Brief communication

MOLECULAR METABOLISM

Microbially produced glucagon-like peptide 1 improves glucose tolerance in mice

Tulika Arora [1], Udo Wegmann [2], Anup Bobhate [2], Ying Shiuan Lee [1], Thomas U. Greiner [1], Daniel J. Drucker [3], Arjan Narbad [2], Fredrik Bäckhed [1,4,*]

ABSTRACT

Objective: The enteroendocrine hormone glucagon-like peptide 1 (GLP-1) is an attractive anti-diabetic therapy. Here, we generated a recombinant *Lactococcus lactis* strain genetically modified to produce GLP-1 and investigated its ability to improve glucose tolerance in mice on chow or high-fat diet (HFD).

Methods: We transformed *L. lactis* FI5876 with either empty vector (pUK200) or murine GLP-1 expression vector to generate LL-UK200 and LL-GLP1, respectively, and determined their potential to induce insulin secretion by incubating primary islets from wild-type (WT) and GLP-1 receptor knockout (GLP1R-KO) mice with culture supernatant of these strains. In addition, we administered these strains to mice on chow or HFD. At the end of the study period, we measured plasma GLP-1 levels, performed intraperitoneal glucose tolerance and insulin tolerance tests, and determined hepatic expression of the gluconeogenic genes *G6pc* and *Pepck*.

Results: Insulin release from primary islets of WT but not GLP1R-KO mice was higher following incubation with culture supernatant from LL-GLP1 compared with LL-UK200. In mice on chow, supplementation with LL-GLP1 versus LL-UK200 promoted increased vena porta levels of GLP-1 in both WT and GLP1R-KO mice; however, LL-GLP1 promoted improved glucose tolerance in WT but not in GLP1R-KO mice, indicating a requirement for the GLP-1 receptor. In mice on HFD and thus with impaired glucose tolerance, supplementation with LL-GLP1 versus LL-UK200 promoted a pronounced improvement in glucose tolerance together with increased insulin levels. Supplementation with LL-GLP1 versus LL-UK200 did not affect insulin tolerance but resulted in reduced expression of *G6pc* in both chow and HFD-fed mice.

Conclusions: The *L. lactis* strain genetically modified to produce GLP-1 is capable of stimulating insulin secretion from islets and improving glucose tolerance in mice.

Keywords *Lactococcus lactis*; Glucose tolerance; Recombinant bacteria; GLP-1

1. INTRODUCTION

Glucagon-like peptide 1 (GLP-1) is released from enteroendocrine L-cells after food intake and stimulates insulin release, reduces appetite and slows down gastric emptying [1]. GLP-1 has a short half-life (~2 min) and is rapidly degraded by dipeptidyl peptidase 4 (DPP4), and currently available GLP-1-based drugs are GLP-1 receptor agonists and DPP4 inhibitors [2]. However, although these drugs offer advantages over traditional anti-diabetic drugs as they promote weight loss and are associated with a lower risk of hypoglycemia [3], GLP-1 receptor agonists are only available as injections and the safety profile of both drug classes for long-term treatment is under debate [4].

There is an increasing interest in the genetic modification of food-grade bacteria to express potentially therapeutic eukaryotic peptides. *Lactococcus lactis* is a homofermentative Gram-positive bacterium used as a starter culture in a wide variety of fermented food products. Its small genome size, the availability of tightly regulated promoter systems, mild proteolytic activity and generally-regarded-as-safe status make it an ideal host for the production, secretion and delivery of peptides directly to the intestine [5]. Recombinant interleukin 10-producing *L. lactis* has been shown to protect against colitis in mouse models [6] and is now being tested in a phase 2a clinical trial in patients with Crohn's disease [7], indicating the potential of recombinant bacterial strains in the treatment of human disease.

[1]Wallenberg Laboratory and Sahlgrenska Center for Cardiovascular and Metabolic Research, Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden [2]Gut Health and Food Safety Programme, Institute of Food Research, Norwich NR4 7UA, UK [3]Department of Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON M5G 1X5, Canada [4]Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

*Corresponding author. Wallenberg Laboratory and Sahlgrenska Center for Cardiovascular and Metabolic Research, Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden. Tel.: +46 31 342 7833; fax: +46 31 82 37 62. E-mail: Fredrik.Backhed@wlab.gu.se (F. Bäckhed).

