



New Applications of Probiotic Products

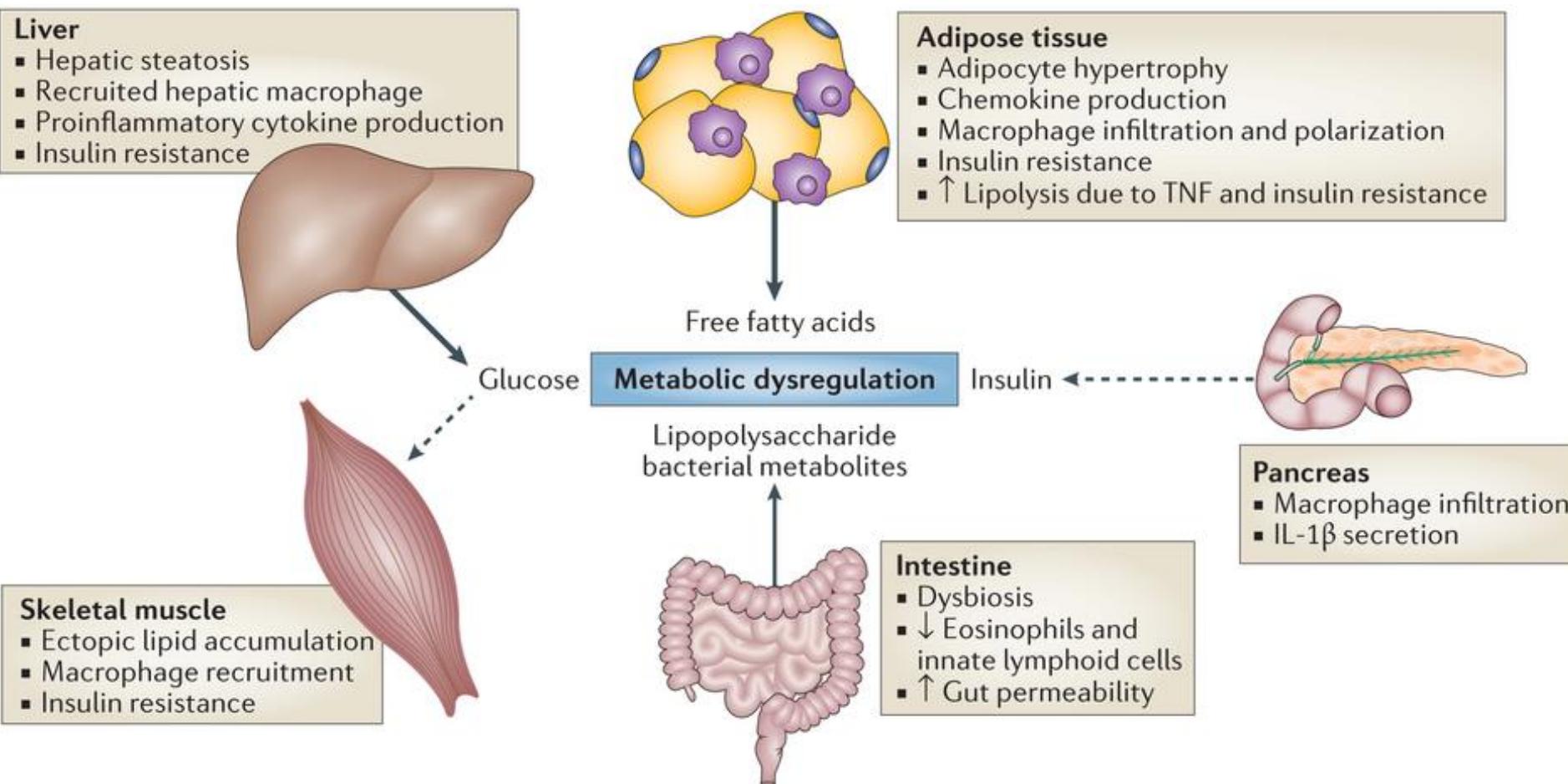
Probiotics in the fight against obesity & metabolic disorder

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The Metabolic Syndrome



The Obesity Epidemic

THE WORLD IS GETTING FATTER



HOW DO I KNOW WHETHER I AM OVERWEIGHT?

Calculate your body mass index (BMI) using this formula

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 (\text{m}^2)}$$



OBESITY KILLS!

7 common diseases due to obesity:

- Arthritis
- Cancer
- Infertility
- Heart Diseases
- Back Pain
- Diabetes
- Stroke

ABC TO OBESITY PREVENTION

SIMPLE RULES TO STAY IN SHAPE

A dopt New Healthy Habits



B alance Your Calorie Intake



C ontrol Your Weight Gain



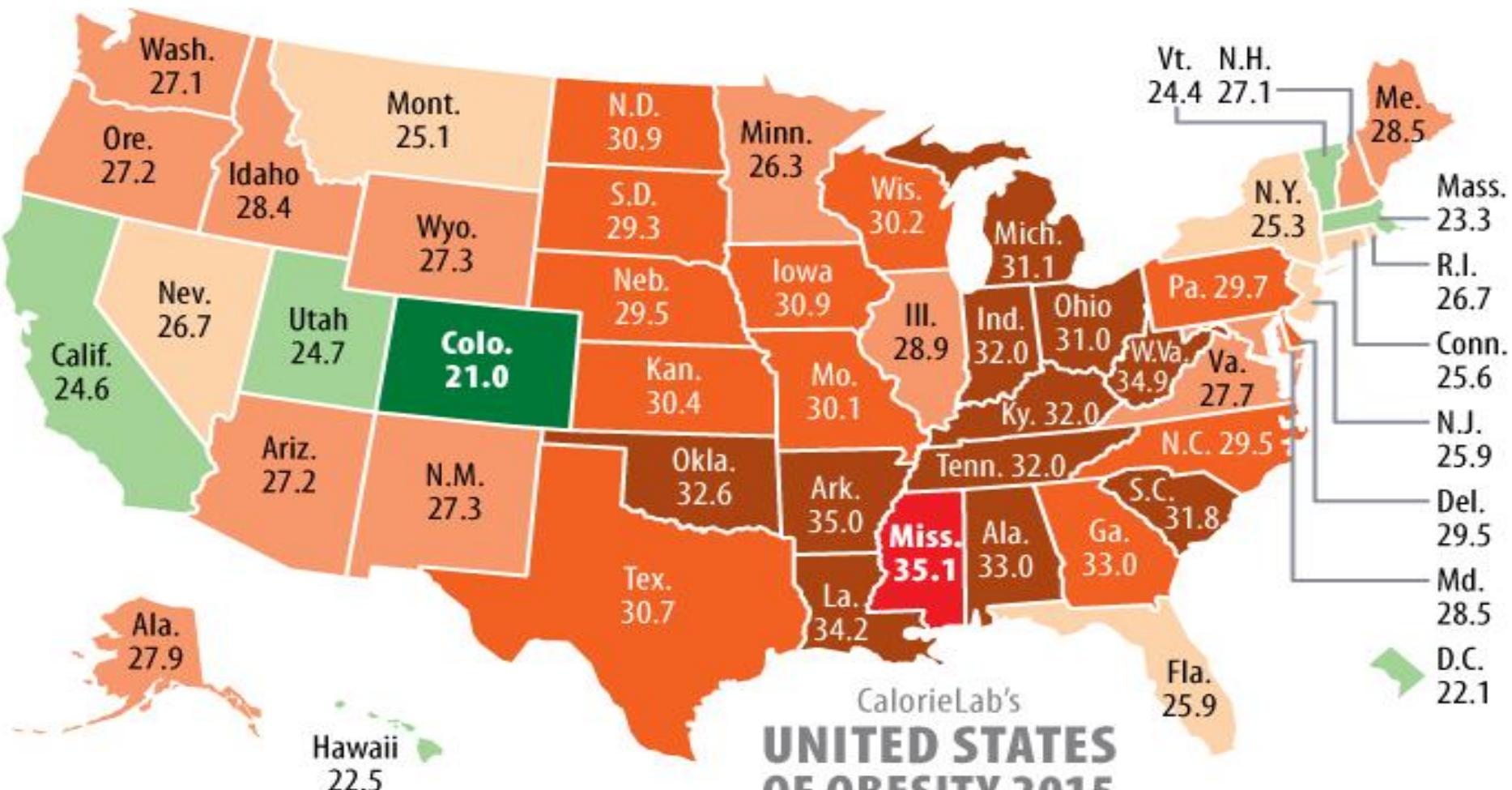
United States of Obesity

Leanest State
Colorado

Percentage of Obese Adult Population

(3-year average from 2012-14 CDC Behavioral Risk Factor Surveillance System data)

Fattest State
Mississippi



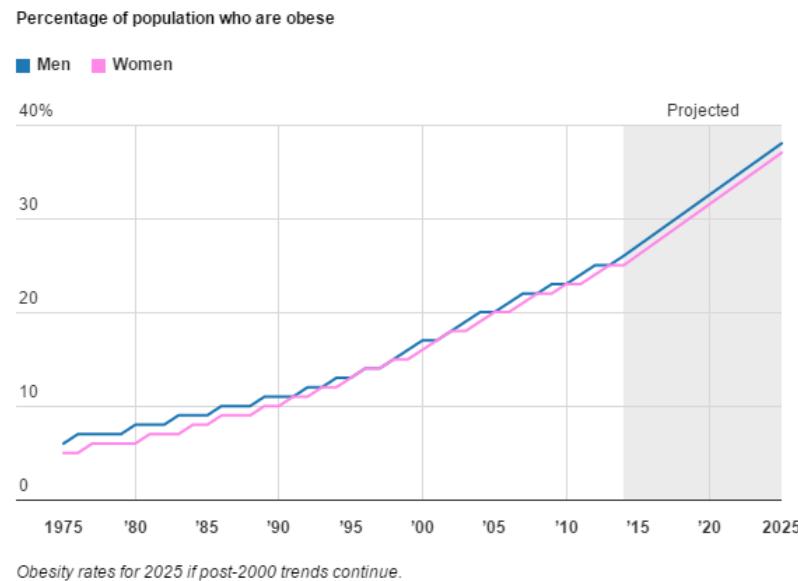
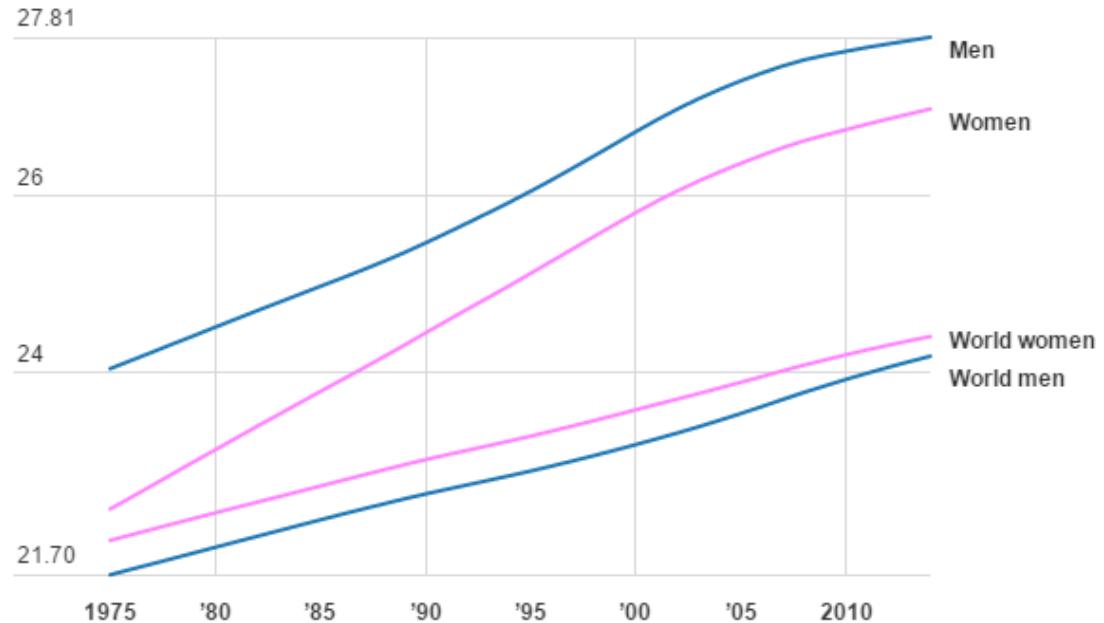
Irelands Obesity Rate Among World's Worst

