Human Gut Microbiota and Health

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Gut Bacteria

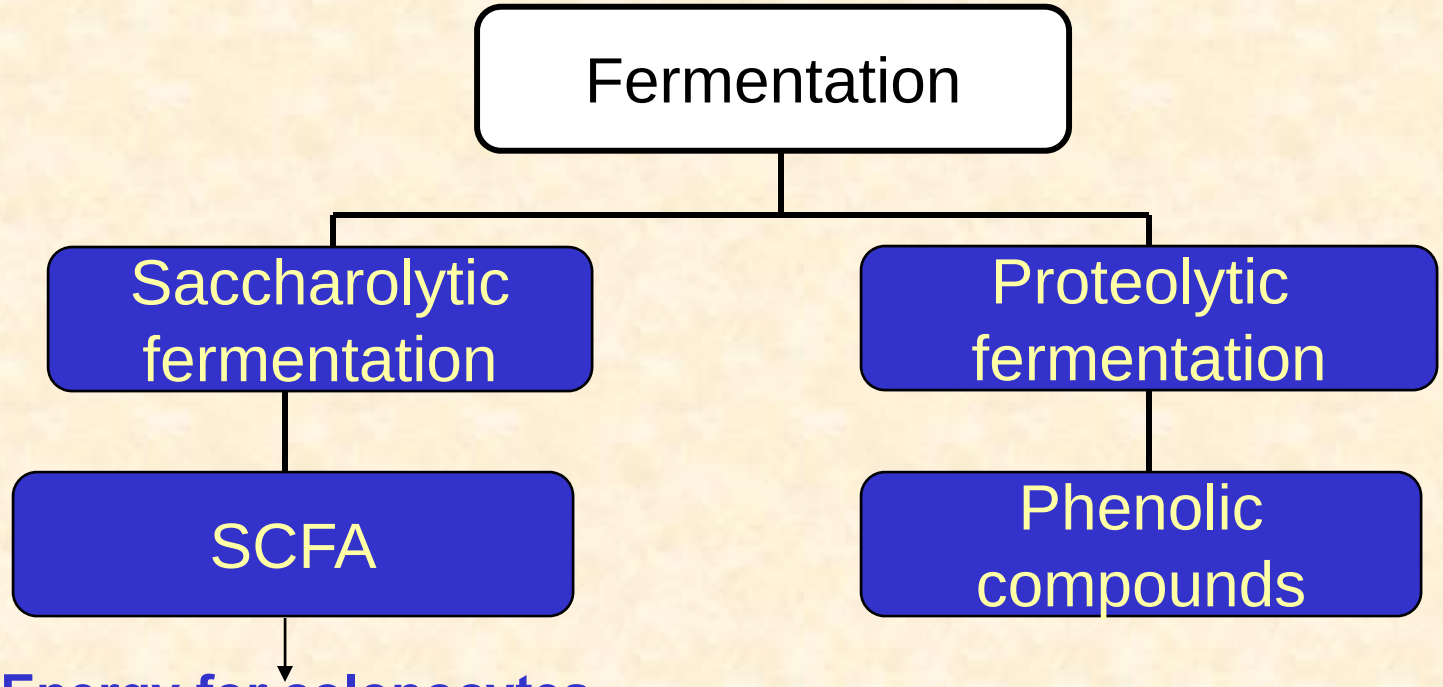
- At birth -digestive tract of humans is sterile.
- Colonised by microbes within the first few days of life
- At first, predominantly bifidobacteria (breast fed infants)
- With the introduction of other foods, a diverse microbial population develops in the gastrointestinal tract.

MAIN FUNCTIONS OF GUT FLORA

- Metabolic & Trophic functions
- Immunomodulatory
- Protective barrier function & Anti-diarrheal

- Antidiabetic ?
- Anticarcinogenic ?
- Hypocholesterolemic ?
- Obesity, metabolic syndrome, cancer?

METABOLIC FUNCTIONS



Energy for colonocytes

Carbohydrate and lipid metabolism

Control of the colonic pH

Maintain integrity of colonic mucosa

Intestinal motility and nutrient absorption.

SACCHAROLYTIC FERMENTATION

Short chain fatty acids

- Acetic acid- used by muscle
- Butyric acid – absorption of fluids, stimulation of proliferation in normal cells
- Propionic acid – decreases production of inflammatory mediators & helps ATP production in liver
- Succinic acid – break- down of acetaldehyde (generates NAD), anti inflammatory action
- SCFA induce lipogenesis

PROTEOLYTIC FERMENTATION

Phytochemicals undergo microbial fermentation – polyphenols, phenolic acids etc.

- **anti-inflammatory**
- **anti-oxidative**
- **anti-aging**
- **cancer preventive**

NUTRITIONAL EFFECTS OF COMMENSAL BACTERIA

1. Recycling: non absorbed nutrients, intestinal secretions, mucus.

   (It could account for 10% of total daily energy)

2. Favouring mineral absorption

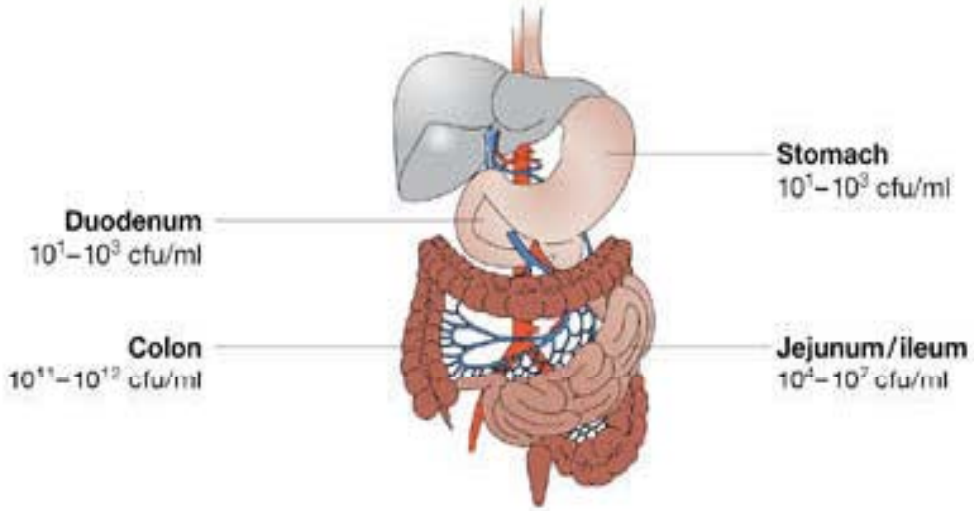
TROPHIC FUNCTIONS

- **Butyrate will affect proliferation in the small intestines and colon.**
- **Intracolonic butyrate may enhance intestinal growth during infancy.** (J. Nutr. 137:916-922, April 2007)
- **Butyrate inhibits cell proliferation and stimulates cells differentiation in cell lines of neoplastic origin.**
- **Butyrate promotes reversion of cells from neoplastic to non-neoplastic phenotypes.**

Commensal Bacteria Prevent Infection

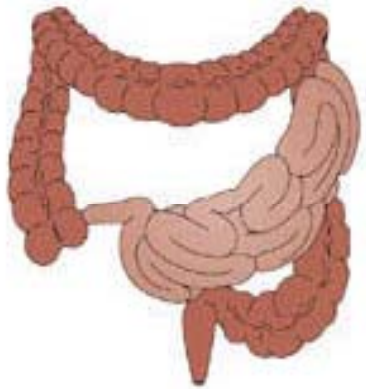
- Competition for attachment sites
- Competition for nutrient availability
- Growth inhibition by productions of antimicrobial substances
- Influence on mucosal barrier
  - mucus production
  - epithelial growth
- Influence on immune function

A

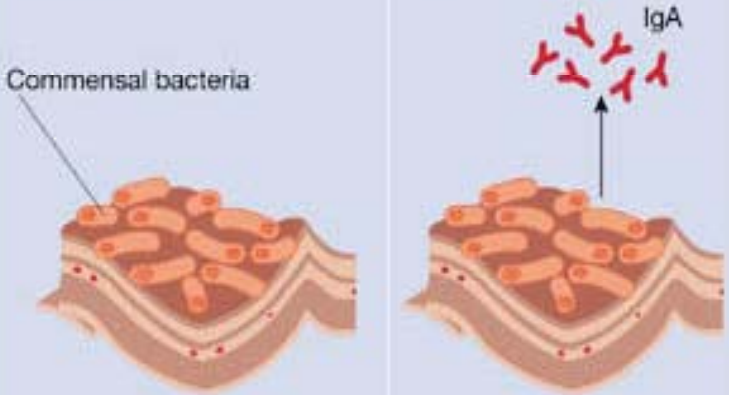


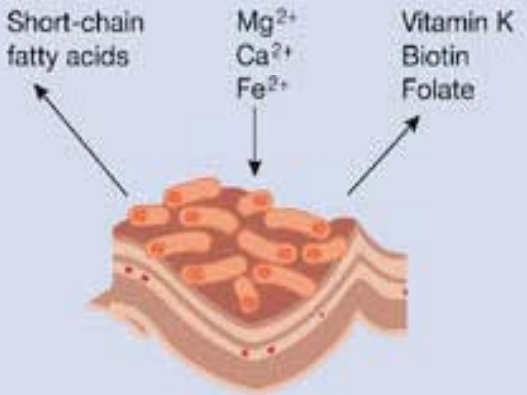
Anaerobic genera	Aerobic genera
Bifidobacterium	*Escherichia*
Clostridium	*Enterococcus*
Bacteroides	*Streptococcus*
Eubacterium	*Klebsiella*

