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N-3 polyunsaturated fatty acids: an innovative strategy against obesity and related metabolic disorders, intestinal alteration and gut microbiota dysbiosis.

by

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ABSTRACT

Obesity is now widely recognized to be associated with low-grade systemic inflammation. It has been shown that high-fat feeding modulates gut microbiota which strongly increased intestinal permeability leading to lipopolysaccharide absorption causing metabolic endotoxemia that triggers inflammation and metabolic disorders. N-3 polyunsaturated fatty acids (PUFAs) have been shown to be associated with anti-obesity properties, but results still remain heterogeneous and very few studies underlined the metabolic pathways involved. Thus, the use of fat-1 transgenic mice allows to better understanding whether endogenous n-3 PUFAs enrichment contributes to obesity and associated metabolic disorders prevention. It specially evidences that such effects occur through modulations of gut microbiota and intestinal permeability. Then, by remodeling gut microbiota, endogenous n-3 PUFAs improve HF/HS-diet induced features of the metabolic syndrome which in turn affects host metabolism. Thus, increasing anti-obesogenic microbial species in the gut microbiota population (*i.e Akkermansia*) by appropriate n-3 PUFAs may represent a promising strategy to control or prevent metabolic disorders.

1. Introduction

Obesity, defined as an abnormal or excessive fat accumulation, is now recognized as an international public health concern. Indeed, in the world, more than 2.1 billion people have a body mass index (BMI) ≥ 25.0 and ≥ 30.0 in more than half a billion of them with no distinction of age or sex. Although obesity is considered as a multifactorial pathology (genetic predisposition, environmental impact, lack of physical activity ...), the leading cause of obesity development is the rising consumption of high-fat/high-sucrose (HF/HS) diets - especially in western countries - and efforts still need to be done to identify factors that would prevent or cure this pathology. Obesity is also associated with increased risk of developing a wide cluster of alterations such as insulin resistance (IR), dyslipidemia, hypertension, diabetes known to be the main components of the metabolic syndrome (1) and of non-alcoholic fatty liver diseases (2).

2. Obesity and low grade inflammation: role of LPS

Nowadays, obesity is linked to low-grade systemic inflammation which is identified as a key factor of its development and of related metabolic disorders (3-5), even if the molecular origin of such inflammation is not fully elucidated. Cani and co-workers (6) have identified major bacterial components, lipopolysaccharides (LPS), mainly found in the outer membrane of Gram-negative bacteria, as a triggering factor of metabolic endotoxemia when it translocates in the bloodstream, which has been associated with an increased risk of obesity and clinical diabetes (7). A rising of concentration of LPS in plasma and both diet-induced metabolic endotoxemia have been shown to be linked to high fat and high energy diets coupled with enhanced Toll-like receptor (TLR) 4 and nuclear factor-kappa B (NF- κ B) expressions (6).

Even if the increased level of LPS in plasma is not fully understood, it is closely correlated with an imbalance in bacterial composition (mainly Bacterioides/Firmicutes ratio and defined as intestinal microbiota dysbiosis) during high-fat (HF) feeding in mice (8) and human (9). Moreover, it has been shown that the gut bacteria changes after HF diet strongly increased gut permeability (10) by downregulating genes encoding for the tight junctions zonula-occludens 1 (ZO-1) and occludin (6). Next, LPS promotes lipid storage in liver and adipose tissue, triggers inflammatory cytokine expression and causes insulin resistance (8). Mice invalidated for TLR4 have been shown to be resistant to HF diet-induced inflammation, obesity, and insulin resistance (11). Moreover, mice invalidated for CD (Cluster of Differentiation) 14

gene (a TLR4 co-receptor) are resistant to the development of LPS-induced inflammation, compared with wild-type (WT) mice (8). These data suggest a major role of LPS in the pathogenesis of metabolic syndrome.

