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RESEARCH ARTICLE

Limosilactobacillus reuteri BIO7251 administration improves metabolic phenotype in obese mice fed a high fat diet: an inter-organ crosstalk between gut, adipose tissue and nervous system

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ABSTRACT

Gut microbiota is implicated in the control of host physiology by releasing bioactive actors that could exert a direct or indirect effect on tissue. A dysfunction of the gut microbiota to tissue axis could participate in the development of pathological states such as obesity and diabetes. The aim of this study was to identify the metabolic effect of *Limosilactobacillus reuteri* (known as *Lactobacillus reuteri*) BIO7251 (*L. reuteri* BIO7251) isolated from Corsican clementine orange. Body weight gain, adiposity, glucose tolerance, glucose absorption and food intake were measured in mice fed a high-fat diet in response to a preventive oral administration of *L. reuteri* BIO7251. This strain of bacteria exerts a beneficial effect on body weight gain by decreasing the subcutaneous adipose tissue mass. The treatment with *L. reuteri* BIO7251 decreases glucose absorption and food intake in obese/diabetic mice. *L. reuteri* BIO7251 could be tested as new probiotic strain that could manage body weight during obesity.

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Probiotic; adipose tissue; glucose absorption; hypothalamus

Introduction

Gut microbiota is now considered as a crucial partner implicated in the inter-organ dialogue impliying the control of physiological processes (de Vos et al. 2022). Intestinal dysbiosis observed in pathological states participates to the development of characteristic symptoms associated disorders. Nowadays, the development of clinical signs such as hyperglycaemia, body weight gain and insulin resistance is clearly linked with changes in the microbiota. Evidence suggests that modulating the gut microbiota population and/or bacterial releasing factors to prevent type 2 diabetes and obesity represents an innovative solution for patients (Van Hul and Cani 2023).

The communication between gut microbiota and host cells is achieved by a multitude of bioactive factors released by bacteria such as short-chain fatty acids (SCFAs), gaseous messengers and bioactive lipids (Cani and Knauf 2021). Thus, gut bacteria and their related mediators may have an impact on the whole body by acting directly on specific tissues and/or indirectly, that is by using hormonal and/or neuronal pathways. Consequently, the plethoric modes of actions of gut microbiota in the host physiology have important repercussions on metabolic functions. For example, gut microbes and their metabolites contribute to control food intake by stimulating the release of glucagon-like peptide 1 (GLP-1) but this hormonal effect also changes gut motility, fat storage, glycaemia and energy expenditure. Alterations of the different mechanisms of communications between gut bacteria and host cells participate in the development of metabolic disorders.

Can we manage glycemic profile and obesity *via* the gut microbiota? Evidence suggests that environmental factors and habits (e.g., diet, drugs, exercise) could have positive repercussions on glycemia and/or body weight loss (Cani et al. 2019; Van Hul and Cani 2023). Over the

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last two decades, among the preventive and therapeutic strategies, several focused on the gut microbiota and the identification of different prebiotics (Van Hul and Cani 2019), probiotics (de Vos et al. 2022) and bacterial-produced metabolites or proteins (Rastelli et al. 2019; Cani and Knauf 2021; Yoon et al. 2021). Although numerous probiotics have been identified and show beneficial effects on different host parameters, few of them have shown effects on obesity and type 2 diabetes.

Among all probiotics, data have shown that some *Limosilactobacillus reuteri* (*L. reuteri*) strains could have a potential interest on obese and/or diabetic patients. In an elegant review, Abuqwider et al. (Abuqwider et al. 2022) have described the complexity (and sometimes contradictory) actions of numerous strains of *L. reuteri* that exist in nature. For example, some strains exert a beneficial impact on body weight (Choi et al. 2021), others on GLP-1 release and insulin sensitivity (Simon et al. 2015), others on hepatic inflammation in response to lipopolysaccharides (Cui et al. 2019). Abuqwider et al. (Abuqwider et al. 2022) conclude that the effects of *L. reuteri* are strain dependent suggesting that future discoveries implicating the potential roles of this probiotic are only at the beginning, and that the identification of novel strains used alone or in combination with others could represent an innovative strategy to improve glycaemia and/or body weight gain. Therefore, in this study, we aimed to identify the impact of *L. reuteri* BIO7251 strain isolated from Corsican clementine in high-fat diet (HFD) fed mice on different metabolic parameters such as body weight, glycaemia, nutrient absorption and food intake.

Materials and methods

Lyophilised L. reuteri BIO7251 strain

L. reuteri BIO7251 was isolated in France from untreated Corsican clementine, identified as being a *L. reuteri* species by 16S rRNA and stored in the Bioprox collection. The strain was sequenced and the genome was deposited in NCBI (Accession: PRJNA980144). *L. reuteri* BIO7251 freeze-dried powder was obtained using a Bioprox proprietary industrial process. After a first fermentation step in 1 liter bioreactor, biomass was harvested at the stationary phase by centrifugation and protected with a proprietary lyoprotectant recipe (1:1; biomass:lyoprotectant) before a last step of freeze-drying. This concentrated powder was diluted by adding more lyoprotectant in order to reach 1×10^9 CFU in 150 µL PBS for daily gavage feeding. A lyophilised placebo was prepared without bacterial biomass but containing the same quantity of lyoprotectant. The needed mg quantities of powders were resuspended extemporaneously in PBS for daily feeding gavage.

Mice

Nine-week-old male C57BL/6J mice (Charles River Laboratory, Saint-Germain-sur-l'Arbresle, France, $n=9$–10 per group) were housed in specific pathogen free conditions and in a controlled environment (room temperature of 22 ± 2 °C, 12 h daylight cycle) with free access to food and water. Mice were fed on High Fat Diet (HFD) containing 20% proteins, 20% carbohydrates, and 60% fats (Research Diet # D12492i, New Brunswick, NJ, USA) for 5 weeks. Mice were treated daily with an oral gavage of 1×10^9 bacterial cells in a volume of 150 µL for the 5 weeks of HFD treatment. The oral gavage was performed each day at the end of the light phase (end of afternoon). All procedures are performed in accordance with the Directive 2010/63/UE recommendations and with French Veterinary Authorities agreement. All animals have been managed similarly, with due regard for their well-being, according to prevailing practices and the current SOPs in force at Enterosys. The design and procedures were approved by Ethical Committee (under ethical protocol APAFIS#11167-2018090712278926).

