



## Full-length Article

## Microbial lysate upregulates host oxytocin



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## ABSTRACT

Neuropeptide hormone oxytocin has roles in social bonding, energy metabolism, and wound healing contributing to good physical, mental and social health. It was previously shown that feeding of a human commensal microbe *Lactobacillus reuteri* (*L. reuteri*) is sufficient to up-regulate endogenous oxytocin levels and improve wound healing capacity in mice. Here we show that oral *L. reuteri*-induced skin wound repair benefits extend to human subjects. Further, dietary supplementation with a sterile lysate of this microbe alone is sufficient to boost systemic oxytocin levels and improve wound repair capacity. Oxytocin-producing cells were found to be increased in the caudal paraventricular nucleus [PVN] of the hypothalamus after feeding of a sterile lysed preparation of *L. reuteri*, coincident with lowered blood levels of stress hormone corticosterone and more rapid epidermal closure, in mouse models. We conclude that microbe viability is not essential for regulating host oxytocin levels. The results suggest that a peptide or metabolite produced by bacteria may modulate host oxytocin secretion for potential public or personalized health goals.

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## 1. Introduction

In a series of prior studies using mice we have shown that the prototype probiotic bacterium *Lactobacillus reuteri* (*L. reuteri*) imparts an array of health benefits (Erdman and Poutahidis, 2014; Ibrahim et al., 2014; Lakritz et al., 2014; Levkovich et al., 2013; Poutahidis et al., 2013a,b, 2014, 2015; Varian et al., 2016a, b, 2014). Aside from its previously described positive contributions in the health of oral (Iniesta et al., 2012; Martin-Cabezas et al., 2016) and gastrointestinal (GI) tract mucosa (Cruchet et al., 2015; Gao et al., 2015; Liu et al., 2012; Schreiber et al., 2009; Varian et al., 2016a; Walter et al., 2011), the probiotic has systemic effects on the immune and hormonal profile of the host animal (Buffington et al., 2016; Erdman and Poutahidis, 2014; Ibrahim et al., 2014; Lakritz et al., 2014; Lee et al., 2016; Levkovich et al.,

2013; Livingston et al., 2010; Poutahidis et al., 2013a,b, 2014, 2015; Simon et al., 2015; Varian et al., 2016a,b, 2014). Mice that consume *L. reuteri* live longer (Ibrahim et al., 2014; Varian et al., 2016a) and resist obesity (Fak and Backhed, 2012; Poutahidis et al., 2013b; Qiao et al., 2015; Varian et al., 2016b, 2014) and age-associated atrophic changes of skeletal muscle (Varian et al., 2016a), testis (Poutahidis et al., 2014), and thyroid (Varian et al., 2014) and thymus glands (Varian et al., 2016a). They also have healthy fur coats (Erdman and Poutahidis, 2014; Levkovich et al., 2013), heal their skin wounds faster (Poutahidis et al., 2013a) and are resistant to intestinal (Varian et al., 2016a), mammary gland, liver, and lung cancer (Lakritz et al., 2014; Poutahidis et al., 2015).

Prompted by the observation that mice consuming *L. reuteri* consistently exhibit increased grooming and maternal care behaviors (Ibrahim et al., 2014), we discovered that the probiotic increases the plasma levels of oxytocin (Ibrahim et al., 2014; Poutahidis et al., 2013a; Varian et al., 2016b). This unprecedented probiotic effect has been proven essential for several of the sys-

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temic beneficial effects of *L. reuteri* as exemplified by experiments using oxytocin deficient mice and immune cell transfers (Erdman and Poutahidis, 2014; Ibrahim et al., 2014; Poutahidis et al., 2013a; Varian et al., 2016b). Interestingly, many of the published beneficial health effects of oxytocin overlap with recognized *L. reuteri* health-promoting attributes. Indeed, as with *L. reuteri* (Britton et al., 2014; Collins et al., 2016; Poutahidis et al., 2013a,b; Varian et al., 2016a,b, 2014; Zhang et al., 2015b), the exogenous administration of oxytocin has been shown to accelerate wound healing (Gavrilenko et al., 2003; Gouin et al., 2010; Vitalo et al., 2009), counteract obesity (Barengolts, 2016; Blevins and Baskin, 2015), suppress uncontrolled inflammation (Wang et al., 2015) and prevent muscle wasting (Costa et al., 2014; Elabd et al., 2014) and bone loss (Colaianni et al., 2014; Sun et al., 2016; Tamma et al., 2009).

A role for oxytocin in promoting good mental health, including social bonding, a general sense of well-being, and improved learning and memory is emerging (Carter, 2014; Donaldson and Young, 2008; Feldman et al., 2016). At the same time, recent evidence supporting the presence of a gut-microbiota-brain axis (Dinan et al., 2013; Kelly et al., 2016; Sherwin et al., 2016) suggests that probiotic bacteria can influence emotions, mood, anxiety and depression (Dinan et al., 2013; Kelly et al., 2016; Sherwin et al., 2016). This places oxytocin (Carter, 2014; Donaldson and Young, 2008; Feldman et al., 2016) and probiotic bacteria among novel approaches for preventing or even treating neuropsychiatric disorders (Dinan et al., 2013; Kelly et al., 2016; Sherwin et al., 2016). Hence, their name psychobiotics (Dinan et al., 2013).