B



Protective functions	Structural functions	Metabolic functions	
Pathogen displacement	Barrier fortification	Control IEC differentiation and proliferation	Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
Nutrient competition	Induction of IgA	Metabolize dietary carcinogens	Ion absorption
Receptor competition	Apical tightening of tight junctions	Synthesize vitamins e.g., biotin, folate	Salvage of energy
Production of anti-microbial factors e.g., bacteriocins, lactic acids	Immune system development		





Mucosal immune system

1st line of Defense- Mucosal surface integrity

- IgA
- Intestinal permeability
- Glycosaminoglycans(mucus!)
- Production of IgA at mucosal surface

2nd Line of Defense

Cell-mediated Immunity –

- TH1 Activation
- Delayed HyperSensitivity
- Macrophage/Phagocytosis

Humoral Immunity –

- TH2 Activation
- Allergic Response
- Antibody Formation

Gut Microbiota and Mucosal Immune Function

1. Commensal microbes have regulatory effects on mucosal immune response.
2. Host immune response to commensals is species/ strain specific.
3. Commensal bacteria have a developmental role in the priming of immune response.

Modulation of the immune response by commensal bacteria

Stimulation of mucosal immunity

- Better oral vaccine responses
- Control infections

Suppression of mucosal immunity

- Reduce allergy
- Reduce inflammation

Gut Microbiota and Mucosal Immune Regulation

Oral Tolerance:

- Bacteria can influence tolerance of Gut immune system to Antigens once ingested.
- This can ↓ the hyperactive immune system in allergies,auto immune disorders,etc

Preventing Allergy:

- Composition of gut flora varies in patients with/without allergies.
- Helpful gut flora stimulate the immune system and 'train' it to respond properly to Ag
- Lack of these bacteria in early life→inadequately trained immune system which overreacts with the Ag.

In allergy: ↓Bacteroides,Bifidobacteria

↑S.aureus, C.difficile

Preventing rotaviral and Antibiotic associated diarrhoea (AAD):

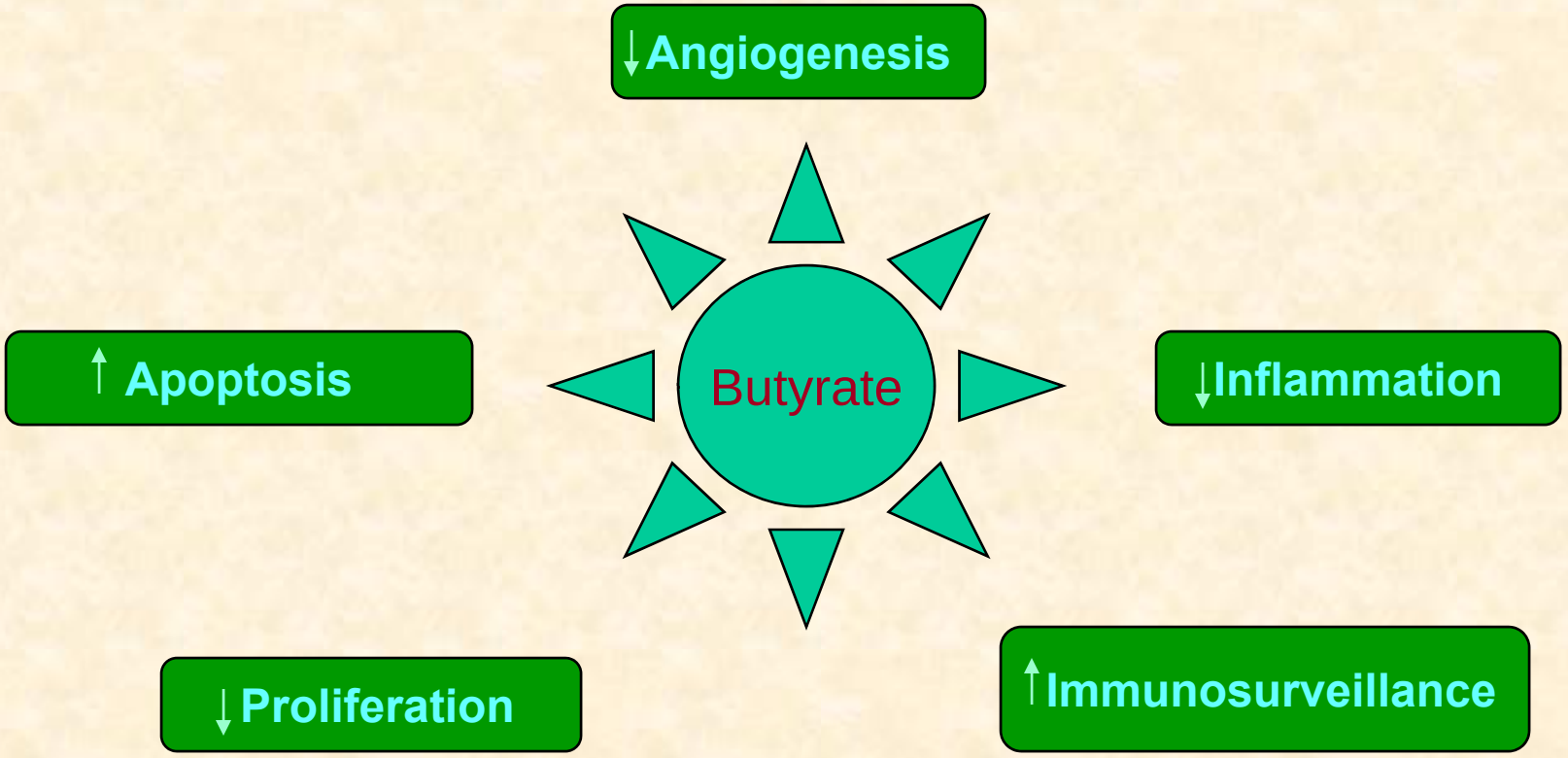
- ❖ Antibiotic can cause AAD by

1. irritating the bowel directly
2. changing gut flora levels
3. allowing pathogenic bacteria to grow
4. or by increasing antibiotic resistant organisms.

- ❖ The mechanism of diarrhoea can be:

1. Inadequate fermentation of CHO or metabolism of bile acids
2. CHO not broken down, absorb much water causing diarrhoea
3. - Lack of SCFA can also cause diarrhoea

Potential Mechanisms of anti-neoplastic action



E. A. Williams *et al. Proceedings of the Nutrition Society* (2003), 62, 107–115

Gut bacteria and Lipid lowering effect:

- Hyperlipidemic subjects- effects are primarily due to reductions in cholesterol.
- Normal lipidemic subjects - effects on serum triglycerides are dominant.

Gut bacteria are different in obese and non obese

- Firmicutes linked with obesity.
- Bifidobacterium and bacteroidetes –normals

Gut Microbes and Obesity

- Young, conventionally reared mice have a higher body fat content (42%) than germ free strains, though they consumed 29% lesser food.
- Microbiota were transferred to these germ free mice, then these mice experienced a 60% increase in body fat in 2 wks without any change in food consumption or energy expenditure.
- Obese mice- More end products of fermentation, and fewer calories in feces led to speculations that gut microbiota in mice help harvest additional calories from ingested food.
- In children from birth to age 7- analyzed stool samples collected at 6 monthly intervals. differences in gut flora precede overweight-obesity

Do Microbial proportions matter?

- Bacteroidetes constituted only 5% of the obese people's gut flora, but 20% in the lean subjects'.

- After a year of either a carbohydrate- or fat-restricted diet, the obese lost weight and the ratio of Firmicutes to Bacteroidetes shifted towards that of their lean counterparts. In the end, Bacteroidetes made up about 15% of their gut flora.

