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Polycystic ovary syndrome and cardiovascular risk. Could trimethylamine N-oxide (TMAO) be a major player? A potential upgrade forward in the DOGMA theory

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ABSTRACT

Several studies reported an increase in cardiovascular risk (CVR) in women with polycystic ovary syndrome (PCOS), considered primarily as the result of the combination of all the clinical features that characterize the syndrome, including hyperandrogenism, insulin resistance, diabetes, obesity chronic low-grade inflammation. Interestingly, in 2012 it has been proposed the so-called DOGMA theory, suggesting the pivotal role played by microbiota alteration in the development of PCOS. Subsequently, several authors evidenced the existence in PCOS women of a marked dysbiosis, which is related to the development of metabolic diseases and cardiovascular complications, mainly due to the production of bacteria-derived metabolites that interfere with various pathways. Among these, trimethylamine-N-oxide (TMAO) is emerging as one of the most important and studied microbiota-derived metabolites related to the increase in CVR, due to its pro-atherosclerotic effect. The purpose of the present review is to summarize the evidence in order to support the hypothesis that, in women with PCOS, dysbiosis might be further involved in enhancement of the CVR via contributing to the increase of circulating TMAO. Although no observational studies on a large number of patients directly investigated the serum levels of TMAO in PCOS women, this manuscript aimed to drive future studies in this field, concurring in providing a novel approach for both comprehension and treatment of the CVR in PCOS.

1. Introduction

A very large body of evidence, during the decades, established that several factors contributed to increasing cardiovascular risk (CVR), including smoking, obesity, hypercholesterolemia, and hyperglycaemia, and numerous studies have been performed in order to clarify the exact role of these risk factors and the main molecular mechanisms underlying this increased risk. Not recently, however, several findings have added a new piece to the complex puzzle of the CVR, suggesting the existence of a novel prognostic biomarker for CVR, trimethylamine N-oxide (TMAO). TMAO is produced by gut microbiota metabolism of dietary L-carnitine, choline, betaine, and phosphatidylcholine, mainly contained in several foods, including eggs, red meat, and fish. Preclinical studies revealed a mechanistic association between TMAO and cardiovascular diseases (CVD), in particular exerting a pro-atherosclerotic effect; furthermore, a meta-analysis demonstrated that TMAO serum levels are positively and dose-dependently associated with cardiovascular events and mortality [1]. Interestingly, TMAO seems to be related to the CVR independently

Abbreviations: CVR, cardiovascular risk; TMAO, trimethylamine N-oxide; CVD, cardiovascular disease; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; IR, insulin-resistance; T2DM, type 2 diabetes mellitus; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CRP, c-reactive protein; EF, endothelial function; IMT, intima-media thickness; TMA, trimethylamine; FMO3, flavin mono-oxygenase 3; LPS, lipopolysaccharides; DOGMA, dysbiosis of gut microbiota.

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of other risk factors, especially in patients with acute coronary syndrome [2].

In the last ten years, studies investigating TMAO significantly increased, as shown in Fig. 1, reflecting the growing interest of scientific research in elucidating the main mechanisms involving TMAO in CVR.

In a very large number of studies, high serum or urinary levels of TMAO have been associated with a plethora of CVD, acting by specific mechanisms, which will be extensively discussed above. Interestingly, evidence also reported high TMAO levels in patients with high-CVR pathologies, such as obesity, metabolic syndrome (MetS) [3] and vitamin D deficiency [4], with the existence of an interesting gender-specific relationship [5]; thus, it is plausible to speculate that TMAO might be also involved in metabolic-endocrine disorders in which coexists a high CVR, representing, once again, a novel interpretation.

The purpose of this review is to summarize the available literature in order to provide evidence about a possible implication of TMAO in polycystic ovary syndrome (PCOS), the most common metabolic-endocrine disorder in fertile age women, which is characterized by a high CVR. A profound literature research has been conducted in the most accredited scientific databases, using several combinations of keywords. No observational studies on a large number of patients directly evaluated the TMAO serum or urinary levels in women with PCOS. However, evidence showed a largely altered microbiota in PCOS women, suggesting the possibility of finding also high levels of TMAO. This paper, thus, aimed to propose a novel field of research in order to provide further explanations for the CVR in women with PCOS, specifically in those also defined as "metabolically healthy".

2. Methods

This review summarizes available evidence on the relationship between PCOS and TMAO, focusing on the role played by hormonal aberrations in dysbiosis development, resulting in increased CVR. The most relevant databases were consulted (including PubMed, Science Direct, Medline, Web of Science, Cochrane) using different keywords alone and/or in combination, such as "Polycystic ovary syndrome", "PCOS", "Trimethylamine-N-oxide", "TMAO", "Cardiovascular diseases", "Cardiovascular risk", "Gut microbiota", "Dysbiosis", "Hyperandrogenism", "Insulin-resistance", "Dysbiosis of Gut Microbiota theory", "DOGMA theory". In vitro studies, animal-based studies and clinical trials were evaluated.

3. PCOS, a metabolic-endocrine disorder with cardiovascular implications

PCOS is an endocrine-metabolic disorder, resulting from the existence of several factors, including genetic and environmental factors [6]. The diagnosis of PCOS can be performed when at least two of the three Rotterdam criteria are present: (i) clinical hyperandrogenism (ii) presence of ultrasound evaluable ovarian cysts and (iii) oligo-amenorrhea with oligo-anovulation [7]. In addition to a mere impairment in gonadic axis-related hormonal status, further pathological conditions coexist, including obesity (mainly central obesity), insulin-resistance (IR) [8] and chronic low-grade inflammation [9]. Besides the well-established vicious circle between obesity, chronic low-grade inflammation, IR and hyperandrogenism, which is responsible for a dramatic worsening of the pathological status [10,11], these factors have been also recognized as main actors in the CVR in PCOS women. Moreover, the high risk to develop MetS and type 2 diabetes mellitus (T2DM) [12–14] strongly increases the risk of cardiovascular events [15,16].

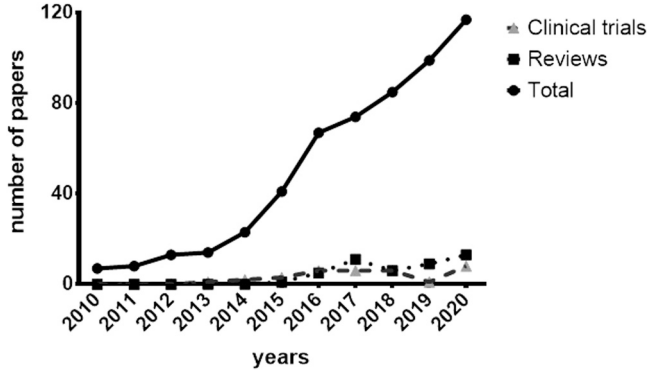

Fig. 1. The number of papers on TMAO per year. Papers have been selected typing the words "trimethylamine N-oxide" in titles.

The etiopathogenesis of PCOS is very complex and sees the involvement of several genetic and environmental factors. Various causes for PCOS development have been identified, including (i) heritable traits/genetic factors (i.e. maternal PCOS, polycystic ovarian morphology, hyperandrogenism, MetS and T2DM, gene variants), (ii) intrauterine environment (i.e. congenital virilization and altered fetal nutrition) and (iii) postnatal environment (i.e. IR, obesity, hyperandrogenism) [17,18]. However, it is well accepted that the most important etiopathogenic factor is an ovarian function dysregulation causing increased steroidogenesis [17,18].

It has been largely documented that metabolic aberrations, including impaired glucose and lipid homeostasis, along with chronic low-grade inflammatory status and hyperandrogenemia, are responsible for women with PCOS to be more prone to develop CVD [15,19], including atherosclerosis, vascular dysfunction, thrombosis and mortality for cardiovascular causes [15,16]. So-called "atherogenic lipoprotein phenotype", characterized by high levels of triglycerides and low-density lipoprotein cholesterol (LDL-c), and oxidized LDL-c and low levels of high-density lipoprotein cholesterol (HLD-c), is a common dyslipidemic pattern in women with PCOS and may be strongly related to the high CVR [20]. IR with compensatory hyperinsulinemia, in turn, leads to excessive production of pro-inflammatory mediators, including C-reactive protein (CRP), plasminogen activator inhibitor-1, white blood cells, endothelin-1 and fibrinogen, resulting in increased atherosclerotic risk, reduced vascular reactivity and impaired endothelial function (EF) [20,21]. Interestingly, there are evidence reporting greater carotid intima-media thickness (IMT) in young PCOS women than in control, also after adjustment for cardiovascular factors [20,21], suggesting that IR might be responsible for increased arterial stiffness and impaired EF, resulting in structural atherosclerosis when combined with features of MetS [21]. Moreover, increased atherosclerosis risk in women with PCOS seems to be also due to a higher coronary artery calcification, whose prevalence has been reported to be higher in this population, compared to controls [20,21]. Another important aspect to consider is that IR-dependent hyperhomocysteinemia commonly occurs in women with PCOS, which further increases CVR, with its well-known role in increasing the risk of atherosclerosis at the cerebral, coronary and peripheral levels [21].

