AgriResearch Conference

Innovating for the future of farming and rural communities

2-3 May, Brussels

Agriculture and other human activities, microbial ecosystems and health

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#AgriResearchEU

European Commission

The vertebrate ecosystem

Vertebrates evolved ~500 million years ago

Microbial partners (Microbiota)

More microbiota in gut than human cells in the body

Much more DNA, metabolic pathways in microbiota than in human genome

Drive development
- Most organs, incl. **brain**
- Sex hormone reuptake

Metabolites
- ~ 20-30 % of small molecules in blood

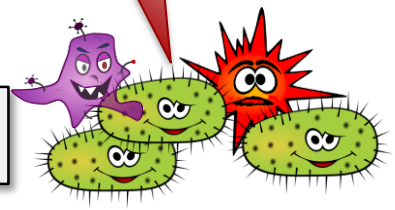
Regulate
- Brain function
- Metabolism
- Endocrine system
- Immune system

Complex **adaptive** immune system

Manage, "farm"

Kill or exclude

Pathogens



Shu et al. (1999) *Nature* **402**:42-6.
McFall-Ngai (2007) *Nature* **445**:153
Pancer & Cooper (2006) *Annu Rev Immunol* **24**:497-518
Fuhrman *et al* (2014) *J Clin Endocrinol Metab* **99**:4632

Organisms with which humans co-evolved : the “Old Friends”

Microbiota
from mother and family
and household

Natural environment
Spores, organisms
(and their genes)

DATA (molecular structures, signals from microbial components), & ORGANISMS

Immune system **at birth**
- hardware
- software
- needs **DATA**

DATA (molecular structures, signals from microbial components), & ORGANISMS

- Epigenetic
- Development
- Repertoire

Attack

Repertoire: biodiversity drives a wide range of “memory” cells that can rapidly recognise pathogens

Immunoregulation

- **Do not attack “forbidden targets”** (self, allergens, gut contents)
- **Turn off redundant inflammation** (metabolic disease, depression, CVD)

Rook (2013) *Proc Natl Acad Sci U S A* **110**:18360 McDade *et al* (2017) *Proc Natl Acad Sci USA* **114**:7611

Distorted microbiota with reduced biodiversity	Risk increased by antibiotics
Immune system attacks forbidden targets	
Autoimmunity	Rosser & Mauri (2016) *J Autoimmun* **74**:85 Clausen *et al* (2016) *PLoS One* **11**:e0161654
Asthma, other allergies	Korpela *et al* (2016) *Nat Commun* **7**:10410 Metsala *et al* (2013) *Epidemiology* **24**:303
Inflammatory bowel disease	Shaw *et al* (2010) *Am J Gastroenterol* **105**:2687 Hviid *et al* (2011) *Gut* **60**:49
Immune system drives redundant inflammation	
Metabolic dysregulation, obesity	Trasande *et al* (2013) *Int J Obes (Lond)* **37**:16 Shao *et al* (2017) *Front Endocrinol* **8**:170 Cassidy-Bushrow *et al* (2017) *Int J Obes (Lond)*
Depression	Slykerman *et al* (2017) *Acta Paediatr* **106**:87 Neufeld *et al* (2017) *J Psychiatr Pract* **23**:25
Cancer (colon and breast)	Cao *et al* (2017) Apr 4 *Gut* Velicer *et al* (2004) *JAMA* **291**:827

Microbiota regulate immune system & metabolism during an *early window* of opportunity

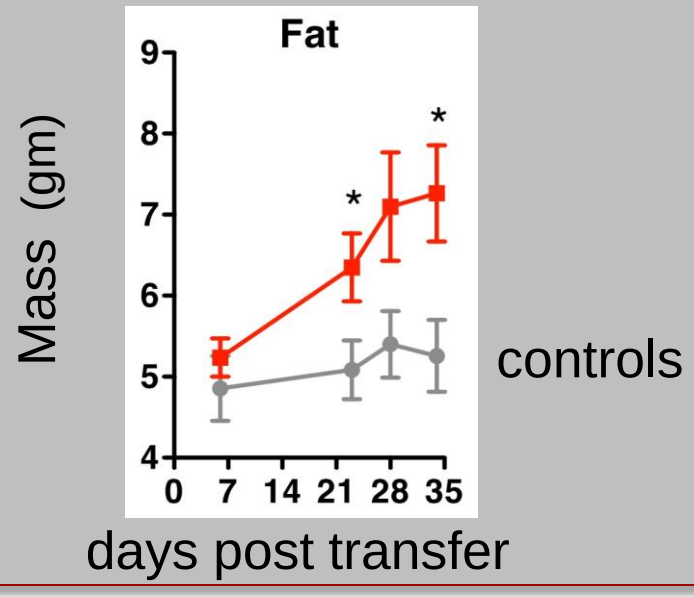
Low dose penicillin while *in utero*, and for first ***4 weeks***

