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

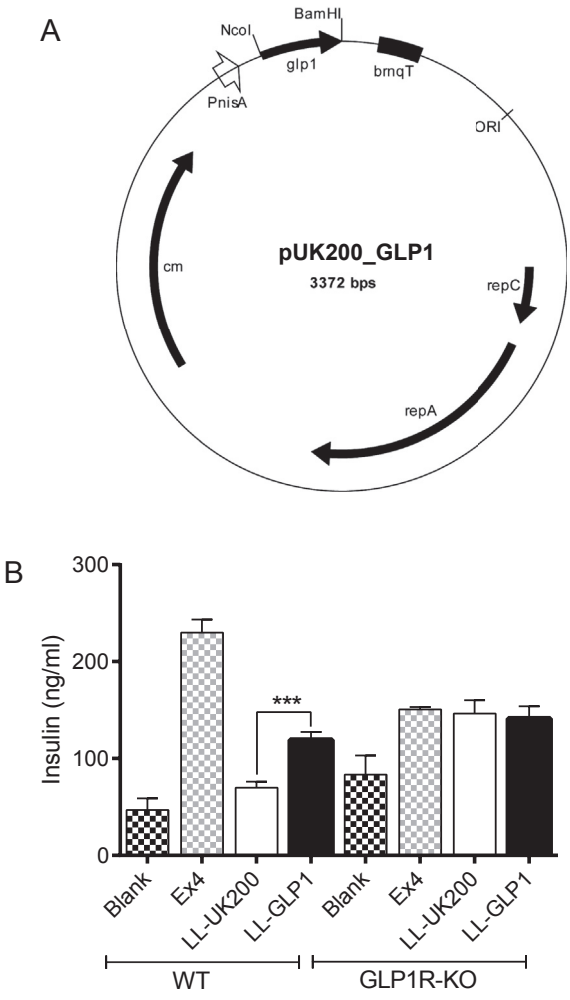

Figure 1: *In vitro* insulin release by recombinant *L. lactis*-derived GLP-1. (**A**) GLP-1 vector expressing murine GLP-1. (**B**) Insulin release from pancreatic islets of wild-type (WT, n = 3) and GLP-1 receptor knock out (GLP1R-KO, n = 3) mice treated with buffer alone (blank), exendin-4 (Ex4, positive 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

the Mesoscale discovery kit. Liver tissue and intestinal contents from ileum, caecum and colon were collected and snap frozen.

2.4. Quantitative PCR for *L. lactis*

Bacterial DNA was extracted from intestinal content of mice using the Machery Nagel NucleoSpin® Soil kit. The DNA was diluted to a concentration of 1 ng/μl and the total number of recombinant *L. lactis* cells was determined in a SYBR green-based qPCR using forward primer 5′-CCTTCTACCCATTATTACAGCAGG-3′ and reverse primer 5′-ACCACGACCTTTAACAAGCC-3′. The colonization level of LL-GLP1 was determined using forward primer 5′-AGCGAAGATGTTGTCTGTTAG-3′ and reverse primer 5′-GGCACTCGGCACTTAATG-3′. Total genomic DNA extracted from LL-GLP1 was used as standard.

2.5. RNA extraction and quantitative PCR

Mouse tissues were homogenised in RLT buffer supplemented with 2-mercaptoethanol using 5 mm steel beads and TissueLyser (Qiagen, Hilden, Germany). RNA was extracted using the RNeasy Kit with on-column DNase I treatment (Qiagen). RNA quantity and quality were examined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). cDNA was synthesised using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR reactions were set up in a 10 μl volume containing 1× SYBR Green Master Mix buffer (Thermo Scientific) and 900 nM gene-specific primers (or 300 nM L32 primers). Reactions were run on a CFX96 Real-Time System (Bio-Rad). Gene expression data were normalized to the ribosomal protein L32 using the ΔΔCT method. The primer sequences for phosphoenolpyruvate carboxykinase (*Pepck*) and glucose 6 phosphatase, catalytic subunit (*G6pc*) are forward 5′-GGCCACAGCTGCTGCAG-3′, reverse 5′- GGTCGCATGGCAAAGGG-3′, and forward 5′-CTGTGAGACCGGACCAGGA-3′, reverse 5′- GACCATAACATAGTATACACCTGCTGC-3′, respectively.

