Sex Differences in Cardiovascular Impact of Early Metabolic Impairment: Interplay between Dysbiosis and Adipose Inflammation

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Abbreviations:
Akt – Protein kinase B
AMPK – AMP-activated protein kinase
AT – Adipose tissue
AT2-R – Angiotensin 2 receptor
BMI – Body mass index
CD14 – Cluster of differentiation 14
COX-2 – Cyclooxygenase 2
CVD – Cardiovascular disease
DIO – Diet-induced obesity
E2 – 17β-estradiol
eNOS – Endothelial nitric oxide synthase
ERβ – Estrogen receptor-β
F/B – Firmicutes/Bacteroidetes
FFAR – Free fatty acid receptor
FIAF – Fasting-induced adipocyte factor
FMO3 – Flavinmonooxygenase 3
FMT – Fecal microbial transplantation
GI – Gastrointestinal
GLP-1 – Glucagon-like peptide 1
GM – Gut microbiota
GPR – G-protein coupled receptor
HDAC – Histone deacetylase
HDL – High density lipoprotein
HF – Heart failure
HFD – High-fat diet
HIF-1α – Hypoxia-inducible factor-1α
HOMA-IR – Homeostatic model assessment-Insulin resistance
HTN – Hypertension
IκBα – Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor α
IL – Interleukin
iNOS – Inducible nitric oxide synthase
IRS-1 – Insulin receptor substrate 1
JNK – Jun NH2-terminal kinase
LDL-c – Low density lipoprotein cholesterol
LPL – Lipoprotein lipase
LPS – Lipopolysaccharide
MD-2 – Myeloid differentiation factor 2
MUC2 – Mucin 2
MyD88 – Myeloid differentiation primary response 88
NADPH oxidase – NOX
NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells
Olf78 – Olfactory receptor 78
OVX – Ovariecotmy
p-IKKβ – phosphor-IK kinase β
P38 MAPK – p38 mitogen-activated protein kinase
PAMPs – Pathogen-associated molecular patterns
PBMC – Peripheral blood monocytes
PCOS – Polycystic ovarian syndrome
PGC-1α – PPARγ gamma coactivator-1α
PK – Protein kinase
PLC – Phospholipase C
PTEN – Phosphatase and tensin homolog
PUFA – Polyunsaturated fatty acids
PVAT – Perivascular adipose tissue
PYY – Peptide YY
RELMβ – Resistin-like molecule β
ROS – Reactive oxygen species
SCFA – Short chain fatty acids
SFA – Saturated fatty acids
SREBP-1 – Sterol response element binding protein-1
T1DM – Type 1 diabetes mellitus
T2DM – Type 2 diabetes mellitus
TIS11B – TNFα mRNA cis-acting AU-rich element-binding protein
TLR4 – Toll-like receptor 4
TMA – Trimethylamine
TMAO – Trimethylamine-N-oxide
TNFα – Tumor necrosis factor α
T<sub>reg</sub> – Regulatory T cells
VSMC – Vascular smooth muscle cell
WAT – White adipose tissue
Abstract:

The Evolving view of gut microbiota has shifted towards describing the colonic flora as a dynamic organ in continuous interaction with systemic physiological processes. Alterations of the normal gut bacterial profile, known as dysbiosis, has been linked to a wide array of pathologies. Of particular interest is the cardiovascular-metabolic disease continuum originating from positive energy intake and high fat diets. Accumulating evidence suggests a role for sex hormones in modulating the gut microbiome community. Such a role provides an additional layer of modulation of the early inflammatory changes culminating in negative metabolic and cardiovascular outcomes. In this review, we will shed the light on the role of sex hormones in cardiovascular dysfunction mediated by high fat diet-induced dysbiosis, together with the possible involvement of insulin resistance and adipose tissue inflammation. Insights into novel therapeutic interventions will be discussed as well.

Significance Statement:

Increasing evidence implicates a role for dysbiosis in the cardiovascular complications of metabolic dysfunction. In this minireview, we summarize the available data on the sex-based differences in gut microbiota alterations associated with dietary patterns leading to metabolic impairment. We propose a role for a differential impact of adipose tissue inflammation across sexes in mediating the cardiovascular detrimental phenotype following diet-induced dysbiosis. Better understanding of this pathway will help introduce early approaches to mitigate cardiovascular deterioration in metabolic disease.
1. Introduction

The gut microbiota (GM) is a complex ecosystem that can be described as a dynamic organ with an active role in human health and disease (Putignani et al., 2014). The microbial community has high plasticity and is sensitive to several stimuli including environmental, hormonal, dietary and stress-related factors (Putignani et al., 2014). Nevertheless, diet remains one of the most vigorous modulators of GM (David et al., 2014) with western-type calorie dense diets driving an imbalance of microorganisms in the gut or dysbiosis (Sen et al., 2017). This is particularly relevant to the steady rise in the prevalence of metabolic disease like diabetes, obesity and their complications, driven by increased caloric intake following the global shift to the western diets, rich in saturated fat and refined sugars (Lutsey et al., 2008; Misra et al., 2010). As such, there has been an increasing interest in studying dysbiosis in these maladies together with the impact of its modification as a therapeutic option.

Significantly, most of the health burden associated with metabolic dysfunction is due to the high risk of cardiovascular mortality and morbidity due to ischemic heart disease, ischemic stroke, cardiac metabolic dysfunction, and heart failure (Ash-Bernal and Peterson, 2006; von Bibra et al., 2016). Of note, cardiovascular risk evoked by metabolic impairment has long been associated with a state of chronic low-grade inflammation (de Rooij et al., 2009). Indeed, under circumstances leading to dysbiosis GM can contribute to this inflammatory state. Normally, the host health/gut bacteria interaction occurs through exposure to either bacterial components known as pathogen-associated molecular patterns (PAMPs), like flagella and cell wall constituents like LPS (Tilg et al., 2019), or to the metabolites produced by bacterial digestion and processing of ingested food, which were shown to have several effects including modulation of the function of immune and autonomic nervous system as will be discussed below. Therefore, dysbiosis outcomes depend on the changes in bacterial Phyla residing in the gut.
Interestingly, considerable sex-dependent differences were reported in inflammatory changes and cardiovascular risk associated with metabolic dysfunction. Recent literature shows that metabolic impairment in humans leads to different inflammatory profiles across sexes with increased production of pro-inflammatory cytokines in males (Horst et al., 2020). Indeed, pre-menopausal females are less prone to adverse cardiovascular events (Mosca et al., 2011), and varying cardiovascular profiles secondary to metabolic deterioration are observed in either sex (Gerdts and Regitz-Zagrosek, 2019). While sex dependent differences in metabolic derived CVDs are typically attributed to estrogen-driven alteration in insulin sensitivity, adiposity, adipocyte size and function, as well as adipose tissue (AT) susceptibility to inflammation (Pradhan, 2014; Ribas et al., 2010; Zore et al., 2018), sex-dependent differences in GM together with its vulnerability to dysbiosis add a new layer of complexity to the paradigm. GM appears to play an important role in mediating the differential patterns observed in diet-induced metabolic and cardiovascular dysfunction across sexes. While the exact mechanism has yet to be comprehensively and systematically investigated, we attempt here to shed the light on the potential mechanisms through which dysbiosis mediates cardiovascular dysfunction in early metabolic impairment in a sex-dependent manner. We explore the sex differences in high-fat diet (HFD) induced dysbiosis and the consequent AT inflammatory changes and cardiovascular dysfunction in the context of early metabolic deterioration. As well, we summarize some of the available evidence regarding possible therapeutic interventions to address these disorders via targeting the gut microbiome homeostasis.

2. Sex-dependent differences in gut microbiota in the healthy state

The assumption of dysbiosis in disease states necessitates a fundamental knowledge of the composition and function of GM in healthy individuals. Nevertheless, a unified healthy GM
profile has not been defined at any profound taxonomic resolution owing to several endogenous and exogenous factors. These include inter-individual host genetic and environmental differences (Abdul-Aziz et al., 2016; Hooper et al., 2001; Rothschild et al., 2018), disparate GM growth rates, strain-level diversities, and variants within microbial genes (Huttenhower et al., 2012; Korem et al., 2015; Zeevi et al., 2019). However, high taxonomic diversity, along with high microbial gene richness and stable microbiome functions represent characteristics of a healthy GM (Huttenhower et al., 2012).

The major bacterial phyla inhabiting the human gut are *Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Actinobacteria, and Fusobacteria*, with *Firmicutes* and *Bacteroidetes* accounting for almost 70% of the total microbiota (Mariat et al., 2009; Zoetendal et al., 2008) and their ratio (*F*/*B*) changing under situations of metabolic impairment (Turnbaugh et al., 2006). The homeostatic state in which the GM is healthy and balanced is referred to as a state of eubiosis (Iebba et al., 2016). Significantly, several studies highlighted sex-dependent variations of the GM in health and disease (Han et al., 2017; Org et al., 2016; Razavi et al., 2019). It was shown that Drosophila melanogaster strains exhibit a differential abundance of microbes across sexes irrespective of nutritional conditions (Han et al., 2017). Interestingly, similar results were also obtained in different strains of mice where the abundance of several taxa exhibited significant sex-dependent differences (Org et al., 2016). Furthermore, such differences were also evident in mice fed either normal chow or HFD suggesting a sex-by-diet interactions (Org et al., 2016).