Body weight, food intake and tissue weights

The body weight, food and water intake were assessed weekly. At the end of the protocol (week 5), after a 2h-fasting period, all animals were weighed. Immediately after cervical dislocation, livers were weighed and sampled for biochemical analyses. Adipose tissues (epididymal, subcutaneous, visceral, and brown adipose tissue) were precisely dissected and weighed. A part of subcutaneous tissue (without lymph node) was sampled for histological analysis and a second part was immediately snap frozen in liquid nitrogen. The brown, visceral and epididymal adipose tissues were immediately snap frozen in liquid nitrogen. Intestinal segments (duodenum), liver and caecum were snap frozen in liquid nitrogen and stored at −80 °C for further analysis. The hypothalami were precisely dissected and stored at −80 °C for further analysis.

Histology

The subcutaneous adipose tissues without lymph node were fixed in 4% paraformaldehyde for 24h at room temperature. Samples were then immersed in ethanol

100% for 24h before processing for paraffin embedding. To determine the adipocyte tissue diameter, paraffin sections of 5 µm were stained with haematoxylin and eosin. The randomly selected fields were analysed for each sample by using an image analyser (ImageJ) to determine the mean adipocyte size and the distribution of the size of adipocytes.

RT-qPCR

The RNAs of the different organs (subcutaneous adipose tissue, duodenum and hypothalamus) were extracted individually using TriReagent, according to the manufacturer's instructions. Quantification analysis of total RNA was performed by analysing 1 µl of each sample in Nanodrop. cDNA was prepared by reverse transcription of 500 ng total RNA using a RT iScript kit (Biorad). Real-time PCR was performed with the Via7 real-time PCR system and software (Applied biotechnology) using SYBR Green Real-Time PCR Master Mixes (Biorad) for detection, according to the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), Beta 2 microglobulin (*b2m*) or ribosomal protein L19 (*rpl19*) were chosen as the housekeeping gene, depending on the organ of interest. All samples were performed in duplicate, and data were analysed according to the $2^{\Delta\Delta CT}$ method. The identity and purity of the amplified product was assessed by melting curve analysis at the end of amplification. All analysed genes were summarised in the following Table 1. Primers for the target and reference genes were designed using Primer designing tool (NCBI), ensuring that they spanned exon-exon junctions. The efficiency of each primer pair was meticulously validated, and only those with efficiencies ranging between 90% and 110% were considered for further analysis.

Plasma assays

Plasma triglycerides, total cholesterol, HDL, LDL and free fatty acids were analysed on blood samples collected at the necropsy using Pentra 400. Plasma leptin and adiponectin levels were analysed on blood samples collected at the necropsy using ELISA assay, according to the manufacturer instructions.

Glucose tolerance, insulin assay and HOMA-index

After 4 weeks of treatment, 6h-fasted mice were orally loaded with glucose (2 g/kg of body weight), around 12h after the last oral administration of vehicle or treatments. Glycaemia was measured at −30, 0 (time of oral glucose loading), +15, +30, +45, +60, +90 and

Table 1. Primers sequences.

Organs	Genes of interest	Sense	Anti-sense
Subcutaneous Adipose Tisse (SAT)	*Lbp*	GTCCTGGGAATCTGTCCTTG	CCGGTAACCTTGCTGTTGTT
	Itgax	GATTTCAGCATCCCAGATCCC	CCAGATCCACCAGTCCATCC
	Mcp1	CCTCGAAACTGGCTTTTGTT	AAGTCGTCCTCCCTGAATGA
	Fabp4	TGTGGGAACCTGGAAGCTTGTC	TCTGACCGGATGGTGACCAAA
	Fasn	ATCCTGGAACGAGAACACGATCT	AGAGACGTGTCACTCCTGGACTT
	Acc1	TGTACAAGCAGTGTGGGCTGGCT	CCACATGGCCTGGCTTGGAGGG
	Plin2	GTGTCGTCGTAGCCGATGC	CCATTTCTCAGCTCCACTCCAC
	Cebpa	CGCAAGAGCCGAGATAAAGC	CGGTCATTGTCACTGGTCAACT
	F4/80	TGACAACCAGACGGCTTGTG	GCAGGCGAGGAAAAGATAGTGT
	CD68	CTTCCCACAGGCAGCACAG	AATGATGAGAGGCAGCAAGAGG
	Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Intestine	*Glut2*	TGGAAGGATCAAAGCAATGTTG	CATCAAGAGGGCTCCAGTCAA
	Glut5	GGCTTCTCCACCTGCCTCATA	TAGGGCATCCAGGAGATGGTGT
	Sglt1	TCCCGTATGATCACTGAGTTTGC	GGCAGTTGCTGGGTTCCAT
	nNos	ACGTCAAGTACGCCACCAACA	GCGAGTTCCACACTCGGAAGT
	Chat	TGATCTTTGCTCGGCAGCACT	TTGGCCCAGTCAGTGGGAATG
	B2m	TGGTCTTTCTGGTGCTTGTCT	TATGTTCGGCTTCCCATTCTC
Hypothalamus	*Npy*	CAGAAAACGCCCCCAGAAC	CGGGAGAACAAGTTTCATTTCC
	Agrp	CGGAGGTGCTAGATCCACAGA	AGGACTCGTGCAGCCTTACAC
	Pomc	AGGCCTGACACGTGGAAGAT	AGCAGGAGGGCCAGCAA
	Cart	TTCCTGCAATTCTTTCCTCTTGA	GGGAATATGGGAACCGAAGGT
	Il1b	TCGCTCAGGGTCACAAGAAA	CATCAGAGGCAAGGAGGAAAAC
	Tnfa	TGGGACAGTGACCTGGAGTGT	TTCGGAAAGCCCATTTGAGT
	nNos	ACGTCAAGTACGCCACCAACA	GCGAGTTCCACACTCGGAAGT
	eNos	GTGGTGACCAGCACATTTGGC	GCCTGACATTTCCATGAGCGC
	iNos	AACTTGAGCGAGGAGCAGGTG	CTGTCAGAGCCTCGTGGCTTT
	Glpr	GTTCACAGAACTCTCCTTCAC	GAAACTCCATCTGGACCTCA
	Lepr	TTCAGTTTTTCGCCAGCTAGGT	GAATAATGTCAAGTCCCCTTTCATC
	Adipor1	AGAAGGTCTCTCGGACTTTT	TCCCCATAATCAGTAGAGCAAT
	Adipor2	CTTTCCACACAACACAAGAATC	CTTTCTTTCTGTCCCTTCTTCT
	Rpl19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT

+120 min with a gluco-meter (Accu-Chek Active, Roche) on blood samples collected from the tip of the tail vein. Blood was also collected from the tail vein at −30 and +15min with heparinised capillaries. The blood was centrifuged at 13000 g during 5 min at 4 °C. At least 15 µL of plasma were stored in a 0.5 ml tube at −80 °C until further analyses. Insulinemia in samples from the OGTT (-30 and 15 min after glucose load) and insulinemia in blood samples taken just prior the start of the protocol on fasted animals were measured using HTRF method (Abot et al. 2018). Homa-index was calculated as follow: insulinemia (mU/L) x glycaemia(mmol/L)/22,5.

