Cell Metabolism
Article

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A Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an Enteric NO-Dependent and Gut-Brain Axis Mechanism

Estelle Grasset,[1,2] Anthony Puel,[1,2] Julie Charpentier,[1,2] Xavier Collet,[1,2] Jeffrey E. Christensen,[1,2] François Tercé,[1,2] and Rémy Burcelin[1,2,3,*]

[1]Institut National de la Santé et de la Recherche Médicale (INSERM), 31024 Toulouse, France
[2]Université Paul Sabatier (UPS), Unité Mixte de Recherche (UMR) 1048, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Team 2: Intestinal Risk Factors, Diabetes, Dyslipidemia, Heart Failure, F-31432 Toulouse, Cedex 4, France
[3]Lead Contact
*Correspondence: remy.burcelin@inserm.fr
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国家健康与医学研究院（INSERM）

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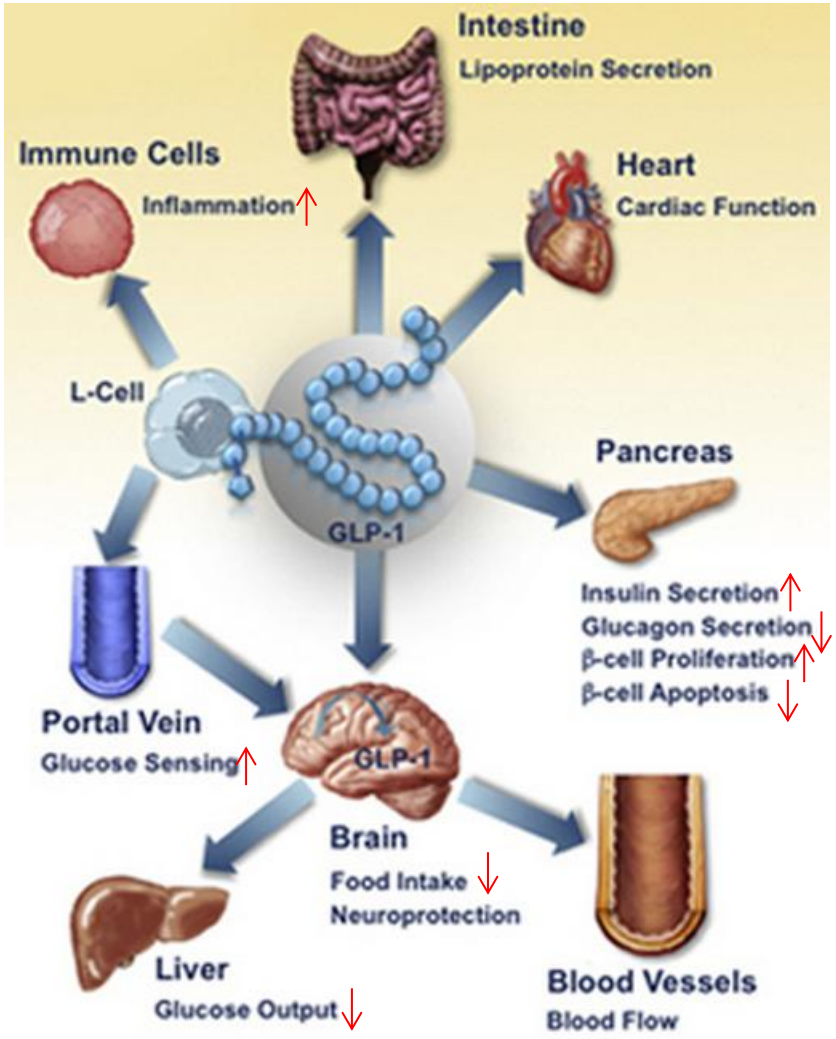
Content

1 Introduction



The function of GLP-1

GLP-1 regulates insulin and glucagon secretions, gastric emptying, food intake, and blood flow.

GLP-1-based therapies of T2D

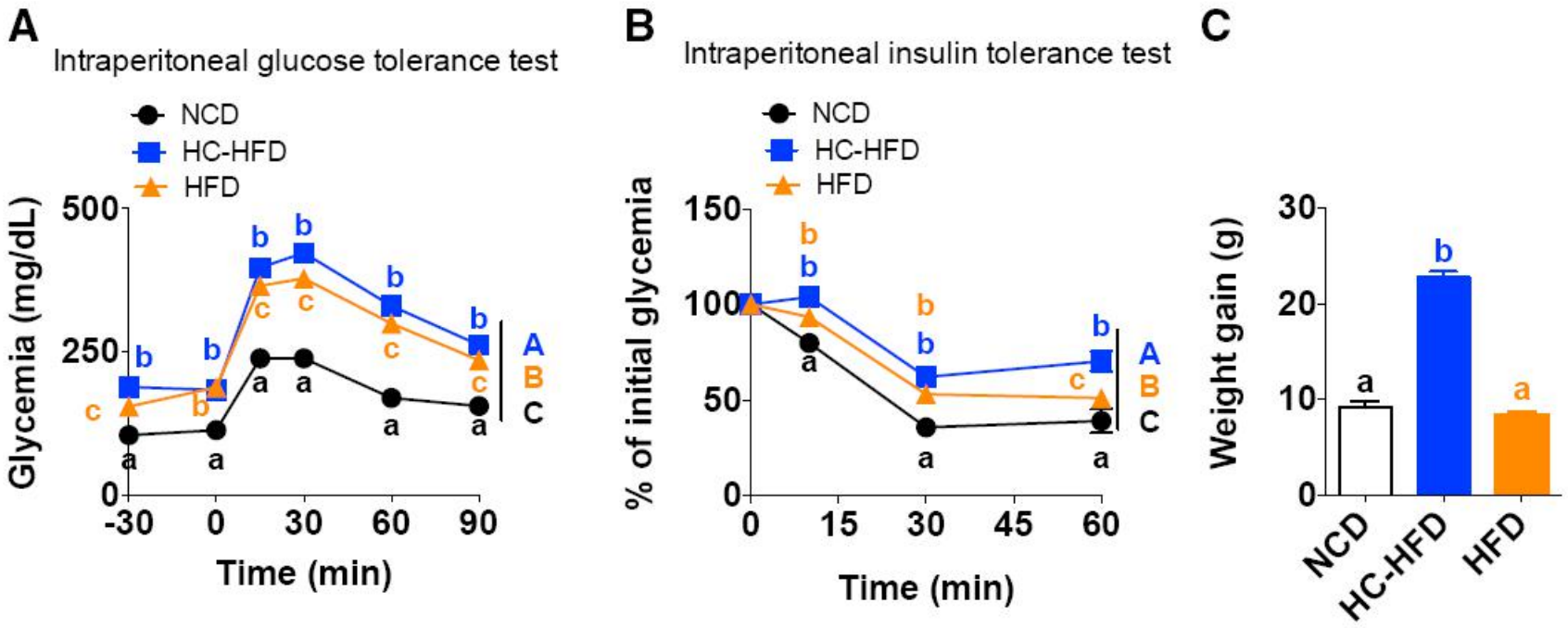
VS

GLP-1 resistance

The mechanisms responsible for GLP-1 unresponsiveness could be related to lipo-glucotoxicity, autonomic neuropathy, and gut microbiota dysbiosis.