Temporary changes in microbiota

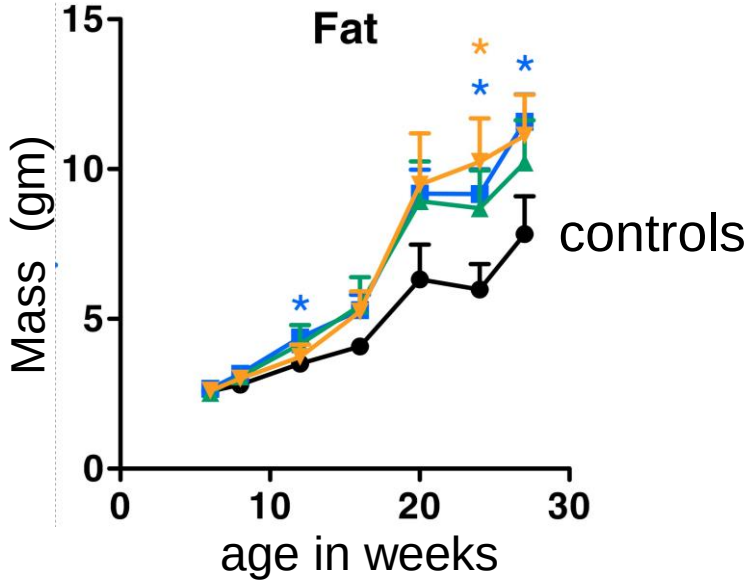
Persistent downregulation of immune function

Persistent changes to hepatic gene expression

Transfer changed microbiota to germ-free mice (no penicillin)



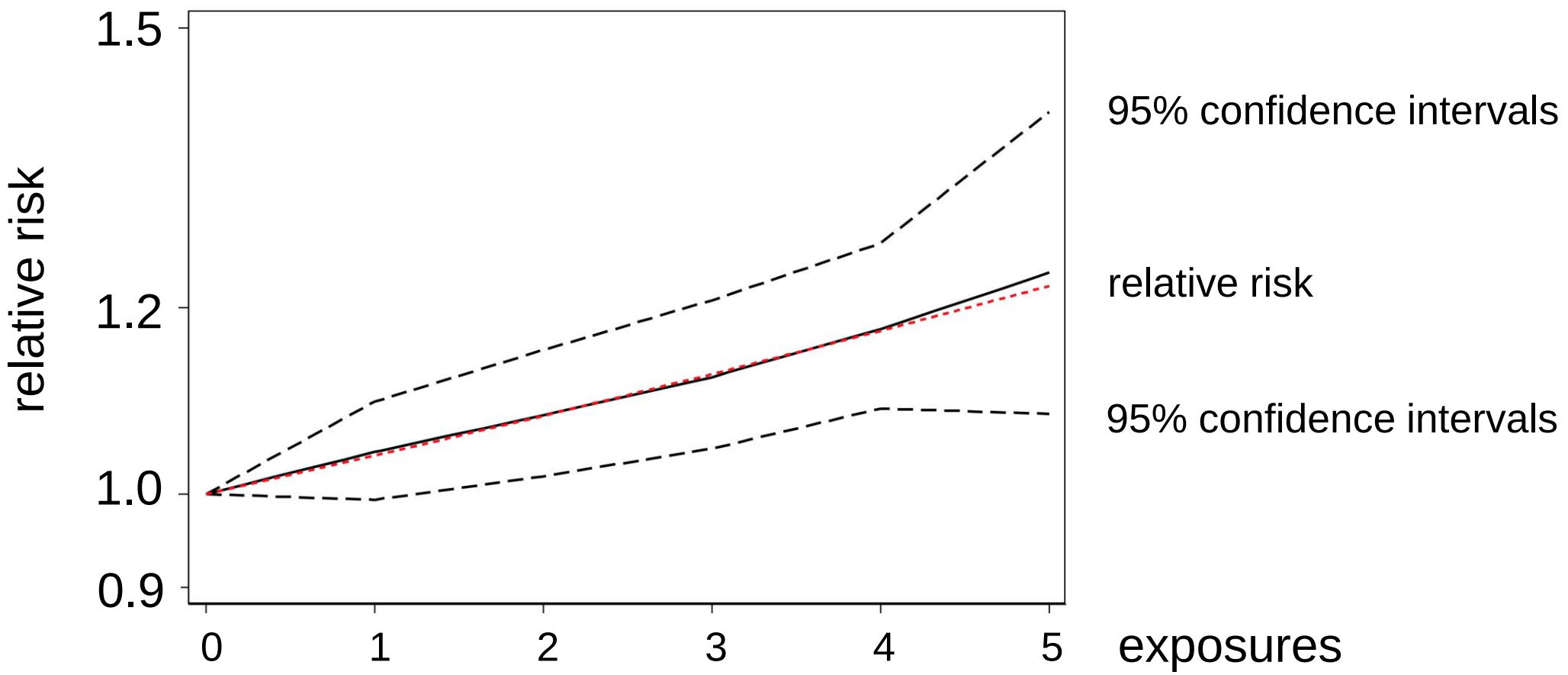
Progressive increase in fat mass, long after microbiota appears normal