THE LANCET

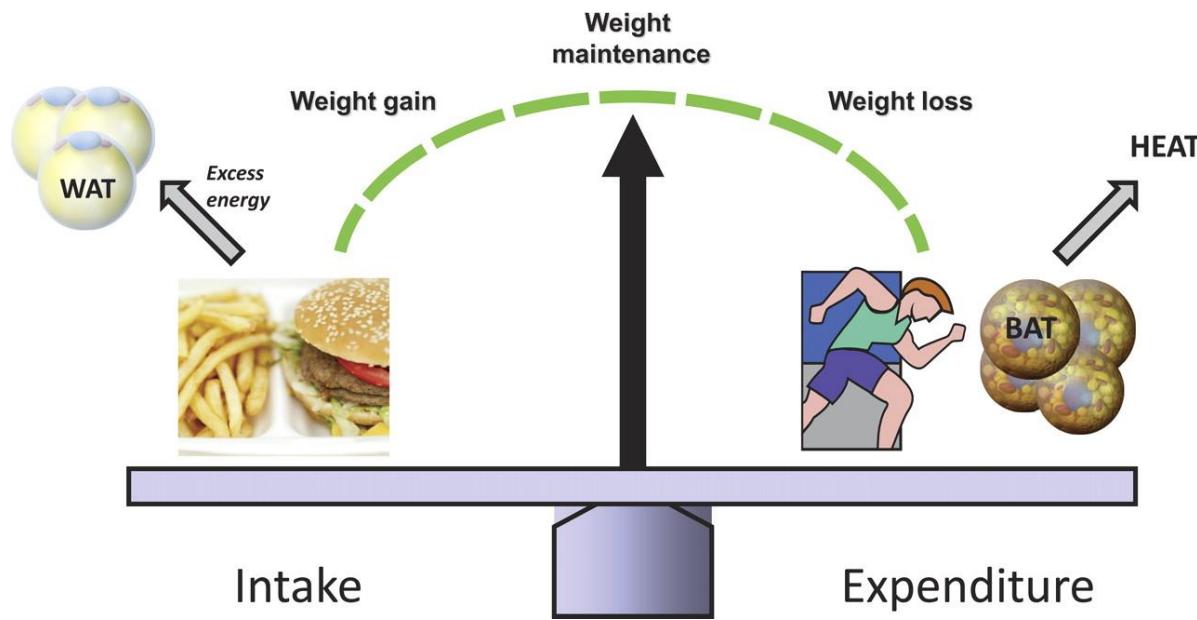
Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants

