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Beneficial Dog Bacteria Up-Regulate Oxytocin and Lower Risk of Obesity

Bernard J Varian[1], Tatiana Levkovich[1], Theofilos Poutahidis[1,2], Yassin M Ibrahim[1], Alison Perrotta[3], Eric J Alm[3,4] and Susan E Erdman[1]*

[1]*Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, United States of America*
[2]*Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece*
[3]*Biological Engineering, Massachusetts Institute of Technology, Cambridge, United States of America*
[4]*Broad Institute of MIT and Harvard, Cambridge, United States of America*

Abstract

Cohabitation with pet dogs imparts diverse health benefits to humans including a slim physique. It is known that neuropeptide hormone oxytocin fundamental in human-canine social bonds regulates appetite and body weight. It was recently shown in mice that consuming *Lactobacillus reuteri* ATCC 6475 from human breast milk lowers body weight and up-regulates oxytocin levels in blood. Here we test the hypothesis that bacteria from dog saliva may similarly modulate recipient host body weight. We find that a *Lactobacillus* spp isolate from dog saliva led to lower body weight when fed to C57BL/6 wild type mice. Mice consuming the canineborne *L. reuteri* also had elevated oxytocin levels in blood plasma, and exhibited body weight in an oxytocin-dependent manner. Interestingly, killed (lysed) canine bacteria were sufficient to achieve the physiological effects. Taken together, these studies provide evidence that dog bacteria modulate oxytocin levels and body weight in recipient mice, and thus may help reduce risk of obesity in individuals cohabitating with pet dogs.

Keywords: Weight; Canine; Microbe; *Lactobacillus*; Model

Introduction

Pets are often considered to be part of the family. Cohabitating with dogs, in particular, has many benefits to humans associated with physical, psychological and social wellbeing [1]. One benefit of human co-habitation with dogs is a more slender physique [2]. This observation is important in the context of the growing obesity epidemic, which has impacted over 78 million adults and 12.5 million children and adolescents in the U.S. in 2009-2010 [3]. In the face of this growing obesity epidemic [3], there is relative inefficiency of existing weight loss strategies. Although a clear relationship between dog ownership and lower risk of obesity exists, study designs have been unable to show a specific link with the hypothesis of increased activity levels due to humans taking more walks or playing with pet dogs [4]. Alternatively, environmental exposures involving pet dogs have been proposed. An understanding of mechanisms through which environmental factors influence obesity is important to develop future interventions [5]. One possible link between dogs and humans is exchange of microbiota. The close cohabitation of dogs and humans may facilitate the transfer of various infectious agents between these species. Lower prevalence of allergic diseases among those living on farms or with pets during childhood support this concept [6-13]. Indeed, this idea coined the 'hygiene hypothesis' theory is based on associations between the decrease in beneficial microbial burdens and the increase with allergies, autoimmune disease and generalized immune dysfunction in modernized societies. A relevant study showed that household dogs may disseminate *Lactobacillus johnsonii* in household dust that lower risk of asthma and other inflammatory disorders in cohabitating humans [14].

The neuropeptide hormone oxytocin is pivotal in the canine-human bond, with studies showing that humans experience higher levels of oxytocin during interactions with pet dogs [15,16]. Importantly, oxytocin has also been convincingly linked with protection from obesity [17-23]. While the nonapeptide oxytocin is historically recognized for its role in parturition [24] and lactation [25] it has gained more recent attention for its apparent effects on prosocial behavior [26,27] and therapeutic potential in the treatment of autism spectrum disorder (ASD) [26,27], schizophrenia [26,28] and obesity [17-23]. A large number of ongoing investigations in humans list oxytocin as the focus in studies on caloric intake, gastric emptying, or obesity, as displayed in the ClinicalTrials.gov registry, National Institutes of Health. Specifically, studies show oxytocin has roles in reducing food intake and body weight in diet-induced obesity [17,19,21-23] in genetically obese rodent models [18,20,21], highlighting potential downstream CNS and peripheral mechanisms. It was also shown that intranasal administration of oxytocin in humans lowers caloric intake and has beneficial metabolic effects, resulting in a shift from carbohydrate to fat utilization and improved insulin sensitivity [29]. Recognizing that oxytocin is important in the mother-infant bond, it was earlier found that ingested *L. reuteri* ATCC 6475 bacteria extracted from human milk serve to up-regulate systemic oxytocin levels in mouse models by a vagus nerve-dependent mechanism [30]. Knowing that oxytocin levels increase in humans after contact with dogs [15], we tested whether exposure to *L. reuteri* bacteria extracted from dog saliva, similar to bacteria collected from human milk, may similarly modulate oxytocin levels and convey benefits of more slender physique and overall good health. We find that *L. reuteri* isolate *2546* from dog saliva fed to C57BL/6 mice leads to higher plasma levels of oxytocin. In addition, mice consuming canine-borne *L. reuteri* exhibit less age-associated weight gain when compared with matched untreated controls, in an oxytocin-dependent manner. Taken together, these studies raise the possibility that microbiota shared between species not only convey mutual survival benefits but also serve to strengthen the human-animal bond.

Animals

C57BL/6 wild type (*wt*), oxytocin-*wt* (*oxt-wt*) and oxytocin-

***Corresponding author:** Susan E Erdman, Division of Comparative Medicine, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, United States of America, Tel: 6172521804; E-mail: serdman@mit.edu

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knockout (*oxt-ko*) B6; 129S-Oxttm1Wsy/J mice (purchased initially from Jackson labs; Bar Harbor, ME) were used in three separate experiments (Figure 1). Mice were housed and handled in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities using techniques and diets including *Lactobacillus reuteri* as specifically approved by Massachusetts Institute of Technology's (MIT) Committee on Animal Care (CAC). Mice were housed under standard 12:12 light cycle conditions with lights on at 7 AM. Mice were fed a standard control chow Purina RMH3000.