Indeed, the impact of gonadectomy and sex hormone replacement on GM is far from settled. It was recently demonstrated that women harbor a more diverse and stable microbiota with no profound alterations associated with menopause (Zhang et al., 2019a). Contrastingly, the microbiota of men was demonstrated to be more variable with correlations to age and testosterone levels. Importantly, sex-dependent differences correlated with lifestyle and were
diminished by age (Zhang et al., 2019a). This is of particular interest in light of the reduced susceptibility of premenopausal women to metabolic and cardiometabolic diseases (Santos-Marcos et al., 2019). Yet, numerous lines of evidence suggested a role for female sex hormones in enhancing the diversity of the GM (Song et al., 2020). A cross-sectional study revealed that while sex-dependent differences in GM were observed before puberty, they tended to increase after puberty with significant differences in $\beta$-diversity (inter-individual dissimilarity) but not in $\alpha$-diversity (intra-individual bacterial diversity) (Yuan et al., 2020). Furthermore, studies have shown a stronger impact of female gonadal hormones on GM. A study in rats showed that while the sex-dependent differences in GM community persisted after gonadectomy, the detrimental impact was more pronounced in female rats, especially when animals were overfed (Santos-Marcos et al., 2020). This is indeed in line with observations in humans demonstrating a shift in the GM profile in post-menopausal women compared to age matched men (Santos-Marcos et al., 2018). Along the same lines, the strong associations observed between sex and $\alpha$-diversity in young adults, though persisted after adjusting for cardiometabolic parameters, tended to diminish after 40 years of age (de la Cuesta-Zuluaga et al., 2019) consistent with an age-related decline in the level of sex hormones. Importantly, the interaction between GM and female sex hormones appear to be bidirectional whereby a study on rats showed that several GM-derived microRNAs were reported to modulate steroid biosynthesis and estrogen signaling (Santos-Marcos et al., 2020).

While less clear in human studies, accumulating evidence from experiments on mice suggests that the differential diversity in GM can drive sexually dimorphic immune responses (Elderman et al., 2018; Felix et al., 2018). It was even suggested that sexual dimorphism in susceptibility to certain autoimmune disorders, like T1DM, was mediated by GM in rodent models, and the alteration of GM at an early age may protect against genetic predisposition to
autoimmune diseases (Candon et al., 2015; Markle et al., 2013; Yurkovetskiy et al., 2013). This sex dependent dysbiosis in disease prognosis was suggested to mediate manganese induced neurotoxicity (Chi et al., 2017). As these factors augment the complexity of the host environment-microbiota interactions, it becomes plausible that diet-induced GM alteration leading to metabolic impairment will trigger distinct inflammatory responses in either sex culminating in disparate cardiovascular consequences.

3. Sex-dependent gut microbiome alterations in early metabolic impairment:

Early metabolic impairment has long been discussed in the literature, yet there has been no consensus on the exact definition and the diagnostic criteria. This is despite the fact that a significant proportion of the global population exhibits suboptimal metabolic health, primarily due to excessive caloric consumption and sedentary lifestyles resulting in the increased prevalence of metabolic diseases such as insulin resistance, obesity, and diabetes (Frost et al., 2020; Jaacks et al., 2019; Le Chatelier et al., 2013; Zheng et al., 2018). This is mirrored by an increased prevalence of metabolic dysfunction-associated cardiovascular diseases (Lakka et al., 2002). Indeed, recent research identified early stages of metabolic deterioration such as prediabetes or metabolically unhealthy normal weight as risk factors of cardiovascular disease (Alderman, 2021; Stefan, 2020). Despite the various pathological mechanisms culminating in the emergence of these disorders, it seems that they are correlated with GM alterations referred to as dysbiosis (Allin et al., 2018; Qin et al., 2012; Qin et al., 2014). Next-generation sequencing of the gut microbiome had a major role in unfolding the involvement of GM in regulating the host metabolism. Metabolic related dysbiosis is usually exemplified by altering the abundance of Bacteroides, Prevotella, Desulfovibrio, Lactobacillus, and Oxalobacter genera in the gut (Clemente et al., 2015; Smits et al., 2017; Tyakht et al., 2013). Nevertheless, the precise dynamics of GM involvement in metabolic
diseases is not fully explored yet. Indeed, whether the disease-associated aberrant microbiota underpins disease causation or represents a secondary phenomenon after disease onset and progression has been widely debated (Bäckhed et al., 2004; Pedersen et al., 2016; Qin et al., 2010). Figure 1 outlines the proposed framework linking dysbiosis in metabolic dysfunction with the pathogenesis of cardiovascular complications together with the interplay with sex-dependent factors as described below.

3.1. Sex-dependent differences in diet-induced dysbiosis:

As diet remains the cornerstone for GM modulation in humans and animal models (Carmody et al., 2015; David et al., 2014), dysbiosis has been the focus of research on diet induced pathologies. HFD was consistently reported to provoke dysbiosis by altering the scale of the major gut phyla; increasing F/B ratio as well as an increase in Proteobacteria, which were ushered with impaired metabolic and cardiovascular function (Moreira et al., 2012; Murphy et al., 2015). Bacteroidetes is considered the most prevalent Gram negative bacteria in the gut and is essentially considered beneficial due to their capacity to modulate caloric absorption through polysaccharide metabolism (Wexler, 2007). On the other hand, Firmicutes are largely Gram positive and are capable of producing various short chain fatty acids (SCFA) (Den Besten et al., 2013). It is generally accepted that a higher F/B ratio is usually observed in overweight and obese subjects (Kasai et al., 2015; Ley et al., 2006; Million et al., 2012), and a reduction in the F/B ratio has been associated with weight loss (Ley et al., 2006; Turnbaugh et al., 2006). However, the opposite was documented as well where HFD and diet-induced obesity (DIO) were associated with decreased F/B ratio in human and animal models in both sexes (Collado et al., 2008; Schwieretz et al., 2010).

Indeed, dysbiosis severity in different situations of metabolic impairment appeared to be sex-dependent (Org et al., 2016). Premenopausal women were shown to have a higher
F/B ratio than postmenopausal women and men (Santos-Marcos et al., 2018). Accumulating evidence suggested that women harbor a higher \( F/B \) ratio in comparison to men, even after adjusting for body mass index (BMI) (Dominianni et al., 2015; Mueller et al., 2006). In fact, \( F/B \) ratio was found to be highly influenced by BMI, and has been used as an indicator of gut dysbiosis, with a higher \( F/B \) ratio indicating a more pronounced dysbiotic microbiome (Kasai et al., 2015). Another human study showed that among subjects with BMI greater than 33, men exhibited a significantly lower \( F/B \) ratio in comparison to women, while the opposite was observed in subjects with a BMI lower than 33 as well as in post-menopausal women (Haro et al., 2016). Sex differential dysbiosis was also observed among lean men and women, as post-menopausal women had a similar GM signature as men, while obesity abolished these differences. The same study emphasized the tight correlation between sex hormones and GM diversity. Premenopausal women had higher \textit{Bifidobacterium} and lower \textit{Bacteroides} than men and post-menopausal women, as GM community could predict testosterone level in humans and recipient mice of human fecal microbiome (Mayneris-Perxachs et al., 2020).

In mechanistic terms, estrogen Receptor -\( \beta \) (ER\( \beta \)) has been proposed to be a modulator of sex-dependent dysbiosis. For instance, ER\( \beta \) knockout female mice on isoflavone and fiber rich feeding achieved a state of eubiosis with an increase in \textit{Bacteroidetes} and a reduction in \textit{Firmicutes} and \textit{Proteobacteria}. However, when these mice were switched to an isocaloric low fiber and simple sugar rich diet, the knockout mice had more pronounced dysbiosis and reduced \textit{Bacteroidetes} in favor of \textit{Proteobacteria} compared to their wild-type counterparts (Menon et al., 2013) suggesting a protective effect of estrogen in diet-induced dysbiosis. Similarly, genetically obese \textit{ob/ob} mice show an elevation of \( F/B \) ratio (Turnbaugh et al., 2006). Interestingly, hormonal treatment with 17\( \beta \)-estradiol (E2) in female mice was found to correct HFD-related dysbiosis in \textit{ob/ob} and wild-type mice by increasing the heterogeneity of GM distribution and reducing the \( F/B \) ratio compared to the
vehicle group (Acharya et al., 2019). Moreover, androgenization of young and adult ovariectomized female Wistar rats induced dysbiosis regardless of dietary intervention. It reduced GM diversity, elevated $F/B$ ratio and impaired overall metabolic function (Moreno-Indias et al., 2016).

While human studies link dysbiosis to obesity, results from experiments on animals implicate HFD as the culprit even in absence of obesity. This was supported with results from RELM$\beta$ knockout female mice, a model that lacks the specific gene for colonic goblet cells, where HFD-fed mice were not obese. These mice presented with the typical HFD-related dysbiosis (Hildebrandt et al., 2009). It is noteworthy that the type of fat affects the microbiome alteration as diets rich in saturated fatty acids (SFA) are thought to contribute to the development of endotoxemia by enhancing the production of LPS, while polyunsaturated fatty acids (PUFAs) are suggested to exert protective effects by influencing systemic endotoxin concentrations, LPS clearance, bile acid metabolism, intestinal alkaline phosphatase activity, intestinal mucosal permeability and microbiota composition diversity (Bellenger et al., 2019; Cândido et al., 2020). Moreover, SFAs were recently demonstrated to act as a non-microbial toll-like receptor 4 (TLR4) agonists, triggering MyD88-dependent or independent inflammatory pathways culminating in the activation of NF-$\kappa$B and the production of inflammatory cytokines, in a similar fashion to LPS (Rocha et al., 2016). Moreover, mice fed HFD rich in omega-6 fatty acids but not omega-3 fatty acids exhibited a more pronounced metabolic endotoxemia (Kaliannan et al., 2015). Transgenic mice over-converting omega-6 to omega-3 in tissues exhibited an augmented production of intestinal alkaline phosphatase, which subsequently alters GM composition, reduces LPS production, maintains gut barrier function, and reduces endotoxemia (Kaliannan et al., 2015). Importantly, linoleic acid and $\alpha$-linolenic acid-enriched HFD fed obese mice exhibited a sex-dependent alleviation of endotoxemia, and systemic and AT inflammation, which was
associated with sex-dependent alterations in GM composition (Zhuang et al., 2018). This suggests that linoleic acid may provide a protective effect against metabolic endotoxemia in female mice, while α-linolenic acid exerts a similar effect in male mice through modulating the gut-adipose tissue axis (Zhuang et al., 2018). These variabilities highlight the complex interplay of sex and diet in shaping the microbiome community and its metabolic consequences.