2 Results

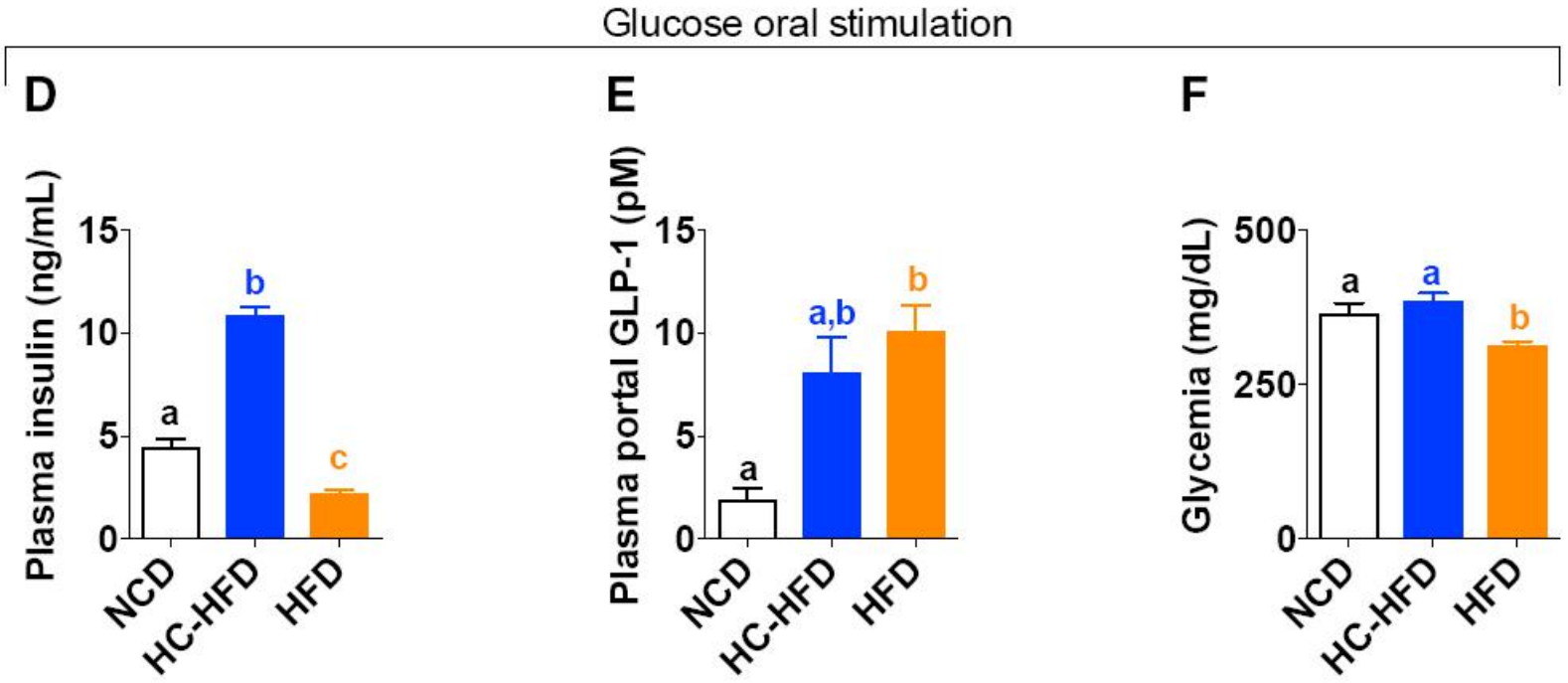
2.1 Animal Models of High-Fat Diet-Induced GLP-1 Resistance



Animal Models of High-Fat-Diet-Induced GLP-1 Resistance

Mice were fed for 3 months with NCD, HC-HFD, or HFD.

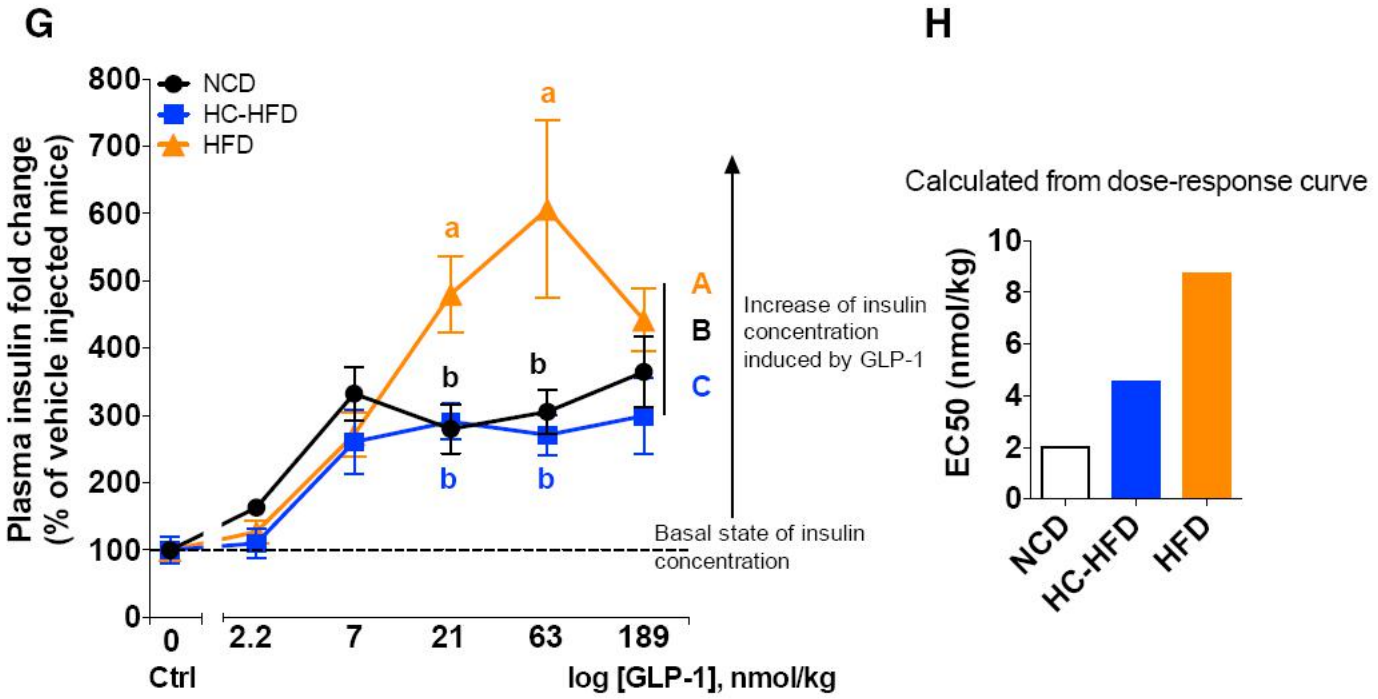
The impact of GLP-1 on insulin secretion



At the 15 min time point after an oral glucose challenge, plasma insulin concentration was twice as high in HC-HFD-fed obese diabetic mice, while it was lower in HFD-fed lean diabetic mice when compared with NCD-fed mice (Figure 1D). Importantly, at the same time point portal vein plasma GLP-1 concentration was three to four times higher in both diabetic models when compared to NCD-fed mice(Figure 1E), while the glycemia were almost similar (Figures 1F)

