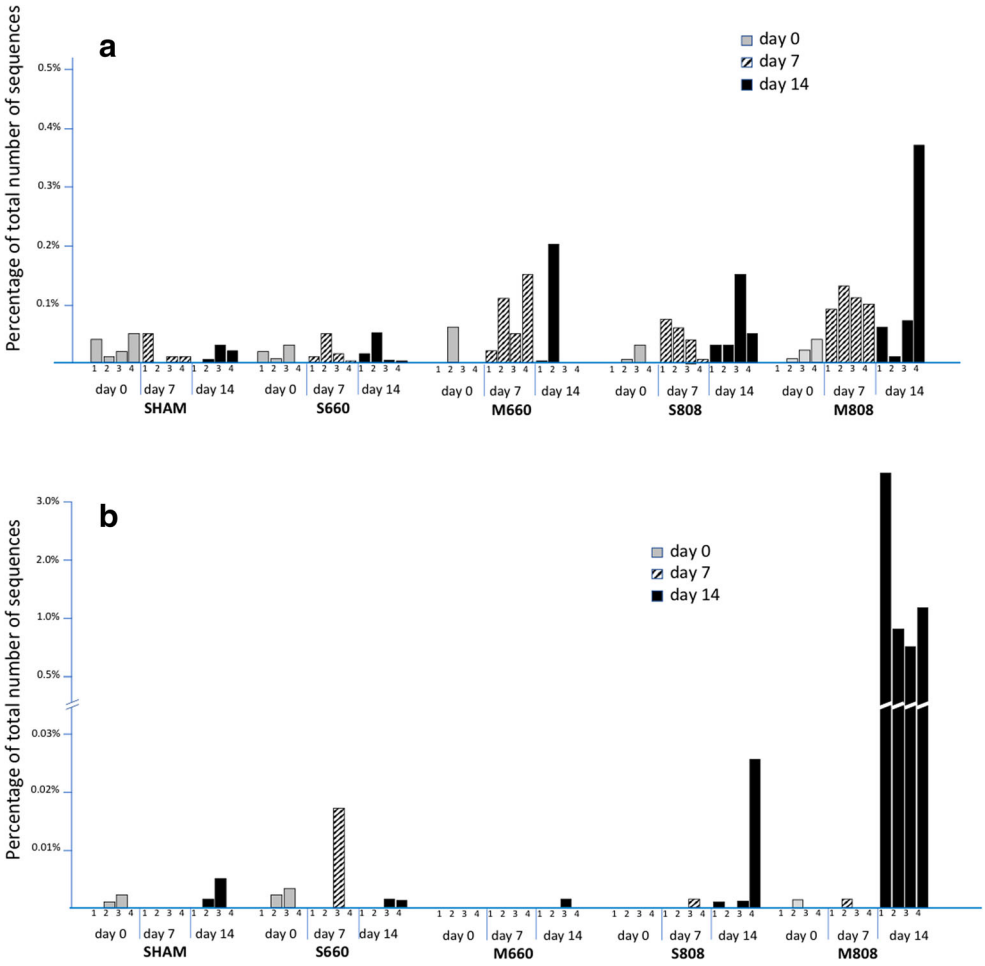

Fig. 4 Changes in sequences in individual mice as a percentage of total microbiome sequences. **a** Sequence corresponding to the chloroplast of a grass (Miscanthus × giganteus) with a BLASTn search. **b** Sequence corresponding to *Allobaculum stercoricanis* with a BLASTn search. SHAM sham treatment; S660 single treatment with red light laser (660 nm); M660 multiple treatments (3 times per week) with red light laser (660 nm); S808 single treatment with infrared light laser (808 nm); M808 multiple treatments (3 times per week) with infrared light laser (808 nm)

metabolic syndrome, but other metabolic, cardiac, and neurological diseases and disorders [54]. A dysregulated gut microbiome is known to be involved in hypertension, atherosclerosis, and cardiovascular disease, including heart failure, due to the persistent systemic inflammation and particularly the circulating trimethylamine N-oxide [4], produced by the gut microbiota metabolism of red meat and egg yolk [55]. Consumption of red meat has also been shown to be significantly correlated with oxidative stress in healthy individuals [56]. In patients with Parkinson's disease, there is a decrease in bacterial genera associated with lowering inflammation (*Blautia*, *Roseburia*, and *Coprococcus*—all SCFA-producing), while pro-inflammatory genera (e.g. *Ralstonia*) are increased. It also appears that a particular microbiome is necessary for the production of the α-synuclein present in Parkinson's disease, as revealed by experiments transplanting human microbiome into α-synuclein susceptible mice [38].