3. N-3 polyunsaturated fatty acids, obesity and related disorders

In this last decade, many studies both in animal and human have focused on the cardioprotective effects of fish oils. Increasing the feeding of n-3 polyunsaturated fatty acids (PUFAs) represents a promising approach (12) against obesity-associated diseases. Thus, the physiological functions and molecular actions of these unsaturated fatty acids have been investigated in the context of obesity, and thanks to their functional properties they could be considered useful to fight metabolic syndrome in obese animal models. Indeed, administration of marine oils containing n-3 PUFAs in the context of obesity and linked disorders has been reported to improve both glucose (13-16), and lipid metabolism (17, 18), and to alleviate liver lipid storage by downregulating lipogenic genes. Interestingly, these beneficial effects of n-3 PUFAs have been associated with reduced adipose inflammation (15, 19, 20), increased expression and secretion of adiponectin - an insulin sensitizing adipokine - (21) raised adipose mitochondrial activity (22), and inhibition of the remodeling of adipose tissue induced by a HF diet (23). Furthermore, beneficial effects of n-3 PUFAs on metabolic syndrome and obesity mitigation could be due to their impact on the regulation of transcriptional factors in liver and adipose tissue (19): among those, n-3 PUFAs, potential endogenous ligands for the peroxisome proliferator activated receptor (PPAR) family nuclear receptors, play a crucial role in adipocyte differentiation and lipid metabolism (21, 24).

In addition, nutrigenomic approaches have shown cardioprotective effects of n-3 PUFAs mediated by a decrease of inflammatory markers, an enhanced synthesis of proresolving lipid mediators and a modulation of genes involved in fatty acid synthesis and oxidation (25). Then, it has been recently shown that feeding eicosapentaenoic acid (EPA) enhances the hepatic content of resolvin (Rv) E1, RvE2, RvD1 and RvD2 in mice fed HF diets, leading to reduction of high-fat diet-induced liver proinflammatory state (26). In addition, other n-3 PUFA-derived mediators (as protectins and maresins) are also known to be specialized lipid mediators able to dampen inflammatory (27) in the context of dietary obesity. Moreover, a recent study demonstrated that administration of protectin D1 (PD1) to obese diabetic *db/db* mice markedly improved their insulin sensitivity (28).

Nevertheless, studies regarding the effects of n-3 PUFAs on obesity remain controversial: some of them argue that these FA are able to significantly decrease body weight and fat mass (29-31) whereas others did not evidence any significant effect on body weight loss even if adipose fat accumulation decreased in n-3-fed animals (32, 33). More recently, it has been demonstrated that the replacement of lard by fish oil in high-fat diet alleviates systemic and tissue inflammation (34). In addition, the authors have shown that n-3 PUFAs increased mitochondrial fatty acid oxidation leading to a lower liver lipid accumulation (35). Nevertheless, very recent meta-analyses did not evidence any positive effects of n-3 (36).

4. N-3 PUFAs, intestinal permeability and microbiota in the context of obesity

Dietary n-3 PUFAs, affecting gut integrity, have been shown to reduce clinical colitis and colonic immunopathology by improving epithelial barrier function in animal models (37, 38). EPA has been shown to up-regulate the expression of tight junctions and to increase the trans-epithelial electrical resistance (TER), thus reducing the permeability of the endothelial cells (39). EPA and DHA, by lowering permeability-induced inflammatory cytokines such as TNF α , IFN γ and IL-4, can maintain gut barrier integrity (40, 41). In agreement, it has been shown that, following intravenous injections of *E. coli* LPS to animal models, dietary DHA supplementation significantly enhanced the expression of intestinal tight junctions as occludin and claudin-1 and decreased intestinal expression of TLR4 (42). Very few studies assessed the impact of n-3 fatty acids on metabolic endotoxemia. Nevertheless, Mani and co-workers (43) have shown in pigs that post-prandial endotoxemia was decreased by fish oil supplementation when it was increased by coconut oil (rich in saturated fatty acids). Recently, enhanced levels of metabolic endotoxemia and systemic low-grade inflammation were observed in high n-6 PUFA-fed mice, whereas these parameters are drastically reduced in transgenic mice able to convert n-6-to-n-3 PUFAs in their tissues (44).