NCD Risk Factor Collaboration (NCD-RisC)*

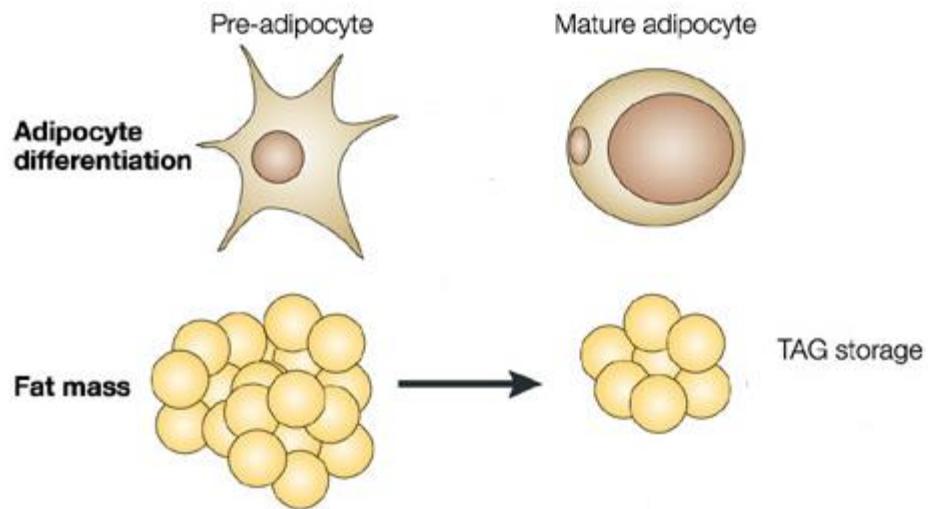
Ireland's growing body mass index



Cellular Mechanisms Involved in Obesity

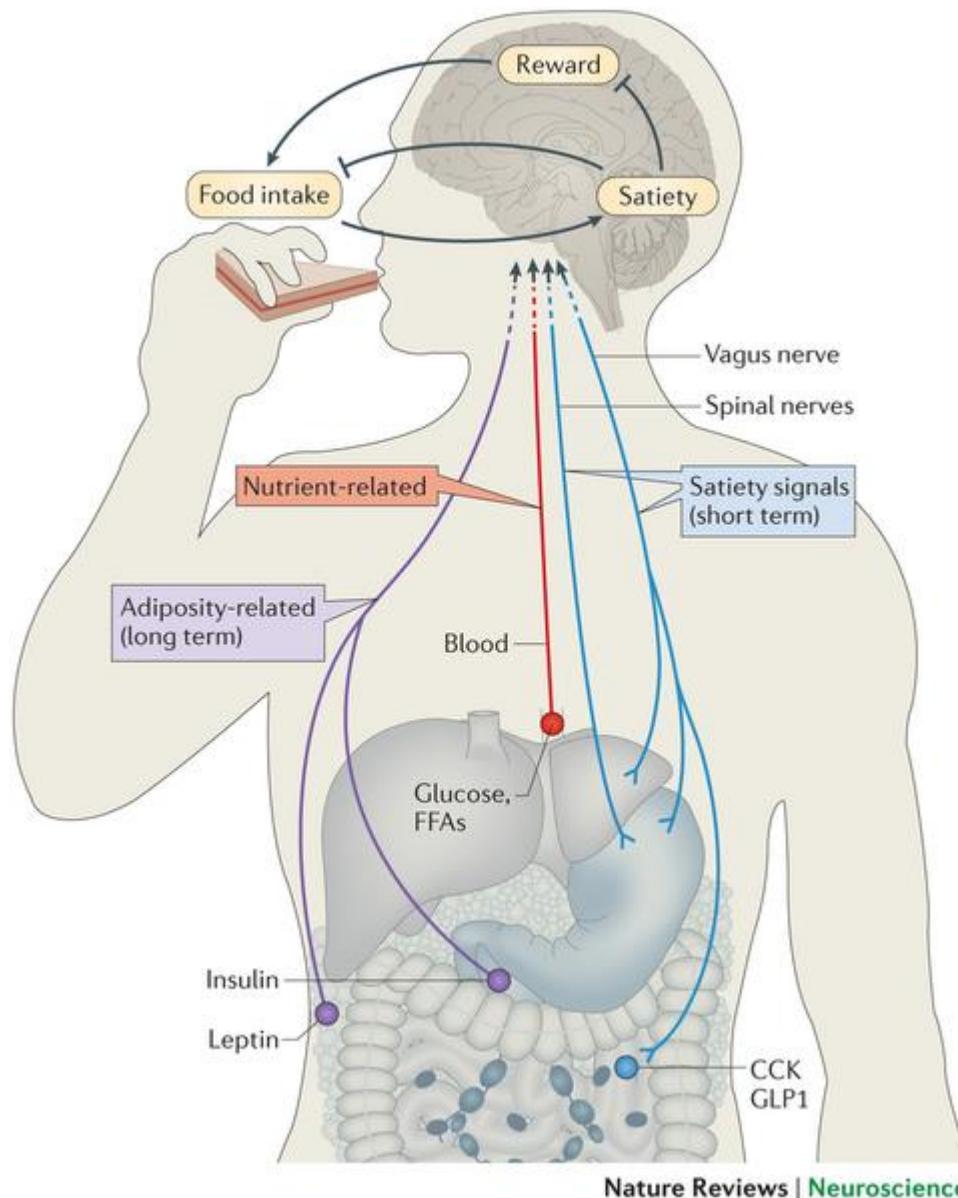


1. Adipogenesis (macrophage recruitment)

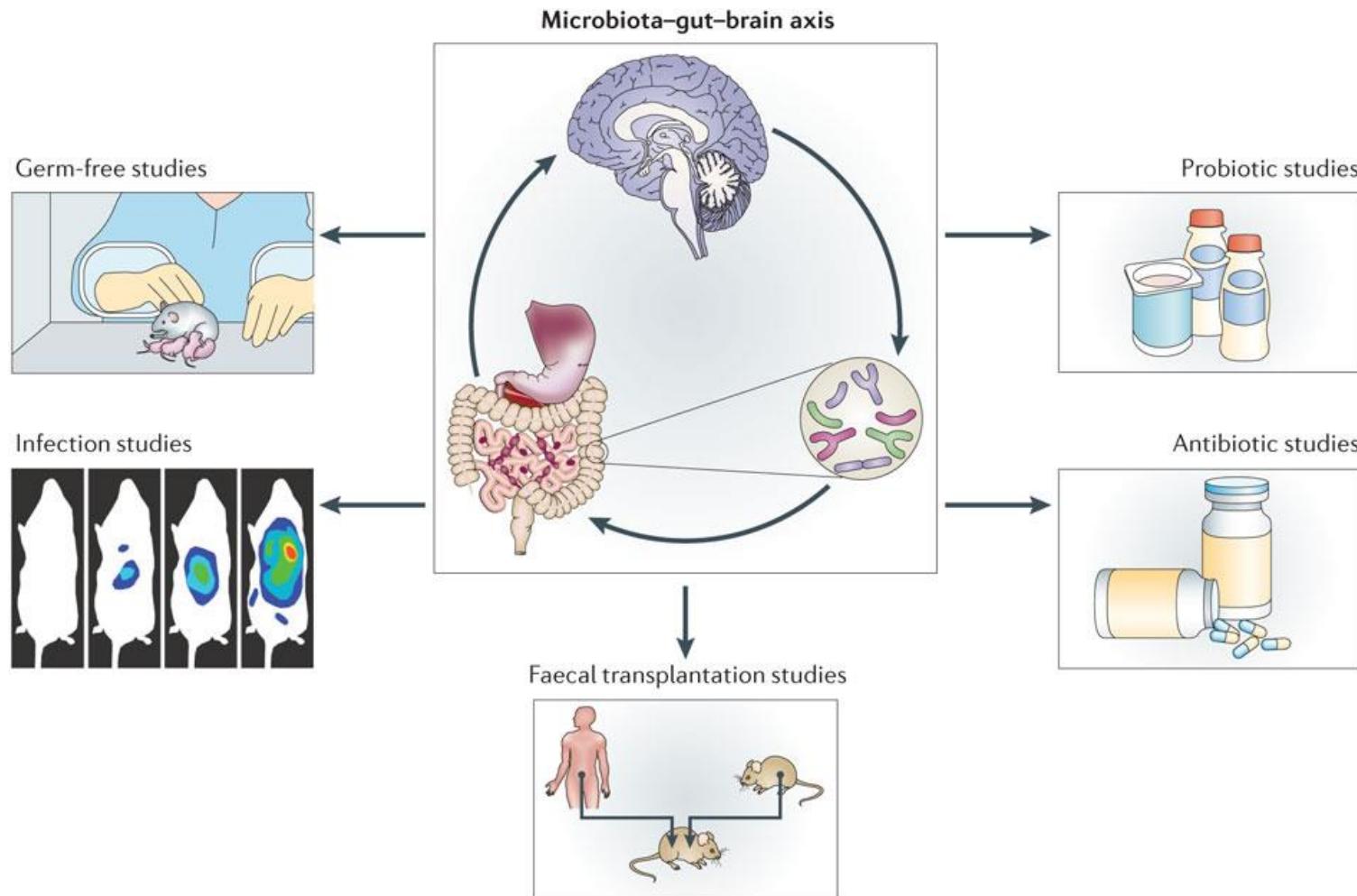


2. Lipid Storage

Gut Hormones & Obesity



Within the APC Microbiome Institute

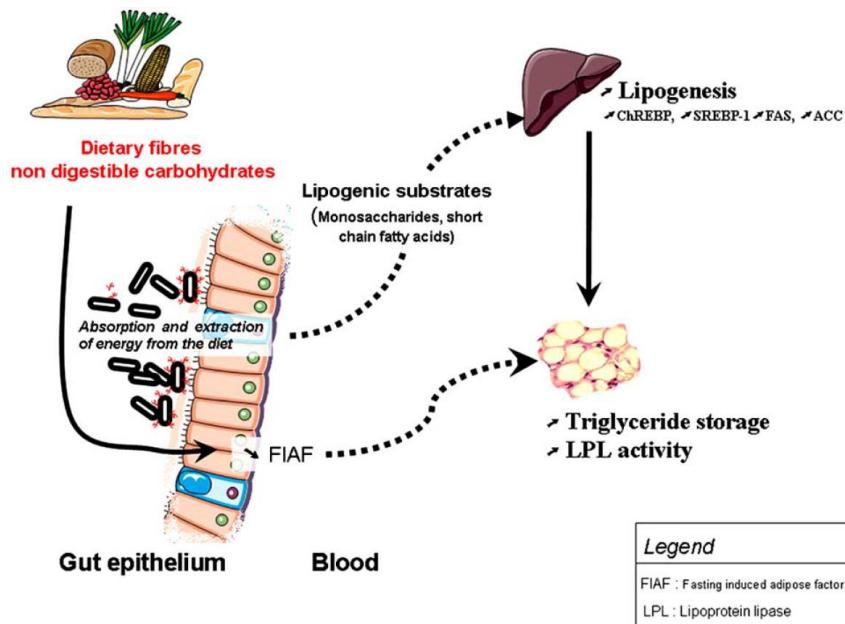


John F. Cryan & Timothy G. Dinan
Nature Reviews Neuroscience (10) 701-712 (October 2012)

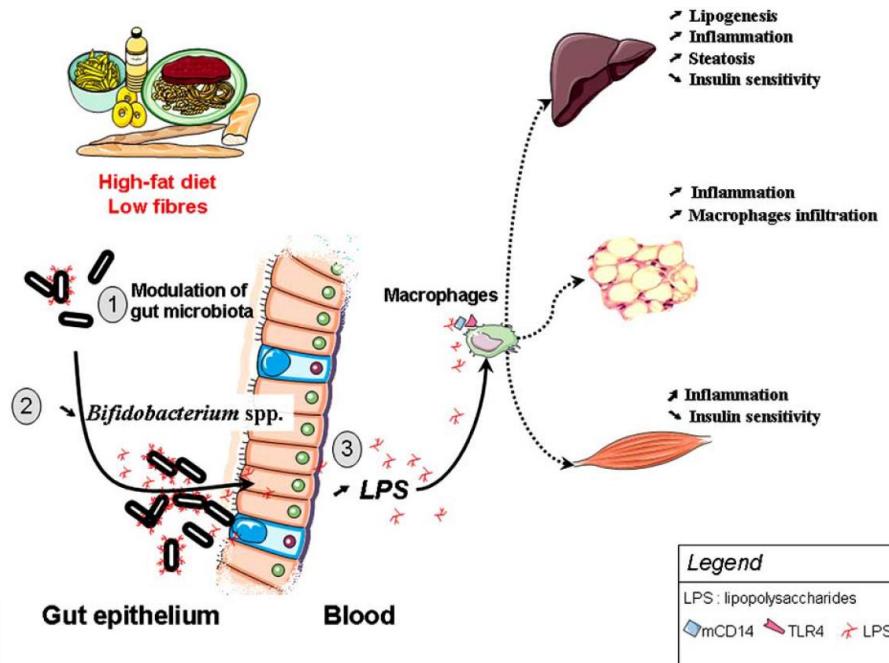
Nature Reviews | Neuroscience

Gut Microbiota and Energy Metabolism

(1)



(2)



Cani and Delzenne (2009) Current Pharmaceutical Design 15, 1546-1558

Can Microbes Make You Fat?

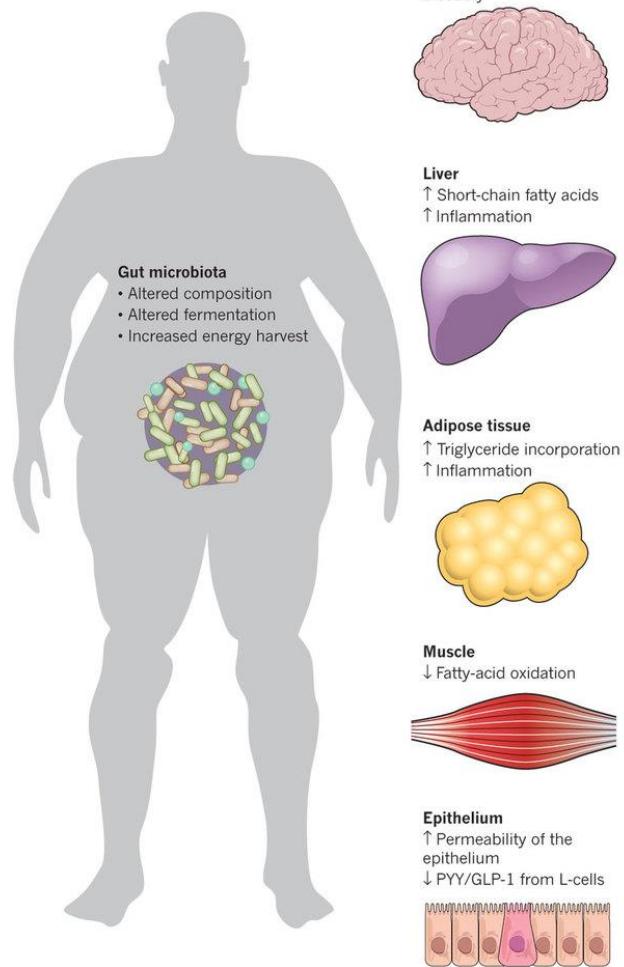
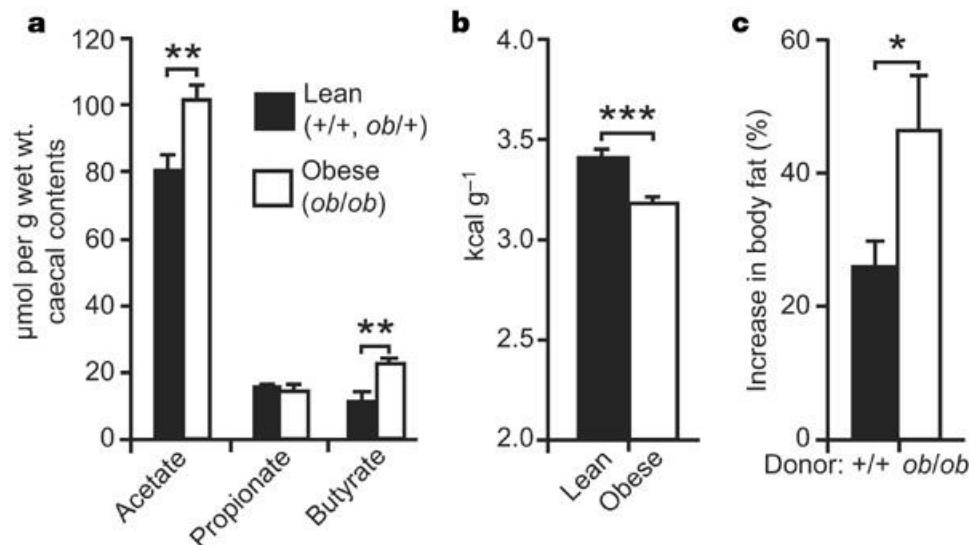
Vol 444 | 21/28 December 2006 | doi:10.1038/nature05414

nature

ARTICLES

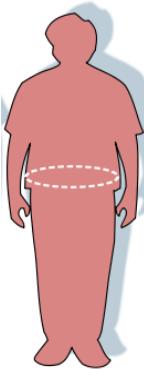
An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh¹, Ruth E. Ley¹, Michael A. Mahowald¹, Vincent Magrini², Elaine R. Mardis^{1,2} & Jeffrey I. Gordon¹



Gut Microbiota and Obesity

- ***Enterobacter cloacae B29*** : obesity & insulin resistance altered Fiaf (fasting induced adipose factor) expression (Fei & Zhao, 2013; ISMEJ)
- **Gram-negative LPS**: fat deposition, insulin resistance in mice (Cani et al., 2007, 2008; Diabetes, de la Serre et al., 2010; AJPG)
- ***Bacteroides thetaiotaomicron-Methanobacter smithii*** cocolonization: increased adiposity in germ-free mice (Samuel & Gordon, 2006; PNAS)