The communication between microbiome and the body is bidirectional, with not only the microbiome affecting the host, but with the host influencing the microbiome. This can be seen in the transmission of key microbiota from the maternal microbiome, in the effect of birthing procedures (caesarean vs vaginal) and in the effects of stress, diet change throughout infancy and adulthood [57], antibiotic use, exercise, and alcohol [58]. There does not appear to be strong effect of genetics (twin studies) although a number of loci were shown to affect the microbiome in a metagenomics analysis of 1514 human subjects [59] (such as genes for immune function, food metabolism, lipid storage), and there appears to be some effect of individual (mostly immune system) genes, such as the MEFV that encodes for pyrin (Mediterranean Fever gene) and the leptin-encoding gene that regulates hunger [60].

Diet has the strongest influence on the microbiome and a changed diet (e.g. high fat, high sugar, plant-based, and meat-based) can change the microbiome within a single day [3], although long-term changes may take months or years [8]. The composition of the microbiome is also affected by prebiotics and probiotics, as well as by drugs such as antibiotics and metformin [29]. The possibility of using diet, prebiotics or probiotics, drug interventions (such as berberine and metformin [29]), or faecal transplants in order to influence the gut microbiota is a therapeutic option that has gained acceptance and is being actively pursued for many metabolic diseases. Faecal transplants are currently being used for *Clostridium*

difficile infection and irritable bowel syndrome and are also being considered for non-intestinal metabolic diseases. PBMt may offer an additional therapeutic option to target the microbiome.

There are a number of potential signalling mechanisms that would allow communication from host to microbiome and thus form a pathway for the indirect effect of PBM on the microbiota: (1) Brain-gut signals via the vagus nerve can influence gut motility and mucin secretion, both of which will affect the microbiome [52]. (2) Immune responses can alter cytokine production in the mucosal immune system and, so, also affect the microbiome. (3) Stress is known to affect the establishment of the gut microbiota in young animals, and stress in adulthood alters microbiome composition, both of which argue for a brain-to-gut regulation of the microbiome. Stress will also activate the HPA axis, leading to an increased gut permeability, increased movement of microbes, and microbial products through the gut wall, triggering an increased immune response [57]. (4) Host-produced signalling molecules, such as catecholamines, serotonin, GABA, and cytokines, can be released into the gut lumen. The effect of these on the microbiome is unknown, although the effect of catecholamines on pathogens has been well studied [52]. Melatonin is another potential microbiome regulator, being highly produced in the gut [61], integral to the immune-inflammatory axis and maintaining the integrity of the intestinal barrier, especially under stress and dietary changes [62]. (5) The gut microbiome is also known affected by host hormone (steroid) levels [51].

The mechanism of the effect of therapeutic light on the microbiome in this study is unknown and deserves further investigation. One possibility is the role of PBMt in reducing inflammation as well as pro-inflammation cytokines, such as IL-1β, IL-6, and TNF-α [13, 23, 63], which may prompt the body to signal to the gut to produce a positive change in microbiome composition. In addition to the well-known inflammatory pathways influenced by PBM, it may be worth considering the kynurenine pathway, which is important in inflammation, cardiovascular disease [64], the blood-brain-barrier [65], autoimmunity, and neurological diseases [66]. PBM has been shown to modulate serotonin [67], which forms a parallel pathway to the kynurenine pathway using the precursor tryptophan. The kynurenine pathway is also influenced by the gut microbiome [68], partly through regulation of tryptophan availability. The effect PBM on the microbiome would be expected to be more pronounced if the mice that were treated were obese, were fed a Westernised diet, or had some other inflammatory condition.