3.2. Sex-dependent differences in dysbiosis consequences: Compromised gut integrity and metabolic endotoxemia

As mentioned in the previous section, the detrimental effects of consumption of HFD are partly mediated by dysbiosis evidenced by an augmented and reduced relative abundance of Proteobacteria and Bacteroidetes, respectively. Disruption of gut barrier function as well as metabolic endotoxemia ensue (Satokari, 2020). While a sizable body of evidence consistently supports an increased F/B ratio under these circumstances, an increased LPS would not be expected given the observed reduction of the Gram negative Bacteroidetes. Paradoxically, increased plasma LPS levels were reported following HFD in animal experiments (Cani et al., 2008; Oliveira et al., 2011) and in obese humans in comparison to their lean controls (Stoll et al., 2004; Trøseid et al., 2013). Such observations can be attributed to the reduced expression of intestinal epithelial tight junction proteins leading to a compromised gut barrier in animals and humans allowing for increased LPS transportation (Saad et al., 2016). As well, chylomicrons, lipoprotein particles mediating intestinal fat absorption, were found to facilitate LPS transport across the intestinal lumen through enterocyte-mediated absorption (Ghoshal et al., 2009) offering an additional mechanism by which HFD can contribute to increase plasma LPS levels.
Expectedly, metabolic endotoxemia will follow, where the transport of microbial associated molecular patterns leads to systemic low-grade and subclinical inflammation via the activation of TLR4 (Cani et al., 2007; Rodriguez et al., 2020; Turnbaugh et al., 2006). Indeed, LPS binds to cluster of differentiation 14 (CD14) and the Toll like receptor 4 (TLR4)/Myeloid differentiation factor 2 (MD-2) receptor complex (Kitchens and Thompson, 2005; Lu et al., 2008). TLR4 cascade was shown to mediate HFD induced inflammation. HFD feeding led to an increased serum and fecal LPS, concurrently with increased F/B ratio in male wild type mice. Moreover, these mice suffered from diet induced colitis indicated by increased intestinal TNFα, IL-1β, iNOS, COX-2, and (p-IKKβ) NF-κB expression and activity together with a compromised gut integrity and reduced expression of tight junctions protein occludin and claudin-1 (Kim et al., 2012). On the other hand, HFD fed TLR4 knockout mice were protected from metabolic endotoxemia, as they had lower serum LPS compared to their low-fat fed counterparts. At the same time, the knockout mouse intestine did not show the same HFD-induced inflammatory consequences (Kim et al., 2012).

Sexual dimorphism in the response to HFD-induced gut barrier permeability and metabolic endotoxemia has been attributed to differential hormonal modulation of these pathological processes. Estrogen has been shown to prevent metabolic endotoxemia and chronic low-grade inflammation, which was suggested to be a contributor to the cardiometabolic privilege of premenopausal women (Santos-Marcos et al., 2019). Indeed, 17β-estradiol-treated male, ovariectomized, and intact female mice exhibited reduced LPS production and lower susceptibility to metabolic endotoxemia and metabolic syndrome partly through upregulation of intestinal alkaline phosphatase (Kaliannan et al., 2018). On the same note, one study highlighted a protective effect of progesterone against endotoxemia, where plasma levels of LPS negatively correlated with plasma progesterone levels but positively correlated with TNF-α plasma levels in pregnant women (Zhou et al., 2019).
This differential effect of sex hormones was shown to be due to the alteration of expression and function of tight junctions and mucin production, thus regulating HFD-induced alteration of gut permeability. For example, progesterone was found to upregulate the expression of the tight junction protein occludin and inhibit NF-κB activation in LPS-stimulated Caco-2 cells (Zhou et al., 2019). Similarly, estrogens promoted gut barrier function through several mechanisms. For instance, ERβ was proposed to play a crucial role in regulating cellular differentiation in colonic tissue as ERβ−/− mice exhibit epithelial hyperproliferation and compromised integrity (Imamov et al., 2004; Wada-Hiraike et al., 2006). This suggests a homeostatic role of estrogen in the maintenance of colon integrity. Furthermore, estrogen-ERβ cascade was suggested to enhance mucin 2 (MUC2) secretion by intestinal goblet cells, which offers epithelial protection. Hence, the deletion of ERβ disrupted the colon mucin layer in female mice (Diebel et al., 2015; Wada-Hiraike et al., 2006). Likewise, female mice during proestrus stage, characterized by high estrogen levels, are protected against intestinal injury in comparison to their male counterparts and female mice in the diestrus stage that is characterized by low circulating estrogen (Homma et al., 2005; Sheth et al., 2010). This can be attributed to the lower mucus thickness observed in the colon of diestrus female mice and male mice compared to the proestrus ones (Elderman et al., 2017). Apart from the induction of MUC2 production, estrogen is also found to upregulate the expression of tight junctions in both male and ovariectomized female rodents as well as in vitro monolayer cultures (Braniste et al., 2009; Homma et al., 2005; Looijer-van Langen et al., 2011). Consistent with these observations, the differential effects of estrogen supplementation on metabolic health observed in pre- and post-menopausal women suggest that the loss of ovarian function may profoundly alter estrogen signaling (Hulley et al., 1998; Rossouw et al., 2002). Indeed, it was shown that ovariectomized mice exhibit temporal and regional changes in GI permeability due to disruption of tight junctions (Collins et al., 2017).
Intriguingly, it was shown in some studies that TLR4 level in female macrophages were higher after OVX (Rettew et al., 2009). Although males tend to have higher metabolic endotoxemia, orchietomized mice were more susceptible to endotoxemia, and isolated macrophages presented higher TLR4 level than intact males. At the same time testosterone treatment attenuated these events suggesting an immunosuppressive effect of testosterone (Rettew et al., 2008). These findings emphasize the complexity of the effect of sex hormones on dysbiosis outcomes. Nevertheless, female sex hormones in animal models consistently exhibited a protective effect on gut integrity and the resulting metabolic endotoxemia, mainly by upregulating intestinal tight junction proteins.

3.3. Sex-dependent differences in dysbiosis-induced insulin resistance and adipose inflammation

Diet induced metabolic derangement and its correlated cardiovascular dysfunction have been suggested to be an outcome of early adipose inflammation and insulin resistance, even in the absence of explicit hyperglycemia (Elkhatib et al., 2019; Gollasch, 2017; Nishimura et al., 2009; Rafeh et al., 2020; Shah et al., 2008). As previously demonstrated, dysbiosis and altered gut permeability precede the emergence of metabolic syndrome (Martínez-Oca et al., 2020). Since HFD consumption increased intestinal permeability by impairing the function of tight junctions (Cani et al., 2008), intestinal hyperpermeability and GM dysbiosis were suggested to further the inflammatory phenotype in AT and to increase the risk of cardiovascular diseases (Clemente-Postigo et al., 2019; Gasmi et al., 2020; Kallio et al., 2015; Serino et al., 2012). A great body of research attempted to establish an understanding of the gut-adipose axis in metabolic dysfunction (Poggi et al., 2007; Samuel et al., 2008; Serino et al., 2012). Accordingly, white adipose tissue (WAT) was identified as a major target of GM. Not only were GM fermentation products shown to regulate WAT energy
balance (Samuel et al., 2008), but also GM regulated fat deposition in AT as well as insulin resistance (Bäckhed et al., 2004; Bäckhed et al., 2007; Velagapudi et al., 2010). Such observations are of relevance given the contribution of WAT to the development of metabolic inflammation leading to insulin resistance and cardiovascular dysfunction (Bouloumié et al., 2008; Bouloumié et al., 2005; Hotamisligil, 2006). In HFD-fed diabetic mice, dysbiosis increased the stromal vascular fraction in WAT, in particular macrophages, lymphocytes, and preadipocytes (Serino et al., 2012). Additionally, LPS migration into the circulation has been suggested to be a contributing factor to the onset of AT inflammation, insulin resistance, obesity, and diabetes (Cani et al., 2007; Cani et al., 2008; Hersoug et al., 2016). Interestingly, HFD consumption had a similar effect to LPS subcutaneous infusion on elevating serum LPS and promoting AT inflammation in male mice (Cani et al., 2007). Significantly, HFD-fed TLR4 knockout mice did not have increased proinflammatory cytokines in the isolated epidydimal AT depot, whereas wild-type mice were hyperinsulinemic and exhibited a proinflammatory response in the epidydimal WAT manifested by increased TNFα, IL-1β, and IL-6, and macrophage infiltration (Kim et al., 2012). Moreover, TLR4 knockout male mice had an increased insulin sensitivity in subcutaneous and epidydimal WAT even in presence of HFD feeding (Poggi et al., 2007). Consistently, HFD-fed male mice showed an improved metabolic status after eight weeks of antibiotic treatment, as serum LPS, insulin, and fasting glucose were reduced. The epidydimal WAT in the treated group had lower TLR4 activation, JNK inactivation, lower IκBα degradation and inhibition of IRS-1 Ser307 phosphorylation. Additionally, the treatment group had improved insulin signaling with increased Akt phosphorylation and reduced macrophage infiltration (Carvalho et al., 2012). It is noteworthy that HFD-fed germ-free mouse models were not vulnerable to HFD-induced insulin resistance (Fleissner et al., 2010; Rabot et al., 2010).
As for sex-based differences, a recent study reported that HFD induced weight gain, and insulin resistance in males but not in female mice with differences in gut microbiome recorded at baseline (Peng et al., 2020). Moreover, E2 treatment in intact female mice improved HFD-induced weight gain, glucose intolerance, and insulin resistance possibly as a result of the downregulation of lipogenic genes, such as sterol response element binding protein-1 (SREBP-1), and leptin and resistin genes expression in WAT (Bryzgalova et al., 2008). Moreover, E2 intervention in ovariectomized female mice prevented HFD-induced obesity (Bless et al., 2014). Administration of E2 in ovariectomized female rats increased leptin sensitivity and led to preferential subcutaneous adiposity, resembling the intact female littermates (Clegg et al., 2006). Notably, subcutaneous fat pads were suggested to be more sensitive to insulin than visceral fat (Chang et al., 2018). This might be one of the many protective roles of estrogens against metabolic impairment related to AT dysfunction, as premenopausal women have more subcutaneous AT than men and postmenopausal women (Lemieux et al., 1993). Furthermore, E2 was proposed to ubiquitinate and degrade HIF1-α eventually reducing adipose inflammation and its subsequent metabolic derangements (Kim et al., 2014). Typically, insulin resistance is associated with hypertrophic adipose expansion leading to increased tissue hypoxia and elevated hypoxia inducible factor 1-α (HIF1-α) expression, which was suggested to contribute the inflammatory cascade (Lumeng et al., 2007; Palmer and Clegg, 2014; Wensveen et al., 2015).

