Life Sciences 235 (2019) 116843

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

N1-methylnicotinamide as a possible modulator of cardiovascular risk markers in polycystic ovary syndrome

Hamid Reza Nejabati[a,b,c], Nasser Samadi[a], Leila Roshangar[b], Mohammad Nouri[b,d,e,*]

[a] Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences Tabriz, Iran
[e] Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Keywords:
Polycystic ovary syndrome
MNAM
Cardiovascular risk markers

ABSTRACT

Polycystic ovary syndrome (PCOS) is a multifactorial disease, which is resulted from the three common features, hyperandrogenism (HA), ovulatory dysfunction (OD), and polycystic ovarian morphology (PCOM). The environmental inducers (like diet, lifestyle, chemicals, drugs, and ageing) and cardiometabolic risk factors (such as insulin resistance, metabolic syndrome, and obesity) are involved in pathogenesis of PCOS. The growing body of evidence has been shown that there exist endothelial cell dysfunction (ECD) in women with PCOS independent of age, weight and metabolic abnormalities. It has been shown that a broad spectrum of cardiovascular risk markers are involved in ECD- induced cardiovascular disease. It is well described that there are no worldwide treatments for PCOS and all of pharmacological treatments are off -label without any approval. MNAM is one of potential therapeutic factor, which produced by nicotinamide *N*-methyltransferase (NNMT) via consumption of S-adenosyl methionine (SAM) and nicotinamide. Only one study has shown higher expression of its producer enzyme, NNMT, in the cumulus cells of women with PCOS. Therefore, we reviewed beneficial effects of MNAM on modulation of cardiometabolic risk factors, which are associated to PCOS and try to describe possible mode of action of MNAM in the regulation of these markers.

1. Introduction

1.1. PCOS

Polycystic ovary syndrome (PCOS) is a multifactorial disease, which is resulted from the three common features, hyperandrogenism (HA), ovulatory dysfunction (OD), and polycystic ovarian morphology (PCOM) [1]. The diagnosis of PCOS is based on the presenting two of the three features of disease [2].

When it comes to aetiology, PCOS could be considered as a multigenic disease in which the combination of various genetic variants collaborates with environmental stimuli to generate diverse PCOS phenotypes [3]. These environmental inducers are diet, lifestyle, chemicals, drugs, and ageing that may lead to PCOS [3]. It is well known that HA could affect body fat dispersion, insulin resistance, and other cardiometabolic risk factors and thereby lead to cardiovascular disease ([4–6]. It has been hypothesized that the wrong circle of androgen excess could favor abdominal adiposity via stimulation of insulin resistance, which thereby assists the progress of androgen secretion in women with PCOS [2,7].

PCOS could accompany the cardiometabolic aberrancies that thereby increase risk of cardiovascular disease [4]. So, it has been suggested some cardiometabolic risk factors such as insulin resistance, and metabolic syndrome, [4]. Insulin resistance is a common characteristic of PCOS that may present in tissues like adipose tissue, liver, and muscles [1]. It could be responsible for about 40% of type 2 diabetes in patients with PCOS ([4,8]. Insulin resistance can cause metabolic syndrome (MS), which has four features - hyperglycemia, central obesity, dyslipidemia and hypertension [9,10]. The prevalence of MS is two or three times higher in patients with PCOS compared to healthy individuals [11,12].