Mice were bred in-house to achieve experimental groups. Each experiment included 5-15 animals per group with one or two replications (total N=10-30 mice examined per group) unless otherwise specified. For the initial studies, C57BL/6 *wt* mice received *Lactobacillus reuteri 2546* isolated from dog saliva. To test putative roles for microbe-induced oxytocin in obesity, *oxt-ko* mice and their *oxt-wt* littermates entered experiments at eight weeks of age. At the conclusion of the study mice were euthanized with CO_2 overdose, and were examined as described below. Eight pet dogs served as saliva microbe donors as approved by the MIT-CAC. Saliva was collected from these dogs in the morning before feeding using sterile swabs [Puritan Sterile Polyester Tipped applicators Guilford, Me Ref: 25-806 1PD] in 1.5 ml centrifuge tubes (Safeseal Microcentrifuge Tubes, Sorenson Bioscience Inc. Salt Lace City, UT Cat# 16070). Bacteria were purified from dog saliva as described in detail below.

PCR of dog saliva for all *Lactobacillus* species

Saliva was collected from eight pet dogs and prepared using High Pure PCR Template kit (Roche Diagnostics) was used without changes to the manufacturer's directions to isolate DNA from the canine saliva. DNA was measured using Nano Drop Spectrophotometer (Thermo Scientific). *Lactobacillus* spp PCR was performed according to the LactoF and LactoR primers (Integrated Data Technologies) described by Byun et al. [31]. LactoF: 5'-TGG AAA CAG RTG CTA ATA CCG -3' and LactoR: 5'- GTC CAT TGT GGA AGA TTC CC -3' with amplification. Initial denaturation was 95 degrees Celsius for 15 minutes, then with 40 cycles of Denaturing at 95 degrees Celsius for 5 seconds, then annealing at 62 degrees Celsius for 1 minute, and Extension: 72 degrees Celsius for 1 minute. A final extension at 72 degrees Celsius for 5 minutes with the resting temperature at 4 degrees Celsius until utilized for gel separation. PCR products were checked on 2% agarose gel (Sigma) using the Kb+ladder (Invitrogen 10787-018) as a molecular weight marker.

Isolation, characterization and confirmation of *L. reuteri 2546*

Saliva from pet dog #3 was cultivated in classical media, with bacteria isolated as previously described [32]. Subsequently, individual colonies were selected and grown on Sheep blood agar plates (Remel Blood Agar TSA w/ Sheep Blood Plate, Lenera, KS Ref# R01202) for further characterization [32]. Isolate *2546* was found to have colony growth characteristics, microscopic morphology, and be positive for Gram stain, indicating the use of the API 50 CHL system for further identification. The identity of the bacteria was further characterized using API 50 CHL (Biomerieux, France) strips, consisting of 50 Biochemical tests to identify Lactobacillus and related genera, was used according to manufacturer's instructions. Specifically, the *2546* isolate was grown according to manufacturer's instructions and collected after 24 hours with a sterile swab and inoculated into the suspension medium (Biomerieux, France). Interpretation of carbohydrate fermentations were dictated by the manufacturer's instructions and analyzed with the APIweb database (Biomerieux, France). Finally, pure bacterial

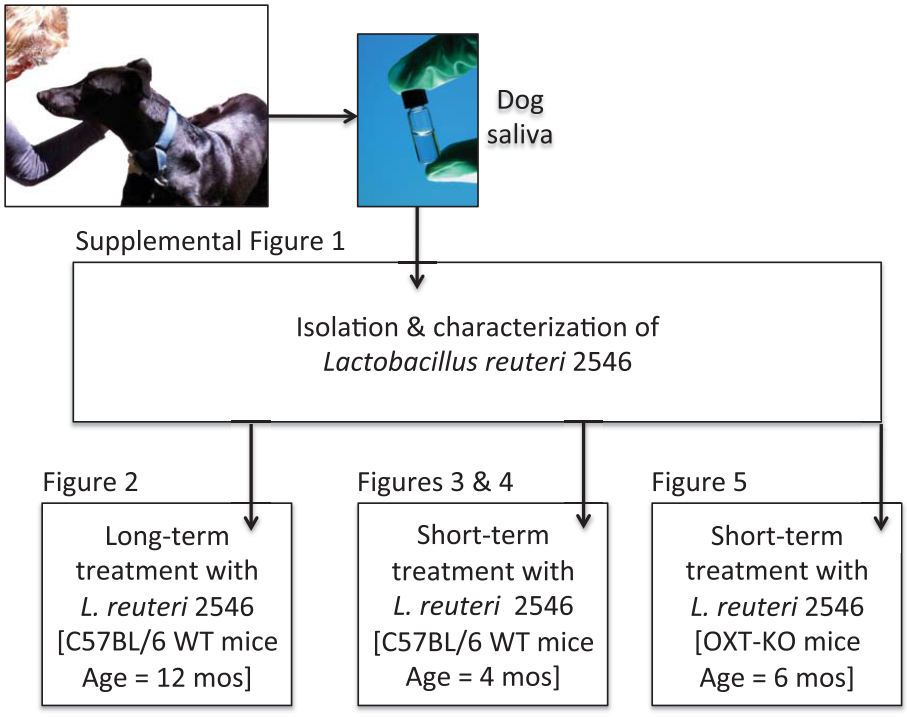

Figure 1: Experimental overview: Canine *L. reuteri 2546* was isolated from pet dog saliva, and then fed to mice in their drinking water. Wild type [*wt*] C57BL/6 mice consuming *L. reuteri 2546* for long-term or short-term intervals were examined for body weight, inflammatory indices, and plasma oxytocin levels. A final experiment tested the hypothesis of whether oxytocin was necessary for slenderizing effects of *L. reuteri 2546*.