2.6. Statistical analysis

Data are shown as mean ± SEM. Unpaired Student's t test (for 2 groups) was performed to determine the significance between groups using GraphPad Prism software. Significance was established at $p < 0.05$.

3. RESULTS

3.1. Effect of LL-GLP1 on *ex vivo* insulin secretion

No GLP-1 was detectable in the culture supernatant of LL-UK200 but we measured 130 pg/ml of active GLP-1 in the culture supernatant of LL-GLP1. No difference in growth rates was observed between the two strains (data not shown). To determine the biological potential of the GLP-1 containing supernatant, we incubated primary islets isolated from WT or GLP1R-KO mice with LL-UK200 or LL-GLP1 culture supernatants (or exendin-4 as a positive control) under conditions of high glucose concentrations. Insulin release from primary islets from WT mice was significantly higher following incubation with LL-GLP1 compared with LL-UK200 culture supernatant (Figure 1B). This difference was not observed in islets from GLP1R-KO mice (Figure 1B), indicating that the biological response to the recombinant peptide was dependent on the GLP-1 receptor.

3.2. Effect of LL-GLP1 on glucose tolerance in chow-fed mice

To investigate the *in vivo* functionality of these strains, we first administered LL-UK200 and LL-GLP1 to WT mice on chow diet once daily for 9 days. By performing qPCR, we showed that the density of total recombinant *L. lactis* and LL-GLP1 was highest in the caecum followed by the colon and both strains were undetectable in the ileum (data not shown). The total recombinant *L. lactis* count in the caecal contents was similar in LL-UK200 and LL-GLP1 administered mice (Figure 2A) whereas LL-GLP1 was only present in the caecum of mice that received LL-GLP1 (Figure 2B). Active GLP-1 levels were significantly higher in the vena porta and showed a tendency to increase in the vena cava of mice treated with LL-GLP1 compared with LL-UK200 (Figure 2C). There was no significant effect of LL-GLP1 versus LL-UK200 administration on body weight (Figure 2D) or fat percentage (Figure 2E) over the study period.

Glucose tolerance (determined by an IPGTT) was improved in WT mice treated with LL-GLP1 compared with mice treated with the LL-UK200 control strain at the end of the study period (Figure 2F). However, there was no significant difference in insulin levels (Figure 2G) nor insulin tolerance (Figure 2H) between the two groups of mice. Hepatic expression of *G6pc* was reduced in LL-GLP1 supplemented mice (Figure 2I).

To determine if the improved glucose tolerance in response to LL-GLP1 administration was dependent on the GLP-1 receptor, we administered LL-UK200 and LL-GLP1 to GLP1R-KO mice on chow diet once daily for 9 days. The density of LL-GLP1 in caecal contents of GLP1R-KO mice (Supplementary Figure 1A) was similar to that observed in WT mice (Figure 2B). Active GLP-1 levels were also significantly higher in the vena porta of GLP1R-KO mice treated with LL-GLP1 compared with LL-UK200 (Supplementary Figure 1B). However, no changes in glucose tolerance were observed in GLP1R-KO mice administered with either LL-GLP1 or LL-UK200 (Supplementary Figure 1C), indicating a requirement of GLP-1 receptor for the biological response to LL-GLP1.