Glucose absorption

The proximal part of the gut was collected then harvested, washed, everted, and filled with in a Krebs–Ringer solution without glucose. Everted intestinal sacs were incubated in Krebs–Ringer with 10 g/L of glucose, for 2 min at 37 °C as published previously [13]. The media of each sac was collected and immediately frozen for subsequent glucose quantification study. Glucose was measured using Glucose GOD FS 10'kit (DiaSys, France).

Statistics

The data were expressed as the mean ± SEM. Differences between the experimental groups were assessed where appropriate using by unpaired Student's, two-way ANOVA, followed by post-hoc test. Data were analysed using GraphPad Prism version 8.00 for Windows (GraphPad Software, San Diego, CA, USA). The results were considered statistically significant at $p < 0.05$.

Results

L. reuteri BIO7251 decreases subcutaneous adipose tissue weight in HFD mice

HFD mice treated with *L. reuteri* BIO7251 present a slightly lower body weight gain along the treatment, but this effect did not reached significance (Figure 1a–d). However, the body weight gain was significantly lower in *L. reuteri* BIO7251 treated mice at week 3 (3.71 ± 0.30 *vs* 2.66 ± 0.34 g, $p < 0.05$, Figure 1b). At the end of the experiment, we observed a significant decrease of subcutaneous adipose tissue weight (4.05 ± 0.25 *vs* 2.53 ± 0.28% body weight, $p < 0.001$, Figure 2a) without impacting other adipose

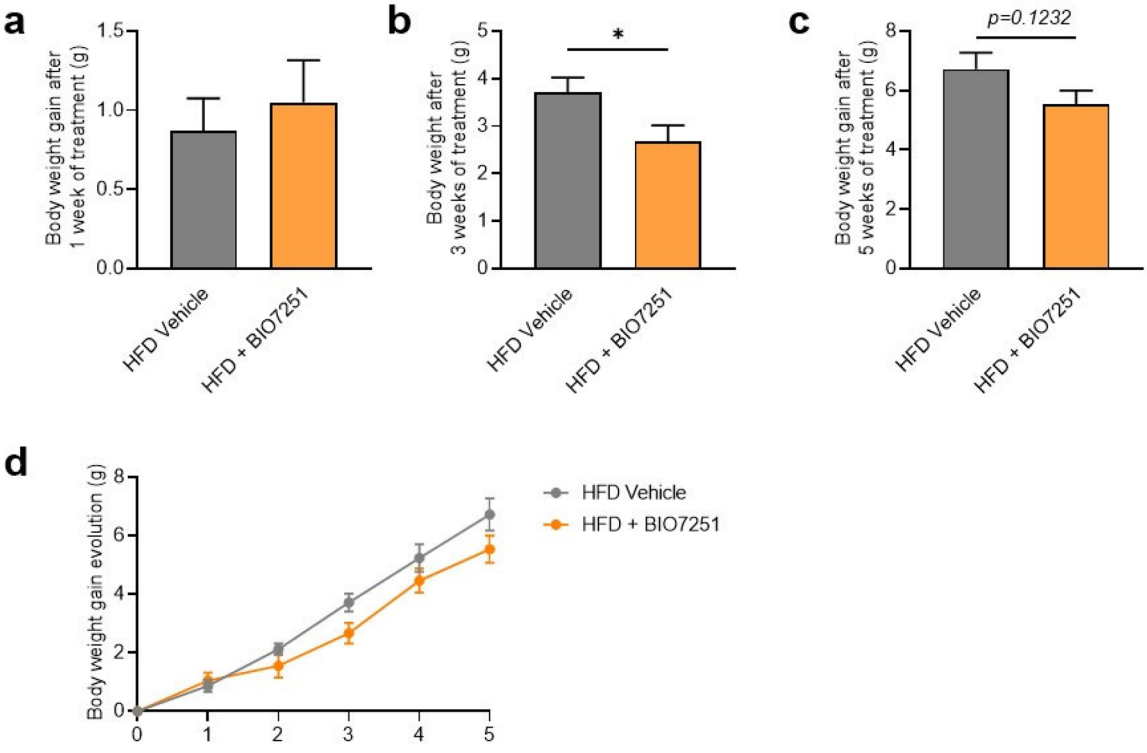

Figure 1. Body weight monitoring. Effects of an oral administration of vehicle or *L. reuteri* BIO7251 on (a) body weight gain after 1 week of treatment; (b) body weight gain after 3 weeks of treatment; (c) body weight gain after 5 weeks of treatment; (d) body weight gain over time. $n = 9–10$ per group. In panel a-c, described p values obtained with a t-test $^*p < 0.05$ *vs* HFD vehicle. In panel d, described p values obtained with 2-way ANOVA followed by Bonferroni's post-hoc test.

tissue (Figure 2b–e), liver (Figure 2f) or caecum (Figure 2g) weights.