suggesting different GLP-1 responsiveness

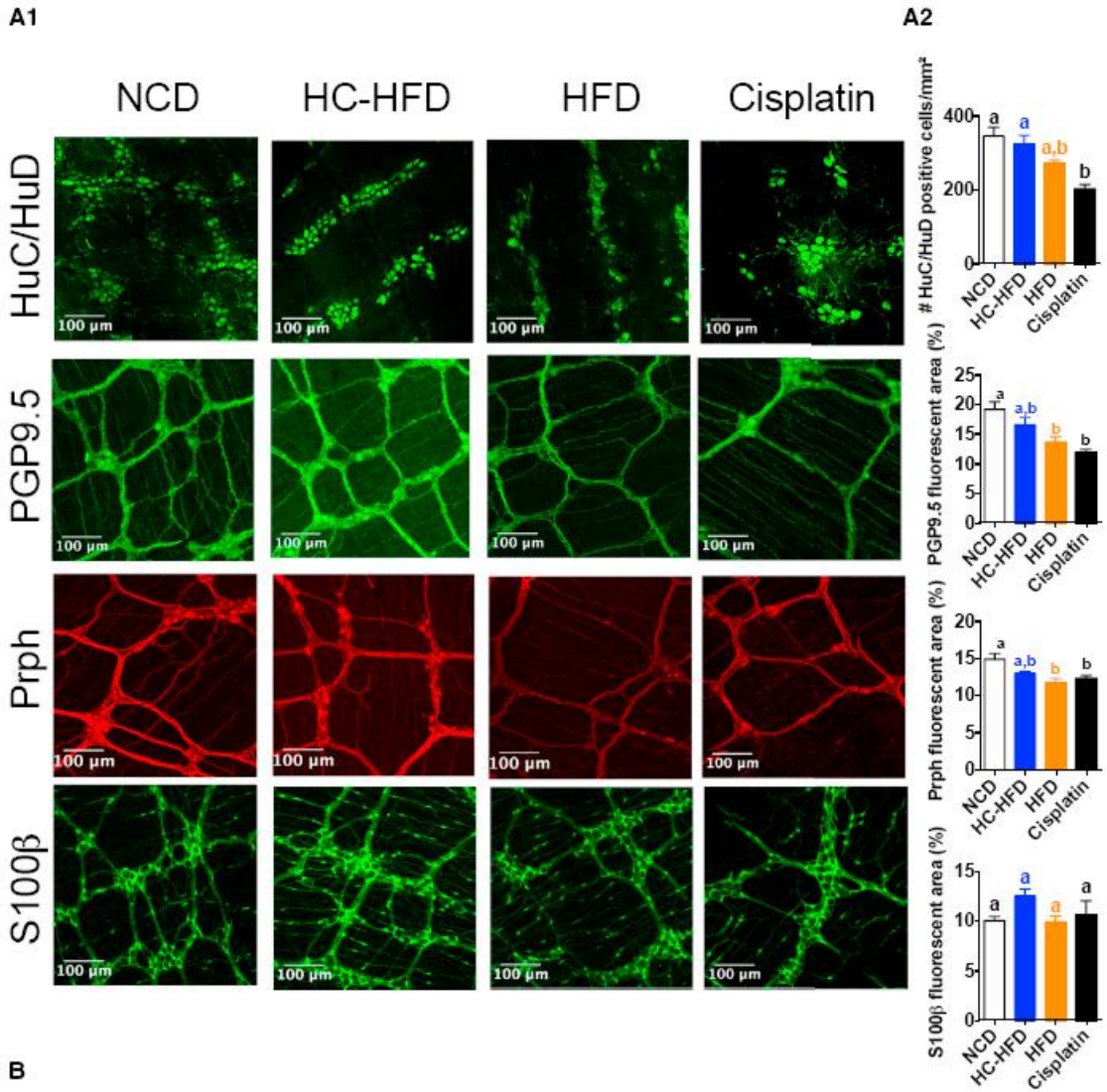
The impact on glucose induced insulin secretion using increasing doses of GLP-1

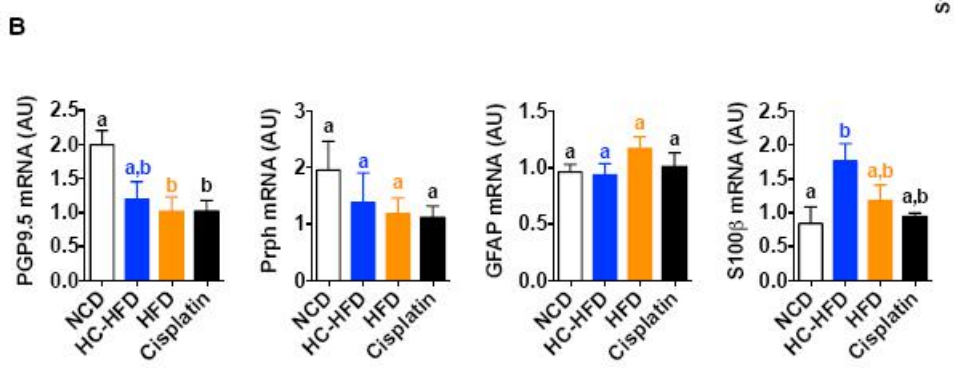


G:the maximal fold change of insulin secretion in NCD and HC-HFD-fed mice was obtained at the dose of 7 nmol/kg of GLP-1, while doses above 21 nmol/kg were required for the HFD-fed mice;H:The calculated EC50 was two and four times higher for the HC-HFD- and the HFD-fed mice, respectively, than for the NCD-fed mice.

This pharmacological analysis further confirmed a state of resistance to GLP-1 in both diabetic models, although to a different extent.

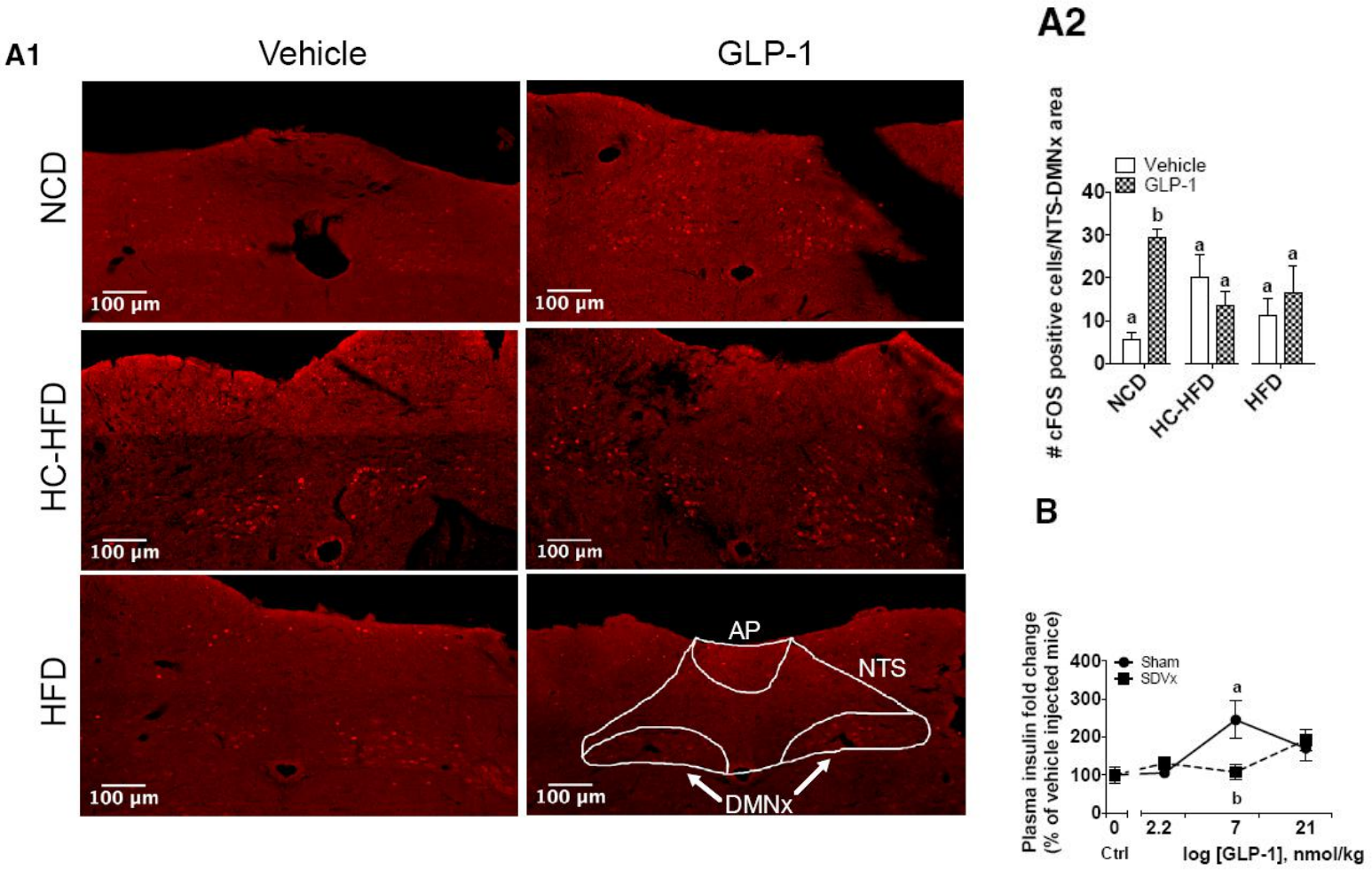
2.2 High-Fat Diet Alters the Enteric Nervous System to Brain Axis and Induces GLP-1 Resistance