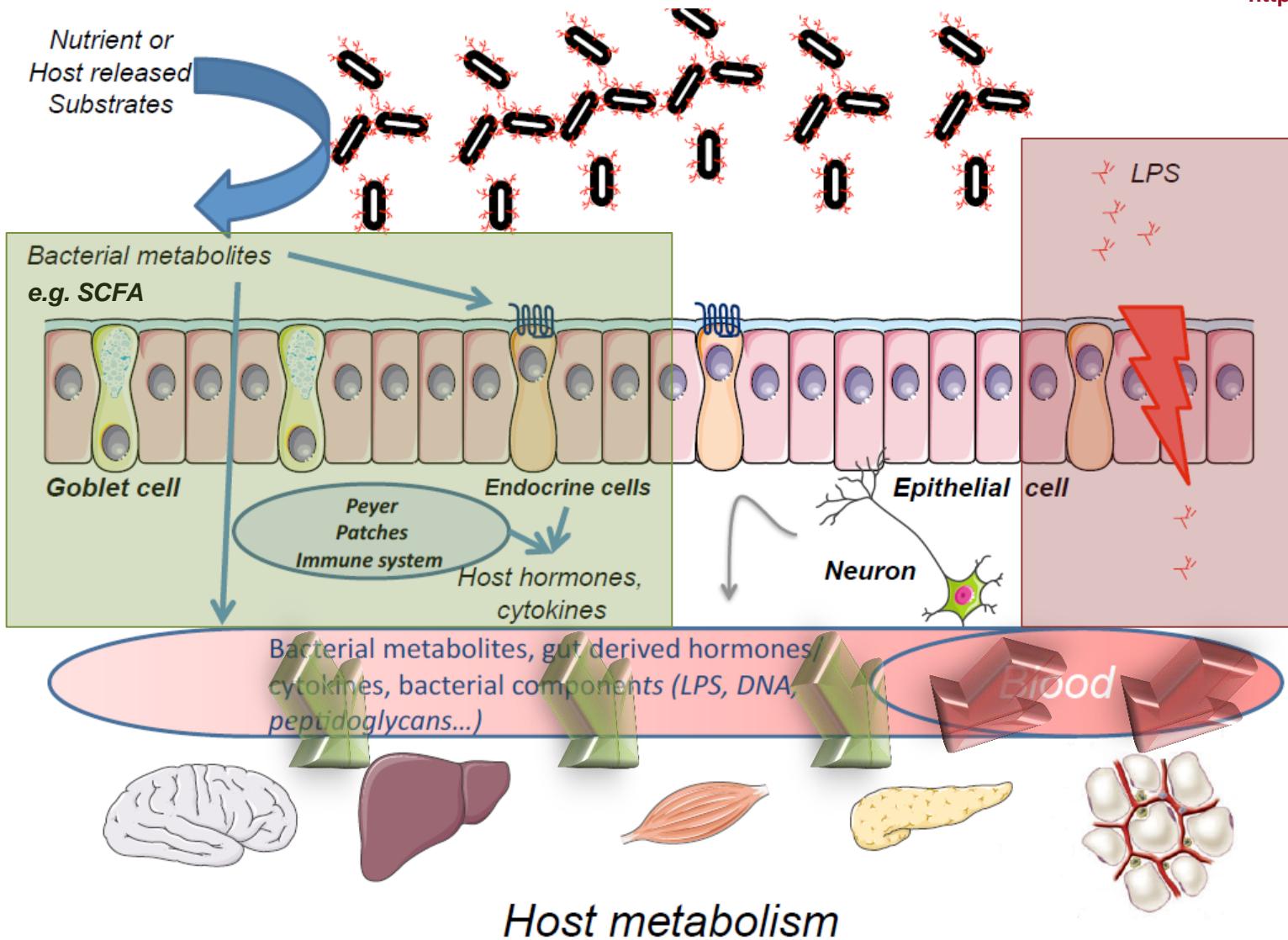
Bacteroidetes : Firmicutes

- Increased ↑
- ***Mollicutes (Erysipelotrichia)***: western diet obese mice (Turnbaugh et al., 2008; cell host microbe)
 - ***Erysipelotrichaceae, Desulfovibrionaceae***: following 6 months of high fat feeding (Zhang et al., 2010; ISMEJ)
 - ***Bacteroides and Staphylococcus***: linked to weight gain during pregnancy (Collado et al., 2008, AJCN)
 - H₂-producing ***Prevotellaceae, Archaea***, methanogenic (Zhang et al., 2009; PNAS)
- Decreased ↓

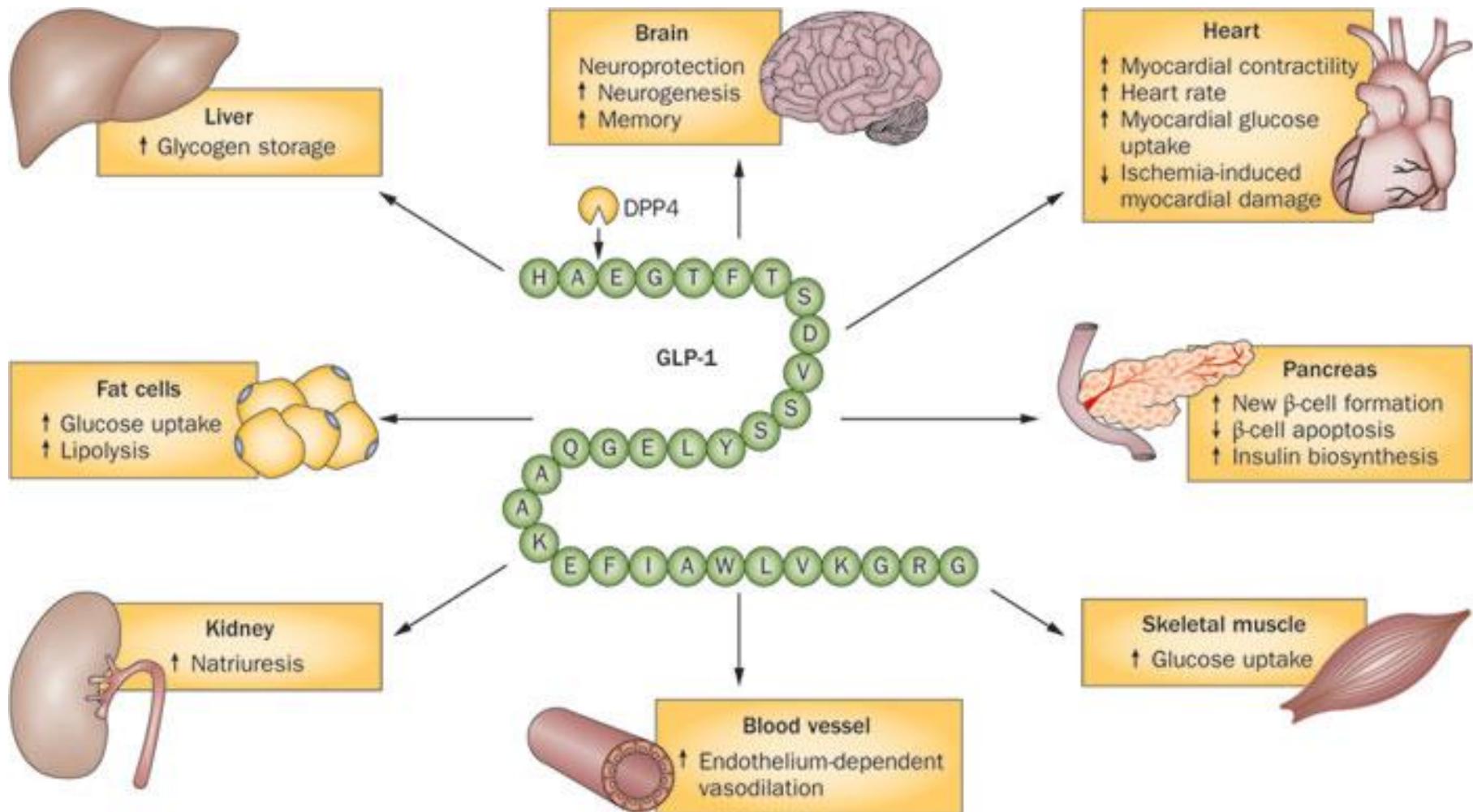
- ***A. muciniphila***: improved metabolic profile, reversed high-fat diet-induced metabolic disorders, including fat-mass gain, insulin resistance (Everard et al., 2013; PNAS)
- ***Enterococcus faecalis FK-23***: anti-obesity, hepatic steatosis (Motonaga et al., 2009; Masatoshi et al., 2014;)
- ***Lactobacillus gasseri SBT2055***: lowered human abdominal adiposity, body weight; gassericin T producer (Kadooka et al., 2010; EJCN)
- ***Bifidobacterium animalis ssp. lactis 420*** prevented weight gain and glucose intolerance in DIO mice (Stenman et al., 2014; Benef microbes)

- bacterial diversity
- ***A. muciniphilia***: in obese and T2D mice (Everard et al., 2013; PNAS)
- ***C. leptum group (Ruminococcus flavefaciens), Bifidobacterium*** (Schwartz et al., 2010; Obesity)
- Butyrate-producing organisms: ***Roseburia spp., Faecalibacterium spp., Eubacterium spp., Faecalibacterium prausnitzii, Haemophilus parainfluenzae, Clostridiales (order)*** : T2D MGWAS study - control enriched organisms (Qin et al., 2012; Nature).

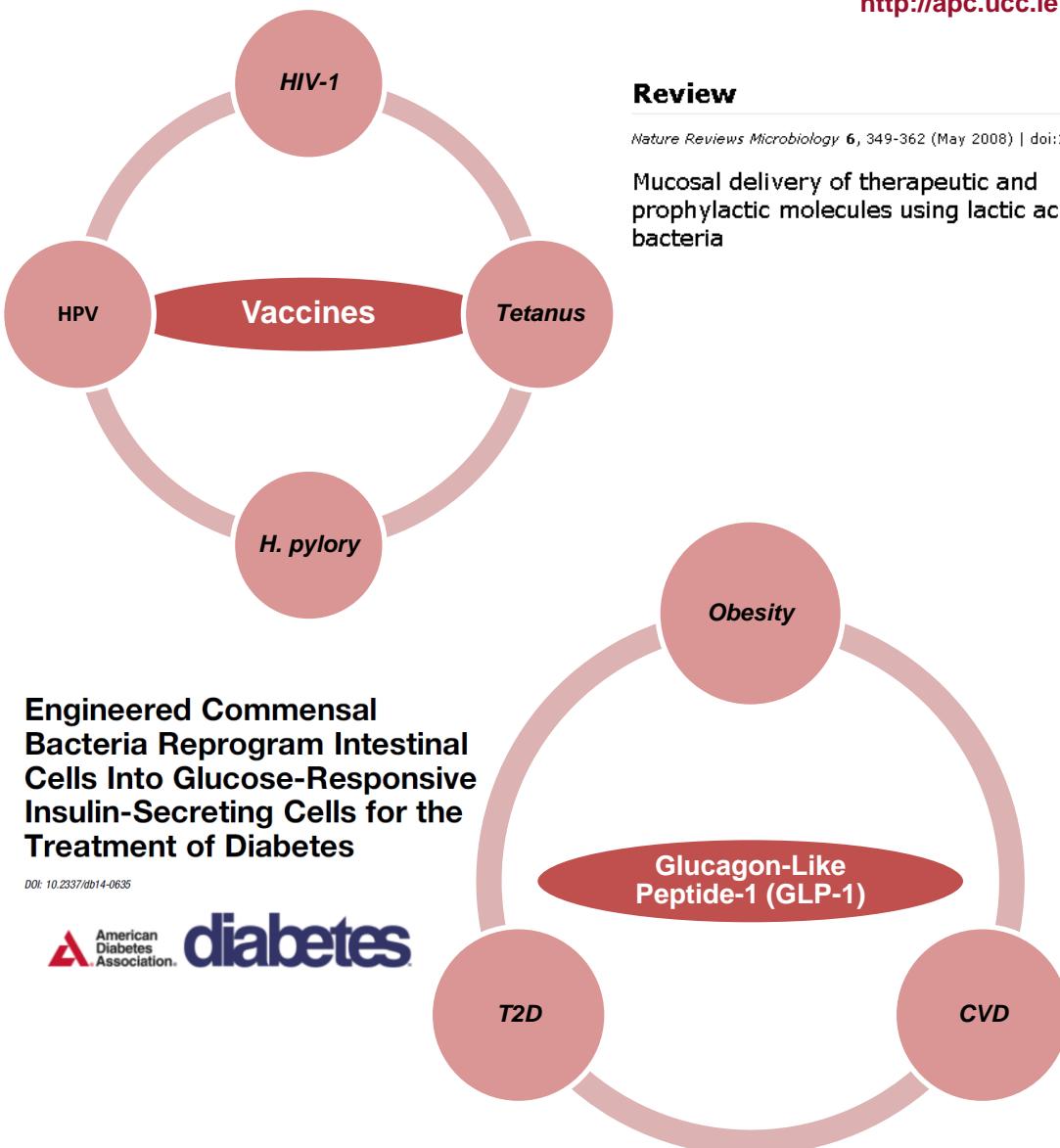
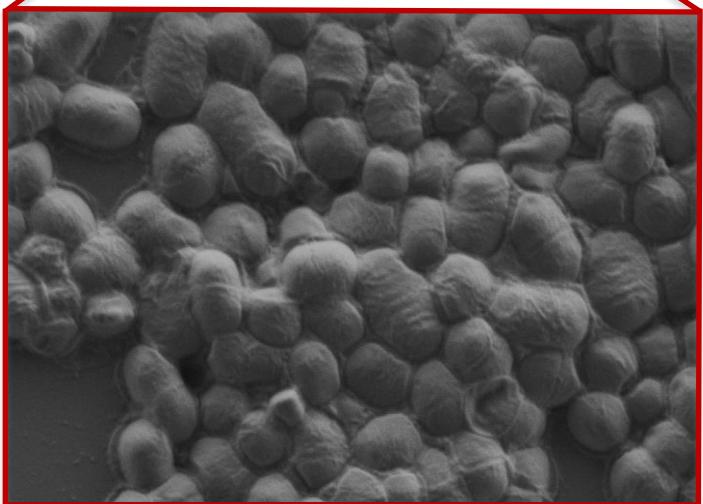
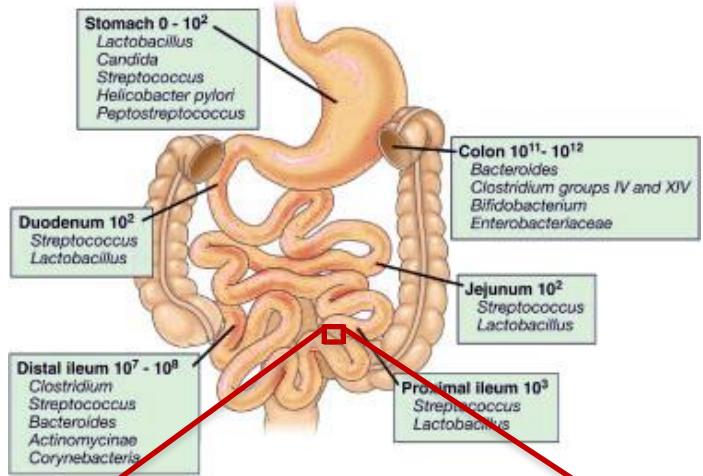
Microbiota Impacts Host Metabolism