L. reuteri BIO7251 reduces adipocyte size and adipose tissue inflammation in HFD mice

Adipocyte hypertrophy is a major factor for obesity. *L. reuteri* BIO7251 supplementation significantly reduced the adipocyte size, including the number of large adipocytes ($9.97\times10^7\pm2.65\times10^7$ *vs* $3.55\times10^7\pm0.40\times10^7$, $p<0.05$) which are also known as adipocyte hypertrophy (Plovier et al. 2017; Van Hul et al. 2018) (Figure 3a). Diabetes and insulin resistance are frequently associated with an altered fatty acid metabolism and adipose tissue inflammation (Plovier et al. 2017; Van Hul et al. 2018). We compared the expression of biomarkers involved in fatty acids biosynthesis (Fatty acid synthase 1, *Fasn1*; acetyl-CoA carboxylase 1, *Acc1*; CCAAT/enhancer-binding protein alpha, *Cepba*), transport and storage (Fatty Acid Binding Protein 4, *Fapb4* and Perilipin 2, *Plin2*). *Acc1* mRNA expression was significantly decreased in response to bacterial supplementation (1.00 ± 0.23 *vs* 0.10 ± 0.01, $p<0.01$, Figure 3b). Furthermore, we measured various inflammation and macrophage infiltration markers. EGF-like module-*F4/80* (1.00 ± 0.13 *vs* 0.59 ± 0.07, $p<0.05$) and *CD68* (1.00 ± 0.15 *vs* 0.38 ± 0.09, $p<0.01$) mRNA expression were reduced by *L. reuteri* BIO7251 supplementation (Figure 3c). *L. reuteri* BIO7251 significantly increases the quantity of free fatty acid in the plasma (0.60 ± 0.02 *vs* 0.79 ± 0.05 mmol/L, $p<0.01$) without any impact on plasma total and HDL cholesterol, triglycerides and adipokines (adiponectin, leptin) levels (Figure 3d). In addition, a decreasing trend to plasma LDL is observed in *L. reuteri* BIO7251 (0.32 ± 0.02 *vs* 0.26 ± 0.01 mmol/L, $p=0.0567$, Figure 3d). Plasma adiponectin and leptin did not vary between the 2 experimental conditions (Figure 3e).

L. reuteri BIO7251 has not significant impact on diabetic phenotype in HFD mice

To investigate the impact of *L. reuteri* BIO7251 on glucose homeostasis, we measured several metabolic parameters in HFD mice treated or not with this probiotic. The treatment with *L. reuteri* BIO7251 in HFD mice did not improve fasted glycaemia (Figure 4a), fasted insulinemia (Figure 4b), HOMA index (Figure 4c) and glucose tolerance (Figure 4d–g).

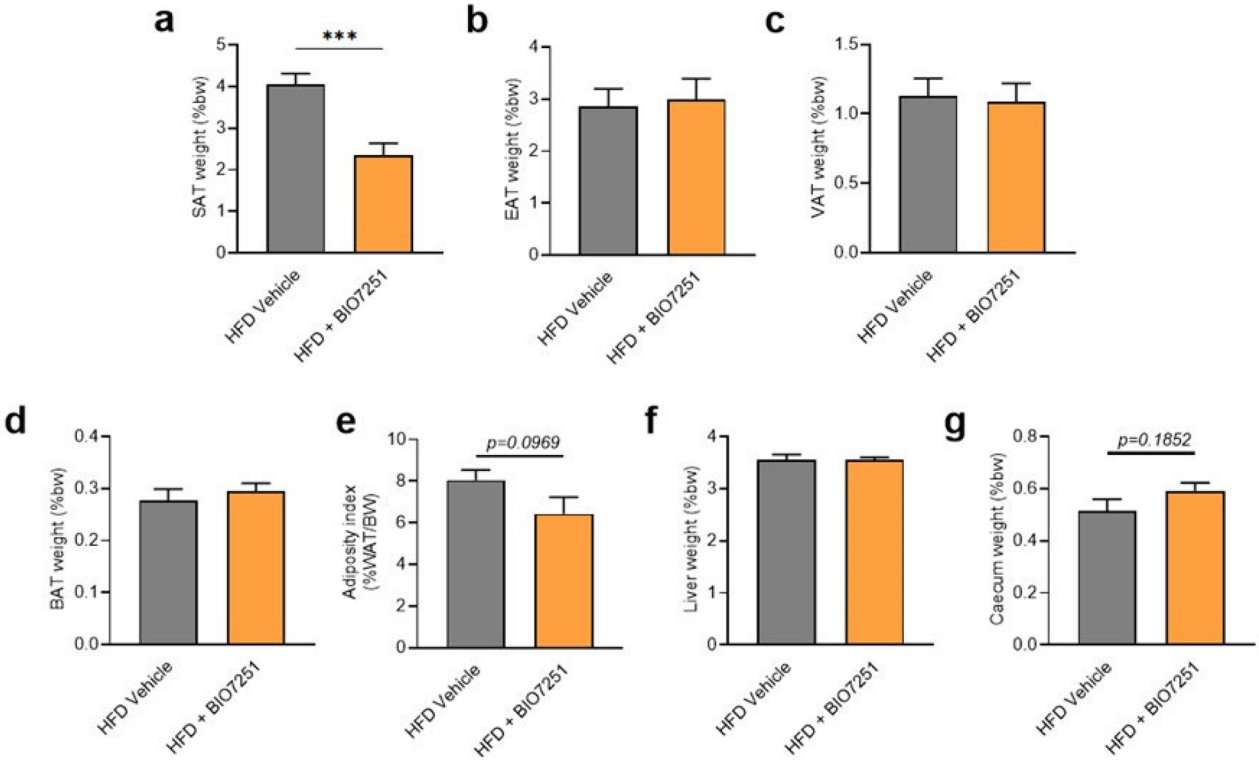

Figure 2. Relative fat mass distribution and relative caecum and liver weight (% of body weight). effects of an oral administration of vehicle or *L. reuteri* BIO7251 for 5 weeks on (a) subcutaneous adipose tissue (SAT); (b) epididymal adipose tissue (EAT); (c) visceral adipose tissue (VAT); (d) brown adipose tissue (BAT); (e) Adiposity index; (f) liver; (g) caecum. $n=9$–10 per group. In panel a, data were significantly different ($p<0.05$) according to a t-test.