A relevant feature of PCOS is the existence of a chronic inflammation, recognized as an important contributor in PCOS pathogenesis. In PCOS, indeed, the levels of various inflammatory biomarkers are found increased. Among these, tumor necrosis factor-α (TNF-α) is considered one of the most important, due to its central role in PCOS-processes, mainly influencing the androgen signaling. In particular, TNF-α is responsible for increased androgen levels via promoting their secretion and up-regulating the expression of CYP17A1 and down-regulating the expression of CYP19A1, two key enzymes involved in synthesis of androgens and androgen-to-estrogen conversion, respectively. This effect of TNF-α results in hyperandrogenism that, in turn, plays a role in causing chronic inflammation in PCOS [22–26].

Overall, this evidence reports the now well-known high CVR in PCOS, which seems to be independent of body weight, affecting not only overweight/obese but also normal-weight women with diagnosed PCOS [19]. However, many authors agree that it is not possible to accurately

predict the CVR in PCOS women [15,19] and that long-term, prospective studies are needed [19]. This might suggest further levels of clinical assessment as a useful tool for a better CVR evaluation in these patients.

4. The relationship between gut microbiota, TMAO and CVR

TMAO is a gut microbiota-derived compound, that is currently recognized as a novel risk factor for CVD, including atherosclerosis [27], heart failure [28], stroke [29–32] and other major adverse cardiovascular events [2,33–38]. Observational studies reported a direct relationship between high serum levels of TMAO and increased risk of infarct relapse [2], rehospitalisation [39] and mortality [2,39] in patients with CVD. Moreover, TMAO has been positively correlated with levels of inflammatory and endothelial dysfunction biomarkers in patients with T2DM and chronic kidney disease [40]. Overall, this evidence suggests that TMAO plays a crucial role in increasing CVR [41]. Interestingly, TMAO has been suggested by several authors as "a prognostic biomarker for CVD beyond the traditional risk factors" [2,28,42,43], suggesting the importance to evaluate its levels for management of CVD. According to several studies, TMAO seems to be involved in various pathways related to the CVR; in particular, evidence reported that TMAO is able to increase recruitment of leukocyte, expression of pro-inflammatory cytokine and adhesion molecules, resulting in enhanced vascular inflammation [41]. Moreover, TMAO is also responsible for the accumulation of cholesterol in peripheral endothelial cells [44], platelet aggregation and adhesion induced by both ADP and thrombin [45]. In this context, the inflammation-promoting effect of TMAO is of relevant importance, also in pathogenesis of PCOS. Recent studies investigated the relationship between TMAO and inflammation, clarifying various mechanisms. In particular, a strong relationship was found between TMAO and TNF-α. It has been reported, indeed, that TMAO is able to enhance the polarization of M1 macrophages via activation of the NLRP3 inflammasome. The M1 macrophage polarization, in turn, mediates the response of the Th1 and Th17 activation [46]. Also, TMAO promotes the TNF-α-induced expression of tissue factor in vascular endothelial cells [47]. On the other and, in the liver, TMAO is responsible for decreased bile acid pool that, in turn, is linked to systemic inflammation [48]. Overall, this evidence remarks the central role played by TMAO in promoting inflammation, one of the main features of PCOS.

Gut microbiota is directly involved in the synthesis of TMAO. In the colon, indeed, bacteria metabolize diet-derived compounds, mainly choline, phosphatidylcholine, carnitine, and betaine, producing trimethylamine (TMA), by the activity of specific TMA lyases [49,50]. Through the portal circulation, TMA reaches the liver where is oxidised by flavin mono-oxygenases 3 (FMO3) to TMAO. Diet, thus, plays a pivotal role in modulation of the TMAO serum levels; in particular, high and prolonged consumption of foods rich in TMA precursors seems to be responsible for increased serum levels of TMAO [51].

There is evidence, however, that in addition to an unhealthy diet, microbiota *per se* contributes to increasing the circulating levels of TMAO [35]. In this sense, studies in animals fed high-carnitine diet showed that antibiotic treatment intended to decimate the commensal microbiota resulted in reduced serum levels of TMAO and abolished cardiovascular consequences, including atherosclerotic lesions, the content of cholesterol in macrophages and foam cell formation [33,34]. Furthermore, the atherosclerotic risk increased or decreased after microbial transplantation with TMA-producing strains or non-TMA producing strains, respectively [52].

Interestingly, specific bacterial strains have been indicated as more responsible than others to produce TMA. In particular, studies demonstrated the existence of a direct relationship between TMAO serum levels and the abundance of *Tenericutes* and *Desulfovibrio* [50]. Further studies reported that bacteria belonging to the *Clostridiaceae* or *Lachnospiraceae* families and *Ruminococcus, Allobaculum* and *Candidatus arthromitus* genera are also implicated, and their abundance results in an increased risk of atherosclerosis and arterial thrombosis via increased TMAO serum levels [45,53]. In line with this evidence, studies investigating human microbiota identified TMA-producing bacterial species, mainly belonging to *Firmicutes* and *Proteobacteria* phyla [54]. It is possible to speculate that the strain-specific ability of such bacteria to produce TMA is mainly due to the fact that some of them predominantly express enzymes involved in the metabolism of TMA precursors, including lyases (*Clostridia* and *Eubacteria*) and carnitine oxygenases (*Proteobacteria*) [33,55]. Notably, a gender-specific diversity has been found, suggesting that in male mice, *Clostridiaceae* play a major role in the microbiota-TMAO relationship, while in the female it is due to *Ruminococci* [53].

FMO3 is directly involved in modulating circulating levels of TMAO, derived from its ability to convert TMA in TMAO in the liver. Evidence reported an interesting relationship between steroid hormones and FMO3 activity. Specifically, it has been demonstrated that 17β-estradiol is able to inhibit the accumulation of Fmo3 mRNA in mouse liver cells [56], and FMO activity in rat hepatocytes [57], suggesting possible gender-specific differences in metabolic pathways underlying the synthesis of TMAO. Moreover, these studies further lead to hypothesize that hormonal status alterations might also act in this way.

5. Altered microbiota in PCOS women

As TMAO is a microbiota-derived metabolite, the hypothesis proposed in the present manuscript is that in women with PCOS an altered gut environment may contribute to increased CVR. The present sections are focused on reporting evidence suggesting the presence of altered microbiota in PCOS women, which role in exacerbating both clinical and subclinical manifestations is well established. As previously reviewed, indeed, dysbiosis is responsible for increased intestinal permeability (the so-called "leaky gut"), promoting the permeation of gut microbiota-derived substances (including lipopolysaccharides, LPS). These, in turn, activate both the immune system and the pro-inflammatory pathways (i.e. via activation of the toll-like receptors 4), resulting in increasing inflammation and IR [58,59]. These mechanisms support the "Dysbiosis of Gut Microbiota (DOGMA) theory" discussed below.

5.1. *In vivo evidence*

A large body of evidence investigated the relationship between PCOS and gut microbiota [60–62], suggesting the existence of a dysbiosis condition which, in turn, is considered the main cause of increased intestinal permeability. This increased permeability favors the entry of bacteria-produced LPS into the bloodstream, resulting in stimulation of the inflammatory pathways. This mild chronic inflammation is responsible for the activation of the immune system, which causes an increase in insulin serum levels altering the physiological functioning of insulin receptors. This mechanism suggests that dysbiosis is crucial in worsening the pathological course in PCOS women [62,63]. Interestingly, in 2012 Tremellen and Pearce proposed the so-called "DOGMA theory", as a microbiological hypothesis for the development of PCOS. In particular, the DOGMA theory suggests that the aforementioned increase in serum insulin caused by LPS-induced inflammation is, in turn, responsible for the alteration of the hormonal status. In the DOGMA paradigm, the dysbiosis is considered as the main cause of the three components of PCOS (hyperandrogenism, ovarian cysts development and altered ovulation) [63].