Glucagon-Like Peptide-1

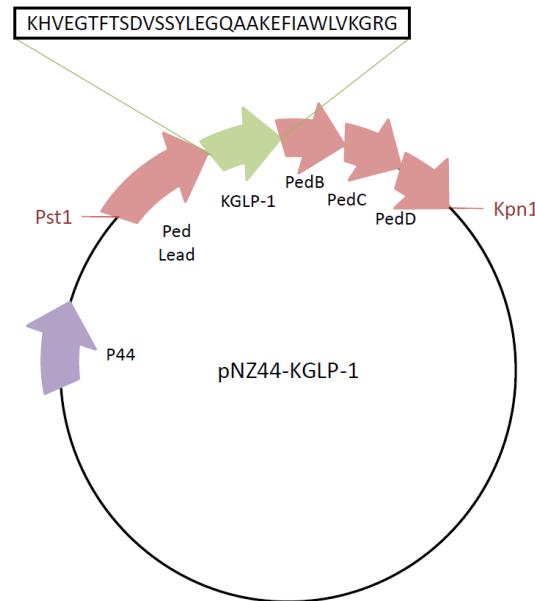


Probiotics as Synthetic Signalling Therapeutics

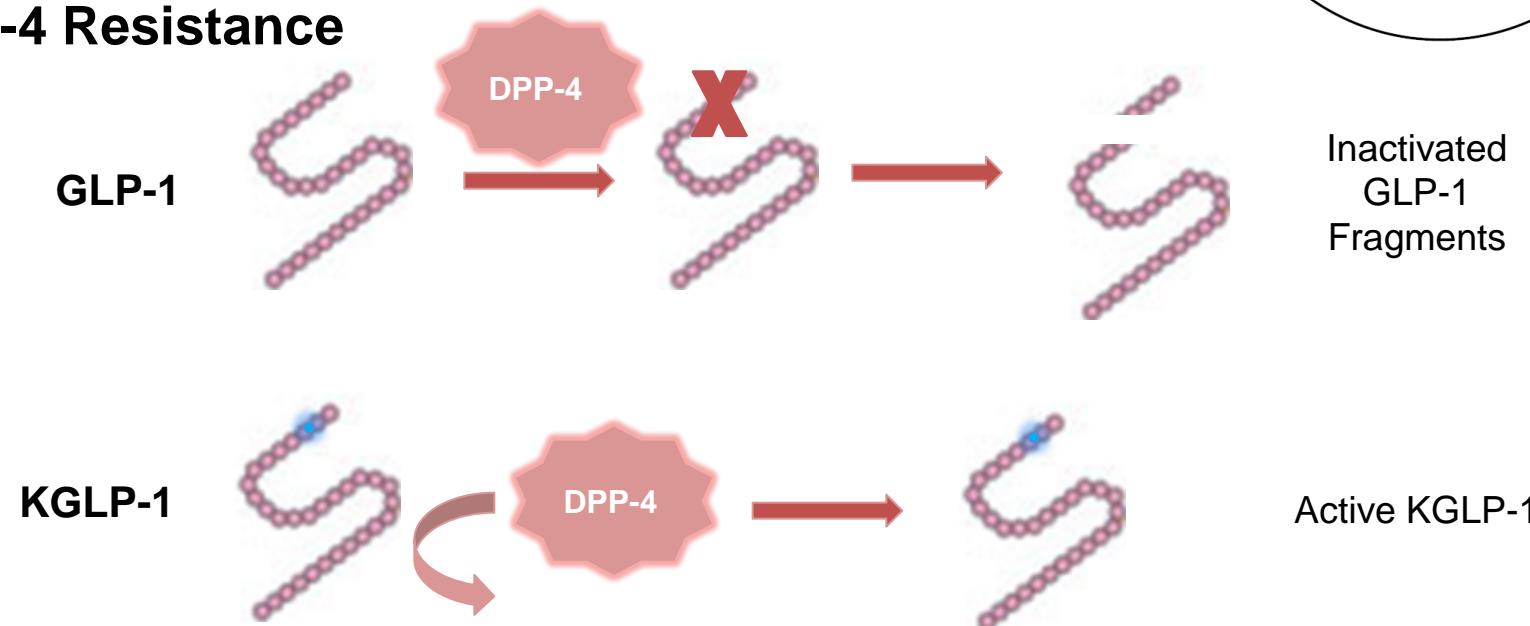


Construct & Peptide

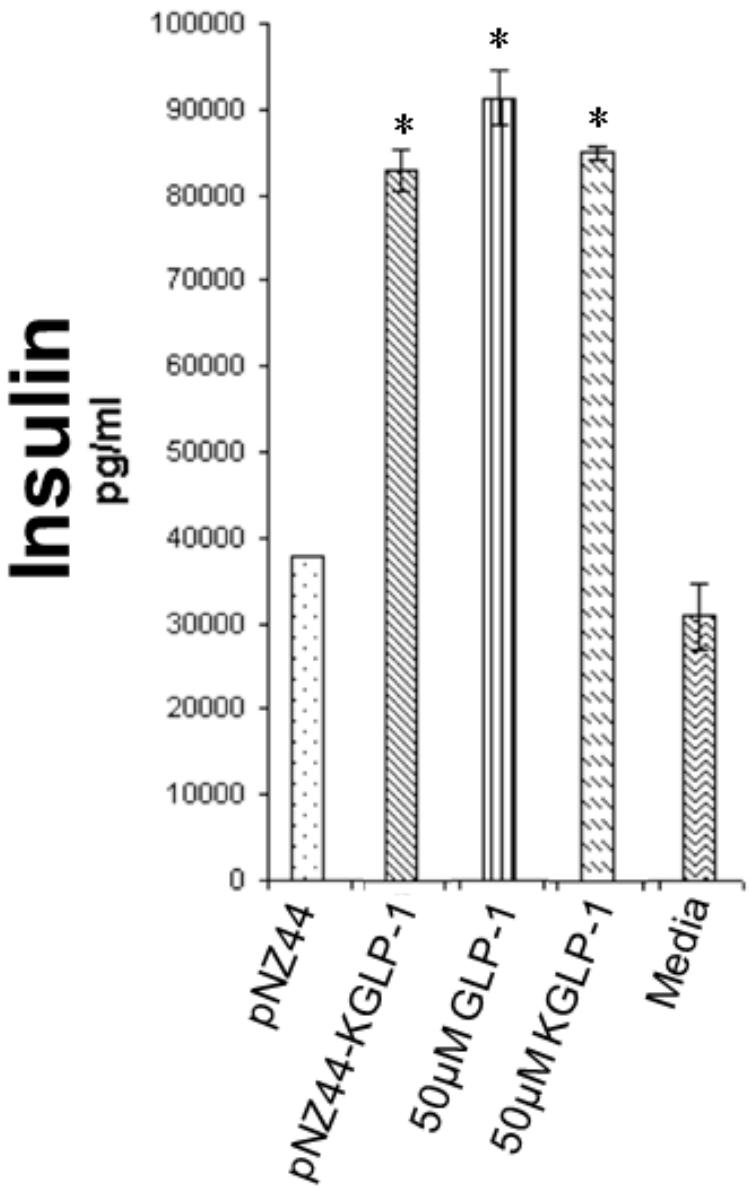
In order to protect the expressed peptide from the proteolytic action of **Dipeptidyl Peptidase-4 (DPP-4)**, the peptide sequence was altered – with addition of a lysine to native GLP-1 before the N-terminal histidine. This peptide was termed **KGLP-1** and expressed within the **pNZ44** plasmid within *Lactobacillus paracasei* NFBC 338.



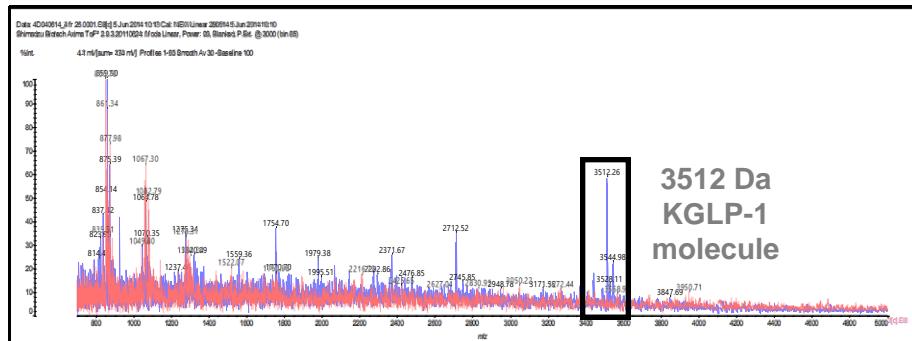
DPP-4 Resistance



In Vitro Insulinotropic activity in Rinnm5F β -cells



MALDI-TOF analysis of overnight culture broth
pNZ44 and *pNZ44-KGLP-1* supernatant

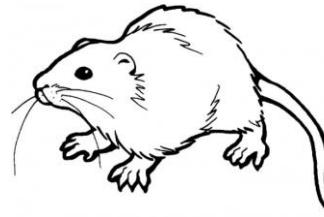


Lb. paracasei NFBC 388 *pNZ44-KGLP-1* cell-free extracts stimulated significant insulin secretion from murine β -cells, at levels comparable to cells treated with 50 μ M of pure GLP-1 or KGLP-1.