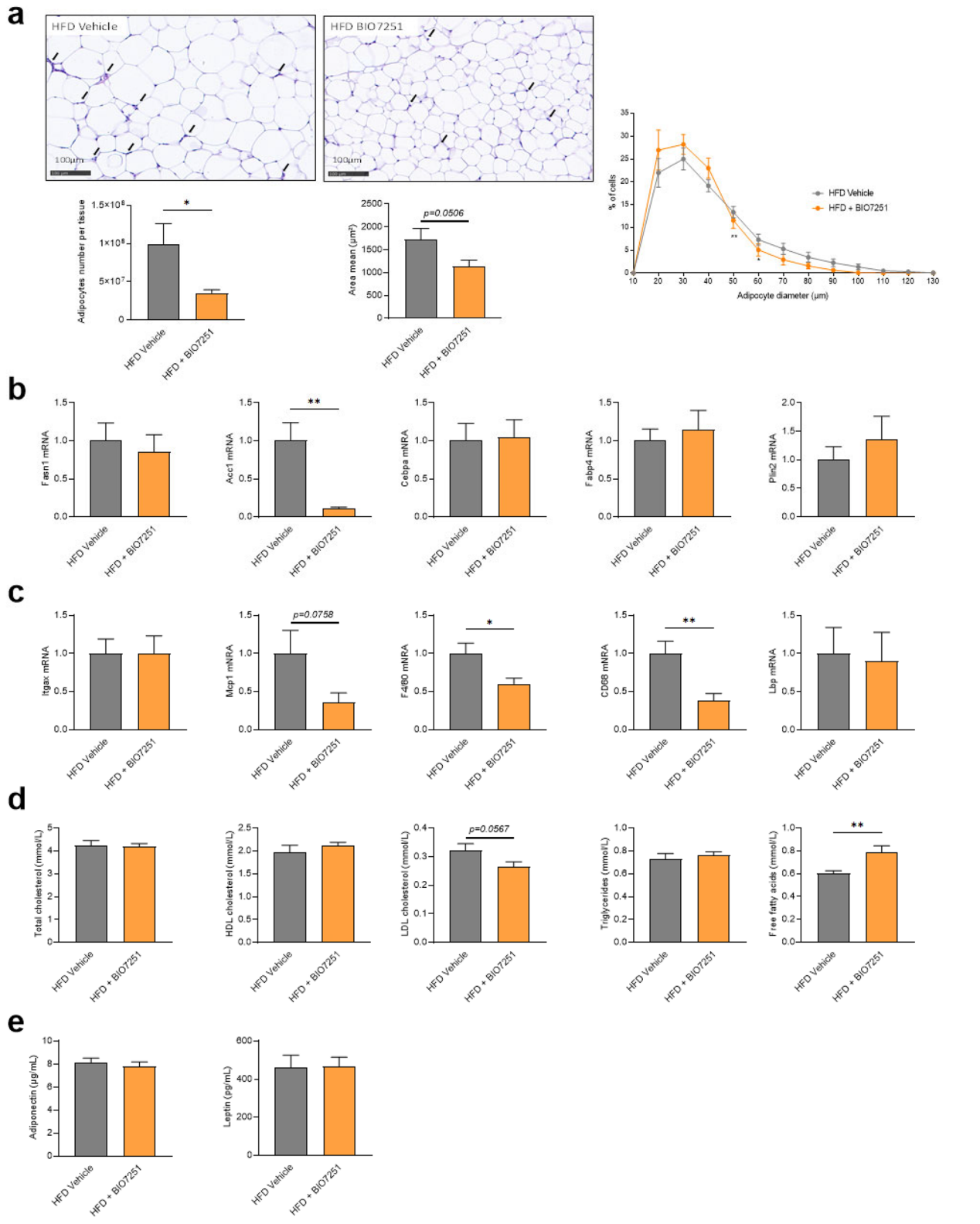

Figure 3. Lipid metabolism and inflammation. Effects of an oral administration of vehicle or *L. reuteri* BIO7251 on (a) Representative histology images of subcutaneous adipose tissue sections with Haematoxylin-Eosin staining and adipocytes number, area, and distribution analysis (scale bars, 100 µm). arrows represents immune cells infiltration; (b) various markers in the subcutaneous adipose tissue (PCR analysis of fold change vs. HFD vehicle group) involved in fatty acids metabolism mRNA relative expression: *Fasn1*, *cebpa*, *Fabp4*, *Plin2*, and *Acc1*; (c) biomarkers involved in inflammation mRNA relative expression: CD11c (*itgax*), lipopolysaccharide Binding protein (*lbp*), monocyte chemoattractant protein-1 (*Mcp1*), *F4/80* and *CD68*; (d) Total plasma lipids level: total cholesterol level, HDL and LDL cholesterol level, triglycerides level and free fatty acids level; (e) total plasma adipokines level: adiponectin and leptin level. $n = 9–10$ per group. In panels a-d, data were significantly different ($p < 0.05$) according to a *t*-test.