Abbreviations: cfu, Colony forming unit; DPP4, Dipeptidyl peptidase 4; G-KRB, glucose-Krebs ringer buffer; GLP-1, Glucagon-like peptide 1; GLP1R-KO, GLP-1 receptor knock out; *G6pc*, glucose 6 phosphatase, catalytic subunit; HFD, high fat diet; IPGTT, Intraperitoneal glucose tolerance test; ITT, Insulin tolerance test; LL-GLP1, GLP-1 producing recombinant strain; LL-UK200, Control vector only strain; *Pepck*, phosphoenolpyruvate carboxykinase; WT, Wild type

Received June 1, 2016 • Revision received June 9, 2016 • Accepted June 14, 2016 • Available online 22 June 2016

http://dx.doi.org/10.1016/j.molmet.2016.06.006

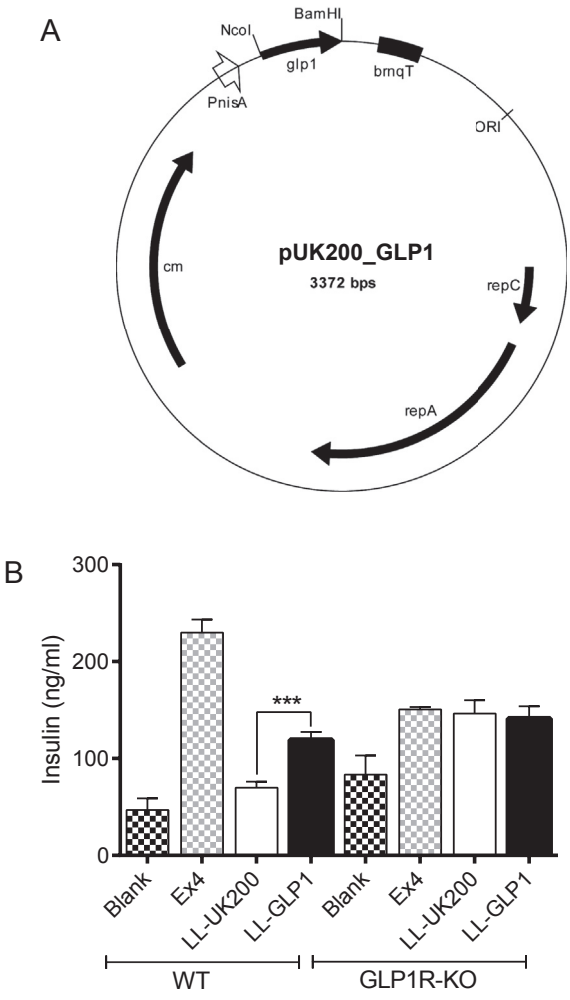

Figure 1: *In vitro* insulin release by recombinant *L. lactis*-derived GLP-1. (**A**) GLP-1 vector expressing murine GLP-1. (**B**) Insulin release from pancreatic islets of wild-type (WT, n = 3) and GLP-1 receptor knock out (GLP1R-KO, n = 3) mice treated with buffer alone (blank), exendin-4 (Ex4, positive control) or culture supernatant from recombinant LL-GLP1 or vector control LL-UK200. All stimulations were performed in duplicate. Data are mean ± SEM. ***$p < 0.001$.

Here, we developed a genetically modified *L. lactis* strain to produce murine GLP-1 and investigated its potential to improve glucose tolerance in wild-type (WT) mice either on chow or high-fat diet (HFD). GLP-1 receptor knock out (GLP1R-KO) mice on chow diet were used as a negative control to assess the specificity of LL-GLP1 in improvement of glucose tolerance.