Cox *et al* (2014) *Cell* **158**:705

Trasande *et al* (2013) *Int J Obes (Lond)* **37**:16

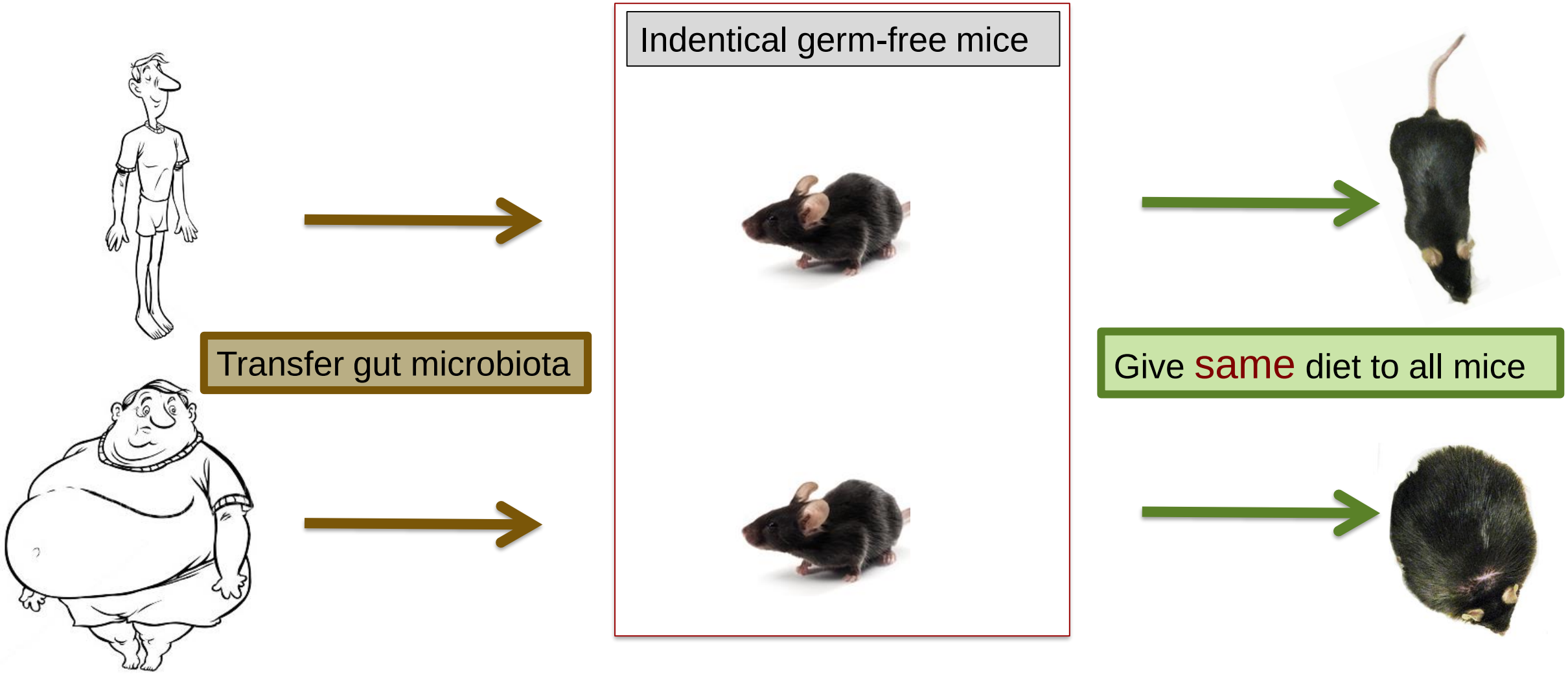
Perinatal (pregnancy or early life) antibiotic exposure & **obesity**