A1:myenteric plexus (LMMP) immunohistochemistry;
A2: quantification of the number of HuC/HuD-positive cells, percentage of PGP9.5 and Prph fluorescent area(as neuronal marker) and percentage of S100b fluorescent area (as glial marker);
B: ileum mRNA concentrations of neuronal markers (PGP9.5 and peripherin-prph) and glial markers (GFAP, S100b)

High-Fat Diet Alters the Enteric Nervous System

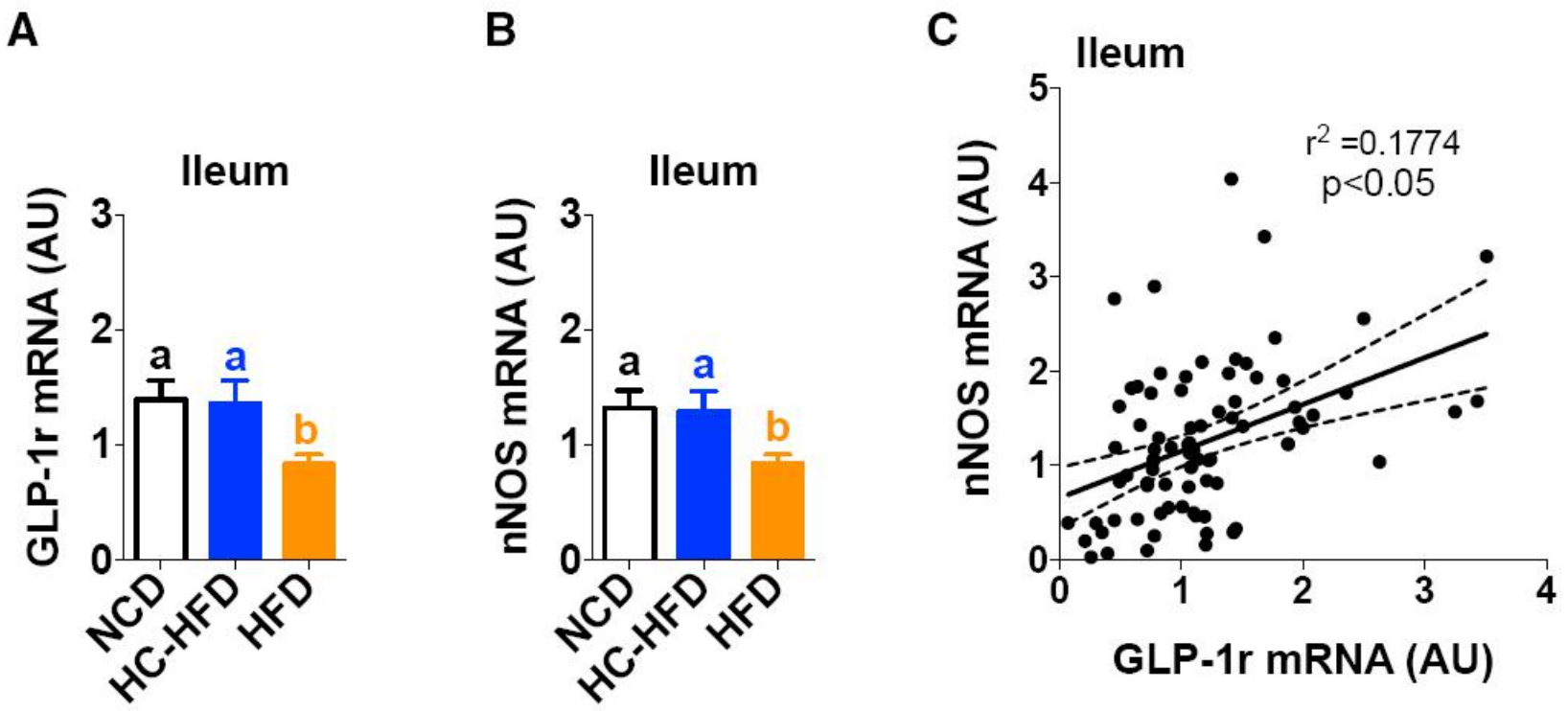


A: the number of cFos-positive neurons increases in response to GLP-1 in NCD mice, but not in HC-HFD and HFD-fed mice

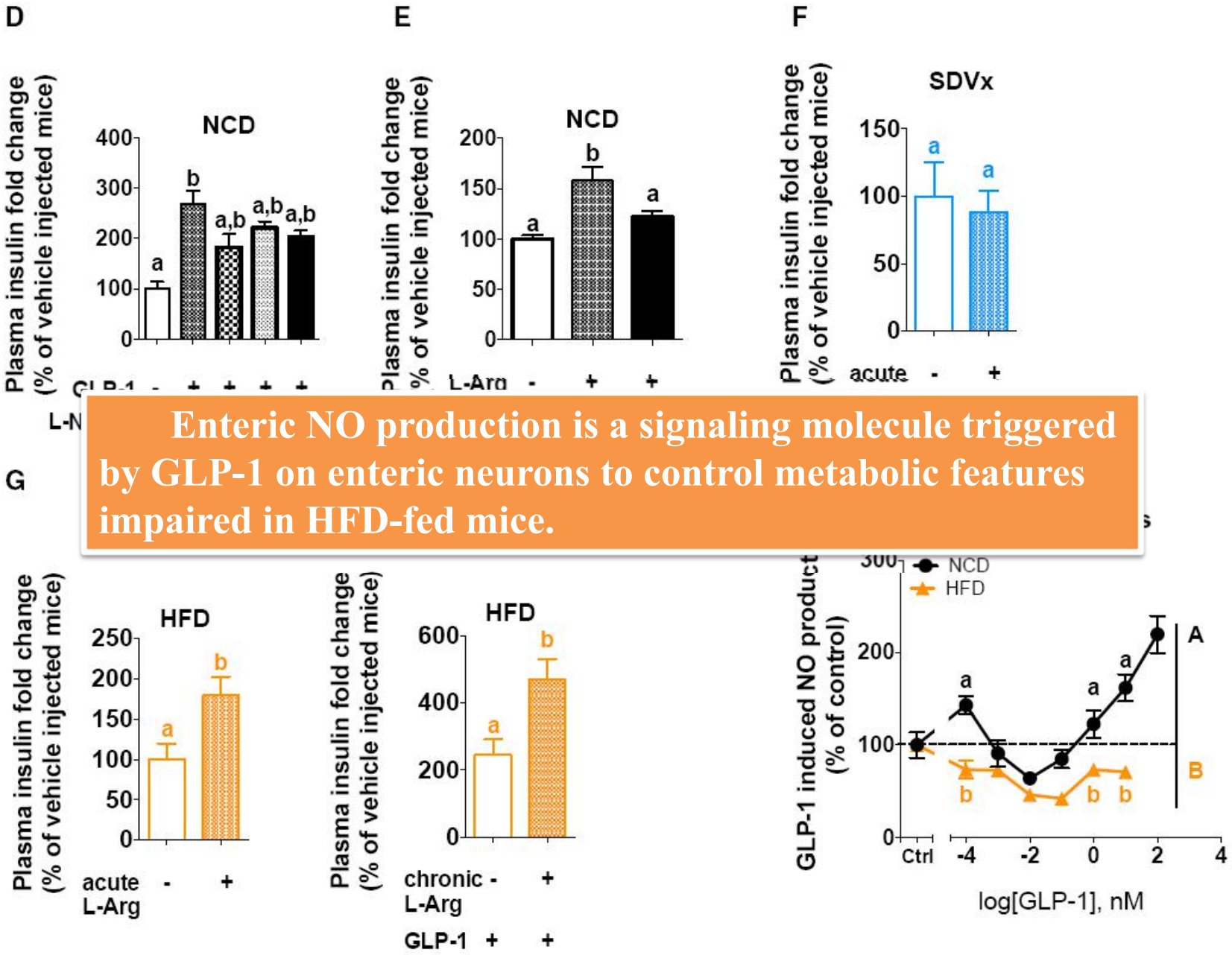
B: GLP-1-induced insulin secretion was reduced by the **vagotomy procedure**

demonstrating the importance of a functional gut-brain axis as the mode of action of GLP-1