culture was tested for genetic identity using PCR with genus specific primers, as below. *Lactobacillus reuteri* PCR was performed according to the L-reu-1 and L-reu-4 primers (Integrated Data Technologies) described by Dommels et al. [33]. L-reu-1: 5'- CAG ACA ATC TTT GAT TGT TTA -3' and L-REU-4: 5'- GTC TGT TGG TTT GGG CTC TTC -3' with Amplification of Initial denaturation 95 degrees Celsius for 5 minutes and then 35 cycles of Denaturing: 95 degrees Celsius for 1 minute, Annealing: 60 degrees Celsius for 1 minute, and Extension: 72 degrees Celsius for 1 minute. A final Extension at 72 degrees Celsius for 8 minutes with the resting temperature at 4 degrees Celsius until utilized for gel separation. PCR products were checked on 2% agarose gel (Sigma) using the Kb+ladder (Invitrogen 10787-018) as a molecular weight marker.

Production of sterile microbe lysate

L. reuteri 2546 was cultivated using methods as previously described [34,35], confirmed for purity by morphology and gram strain, and then suspended in sterile 1xPBS and measured for concentration with a spectrophotometer. A bacteria pellet was obtained by centrifugation for 10 minutes at 14,000 rpm and then resuspended and incubated in a Lysozyme STET buffer for 4 hours at 37 degrees Celsius. Bacteria buffer was centrifuged for 10 minutes at 7,500 rpm to obtain a pellet, that was subsequently washed 2X and then resuspended in 1xPBS before lysing by sonication in an ice water bath at 20 kHz and the amplitude of 30% intensity for one-minute-on-thenone-minute-off for 25 minutes. Lysed bacteria was then centrifuged for 15 minutes at 4,000 rpm with the supernatant being collected as the final product. The supernatant was then confirmed to be sterile using growth by the streak plate method with no growth after three days. Bacterial lysate was stored in 1 ml aliquots in a -80 degrees Celsius until use.

Special microbial treatments for animals

Mice were fed standard rodent chow (RMH 3000; Purina Labs, St Louis MO). Subsets of animals were supplemented orally with a strain of *L. reuteri 2546*, originally isolated from dog saliva, and subsequently cultivated as described elsewhere [34,35], using a supply dosage of 3.5×10^5 organisms/mouse/day continuously in drinking water. For the initial studies, C57BL/6 *wt* mice received *L. reuteri* as above, or, alternatively, regular drinking water. For experiment #2, lysate was delivered at the same concentration in drinking water. For subsequent studies, *oxt-ko* and their littermate *oxt-wt* mice began drinking *L. reuteri 2546* organisms, as above, starting at 6-8 weeks of age, and then underwent analyses at 24 weeks of age. Drinking water was replaced twice weekly to minimize variability in microbial exposure levels. Control animals received regular drinking water.

Experimental design

Experiment 1: To probe the roles of dog bacteria in weight gain of cohabitating animals, 12 eight-week-old C57BL/6 *wt* mice were randomly subdivided into groups of six mice per treatment. Mice treated with the canine isolate *L. reuteri 2546* received it in their drinking water continuously until twelve-months-of-age. Body weight, whole blood cell counts, and body fat histology were evaluated. Terminal blood collections for mice were performed mid-day for all subjects in order to minimize variability due to Circadian rhythms. Animals were housed under 12:12 light cycle conditions and lights turned on at 7 AM.

Experiment 2: To test whether oral therapy with killed [sterile] *L. reuteri 2546* lysate was sufficient for physiological effects, we examined 18 eight-week-old C57BL/6 *wt* mice. Experimental mice were divided into groups of six (N=6/treatment group) and then received in their drinking water *L. reuteri 2546* or lysate of *L. reuteri 2546* starting at eight-weeks-of-age until fourteen-weeks-of-age. Body weight, whole blood cell counts, plasma oxytocin levels, and body fat histology were evaluated.

Experiment 3: To test whether oxytocin is required for fat-inhibiting benefits of oral therapy with canine source *L. reuteri 2546*, we examined 16 oxytocin-*wt* (*oxt-wt*) and 16 knockout (*oxt-ko*) B6;129S-Oxttm1Wsy/J mice. Experimental mice were randomly subdivided into groups of eight mice and then received in their drinking water *L. reuteri 2546* starting at eight-weeks-of-age for a duration of sixteen weeks.

Complete blood cell counts

Whole blood was collected by cardiac puncture from unconscious animals prior to necropsy and suspended in EDTA to prevent clotting. Automated neutrophil counts were then performed using mouse parameters in a HemaVet 950FS (Drew Scientific, Oxford CT). Terminal blood collections for mice were performed mid-day for all subjects in order to minimize variability due to Circadian rhythms.

Measurement of plasma oxytocin levels

Whole blood was collected terminally by cardiac puncture under general anesthesia to obtain plasma. Blood was collected into pre-chilled 5 ml EDTA tubes with 250 KIU of aprotinin, and refrigerated until processing. Plasma was isolated by centrifugation at 1800 g, 15 minutes, 4°C, and then stored in aliquots at !70°C. Plasma was then tested commercially by an outside laboratory with internal validations (AniLytics,. Inc., Gaithersburg, MD). Euthanasia for mice was performed mid-day for all subjects (n=10 per group) to minimize variability due to Circadian rhythms.