3.3. Effect of LL-GLP1 on glucose tolerance in HFD-fed mice

To investigate the functionality of LL-GLP1 in a model of impaired glucose tolerance, we tested the effect of LL-UK200 and LL-GLP1 in mice challenged with a HFD. LL-UK200 and LL-GLP1 were administered once daily for 3 weeks to mice on a chow diet and then for a further 3 weeks to the same mice on a HFD. Similar to the mice that received the strains for 9 days on a chow diet, the total recombinant *L. lactis* count in the caecal contents was similar in LL-UK200 and LL-GLP1 administered mice (Figure 3A), whereas LL-GLP1 was only present in the caecum of mice that received LL-GLP1 (Figure 3B). Active GLP-1 levels showed a tendency to increase in the vena porta of mice treated with LL-GLP1 compared with LL-UK200 (Figure 3C). There was no significant effect of LL-GLP1 versus LL-UK200 administration on body weight (Figure 3D) or fat percentage (Figure 2E) over the 6-week study period.

As expected, mice treated with the LL-UK200 control strain and fed a HFD for the final 3 weeks of the study had impaired glucose tolerance together with high fasting glucose and insulin levels (Figure 3F,G) compared with mice on chow (Figure 2F,G). Administration of LL-GLP1 promoted a significant improvement in glucose tolerance together with increased fasting insulin levels, and increased insulin levels 15 and 30 min after the glucose injection for the IPGTT (Figure 3F,G). Insulin tolerance did not differ between the LL-GLP1 and LL-UK200 groups (Figure 3H). Expression of *Pepck* was slightly increased whereas *G6pc* was reduced in the liver of LL-GLP1 supplemented mice on a HFD (Figure 3I).

4. DISCUSSION

In the present study, we generated a recombinant *L. lactis* strain that produced GLP-1 and demonstrated its biological efficacy in stimulating insulin secretion from primary islets of WT but not GLP1R-KO mice. In