Dose–response meta-analysis of the association between antibiotic exposure in early life and **childhood obesity**

Shao *et al* (2017) *Front Endocrinol* **8**:170

The gut microbiota can influence weight gain



Turnbaugh et al (2006) *Nature* **444**:1027-31.

Ridaura et al. (2013) *Science* **341**:1241214.

Allergies and exposure to environmental microbiota

Epidemiology

Riedler *et al* (2001) *Lancet* **358**:1129
Aichbhaumik *et al* (2008) *CEA* ***38***:1787
Sozanska *et al* (2013) *JACI* **133**:1347
Song *et al* (2013) *Elife* **2**:e00458
Lynch *et al* (2014) *JACI* **134**:593

- Farms, cowsheds
- Dogs *in the home*
- Traditional rural versus urban
- Microbe-rich house dust
- Spores of human-adapted strains

plants
soil
animals
outside air

Identify candidate organisms

Ege *et al* (2012) *Allergy* **67**:1565
Hanski *et al* (2012) *PNAS* **109**:8334
Karvonen *et al* (2014) *Allergy*
Lynch *et al* (2014) *JACI*
Browne *et al* (2016) *Nature* **533**:543

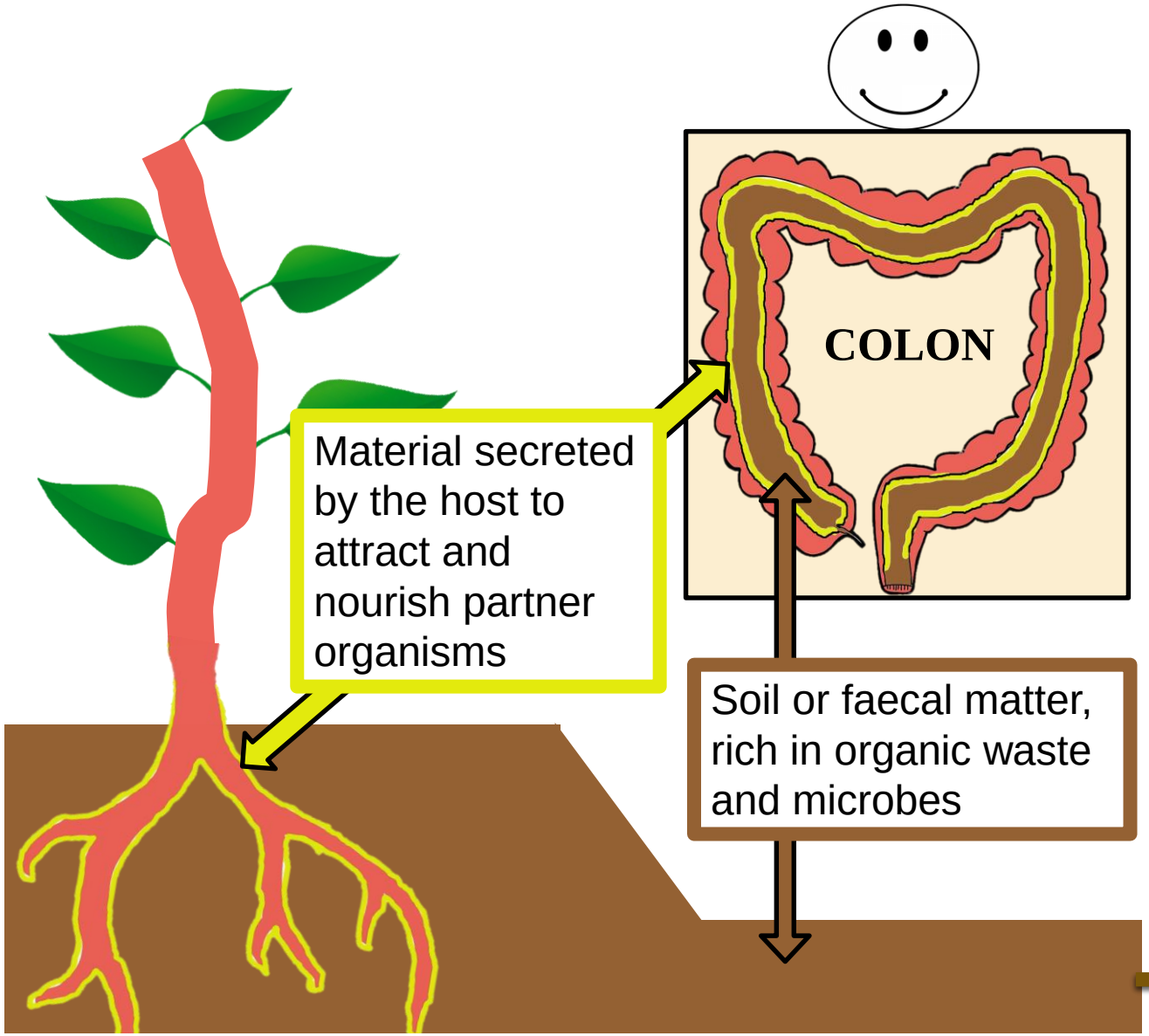
Test in animal models

Debarry *et al* (2007) *JACI.* **119**:1514