Since adiposity patterns, insulin sensitivity, LPS levels, TLR activation, hypoxia, and AT inflammation were all ameliorated in the presence of estrogen, its enhancement of AT metabolic state via GM modulation can be one of the protective roles female sex hormones possess against cardiometabolic dysfunction. Interestingly, HFD-fed androgen receptor knockout male mice had increased obesity, visceral adiposity and adipocyte hypertrophy, glucose intolerance and insulin resistance compared to control males and HFD-fed females.
These derangements were linked to dysbiosis as antibiotic treatment corrected the dysfunction (Harada et al., 2020). These observations further emphasize the complexity and the importance of sex hormone contribution to the GM-cardiometabolic interaction, particularly the female sex hormones playing a modulatory role on several intermediary factors of cardiometabolic insults as outlined above. Figure 2 depicts the different pathways triggered by GM leading to WAT inflammation as well as their modulation by sex hormones.

Nonetheless, it is prudent to mention that accumulating evidence recognize sex hormone independent differences in metabolism and response to metabolic challenge between males and females (Manwani et al., 2015). Differences in consequences of the genetic disparity between the XX and XY chromosome combination extend well beyond sex hormone production to comprise variable levels of X-linked gene imprinting and expression, in addition to a considerable dimorphism in the complement of noncoding RNA molecules production (Link et al., 2013). The study of the metabolic sequela of such differences was hampered for a long time by the lack of ability of dissociating the concurrence of the XX or the XY combination with the corresponding gonadal hormone production. Evidence from transgenic mouse models expressing different combinations of sex chromosomes in presence and absence of the corresponding gonads showed a remarkable impact of the XX chromosome combination on preferential subcutaneous AT distribution regardless of the gonadal hormone status (Chen et al., 2012). However, the increased sub-cutaneous adiposity in this model was associated with increased insulin resistance thus emphasizing the protective effect of estrogen in this regard. More recently, genetic association studies were conducted on humans to detect the effect of X-chromosome on metabolic and cardiovascular disease, but had conflicting results. While some studies found differential associations with insulin resistance, atherosclerosis and coronary artery disease between sexes (Traglia et al., 2017; Tukiainen et al., 2014), others failed to detect these differences and attributed the
observations to gonadal hormones (Manwani et al., 2015). However to our knowledge, the interaction between sex chromosomes and dysbiosis did not receive much attention and warrants detailed investigation in the future.

3.4. Sex-dependent differences in alteration of short chain fatty acids production and consequent metabolic dysfunction:

Another important component of HFD-induced dysbiosis is the decline in SCFA generation; mainly acetate, propionate and butyrate, which were correlated with numerous metabolic and cardiovascular disorders (Canfora et al., 2015; Chambers et al., 2018). Despite the extensive research on the role of SCFA in the host wellbeing, the exact role of SCFAs in regulating metabolic function remains debatable. For instance, some evidence suggested a negative correlation of fecal SCFAs levels with the host’s metabolic function (de la Cuesta-Zuluaga et al., 2018; Teixeira et al., 2013) with studies showing that SCFA levels were higher in overweight or obese human subjects (Rahat-Rozenbloom et al., 2014).

SCFAs are byproducts of fermentation of complex carbohydrates or insoluble fiber that are mainly produced in the cecum and proximal colon (Canfora et al., 2015; Morrison and Preston, 2016). Their concentration varies across the large intestines, however, fecal concentration of SCFAs is reflective of the values in cecum and colon (Canfora et al., 2015). SCFAs derive their importance from being natural ligands for free fatty acid receptors 2 and 3 (FFAR 2 and 3), which are a G-protein coupled receptor 43 and 41 respectively (GPR43 and 41) (Ang and Ding, 2016; Canfora et al., 2015; Kimura et al., 2013). SCFAs were proposed to exert anti-inflammatory functions upon the binding to GPR41 and 43, where the downstream effect leads to the inhibition of NF-κB (Saad et al., 2016). On one hand, GPR43
KO mice exhibited an obese phenotype in response to regular chow. On the other hand, overexpressing GPR43 in AT in HFD-fed mice induced a lean phenotype with reduced adiposity and suppressed insulin-mediated Akt phosphorylation via PTEN activation and G(i/o)βγ–PLC–PKC signaling (Kimura et al., 2013).

Butyrate was proposed to activate T regulatory cells (Tregs) and the production of IL-10 (Saad et al., 2016). Of note, a low count of Tregs was observed in Visceral AT (VAT) isolated from male mice in which HFD induced insulin resistance. Along the same lines, VAT Treg cell ablation promoted pro-inflammatory cytokine production. At the same time, injecting HFD-reared mice with Treg cells improved IL-10 production and lowered HOMA-IR compared to their HFD-fed non-treated littermates (Feuerer et al., 2009). Additionally, butyrate was able to exert some anti-inflammatory functions in human isolated monocytes, as it inhibited IL-12 while promoting IL-10 production (Säemann et al., 2000). At the same time, butyrate was shown to be a potent regulator of TNF-α mRNA synthesis in primary synoviocytes, murine macrophages and human peripheral blood monocytes (PBMCs), as it facilitates degradation of TNFα mRNA cis-acting AU-rich element (ARE)-binding protein (TIS11B) (Fukae et al., 2005). Several studies highlighted the role of butyrate in inhibiting NF-κB binding to DNA in human colonic cells (Inan et al., 2000) and PMBCs (Segain et al., 2000). This can be attributed to the hyperacetylation that may occur from high doses of butyrate, since it is considered as a strong inhibitor of histone deacetylase (HDAC) (Boffa et al., 1978; Chriett et al., 2019; Vidali et al., 1978). Furthermore, oral supplementation of butyrate attenuated HFD-induced metabolic derangements in male mice enhancing insulin sensitivity (lower HOMA-IR) and fatty acid oxidation while reducing adiposity, correcting serum lipid profile, increasing uncoupling protein 1 (UCP1) and PGC-1α in BAT on mRNA and protein levels. It’s worth mentioning that butyrate activated AMPK and p38 as well, in the liver and muscle tissues of these mice (Gao et al., 2009). Butyrate was also proposed to
directly and indirectly affect PGC-1α, either by increasing PGC-1 α expression and protecting it from degradation, by being a potent HDAC inhibitor, or by activating kinases such as AMPK and p38 which can phosphorylate PGC-1α and induce its activity.

As for acetate and propionate, it is suggested that they offset LPS induced endotoxemia by lowering TNF-α and NF-κB production as shown in human neutrophils and macrophages *in-vitro* experiments (Canfora et al., 2015). These two SCFAs were shown to be effective in reducing TNFα production in LPS-activated neutrophils, whilst repressing the activity of NF-κB receptor in a human colon adenocarcinoma cell line (Tedelind et al., 2007). Consistently, *in vitro* treatment of human omental and subcutaneous adipocytes with propionate, reduced mRNA expression of the proinflammatory factor resistin, and stimulated leptin mRNA expression (Al-Lahham et al., 2010). Another study on human omental AT supported the previous findings, as propionate treatment declined both mRNA and protein levels of proinflammatory cytokines like IL-4 and TNF-α (Al-Lahham et al., 2012). Additionally, animal models reared on HFD and treated with propionate had an improved insulin sensitivity, glucose tolerance, thermogenesis and mitochondrial function, besides an improved metabolic state in BAT, liver and muscles (Brial et al., 2018; Liang and Ward, 2006). In humans, rectal administration of sodium acetate reduced serum TNF-α in obese females (Freeland and Wolever, 2010). Another trial on acute intravenous infusion of acetate in women with hyperinsulinemia and overweight improved serum PYY, GLP-1 and reduced circulating TNFα and ghrelin (Freeland and Wolever, 2010). Pathways modulated by SCFAs are summarized in Figure 3.