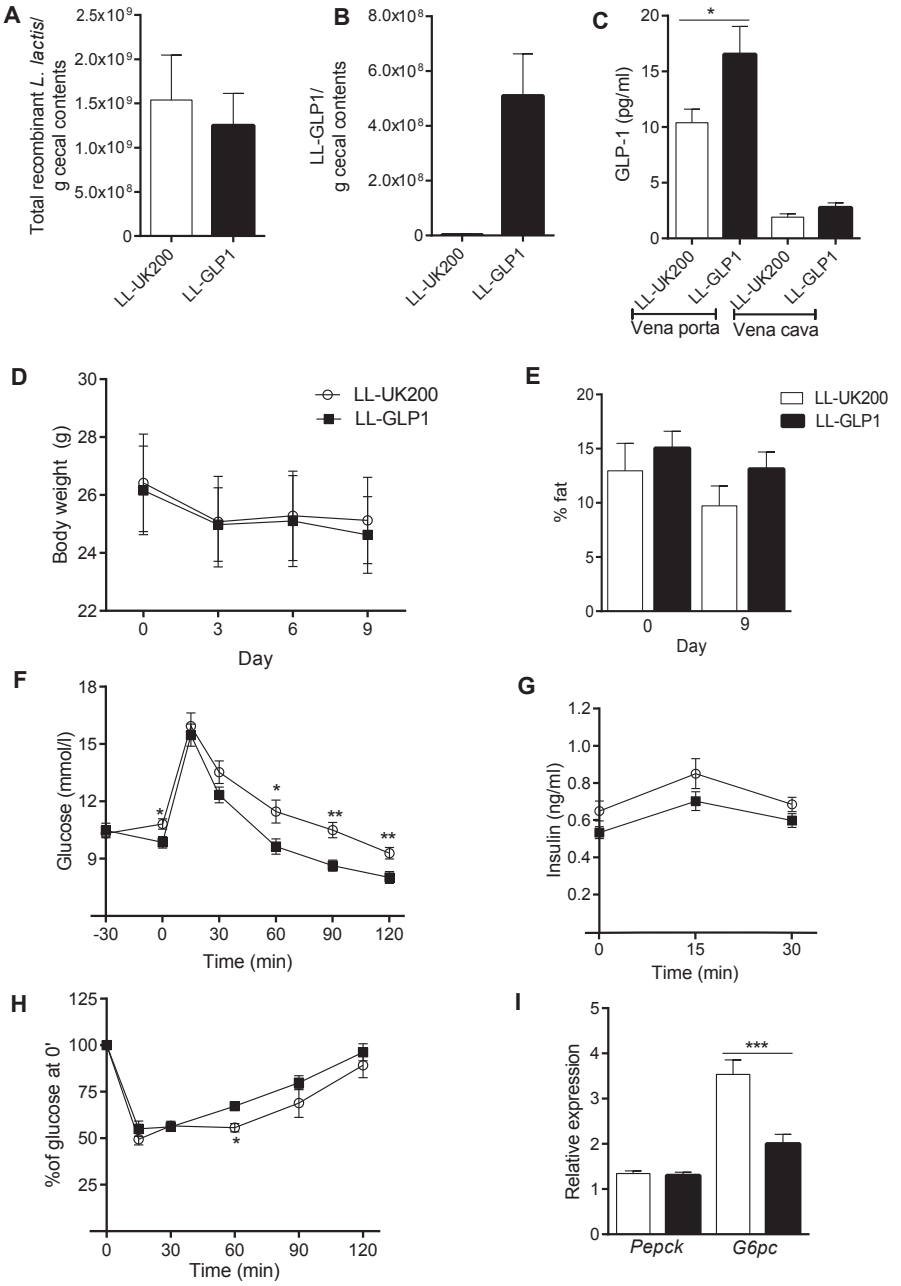

Figure 2: Effect of recombinant LL-GLP-1 in chow-fed mice. Copies of (**A**) total recombinant *L. lactis* and (**B**) LL-GLP1 in caecal contents of chow-fed mice (n = 12) supplemented with LL-GLP1 or the vector control LL-UK200 for 9 days. (**C**) GLP-1 levels in vena porta and vena cava plasma of chow-fed mice (n = 7) supplemented with LL-GLP1or LL-UK200 for 9 days. (**D**) Body weight curves and (**E**) fat percentage before and after 9 days of treatment in chow-fed mice (n = 5) supplemented with LL-UK200 or LL-GLP1. (**F**) Glucose and (**G**) insulin levels before and during an intraperitoneal glucose tolerance test and (**H**) glucose excursions during an insulin tolerance test in chow-fed mice (n = 11) supplemented with LL-UK200 or LL-GLP1 for 9 days. (**I**) Relative expression of *Pepck* and *G6pc* in the liver of chow-fed mice (n = 12) supplemented with LL-UK200 or LL-GLP1 for 9 days. Data are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, LL-GLP1 versus LL-UK200.

mice on a chow diet, glucose tolerance was improved following administration of LL-GLP1 versus the control LL-UK200 in WT but not in GLP1R-KO mice, indicating a requirement for the GLP-1 receptor. Similarly, supplementation with LL-GLP1 promoted a pronounced improvement in glucose tolerance together with increased insulin levels in mice fed HFD. 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.

A previous study has shown that *L. lactis* genetically modified to produce GLP-1 could stimulate insulin release from a pancreatic beta cell line HIT-T15 [11]. However, an advantage of our study is that we used islets from WT and GLP1R-KO mice, and could thus show that the effect of LL-GLP1 on insulin secretion was specifically mediated through the GLP-1 receptor. Furthermore, we showed that mice on a chow diet treated with LL-GLP1 versus LL-UK200 had higher vena porta levels of GLP-1. Although we cannot differentiate between