1.2. Cardiovascular disease and PCOS

The growing body of evidence has been shown that there is confirmed relationship between PCOS and vascular disorders and also there

* Corresponding author at: Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail address: nourimd@yahoo.com (M. Nouri).

https://doi.org/10.1016/j.lfs.2019.116843
Received 14 July 2019; Received in revised form 20 August 2019; Accepted 5 September 2019
Available online 05 September 2019
0024-3205/

exist endothelial cell dysfunction (ECD) in women with PCOS independent of age, weight and metabolic abnormalities ([4,13–15]. It has also been reported higher intima-media thickness of carotid arteries in women with PCOS and it can be indicator of higher risk of atherosclerosis in these patients [16]. The numerous studies have demonstrated higher risk of cardiovascular disease in PCOS patients especially when have dyslipidemia, hypertension, obesity, diabetes, hypercholesterolemia, hypertriglyceridemia, infertility, history of using oral contraceptives as one study reported ([4,17–19] that the incidence of coronary artery disease is 63% higher in PCOS patients compared with healthy ones [17]. However, there are some studies that declared insignificant results regarding the association of cardiovascular problems and PCOS [20–22]. Therefore, it is valuable to study potential risk factors and markers of cardiometabolic risks in PCOS cases.

1.3. Cardiovascular markers in PCOS

The various studies have been indicated that some cardiovascular risk markers could be associated to PCOS [23]. As almost all of these markers are related to ECD that could lead to cardiovascular disease and until now two major theories for ECD-induced vascular disease have been proposed; “Response to injury” and “inflammation” [23,24].

1.3.1. Endothelial cell dysfunction (ECD)

Vascular endothelial cells have permeable and selective characteristics, which involve in transfer of fluids and macromolecules [25–27]. In addition, they exert various roles such as anticoagulation, production of cytokines, hormones, vasoactive mediators, and growth factors [26].

ECD could be considered as a major cause of cardiovascular disease such as atherosclerosis [28]. “Response-to-Injury Hypothesis” is defined as initiation of atherogenesis in response to intense injury to endothelium via broad spectrum of reasons including oxidized cholesterol, hyperglycemia, cigarette smoke, hyperhomocysteinemia [29]. Nitric oxide (NO) is one of the major endothelial-derived factors that is produced by endothelial NO synthase (eNOS) [30] as *eNOS*-knockout causes thrombosis, and manifestations of atherosclerosis ([31,32]. Moreover, hypercholesterolemia can stimulate endothelial cells to secrete greater NO in response to ECD [26,28,33].

In the pathological circumstances such as atherosclerosis, NO bioavailability is diminished as compensatory increase of eNOS could cause eNOS uncoupling, that defined as changeover of eNOS from NO generating enzyme to superoxide producing molecule [34–37]. It has been proposed wide range of mechanisms involved in eNOS uncoupling like elevation of asymmetrical dimethylarginine (ADMA) —endogenous NOS inhibitor—inadequecy of tetrahydrobiopterin (BH4), and reduction of L-arginine [34,36].

1.3.2. Proinflammatory activation and ECD

It is well documented that infection and injury (type I inflammatory response) and damages, pathogens, and inflammatory cytokines (type II inflammatory response) could stimulate endothelial cells to changing the phenotype of endothelial cells to proinflammatory condition. [26,38–41]. The close association between inflammation and atherosclerosis has been confirmed by many studies [41–43] as lead to alteration of old idea from “Response-to-Injury Hypothesis” to “Inflammatory Hypothesis of Atherothrombosis” [44–47]. It has been shown that a broad spectrum of cardiovascular risk markers are involved in ECD- induced cardiovascular disease including asymmetric dimethylarginine (ADMA), CRP (C - reactive protein), homocysteine, plasminogen activator inhibitor-I (PAI-I), interleukin-6 (IL-6), and visfatin [26,39–41].

1.3.3. ADMA

ADMA is known as endogenous NOS inhibitor, which is produced through post-translation modification (PTM) [48]. The two enzymes—protein-arginine methyltransferases (PRMTs) and dimethylarginin dimethylaminohydrolase (DDAH) — are involved in its biosynthesis and catabolism, respectively [49,50]. Also, ADMA could reduce NO bioavailibity and thereby affect vascular biology as its roles in some diseases like cardiovascular disease and diabetes have been reported [23].