It has been recently suggested that n-3 PUFAs may influence the content of gut microbiota (45-47) when another study showed no prevention of gut microbiota dysbiosis in EPA- and DHA-treated mice (48). N-3 PUFAs, brought by diet, are able to attenuate the decrease of the Bacterioides/Firmicutes ratio observed in high fat-fed mice (46). These results agree with dietary experimentation conducted by Andersen et al. who reported that, compared with sunflower oil, dietary intake of fish oil impacted most of bacterial phyla (49). Moreover, Yu et al. showed that fish oil is capable of producing significant changes in gut microbiota, especially suppression of *Helicobacter* and Firmicutes growth (47), known to be highly

involved in the etiology of peptic ulcer disease and obesity respectively. Finally, a just recently published paper showed that a host-microbiome relationship mediates the opposing effects of n-6 and n-3 FA on metabolic endotoxemia (44).

A large amount of interest has been generated these last years by the bacterium *Akkermansia muciniphila*, a highly represented species in human gastrointestinal tract with 5% of the total bacteria in basal conditions. Interestingly, besides the fact that *Akkermansia* uses mucins, it seems to be crucial for the maintenance of mucus layer integrity (49, 50). Increased intestinal abundance of *Akkermansia* can protect against obesity-linked metabolic syndrome, and reduce (brought as a probiotic) systemic LPS levels in high-fat-fed mice (50-53). Possibly, it occurs through mucus layer thickness prevention, and then reduction of gut permeability and LPS leakage (50). Moreover, in a study on the effects of a polyphenol-rich cranberry extract, Anhê et al. showed that the putative effects of *Akkermansia* on dysbiosis induced by obesity arises with no major changes in the proportions of Firmicutes and Bacteroidetes (51). The mechanism by which *Akkermansia* could protect against obesity is not fully elucidated, but recent data indicate that a membrane protein from *Akkermansia muciniphila* (Amuc_1100*) - more than the bacteria by itself - could be involved in the protective effects. This hypothesis has been reinforced by the fact that data obtained with pasteurized bacteria have shown similar effects (54). Collectively, these data indicate that modulation of intestinal tight-junction barrier by n-3 PUFAs remains not fully understood and the means by which n-3 PUFAs are able to reduce LPS-induced permeability still needs to be evaluated and more particularly under diet-induced obesity (DIO) conditions. In addition, the complexity to point out the specific effects of n-3 PUFAs per se is reinforced by many potential confounding factors provided by the dietary procedures.

5. The fat-1 mice: an appropriate model for studying obesity

In obesity-related studies, fat-1 mice represent an innovative animal model that overcomes the use of dietary manipulation helpful for avoiding any confounding dietary elements brought by diets. Indeed, these transgenic mice ubiquitously express a gene coming from *C. Elegans*, which encodes for a n-3 fatty acid desaturase able to convert n-6 to n-3 PUFAs (55) leading to a remarkable tissue enrichment in n-3 PUFAs compared with WT. Moreover, in this mouse model, tissue contents in n-3 PUFAs not only increase but the levels of excessive endogenous n-6 PUFAs also decrease resulting in a balanced n-6-to-n-3 ratio (56). This endogenous synthesis of n-3 PUFAs allows getting away from the use of dietary intervention introducing potential confounding factors and is more effective in balancing the

n-6-to-n-3 ratio. This n-3 PUFA enrichment is ideal for identifying their specific roles and addressing nutrient-gene interactions. Then, this mouse model represents a useful in vivo system for giving new insights of the role of the n-6-to-n-3 fatty acid ratio in the context of obesity and associated metabolic disorders.

We recently took advantage of this model to answer the following questions: does n-3 fatty acids tissue enrichment prevent obesity and associated metabolic disorders? Does such prevention occur through alleviation of intestinal permeability (tight junction protein expression, gut permeability) and metabolic endotoxemia, in association with gut microbiota remodeling?

6. Remodeling gut microbiota by endogenously synthesized n-3 fatty acids prevents high fat/high sucrose diet-induced metabolic endotoxemia and associated metabolic disorders

In the context of obesity, remodeling the gut microbiome could be a promising therapeutic target. Then, it has recently been pointed out in mice the relative contribution of endogenously synthesized n-3 PUFAs in altering gut microbiota in order to change metabolic parameters (28, 57).