Histopathology and histomorphometry

Formalin-fixed tissues were embedded in paraffin, cut at 4-5 μm, and stained with hematoxylin and eosin (HE). CLS counting in abdominal fat sections and measurements of subcutaneous fat thickness were done as previously described [39]. Briefly, multiple images of comparable histological fields were taken at x10 (for crown-like structures=CLS) or x4 (subcutaneous fat) magnification. Twenty images per experimental group were randomly selected and used for assessments using the Image J image processing and analysis program (NIH, Bethesda, MD).

Statistical analyses

For all statistical analyses the Mann-Whitney U test (Graphpad Prism version 4.0 for windows, Graph-Pad software, San Diego, CA, USA) was used. Effects were considered to be significant at $p<0.05$.

Results

Canine oral bacterial flora includes *Lactobacillus* spp

To test our hypothesis that pet dogs may harbor bacteria beneficial for human body weight control, we began by sampling canine saliva. We chose saliva because one fundamental aspect of the human-canine bond is the gesture of licking that spreads oral cavity microbes on the recipient's skin surface. Recognizing that *L. reuteri* ATCC 6475 bacteria collected from human milk was found to up-regulate oxytocin when fed to mouse models [30], and that oxytocin is pivotal in canine-human bonds and weight control [2,36-38], we postulated that dogs may harbor and spread similar microbes that modulate oxytocin and impart a slim physique in the recipient. To test this possibility, we first interrogated the canine oral microbiome using molecular assays and microbial culture (Figure 1). Using generic PCR primers to amplify

all *Lactobacillus* spp in dog saliva, we found nonspecific evidence of *Lactobacillus* spp in the oral cavity samples of eight [8/8] pet dogs that were examined (Supplemental Figure 1A). Elsewhere, it has already well-established that pet dogs may disseminate organisms such as *L. johnsonii* in household dust that lower risk of asthma and other inflammatory disorders in cohabitating humans [14].

To determine whether canine oral bacteria may impart health benefits such as slender physique to a cohabitating animal host, we isolated candidate *Lactobacillus* spp using standard microbiology techniques. Based upon colony growth properties, and microscopic morphology of short rods forming chains, a tractable gram-positive rod isolate was confirmed to be *L. reuteri* based on the composite of gram stain/morphology, biochemical tests, and molecular tests (Supplemental Figures 1B-1D). Afterwards, C57BL/6 wild type mice were fed this purified microbe as a surrogate to mimic canine-human contact. The *L. reuteri* isolate *2546* was cultivated as previously described [34,35], and fed 3×10^5 CFU per day to C57BL/6 mice in their regular drinking water to test the bacteria-body weight hypothesis. Age-matched controls received regular drinking water.

Mice exposed to bacteria from dog saliva are more slender than controls

Based on our knowledge that consumption of *Lactobacillus* ATCC 6475 is sufficient to inhibit inflammation and age-associated obesity in mouse models [39], and *Lactobacillus rhamnosus* CGMCC1.3724 stimulates weight loss in obese humans [40], we tested the microbe-obesity hypothesis using mice exposed orally to the canine-source *L. reuteri 2546* and compared them with untreated control animals. After nine months of daily feeding with *L. reuteri*, we discovered that *L. reuteri 2546*-treated mice had significantly lower body weight than control animals (Figure 2A). Further, abdominal fat (Figure 2B) and subcutaneous fat (Figure 2C) was significantly less in mice receiving canine source *L. reuteri 2546* in drinking water.

To determine whether body fat pathology was altered by exposure to the dog microbe, we microscopically examined abdominal fat from mice of both groups. Similar to what was reported previously using a different strain of *L. reuteri* [39], we found that the canine *L. reuteri* isolate *2546* protected mice from adipose tissue lesions characteristic of obese or aged mice. The histological analysis of abdominal fat revealed that *L. reuteri 2546*-treated mice had significantly fewer "crown-like structures" (CLS), which is the typical lesion of adipocyte death-related inflammation, and focal pyogranulomatous inflammation (Figure 2D).

Lysed (sterile) bacteria are sufficient for physiological effects in mice

Studies involving pet dogs show reduced risk for asthma in humans due to exposure of dust when cohabitating with pet dogs [14]. To test whether living bacteria are actually required for beneficial effects, purified canine microbe *2546* was rendered sterile by lysis before being fed to mice in their drinking water for six weeks duration. Interestingly, we found that exposure to sterile lysed forms of the same bacteria were sufficient for lower body weight and reduced subcutaneous and visceral fat (Figures 3 and 4). In earlier studies it was determined that routinely consuming *L. reuteri* ATCC 6475 also lowered systemic inflammatory tone [39]. To test this possibility, examination of whole blood counts revealed that circulating neutrophils were significantly fewer in mice undergoing treatment with *L. reuteri 2546* for four weeks (Figure 4C). The finding that lysates were sufficient for physiological effect raises the