Importantly, recent literature reveals that interventions targeting GM have a differential impact on SCFA generation across sexes. For instance, ciprofloxacin-metronidazole treatment reduced SCFA production only in female mice consistent with a reduction in the relative abundance of *Firmicutes* (Gao et al., 2019). Alternatively, prebiotic supplementation
was shown to increase fecal butyrate output only in male but not female rats (Shastri et al., 2015). A similar study in humans demonstrated a differential effect whereby beta-glucan supplementation led to an increased butyrate production in female subjects but not in males (Trimigno et al., 2017). On the other hand, SCFAs were proposed to promote the storage of triglycerides through the activation of lipogenic hepatic enzymes including SREBP, which show a positive differential expression in women (Bäckhed et al., 2004; Jiang et al., 2016). This is suggested to reflect in an increased microbiota-dependent lipid storage and obesity risk in women. SCFAs also are known to suppress the fasting-induced adipocyte factor (FIAF), an inhibitor of lipoprotein lipase (LPL) (Bäckhed et al., 2004; Khan et al., 2016). This increased LPL activity may lead to microbiota-dependent augmentation in fat storage, that may contribute in part to sex differences in body composition (Bäckhed et al., 2004). Henceforth, sex-differential response to GPR41 may contribute to microbiota-associated body weight sexual dimorphism (Inoue et al., 2014). This is particularly important as male but not female GPR41 knockout mice exhibited an increased body fat mass and a decreased energy expenditure (Bellahcene et al., 2013). Moreover, further sex-specific interactions with SCFAs were reported where butyrate was shown to increase estrogen secretion in granulosa cell culture models (Lu et al., 2017). Indeed, conclusions about the involvement of SCFAs in cardiometabolic derangements in sex dependent manner are hard to be drawn, and further studies are highly needed for more solid evidence.

4. **Sex-dependent impact of gut microbiome on metabolically induced cardiovascular dysfunction:**

While traditional cardiovascular risk factors appear to be related to the development of cardiovascular disease in either sex, research has long recognized significant complexity in their differential roles and relative weights (Njoelstad et al., 1996). For instance, analysis of a
large case-control study showed that the impact of diabetes and hypertension was more pronounced on the development of myocardial infarction in women than in men (Anand et al., 2008). Yet, the impact of these two factors appeared to differ by age, being stronger in younger men, leading to an age difference of cardiovascular disease onset by about nine years. This further implicates the role of sex hormones in the observed protective effect in pre-menopausal females, as the incidence of both hypertension (Lima et al., 2012) and diabetes (Heianza et al., 2013) in post-menopausal females appear to exceed that in men. Interestingly, it is well recognized that both disorders have strong mechanistic links to adipose tissue dysfunction, particularly PVAT, observed in metabolic impairment (Saxton et al., 2019). Significantly, female-specific risk factors for cardiovascular disease such as polycystic ovary syndrome and preeclampsia appear to have a strong metabolic impairment component carrying the hallmarks of a dysfunctional adipose tissue (Huda et al., 2017; Leon et al., 2019; Osibogun et al., 2020). In the below sections, we examined the impact of sex-based differences in the interconnection among dysbiosis, metabolic impairment and adipose inflammation on incidence of cardiovascular disease.

4.1. Dysbiosis and cardiovascular dysfunction:

HFD is known to induce cardiovascular dysfunction (Aghajani et al., 2017; Martins et al., 2015). Since HFD stimulates dysbiosis, the microbiome-cardiovascular axis was extensively studied and dysbiosis was linked to several diseases such as hypertension (HTN), atherosclerosis, and heart failure (HF) among others (Kappel and Federici, 2019; Razavi et al., 2019; Tang and Hazen, 2017; Taylor and Takemiya, 2017). For instance, atherosclerotic plaques were found to contain bacterial DNA, and these bacterial taxa were also present in the gut of the same individuals (Koren et al., 2011; Ott et al., 2006) proposing a possible role of microbial communities in plaque instability and the subsequent adverse effects (Koren et al., 2011). In patients with HF, both metabolites and gut flora print were significantly
Determinantal compared to healthy subjects, and were even worse in patients with decompensated heart failure (Hayashi et al., 2018). Significantly, gut dysfunction involving disturbances in intestinal motility and villi absorption, in addition to an impaired tissue perfusion and edema was also observed in HF patients (Krack et al., 2005; Sandek et al., 2012). Undeniably, the HFD-induced impairment of gut integrity and gut hyperpermeability were linked to the aforementioned cardiovascular insults (Lewis and Taylor, 2020).

Gut microbial signature in HTN was heavily investigated in the last few decades (Kim et al., 2018; Mell et al., 2015). Interestingly, fecal microbial transplantation from hypertensive patients to germ-free mice induced HTN in these mice (Li et al., 2017). Moreover, these results were also observed in germ-free rats receiving GM from spontaneously hypertensive rats (Shi et al., 2021). However, the impact of GM appears to be complex as germ-free rats demonstrated a reversal of poor vascular contractility and reduced blood pressure control upon acquisition of normal GM (Joe et al., 2020). Indeed, a decrease in microbial richness and diversity in pre-hypertensive and hypertensive human subjects were recorded as well (Li et al., 2017). Additionally, spontaneously hypertensive and chronic angiotensin-II-induced hypertensive rat models presented dysbiosis manifested by an increase in F/B ratio compared to the normotensive controls (Santisteban et al., 2017a; Yang et al., 2015). Moreover, high-salt diet was shown to deplete a strain of *Lactobacillus*, however, treating these mice with this strain attenuated salt-sensitive hypertension (Wilck et al., 2017). Furthermore, HFD-induced dysbiosis was shown to have a role in the development of the obstructive sleep apnea-induced HTN (Durgan et al., 2016). HFD-induced dysbiosis was also correlated with increased serum LPS-binding protein, IL-6, endothelial dysfunction, arterial stiffness, aortic phosphorylated NF-κB and NADPH oxidase (NOX) in perivascular adipose tissue (PVAT) leading to a positive oxidative state. Interestingly, all these insults were attenuated with antibiotic treatment (Battson et al., 2018). Not only had been NOX-related ROS shown to be
detrimental in CVDs (Brandes et al., 2010), PVAT inflammatory and oxidative changes were consistently reported to contribute to vascular and cardiac autonomic dysfunction in HFD-fed rats even prior to the development of overt metabolic impairment (Al-Assi et al., 2018; Elkhatib et al., 2019; Rafeh et al., 2020) implicating the GM-metabolic-AT-CVD axis, which will be discussed later.

SCFAs may also contribute to the reduction of systemic blood pressure and serum cholesterol levels (Den Besten et al., 2013; Mariño et al., 2017). High-fiber diet, diet supplemented with SCFA, or parenteral injection of SCFA improved cardiometabolic health in several murine models by reducing blood pressure and cardiac fibrosis (Brial et al., 2018). It was proposed that the role of SCFA in BP regulation might be mediated by the activation of GPR41 in the vascular endothelium (Jonsson and Bäckhed, 2017). As well, SCFAs were found to hold a strong vasorelaxant properties (Poll et al., 2020). Interestingly, they were also discovered to be ligands for Olfactory receptor (Olfr78), a G protein-coupled receptors expressed in the vasculature, which plays an important role in vasoregulation and renin release, and is activated mainly by acetate and propionate. Olfr78 knockout mice had basal hypotension and low serum renin level, possibly indicative of the opposing response of GPR41 to SCFAs (Pluznick, 2014; Pluznick et al., 2013).