6.1. Fat-1 mice were protected against obesity, glucose intolerance, and hepatic steatosis

Homozygous fat-1 transgenic mice have been shown totally protected against HF/HS diet-induced obesity (DIO), glucose intolerance and severe hepatic steatosis occurring in obesogenic diet-fed mice (Fig 1). Moreover, in accordance with such a liver steatosis prevention, the mRNA liver expressions of FATCD36 (a fatty acid transport protein) and PPAR γ (an adipogenic marker) were not altered as usually observed in DIO (57). These data extend those of Li et al. (28) and Kim et al.(58), who showed that fat-1 gene expression provided protection against hypercholesterolemia and hepatic steatosis when mice are fed a high-fat diet. The homozygous status has been revealed essential for obesity protection, as if fat-1 heterozygous mice were protected against obesity-linked inflammation and insulin resistance and exhibited amelioration of non-alcoholic fatty liver disease, no change was observed in their body weight compared to WT mice under DIO (15).

The protection of fat-1 mice against metabolic disorders associated to obesity might be related to the release of anti-inflammatory n-3 PUFAs mediators, as shown by White et al. (15) who

pointed out an increase of docosanoid biosynthesis, *i.e* the pro-resolving lipid mediator, PD1 (10*R*,17*S*-dihydroxydocosa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-hexaenoic acid) in DIO.

6.2. Unlike WT mice, fat-1 mice maintained a normal barrier function and exhibited a significantly lower metabolic endotoxemia

Low-grade systemic inflammation is now recognized as a crucial factor underlying obesity and type-2 diabetes (59) where significant alterations in the intestinal barrier take place (8, 60, 61). Until now, very few *in vivo* studies have investigated the effects of n-3 PUFAs on intestinal permeability and metabolic endotoxemia (42, 43). A recent study showed high levels of metabolic endotoxemia and systemic low-grade inflammation in WT mice fed a diet high in n-6 FA, whereas endotoxemic and inflammatory status were alleviated in fat-1 mice (44). We have also reported (57) that fat-1 mice exhibited a decrease of intestinal permeability and metabolic endotoxemia compared with WT mice (Fig 1).

6.3. Transplantation of fat-1 microbiota to HF/HS-fed wild-type animals decreases metabolic syndrome disorders and maintains gut barrier integrity

The use of reciprocal microbiota transplantation represents an appropriate approach to validate the fact that n-3 PUFA-enrichment in fat-1 mice impacts their gut microbiota by enhancing the proliferation of beneficial bacteria leading to DIO and associated disorders prevention. The transfer of fat-1 fecal microbiota to WT mice was able to strikingly prevent obesity induced by a HF/HS diet and to regularize glucose tolerance and intestinal permeability (57), evidencing that a lean phenotype can be transferred from fat-1 to WT mice and is probably mediated by changes of gut microbiota by n-3 PUFAs (Fig 2).

7. Conclusions and future prospects

Altogether, these data evidence that n-3 PUFAs represent a promising approach in the prevention and treatment of obesity and associated disorders. Indeed, by the use of transgenic fat-1 mice avoiding nutritional confounding factors, tissue n-3 PUFAs enrichment indisputably demonstrates that these functional fatty acids prevent weight gain, glucose intolerance, liver steatosis inflammation and leaky gut. Moreover such tissue enrichment unexpectedly change gut microbiota composition (a peculiar increase of Akkermansia) in favor of a lean phenotype. Remarkably, transplanting gut microbes from fat-1 mice into WT replicated this protection against DIO and linked metabolic disorders, unequivocally showing

that n-3 PUFAs impact intestinal microbiota that will consequently influence host metabolism.

Although more studies are required to decipher the role of other intestinal components implicated in gut barrier integrity (*i.e* colonic mucus layer) and the molecular mechanisms involved. Fecal microbiota characterization of obese patients may become a relevant and usable means to evaluate the positive effect of n-3 PUFAs in individualized medicine.

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Figure legends:

Figure 1:

Fat-1 transgenic mice maintain a lean phenotype when fed an HF/HS-diet

The increase of endogenously synthesized n-3 PUFAs in their tissues protects fat-1 mice against obesity induced by high-fat/high-sucrose diet. Moreover, these mice are also protected against liver steatosis and adipose tissue inflammation. Compared with wild-type mice, fat-1 mice fed a HF/HS diet maintained gut epithelium permeability and then exhibit a lower plasma LPS level.