Vogel *et al* (2008) *JACI* **122**:307
Conrad *et al* (2009) *JEM* **206**:2869
Hagner *et al* (2013) *Allergy* **68**:322

Mechanisms

Treg (regulatory T lymphocytes), earlier maturation of Th1, IL-10, DCreg

Soil, plant and vertebrate microbiota

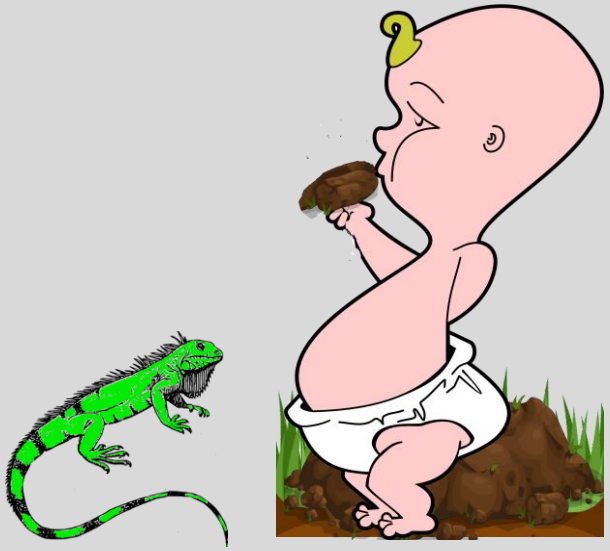


Flandroy et al (2018) *Sci Tot Env* **627**:1018-1038

Reconstitute the gut microbiota of germ-free mice with soil microbiota

Seedorf *et al* (2014) *Cell* **159**:253

Young vertebrates eat soil



Ngure *et al* (2013) *Am J Trop Med Hyg* **89**:709
Troyer (1984) *Behav Ecol Sociobiol* **14**:189

Farmers, immunoregulation and the natural environment

Amish

Hutterite

	Allergies	
4x lower	Asthma	4x higher
6x lower	Allergic sensitisation	6x higher

Findings in blood

Higher ← Immunoregulatory gene *TNFAIP3* (A20) in blood

HLA DR on monocytes, biomarker of inflammation → Higher

Amish house dust ↓ Effect on an asthma model in allergic mice ↑ Hutterite house dust

Stein et al. (2016) *New Engl J Med* **375**:411-421 Schuijs *et al* (2015) *Science* **349**:1106