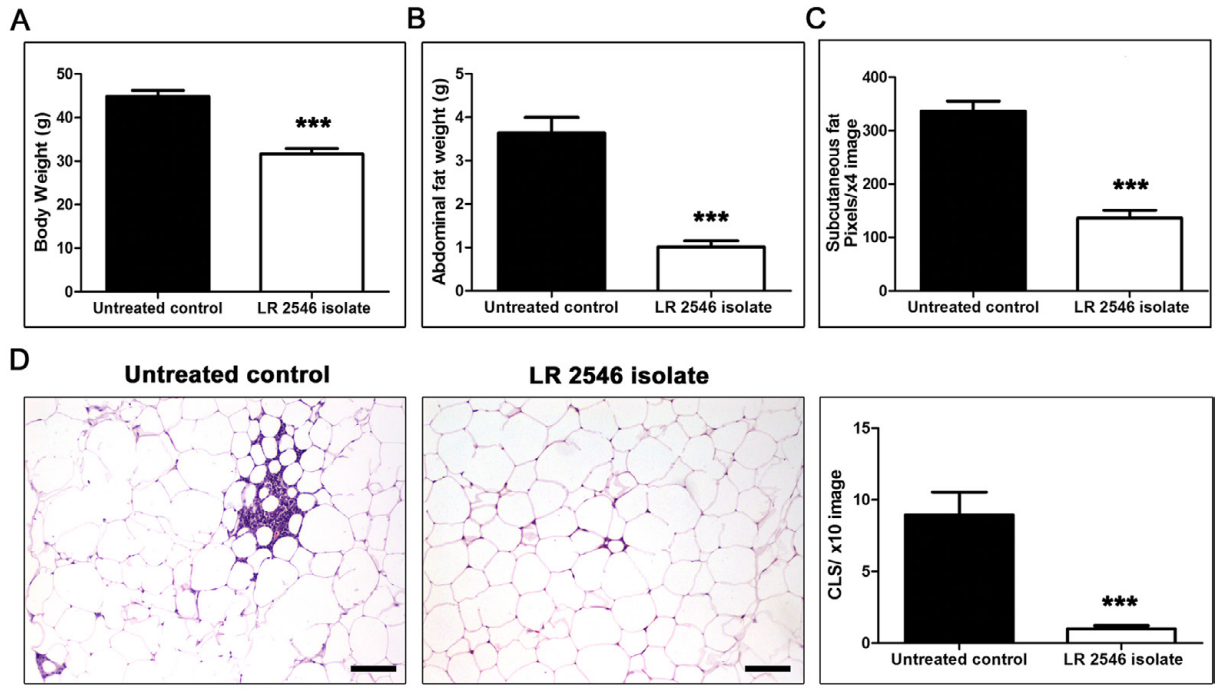

Figure 2: The canine *L. reuteri 2546* isolate recapitulates key probiotic health benefits in mice. Mice consuming *L. reuteri 2546* for nine months had significantly (A) lower body weights, and reduced accumulations of (B) abdominal and (C) subcutaneous fat compared to untreated age-matched control mice. (D) The abdominal fat of control mice had readily recognizable crown-like structure [CLS] lesions that often coalesce to form sizable pyogranulomatous lesions. By contrast, CLS were rare in *L. reuteri*-treated mice. The analysis of histomorphometrical counts of CLS in the abdominal fat of mice shows that the anti-inflammatory effect of probiotic treatment is statistically significant. (D) Hematoxylin and Eosin. Scale bars: 100 μm. Numbers on the y-axis of bar graphs correspond to the mean ± SEM of the parameters assessed; ***p<0.0001.

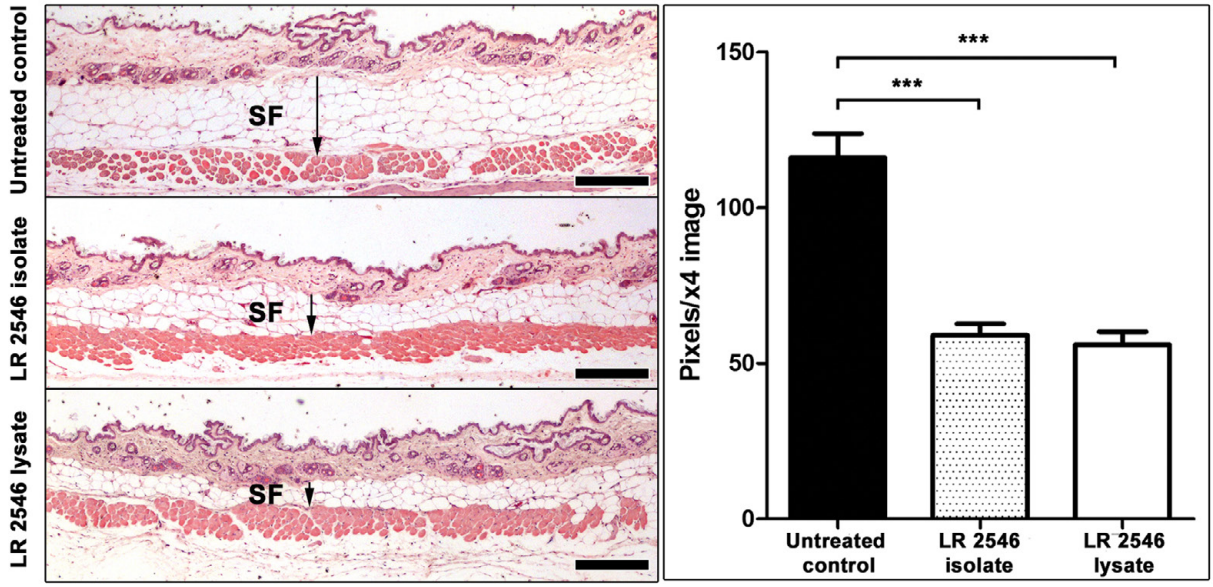

Figure 3: A single month of *L. reuteri 2546* treatment results in reduced subcutaneous fat in mice. The histomorphometrical assessment of subcutaneous fat thickness in mice shows that both live and killed *2546* bacterial cells when orally consumed act to reduce the thickness of the subcutaneous fat (SF) layer at statistically significant levels. Hematoxylin and Eosin. Scale bars: 250 μm. Numbers on the y-axis of bar graph corresponds to the mean ± SEM of SF layer thickness measured in image pixels; ***p<0.0001.