Cincinnati Metabolic Disease Institute Rat Study



20 Long-
Evans Rats



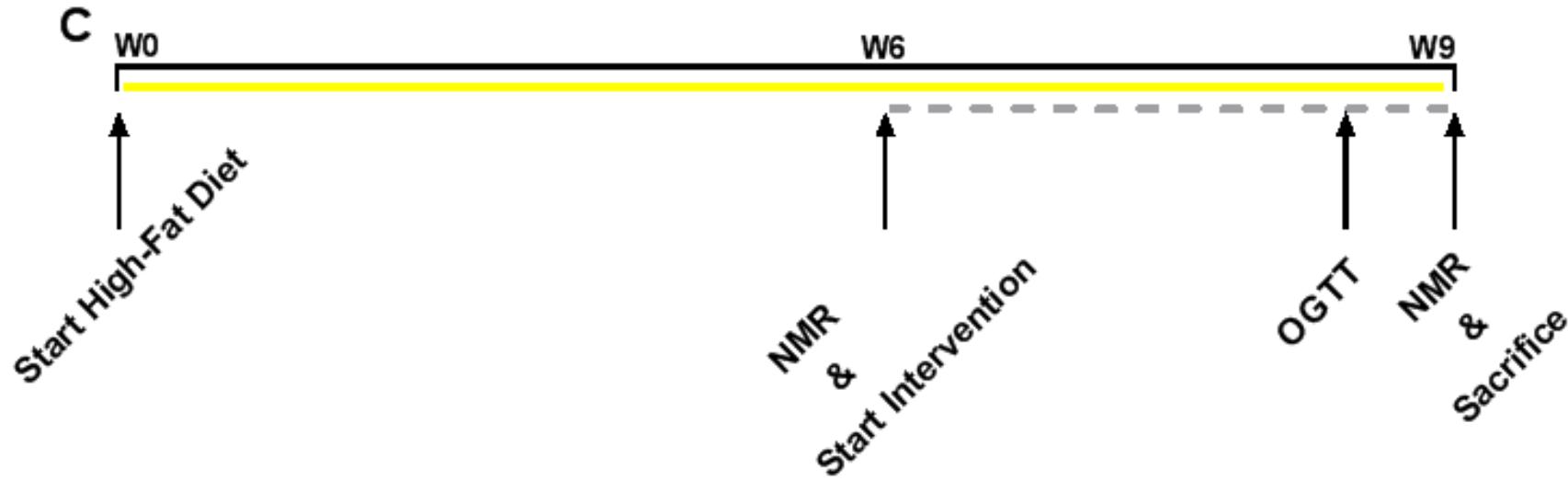
Paul Ryan

pNZ44

10^9 CFU/day

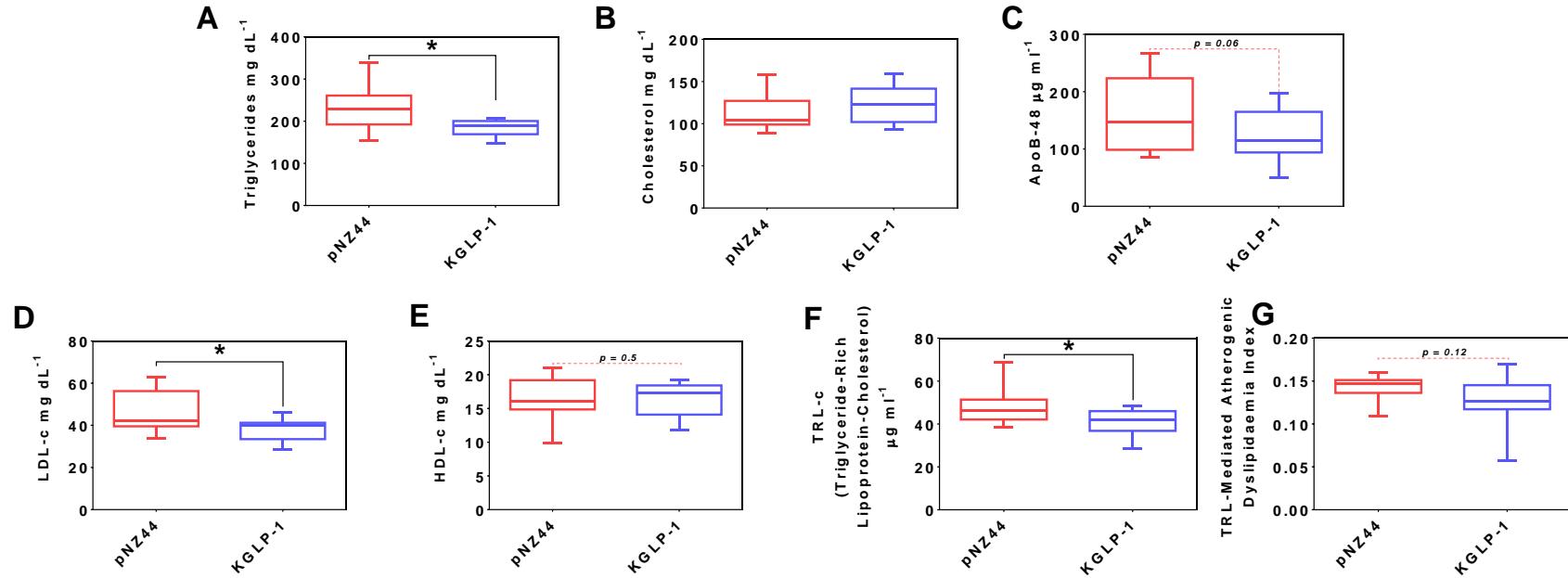
KGLP-1

10^9 CFU/day

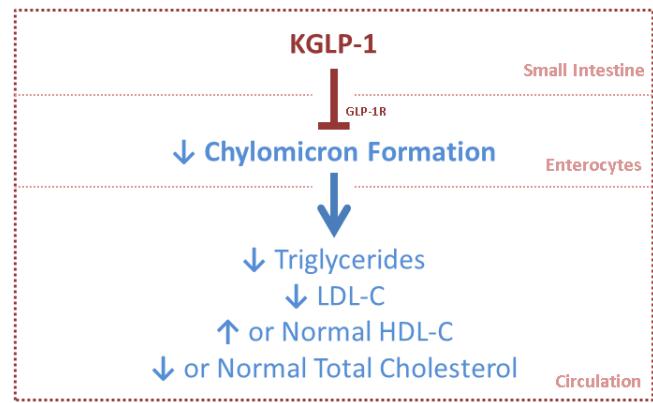
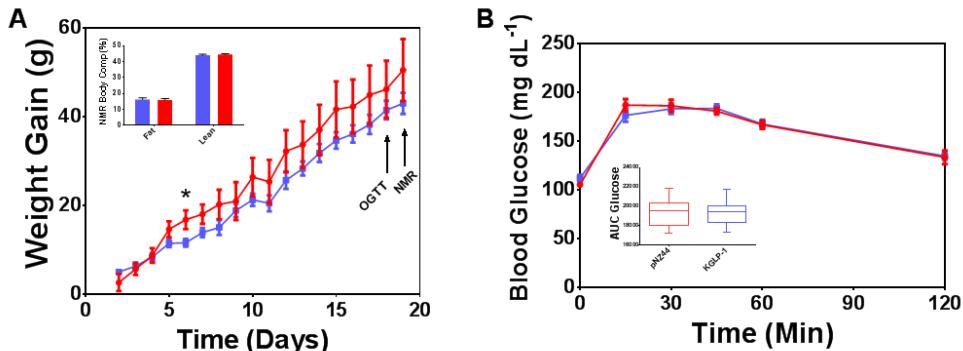


Lb. paracasei pNZ44 KGLP-1 Corrects Dyslipidaemia

Serum Lipid Profile

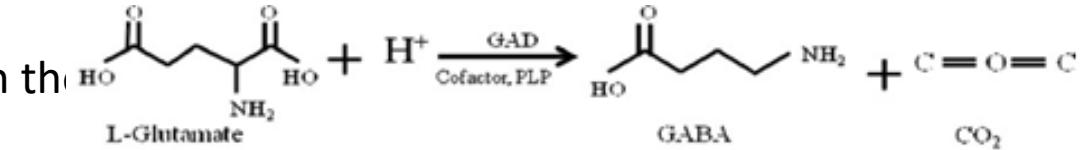


Weight Gain & Glucose Tolerance

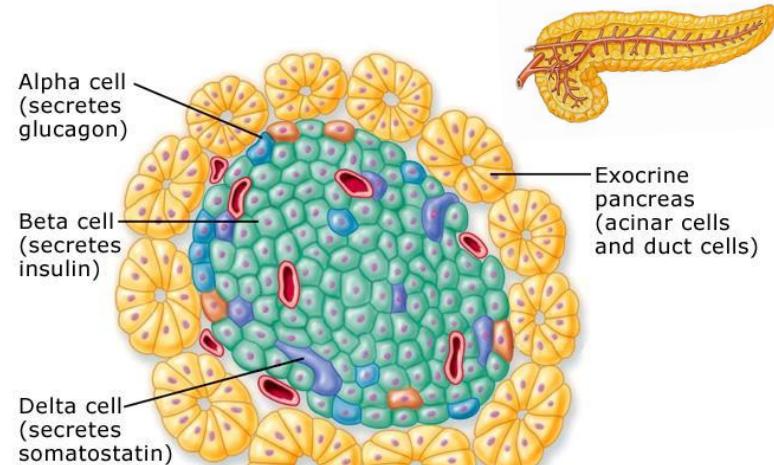


Gamma-Aminobutyric Acid

- γ -aminobutyric acid (GABA) is synthesised through decarboxylation of glutamic acid by glutamic acid decarboxylase (GAD)



- main inhibitory neurotransmitter in the brain
- biological functions of GABA are mediated by activation of GABA receptors in the brain and periphery (gastrointestinal tract, ovaries, uterus, and **pancreas**)
- α -cells, GABA induces membrane hyperpolarisation and \downarrow glucagon secretion
- β -cells, GABA induces membrane depolarisation and \uparrow insulin secretion
- GABA exerts β -cell regenerative effects, stimulates β -cell proliferation, protects β -cells against apoptosis and has anti-inflammatory and immunoregulatory activities



GABA vs Diabetes

OPEN  ACCESS Freely available online



Oral Treatment with γ -Aminobutyric Acid Improves Glucose Tolerance and Insulin Sensitivity by Inhibiting Inflammation in High Fat Diet-Fed Mice

Jide Tian*, Hoa N. Dang, Jing Yong, Wing-Sheung Chui, Matthew P. G. Dizon, Catherine K. Y. Yaw,
Daniel L. Kaufman

Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, California, United States of America

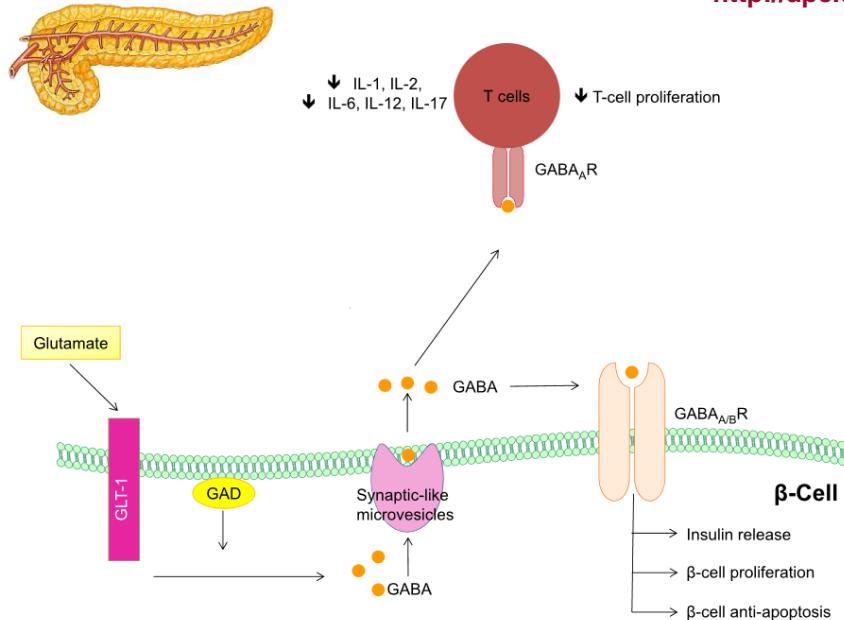
OPEN  ACCESS Freely available online



Combining Antigen-Based Therapy with GABA Treatment Synergistically Prolongs Survival of Transplanted β -Cells in Diabetic NOD Mice

Jide Tian, Hoa Dang, Daniel L. Kaufman*

Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, California, United States of America



GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes

Neftali Soltani^{a,b,1,2}, Hongmin Qiu^{a,b,2,3}, Mila Aleksić^b, Yelena Glinka^c, Fang Zhao^{a,b}, Rui Liu^{a,d}, Yiming Li^d, Nina Zhang^{a,b}, Rabindranath Chakrabarti^d, Tiffany Ng^{a,b}, Tianru Jin^b, Haibo Zhang^{b,e}, Wei-Yang Lu^f, Zhong-Ping Feng^b, Gerald J. Prud'homme^c, and Qinghua Wang^{a,b,d,4}