Guo et al. [62] demonstrated the existence of differences in gut microbiota between letrozole-induced PCOS rats and controls. In particular, they found a relative abundance of *Pseudomonas, Roseburia* and *Prevotella*, and reduced amount of *Lactobacillus, Ruminococcus* and *Clostridium* in the PCOS group compared to the control [62]. Similar results have been reported by Kelley and coworkers (2016) demonstrating that the altered microbiota in letrozole-induced mouse resulted in changes in relative abundance of *Bacteroidetes* and *Firmicutes*, in particular with reduction in *Bacteroidales* and increase in *Clostridiales*

amount [64]. The reduced amount of *Bacteroidetes* and increased amount of *Firmicutes* have been also found in dihydrotestosterone-induced PCOS rats which, remarkably, presented a microbiota profile similar to that of rats fed a high-fat diet. Additionally, in PCOS group was found an abundance of *Prevotella,* which amount has been reported to be higher in males than in females, suggesting the influence of sex hormones [65].

In addition to animal-based studies, various authors investigated the above-mentioned relationship between PCOS and altered microbiota in humans, demonstrating the existence of a dysbiosis [60,61,66–68]. Similar to the studies conducted on rats and mice, in women with PCOS two main features have been found: a microbiota profile similar to that of obesity, both in obese and non-obese PCOS women [60] and the reduced relative abundance of specific phyla, including *Bacteroidetes* [60,66,67] and *Tenericutes* [61] and increased genera, including *Streptococcus* [60], *Catenibacterium, Kandleria* [61], *Prevotella* and *Collinsella* [68]. Interestingly, in PCOS women, lower abundance of *Lactobacillus* [67], *Bifidobacterium* [67,68] and *Faecalibacterium prausnitzii* [68] has been found, which are also referred to as "good bacteria" [68].

5.2. The role of hyperandrogenism in microbiota alterations

Besides the DOGMA theory proposed a microbiological point of view for the pathogenesis of PCOS, a large body of studies provided evidence on a clear and marked effect of hormones on the microbiota. In particular, many authors demonstrated that alterations in hormonal status are reflected in changes in the normal distribution of bacteria in the gut environment, resulting in the development of dysbiosis. This is clearly observable in PCOS women, where hyperandrogenism may cause dysbiosis. In this way, therefore, a new vicious cycle is emerging in the PCOS scenario, which underlines the bi-directional relationship between the altered hormonal status and microbiota, with the consequent impaired metabolism and inflammation, characteristic in PCOS.

Evidence about the influence of hormones in microbiota changes is provided by both animal-based and human studies. During their studies on letrozole-induced PCOS rats, Guo and colleagues demonstrated a linear correlation between increased levels of androgens and copy number of *Prevotella*, and between reduced levels of estrogens and colonization of *Lactobacillus*, suggesting that dysbiosis and sex hormones were associated [62]. In this animal model, the hypothesis that the hyperandrogenism may be responsible for a microbiota dysbiosis arises from the fact that letrozole is an aromatase inhibitor acting, therefore, on the reduction of the testosterone to estrogen conversions, and resulting in increased circulating levels of androgens. The observed dysbiosis in letrozole-induced animals, compared to control, may support the hypothesis [64]. Interestingly, in dihydrotestosterone-induced PCOS rats the treatment with cyproterone acetate, a progesterone agonist and androgen antagonist, ameliorated the microbiota profile, in particular reducing the amount of *Prevotella* [65].

The relationship between sex hormone levels and microbiota were also found in humans, suggesting that both androgens and estrogens may be involved in a sexual dimorphism related to the gut environment, resulting in the so-called "microgenderome" [69], which spotlights on the existence of the mutual relationship between sex hormones and microbiota. In addition, it has been reported that in men the relative abundance of *Bacteroidetes* is higher than in women [70–73], while the abundance of *Desulfovibrio, Methanobrevibacter* and *Clostridia* is lower [74].

Notably, in PCOS women interesting correlations have been found between *Prevotella* and circulating testosterone and between *Kandleria* and circulating androstenedione, while *Prevotella* negatively correlated with circulating estradiol [61]. Similarly, both alpha- (the overall species richness) and beta- (the composition of the microbial community) diversity have been found correlated with hyperandrogenism in women with PCOS [75].

In addition, to further support the influence of sex hormones on microbiota, an interesting study using an animal model reported that the prenatal androgen exposure caused dysbiosis in the offspring [76], suggesting that hyperandrogenism in PCOS women may also act during pregnancy in modulating not only maternal microbiota but also that of unborn.

To understand the mechanisms at the base of this relationship, it is necessary to underline the numerous activities of bacteria, and among these their ability to act in altering the conjugated/deconjugated status of several host-derived compounds, including sex hormones [73,77]. Gut bacteria are also able to synthesize beta-glucuronidase that deconjugates molecules, facilitating their re-absorption throughout the entero-hepatic circulation, and releasing glucuronic acid, used as a source of energy. It has been speculated that changing the levels of substrate, sex steroids may be responsible for the alteration of both beta-glucuronidase activity and the production of energy, resulting in the modification of the gut microbiota [73]. In addition to this mechanism, sex hormones may also activate steroid receptors, also present in the gastrointestinal tract, resulting in a further modulation of the gut microbiota. In particular, both estrogen and androgen receptors have been reported to be expressed in intestinal epithelial cells [78,79] and human colon mucosa [80,81], respectively. Intriguing, it has been demonstrated that estrogen receptor knockout mice presented an altered microbiota compared to wild-type [79], suggesting the marked influence of sex hormones in modulating the gut microbiota.

6. Increased TMAO serum levels in PCOS women

A very small body of evidence directly investigated the role of TMAO in both pathogenesis and increased CVR in PCOS women. However, such evidence are concord in demonstrating higher serum levels of TMAO in this kind of patients compared to age- and BMI-matched controls.

In 2019, Eyupoglu and colleagues investigated for the first time in this sense. They demonstrated that PCOS women have higher levels of circulating TMAO compared to control (2.39 (2.15–4.49) and 2.05 (1.39–3.33), $p = 0.042$, PCOS and control, respectively). Interestingly, they observed that 3-month oral contraceptive and healthy lifestyle (diet and regular exercise) contributed to decrease TMAO levels, from 3.35 (2.18–4.93) to 2.05 (1.7–2.93), $p = 0.002$. Authors concluded that increased TMAO serum levels were associated with hyperandrogenism [82]. The same research group conducted another interesting study aimed to investigate differences in gut microbiota composition between overweight/obese PCOS women and age- and BMI-matched controls. Authors observed the abundance of *Ruminococcaceae* in PCOS subjects [83], a microbe family positively associated with plaque size and TMAO serum levels in animals [53]. Interestingly, this diversity in microbiota composition was associated with hyperandrogenism [83]. In line with the results published by Eyupoglu and coworkers, a recent observational study conducted on 27 PCOS women reported higher TMAO serum levels compared to non-PCOS women [84]. Notably, a not recent cross-sectional study demonstrated that PCOS women presented significantly higher serum levels of dimethylamine than healthy women [85], providing further evidence for the predominant role played by microbiota in this class of patients.

Overall, this evidence allows concluding that in PCOS women alterations in microbiota composition (probably due by endocrine aberrations, i.e. hyperandrogenism) are responsible for overproduction of specific metabolites (i.e. TMA as a precursor of TMAO) that, in turn, may increase the risk of CVD development. However, observational studies on a larger number of patients are needed to confirm the presence of increased circulating TMAO levels in PCOS women.

7. Conclusion and future perspectives

In 2012, the DOGMA theory provided a novel and captivating approach for the comprehension of the complex PCOS pathogenesis, suggesting the pivotal role played by altered microbiota in the

development of both the syndrome and related metabolic complications. A large amount of evidence suggests how dysbiosis may be considered the trigger of many diseases, including obesity, T2DM, MetS and CVD [33,86–95].

According to this evidence, dysbiosis may be considered the keystone for a novel approach in both comprehension and study of the CVR also in PCOS women. As described above, altered microbiota is responsible for increased serum levels of TMAO, which has been considered as one of the main causes of enhanced CVR, due to its pro-atherosclerotic effect, as schematically represented in Fig. 2.

What has been proposed in the present manuscript is a hypothesis, based on the analysis of the available literature, and further investigations in this field are undoubtedly needed in order to confirm or disprove it. Moreover, this review aimed to provide the basis to establish novel approaches for the management of PCOS, including the use of probiotics, to restore a normal microbiota, or nutraceutical to contrast the increased serum levels of TMAO.