Pollutant (or metabolites)	**µg/L in human urine [1]**	**Effect on microbiota**
Permethrin	0.4	[2]
Chlorpyrifos (2009-2010)	0.8	[3]
Glyphosate (not in CDC report)	0.5-7.20 (from ref [14])	[4]
Phthalates	30-40	[5]
Methylparaben	>40	[5]
Bisphenol A	~ 2	[6]
Triclosan	10-20	[5, 7]
Lead	0.5	[8, 9]
Arsenic	10	[10]
Cadmium	0.2	[8, 11]
Polycyclic aromatic hydrocarbons	5 (Non smokers)	[12, 13]

macrolides, sulphonamides, trimethoprim	low µg/L in **drinking water reservoirs**	[15]

1. Centers for Disease Control and Prevention (2018) http://www.cdc.gov/exposurereport

2. Nasuti *et al* (2016) *Env Sci Pollut Res* **23**:10930
3. Joly *et al* (2013) *Environ Sci Pollut Res Int* **20**:2726
4. Lozano *et al* (2018) *Toxicology Reports* **5**:96
5. Hu *et al* (2016) *Microbiome* **4**:26
6. Lai *et al* (2016) *Environ Pollut* **218**:923
7. Ribado *et al* (2017) *EMBO Mol Med* **9**:1732
8. Breton *et al* (2013) *BMC Pharmacol Toxicol* **14**:62
9. Xia *et al* (2018) *Sci Tot Env* **631-632**:439
10. Dong *et al* (2017) *PLoS ONE* **12**:e0188487
11. Liu *et al* (2014) *PLoS One* **9**:e85323
12. Parajuli *et al* (2017) *PLoS One* **12**:e0187852
13. Roslund *et al* (2018) *PeerJ* **6**:e4508
14. Parvez *et al* (2018) *Environ Health* **17**:23
15. Kümmerer (2009) *Chemosphere* **75**:417

More reasons to worry about low level pollutants and microbiota

Many “endocrine disruptors” target the microbiota

Snedeker&Hay (2012) *Env. Health Perspect.* **120**:332
Velmurugan *et al* (2017) *Trends Endocrinol Metab* **28**:612
Jin *et al* (2017) *Environ Pollut* **222**:1

Sub-inhibitory concentrations of antibiotics (as low as 1/100,000 of the MIC) as found in environmental water, can modify transcription and change biofilm architecture

Bruchmann *et al* (2013) *Env. Sci. Pollut. Res.* **20**:3539
Gullberg *et al* (2011) *PLoS Pathog* **7**:e1002158

Accumulative effects of sub-threshold doses of multiple active molecules, even of different classes, and with different sites of action

Kortenkamp (2007) *Env. Health Perspect.* **115**:98
Thrupp *et al* (2018) *Sci. Tot. Env.* **619-620**:1482

Early life exposure to low dose pollutant mixtures

Diethyl phthalate (DEP), Methylparaben (MPB), Triclosan (TCS)