Importantly, SCFAs may also participate in regulating the sympathetic tone. Actually, in GPR41 knockout male mice, propionate was found to be a potent activator of sympathetic ganglia through Gβγ-PLCβ-MAPK pathway rather than cAMP synthesis inhibition (Kimura et al., 2011). Interestingly, propionate was able to trigger epinephrine secretion in sympathetic neurons. On the other hand, wild-type mice did not have GPR41 expression in the mesenteric fat pad and only presented GPR43 whose activation seemed to increase leptin production. Henceforth, it was suggested that GPR43 mediate sympathetic stimulation by adipocytes activation and leptin overexpression (Kimura et al., 2011).
recent study on Wistar-Kyoto and spontaneously hypertensive male rats revealed the strong association between dysbiosis and sympathetic activation through the induction of inflammation and oxidative stress in the brain. Not only blood pressure was corrected after fecal microbial transplantation from Wistar-Kyoto to spontaneously hypertensive rats, but also inflammation and oxidative stress in the paraventricular nucleus were improved. However, fecal microbial transplantation in the opposite direction deteriorated the inflammatory, oxidative and blood pressure state of the Wistar-Kyoto rats simultaneously with poor gut integrity and increased colonic TNF-α and circulating LPS, which were attenuated in the former transplant. Worth mentioning, spontaneously hypertensive rats had a lower butyrate receptor expression in the hypothalamus, alongside a higher Th17 cells and macrophage infiltration in the paraventricular nucleus (Toral et al., 2019). Moreover, LPS infusion was found to induce HTN in normotensive rats, by provoking neuroinflammation in the rostral ventrolateral medulla, which is considered as an important part in intensifying the sympathetic stream to the blood vessels (Wu et al., 2012). Interestingly, another study on the same model indicated a similar pattern of dysbiosis; increased F/B ratio, in addition to a notable reduction in acetate, increased gut sympathetic outflow, elevated blood pressure, impaired gut integrity, impaired endothelial-dependent relaxation to acetylcholine, and NOX overactivation. Treating spontaneously hypertensive rats with losartan, an angiotensin 2 receptor (AT2-R) antagonist, preserved gut integrity and enhanced functionality and immune response in the vasculature improving acetylcholine-induced relaxation and increased T\textsubscript{regs} infiltration (Robles-Vera et al., 2020; Santisteban et al., 2017a). Indeed, these lines of evidence support the involvement of dysbiosis in inducing diet related HTN and sympathetic overactivation, alongside with neuroinflammation and oxidation, that is proposed to be corrected by SCFAs. Figure 4 depicts the role of GM in the interaction between the dysbiosis, cardiometabolic dysfunction and neuroinflammation.
On another note, accumulating evidence highlighted the role of dysbiosis in augmenting the production of the bacterial metabolite trimethylamine-N-oxide (TMAO), which is suggested to be an indicator of CVDs (Moludi et al., 2020). TMAO is a plasma metabolite formed through a metaorganismal pathway and its level is depending on dietary intake mainly from animal protein sources like red meat, egg yolk and seafood, which are abundant in choline, phosphatidylcholine, and L-carnitine (Anders et al., 2013; Tang et al., 2015). These compounds are initially metabolized by GM to form Trimethylamine (TMA), and then converted by the host liver enzyme flavinmonooxygenase 3 (FMO3) to form TMAO (Bennett et al., 2013). Noteworthy, FMO3 Knockdown in female mice protected from diet-induced obesity and reduced hypertrophy and adiposity in WAT while improving total metabolic health (Schugar et al., 2017). Increased TMAO has been anticipated to induce insulin resistance and adipose inflammation in mice, as well as increasing the risk for type II diabetes in human subjects (Tang et al., 2017). Circulating TMAO was linked to elevated vascular inflammation through the incitement of pro-inflammatory cytokines expression and leukocytes recruitment (Seldin et al., 2016). Also, dietary supplementation of choline in mice increased TMAO levels, macrophage foam cell formation, and atherosclerosis incidence (Wang et al., 2011). Moreover, it prompted platelet hyperactivity (Marcucci et al., 2014; Trip et al., 1990) and enhanced thrombosis (Zhu et al., 2016), which are considered major risk factors for developing CVDs. Indeed, serum TMAO level was tightly correlated with atherosclerosis (Tang and Hazen, 2017). In the same context, a human study revealed that subjects with higher serum TMAO levels had a two-fold risk increase for developing major cardiovascular events compared to subjects with low TMAO (Tang et al., 2013). TMAO levels had also been suggested to be an accurate indicator for heart failure diagnosis (Tang et al., 2014). It was found to fuel endothelial dysfunction as well by upregulating vascular adhesion molecule-1, monocyte attraction and NF-κB activation (Ma et al., 2017).
As stated before, HFD induces AT dysfunction, which predispose to metabolic and cardiovascular disorders. The AT surrounding the vascular bed, referred to as perivascular AT (PVAT), which has been identified as a crucial component of the vascular regulatory machinery. It is worth mentioning that PVAT has been suggested to be one of the most sensitive AT depots to positive energy intake and the first to undergo negative remodeling including hypertrophy, inflammation and hypoxia which were observed in early metabolic impairment (AlZaim et al., 2020; Elkhatib et al., 2019). Moreover, resistance arterioles from obese mice showed a PVAT-dependent impairment in insulin/Akt-mediated vasodilatation due to reduced adiponectin and AMPK downstream effects, which was restored with Jun NH2-terminal kinase (JNK) inhibition (Meijer et al., 2013). HFD induced PVAT dysfunction might be mediated via sympathetic overactivation and insulin resistance that have been linked to a wide range of subclinical cardiovascular insults such as endothelial dysfunction and cardiac autonomic neuropathy (Akoumianakis et al., 2017; Alaaeddine et al., 2019; AlZaim et al., 2020; Bakkar et al., 2020; Britton and Fox, 2011; Bulloch and Daly, 2014; Greenstein et al., 2009; Rafeh et al., 2020). As such, it becomes plausible that PVAT inflammation might mediate the effect of HFD-induced dysbiosis on early metabolic impairment and cardiovascular dysfunction. This hypothesis is presented in Figure 5. However, limited studies have explored the association between PVAT and HFD induced dysbiosis. One recent study examined the role of PVAT FMO3 in response to direct TMA stimulation in tone regulation in excised aortas from male rats. TMA exerted a contractile effect through activating L-type voltage-gated calcium channels that was found to be dependent on endothelium rather than PVAT, suggesting that TMAO and TMA modulate vascular tone by a direct effect of VSMCs (Restini et al., 2020). However, to our knowledge no studies were conducted to investigate the impact of dysbiosis or SCFAs on PVAT modulation and thus the HFD-dysbiosis-PVAT axis remains elusive.
4.2. Sex-dependent impact of dysbiosis on cardiovascular dysfunction:

Sex-dependent cardiovascular risk and pathology are well documented in the literature, as men have a higher absolute risk compared to premenopausal women, a difference that diminishes after menopause indicating the important role of sex hormones in CVDs (Chella Krishnan et al., 2018; Kim and Reaven, 2013; Pei et al., 2017; WHO, 2017). While metabolic disorders and cardiovascular diseases have long been intertwined, early metabolic impairment has been the focus of interest as it imparts predisposition to inevitable CVDs. This effect is mainly mediated by subclinical events such as metabolic endotoxemia, AT inflammation, and insulin resistance (Heilbronn and Campbell, 2008; Kallio et al., 2015; Nishimura et al., 2009; Shah et al., 2008; Wensveen et al., 2015). Interestingly, GM has been identified as a major driver of these anomalies. Since sex-dependent differences exist in dysbiosis, the differential GM effect is expected to be extrapolated to CVDs. As discussed previously, estrogens attenuate HFD-induced gut hyperpermeability and LPS transport either through leaky membranes or chylomicrons together with the consequent metabolic endotoxemia, Th17 cell activation and T_{reg} inhibition (Cani et al., 2007), which were found to be higher in men. As stated previously, LPS activates TLR4 on target tissues including AT and macrophages triggering proinflammatory cascade and activating NF-κB. AT overactivation and hypertrophied expansion due to dysbiosis was linked to increased leptin production, which in turn will activate sympathetic outflow. In parallel, LPS induced neuroinflammation triggers sympathetic firing. The resultant sympathetic overactivation and insulin resistance, which are expected to be higher in males, will lead to early inflammation and negative remodeling of PVAT precipitating a wide range of subclinical cardiovascular insults (Akoumianakis et al., 2017; Alaaeddine et al., 2019; Bakkar et al., 2020; Britton and Fox, 2011; Bulloch and Daly, 2014; Greenstein et al., 2009; Khatib et al., 2018). Since
estrogen holds a protective effect against hypoxia in AT, possibly estrogen will block
dysbiosis-mediated dysfunction cardiovascular dysfunction by interfering with PVAT
inflammation. Additionally, progesterone was found to have an anti-inflammatory effect
against LPS mediated neuroinflammation (Lei et al., 2014).

The evidence regarding sexual dimorphism in HFD-induced PVAT remodeling is scarce,
however, females tend to have a more functional PVAT compared to males. In this regard,
ovariectomy in murine models instigated endothelial and PVAT dysfunction mediated by
increased ROS when compared to their sham littermate (Taylor and Sullivan, 2016; Wang et
al., 2014). Additionally, an enhanced anticontractile role of PVAT was observed in female
pigs and was attributed to a higher sensitivity of adiponectin receptor in coronary artery
(Ahmad et al., 2017). Taken together these observations suggest a possible role of PVAT in
mediating a sex-dependent cardiovascular impact of dysbiosis in early metabolic dysfunction.

On another note, the protective effect of estrogen on HTN development is mainly through
inducing eNOS mediated vasorelaxation (Bucci et al., 2002; Sobrino et al., 2017), while at
the same time inhibiting vasoconstricting agents such as angiotensin-II (Schunkert et al.,
1997). However, mounting evidence suggests the role of immune responses in mediating sex-
dependent GM-HTN axis. For instance, dysbiosis was linked to an increased activity of Th17
cells, which had a role in initiating arterial hypertension (Guzik et al., 2007; Ivanov et al.,
2009; Wenzel et al., 2016). In this regard, hypertensive male rats had higher Th17 activity
compared to females (Gillis and Sullivan, 2016).

5. Therapeutic interventions for cardiometabolic consequences of dysbiosis:

Bidirectional interactions between GM and cardiovascular drugs have been reported for
quite some time. Indeed, not only has the gut bacterial community been implicated in altering
the pharmacokinetics of some cardiovascular drugs, but treatment with certain drug classes
has also been associated with favorable changes in GM populations. For instance, certain phyla of gut bacteria were shown to metabolize digoxin and amlodipine reducing their availability at target tissues, while others were proposed to decrease the absorption of simvastatin and captopril (Tuteja and Ferguson, 2019). In parallel, one human study showed that the LDL-C lowering effect of a 4-8-week rosuvastatin treatment was associated with a change in the abundance of Firmicutes (Liu et al., 2018). As for animal studies, atorvastatin therapy appeared to reverse HFD-induced dysbiosis in male rats (Khan et al., 2018). Similarly, captopril treatment reduced dysbiosis and improved gut permeability associated with hypertension in spontaneously hypertensive rats (Santisteban et al., 2017a; Santisteban et al., 2017b). However, none of these interventions has been examined systematically and the underlying mechanisms remain unclear. From a different perspective, tailored pharmacological interventions targeting GM with the purpose of imparting a protective cardiovascular research have been proposed. Indeed, as bacterial metabolic reactions have been thoroughly recognized, selective approaches could be designed to modify harmful metabolite production. As such, small molecule inhibitors of TMAO synthesis were designed and proposed to treat atherosclerosis (Wang et al., 2015). Moreover, nanoparticle-based approaches were proposed either to deliver useful bacterial species associated with increased SCFA production or reduced LPS, or to scavenge TMAO and pro-inflammatory cytokines (Kazemian et al., 2020). Nevertheless, all these interventions remain in early stages and the current viable options for prevention of detrimental outcomes of gut bacterial alteration remain related to direct manipulation of bacterial population using probiotics, antibiotics, fecal microbial transplantation or using bacterial metabolites such as SCFAs as described below. The impact of these interventions on dysbiosis triggered pathways are demonstrated throughout Figures 1-5.
5.1. Probiotics:

Probiotics are nonpathogenic strains of bacteria, usually belonging to *Lactobacilli*, and *Bifidobacteria*, which have been used to reset microbiome dysbiosis (Holzapfel and Schillinger, 2002; Isolauri et al., 2004; Williams, 2010). Some clinical trials attempted to explore probiotics as a potential intervention with the symptoms in some neurological and psychological diseases such as amyotrophic lateral sclerosis and schizophrenia (Mazzini et al., 2018; Severance et al., 2017), while others focused on using different strains of probiotic bacteria as a potential therapy and early preventive technique for cardiovascular and metabolic disorders. However, these trials have had controversial findings. On one hand, several studies showed that probiotic administration improved cardiometabolic and inflammatory parameters not only in metabolically impaired but also in borderline individuals. For instance a double blind placebo-controlled trial, showed that daily ingestion of *Lactobacillus plantarum* in hypercholesteremic individuals for 12 weeks significantly improved blood pressure, reduced serum total cholesterol, LDL, and triglycerides, while increasing HDL level (Costabile et al., 2017). The same strain was used in smoking subjects for 6 weeks and had similar findings reducing CV risk factors (Naruszewicz et al., 2002). In post-menopausal women with metabolic syndrome, supplementation with the same strain for 90 days decreased blood glucose and homocysteine (Barreto et al., 2014). On the same note, *Lactobacillus plantarum* 299v supplementation for 6 weeks in men with stable coronary artery disease significantly improved endothelium dependent vasodilatation, induced some changes in GM by enriching *Lactobacillus* genus, decreased plasma propionate, leptin and IL-8 and 12, without changing blood glucose, lipid profile and body weight (Malik et al., 2018). Another strain of bacteria, *Bifidobacterium longum* BB536, exhibited beneficial effects after 12 weeks of blinded controlled intervention of food supplement intake containing; red yeast extract, niacin, and coenzyme Q10, on individuals with low score of CV conditions.
risk. The results showed in improved levels of atherogenic lipid profile (Ruscica et al., 2019). Men with mild hypercholesteremia were treated with isoflavone-supplemented soy product fermented with Enterococcus faecium CRL 183 and Lactobacillus helveticus 416 for 42 days, and showed an improved serum lipid profile, but neither CRP nor fibrinogen (Cardoso Umbelino Cavallini et al., 2016). Another study examined the role of Bifidobacterium lactis in metabolic syndrome patients. The organism was supplemented in fermented milk and given for 45 days. Treated subjects showed a reduction in BMI, serum TNF-α and IL-6, while improving lipid profile (Bernini et al., 2016). A two-month treatment with yogurt supplemented with a probiotic mix (Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12) in men and women with metabolic syndrome improved fasting blood glucose and insulin sensitivity. Importantly, it improved some vascular and endothelial function markers, like vascular cell adhesion molecule 1 (VCAM1) and plasminogen activator inhibitor 1 (Rezazadeh et al., 2019). Healthy subjects with BMI at the upper limit of the healthy range randomized for a 12-week treatment with Bifidobacterium lactis with arginine supplementation appeared to have a better endothelial function and hence a reduced risk of developing atherosclerosis (Matsumoto et al., 2019). This suggests a possible therapeutic and preventive effect of probiotics on endothelial function and CV risk. However, conclusions should be drawn carefully from these results given the possible contribution of arginine supplementation to endothelial improvement and the intervention being on low-risk subjects. Interestingly and worth investigating is the interplay between sex hormones and probiotics, as these positive outcomes seem to be independent of the status of sex hormones. In obese postmenopausal women, administration of multispecies probiotic for 12 weeks seemed to improve metabolic parameters; serum insulin, glucose, LPS, total lipid profile, uric acid and HOMA-IR in both high and low dose groups, however, reduced adiposity was only observed in the high dose arm, suggesting an improved gut permeability and reduced cardiometabolic
risk factors (Szulińska et al., 2018). Similarly, premenopausal women diagnosed with polycystic ovarian syndrome (PCOS) were treated with pomegranate juice with and without probiotics mix (Lactobacillus rhamnosus GG, bacillus koagolans and indicous) for 8 weeks. The group receiving probiotics showed an improved metabolic and inflammatory function alongside reduction in blood pressure (Esmaeilinezhad et al., 2020). Women aged 20-50 years with arterial HTN treated with a probiotic cocktail (Lactobacillus para casei LPC-37, Lactobacillus rhamnosus HN001, Lactobacillus acidophilus NCFM, and Bifidobacterium lactis HN019) for eight weeks showed an improved fasting blood glucose, cholesterol and elevated HDL level compared to baseline. Interestingly, probiotics improved autonomic function and heart rate variability by reducing the low frequency (LF) domain, without significantly changing blood pressure, yet systolic BP was reduced by 5 mmHg compared to the placebo (da Silva et al., 2020).

On the other hand, several clinical trials failed to record therapeutic benefits of probiotics on metabolic and cardiovascular outcomes. For instance, the commercial probiotic VSL#3®, which contains 8 different strains of lactic acid bacteria, was used in a twice daily intervention for 10 weeks in men and women with non-alcoholic fatty liver disease. It did not appear to improve cardiovascular risk factors and liver injury scores. Nevertheless, it improved HOMA-IR (Chong et al., 2021). Another randomized controlled cross-over study showed that metabolic syndrome symptoms were not alleviated by a daily intervention with Lactobacillus reuteri V3401 strain for 12 weeks. Yet, this intervention was able to reduce some inflammatory markers, IL-6 and VCAM1 (Tenorio-Jiménez et al., 2019). Although probiotics exhibited some beneficial metabolic outcomes in human trials, results seemed to be dependent on the bacterial species used. As such, some probiotics did not seem to change dysbiosis-related parameters such as gut permeability compared to the control groups (Grąt et
al., 2017; Ivey et al., 2015; Leber et al., 2012; Stadlbauer et al., 2015). For instance, using *Lactobacillus casei* Shirota for 12 weeks in subjects with metabolic syndrome did not correct dysbiosis nor improve gut integrity (Stadlbauer et al., 2015). Thus, conclusions drawn from probiotics intervention must be specified to the strains and concentrations used. Another important note is that not only different strains have been used in probiotics studies, but even some trials used different approaches in implementing the interventions, such as using probiotics with other dietary components (Cardoso Umbelino Cavallini et al., 2016; Rezazadeh et al., 2019; Ruscica et al., 2019; Scorletti et al., 2018), or other dietary interventions and lifestyle modifications such as calorie restriction and physical activity (Behrouz et al., 2017). Therefore, the role of probiotics in combating metabolic and cardiovascular insults must be carefully investigated, and studies should be accurately designed to limit other confounding factors such as dietary and lifestyle modifications. However, controlled use of probiotics can be safe and useful in preventing CVDs and metabolic derangements in low-risk individuals alone or in combination with other compounds such as prebiotics (Behrouz et al., 2017; Trotter et al., 2020).

### 5.2. Antibiotics:

Antibiotics were proposed to be one of the interventions to achieve eubiosis (Ianiro et al., 2016). However, few studies explored the efficacy of antibiotics in ameliorating dysbiosis related cardiometabolic dysfunction. For example, one study used antibiotics to reset GM community in patients with T2DM and obesity. Yet, major metabolic parameters such as insulin sensitivity, systemic inflammation, gut permeability, adipocyte size did not change positively in response to 7 days treatment of amoxicillin, vancomycin, or placebo (Reijnders et al., 2016). One case study on a post-menopausal woman suffering from chronic resistant HTN for 3 years, which was uncontrolled on more than 3 antihypertensive drugs, in addition
to a history of metabolic and immune pathologies including diabetes and arthritis, reported a temporary (six-month) improvement of her HTN control upon treatment with a post-operative antibiotic mix (IV vancomycin, rifampin and ciprofloxacin orally) (Qi et al., 2015). Given the adverse effects associated with antibiotics use and the risk of development of antibacterial resistance, this might be the least desirable intervention to correct dysbiosis and related pathologies.

5.3. Fecal microbial transplantation (FMT):

FMT is a novel method that has been recently suggested to induce eubiosis and alleviate pathologies mediated by disturbed gut microbiome. It is the process of isolating GM from healthy donors to transplant it into diseased subjects. FMT can be done through various methods that are relatively safe and non-invasive; rectally like enema, naso-gastric route or orally by capsules (Lagier, 2014; Wang et al., 2016). Transplanted microbiota can be homologous from the same person and heterologous/allogenic from first degree relatives or other healthy subjects. Interestingly, allogenic GM transplantation was found to be more effective than homologous interventions (Grehan et al., 2010; Schepici et al., 2019; Wang et al., 2016). Adverse effects reported after FMT are not serious. The side effects reported were mainly abdominal discomfort and diarrhea for a few hours after the procedure (Lagier, 2014). However, there remains concerns about the potential safety/side effects related to non-bacterial component of the fecal material transferred (Bojanova and Bordenstein, 2016). A preliminary report observed that sterile protein isolates from donor fecal material were able to induce the required response in recipients (Ott et al., 2017), however, future investigation will be required to determine the possibility of fractionation and reducing the content being transplanted to the necessary organisms only.
FMT has been considered as one of the important lines of life-saving treatments for patients with *Clostridium difficile* infections as these patients had improved outcomes and less chances for reoccurrence than those receiving conventional treatments (Kelly et al., 2016; Lee et al., 2016; Van Nood et al., 2013). It was even suggested that FMT can be promising in eradicating multidrug resistant microorganisms (Saha et al., 2019). Since GM has been identified as an important variable in the pathogenicity and prognosis of a large set of metabolic and cardiovascular diseases, FMT might be effective in correcting and alleviating dysbiosis related dysfunctions, especially the ones starting early on and having no clear treatment regimens.