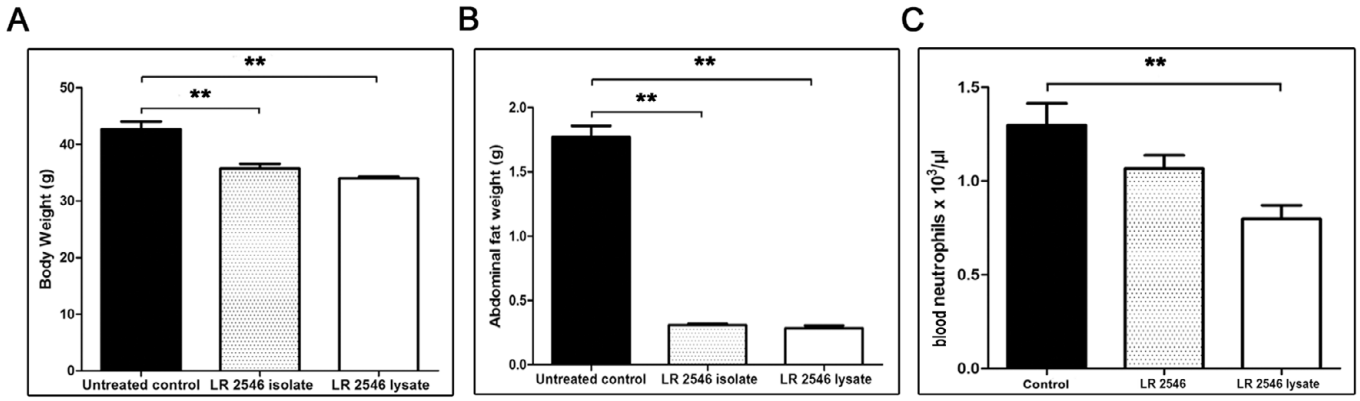

Figure 4: Short-term treatment with *L. reuteri 2546* contributes to slimmer phenotypes and lower systemic inflammatory tone in mice. The oral consumption of either live or lysed probiotic bacteria cells reduces the (A) body weight, (B) abdominal fat weight and (C) numbers of neutrophils in the blood of mice. Numbers on the y-axis of bar graph corresponds to the mean ± SEM of SF layer thickness measured in image pixels; **p<0.001.

possibility that colonization with microbes or influence of microbial communities is not necessarily required for benefits shared between cohabitating hosts.

Canine bacteria induce neuropeptide hormone oxytocin

Knowing that oxytocin inhibits weight gain in rodent models [21,41], and that oxytocin modulates appetite in human subjects [29], plasma levels of oxytocin were tested in mice of long- and short-term experiments. We found significantly elevated blood plasma oxytocin levels in C57BL/6 mice getting canine-source *L. reuteri 2546* in their water, when compared with age-matched controls drinking regular water (Figures 5A and 5B). These observations matched earlier reports of elevated plasma oxytocin levels after feeding another *L. reuteri* isolate from human milk (ATCC 6475) that was demonstrated to improve systemic wound healing capacity [30]. Interestingly, lysed *L. reuteri 2546* was also potent for increasing the systemic levels of plasma oxytocin in mice (Figure 5B). Altogether, these findings suggest novel microbe-based strategies for body weight control and psychological well-being. The consistent up-regulation of oxytocin after eating *L. reuteri 2546* led us to test whether oxytocin is required for the lowered body weight phenomenon.

Consumption of *L. reuteri* reduces risk for obesity in an oxytocin-dependent manner

Recognizing that oxytocin inhibits weight gain in rodent models [21,41] and in humans [29], we challenged oxytocin-deficient B6;129S-Oxttm1Wsy/J mutant mice with canine source *L. reuteri 2546* to determine whether this neurotropic hormone oxytocin is essential for *L. reuteri 2546*-induced weight control. We found that mice globally lacking oxytocin did not benefit from microbe-induced body weight