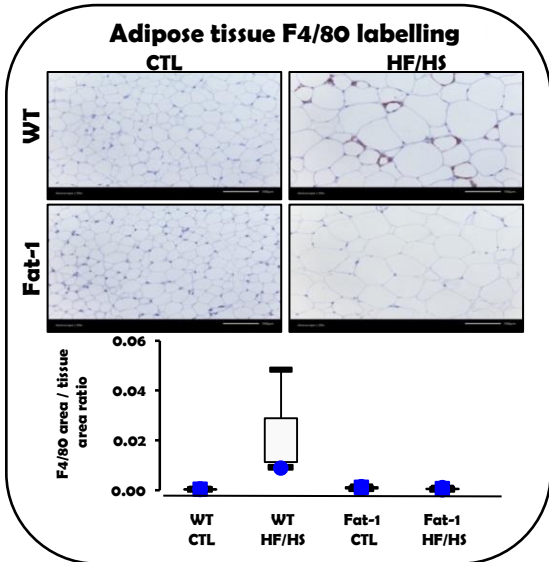
Figure 2:

Fecal transplantation of the n-3-modified microbiome as a relevant way to preserve a lean phenotype in mice fed the HF/HS diet

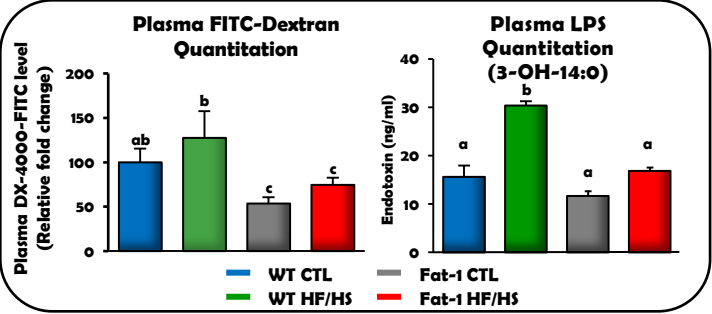
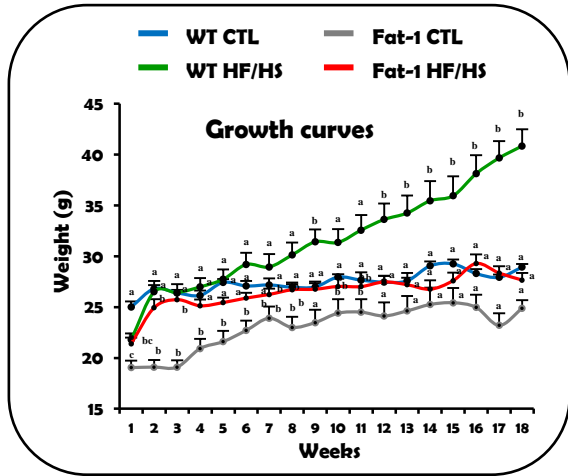
High fat/high sucrose diet is known to be associated with gut microbiota dysbiosis and to lead to increased intestinal paracellular permeability. These alterations will allow the leakage of microbial products (LPS) into the systemic circulation (named as metabolic endotoxemia) resulting in a low-grade systemic inflammation, considered as an important component of the evolution of obesity.

The transfer of fat-1 fecal microbiota to WT mice strikingly prevented metabolic endotoxemia and subsequent low-grade inflammation as well as obesity and associated metabolic disorders. Then, preventing a “dysbiotic” microbiota by n-3 PUFAs affects host metabolism and therefore potentially offers an interesting target for therapeutic intervention.