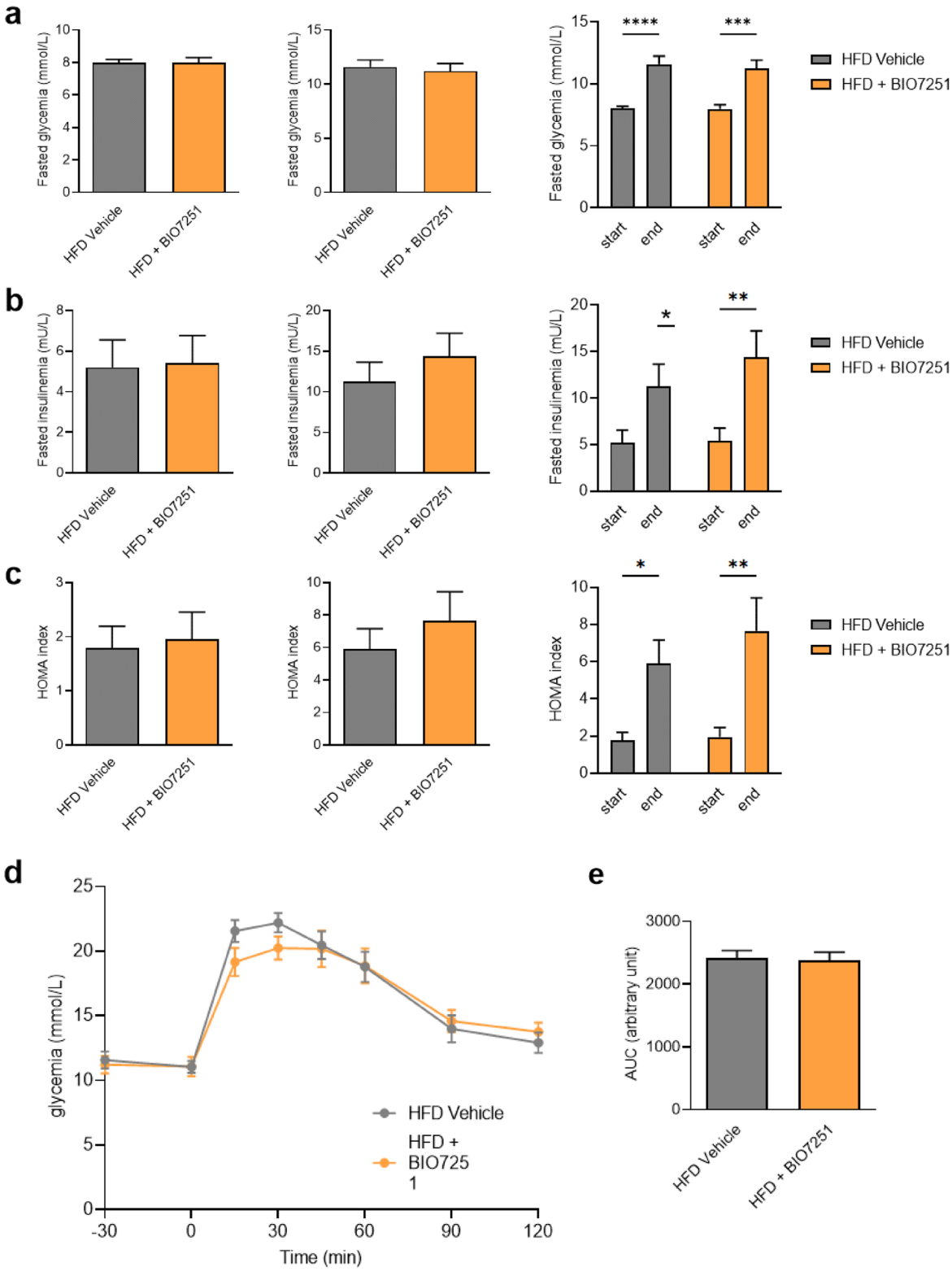

Figure 4. Glucose metabolism. Effects of an oral administration of vehicle or *L. reuteri* BIO7251 on (a) fasted glycaemia at the beginning, the end of the protocol and the evolution, (b) fasted insulinemia at the beginning, the end of the protocol and the evolution, (c) Homa index calculated as insulinemia (mU/L) x glycaemia (mmol/L)/22,5 at the beginning, the end of the protocol and the evolution, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ start vs. end of the protocol using a 2-way ANOVA followed by bonferroni's post-hoc test; (d) Oral glucose tolerance test (OGTT) after 4 weeks of treatment and glycaemia regulation before and after an oral load of glucose (2 g/kg of body weight); (e) the average area under the curve (AUC); (f) fasted glycaemia at T= −30min; (g) insulinemia at t-30min and $t + 15$ min the oral load of glucose; (h) gene expression (PCR analysis of fold change *vs*. HFD vehicle group) of various markers of the glucose and fructose absorption in the duodenum *Glut2*, *Glut5* and *sglt-1* mRNA relative expression; (i) Gene expression (PCR analysis of fold change *vs*. HFD vehicle group) of biomarkers of enteric nervous system activity in the duodenum, *nNos* and *Chat* mRNA relative expression; (j) *ex vivo* glucose absorption in duodenal everted sacs. $n = 9$-10 per group. In panels a–c, i and j, data were significantly different ($p < 0.05$) according to a *t*-test.

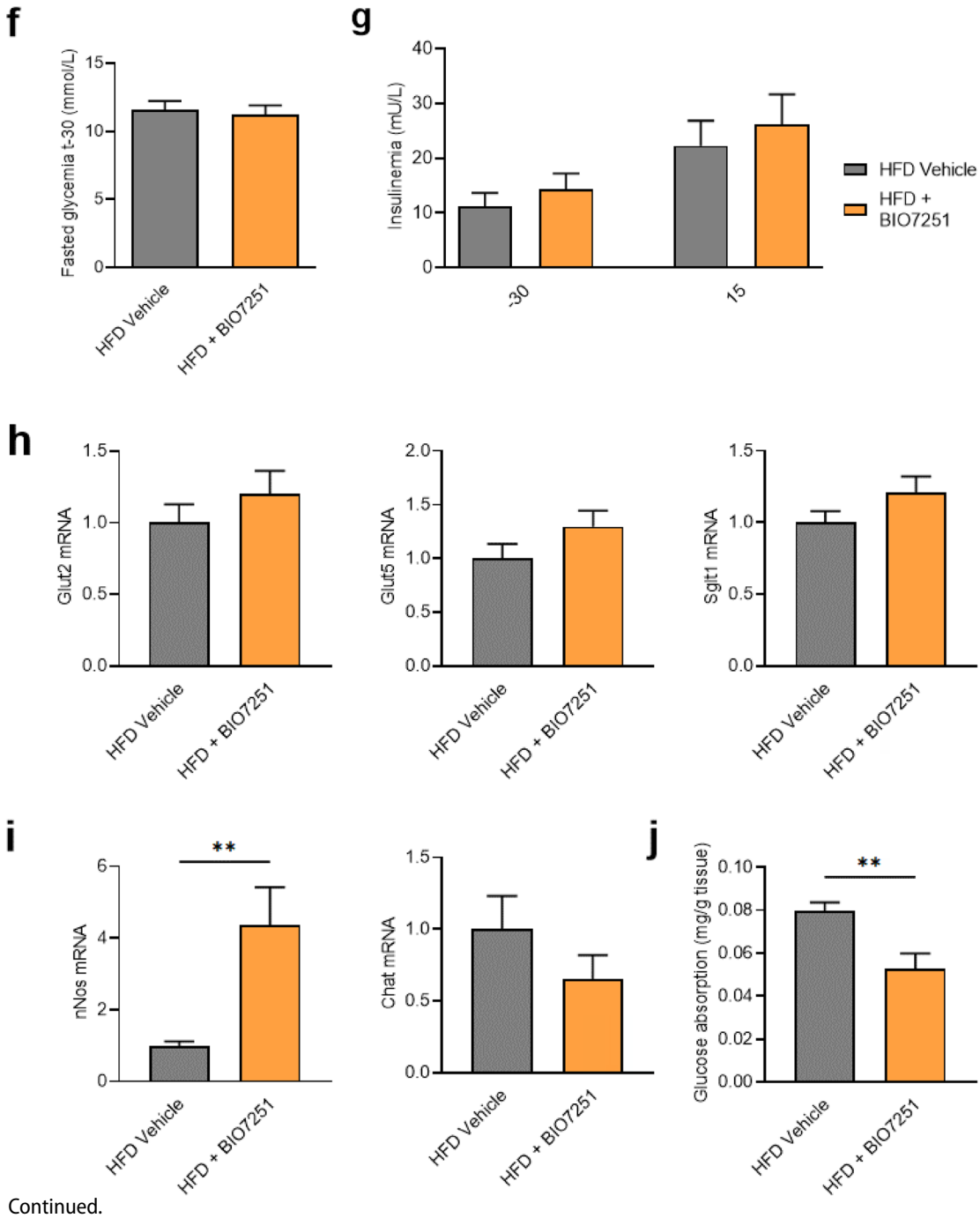

Figure 4. Continued.