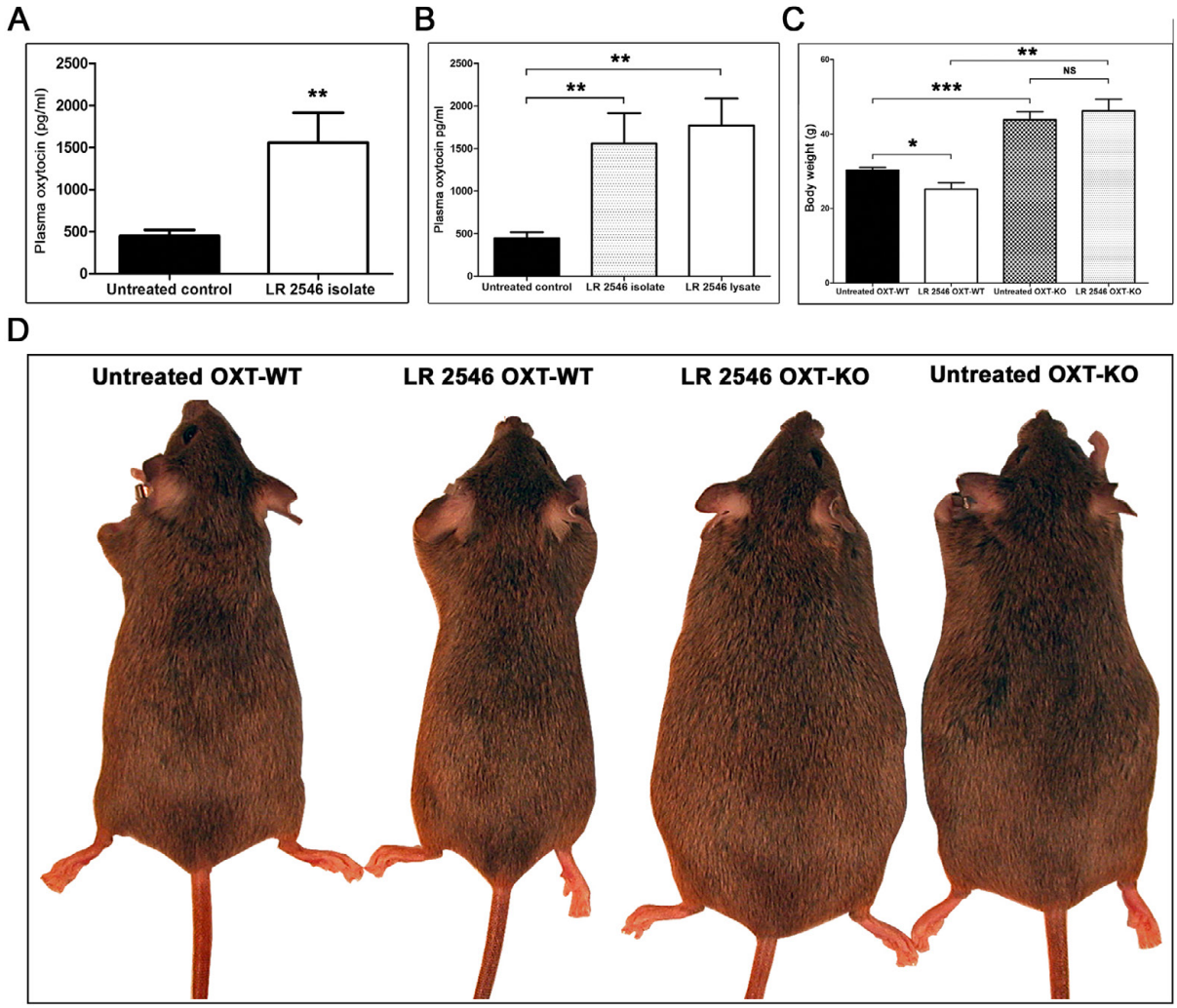

Figure 5: The health benefits of oral *L. reuteri 2546* consumption depend on oxytocin, Short (A) and long term (B) consumption of *L. reuteri 2546* cells, whether live (A) or killed (B), upregulate plasma oxytocin levels at statistically significant levels. (C) Using oxytocin-deficient mice and their wild-type controls, we find that the statistically significant effect of probiotics in reducing body weight of mice is negated in the absence of oxytocin. (D) Whole mouse body representative images are provided for a side-by-side comparison. Shown are oxytocin-deficient and wild-type mice that were either treated with *L. reuteri* or remained untreated. Note that the *L. reuteri* treatment correlates with a slender phenotype in wild-type but not oxytocin-deficient mice. Numbers on the y-axis of bar graphs correspond to the mean ± SEM of the parameters assessed, **p<0.001, ***p<0.0001.

effects (Figures 5C and 5D). This is consistent with the other data; in particular, using oxytocin-deficient B6; 129SOxttm1Wsy/J mutant mice, it was previously shown that inflammation, and specifically neutrophils, have a reciprocal relationship with oxytocin in the wound repair process [30]. The apparent requirement for oxytocin-competency in this model system led us to conclude that microbe-driven oxytocin contributed to the lean outcome of mice.

Discussion

Here we tested whether common commensal bacteria in pet dogs may help explain the leaner body weight of dog owners. Using a C57BL/6 wild type mouse model as a surrogate for human subjects, we found that mice consuming *L. reuteri 2546* isolated from pet dog saliva exhibited less age-associated weight gain in an oxytocin-dependent manner. Interestingly, lysed (sterile) forms of the same bacteria were also sufficient to up-regulate mouse plasma oxytocin and lower circulating neutrophils, fat pathology, and body weights, suggesting future therapeutic possibilities for sterile microbial fractions in good physical and mental health. Taken together, these studies in mice provide evidence that canine microbiota may contribute to lower body weights-and simultaneously serve to strengthen the human-animal bond - due to microbe-induced activities of the hormone oxytocin. It remains to be proven whether these effects exist in human subjects.

Epidemiological data showing lower prevalence of allergic diseases among those living on farms or with pets during childhood support this beneficial microbe concept, thus sparking intense research interest in this topic [6-13]. The 'hygiene hypothesis' theory is based on associations between the modern living-associated decrease in infectious agent exposures and the commensurate increase in allergies and autoimmune diseases. Earlier work from our own lab [39] and other labs [42-53] begin to connect-the-dots between microbes, inflammation, and obesity. Indeed, it was previously shown that exposure to dietary *L. reuteri* strains ATCC 6475 [39] or ATCC 4659 [53] led to less weight gain in mouse models. Another recent study showed that household dogs, specifically, may disseminate *Lactobacillus*

spp in household dust that lower risk of asthma and other inflammatory disorders in cohabitating humans [14,54]. It is an attractive idea that certain microbes can be strategically applied to stimulate beneficial host pathways as a replacement for microbes lost due to antibiotics and routine sanitary practices.