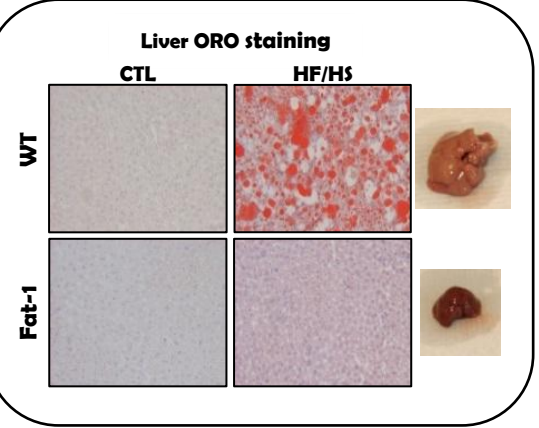
Adipose tissue inflammation



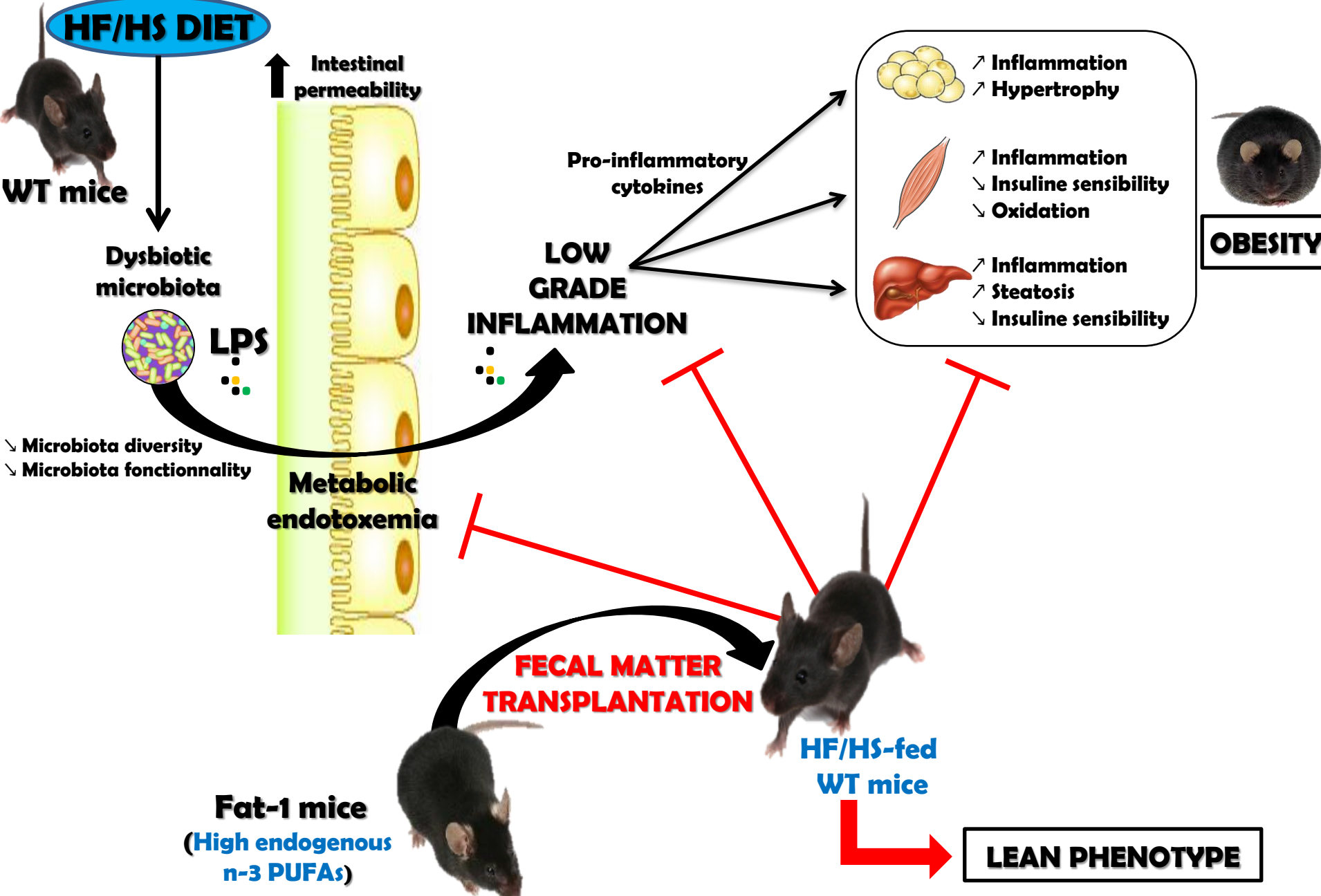
Obesity



Intestinal permeability



Liver steatosis



According to Bidu C. et al., *Diabetes*, 2018