2.3 Enteric GLP-1 Sensitivity Requires the Production of NO by Enteric Neurons and Is Impaired in HFD-Fed Mice

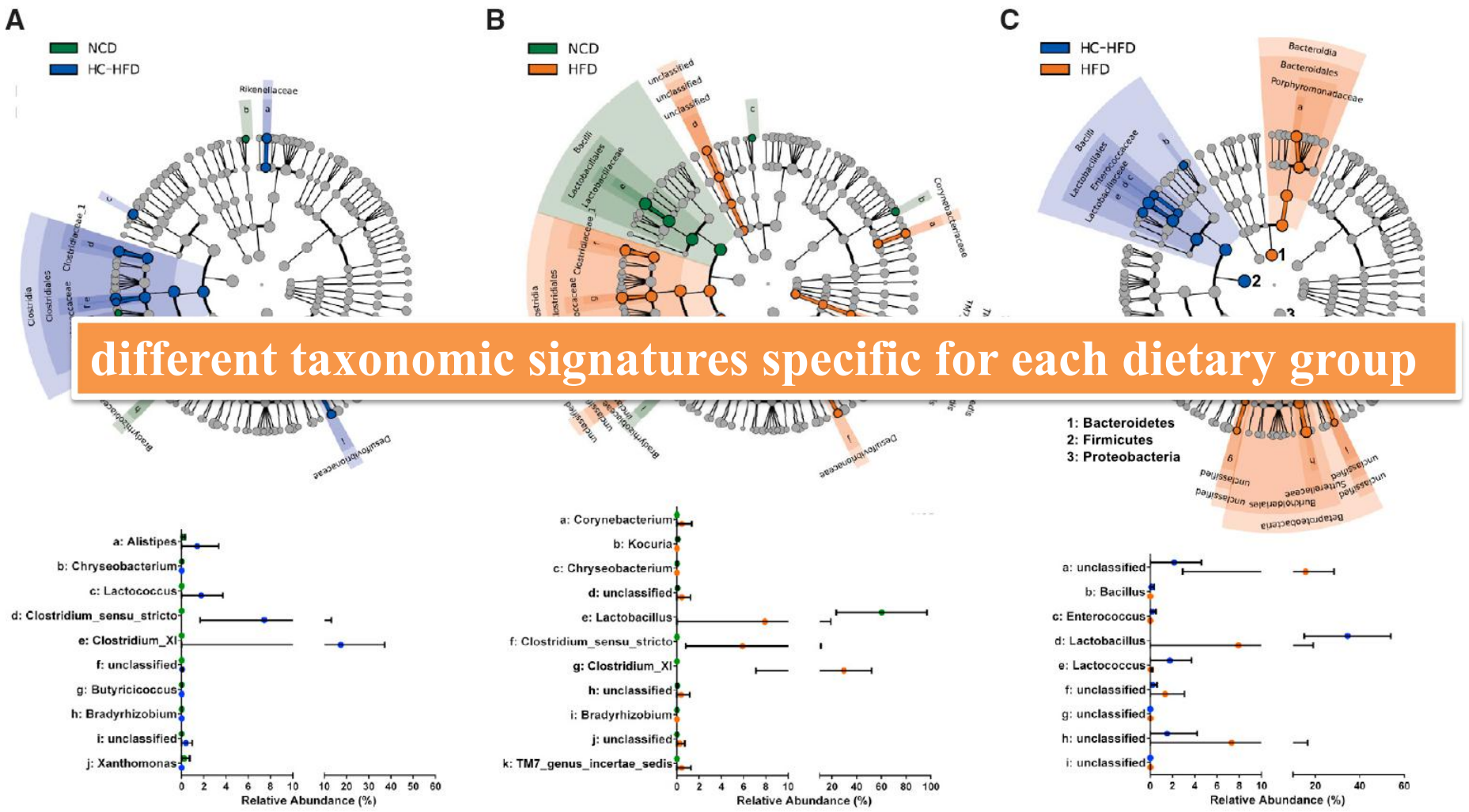


The mRNA concentration analyses suggested that HFD-induced GLP-1 resistance might be linked to an impaired GLP-1r expression and the corresponding induction of NO production through nNOS signaling.