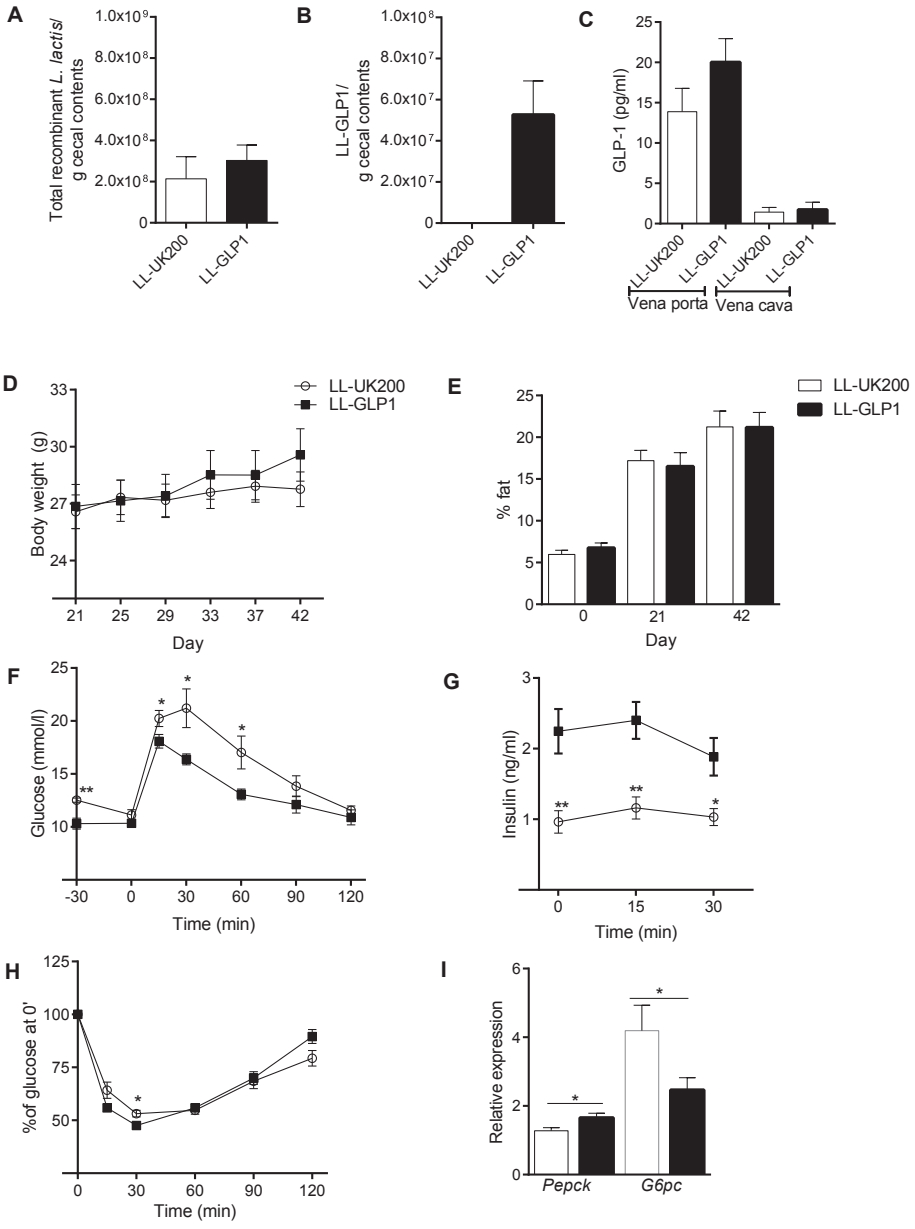

Figure 3: Effect of recombinant LL-GLP1 in HFD-fed mice. Copies of (**A**) total recombinant *L. lactis* and (**B**) LL-GLP1 in caecal contents of mice (n = 7–8) supplemented with LL-UK200 or LL-GLP1for 3 weeks on chow followed by 3 weeks on a HFD. (**C**) GLP-1 levels in vena porta and vena cava plasma at the end of the study, (**D**) body weight curves for the final 3 weeks of the study and (**E**) fat percentage at the indicated times in mice (n = 7–8) supplemented with LL-UK200 or LL-GLP1 for 3 weeks on chow followed by 3 weeks on a HFD. (**F**) Glucose and (**G**) insulin levels before and during an intraperitoneal glucose tolerance test, (**H**) glucose excursions during an insulin tolerance test, and (**I**) relative expression of *Pepck* and *G6pc* in the liver of mice (n = 7–8) supplemented with LL-UK200 or LL-GLP1for 3 weeks on chow followed by 3 weeks on a HFD. Data are mean ± SEM. *p < 0.05, **p < 0.01, LL-GLP1 versus LL-UK200.

endogenous and recombinant *L. lactis*-derived GLP-1, this observation suggests that GLP-1 was constitutively synthesised by LL-GLP1 and continuously diffused into the portal circulation. Our results are in accordance with a previous report that showed increased circulating levels of biologically active interleukin 12 after administration of genetically engineered *L. lactis* [12].

Although mice on a chow diet exhibited improved glucose tolerance in an IPGTT following supplementation with LL-GLP1 versus LL-UK200, no difference in insulin levels was observed. This result was surprising as many studies have reported that GLP-1 has a post-prandial insulinotropic effect under physiological conditions [13]. However, in light of its short half-life and non-proximity of L-cells with the pancreas, it has been proposed that GLP-1 is also sensed by the hepato-portal GLP-1 receptor that stimulates insulin-independent glucose clearance [14]. The presence of the GLP-1 receptor in extra pancreatic tissues such as the liver is still debated [15], but GLP-1 therapy has been reported to reduce expression of genes involved in hepatic gluconeogenesis, thereby reducing hepatic glucose production [16]. In agreement with this, we observed decreased expression of *G6pc* in mice supplemented with LL-GLP1, which may contribute to the improved glucose tolerance in the absence of altered insulin levels in mice on a chow diet.