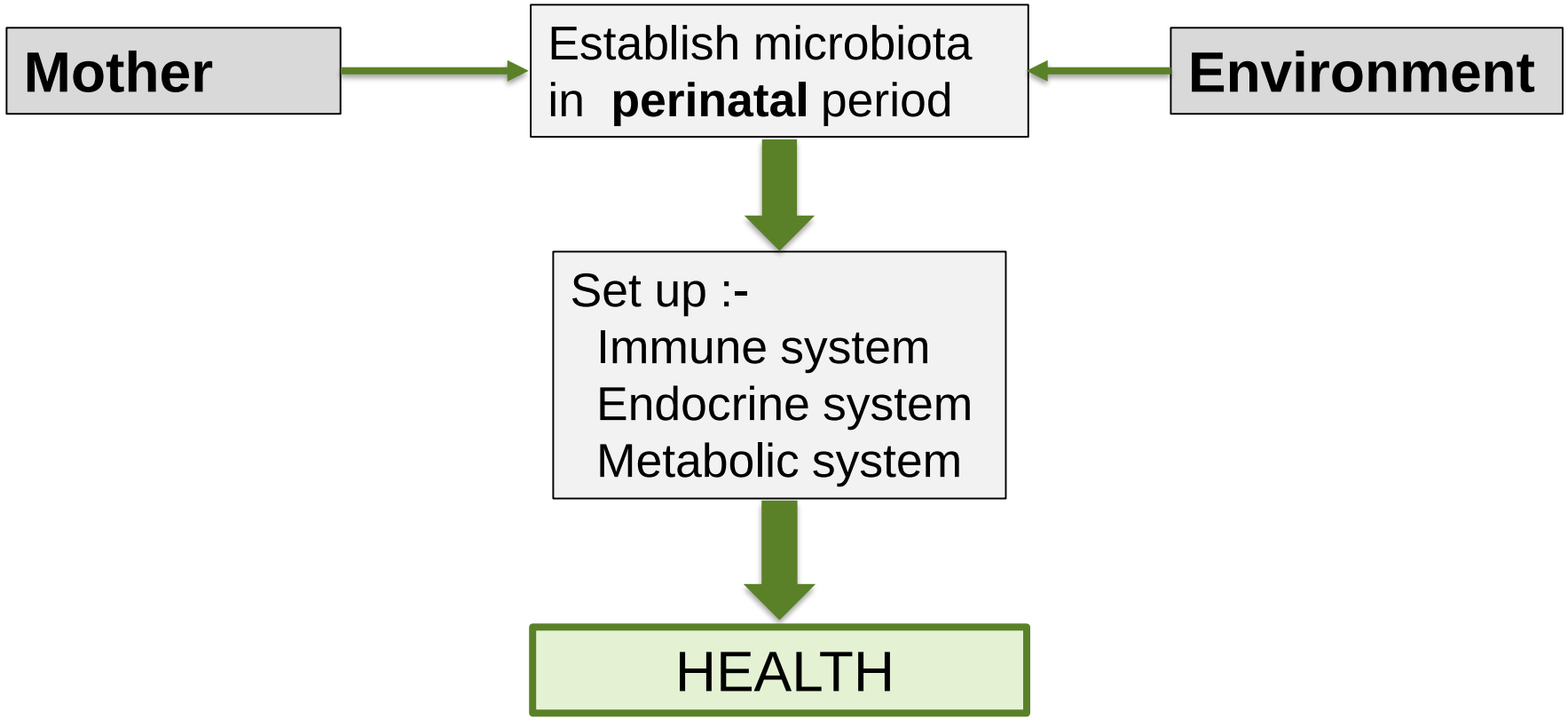
By gavage to the mothers
from parturition for 4 weeks

After weaning at 4 weeks,
administered to the ♀ pups

DEP, MPB & TCS levels in urine similar to those in the US population

Significant changes in the overall bacterial composition,
but in ***day 62* adolescent rats only**.

Hu *et al* (2016) *Microbiome* **4**:26



Rook (2013) *Proc Natl Acad Sci U S A* **110**:18360 McDade *et al* (2017) *Proc Natl Acad Sci USA* **114**:7611

Mother

↑ caesareans,
↓ breast-feeding
↑ antibiotics,
↑ bad diet

(-)

Establish microbiota in **perinatal** period

(-)