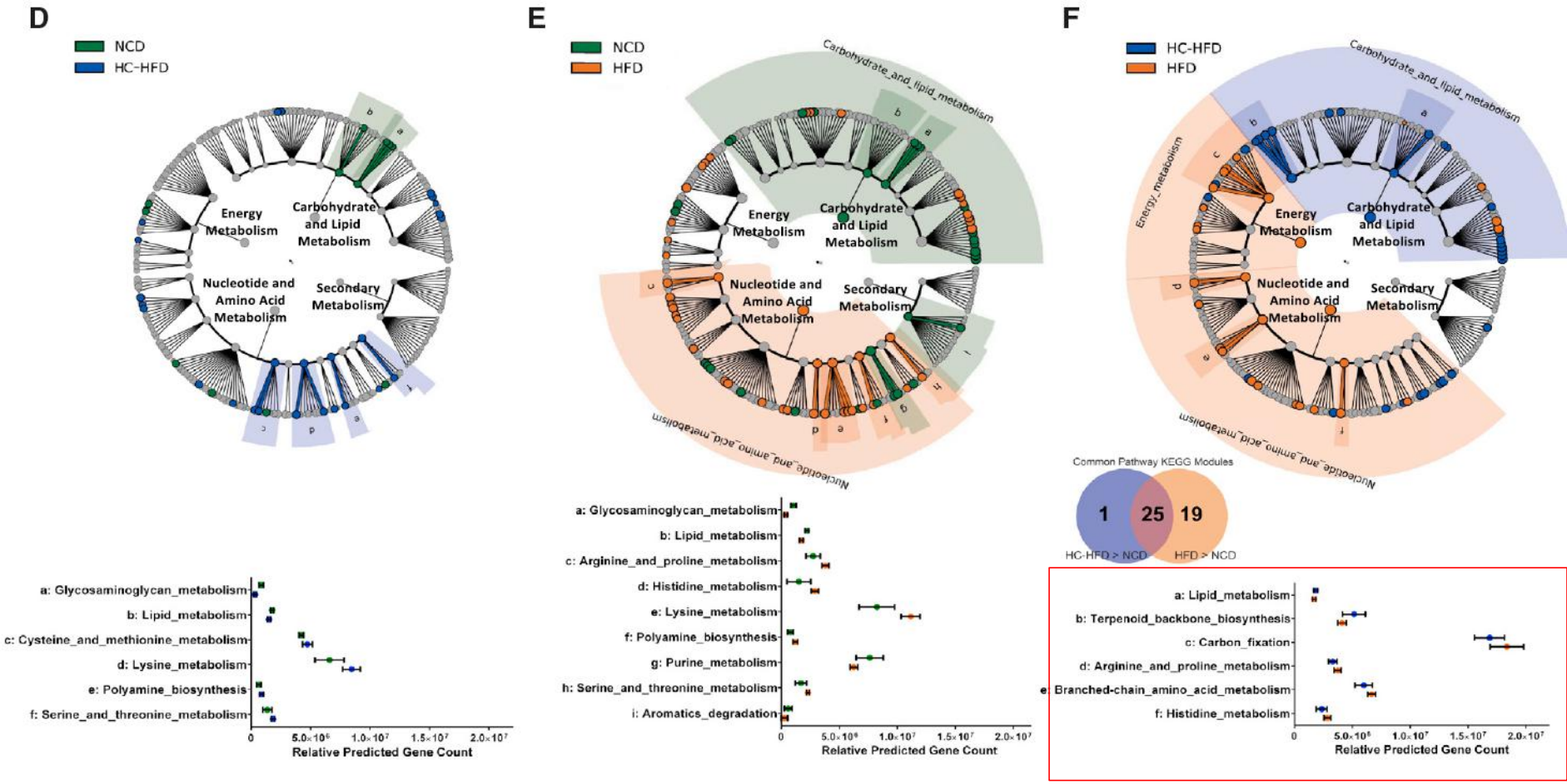


Enteric NO production is a signaling molecule triggered by GLP-1 on enteric neurons to control metabolic features impaired in HFD-fed mice.

2.4 Gut Microbiota Dysbiosis Is Responsible for the GLP-1 Resistance

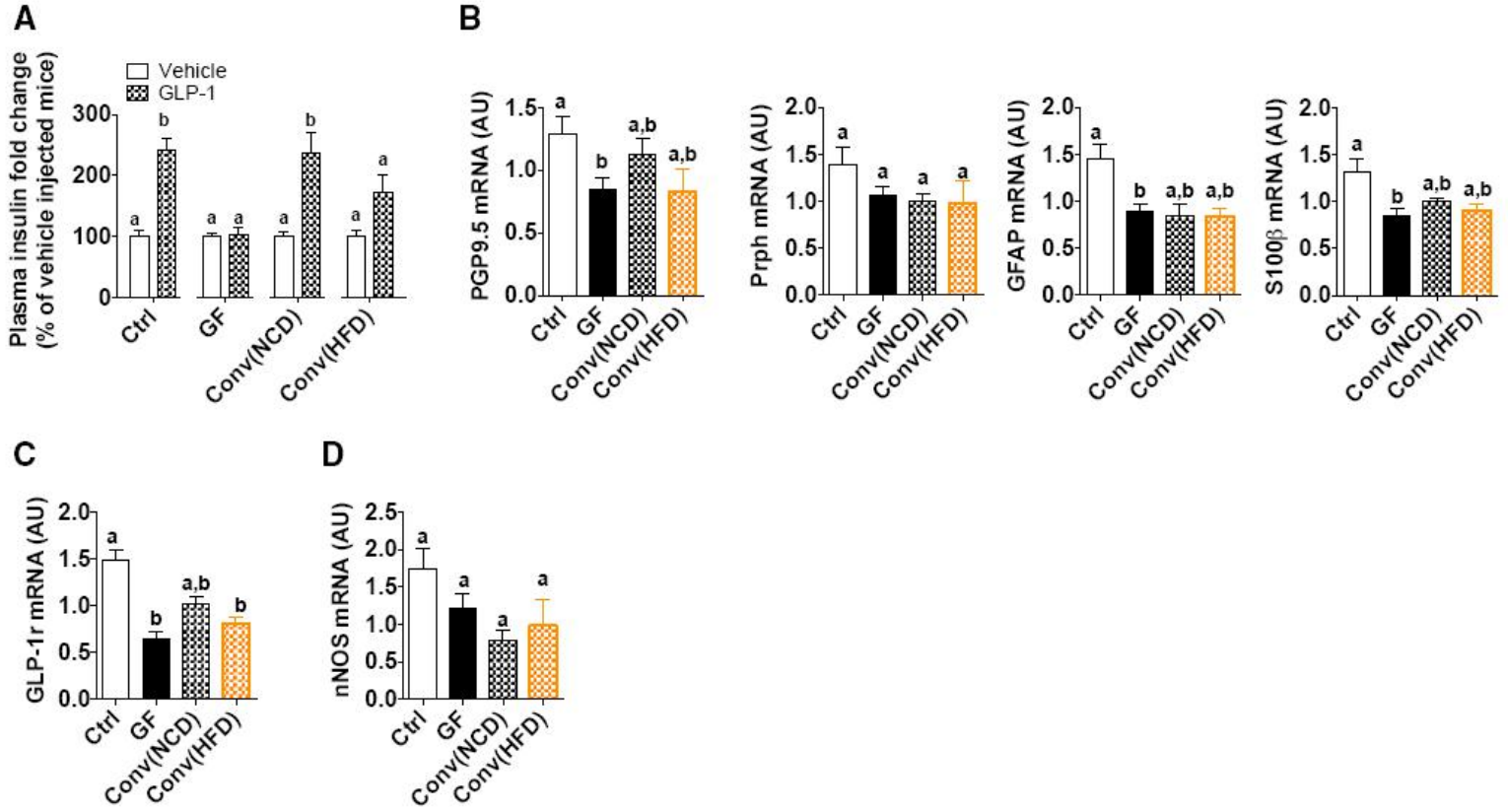


different taxonomic signatures specific for each dietary group



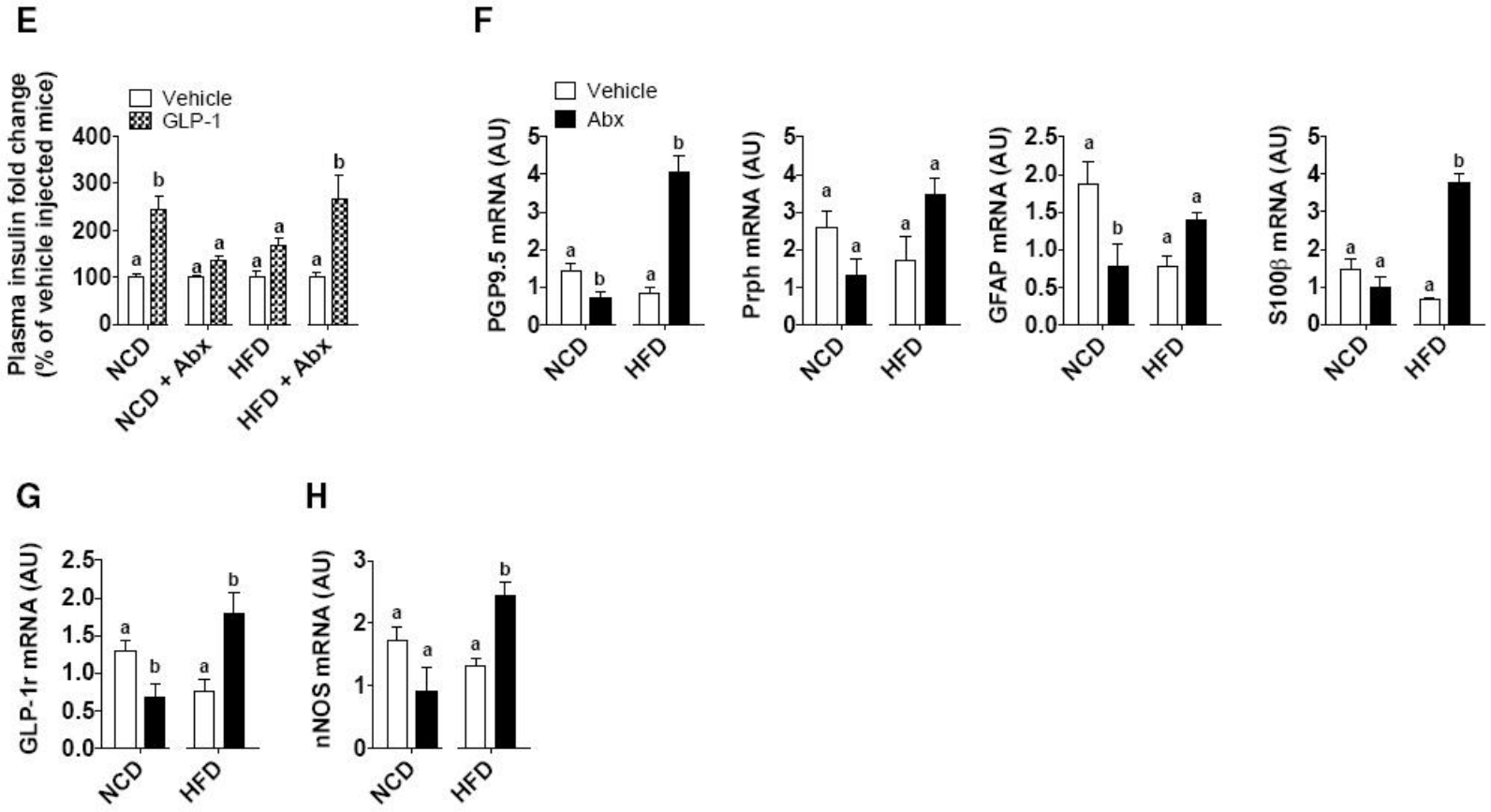
the frequency of the bacterial genes involved in the metabolism of nucleotides and amino acids was increased in the diabetic mice