We also investigated the effect of LL-GLP1 and LL-UK200 supplementation in HFD-fed mice, which is a robust model for impaired glucose tolerance [17]. Using this model, we observed a pronounced improvement of glucose tolerance together with increased insulin levels in mice supplemented with LL-GLP1 versus LL-UK200. Similar improvement in glucose tolerance (mixed meal over 8 h) coupled with high fasting and 8 h insulin levels was observed in patients with type 2 diabetes when they were assigned continuous subcutaneous infusion of GLP-1 for 6 weeks [18]. HFD is known to reduce the incretin effect, and earlier studies in mice have shown that HFD reduces the colonic expression of proglucagon, the gene that expresses GLP-1, and circulating GLP-1 levels [10,19]. Our results suggest that the constitutive synthesis of GLP-1 by the modified *L. lactis* strain can compensate for the reduction in incretin response induced by HFD. Additionally, reduced expression of *G6pc* may also account for reduced glucose output from liver thus contributing to an improved glucose metabolism.

Taken together, we demonstrated the efficacy of a *L. lactis* strain genetically modified to produce GLP-1 in stimulating insulin secretion in isolated mouse islets and improving glucose tolerance in mice on either chow or a HFD. In summary, these findings provide evidence that recombinant *L. lactis* strains may have potential clinical applications, although further research is clearly required.

ACKNOWLEDGEMENTS

We thank Rosie Perkins for editing the manuscript and are grateful to Carina Arvidsson, Sara Nordin-Larsson, Ulrica Enqvist, Caroline Wennberg and Zakarias Gulic for excellent animal husbandry. This study was supported by the Swedish Research Council Formas, Torsten Söderberg's Foundations, Ingabritt and Arne Lundberg's Foundation, Swedish Foundation for Strategic Research, Knut and Alice Wallenberg Foundation, and the regional agreement on medical training and clinical research (ALF) between Region Västra Götaland and Sahlgrenska University Hospital. FB is a recipient of ERC Consolidator Grant (European Research Council, Consolidator grant 615362 — METABASE). AN was supported by Biotechnology and Biological Sciences Research Council (BBSRC) strategic grant and both FB and AN received funding from EU FP7 TORNADO programme.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.06.006.