Since TMAO is a gut microbiota-derived metabolite, it appears clear that the first approach for control of its serum levels may reside in the use of probiotic supplements. However, available literature in this sense is contradictory [96]. On one hand, clinical studies reported that supplementation with *L. plantarum 229v* (20 billion CFU daily for 6 weeks) [97], *L. casei Shirota* (6.5×10^9 CFU daily, 3 times for 12 weeks) [98] or mix of probiotics (900 billion live bacteria for 4 weeks) [99] did not change significantly the serum levels of TMAO. In contrast, an animal-based study conducted on ApoE$^{-/-}$ choline-fed mice reported that supplementation with *L. plantarum ZYDO4* was able to inhibit the development of atherosclerosis induced by TMAO [100]. Our research group in a clinical trial also observed the TMAO-reducing effect of probiotic supplementation (lactofermented annurca apple puree containing 3×10^8 CFU *L. rhamnosus LRH11* and *L. plantarum SGL07*) in high CVR subjects [101]. Similarly, it has been reported that *B. animalis* subsp. *Lactis LKM512* reduced the levels of TMA in healthy subjects [102].

Notably, recent studies demonstrated that grape pomace polyphenolic extract-based nutraceuticals significantly reduced TMAO circulating levels both in healthy young people [103] and overweight/obese middle-aged subjects [104], probably acting not only in modulating microbiota but also in exerting antioxidant activity, resulting in a reduction of circulating TMAO to TMA at serum level.

The travel proposed in this manuscript would suggest that every step forward in medical research should not be experienced with fear but understood with the knowledge that no certainty remains unchanged over time, and it is always possible to obtain new tools to manage already widely known diseases.

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CRediT authorship contribution statement

Giuseppe Annunziata: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Roberto Ciampaglia:** Writing – original draft. **Xavier Capò:** Writing – original draft. **Fabrizia Guerra:** Writing – original draft. **Antoni Sureda:** Visualization, Supervision. **Gian Carlo Tenore:** Visualization, Supervision. **Ettore Novellino:** Visualization, Supervision.

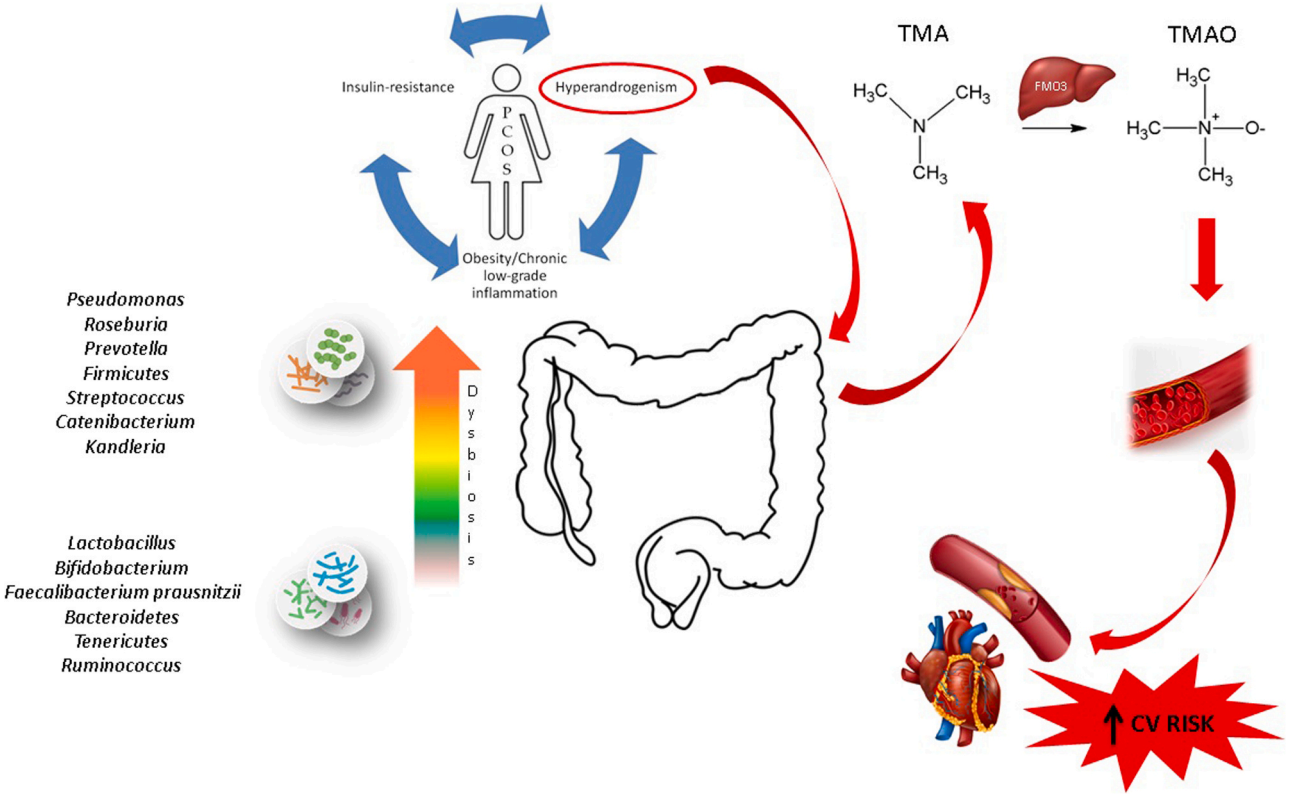

Fig. 2. Schematic representation for the hypothesis of the high CVR due to increased circulating TMAO levels via altered microbiota. Among the features of PCOS representing the characteristic triad (excess of androgens, IR and obesity with chronic low-grade inflammation), hyperandrogenism may be considered one of the most important actors in the development of dysbiosis, characterized by reduced levels of so-called "good bacteria" and increased levels of "bad bacteria". Dysbiosis, in turn, is responsible for the elevated production of bacteria-derived metabolites, including TMA. Through the entero-hepatic circulation, TMA reaches the liver where is oxidised by FMO3 to TMAO which, in turn, exerts a pro-atherosclerotic effect in the bloodstream, resulting in increasing the CVR.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] G.G. Schiattarella, A. Sannino, E. Toscano, G. Giugliano, G. Gargiulo, A. Franzone, B. Trimarco, G. Esposito, C. Perrino, Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis, Eur. Heart J. 38 (2017) 2948–2956, https://doi.org/10.1093/eurheartj/ehx342.

[2] X.S. Li, S. Obeid, R. Klingenberg, B. Gencer, F. Mach, L. Räber, S. Windecker, N. Rodondi, D. Nanchen, O. Muller, M.X. Miranda, C.M. Matter, Y. Wu, L. Li, Z. Wang, H.S. Alamri, V. Gogonea, Y.M. Chung, W.H.W. Tang, S.L. Hazen, T. F. Lüscher, Gutmicrobiota-dependent trimethylamine N-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors, Eur. Heart J. 38 (2017) 814–824, https://doi.org/10.1093/eurheartj/ehw582.

[3] L. Barrea, G. Annunziata, G. Muscogiuri, C. Di Somma, D. Laudisio, M. Maisto, G. de Alteriis, G.C. Tenore, A. Colao, S. Savastano, Trimethylamine-N-oxide (TMAO) as novel potential biomarker of early predictors of metabolic syndrome, Nutrients 10 (2018) 1971, https://doi.org/10.3390/nu10121971.

[4] L. Barrea, G. Muscogiuri, G. Annunziata, D. Laudisio, G.C. Tenore, A. Colao, S. Savastano, A new light on vitamin d in obesity: a novel association with trimethylamine-n-oxide (tmao), Nutrients 11 (2019) 1310, https://doi.org/10.3390/nu11061310.

[5] L. Barrea, G. Annunziata, G. Muscogiuri, D. Laudisio, C. Di Somma, M. Maisto, G. C. Tenore, A. Colao, S. Savastano, Trimethylamine N-oxide, Mediterranean diet, and nutrition in healthy, normal-weight adults: also a matter of sex? Nutrition 62 (2019) 7–17, https://doi.org/10.1016/j.nut.2018.11.015.

[6] J.S. Laven, B. Imani, M.J. Eijkemans, B.C. Fauser, New approach to polycystic ovary syndrome and other forms of anovulatory infertility, Obs. Gynecol. Surv. 57 (2002) 755–767, https://doi.org/10.1097/00006254-200211000-0002.

[7] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, Fertil. Steril. 81 (1) (2004) 19–25, https://doi.org/10.1016/j.fertnstert.2003.10.004.