It has been indicated that oxidative stress and oxidized LDL increase ADMA accumulation via elevation of PRMT and reduction of DDAH [50]. Higher levels of ADMA have been reported in atherosclerosis and considered as independent marker of ECD and cardiovascular disease [51]. Since circulating ADMA levels are increased in women with PCOS, these patients are possibly at the risk of cardiovascular complications. Furthermore, the positive and significant correlations were reported between ADMA levels and CRP, BMI, and fasting insulin [52]. Therefore, it is obvious that ADMA could be a reliable candidate for evaluation of cardiometabolic risks in PCOS patients.

1.3.4. CRP

CRP has been considered as an inflammation biomarker, owing to its higher levels in inflammatory conditions like obesity, PCOS, and cardiovascular disease ([23,53]. In addition, CRP impairs fibrinolysis and homeostasis of endothelial cells via decrease the eNOS expression, NO production, destabilization of prostacyclin, and elevation of PAI-1, and thereby could increase risk of cardiovascular thrombosis and predict future cardiovascular events ([23,54–56].

1.3.5. Homocysteine

Hyperhomocysteinemia provokes atherosclerosis via oxidative stress, endothelial dysfunction, and thrombosis and led the researchers to consider it as an independent risk marker for development of atherosclerosis [23,57]. Some studies have confirmed higher levels of homocysteine in PCOS patients, independent of insulin resistance, HA, and obesity [58]. On the other hand, it is believed that deteriorative effects of ADMA in ECD, at least partly is through homocysteine, because the main source of methylation in ADMA, is S-adenosylmethionine, which is mediator of homocystein metabolism [59]. Since ADMA and homocysteine are elevated in PCOS patients and also are the main players of ECD-induce cardiovascular problems, they could be possible candidate biomarkers for assessment of cardiovascular risks in women with PCOS [23,58].

1.3.6. PAI-I

PAI-1 inhibits urokinase plasminogen activator and tPA, and by that blocks fibrinolysis [60]. The growing body of evidence indicates that different kind of factors may increase adipose tissue-derived PAI-1 levels such as transforming necrosis factor (TNF-a), angiotensin-II, transforming growth factor- β (TGF-β), insulin, and leptin [61].

The accumulating body of literature shows the elevated serum levels of PAI-1 in different pathological states like obesity, type 2 diabetes, insulin resistance, and PCOS [62]. Due to association between higher PAI and insulin resistance in PCOS patients, it could be consider as predicator of insulin resistance and also cardiometabolic risks in PCOS

1.3.7. Visfatin

It is well recorded that visfatin stimulates vascular inflammation as a result of changing the expression of inflammatory cytokines like TNF-a, and IL-6 [23]. The relationship between circulating visfatin levels and PCOS is controversial and inconsistent as higher levels of visfatin in PCOS patients have been noted and even consider it as specific markers of insulin sensitivity in PCOS [63]. Furthermore, it was reported that circulating visfatin levels are significantly correlated to CRP and could predicate ECD in women with PCOS [63,64]. The higher gene expression of visfatin in adipose tissue of women with PCOS and its correlation with insulin resistance and BMI have been reported [63,65]. On the other hand, some studies declared that there are no significant differences between circulating visfatin levels and PCOS [23,63].

2. Management and treatment

It is well described that there is no worldwide treatment for PCOS and all of pharmacological treatments are off -label without any approval [2]. Nowadays, almost all of treatments have focused on the three important facets of PCOS including HA, infertility and metabolic dysfunction [1,2].

2.1. Hyperandrogenism(HA)

It has been thoroughly reported that using oral contraceptive pills (OCPs) and anti-androgens like flutamide, spironolactone, finasteride, and cyproterone acetate are recommended for treatment of HA in PCOS patients ([1,2,66,67]. It is also known that estrogen and progestin of OCPs reduce androgen levels through induction of hepatic sex hormone binding globulin (SHBG) and direct androgen biosynthesis, respectively ([1,68].