L. reuteri BIO7251 decreases glucose absorption in HFD mice

Glucose absorption could be under the influence of the quantity of glucose transporters on enterocytes (Wemelle et al. 2022), but also under the dependence of the intestinal motility (Fournel et al. 2017; Abot et al. 2018). In fact, during feeding, an increase of intestinal motility is associated with an increase of glucose absorption by a mechanical physical interaction that increases the contact surface between nutrients and intestinal epithelium (Sababi and Bengtsson 2001). As shown in Figure 4h, *L. reuteri* BIO7251 did not modify the expression of nutrients (glucose, fructose) transporters in the intestine of HFD mice compared to its control. Interestingly, HFD mice treated with *L. reuteri* BIO7251 presented a significant increase of mRNA expression of *nNos* in the intestine (1.00 ± 0.11 *vs* 4.37 ± 1.04, $p < 0.01$, Figure 4i), an enzyme that produces the major inhibitory neurotransmitter of

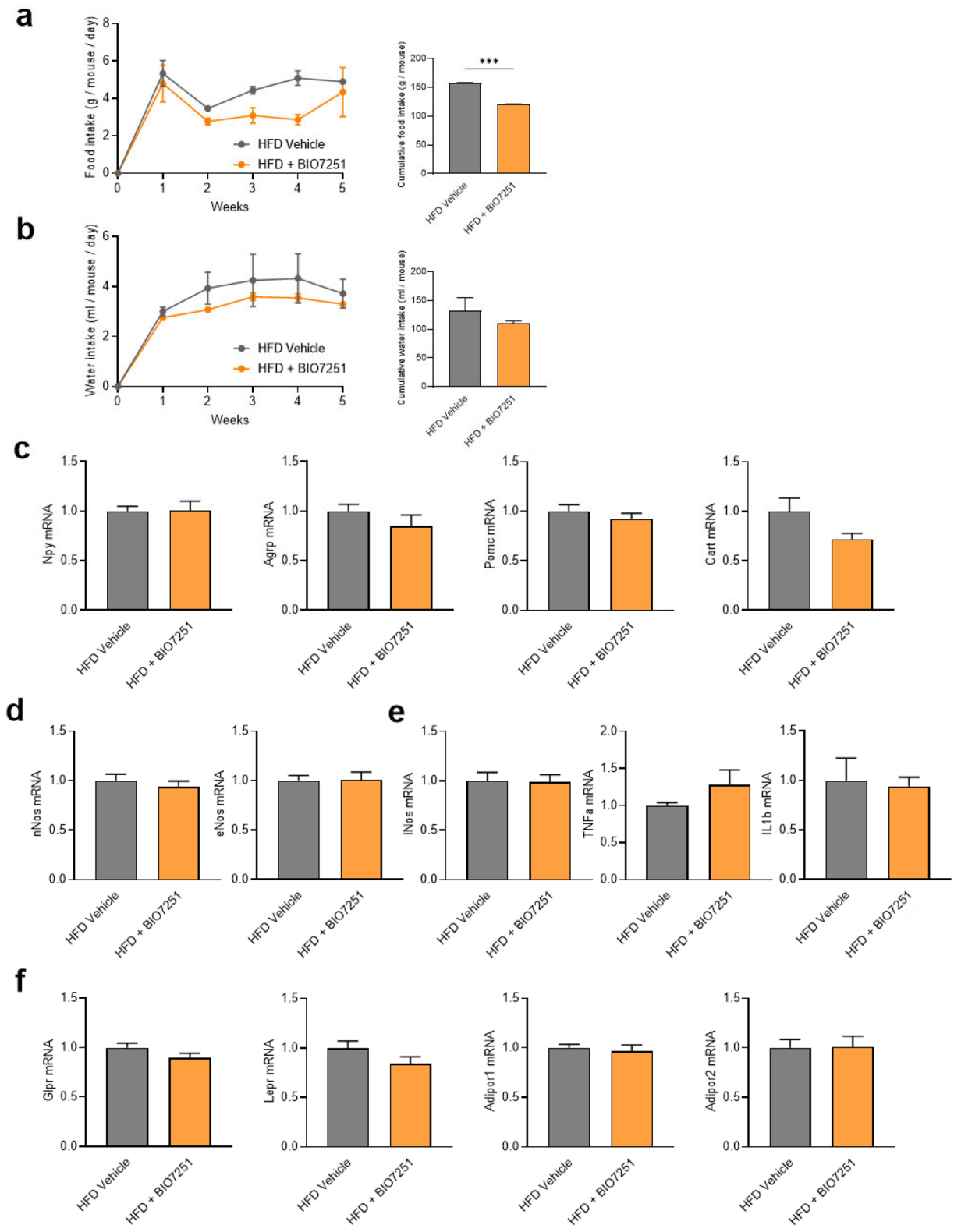

Figure 5. Food and water intake monitoring. Effects of an oral administration of vehicle or *L. reuteri* BIO7251 on (a) the food intake over the time and cumulative food intake; (b) the water intake over the time and cumulative water intake, no significant difference was observed; (c) Orexigenic (*Npy* and *Agrp*) and anorexigenic (*Pomc* and *Cart*) neuropeptides mRNA relative expression in the hypothalamus, no significant difference was observed; (d) *nNos* and *eNos* mRNA relative expression in the hypothalamus, no significant difference was observed; (e) inflammatory biomarkers, *iNos*, *tnfa* and *IL1β* in the hypothalamus, no significant difference was observed; (f) biomarkers involved in endocrine signalling pathways, *glpr*, *Lepr*, *Adipor1* and *Adipor2* in the hypothalamus, no significant difference was observed. $n = 9–10$ per group; 2 cages/group. In panels a, data were significantly different ($p < 0.05$) according to a *t*-test, in panels a and b, for food and water intake over the time, a two-way ANOVA following a Bonferroni's post-hoc test was performed.

intestinal contraction i.e. Nitric Oxide (NO). No modification of the mRNA expression of *Chat*, an enzyme that produces acetylcholine which has a powerful stimulatory effect on gut motility (Figure 4i). This molecular action of *L. reuteri* BIO7251 in the intestine of HFD mice was associated with a decrease of glucose absorption compared to HFD control mice (0.079 ± 0.004 *vs* 0.052 ± 0.007 mg per g of tissue, $p < 0.01$, Figure 4j).