[8] E. Diamanti-Kandarakis, F. Economou, S. Palimeri, C. Christakou, Metformin in polycystic ovary syndrome, Ann. N. Y. Acad. Sci. 1205 (2010) 192–198, https://doi.org/10.1111/j.1749-6632.2010.05679.x.

[9] F. González, Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction, Steroids 77 (2012) 300–305, https://doi.org/10.1016/j.steroids.2011.12.003.

[10] R. Deswal, A. Yadav, A.S. Dang, Sex hormone binding globulin – an important biomarker for predicting PCOS risk: a systematic review and meta-analysis, Syst. Biol. Reprod. Med. 64 (2018) 12–24, https://doi.org/10.1080/19396368.2017.1410591.

[11] P. Moghetti, F. Tosi, R. Castello, C.M. Magnani, C. Negri, E. Brun, L. Furlani, M. Caputo, M. Muggeo, The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women, J. Clin. Endocrinol. Metab. 81 (1996) 952–960, https://doi.org/10.1210/jcem.81.3.8772557.

[12] J. Lord, R. Thomas, B. Fox, U. Acharya, T. Wilkin, The central issue? Visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome, BJOG 113 (2006) 1203–1209, https://doi.org/10.1111/j.1471-0528.2006.00973.x.

[13] B.C. Fauser, B.C. Tarlatzis, R.W. Rebar, R.S. Legro, A.H. Balen, R. Lobo, E. Carmina, J. Chang, B.O. Yildiz, J.S. Laven, J. Boivin, F. Petraglia, C. N. Wijeyeratne, R.J. Norman, A. Dunaif, S. Franks, R.A. Wild, D. Dumesic, K. Barnhart, Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group, Fertil. Steril. 97 (2012) 28–38, https://doi.org/10.1016/j.fertnstert.2011.09.024.

[14] S.A. Geronikolou, F. Bacopoulou, D. Cokkinos, Bioimpedance measurements in adolescents with polycystic ovary syndrome: a pilot study, Adv. Exp. Med. Biol. 987 (2017) 291–299, https://doi.org/10.1007/978-3-319-57379-3_26.

[15] G. Papadakis, E. Kandaraki, O. Papalou, A. Vryonidou, E. Diamanti-Kandarakis, Is cardiovascular risk in women with PCOS a real risk? Current insights, Minerva Endocrinol. 42 (2017) 340–355, https://doi.org/10.23736/S0391-1977.17.02609-8.

[16] P. Ravn, New paradigms in PCOS: impaired glucose tolerance and cardiovascular risk. Clinical approach, Minerva Ginecol. 67 (2017) 217–223.

[17] R. Rosenfield, D.A. Ehrmann, The pathogenesis of Polycystic Ovary Syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited, Endocr. Rev. 37 (2016) 467–520, https://doi.org/10.1210/er.2015-1104.

[18] S. Bednarska, A. Siejka, The pathogenesis and treatment of polycystic ovary syndrome: what's new? Adv. Clin. Exp. Med. 26 (2017) 359–367, https://doi.org/10.17219/acem/59380.

[19] C. Christakou, E. Diamanti-Kandarakis, Structural, biochemical and non-traditional cardiovascular risk markers in PCOS, Curr. Pharm. Des. 19 (2013) 5764–5774, https://doi.org/10.2174/1381612811319320011.

[20] R.A. Wild, E. Carmina, E. Diamanti-Kandarakis, A. Dokras, H.F. Escobar-Morreale, W. Futterweit, R. Lobo, R.J. Norman, E. Talbott, D.A. Dumesic, Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society, J. Clin. Endocrinol. Metab. 95 (2010) 2038–2049, https://doi.org/10.1210/jc.2009-2724.

[21] H.J. Teede, S. Hutchison, S. Zoungas, C. Meyer, Insulin resistance, the metabolic syndrome, diabetes, and cardiovascular disease risk in women with PCOS, Endocrine 30 (2006) 45–53, https://doi.org/10.1385/ENDO:30:1:45.

[22] Q. Lang, X. Yidong, Z. Xueguang, W. Sixian, X. Wenming, Z. Tao, ETA-mediated anti-TNF-α therapy ameliorates the phenotype of PCOS model induced by letrozole, PLoS One 14 (2019), 0217495, https://doi.org/10.1371/journal.pone.0217495.

[23] M. Szczuko, M. Zapałowska-Chwyć, A. Drozd, D. Maciejewska, A. Starczewski, P. Wysokiński, E. Stachowska, Changes in the IGF-1 and TNF-α synthesis pathways before and after three-month reduction diet with low glicemic index in women with PCOS, Ginekol. Pol. 89 (2018) 295–303, https://doi.org/10.5603/GP.a2018.0051.

[24] R. Bhatnager, J. Jalthuria, R. Sehrawat, S. Nanda, A.S. Dang, Evaluating the association of TNF α promoter haplotype with its serum levels and the risk of PCOS: a case control study, Cytokine 114 (2019) 86–91, https://doi.org/10.1016/j.cyto.2018.11.004.

[25] M. Szczuko, M. Zapałowska-Chwyć, A. Drozd, D. Maciejewska, A. Starczewski, E. Stachowska, Effect of IGF-I and TNF-α on intensification of steroid pathways in women with PCOS phenotypes are not identical. Enhancement of progesterone pathway in women with PCOS increases the concentration of TNF-α, Gynecol. Endocrinol. 32 (2016) 714–717, https://doi.org/10.3109/09513590.2016.1159672.

[26] M. Szczuko, M. Zapałowska-Chwyć, D. Maciejewska, A. Drozd, A. Starczewski, E. Stachowska, High glycemic index diet in PCOS patients. The analysis of IGF I and TNF-α pathways in metabolic disorders, Med. Hypotheses 96 (2016) 42–47, https://doi.org/10.1016/j.mehy.2016.09.016.

[27] E. Randrianarisoa, A. Lehn-Stefan, X. Wang, M. Hoene, A. Peter, S.S. Heinzmann, X. Zhao, I. Königsrainer, A. Königsrainer, B. Balletshofer, J. Machann, F. Schick, A. Fritsche, H.U. Hring, G. Xu, R. Lehmann, N. Stefan, Relationship of serum trimethylamine N-oxide (TMAO) levels with early atherosclerosis in humans, Sci. Rep. 6 (2016) 26745, https://doi.org/10.1038/srep26745.

[28] W.H.W. Tang, Z. Wang, Y. Fan, B. Levison, J.E. Hazen, L.M. Donahue, Y. Wu, S. L. Hazen, Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: Refining the gut hypothesis, J. Am. Coll. Cardiol. 64 (2014) 1908–1914, https://doi.org/10.1016/j.jacc.2014.02.617.

[29] Z. Liang, Z. Dong, M. Guo, Z. Shen, D. Yin, S. Hu, X. Hai, Trimethylamine N-oxide as a risk marker for ischemic stroke in patients with atrial fibrillation, J. Biochem. Mol. Toxicol. 33 (2019) 22246, https://doi.org/10.1002/jbt.22246.

[30] J. Nie, L. Xie, B.X. Zhao, Y. Li, B. Qiu, F. Zhu, G.F. Li, M. He, Y. Wang, B. Wang, S. Liu, H. Zhang, H. Guo, Y. Cai, Y. Huo, F.F. Hou, X. Xu, X. Qin, Serum trimethylamine N-oxide concentration is positively associated with first stroke in hypertensive patients, Stroke 49 (2018) 2021–2028, https://doi.org/10.1161/STROKEAHA.118.021997.

[31] A. Haghikia, X.S. Li, T.G. Liman, N. Bledau, D. Schmidt, F. Zimmermann, N. Kränkel, C. Widera, K. Sonnenschein, A. Haghikia, K. Weissenborn, D. Fraccarollo, M.M. Heimesaat, J. Bauersachs, Z. Wang, W. Zhu, U. Bavendiek, S. L. Hazen, M. Endres, U. Landmesser, Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes, Arterioscler. Thromb. Vasc. Biol. 38 (2018) 2225–2235, https://doi.org/10.1161/ATVBAHA.118.311023.

[32] C. Wu, C. Li, W. Zhao, N. Xie, F. Yan, Y. Lian, L. Zhou, X. Xu, Y. Liang, L. Wang, M. Ren, S. Li, X. Cheng, L. Zhang, Q. Ma, H. Song, R. Meng, X. Ji, Elevated trimethylamine n-oxide related to ischemic brain lesions after carotid artery stenting, Neurology 90 (2018) e1283–e1290, https://doi.org/10.1212/WNL.0000000000005298.