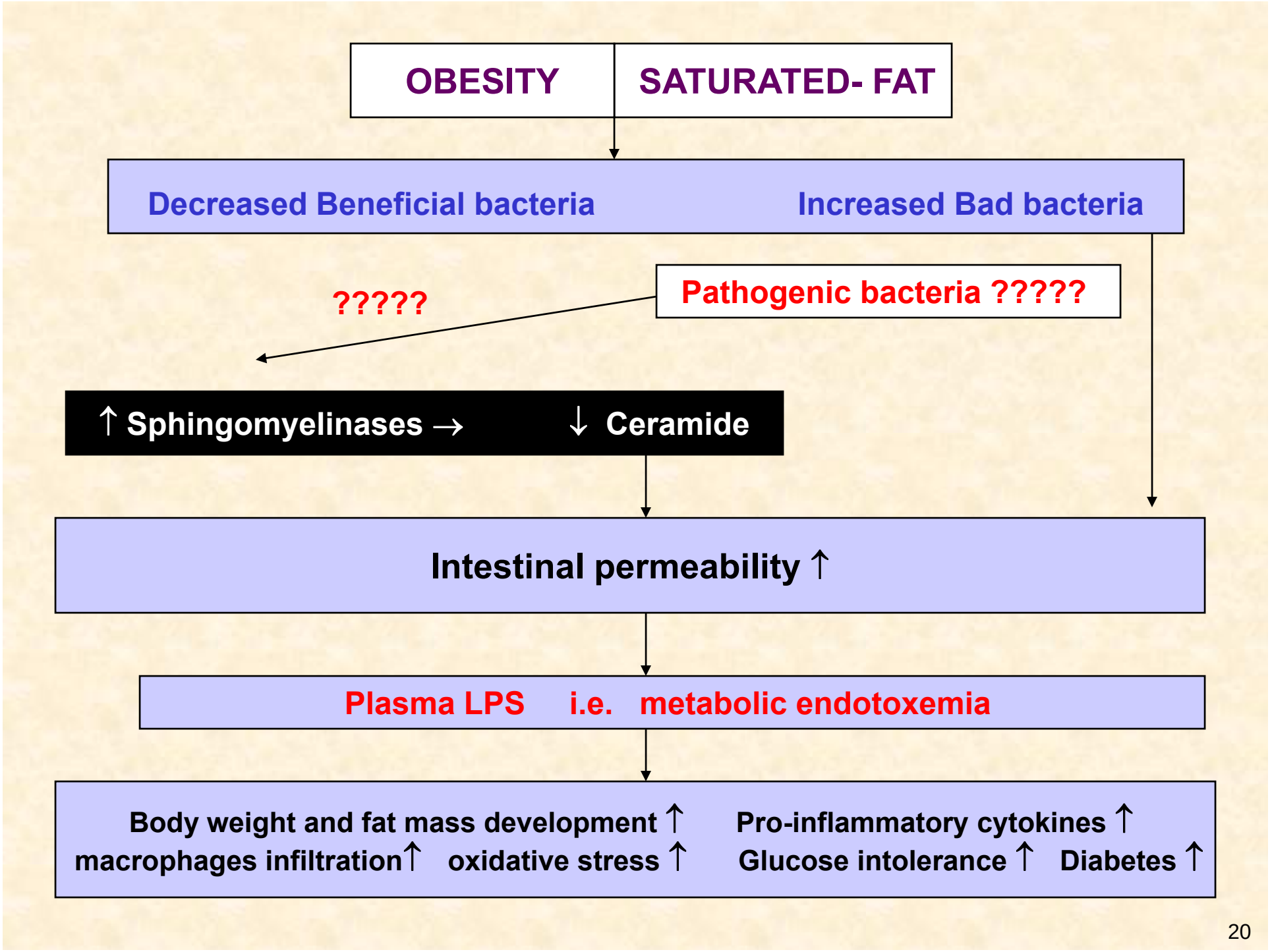
- These changes are irrespective of diet and were proportional to the amount of wt lost.

Microbial Proportions Matter

BMI

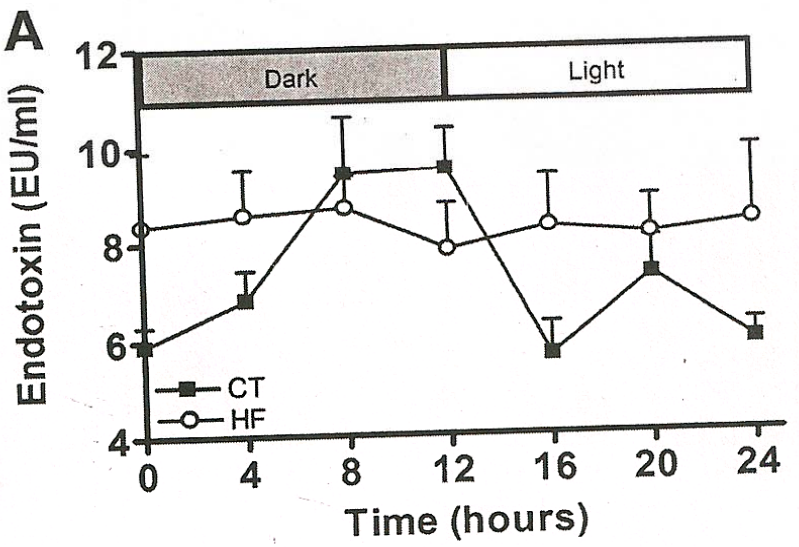
Lactobacilli
Bifidobacteria
Enterobacilli

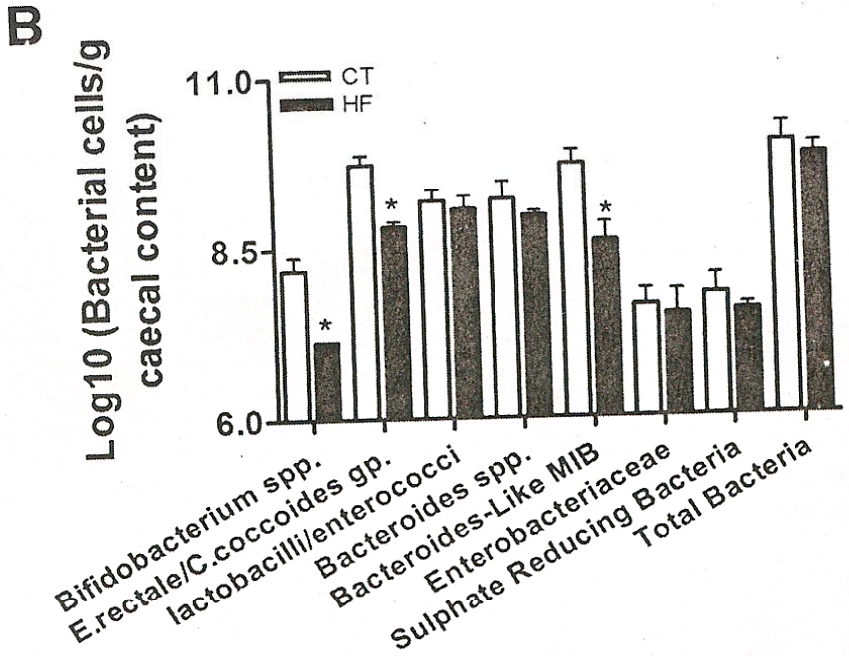
Firmicutes


OBESITY
SATURATED- FAT
Decreased Beneficial bacteria
Increased Bad bacteria
?????
Pathogenic bacteria ?????
↑ Sphingomyelinases →
↓ Ceramide
Intestinal permeability ↑
Plasma LPS i.e. metabolic endotoxemia
Body weight and fat mass development ↑
Pro-inflammatory cytokines ↑
macrophages infiltration↑
oxidative stress ↑
Glucose intolerance ↑
Diabetes ↑

High fat feeding induced Metabolic endotoxemia and changed intestinal bacteria

Patrice D.Canietal Diabetes,57, 2008





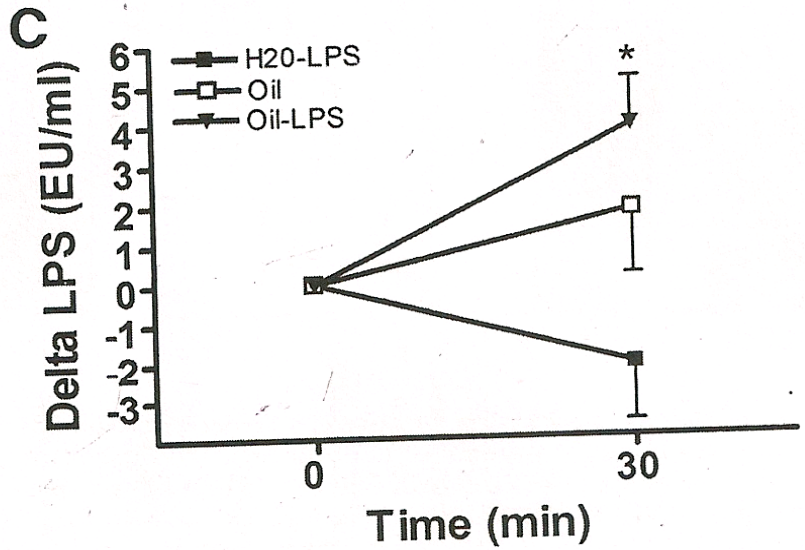


FIG. 1. High-fat feeding increased endotoxemia and changed intestinal microbiota. *A*: Plasma LPS concentration (EU/ml) was assessed every 4 h throughout the day in normal diet (CT; $n = 9$) (■) and 4-week high-fat–fed (HF; $n = 9$) (○) mice. *B*: Groups of bacteria in the caecal content of mice fed the normal diet (CT; $n = 8$) or the high-fat diet (HF; $n = 8$) for 4 weeks. Bacterial numbers are expressed as $\log_{10}$ (bacterial cells per gram caecal content wet weight). $*P < 0.05$ vs. CT. *C*: Delta plasma LPS concentration in (EU/ml) in mice before and 30 min after an oral administration of LPS diluted in corn oil ($n = 6$) (oil-LPS) or in water ($n = 6$) (H_2O-LPS) or an administration of oil alone ($n = 6$) (oil). $*P < 0.05$ vs. H_2O-LPS. Data are means ± SE.