Light has a modulating effect on all types of organisms from bacteria to humans. In humans, this can be seen as increased depression in winter (seasonal affective disorder—SAD), in neonate jaundice, in the healing of wounds, in the increase in cardiac events after clock changes for daylight saving, in the latitude and month of conception for multiple sclerosis risk, and in the progression of Alzheimer's disease in nursing homes with and without natural light [16]. Light can be in the form of naturally occurring light or therapeutic applications such as bright light therapy for SAD, blue light for bilirubin, UVB for multiple sclerosis therapy, and PBMt (laser and LED). There are also reports that light can have an indirect influence on the microbiome, via circadian rhythms. Deletion of the *Bmal1* clock gene in mice results in changes to microbiome composition [28]. There is also an interaction between circadian rhythm and obesity, with diet influencing circadian rhythm and changing rhythm leading to obesity and metabolic syndrome [69]. Each organ has its own daily oscillations, and the liver's circadian rhythm is also related to food intake. The gut microbiome controls nutrient absorption and the rhythm and amplitude of daily oscillations in the liver and intestine, which in turn regulates clock genes and biomarkers such as HDL cholesterol, free fatty acids, FGF-21, bilirubin, and lactate [7]. The circadian rhythm of the liver, as well as the timing of food input, imparts a diurnal rhythm to the microbiome [70].

Conclusion

It has been demonstrated for the first time that PBM delivered as low-laser light to the abdomen of healthy mice can produce a significant change in the microbiome. PBM significantly altered the microbial diversity of the microbiome, an effect most pronounced in mice treated three times per week with infrared light and not apparent with a single treatment of red light. PBM also significantly increased the percentage of the beneficial bacterium *Allobaculum* in the microbiota of mice after 14 days of treatment with infrared (904 nm) light. The alteration of the microbiome was most probably due to a secondary effect of the PBM altering the mouse inflammatory response and in turn affecting the gut microbiota. It is hypothesised that this effect may be due to the effect of PBM on inflammation. The alteration of mouse microbiome after light treatment suggests that this may also occur during a number of PBM therapies, including treatment of the abdomen for obesity and metabolic diseases and (possibly) the abscopal treatment for cardiothoracic and neurological conditions. This raises the prospect of the using PBMt as a safe and non-invasive adjunct therapy to promote changes in the microbiome for a number of inflammatory and neurological diseases, such as Parkinson's disease and cardiovascular disease.

This experiment is preliminary and needs to be repeated. The number of mice in each treatment group was small (4), and the method carries the inherent biases of 16S rDNA sequencing with primers. In addition, the assumption that faeces samples are representative of the microbiome ignores

the population of microorganisms more firmly adhering to the intestinal mucosa, potentially important in the microbiome.

The treatment of obesity, its related metabolic diseases (such as metabolic syndrome), neurological disorders, and neurodegenerative and cardiovascular diseases require new strategies to alter homeostatic mechanisms. If it is indeed possible to use PBM to affect the microbiome, we may have another potential therapy to target these problematic diseases and, so, decrease systemic inflammation and its sequelae. Alterations to the microbiome would improve host redox balance, lipid metabolism, and protein turnover and result in many beneficial downstream effects. If confirmed by future experimental and clinical studies, this tool may complement other strategies, such as diet and exercise, to prevent and treat lifestyle-related diseases.

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Author contribution BB, AL, and DJ—design and implementation the study; BB and DJ—acquisition of data; BB—analysis and interpretation; BB, AL, and HK—drafting of manuscript; all authors—revision and approval.

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Compliance with ethical standards

Conflict of interest BB is an agent for Spectro Analytic Irradia AB, the company that manufactures the Irradia laser products supplied for this experiment. The other authors declare that they have no conflict of interest.

Ethics approval All experiments were approved by the Animal Ethics Committee of University of Sydney (Protocol Number: 2017/1128).

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