One clinical trial on male subjects with metabolic derangements including hyperinsulinemia, BMI above 30, elevated waist circumference, and increased adiposity treated with purified GM from lean and healthy donors (allogenic transfer) matched in sex and age through a duodenal tube over a 6 weeks period showed improved insulin sensitivity and microbial diversity favoring butyrate producing bacteria such as *Roseburia intestinalis* compared to the control group receiving an autologous transfer (Vrieze et al., 2012). However, another double blinded randomized clinical trial using FMT delivered through capsules once per week from healthy donors to subjects with obesity and insulin resistance, did not record any difference in either parameter after 12 weeks of intervention (Reijnders et al., 2016). Similar results were recorded from a randomized trial of obese adolescents, after ingestion of 28 capsules of lean donors GM. Up to 26 weeks post intervention recipients of either sex did not show any evidence of improvement neither in metabolic parameters such as insulin sensitivity nor in obesity, though central to peripheral fat ratio was reduced in the FMT arm only (Leong et al., 2020). Furthermore, FMT in patients with non-alcoholic fatty liver disease who suffered from insulin resistance delivered directly to the colon from autologous and allogenic sources did not improve insulin resistance, however allogenic FMT
improved gut permeability (Craven et al., 2020). Also, FMT in patients with metabolic syndrome from a healthy vegan donor did not alleviate TMAO levels and vascular inflammation (Smits et al., 2018). Although FMT has been revolutionary in treating diseases like *Clostridium difficile* infections, ulcerative colitis, and others, its role in cardiometabolic dysfunction is not fully understood, requires further investigation, and its long term efficacy has yet to be established (Zhang et al., 2019b).

### 5.4. Short Chain Fatty Acids:

In human studies, SCFAs were measured as a secondary outcome of dietary intervention rather than being the treatment *per se*, and most evidence is drawn from animal studies. One recent study using rectal capsule delivery of SCFAs in a triple blinded randomized trial examined the effect of one week of SCFAs administration on psychosocial stress of 66 healthy men. The two intervention arms; low and high SCFAs were equally successful in reducing cortisol levels in response to psychosocial stress, both had an increased serum SCFAs as well compared to the placebo arm (Dalile et al., 2020). The OmniHeart study, which included 164 adults, assessed the role of macronutrients on serum SCFAs levels. Three isocaloric and high fiber diets rich in either carbohydrate, protein, or unsaturated fat were applied for 6 weeks. The results indicated differences in SCFAs serum levels in response to different diets, which were correlated with some cardiometabolic aspects. For instance, butyrate level was only increased by high protein diet and was associated with decreased HDL levels and ghrelin and increased insulin and glucose levels (Mueller et al., 2020). A lot of questions and concerns arise regarding the use of SCFAs for therapeutic purposes in humans, especially in cardio and metabolic pathologies. Specifically, therapeutic dose selection, safety and efficacy of single or combined use, and most importantly the long-term effects of their use. Henceforth, more clinical studies should be done using SCFAs as a therapy for early metabolic derangements in a sex-dependent fashion.
6. Conclusion:

Dysbiosis is a common occurrence in patients suffering from cardiometabolic conditions. Not only do GM alterations in these patients appear to be driven by the same risk factors of the other pathologies, but they also seem to contribute to and drive the molecular changes leading to cardiovascular involvement including AT inflammation, particularly in PVAT. Sexual dimorphism is evident in several steps starting at the differential effect of sex hormones on GM diversity and stability, encompassing sex-dependent effects on GM metabolite production, gut permeability, vulnerability of AT to inflammatory changes, and culminating in a different susceptibility to CVD incidence. Future investigation utilizing systematic approaches is required for better understanding of the pathways involved to allow for tailored therapy for effective management of early cardiometabolic dysfunction in either sex.

7. Authorship contributions:
Contributed to writing the manuscript: Dwaib, AlZaim, Ajouz, Eid, El-Yazbi
8. References:


9. **Footnotes:**

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10. Figure legends:

**Figure 1: High fat diet-mediated gut dysbiosis and links to cardiovascular disease and sex-dependent factors.** Significant increase in the F/B ratio occurs after the consumption of a high fat diet. The ensuing gut microbiota dysbiosis detrimentally affects the adipose tissue and the cardiovascular system through intricate pathways. Mechanistically, gut microbiota dysbiosis enhances lipopolysaccharide production, as well as its chylomicron-mediated transport and paracellular diffusion. The latter being possible due to gut microbiota dysbiosis-mediated dysfunction of tight junctions leading to a compromised gut integrity. Locally, LPS activates, through TLR4, the pro-inflammatory NF-κB pathway. Systemically, the increased levels of serum LPS results in endotoxemia. Importantly, sex hormones are partly responsible
for the differential modulation of these pathways in either sex as indicated. Pathways involved in gut microbiota dysbiosis are presented in red, while those counteracting them in black. AT, Adipose tissue; COX2, Cyclooxygenase 2; ER-β, Estrogen receptor β; F/B, Firmicutes/Bacteroidetes; GM, Gut microbiota; HFD, High-fat diet; IAP, Intestinal alkaline phosphatase; IL, Interleukin; iNOS, Inducible nitric oxide synthase; IR, Insulin resistance; LPS, Lipopolysaccharide; MetS, Metabolic Syndrome; MUC2, Mucin2; MyD88, Myeloid differentiation factor 88; NF-kB, Nuclear factor kappa B; PUFA, Polyunsaturated fatty acid; TLR4, Toll-like receptor 4; TNF-α, Tumor necrosis factor α.

**Figure 2: Detrimental effects of dysbiosis on adipose tissue homeostasis.** Gut microbiota dysbiosis amplifies high fat diet-mediated adipose tissue dysfunction through increasing energy absorption, weight gain, and adiposity. The subsequent development of insulin resistance and adipose tissue inflammation leads to the development of the metabolic syndrome linked to the emergence of cardiovascular diseases. Testosterone and estrogen inhibit TLR4 signaling. Estrogen decreases the level of circulating LPS, enhances leptin sensitivity, decreases insulin resistance, and limits consequences of hypoxia by induction of the proteasomal degradation of HIF-1α. Several therapeutic interventions such as antibiotic treatment and fecal microbiota transfer also positively modulate the depicted pathways. Pathways implicated in gut microbiota dysbiosis are presented in red, while those counteracting them are presented in black. AT, Adipose tissue; ATB, Antibiotic; CVD, Cardiovascular disease; FMT, Fecal microbiota transfer; GM, Gut microbiota; HFD, High fat diet; HIF-1α, Hypoxia-inducible factor 1α; IL, Interleukin; MyD88, Myeloid differentiation factor 88; NF-kB, Nuclear factor kappa B; TLR4, Toll-like receptor 4; TNF-α, Tumor necrosis factor α.
Figure 3: Short chain fatty acids regulate lipogenic, inflammatory and neuronal pathways that are dysregulated in states of metabolic dysfunction. The three major short chain fatty acids are acetate, propionate, and butyrate. They improve cardiometabolic health through several pathways as indicated, thus counteracting HFD-induced metabolic dysfunction. Akt, Protein kinase B; BP, Blood pressure; FIAF, Fasting-induced adipose factor; FMT, Fecal microbiota transplant; GPR, G-protein couples receptor; HFD, High fat diet; IL, Interleukin; LPL, Lipoprotein lipase; LPS, Lipopolysaccharide; MAPK, Mitogen-activated protein kinase; NF-kB, Nuclear factor kappa B; PKC, Protein kinase C; PLC; Phospholipase C; PTEN; Phosphatase and tensin homolog; SCFA, Short chain fatty acids; SREBP-1, Sterol regulatory element-binding protein 1; TNF-α, Tumor necrosis factor α; UCP-1, Uncoupling protein 1.

Figure 4: Gut microbiota dysbiosis in the metabolic dysfunction-neuroinflammation-cardiovascular disease continuum. Gut microbiota increases the F/B ratio resulting in oxidative stress in the brain leading to neuroinflammation and sympathetic overactivation. The latter consequently increases blood pressure and predispose to the development of hypertension. Gut microbiota dysbiosis also leads to endotoxemia and increases Th17 and macrophage brain infiltration, both leading to arterial hypertension. Gut microbiota dysbiosis also detrimentally accelerates adipose tissue dysfunction leading to an increased production of leptin which further augments the activation of the sympathetic system. Additionally, endotoxemia inhibits the rather beneficial accumulation of Treg cells in the brain which further augments inflammation. Estrogen inhibits the development of hypertension through an eNOS-mediated vasodilatory effect and through decreasing serum levels of AngII. Progesterone, as well as fecal microbiota transfer inhibits neuroinflammation and its downstream consequences. The antagonism of AT2-R also reverses immune cell profile
alterations mediated by gut microbiota dysbiosis. Pathways involved in gut microbiota dysbiosis are presented in red, while those counteracting them in black. AngII, Angiotensin II; AT, Adipose tissue; AT2-R, Angiotensin AT2 receptor; BP, Blood pressure; eNOS, Endothelial nitric oxide synthase; F/B, Firmicutes/Bacteroidetes; FMT, Fecal microbiota transfer; GM, Gut microbiota; LPS, Lipopolysaccharide; Th17, T helper cell 17; Treg, Regulatory T cell.

**Figure 5: Perivascular adipose tissue dysfunction: Novel mechanisms of gut microbiota dysbiosis-mediated cardiovascular derangements.** A healthy perivascular adipose tissue secretes adiponectin, which elicits an AMPK-mediated anti-contractile effect. The consumption of a high fat diet causes sympathetic overactivation and insulin resistance leading to perivascular adipose tissue expansion, adipocyte hypertrophy, and adipose tissue inflammation, thus jeopardizing the anti-contractile activity of perivascular adipose tissue. Importantly, the consumption of a high fat diet causes gut microbiota dysbiosis which enhances NOX-mediated production of ROS, a pathway that is augmented following ovariectomy. Additionally, gut microbiota dysbiosis increases aortic NF-kB signaling leading to arterial stiffness. Microbial metabolism products including TMA and TMAO participate in gut microbiota dysbiosis-caused perivascular adipose tissue dysfunction. TMA, through its activity on VSMCs induces L-type voltage-gated calcium channels which counteracts perivascular adipose tissue-mediated anti-contractile effect. AMPK, 5’ AMP-activated protein kinase; GM, Gut microbiota; HFD, High fat diet; NOX, Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase; PVAT, Perivascular adipose tissue; ROS, Reactive oxygen species; TMA, Trimethylamine; TMAO, Trimethylamine N-oxide; VCAM-1, Vascular cell adhesion protein 1; VSMCs, Vascular smooth muscle cells.