Chronic experimental metabolic endotoxemia induces obesity,diabetes and insulin resistance

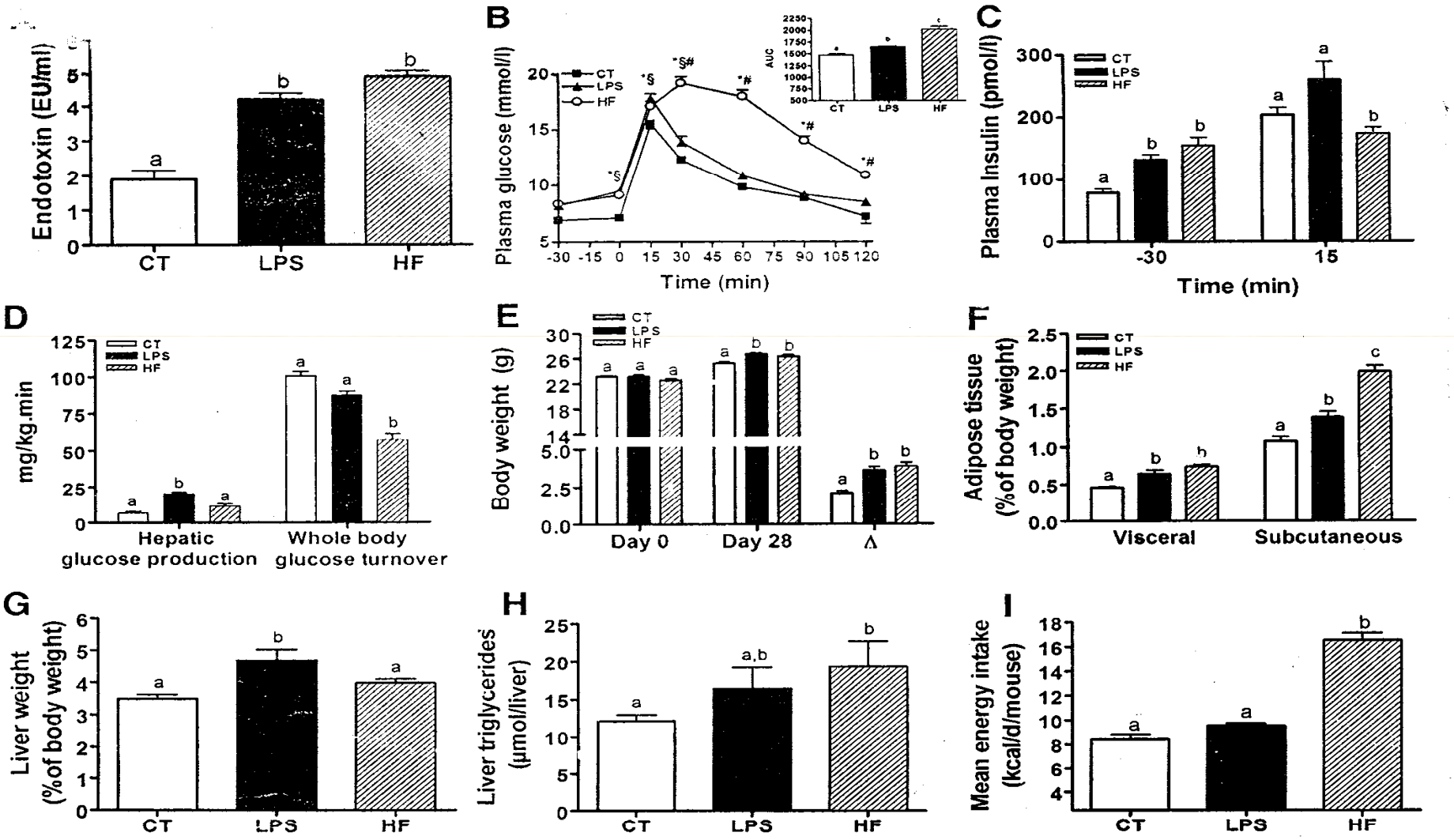


FIG. 2. Chronic experimental metabolic endotoxemia induces obesity and diabetes. *A*: Plasma endotoxin concentration (EU/ml) in WT mice infused with saline (CT; $n = 18$) or LPS ($n = 18$) for 4 weeks using subcutaneous osmotic pumps and compared with mice fed a high-fat diet for 4 weeks (HF; $n = 18$). *B*: Plasma glucose (mmol/l) following an oral glucose load (3 g/kg) in control (CT; $n = 24$), LPS ($n = 13$), or high-fat diet (HF; $n = 24$) mice. The *inset* represents the area under curve for each group. *$P < 0.05$ vs. CT; §LPS vs. CT; #HF vs. LPS. *C*: Plasma insulin (pmol/l) concentrations 30 min before (−30) and 15 min after (15) an oral glucose load in control (CT; $n = 24$), LPS ($n = 13$), or high-fat diet–fed (HF; $n = 24$) mice. *D*: Hepatic glucose production and whole-body glucose turnover rates ($mg \cdot kg^{-1} \cdot min^{-1}$) in control (CT; $n = 5$), LPS ($n = 5$), or high-fat diet–fed (HF; $n = 5$) mice. *E*: Body weight (g) before (day 0) and after a 28-day treatment period (day 28) and body weight gain (Δ) in

CD14 mice are protected against LPS induced inflammation

- To demonstrate the causal link between LPS and obesity/Diabetes,CD 14 mutant mice were studied.

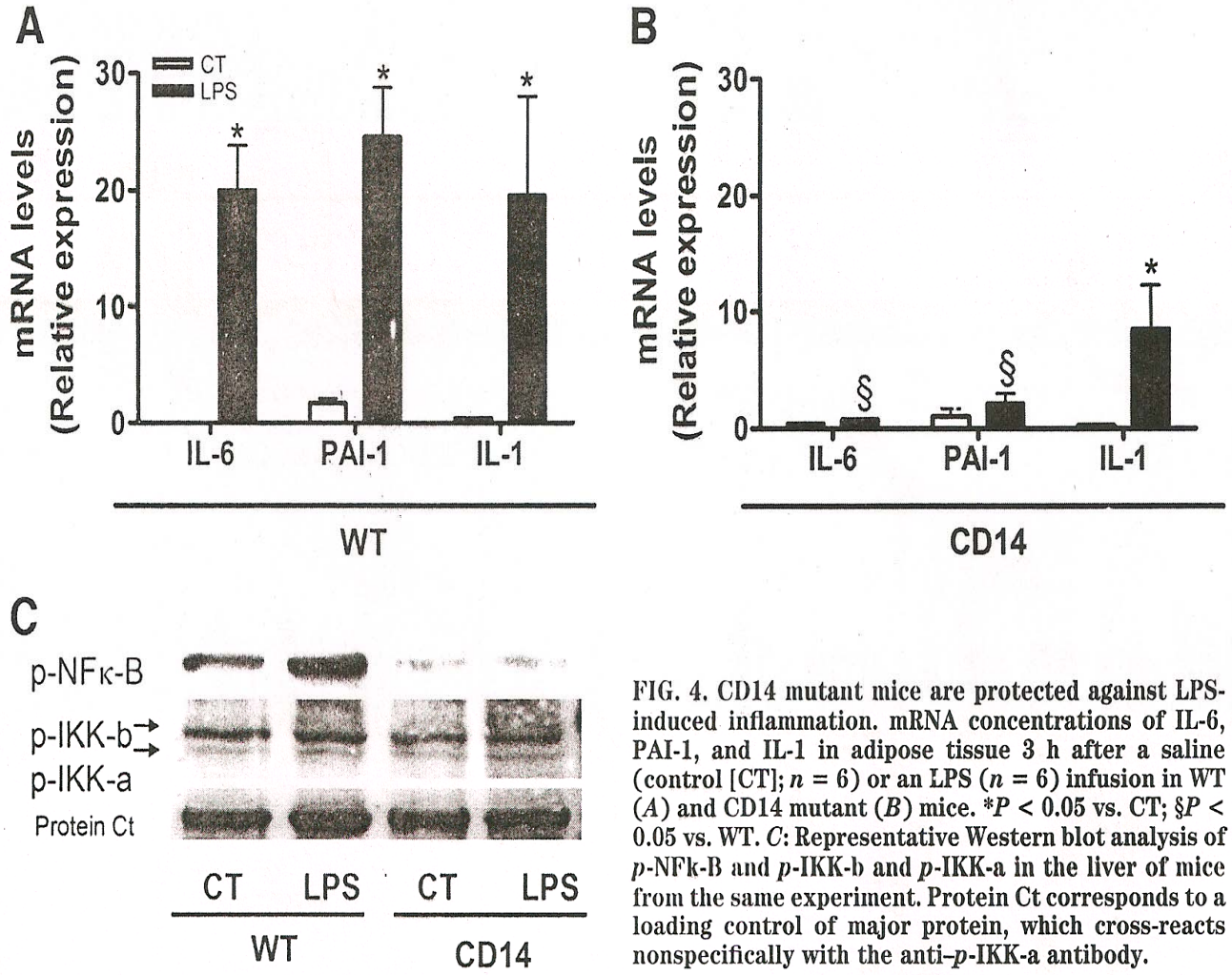


FIG. 4. CD14 mutant mice are protected against LPS-induced inflammation. mRNA concentrations of IL-6, PAI-1, and IL-1 in adipose tissue 3 h after a saline (control [CT]; $n = 6$) or an LPS ($n = 6$) infusion in WT (A) and CD14 mutant (B) mice. *$P < 0.05$ vs. CT; §$P < 0.05$ vs. WT. C: Representative Western blot analysis of p-NFk-B and p-IKK-b and p-IKK-a in the liver of mice from the same experiment. Protein Ct corresponds to a loading control of major protein, which cross-reacts nonspecifically with the anti-p-IKK-a antibody.