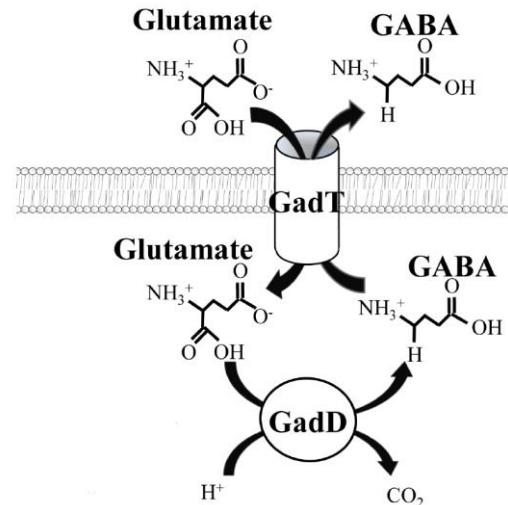
GABA Promotes Human β -Cell Proliferation and Modulates Glucose Homeostasis

Indri Purwana^{1,2}, Juan Zheng^{1,2}, Xiaoming Li^{1,2}, Marielle Deurloo², Dong Ok Son^{1,2}, Zhaoyun Zhang³, Christie Liang^{1,2}, Eddie Shen^{1,2}, Akshaya Tadkase¹, Zhong-Ping Feng², Yiming Li³, Craig Hasilo⁴, Steven Paraskevas⁴, Rita Bortell⁵, Dale L. Greiner⁵, Mark Atkinson⁶, Gerald J. Prud'homme⁷ and Qinghua Wang^{1,2,3,4}

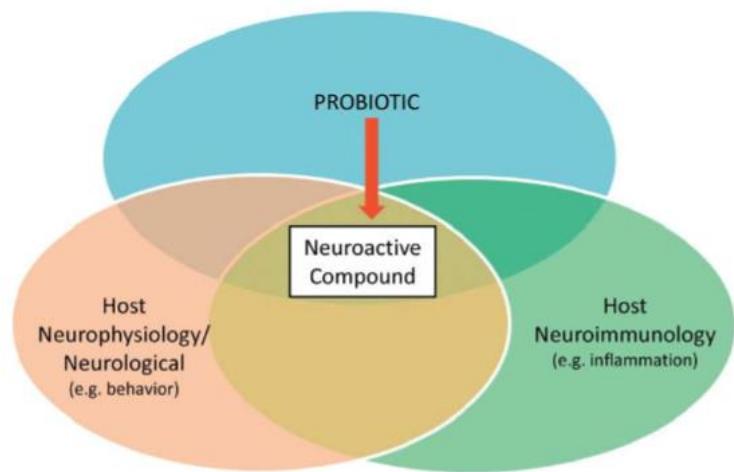
GABA = Microbial Metabolite vs Diabetes

- The GAD system in micro-organisms is mainly related with resistance against acidic conditions
- Can be considered a probiotic factor, as this system enables organisms to pass through the acidic conditions of the stomach barrier

Species	Strain source	GABA (mg ml^{-1}) converted from increasing concentrations of MSG (mg ml^{-1})				
		10 (mg ml^{-1}) MSG	20 (mg ml^{-1}) MSG	30 (mg ml^{-1}) MSG	40 (mg ml^{-1}) MSG	50 (mg ml^{-1}) MSG
<i>Lactobacillus brevis</i> DPC6108	Infant faeces	5.51 ± 0.09	10.23 ± 0.04	14.01 ± 0.07	15.19 ± 0.16	16.16 ± 0.41



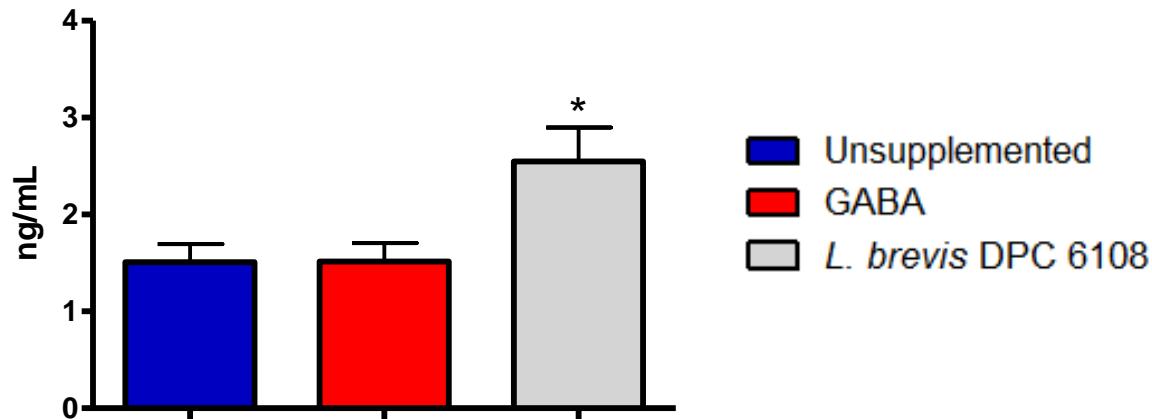
91 intestinally-derived bacteria assessed, one *Lactobacillus brevis* strain (*Lb. brevis* DPC 6108) could convert up to 90% MSG to GABA *in vitro*