Environment

↑ urban lifestyle
↓ exposure to nature
↑ chemical pollutants

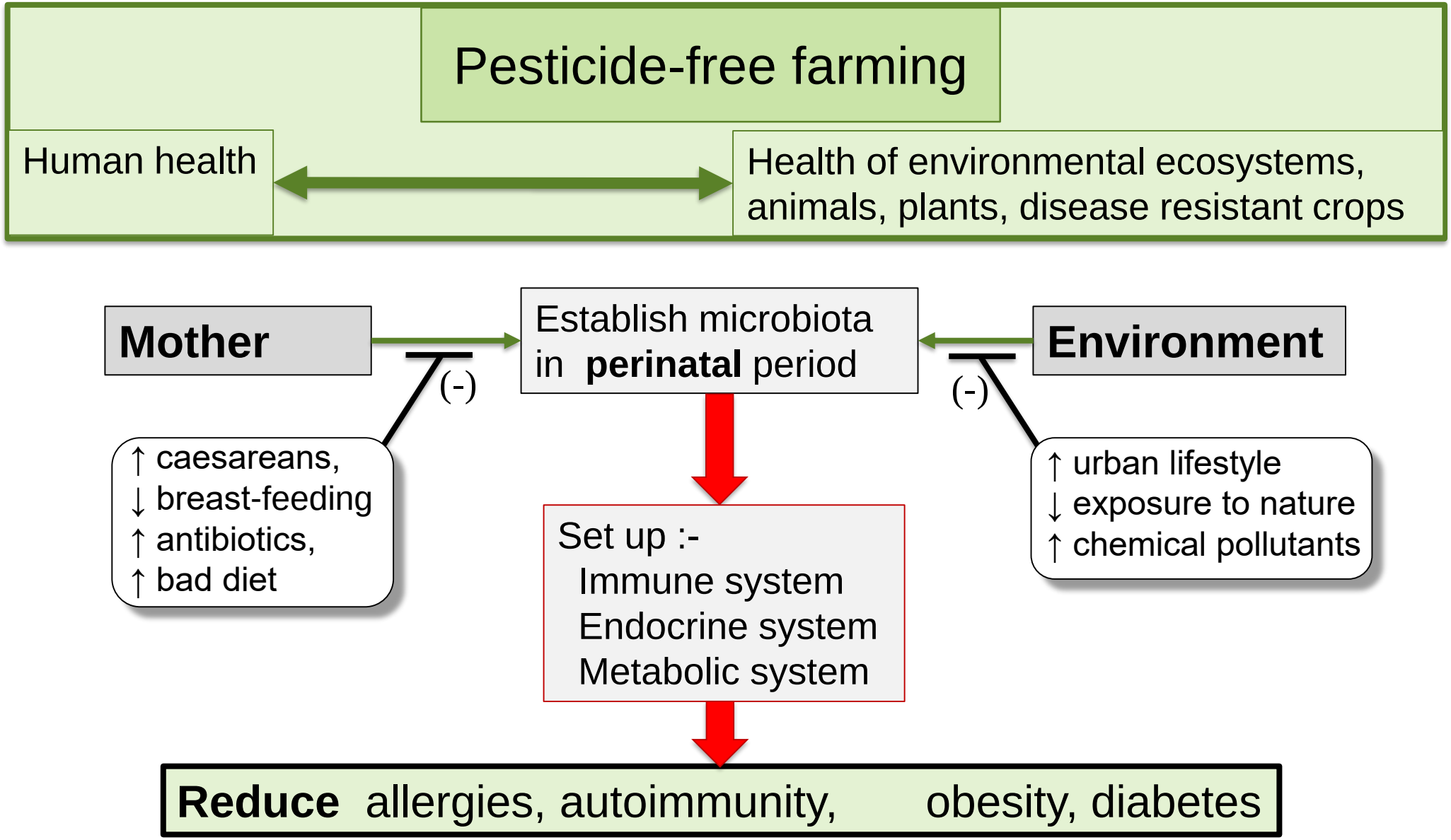
Failure to set up :-
Immune system
Endocrine system
Metabolic system

allergies, autoimmunity — (Forbidden targets)

obesity, diabetes — (Persistent inflammation)

Diabetic population in India doubled between 1980–2014

Velmurugan *et al* (2017) *Trends Endocrinol Metab* **28**:612
Claus *et al* (2016) *npj Biofilms & Microbiomes* **2**:16003
Lori *et al* (2017) *PLoS One* **12**:e0180442
Gullberg *et al* (2011) *PLoS Pathog* **7**:e1002158



Velmurugan *et al* (2017) *Trends Endocrinol Metab* **28**:612
Claus *et al* (2016) *npj Biofilms & Microbiomes* **2**:16003
Lori *et al* (2017) *PLoS One* **12**:e0180442
Gullberg *et al* (2011) *PLoS Pathog* **7**:e1002158

Conclusions

1) Microbial inputs to humans, especially in *early life*, are essential but failing
2) Reduced inputs predispose to inflammatory and metabolic disorders
3) Chemical pollutants are a likely cause
4) The gut microbiota may be the major target of many chemical pollutants

1) Define the contribution of **soil, plant & animal** microbiota to human microbiota

2) Study agricultural chemicals in **relevant** experimental systems

- test mixtures of multiple agricultural chemicals in animal models
- concentrations encountered by humans
- administer during pregnancy and early life

→ Investigate the gut **microbiota** in early life

- study microbiota of human babies and animals (wild and domesticated) in pesticide-free versus conventional farms.