[33] Z. Wang, E. Klipfell, B.J. Bennett, R. Koeth, B.S. Levison, B. Dugar, A.E. Feldstein, E.B. Britt, X. Fu, Y.M. Chung, Y. Wu, P. Schauer, J.D. Smith, H. Allayee, W.H. W. Tang, J.A. Didonato, A.J. Lusis, S.L. Hazen, Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, Nature 472 (2011) 57–65, https://doi.org/10.1038/nature09922.

[34] R.A. Koeth, Z. Wang, B.S. Levison, J.A. Buffa, E. Org, B.T. Sheehy, E.B. Britt, X. Fu, Y. Wu, L. Li, J.D. Smith, J.A. Didonato, J. Chen, H. Li, G.D. Wu, J.D. Lewis, M. Warrier, J.M. Brown, R.M. Krauss, W.H.W. Tang, F.D. Bushman, A.J. Lusis, S. L. Hazen, Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis, Nat. Med. 19 (2013) 576–585, https://doi.org/10.1038/nm.3145.

[35] W.H.W. Tang, Z. Wang, B.S. Levison, R.A. Koeth, E.B. Britt, X. Fu, Y. Wu, S. L. Hazen, Intestinal microbial metabolism of phosphatidylcholine and

cardiovascular risk, N. Engl. J. Med. 368 (2013) 1575–1584, https://doi.org/10.1056/NEJMoa1109400.
[36] M. Lever, P.M. George, S. Slow, D. Bellamy, J.M. Young, M. Ho, C.J. McEntyre, J. L. Elmslie, W. Atkinson, S.L. Molyneux, R.W. Troughton, C.M. Frampton, A. M. Richards, S.T. Chambers, Betaine and trimethylamine-N-oxide as predictors of cardiovascular outcomes show different patterns in diabetes mellitus: an observational study, PLoS One 9 (2014), 114969, https://doi.org/10.1371/journal.pone.0114969.
[37] A. Mente, K. Chalcraft, H. Ak, A.D. Davis, E. Lonn, R. Miller, M.A. Potter, S. Yusuf, S.S. Anand, M.J. McQueen, The relationship between trimethylamine-N-oxide and prevalent cardiovascular disease in a multiethnic population living in Canada, Can. J. Cardiol. 31 (2015) 1189–1194, https://doi.org/10.1016/j.cjca.2015.06.016.
[38] M. Trøseid, T. Ueland, J.R. Hov, A. Svardal, I. Gregersen, C.P. Dahl, S. Aakhus, E. Gude, B. Bjørndal, B. Halvorsen, T.H. Karlsen, P. Aukrust, L. Gullestad, R. K. Berge, A. Yndestad, Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure, J. Intern. Med. 277 (2015) 717–726, https://doi.org/10.1111/joim.12328.
[39] T. Suzuki, Y. Yazaki, A.A. Voors, D.J.L. Jones, D.C.S. Chan, S.D. Anker, J. G. Cleland, K. Dickstein, G. Filippatos, H.L. Hillege, C.C. Lang, P. Ponikowski, N. J. Samani, D.J. van Veldhuisen, F. Zannad, A.H. Zwinderman, M. Metra, L.L. Ng, Association with outcomes and response to treatment of trimethylamine N-oxide in heart failure: results from BIOSTAT-CHF, Eur. J. Heart Fail. 21 (2019) 877–886, https://doi.org/10.1002/ejhf.1338.
[40] M. Al-Obaide, R. Singh, P. Datta, K. Rewers-Felkins, M. Salguero, I. Al-Obaidi, K. Kottapalli, T. Vasylyeva, Gut microbiota-dependent trimethylamine-N-oxide and serum biomarkers in patients with T2DM and advanced CKD, J. Clin. Med. 6 (2017) 86, https://doi.org/10.3390/jcm6090086.
[41] M.H. Janeiro, M.J. Ramírez, F.I. Milagro, J.A. Martínez, M. Solas, Implication of trimethylamine n-oxide (TMAO) in disease: potential biomarker or new therapeutic target, Nutrients 10 (2018) 1398, https://doi.org/10.3390/nu10101398.
[42] V. Senthong, Z. Wang, Y. Fan, Y. Wu, S.L. Hazen, W.H.W. Tang, Trimethylamine N-oxide and mortality risk in patients with peripheral artery disease, J. Am. Heart Assoc. 5 (2016), e004237, https://doi.org/10.1161/JAHA.116.004237.
[43] Z. Dong, Z. Liang, M. Guo, S. Hu, Z. Shen, X. Hai, The association between plasma levels of trimethylamine N-oxide and the risk of coronary heart disease in Chinese patients with or without type 2 diabetes mellitus, Dis. Mark. 2018 (2018), 1578320, https://doi.org/10.1155/2018/1578320.
[44] M.M. Seldin, Y. Meng, H. Qi, W.F. Zhu, Z. Wang, S.L. Hazen, A.J. Lusis, D.M. Shih, Trimethylamine N-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-κb, J. Am. Heart Assoc. 5 (2016), e002767, https://doi.org/10.1161/JAHA.115.002767.
[45] W. Zhu, J.C. Gregory, E. Org, J.A. Buffa, N. Gupta, Z. Wang, L. Li, X. Fu, Y. Wu, M. Mehrabian, R.B. Sartor, T.M. McIntyre, R.L. Silverstein, W.H.W. Tang, J. A. Didonato, J.M. Brown, A.J. Lusis, S.L. Hazen, Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk, Cell 165 (2016) 111–124, https://doi.org/10.1016/j.cell.2016.02.011.
[46] K. Wu, Y. Yuan, H. Yu, X. Dai, S. Wang, Z. Sun, F. Wang, H. Fei, Q. Lin, H. Jiang, T. Chen, The gut microbial metabolite trimethylamine N-oxide aggravates GVHD by inducing M1 macrophage polarization in mice, Blood 136 (2020) 501–515, https://doi.org/10.1182/blood.2019003990.
[47] X. Cheng, X. Qiu, Y. Liu, C. Yuan, X. Yang, Trimethylamine N-oxide promotes tissue factor expression and activity in vascular endothelial cells: a new link between trimethylamine N-oxide and atherosclerotic thrombosis, Thromb. Res. 177 (2019) 110–116, https://doi.org/10.1016/j.thromres.2019.02.028.
[48] S. Rohrmann, J. Linseisen, M. Allenspach, A. von Eckardstein, D. Müller, Plasma concentrations of trimethylamine-N-oxide are directly associated with dairy food consumption and low-grade inflammation in a German adult population, J. Nutr. 46 (2016) 283–289, https://doi.org/10.3945/jn.115.220103.
[49] S. Ascher, C. Reinhardt, The gut microbiota: an emerging risk factor for cardiovascular and cerebrovascular disease, Eur. J. Immunol. 48 (2018) 564–575, https://doi.org/10.1002/eji.201646879.
[50] H.S. Nam, Gut microbiota and ischemic stroke: the role of trimethylamine N-oxide, J. Stroke 21 (2019) 151–159, https://doi.org/10.5853/jos.2019.00472.
[51] M.T. Velasquez, A. Ramezani, A. Manal, D.S. Raj, Trimethylamine N-oxide: the good, the bad and the unknown, Toxins 8 (2016) 326, https://doi.org/10.3390/toxins8110326.
[52] J.C. Gregory, J.A. Buffa, E. Org, Z. Wang, B.S. Levison, W. Zhu, M.A. Wagner, B. J. Bennett, L. Li, J.A. DiDonato, A.J. Lusis, S.L. Hazen, Transmission of atherosclerosis susceptibility with gut microbial transplantation, J. Biol. Chem. 290 (2015) 5647–5660, https://doi.org/10.1074/jbc.M114.618249.
[53] Z. Wang, A.B. Roberts, J.A. Buffa, B.S. Levison, W. Zhu, E. Org, X. Gu, Y. Huang, M. Zamanian-Daryoush, M.K. Culley, A.J. Didonato, X. Fu, J.E. Hazen, D. Krajcik, J.A. Didonato, A.J. Lusis, S.L. Hazen, Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis, Cell 163 (2015) 1585–1595, https://doi.org/10.1016/j.cell.2015.11.055.