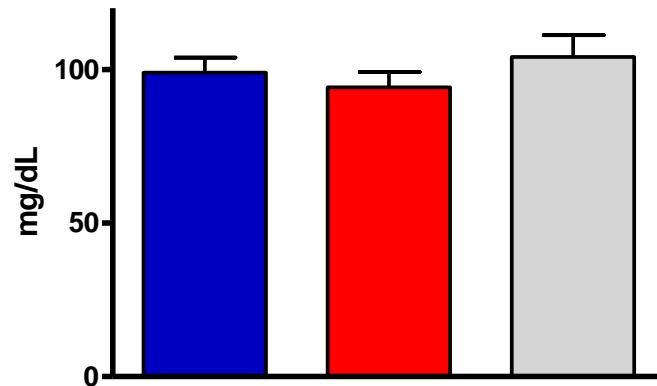
GABA producing *Lb. brevis* DPC 6108

Healthy Rats

Insulin

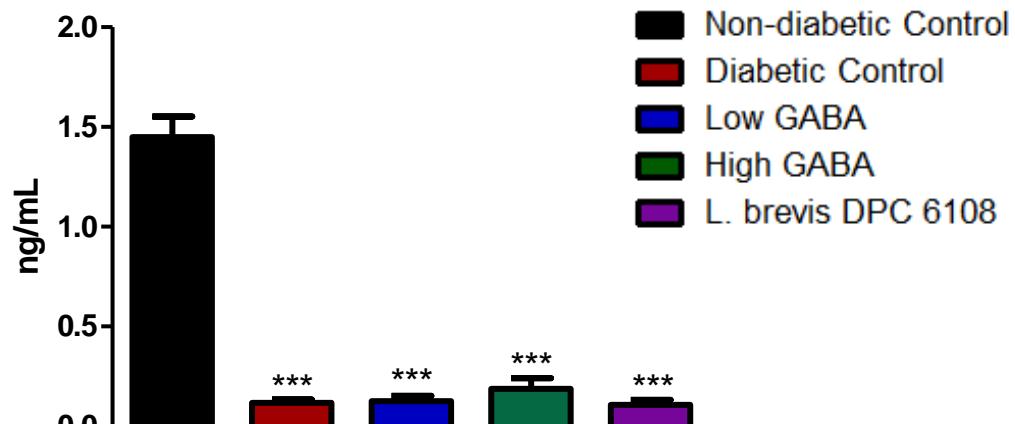


Glucose

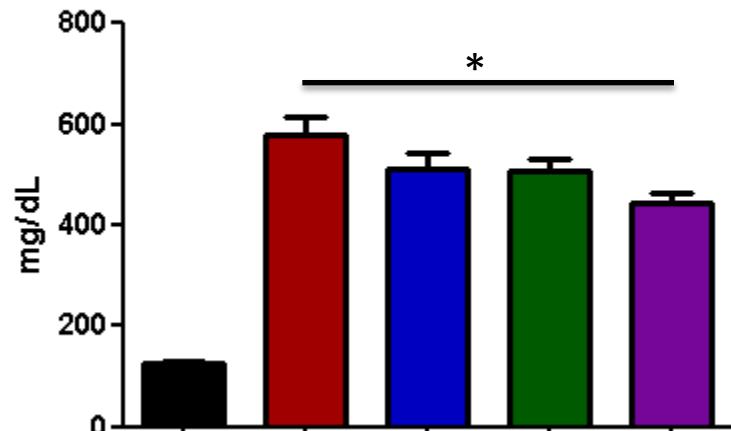


Unhealthy / T1D Rats

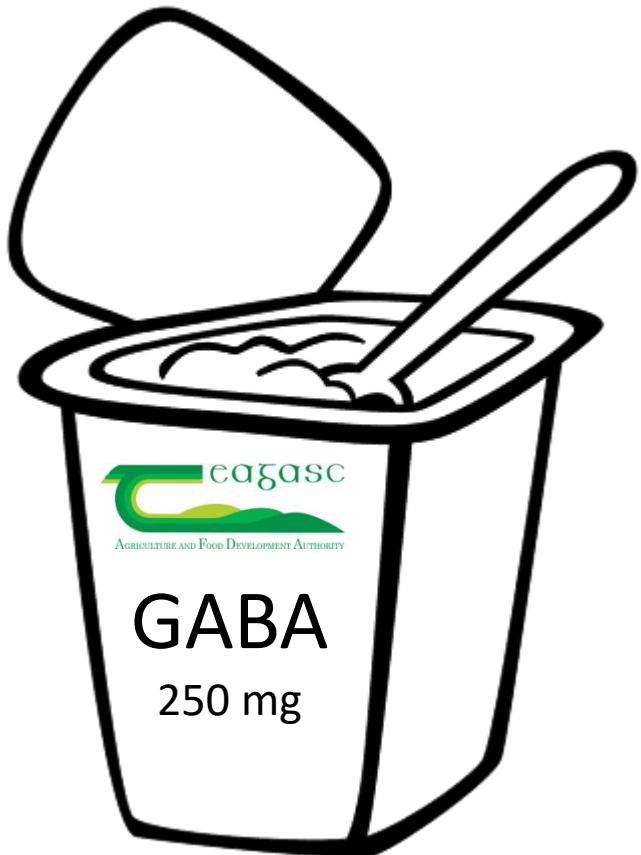
Insulin



Glucose



GABA Yoghurt



 *Lactobacillus bulgaricus* CH

 *Streptococcus thermophilus* GABA⁺



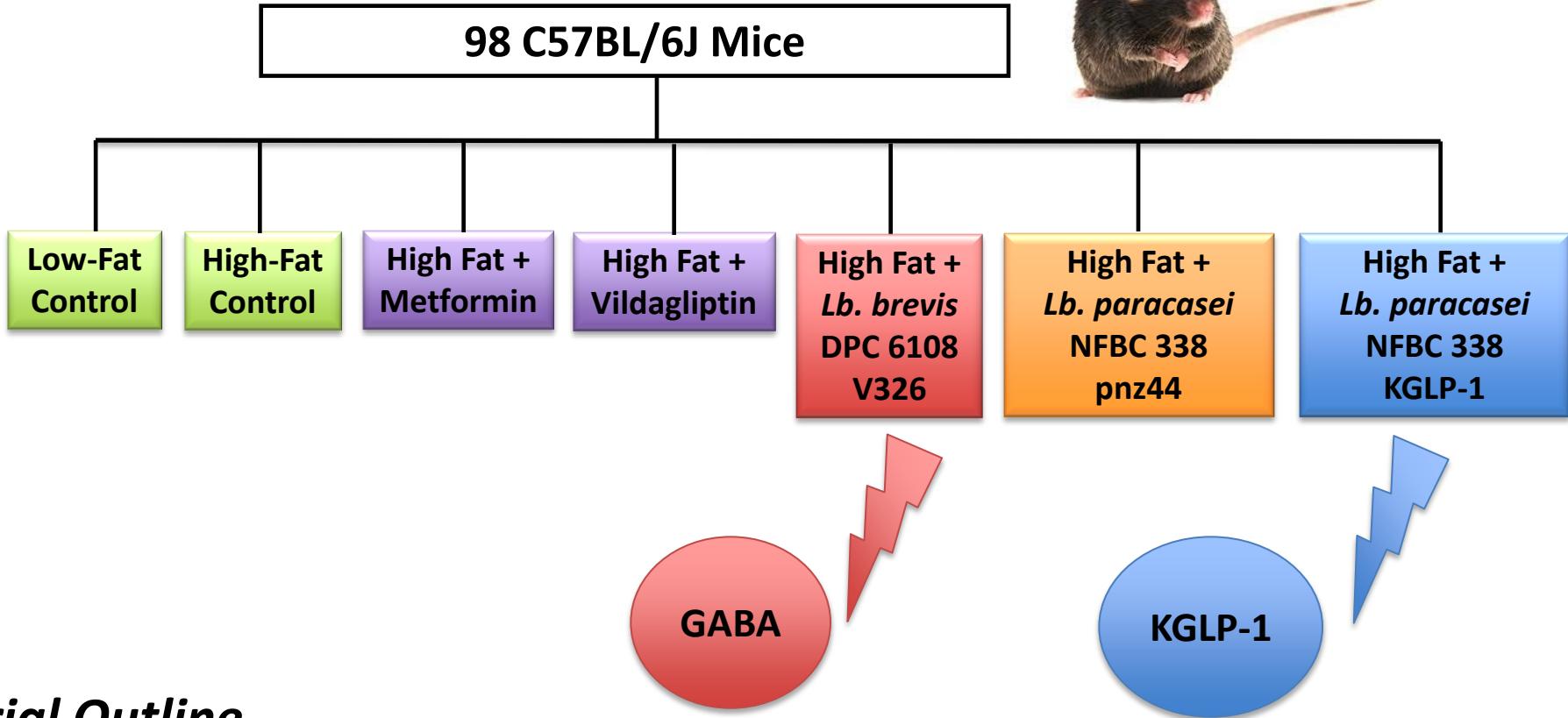
 *Lactobacillus bulgaricus* CH

 *Streptococcus thermophilus* CH



Dr. Daniel
Linares

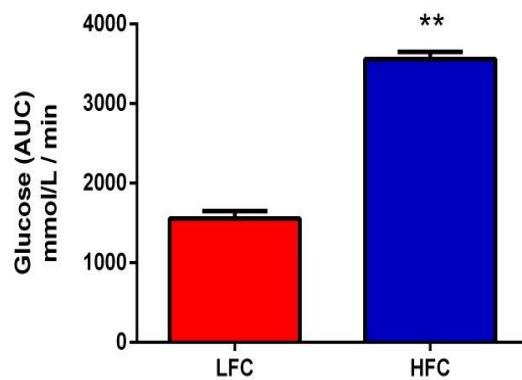
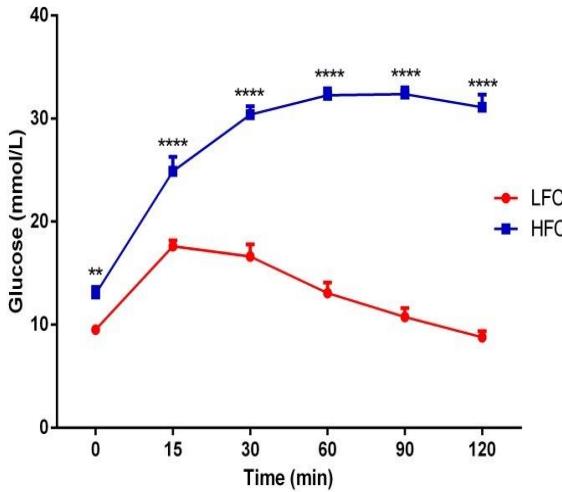
Anti-Obesity / Diabetes Probiotics - What's next?



Trial Outline



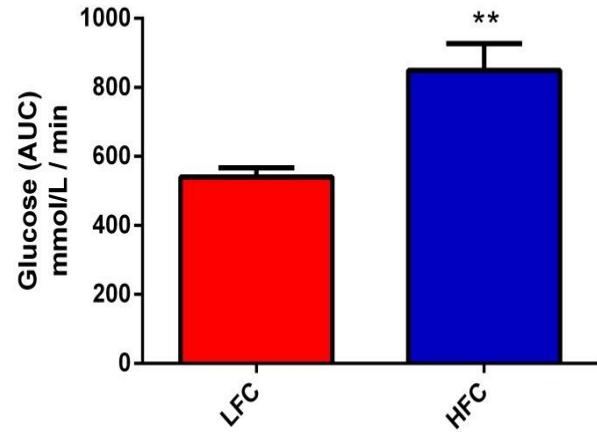
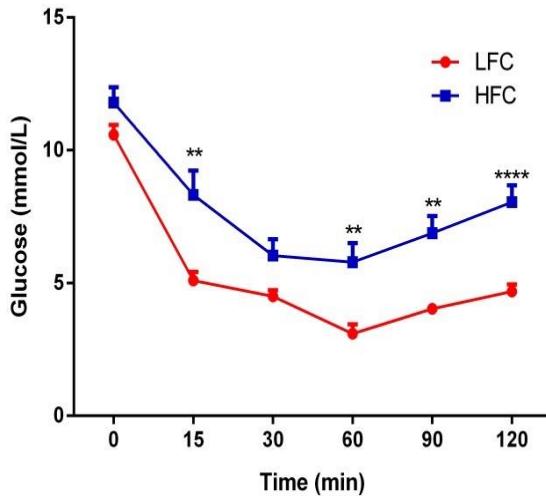
Glucose Intolerance & Insulin Sensitivity



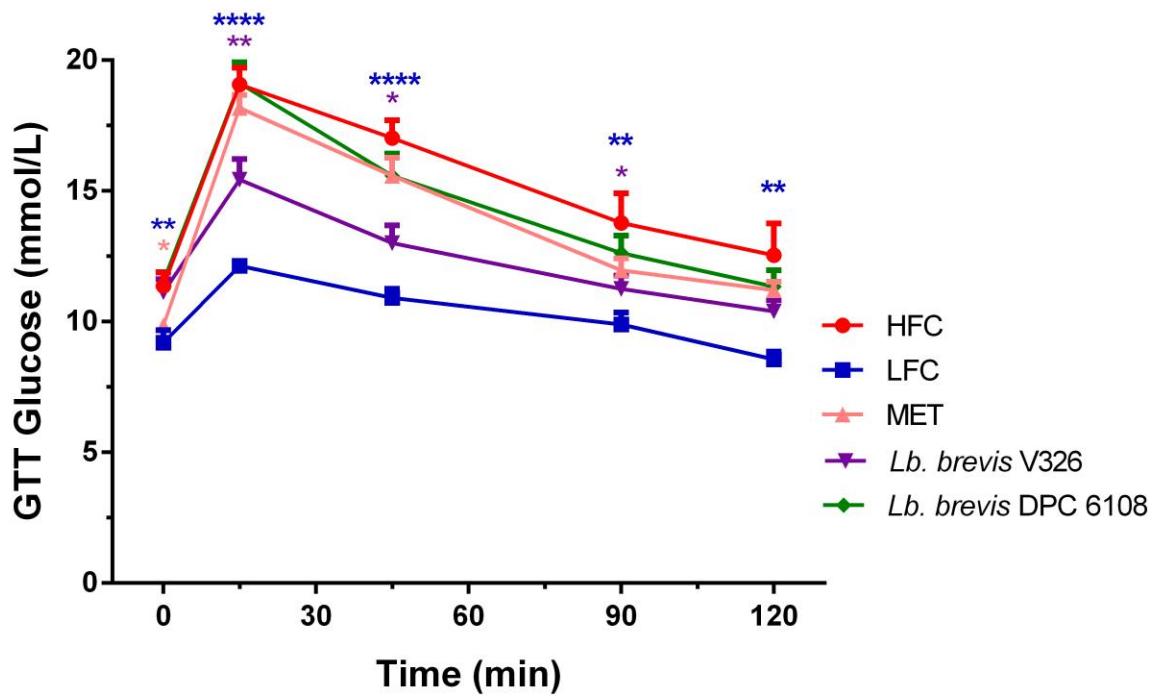
Inability to clear blood glucose after 12 weeks HF feeding



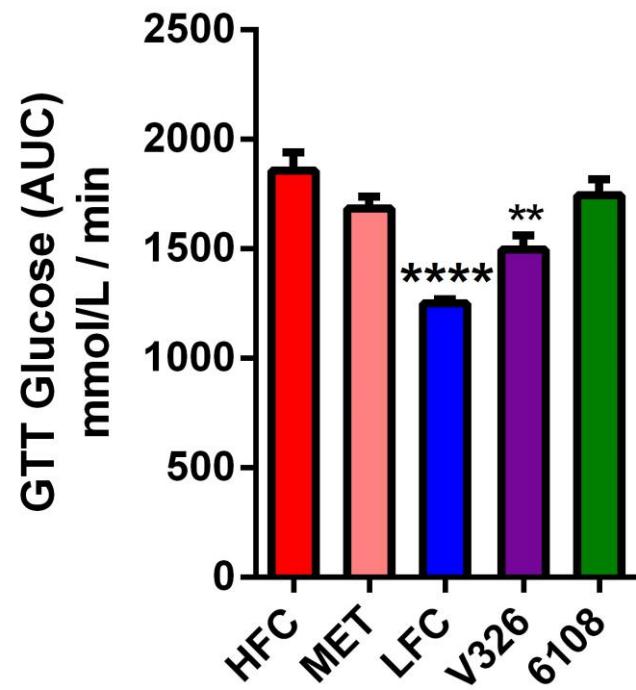
Reduced insulin sensitivity after 12 weeks HF feeding



Glucose Intolerance & Insulin Sensitivity



Lb. brevis V326 significantly improved glucose tolerance after 10 weeks feeding



Conclusions

- Over 37% of Irish men & women will be obese by 2025
- The gut microbiota alters the energy harvesting capacity of the host
- Altered gut microbial composition in obesity and diabetes
- Microbial metabolites can regulate appetite and energy balance
- GABA & GLP-1 could prove effective metabolites for the treatment of high fat dietary induced hyperglycaemia
- Could be used as functional food ingredients for yoghurt, dairy drinks, formula drinks for healthy & diabetic individuals to control blood glucose

Acknowledgments



Mr. Paul Ryan

Dr. Tatiana Marques

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Dr. Daniel Linares



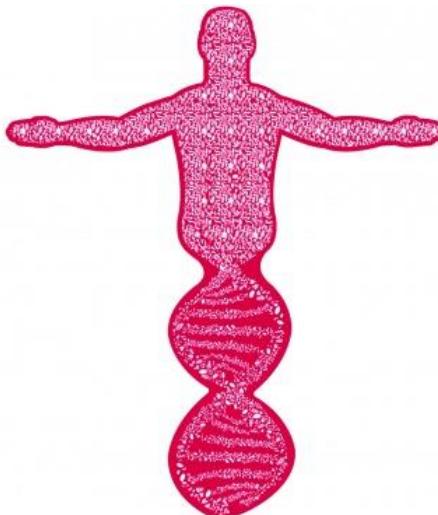
Prof. Catherine Stanton

Prof. Paul Ross

Prof. John Cryan

Prof. Ted Dinan

Prof. Fergus Shanahan



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Dr. Bernadette Grayson

Prof. Randy J Seeley