germ-free(GF) mice and ileum microbiota transplant mice



A, the absence of microbiota prevented GLP-1-induced insulin secretion; the GLP-1 sensitivity to glucose-induced insulin secretion was completely reversed in the NCD-conventionalized mice but conv(HFD) mice
B, The GLP-1-resistant state in GF mice can be linked to a decreased of enteric neuron (PGP9.5, prph) and glial cell (S100b) mRNA in the ileum
C, the concentration of GLP-1r was dramatically reduced in GF mice
D, the concentration of nNOS mRNA reduced

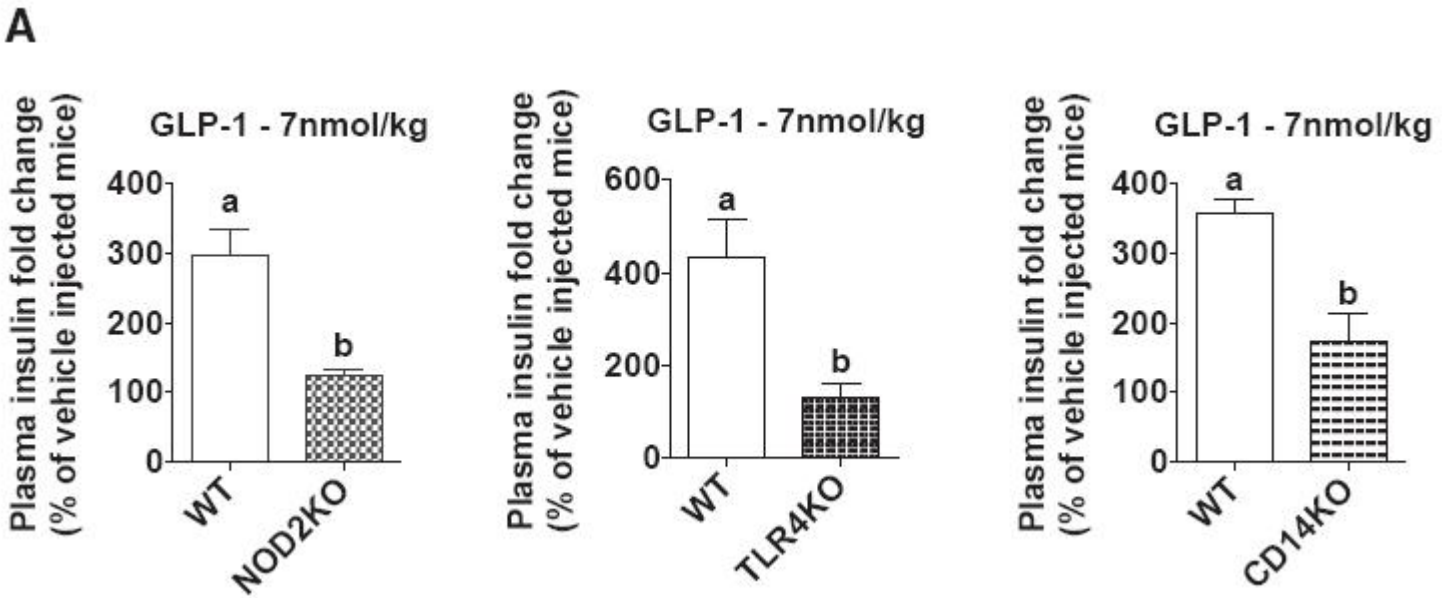
antibiotics (Abx) treated



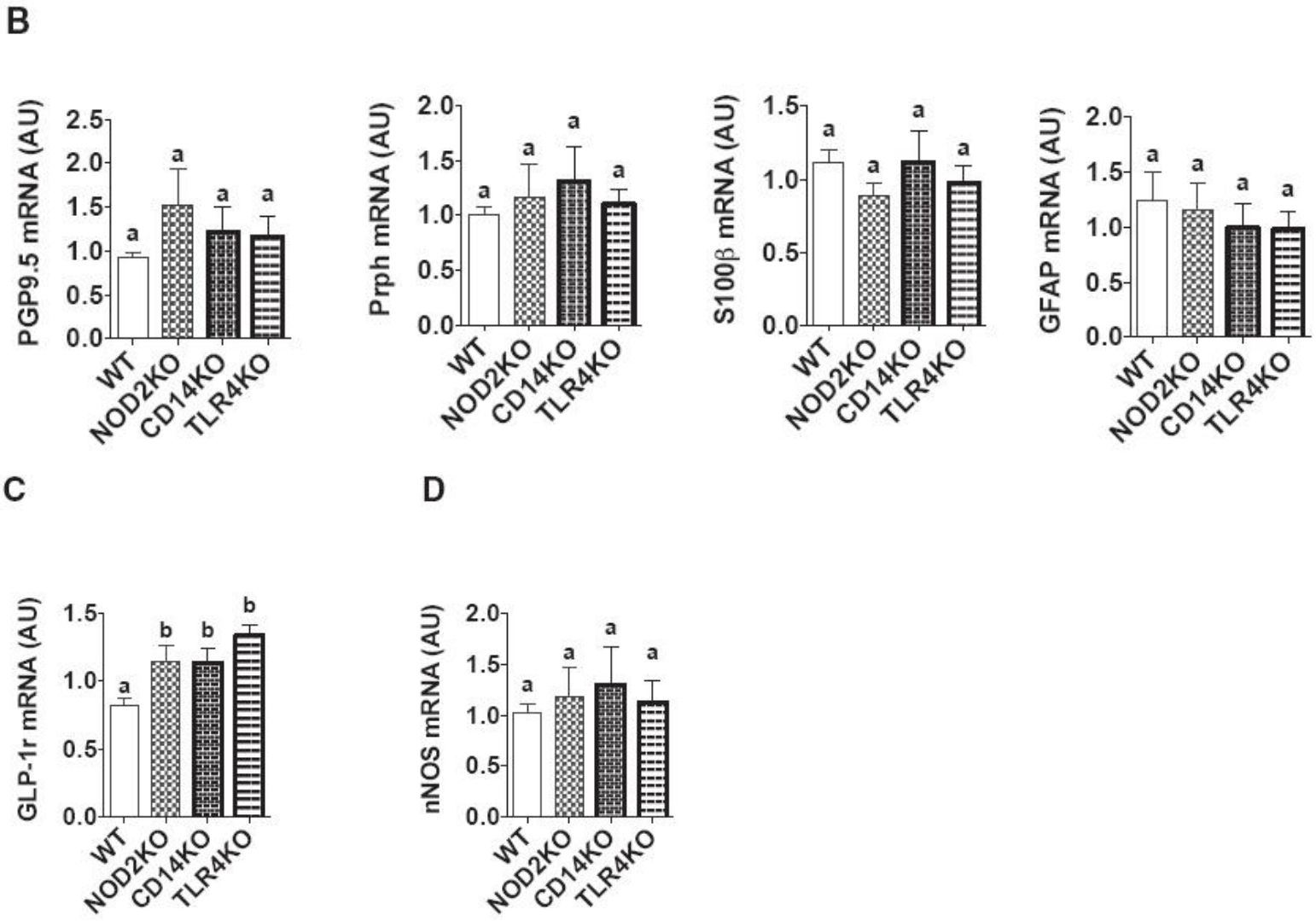
E, GLP-1 Induced insulin secretion is blocked by Abx; Conversely, **HFD-fed mice** was **reversed** when the dysbiotic microbiota were eliminated by the antibiotic treatment