CD14 null mutation prevents the effect of LPS induced obesity and diabetes

- Body wt gain, visceral and subcutaneous adipose depot wts were increased in WT (Wild type), but unchanged in CD14 mutants(5a,b)
- Fasted and glucose stimulated glycaemias were augmented in WT-LPS compared to WT-C(5c)
- The area under the curve was inc in response to LPS infusion in WT only(5c inset)
- Plasma insulin conc were similar in basal and glucose stimulated conditions for all groups(5d)
- Chr LPS infusions increased liver wt of WT mice only(5e)
- Triglycerides were increased by 30% in WT-LPS mice's liver only, but were not statistically significant(5f)

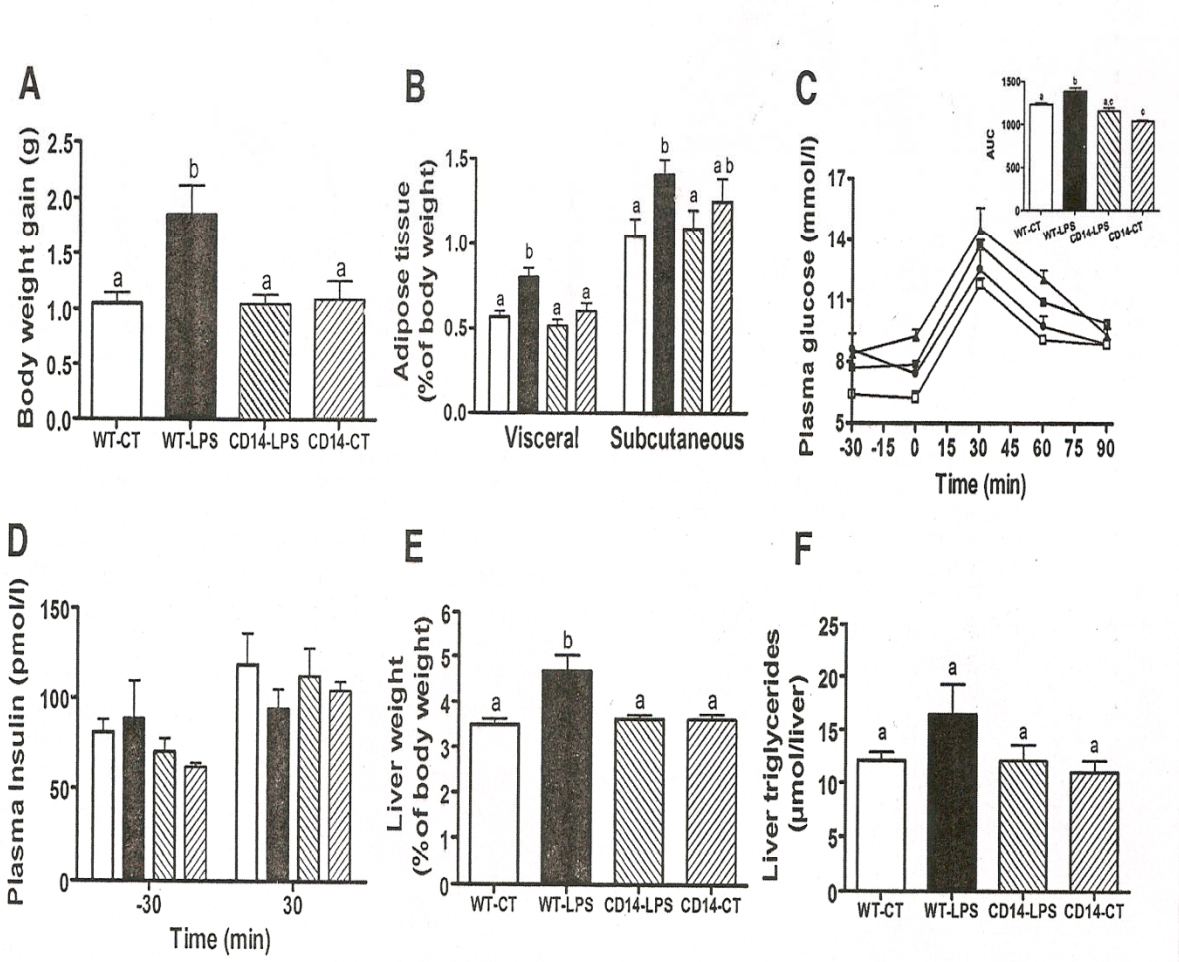
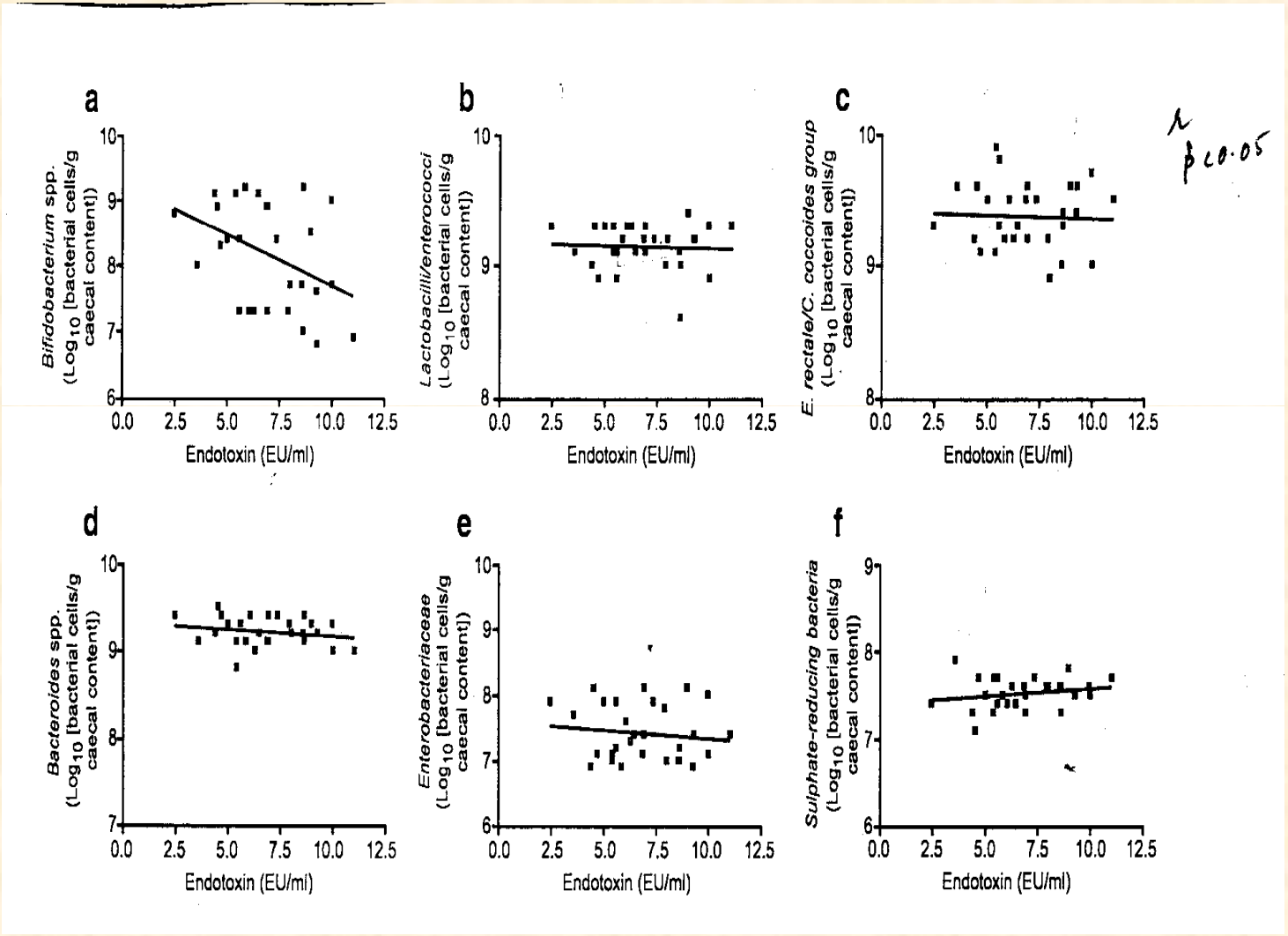