At the same time that bacteria in dog saliva, or microbiota from other pet or farm animals, may have beneficial properties [55], caution is warranted involving zoonotic organisms that readily transmit diseases between species. Common examples include plague from infected fleas [56]. Also, significantly higher infection rates of Chagas disease were evident in humans who slept with their pet dog [57]. *Bartonella hensalae* infection was confirmed by serologic testing of a 50-year- old man from Japan, who lived with a dog that often licked his face [58]. Pasturella sp infections have also been associated with dogs licking human faces [59,60]. Cases exist where organisms from dog saliva have identical biochemical patterns and genotypic similarities with isolates in human infections [61-63], supporting that dog saliva is the mode of bacterial transmission [64]. Thus, microbe strategies that maximize the benefits of bacteria exposures and simultaneously lower risks of zoonotic diseases are a practical goal.

These present findings linking *Lactobacillus* spp with a more slender physique are not entirely surprising since consumption of *L. reuteri* ATCC 6475 was proven sufficient to inhibit inflammation and age-associated obesity in mouse models [39]. Similar weight loss was shown in obese humans who consumed purified *L. rhamnosus* [40]. It was also previously shown that microbe-induced oxytocin modulates host immunity by inducing a more rapid return to health after injury [30]. Most notable in those earlier studies were expedited influxes of neutrophils with more rapid wound repair afterwards when treated with *L. reuteri* ATCC 6475, a phenomenon that was reliant upon oxytocin as shown in B6;129S-Oxttm1Wsy/J mutant mice [30,65]. In those studies, central in beneficial effects of feeding *L. reuteri* ATCC 6475 was recruitment of homeostatic CD4+CD25+Foxp3+regulatory T (Treg) cells that are otherwise known to suppress deleterious inflammatory responses [66]. The superior physiological role of Treg cells is to prevent immunopathology after a host insult [67], a feature that can be utilized to host benefit in maintaining immune homeostasis. Many questions remain to be answered about the host range and other physiological properties of canine *L. reuteri 2546*.

In earlier studies, *L. reuteri* ATCC 6475 -induced up-regulation of plasma oxytocin was a vagus nerve- dependent phenomenon, suggesting central nervous system (CNS) involvement [30]. Other work has shown a release of oxytocin from somatodendrites and axonal terminals within the CNS implicated in both control of energy balance the formation of prosocial behaviors [41]. Romero et al. and Nagasawa et al. [16,68] found that giving dogs exogenous oxytocin supplements causes them to display stronger social bonding behavior, both with people and other dogs. To the same extent, oxytocin has been shown to benefit antisocial behaviors in autism spectrum disorder (ASD) in humans [69,70]. Interestingly, mice eating *L. reuteri* ATCC 6475 in earlier studies were also shown to improve maternal care with higher infant survival rates [71]. Unlike the short half-life of exogenous supplements of oxytocin, the plasma elevations seen in the present mice are a consistent and reproducible effect [30], making bacteria or bacterial products a possible therapy for mental health. It remains to be proven whether microbe-induced oxytocin in these murine models originates primarily from the hypothalamus or from other peripheral sources [72-76]. Nonetheless, our results suggest that gut bacteria-induced oxytocin may explain data linking gut microbiome dysbiosis with neuropsychological disorders, including autism [77,78].

One interesting question is whether microbiota or microbe-stimulated oxytocin inhibit weight gain at the expense of host muscle mass. Indeed, emerging work shows oxytocin does exactly the opposite, that oxytocin helps build host muscle mass [79]. Feeding of a human isolate of *L. reuteri* (ATCC 6475) to mice was also shown to inhibit muscle wasting disorders, associated with an increase in growth hormone levels and also a larger thymus gland size [80]. Likewise, the same strain of *L. reuteri* ATCC 6475 was previously shown to stimulate an increase in serum thyroid hormone T4 levels in mice [81] commensurate with more slender physique. Taken together, there is precedent for microbiota, and *L. reuteri* isolates in particular, to stimulate systemic hormone secretion that re-directs energy toward muscle growth and away from fat storage.

Recognizing that bacteria from dogs and other cohabitating pet, food and fiber animals carry zoonotic risks, a potentially important finding in the present study involves benefit of exposures to lysed sterile forms of bacteria. These intriguing data also raise the possibility that colonization with live microbes or microbial communities is not required for physiological benefits, whether at an individual level or shared between cohabitating hosts. An additional benefit is that sterile extracts of microbes have fewer health risks for immune-compromised patients, lowering risk of microbial overgrowth in patients who may otherwise suffer inappropriate immune responses. Some earlier work has suggested that killed bacteria or their extracts have healthful anti-inflammatory properties, in particular during inflammatory bowel conditions [82-86]. Precise characterization of the dog bacterial extract and potential in human subjects remains to be determined. Nonetheless, these data reveal vast potential for sterile microbe extracts in good physical, social and mental health.

In conclusion, these data build upon earlier studies in mice showing that *L. reuteri* ATCC 6475 from human breast milk lowers body weight and up-regulates oxytocin levels in blood. We found that bacteria isolated from dog saliva, *L. reuteri 2546*, may regulate inflammation and host body weight involving mechanisms of oxytocin, raising interesting evolutionary cohabitation questions and therapeutic possibilities. The discovery that sterile microbial products also achieve similar benefits paves the way for novel therapeutics for good health.

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Competing Interests

There are no competing financial or commercial interests.

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