2. METHODS

2.1. Construction of recombinant GLP-1-producing *L. lactis*

A 206-bp synthetic gene construct encoding the murine GLP-1 peptide (1—37) N-terminally fused to the lactococcal Usp45 signal peptide was created *in silico* and its codon usage was optimised for lactococcal expression. The resulting gene cassette was obtained through gene synthesis and subsequently cloned into the *E. coli* plasmid pEX-A (Eurofins, Germany). The cassette contains NcoI and BamHI restriction sites at its 5′ and 3′ ends, respectively, allowing for the translational fusion of the gene to the *nisA* start codon in the lactococcal expression vector pUK200 [8]. The gene was excised from pEX-A using NcoI and BamHI and ligated into pUK200, which had been restricted in the same way, resulting in plasmid pUK200_GLP1. The empty vector and the nisin-inducible GLP-1 expression vector (Figure 1A) were then transformed into the nisin-producing strain *L. lactis* FI5876 [9] to generate strains FI10936 (LL-UK200) and FI10937 (LL-GLP1), respectively.

2.2. Culture conditions

Both *L. lactis* strains were grown overnight at 30 °C for 16 h in M17 media (pH 7.0) supplemented with glucose (2% wt/vol). Thereafter, the cultures were pelleted and subsequently resuspended in fresh M17 media (pH 8.5) supplemented with glucose (2% wt/vol). Growth was observed for an additional ~3 h until the pH reached 7.0. Active GLP-1 in the culture supernatant was measured at this time point using the Mesoscale discovery kit.

2.3. Mice

All procedures in mice were approved by the Ethics Committee on Animal Care and Use in Gothenburg, Sweden. Male C57/bl6 and GLP1R-KO mice aged 10—12 weeks of age were used mice were either bred in-house (for chow study) or purchased from Taconic, Denmark (for HFD study).

2.3.1. Glucose-stimulated insulin release from isolated islets

Islets were isolated from WT and GLP1R-KO mice using collagenase perfusion as described before [10]. Five islets per group were incubated with 16 mmol/l glucose-Krebs ringer buffer (G-KRB), 2 μmol/l exendin-4 prepared in G-KRB (positive control) or cell-free supernatants from LL-UK200 or LL-GLP1 cultures diluted 1:4 in G-KRB at 37 °C for 60 min. After incubation, islets were sedimented and insulin was measured in the supernatants using the insulin ELISA kit (Crystal Chem). All stimulations were performed in duplicate and repeated three times independently.

2.3.2. *In vivo* study

We performed two *in vivo* studies to investigate the effect of LL-GLP1 on glucose tolerance. In the first study, WT or GLP1R-KO mice were randomised on an autoclaved low-fat polysaccharide-rich chow diet (LabDiet, St Louis, MO, USA) *ad libitum* and they received 200 μl oral daily gavages of fresh bacterial culture containing 1×10^{10} colony forming units (cfu) of either LL-UK200 or LL-GLP1 cultures for 9 days. In the second study, WT mice were randomised to receive 200 μl oral daily gavages of fresh LL-UK200 or LL-GLP1 cultures for 3 weeks on chow followed by 3 weeks onto a high-fat, high-sugar western diet with 40% of calories from fat (Adjusted Fat Diet TD.96132, Harlan Teklad, Indianapolis, IN, USA). Body weight and body fat (measured by whole body magnetic resonance imaging) were measured at the indicated time points.

Intraperitoneal glucose tolerance tests (IPGTT) and insulin tolerance tests (ITT) were performed at the end of each study. For IPGTT, mice were fasted for 4 h and injected intraperitoneally with a 20% glucose solution prepared in PBS (1 mg/g body weight). Blood was drawn from the tail vein before and after the glucose injection (at −30, 0, 15, 30, 60, 90 and 120 min) and blood glucose was measured using a Bayer glucometer. Additional blood was collected for insulin measurement at 0, 15 and 30 min. Insulin was measured by ELISA (CrytalChem). For ITT, mice were fasted for 4 h and injected intraperitoneally with insulin (0.75 U/kg body weight). Blood was drawn from the tail vein at 0, 15, 30, 60, 90 and 120 min for blood glucose measurements.

Blood from the vena porta and the vena cava was collected at the end of all experiments; 5 μl DPP4 inhibitor and 2 μl aprotinin (Millipore) were added to EDTA tubes and syringes before blood collection to prevent the degradation of GLP-1. Active GLP-1 was measured using