FIG. 5. The CD14 null mutation prevents the effect of LPS-induced obesity and diabetes. *A*: Body weight gain (g) in WT mice infused with saline (WT-CT; $n = 13$) or LPS (WT-LPS; $n = 14$) and CD14 mutant mice infused with saline (CD14-CT; $n = 13$) or LPS (CD14-LPS; $n = 12$) for 4 weeks using subcutaneous osmotic pumps. *B*: Visceral and subcutaneous adipose tissue weight (percentage of body weight) in WT-CT ($n = 13$) (□), WT-LPS ($n = 14$) (■), CD14-CT ($n = 13$) (▨), and CD14-LPS ($n = 12$) (▧) mice. *C*: Plasma glucose concentration (mmol/l) following an intraperitoneal glucose load (1 g/kg) in WT-CT ($n = 6$) (■), WT-LPS ($n = 6$) (▲), CD14-CT ($n = 5$) (□), and CD14-LPS ($n = 6$) (●) mice. The *inset* represents the area under curve of the same groups. *D*: Plasma insulin (pmol/l) concentration 30 min before (−30) and 30 min after (30) intraperitoneal glucose administration in WT-CT ($n = 6$) (□), WT-LPS ($n = 6$) (■), CD14-CT ($n = 5$) (▧), and CD14-LPS ($n = 6$) (▨) mice. *E*: Liver weight (percentage of body weight) in WT-CT ($n = 13$), WT-LPS ($n = 13$), CD14-CT ($n = 12$), and CD14-LPS ($n = 13$) mice. *F*: Liver triglycerides (µmol/liver) in WT-CT ($n = 12$), WT-LPS ($n = 9$), CD14-CT ($n = 5$), and CD14-LPS ($n = 6$) mice. Data are means ± SE. Data with different superscript letters are significantly different at $P < 0.05$, according to the post hoc ANOVA statistical analysis.

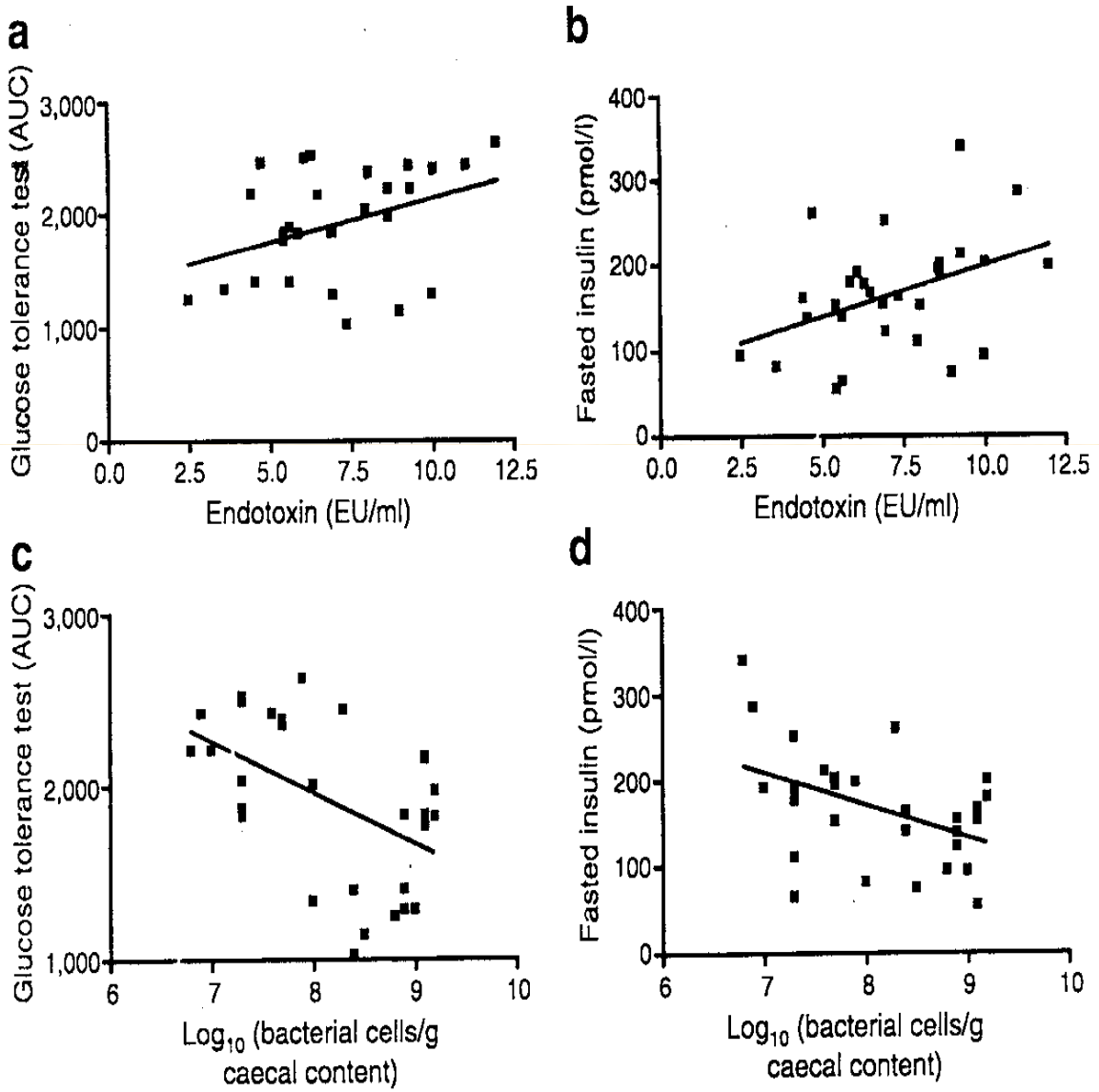
Bifidobacterium Decreases Endotoxemia (LPS)



Multiple correlation analysis between major Gram +ve and Gram-ve bacteria in the caecal contents of mice was done to identify whether one specific group of gut bacteria was involved in the determination of endotoxemia.

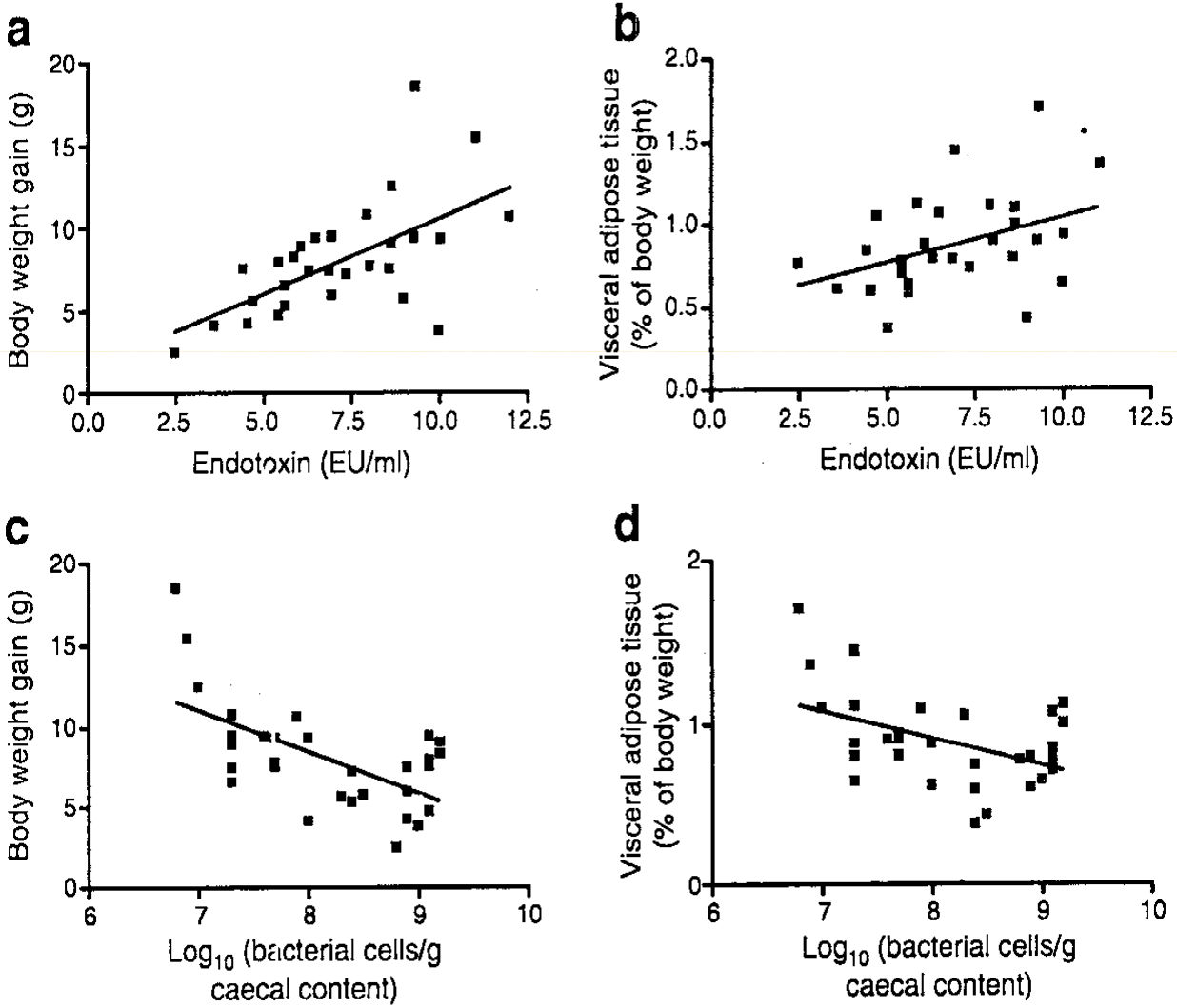
Bifidobacterium Decreases Blood Glucose and Insulin Levels