L. reuteri BIO7251 decreases food intake in HFD mice

Food intake (157.11 ± 0.99 *vs* 120.27 ± 0.23 g/mouse, $p < 0.001$, Figure 5a), but not water intake (Figure 5b), is significantly decreased in HFD mice treated with *L. reuteri* BIO7251 compared to HFD mice. However, none of the mRNA coding for orexigenic (Neuropeptide Y, *Npy*; Agouti Related Protein, *Agrp*) and anorexigenic (Proopiomelanocortin, *Pomc*; Cocaine and Amphetamine Related Transcript, *Cart*) factors were changed in the hypothalamus (Figure 5c). In addition, the mRNA expression of neuronal Nitric Oxide Synthase (*nNos*) and endothelial NOS (*eNos*) that released Nitric Oxide (NO) in the hypothalamus did not varied (Figure 5d). This is also the case for mRNA expression of proinflammatory markers (inducible NOS, *iNos*; Tumour necrosis factor alpha, *Tnfa* and Interleukin 1 beta, *Il1b*) (Figure 5e), GLP-1 receptor (*Glp1r*), leptin receptor (*Lepr*) and adiponectin receptors (*Adipor1* and *Adipor2*) (Figure 5f).

Discussion

The probiotic approach to preventing metabolic diseases is constantly evolving, given the number of new bacteria discovered every year. Among all known bacteria, *L. reuteri* is widely studied in the literature for its effects on obesity, insulin sensitivity, liver disorders and gut-brain axis (for review (Abuqwider et al. 2022)). Depending on the strain, *L. reuteri* could exert beneficial effects on one or more physiological parameters listed above. Identifying a new strain to use alone or in combination with another strain (with a potential different activity) could bring novel solution for obese and/or diabetic patients.

Here, we have studied the role of *L. reuteri* BIO7251 on numerous physiological parameters in obese and diabetic mice fed a 60% HFD. So far, nothing was known about its potential action on metabolic functions. Here, we discovered that this strain reduced subcutaneous fat accumulation. In addition, *L. reuteri* BIO7251 treatment decreased the size of adipocytes in SAT of HFD mice. This was associated with a decrease of mRNA expression of *Acc1* (an enzyme implicated in the de novo biosynthesis of long-chain fatty acids) and *CD68* and *F4/80* (markers of macrophage activity). Our results tend to show that *L. reuteri* BIO7251 could mitigate some of the classical hallmarks of obesity when this strain is used as a preventive approach. These effects were reinforced by the fact that *L. reuteri* BIO7251 decreased food intake in HFD mice compared to control. This effect was not associated with changes in the expression of several genes well-known to code for orexigenic and anorexigenic neurotransmitters or on other gaseous messenger like hypothalamic NO which is known to control glucose homeostasis (Abot et al. 2022). However, it has been demonstrated that these genes are under a circadian rhythm, and we may not exclude that the timing of tissue collection were adequate to precisely study this parameter (Paschos et al. 2012; Cani 2019; Challet 2019). Moreover, we cannot exclude that the decreased food intake observed with *L. reuteri* BIO7251 was the result of only other neurotransmitters released *via* an afferent nervous message, a hormonal signal or *via* a metabolite as described in the literature for probiotics (Rastelli et al. 2018, 2019).

The strain *L. reuteri* BIO7251 seems to have a better effect on fat mass than on glucose metabolism. Indeed, we failed to observe any effect on the bacteria on fasted glycaemia, fasted insulinemia and glucose tolerance. We cannot completely rule out an impact on glycaemia since we have demonstrated in this study that the probiotic exerts an inhibitory function on intestinal glucose absorption which is measured after a very short-term period of fasting i.e. after 2 h mimicked the post-prandial conditions, whereas the OGTT is performed after 6 h of fasting, a distance from the last oral gavage and with a relatively high-dose of glucose. Nevertheless, this inhibitory effect on glucose absorption was linked with the increased expression of *nNos* mRNA, an enzyme expressed in some neurons of the enteric nervous system (ENS) that releases NO and slows down gut motility (Knauf et al. 2020). We did not measure the contraction of the proximal part of the intestine since the large majority of studies that imply gut motility during diabetes and obesity are performed on a HFD45% model (Fournel et al. 2017; Abot et al. 2018, 2021; Wemelle et al. 2022). In fact, intestinal contractions are controlled by the ENS composed notably of ChAT and nNOS motoneurons. Increase the release of intestinal NO is associated with a restoration of the

gut-brain axis coupled to an improvement of insulin sensitivity in mice fed a HFD45% (Fournel et al. 2017; Abot et al. 2018, 2021; Wemelle et al. 2022). Molecules named enterosynes could modify the activity of ENS neurons to decrease gut motility in order to decrease glucose absorption and/or stimulate glucose utilisation *via* the gut-brain axis is considered as a novel potential approach to treat diabetes (Knauf et al. 2020). How *L. reuteri* BIO7251 could have an impact in the gut-brain axis? Our results show that there is no modification of glucose homeostasis in probiotic treated mice, but the decrease of food intake observed tends to show the existence of a '*L. reuteri* BIO7251- to hypothalamus' axis. Nothing is known concerning the impact on gut motility on food intake. At the opposite, numerous articles show that probiotic can modulate the release of gut hormones, metabolites or vagal afferences stimulation that could modify the activity of hypothalamic neurons (Asadi et al. 2022). Many avenues are therefore open to identify the strain's mode of action on food intake. To finish, experiments with oral gavage of *L. reuteri* BIO7251 could be performed during a longer period of treatment or with less stringent diet such as HFD45%. Another possibility could be the use of *L. reuteri* BIO7251 with other strain known to have anti-diabetic effect and measure the potential additional action of the combo of parameters studied here.

Disclosure statement

PDC and CK were co-founders of Enterosys SAS (France), AA, NP and GA are employed by Enterosys SAS (France), NR is employed by Biodis (France). PDC was co-founder of The Akkermansia company SA and coinventor on patents dealing with gut microbes and health.

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Data availability statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the industrial properties of the data.

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