Apart from findings showing that *L. reuteri* upregulates oxytocin and enhances maternal care behaviors (Ibrahim et al., 2014; Poutahidis et al., 2013a; Varian et al., 2016b), until recently there were no other data suggesting that this bacterium could be considered among suspected psychobiotics (Dinan et al., 2013). However, a recent study in mice (Buffington et al., 2016) supports this notion and expands prior findings connecting maternal obesity-induced gut microbiota dysbiosis with detrimental effects in the offspring. A gut microbiota that is poor in *L. reuteri* associates with adverse social behavior effects in offspring mice. Conversely, supplementary consumption of *L. reuteri* upregulates oxytocin in the hypothalamus, stimulates the mesolimbic dopamine reward system and promotes prosocial behavior (Buffington et al., 2016). This matches earlier multigenerational findings involving feeding of special diets and *L. reuteri* ATCC 6475 in mice (Poutahidis et al., 2015).

Among the newly recognized actions of oxytocin, its connection with the immune system, is particularly important (Wang et al., 2015). Indeed, oxytocin contributes to the maturation and selection of T-lymphocytes in the thymus (Hansenne et al., 2009, 2005; Wang et al., 2015), which is integral for immune system homeostasis and overall health. We have recently reported that mice consuming *L. reuteri* retain a sizable thymus in adulthood and that this effect is mediated by the probiotic-induced upregulation of the Forkhead Box N1 (FoxN1) in thymic epithelial cells (Varian et al., 2016a). FoxN1 is essential for normal thymus development and a competent host immune system as exemplified by mice deficient in FoxN1 (athymic nude mice), which lack functional T-lymphocytes (Nehls et al., 1994; Romano et al., 2013; Zhang et al., 2012), and as a result are highly susceptible to infections and developing cancer.

Another well-established effect of oxytocin in the immune system relates with its ability to down-regulate neutrophilic pro-inflammatory responses (Al-Amran and Shahkolahi, 2013; Biyikli et al., 2006; Iseri et al., 2005a,b; Petersson et al., 2001; Wang et al., 2015). Studies using *L. reuteri* consumption in mice consistently show a downregulation of circulating neutrophils in the

context of maintaining homeostasis during wound repair and slim physique (Poutahidis et al., 2013a; Varian et al., 2016a,b). Ways in which *L. reuteri* contributes to reducing neutrophils and consequently lowering systematic inflammatory tone are particularly important in the light of recent evidence that neutrophils are an important cellular component of chronic smoldering subclinical systemic inflammation that associates with increased risk for carcinogenesis, metabolic disorders and cardiovascular disease (Coffelt, 2016; Manda-Handzlik and Demkow, 2015; Paquissi, 2016; Seijkens et al., 2014). However, while oxytocin has apparent beneficial effects, repeated pharmaceutical oxytocin administration may have unwanted effects under certain circumstances in humans. It may be anxiogenic and promote aggressiveness towards others that are being thought as outsiders or competitors (De Dreu et al., 2011). Also, in patients suffering from borderline personality disorder, oxytocin decreased trust and cooperation (Bartz et al., 2011).

Given the challenges raised regarding the efficacy and safety of exogenous oxytocin administration (Lefevre and Sirigu, 2016; Leng and Ludwig, 2016), the likelihood that microbial supplementation may endogenously increase oxytocin levels appears appealing. In order to go deeper into the mechanistic analysis of *L. reuteri* mode of action, including oxytocin induction and eventually therapeutic strategies, however, a basic question arises. Is it required to ingest live *L. reuteri* organisms to achieve up-regulation of oxytocin and associated health benefits?

In the present study, we show that oral dosing of *L. reuteri* leads to improved wound repair capacity in mouse models and human subjects. We provide direct evidence that dietary *L. reuteri* lysate is sufficient for wound healing improvements and increases the number of oxytocin-positive cells in the caudal portion of the paraventricular nucleus of the hypothalamus (PVN) in mice. The increase in oxytocin-positive cells in the PVN is correlated with lowered circulating levels of stress hormone corticosterone in mice consuming *L. reuteri*. Microbe-induced oxytocin is necessary for the induction of important beneficial host effects, including proficient wound repair. Finally, sterile *L. reuteri* lysate prepared by sonication is sufficient for achieving up-regulation of oxytocin and health benefits in mice, suggesting that these phenomena are triggered by a bacterial component, rather than live probiotic bacteria.

## 2. Methods

### 2.1. Animal models

Female outbred Swiss stock CD-1 female mice (Charles River, Wilmington MA), C57BL/6 wild type (*wt*), oxytocin-*wt* [*ot-wt*] and oxytocin-knockout (*ot-ko*) B6;129S-Ox<sup>tm</sup>1Wsy/J mice (purchased initially from Jackson labs; Bar Harbor, ME) were used in three separate experiments. Mice were housed and handled in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities using techniques and diets including *L. 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 7AM. Mice were fed a standard control chow Purina RMH3000.

Mouse models were bred in-house to achieve experimental groups. Mice were randomly assigned to experimental groups, and group housed with 4–5 mice per cage. Each experiment included 5–11 animals per group as specifically enumerated within the text. Mice received in their drinking water *L. reuteri* ATCC-PTA-6475 originally isolated from human breast milk.

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