- Fasted insulin and glycaemic response were positively correlated with plasma endotoxin levels(4a,b)
- And negatively correlated with Bifidbacteria(4 c,d)



Bifidobacterium associated with low body weight and visceral fat

- Body weight and visceral fat mass correlated positively with plasma endotoxin levels(5a,b)
- Correlated negatively with Bifidobacteria. (5c,d)



Markers of metabolic syndrome

- Glucose Intolerance
- Fasted Insulinemia
- Inflammatory Markers
- Adipose Tissue &
- Body Weight Gain

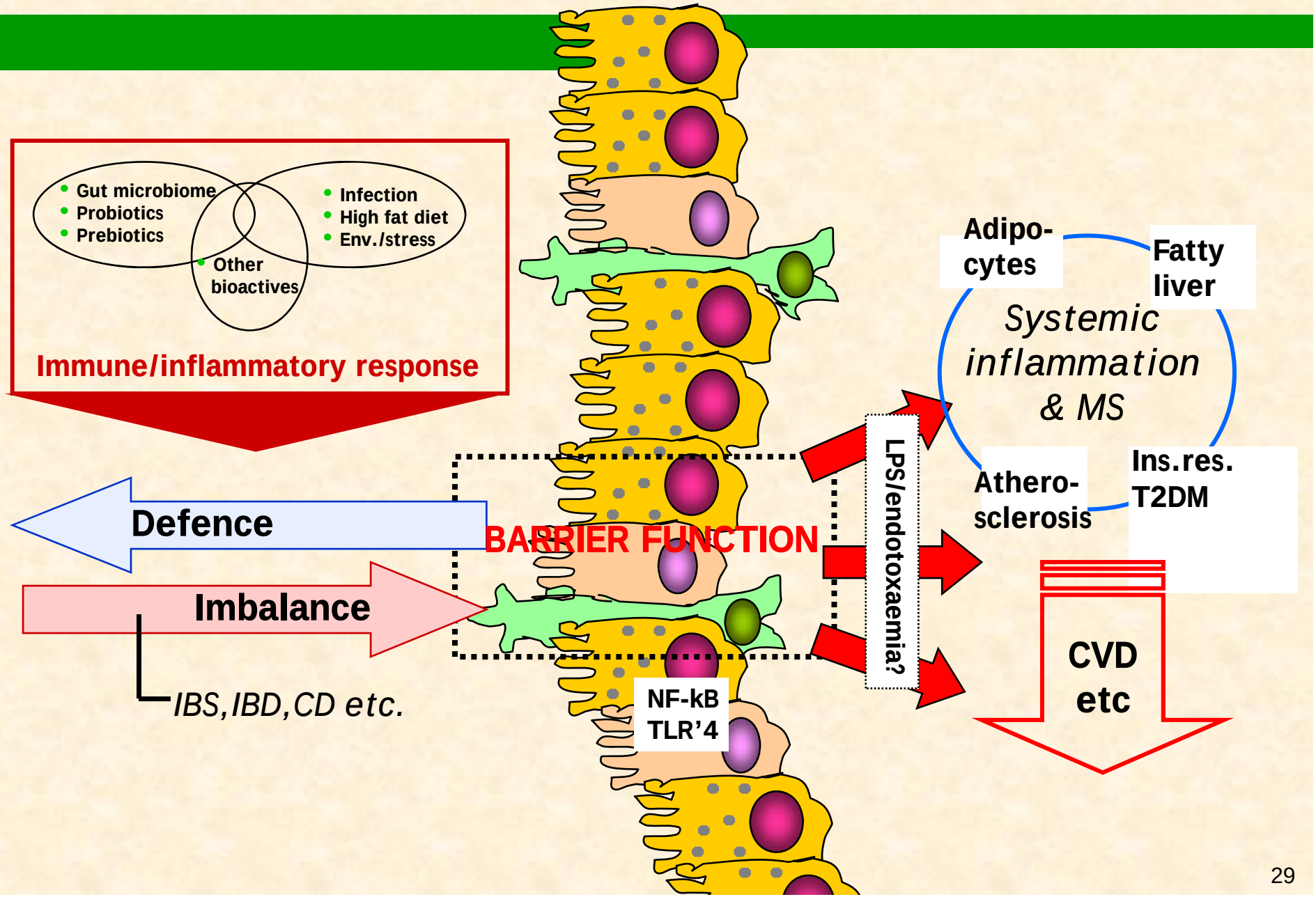
Increase with

Endotoxemia

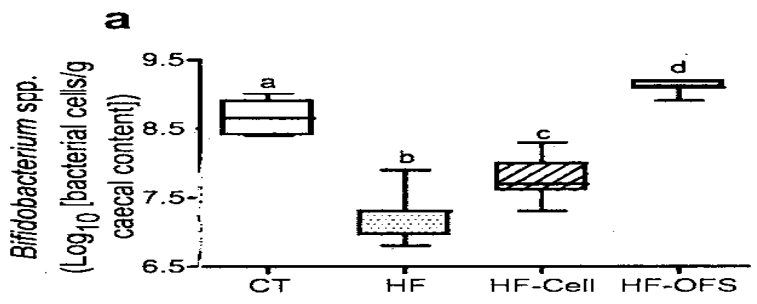
Decrease with

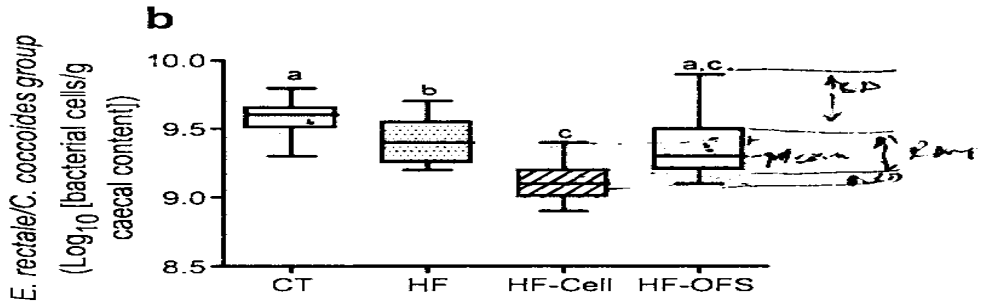
Bifidobacteria

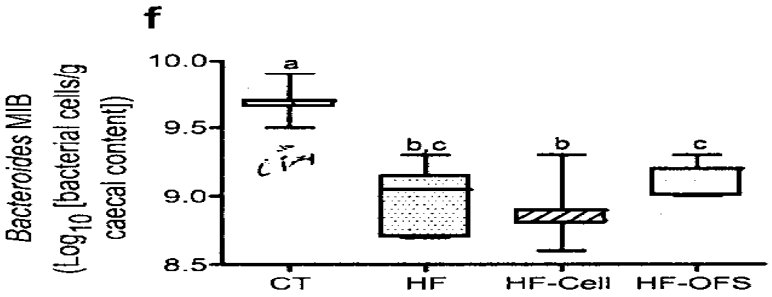
LPS leaks through epithelial barrier!