This last set of data strongly supports the notion that a eubiotic gut microbiota enhances GLP-1 sensitivity while a dysbiotic microbiota reduces it.

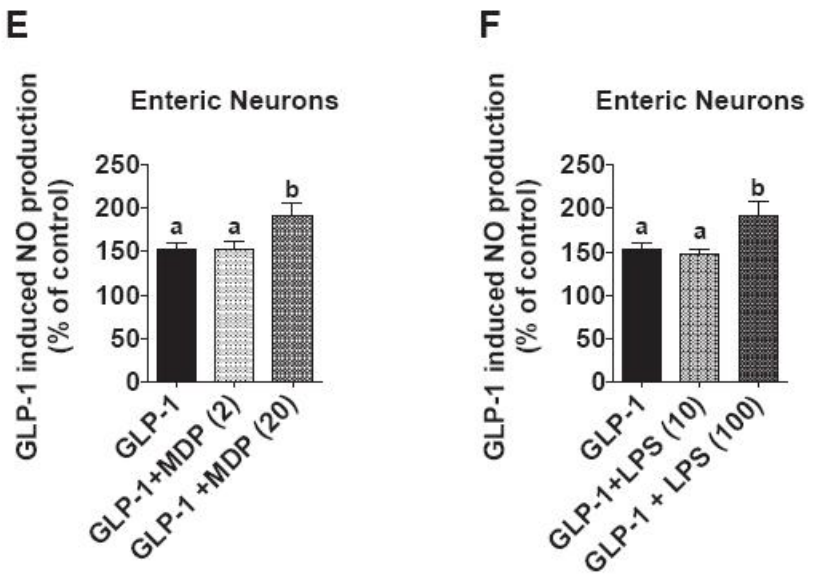
2.5 The Microbial-Associated Molecular Pattern Receptors NOD2, CD14, and TLR4 Control GLP-1 Sensitivity



GLP-1-induced insulin secretion was dramatically reduced in NOD2, TLR4, or CD14 KO when compared to WT mice

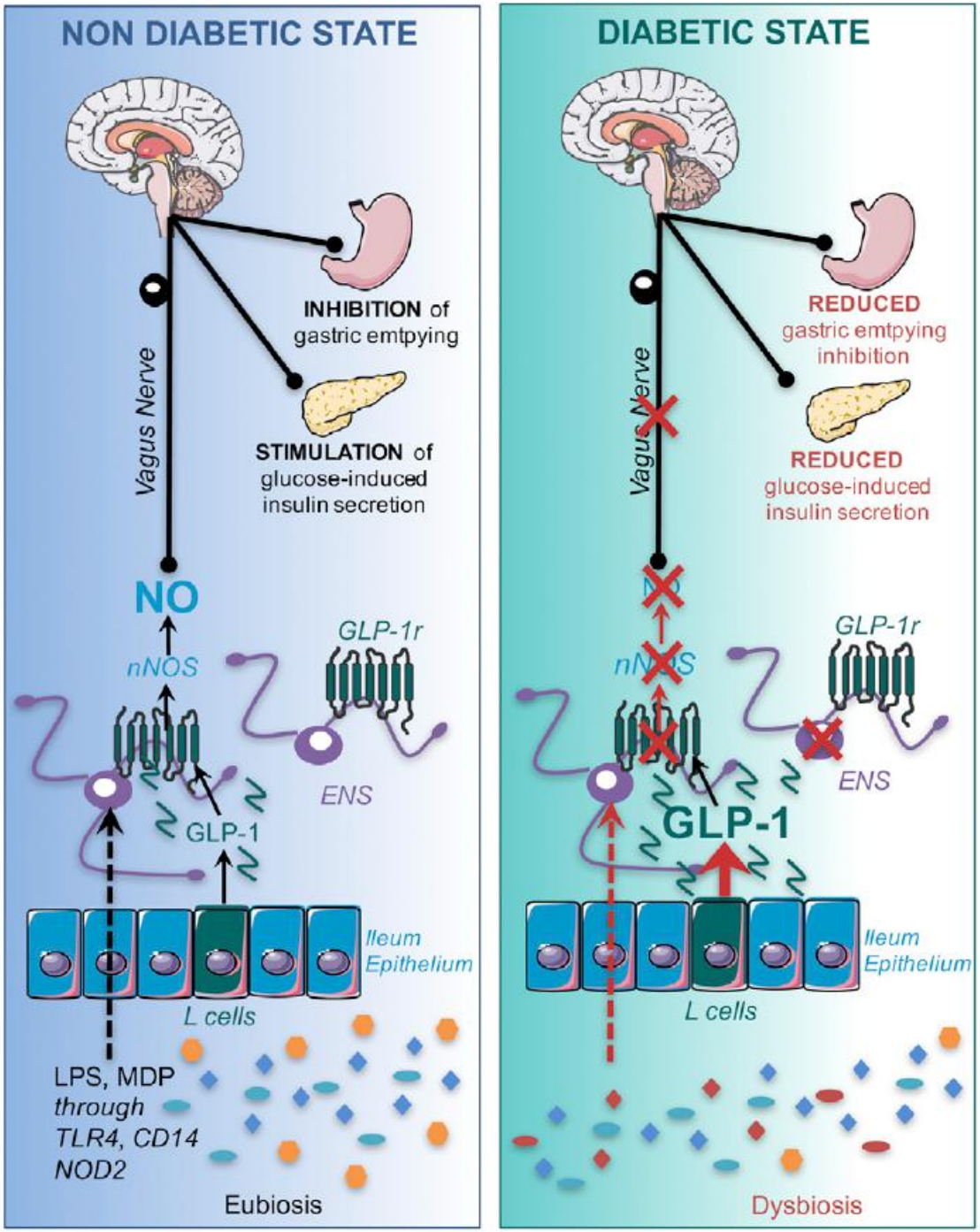


in the KO mice the mRNA of the GLP-1r was increased (C), while the mRNA encoding for nNOS remained unchanged (D). PGP9.5, prph, GFAP, and S100b in the ileum remained similar to WT mice (B), suggesting that **the impaired bacterial signaling is upstream to the ENS**.



To functionally address the role of bacterial determinant on the control of GLP-1 sensitivity, we studied the impact of MDP (NOD2 agonist) and LPS (CD14/TLR4 agonist) on NO production by enteric neurons. The data show that GLP-1-induced NO production was enhanced by high doses of both microbial-associated molecular patterns .

Conclusion


NON DIABETIC STATE
INHIBITION of
gastric emtpying
STIMULATION of
glucose-induced
insulin secretion
Vagus Nerve
NO
nNOS
GLP-1r
ENS
GLP-1
Ileum
Epithelium
L cells
LPS, MDP
through
TLR4, CD14
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Dysbiosis

Thanks for your attention!