REFERENCES

[1] Drucker, D.J., 2007. The role of gut hormones in glucose homeostasis. The Journal of Clinical Investigation 117:24—32.

[2] Tomkin, G.H., 2014. Treatment of type 2 diabetes, lifestyle, GLP1 agonists and DPP4 inhibitors. World Journal of Diabetes 5:636—650.

[3] Uccellatore, A., Genovese, S., Dicembrini, I., Mannucci, E., Ceriello, A., 2015. Comparison review of short-acting and long-acting glucagon-like peptide-1 receptor agonists. Diabetes Therapy 6:239—256.

[4] Akiyode, O.F., desoye, A.A.A., Adverse effects associated with newer diabetes therapies: a review article *Journal of Pharmacy Practice* [Epub ahead of print].

[5] Kunji, E.R., Slotboom, D.J., Poolman, B., 2003. *Lactococcus lactis* as host for overproduction of functional membrane proteins. Biochimica et Biophysica Acta 1610:97—108.

[6] Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., et al., 2000. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science 289:1352—1355.

[7] Braat, H., Rottiers, P., Hommes, D.W., Huyghebaert, N., Remaut, E., Remon, J.P., et al., 2006. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clinical Gastroenterology and Hepatology 4: 754—759.

[8] Wegmann, U., Klein, J.R., Drumm, I., Kuipers, O.P., Henrich, B., 1999. Introduction of peptidase genes from *Lactobacillus delbrueckii* subsp. lactis into *Lactococcus lactis* and controlled expression. Applied and Environmental Microbiology 65:4729—4733.

[9] Dodd, H.M., Horn, N., Hao, Z., Gasson, M., 1992. A lactococcal expression system for engineered nisins. Applied and Environmental Microbiology 58: 3683—3693.

[10] Wichmann, A., Allahyar, A., Greiner, T.U., Plovier, H., Lunden, G.O., Larsson, T., et al., 2013. Microbial modulation of energy availability in the colon regulates intestinal transit. Cell Host & Microbe 14:582—590.

[11] Agarwal, P., Khatri, P., Billack, B., Low, W.K., Shao, J., 2014. Oral delivery of glucagon like peptide-1 by a recombinant *Lactococcus lactis*. Pharmaceutical Research 31:3404—3414.

[12] Fernandez, A., Horn, N., Wegmann, U., Nicoletti, C., Gasson, M.J., Narbad, A., 2009. Enhanced secretion of biologically active murine interleukin-12 by *Lactococcus lactis*. Applied and Environmental Microbiology 75:869—871.

[13] Drucker, D.J., 2006. The biology of incretin hormones. Cell Metabolism 3: 153—165.

[14] Burcelin, R., Da Costa, A., Drucker, D., Thorens, B., 2001. Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. Diabetes 50:1720—1728.

[15] Cantini, G., Mannucci, E., Luconi, M., 2016. Perspectives in GLP-1 research: new targets, new receptors. Trends in Endocrinology & Metabolism 27:427—438.

[16] Lee, Y.-S., Shin, S., Shigihara, T., Hahm, E., Liu, M.-J., Han, J., et al., 2007. Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. Diabetes 56:1671—1679.

[17] Winzell, M.S., Ahrén, B., 2004. The high-fat diet-fed mouse. A model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 53:S215—S219.

[18] Zander, M., Madsbad, S., Madsen, J.L., Holst, J.J., 2002. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. The Lancet 359: 824—830.

[19] Kappe, C., Zhang, Q., Nyström, T., Sjöholm, Å., 2014. Effects of high-fat diet and the anti-diabetic drug metformin on circulating GLP-1 and the relative number of intestinal L-cells. Diabetology & Metabolic Syndrome 6:1—8.