[54] K.A. Romano, E.I. Vivas, D. Amador-Noguez, F.E. Rey, Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide, MBio 6 (2015) 02481, https://doi.org/10.1128/mBio.02481-14.
[55] S. Rath, B. Heidrich, D.H. Pieper, M. Vital, Uncovering the trimethylamine-producing bacteria of the human gut microbiota, Microbiome 5 (2017) 54, https://doi.org/10.1186/S40168-017-0271-9.
[56] T. Esposito, B. Varriale, R. D'Angelo, A. Amato, A. Sidoti, Regulation of flavin-containing mono-oxygenase (Fmo3) gene expression by steroids in mice and humans, Horm. Mol. Biol. Clin. Invest. 20 (2014) 99–109, https://doi.org/10.1515/hmbci-2014-0012.
[57] S. Coecke, G. Debast, I.R. Phillips, A. Vercruysse, E.A. Shephard, V. Rogiers, Hormonal regulation of microsomal flavin-containing monooxygenase activity by sex steroids and growth hormone in co-cultured adult male rat hepatocytes, Biochem. Pharm. 56 (1998) 1047–1051, https://doi.org/10.1016/s0006-2952(98)00104-x.
[58] F.F. He, Y.M. Li, Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: a review, J. Ovarian Res. 13 (2020) 73, https://doi.org/10.1186/s13048-020-00670-3.
[59] P. Giampaolino, V. Foreste, C. Di Filippo, A. Gallo, A. Mercorio, P. Serafino, F. P. Improda, P. Verrazzo, G. Zara, C. Buonfantino, M. Borgo, G. Riemma, C. Angelis, B. Zizolfi, G. Bifulco, L. Della Corte, Microbiome and PCOS: state-of-art and future aspects, Int. J. Mol. Sci. 22 (2021) 2048, https://doi.org/10.3390/ijms22042048.
[60] R. Liu, C. Zhang, Y. Shi, F. Zhang, L. Li, X. Wang, Y. Ling, H. Fu, W. Dong, J. Shen, A. Reeves, A.S. Greenberg, L. Zhao, Y. Peng, X. Ding, Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome, Front. Microbiol. 8 (2017) 324, https://doi.org/10.3389/fmicb.2017.00324.
[61] M. Insenser, M. Murri, R. Del Campo, M.Á. Martínez-García, E. Fernández-Durán, H.F. Escobar-Morreale, Gut microbiota and the polycystic ovary syndrome: influence of sex, sex hormones, and obesity, J. Clin. Endocrinol. Metab. 103 (2018) 2552–2562, https://doi.org/10.1210/jc.2017-02799.
[62] Y. Guo, Y. Qi, X. Yang, L. Zhao, S. Wen, Y. Liu, L. Tang, Association between polycystic ovary syndrome and gut microbiota, PLoS One 11 (2016), 0153196, https://doi.org/10.1371/journal.pone.0153196.
[63] K. Tremellen, K. Pearce, Dysbiosis of gut microbiota (DOGMA)–a novel theory for the development of polycystic ovarian syndrome, Med. Hypotheses 79 (2012) 104–112, https://doi.org/10.1016/j.mehy.2012.04.016.
[64] S.T. Kelley, D.V. Skarra, A.J. Rivera, V.G. Thackray, The gut microbiome is altered in a letrozole-induced mouse model of polycystic ovary syndrome, PLoS One 11 (2016), 0146509, https://doi.org/10.1371/journal.pone.0146509.
[65] F. Zhang, T. Ma, P. Cui, A. Tamadon, S. He, C. Huo, G. Yierfulati, X. Xu, W. Hu, X. Li, L.R. Shao, H. Guo, Y. Feng, C. Xu, Diversity of the GUt Microbiota in Dihydrotestosterone-induced PCOS rats and the pharmacologic effects of diane-35, probiotics, and berberine, Front. Microbiol. 10 (2019) 175, https://doi.org/10.3389/fmicb.2019.00175.
[66] L. Lindheim, M. Bashir, J. Münzker, C. Trummer, V. Zachhuber, B. Leber, A. Horvath, T.R. Pieber, G. Gorkiewicz, V. Stadlbauer, B. Obermayer-Pietsch, Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with Polycystic Ovary Syndrome (PCOS): a pilot study, PLoS One 12 (2017), 0168390, https://doi.org/10.1371/journal.pone.0168390.
[67] B. Zeng, Z. Lai, L. Sun, Z. Zhang, J. Yang, Z. Li, J. Lin, Z. Zhang, Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): a pilot study, Res. Microbiol. 170 (2019) 43–52, https://doi.org/10.1016/j.resmic.2018.09.002.
[68] J. Zhang, Z. Sun, S. Jiang, X. Bai, C. Ma, Q. Peng, K. Chen, H. Chang, T. Fang, H. Zhang, Probiotic bifidobacterium lactis V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis, MSystems 4 (2019) e00017–e00019, https://doi.org/10.1128/mSystems.00017-19.
[69] M.B. Flak, J.F. Neves, R.S. Blumberg, Immunology. Welcome to the microgenderome, Science (80-) 339 (2013) 1044–1045, https://doi.org/10.1126/science.1236226.
[70] S. Mueller, K. Saunier, C. Hanisch, E. Norin, L. Alm, T. Midtvedt, A. Cresci, S. Silvi, C. Orpianesi, M.C. Verdenelli, T. Clavel, C. Koebnick, H.J. Zunft, J. Doré, M. Blaut, Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study, Appl. Environ. Microbiol. 72 (2006) 1027–1033, https://doi.org/10.1128/AEM.72.2.1027-1033.2006.
[71] M. Li, B. Wang, M. Zhang, M. Rantalainen, S. Wang, H. Zhou, Y. Zhang, J. Shen, X. Pang, M. Zhang, H. Wei, Y. Chen, H. Lu, J. Zuo, M. Su, Y. Qiu, W. Jia, C. Xiao, L.M. Smith, S. Yang, L. Zhao, Symbiotic gut microbes modulate human metabolic phenotypes, Proc. Natl. Acad. Sci. USA 105 (2008) 2117–2122, https://doi.org/10.1073/pnas.0712038105.
[72] C. Dominianni, R. Sinha, J.J. Goedert, Z. Pei, L. Yang, R.B. Hayes, J. Ahn, Sex, body mass index, and dietary fiber intake influence the human gut microbiome, PLoS One 10 (2015), 0124599, https://doi.org/10.1371/journal.pone.0124599.
[73] V.G. Thackray, Sex, microbes, and polycystic ovary syndrome, Trends Endocrinol. Metab. 30 (2019) 54–65, https://doi.org/10.1016/j.tem.2018.11.001.
[74] G. Falony, M. Joossens, S. Vieira-Silva, J. Wang, Y. Darzi, K. Faust, A. Kurilshikov, M.J. Bonder, M. Valles-Colomer, D. Vandeputte, R.Y. Tito, S. Chaffron, L. Rymenans, C. Verspecht, L. De Sutter, G. Lima-Mendez, K. D'hoe, K. Jonckheere, D. Homola, R. Garcia, J. Raes, Population-level analysis of gut microbiome variation, Science (80-) 352 (2016) 560–564, https://doi.org/10.1126/science.aad3503.
[75] P.J. Torres, M. Siakowska, B. Banaszewska, L. Pawelczyk, A.J. Duleba, S.T. Kelley, V.G. Thackray, Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism, J. Clin. Endocrinol. Metab. 103 (2018) 1502–1511, https://doi.org/10.1210/jc.2017-02153.
[76] S.B. Sherman, N. Sarsour, M. Salehi, A. Schroering, B. Mell, B. Joe, J.W. Hill, Prenatal androgen exposure causes hypertension and gut microbiota dysbiosis,