Specific Increase of Bifidobacteria by Prebiotics



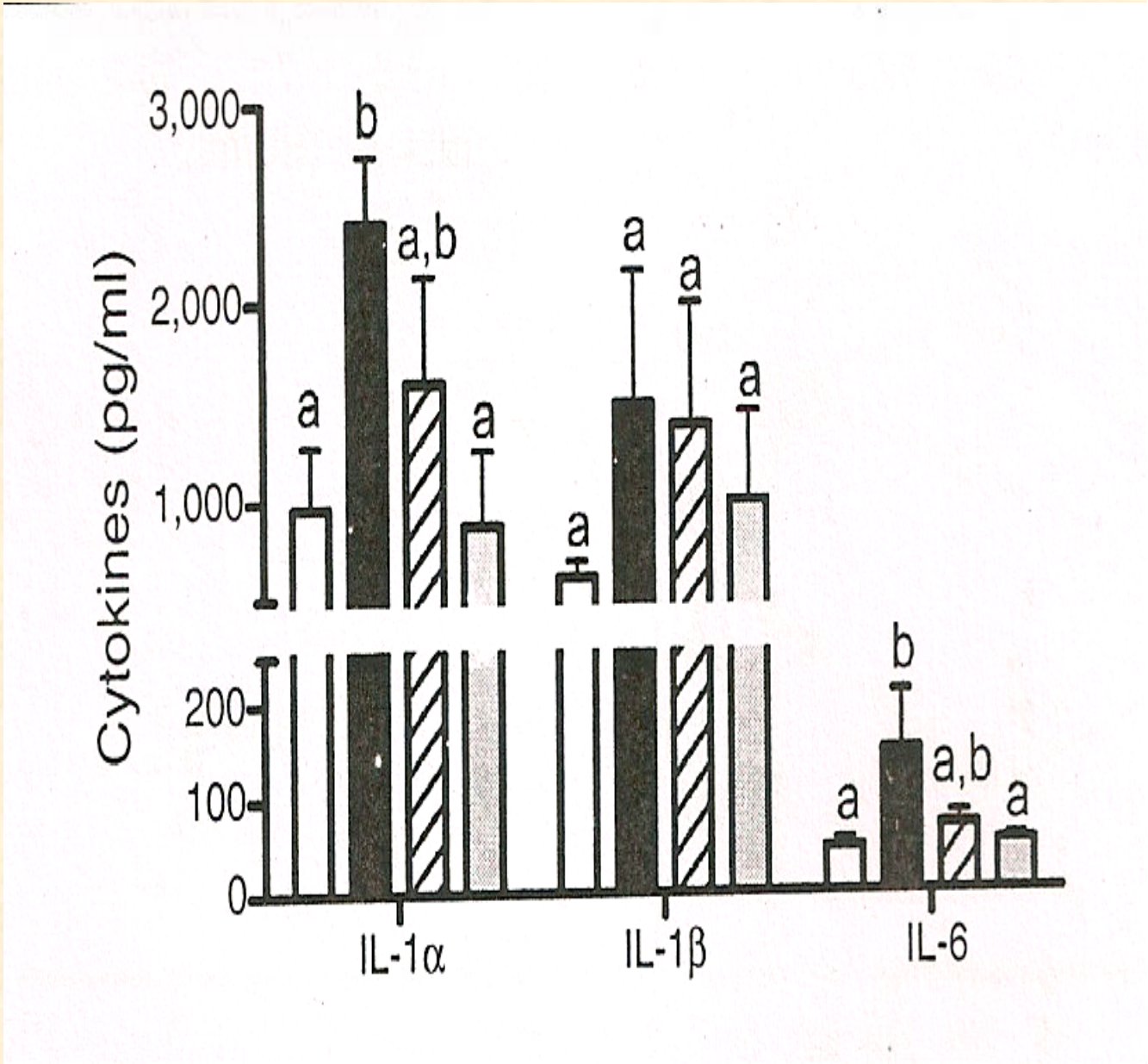




Prebiotics Control High Fat Diet Induced Inflammation

- IL-1α, IL-1β, and IL-6 were increased in HF mice compared to control
- HF-OFS had significantly normalized IL-1α and IL-6 cytokines plasma levels compared with HF and decreased IL-1β
- HF –Cell mice showed intermediary levels.

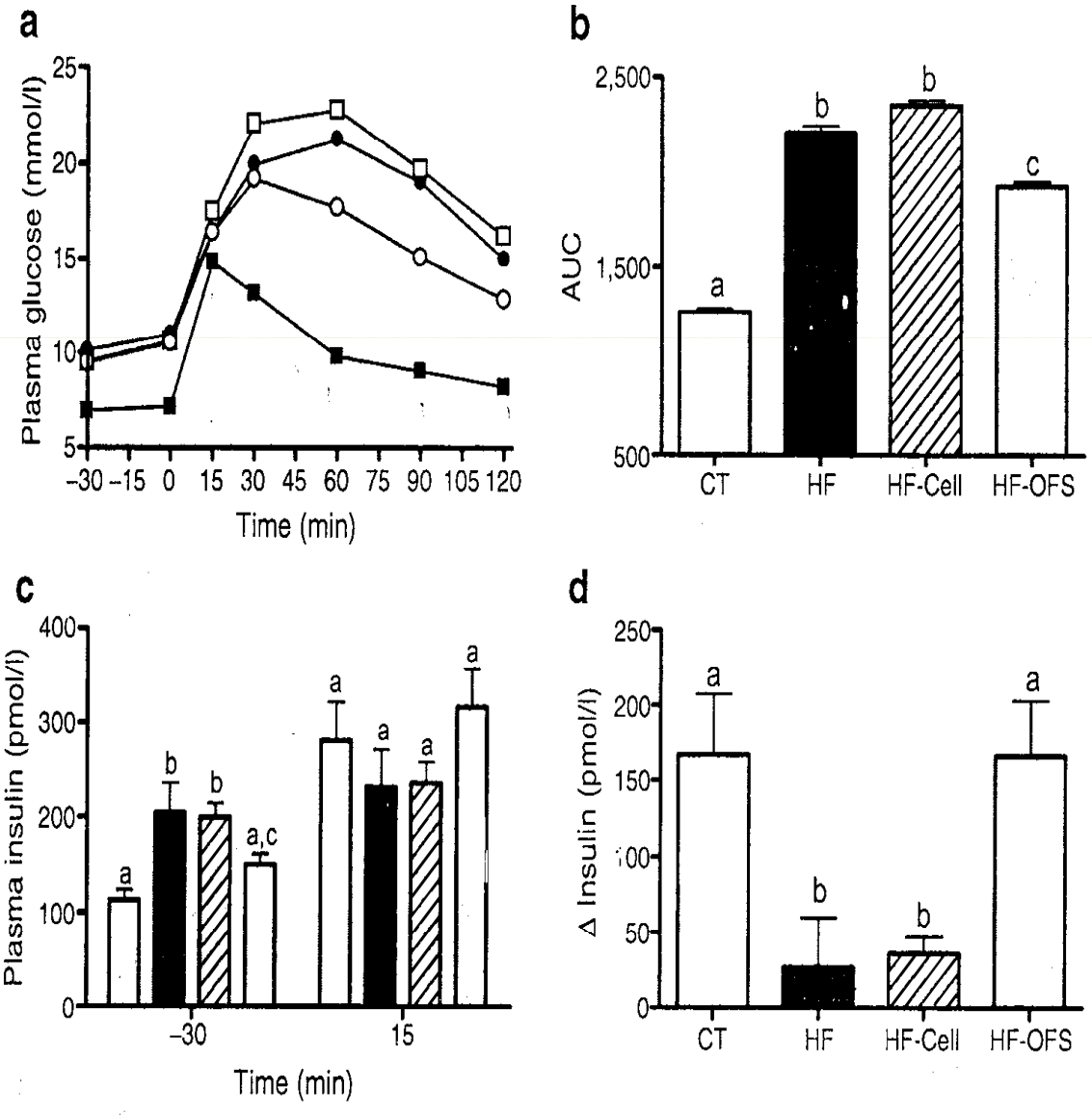
C- open bars
HF-closed bars
HF-Cell- hatched bars
HF-OFS- grey bars



Prebiotics Improve Glucose Tolerance and Restore Glucose Induced Insulin Secretion

- HF mice showed strong glucose intolerance(3a,b)
- Fasted insulinemia was significantly increased in HF and HF-Cell mice compared to control(3c)
- Insulin secretion following glucose load was almost absent in HF and HF-Cell mice(3c,d). In contrast HF-OF mice showed normal fasting plasma insulin levels and restored glucose-insulin secretion.

3a: C- closed squares
HF- closed circles
HFCell-open squares
HF-OFS-open circles



Food Sources of Prebiotics

- Chicory
- Oatmeal
- Barley
- Whole grains
- Onions, garlic
- Greens (spinach, mustard green)
- Berries, banana, other fruits
- Legumes (lentils,kidney beans, chickpeas)

Can Bifidobacteria help prevent development of metabolic syndrome?

High fat diet	High fat diet + oligofructose
↑ **Endotoxaemia (plasma LPS**	↓ **Endotoxaemia (plasma LPS**
↓ **Caecal counts - Gram positives - Bifidobacteria**	↑ **Caecal counts - Gram positives - Bifidobacteria**

Selective Increases of Bifidobacteria in Gut Microflora Improve High-Fat-Diet-Induced Diabetes in Mice Through a Mechanism Associated with Endotoxaemia

Cani PD et al. 2007, Diabetologia,

Modifying the gut microbiota in favour of Bifidobacteria may prevent deleterious effects of high-fat-diet-induced metabolic diseases...

THE FUTURE

- Studies on gut microbiota interactions with metabolic phenotypes (so-called functional metagenomics)
- Understanding of microbiota diversity on a population level and across various cultural and ethnic group.
- To standardize the microbiota analysis methodology, sample collection, storage, analysis methods.
- Correlating microbiota composition with disease risk, require large prospective epidemiological studies.

THANK YOU