Gut Microbes 9 (2018) 400–421, https://doi.org/10.1080/19490976.2018.1441664.
[77] S.J. Pellock, M.R. Redinbo, Glucuronides in the gut: sugar-driven symbioses between microbe and host, J. Biol. Chem. 292 (2017) 8569–8576, https://doi.org/10.1074/jbc.R116.767434.
[78] M. Looijer-van Langen, N. Hotte, L.A. Dieleman, E. Albert, C. Mulder, K. L. Madsen, Estrogen receptor-beta signaling modulates epithelial barrier function, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G621–G626, https://doi.org/10.1152/ajpgi.00274.2010.
[79] R. Menon, S.E. Watson, L.N. Thomas, C.D. Allred, A. Dabney, M.A. Azcarate-Peril, J.M. Sturino, Diet complexity and estrogen receptor beta status affect the composition of the murine intestinal microbiota, Appl. Environ. Microbiol. 79 (2013) 5763–5773, https://doi.org/10.1128/AEM.01182-13.
[80] M.G. Catalano, U. Pfeffer, M. Raineri, P. Ferro, A. Curto, P. Capuzzi, F. Corno, L. Berta, N. Fortunati, Altered expression of androgen-receptor isoforms in human colon-cancer tissues, Int. J. Cancer 86 (2000) 325–330, https://doi.org/10.1002/(sici)1097-0215(20000501)86:3<325::aid-ijc4>3.0.co;2-g.
[81] P. Waliszewski, M. Blaszczyk, E. Wolinska-Witort, M. Drews, M. Snochowski, R. E. Hurst, Molecular study of sex steroid receptor gene expression in human colon and in colorectal carcinomas, J. Surg. Oncol. 64 (1997) 3–11, https://doi.org/10.1002/(sici)1096-9098(199701)64:1<3::aid-jso2>3.0.co;2-g.
[82] N.D. Eyupoglu, E. Caliskan Guzelce, A. Acikgoz, E. Uyanik, B. Bjørndal, R. K. Berge, A. Svardal, B.O. Yildiz, Circulating gut microbiota metabolite trimethylamine N-oxide and oral contraceptive use in polycystic ovary syndrome, Clin. Endocrinol. 91 (2019) 810–815, https://doi.org/10.1111/cen.14101.
[83] N.D. Eyupoglu, K. Ergunay, A. Acikgoz, Y. Akyon, E. Yilmaz, B.O. Yildiz, Gut microbiota and oral contraceptive use in overweight and obese patients with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 105 (2020) dgaa600, https://doi.org/10.1210/clinem/dgaa600.
[84] J. Huang, L. Liu, C. Chen, Y. Gao, PCOS without hyperandrogenism is associated with higher plasma trimethylamine N-oxide levels, BMC Endocr. Disord. 20 (2020) 3, https://doi.org/10.1186/s12902-019-0486-9.
[85] L. Sun, W. Hu, Q. Liu, Q. Hao, B. Sun, Q. Zhang, S. Mao, J. Qiao, X. Yan, Metabonomics reveals plasma metabolic changes and inflammatory marker in polycystic ovary syndrome patients, J. Proteome Res. 11 (2012) 2937–2946, https://doi.org/10.1021/pr3000317.
[86] R.E. Ley, P.J. Turnbaugh, S. Klein, J.I. Gordon, Microbial ecology: human gut microbes associated with obesity, Nature 444 (2006) 1022–1023, https://doi.org/10.1038/4441022a.
[87] P.C. Moran, F. Shanahan, Gut microbiota and obesity: role in aetiology and potential therapeutic target, Best. Pract. Res. Clin. Gastroenterol. 28 (2014) 585–597, https://doi.org/10.1016/j.bpg.2014.07.005.
[88] P.J. Turnbaugh, M. Hamady, T. Yatsunenko, B.L. Cantarel, A. Duncan, R.E. Ley, M.L. Sogin, W.J. Jones, B.A. Roe, J.P. Affourtit, M. Egholm, B. Henrissat, A. C. Heath, R. Knight, J.I. Gordon, A core gut microbiome in obese and lean twins, Nature 457 (2009) 480–484, https://doi.org/10.1038/nature07540.
[89] N. Larsen, F.K. Vogensen, F.W. van den Berg, D.S. Nielsen, A.S. Andreasen, B. K. Pedersen, W.A. Al-Soud, S.J. Sørensen, L.H. Hansen, M. Jakobsen, Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults, PLoS One 5 (2010) 9085, https://doi.org/10.1371/journal.pone.0009085.
[90] C.A. Lozupone, J.I. Stombaugh, J.I. Gordon, J.K. Jansson, R. Knight, Diversity, stability and resilience of the human gut microbiota, Nature 489 (2012) 220–230, https://doi.org/10.1038/nature11550.
[91] F. D'Aversa, A. Tortora, G. Ianiro, F.R. Ponziani, B.E. Annicchiarico, A. Gasbarrini, Gut microbiota and metabolic syndrome, Intern. Emerg. Med. 8 (Suppl. 1) (2013) S11–S15, https://doi.org/10.1007/s11739-013-0916-z.
[92] A. Everard, P.D. Cani, Diabetes, obesity and gut microbiota, Best. Pract. Res. Clin. Gastroenterol. 27 (2013) 73–83, https://doi.org/10.1016/j.bpg.2013.03.007.
[93] F.H. Karlsson, V. Tremaroli, I. Nookaew, G. Bergström, C.J. Behre, B. Fagerberg, J. Nielsen, F. Bäckhed, Gut metagenome in European women with normal, impaired and diabetic glucose control, Nature 498 (2013) 99–103, https://doi.org/10.1038/nature12198.
[94] J.G. Markle, D.N. Frank, S. Mortin-Toth, C.E. Robertson, L.M. Feazel, U. Rolle-Kampczyk, M. von Bergen, K.D. McCoy, A.J. Macpherson, J.S. Danska, Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity, Science (80-) 339 (2013) 1084–1088, https://doi.org/10.1126/science.1233521.
[95] J. Qin, Y. Li, Z. Cai, S. Li, J. Zhu, F. Zhang, S. Liang, W. Zhang, Y. Guan, D. Shen, Y. Peng, D. Zhang, Z. Jie, W. Wu, Y. Qin, W. Xue, J. Li, L. Han, D. Lu, P. Wu, J. Wang, A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (2012) 55–60, https://doi.org/10.1038/nature11450.
[96] B. Olas, Probiotics, prebiotics and synbiotics–a promising strategy in prevention and treatment of cardiovascular diseases? Int. J. Mol. Sci. 21 (2020) 9737, https://doi.org/10.3390/ijms21249737.
[97] M. Malik, T.M. Suboc, S. Tyagi, N. Salzman, J. Wang, R. Ying, M.J. Tanner, M. Kakarla, J.E. Baker, M.E. Widlansky, Lactobacillus plantarum 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease, Circ. Res. 123 (2018) 1091–1102, https://doi.org/10.1161/CIRCRESAHA.118.313565.
[98] N.J. Tripolt, B. Leber, A. Triebl, H. Köfeler, V. Stadlbauer, H. Sourij, Effect of Lactobacillus casei Shirota supplementation on trimethylamine-N-oxide levels in patients with metabolic syndrome: an open-label, randomized study, Atherosclerosis 242 (2015) 141–144, https://doi.org/10.1016/j.atherosclerosis.2015.05.005.
[99] N.E. Boutagy, A.P. Neilson, K.L. Osterberg, A.T. Smithson, T.R. Englund, B. M. Davy, M.W. Hulver, K.P. Davy, Probiotic supplementation and trimethylamine-N-oxide production following a high-fat diet, Obesity 23 (2015) 2357–2363, https://doi.org/10.1002/oby.21212.
[100] L. Qiu, X. Tao, H. Xiong, J. Yu, H. Wei, Lactobacillus plantarum ZDY04 exhibits a strain-specific property of lowering TMAO via the modulation of gut microbiota in mice, Food Funct. 9 (2018) 4299–4309, https://doi.org/10.1039/c8fo00349a.
[101] G.C. Tenore, D. Caruso, G. Buonomo, M. D'Avino, R. Ciampaglia, M. Maisto, C. Schisano, B. Bocchino, E. Novellino, Lactofermented annurca apple puree as a functional food indicated for the control of plasma lipid and oxidative amine levels: results from a randomised clinical trial, Nutrients 11 (2019) 122, https://doi.org/10.3390/nu11010122.
[102] M. Matsumoto, Y. Kitada, Y. Shimomura, Y. Naito, Bifidobacterium animalis subsp. lactis LKM512 reduces levels of intestinal trimethylamine produced by intestinal microbiota in healthy volunteers: a double-blind, placebo-controlled study, J. Funct. Foods 36 (2017) 94–101, https://doi.org/10.1016/j.jff.2017.06.032.
[103] G. Annunziata, M. Maisto, C. Schisano, R. Ciampaglia, V. Narciso, G.C. Tenore, E. Novellino, Effects of grape pomace polyphenolic extract (Taurisolo®) in reducing tmao serum levels in humans: preliminary results from a randomized, placebo-controlled, cross-over study, Nutrients 11 (2019) 139, https://doi.org/10.3390/nu11010139.
[104] G. Annunziata, M. Maisto, C. Schisano, R. Ciampaglia, V. Narciso, S.T.S. Hassan, G.C. Tenore, E. Novellino, Effect of grape pomace polyphenols with or without pectin on TMAO serum levels assessed by LC/MS-based assay: a preliminary clinical study on overweight/obese subjects, Front. Pharmacol. 10 (2019) 575, https://doi.org/10.3389/fphar.2019.00575.