2.2. Infertility

It has been confirmed that reversing the ovulation is a first-line treatment of infertility in PCOS patients [1]. The main target of such treatments is modulation of insufficient estrogen feedback using drugs like clomiphene citrate (selective estrogen receptor antagonist), and letrozole (aromatase inhibitor) ([1,69]. The second-line treatment is gonadotropin therapy, which includes LH, FSH, or both and low dose should be used to reduce risk of multiple pregnancies and OHSS (ovarian hyperstimulation syndrome) [70,71]. Although gonadotropin therapy has higher pregnancy rates compared to first-line treatments [72], higher costs and vital risks such as OHSS forwarded clinicians to first line treatment [1]. Third-line treatment is in vitro fertilization (IVF), as when applied to PCOS women; their pregnancy rates are similar to other women with different indications ([1,73]. Two common corrections are suggested for IVF procedure in PCOS patients; in vitro maturation (IVM) of immature oocytes (process without gonadotropin therapy) and freeze-all policy (transfer of cryopreserved embryos in a following embryo transfer cycle) ([1,74]. It has been shown that pregnancy rate in IVM procedure is lower than conventional IVF; however, non-use of gonadotropin during IVM could eliminate OHSS risks in these patients [75,76]. Furthermore, PCOS patients in freeze-all process have better pregnancy rates compared to fresh embryo transfer cycle [77].

2.3. Metabolic dysfunction

A growing body of literature has suggested some treatment options for metabolic dysfunctions such as lifestyle modification, bariatric surgery, and medical treatment [1].

It has been demonstrated that lifestyle alteration is an initial treatment of metabolic dysfunction in PCOS and by that promotes fertility outcomes like ovulation and pregnancy rates ([78,79]. Furthermore, physical activity decreases cardiovascular risks in PCOS patients through increase insulin sensitivity, and improvement of body fat dispersion [80]. Bariatric surgery is another reliable treatment option, in which its actions have confirmed in the context of cardiovascular risks in PCOS [81].

The wide spectrum of drugs such as metformin, thiazolidinediones, inositol isomers, orlistat, vitamin D, and statins are used for treatment of metabolic dysfunction [1]. Metformin is an approved drug for type II diabetes as could improve insulin sensitivity in women with PCOS [82]. Furthermore, it has been demonstrated that beneficial effects of metformin plus life style modifications on the reduction of BMI and subcutaneous adipose tissue in PCOS patients [83]. Due to lack of FDA-approved medications for PCOS patients, many research groups have focused on the discovery of new therapeutic factors to targeted different aspects of this multifactorial syndrome. There is no specific suggested drug to minimize cardiometabolic risk in PCOS patients. In the following sections, we introduce N1-methylnicotinamide (MNAM) as a possible therapeutic factor to modulate such risks and discuss its possible mode of actions.

3. N1-methylnicotinamide (MNAM)

MNAM is produced by nicotinamide *N*-methyltransferase (NNMT) via consumption of S-adenosyl methionine (SAM) and nicotinamide (NAM) [84]. It is confirmed that epigenome of a few cancer cells [85,86], adipose tissue [87], and human embryonic stem cells (hESCs) [88], are affected by NNMT activity owing to its need for using SAM [89]. Therefore, using MNAM instead of NAM and nicotinic acid (NA) could eliminate risk of methyl donor imbalance in targeted cells/tissues as it has been considered as a safe food according to UK food standards agency.

3.1. MNAM and hyperhomocysteinemia

It has been demonstrated that exposure of white adipose tissue (WAT) to NAM could increase homocystein secretion through activation of NNMT [87]. In contrast, MNAM, as inhibitor of NNMT activity, decreases release of homocysteine around 50% and this fact led the scientists to think that one half of homocysteine production of WAT is possibly belongs to NNMT activity [87]. Owing to positive association of NNMT with insulin resistance [90,91], higher NNMT activity of WAT could lead to hyperhomocysteinemia, and being potential linker between insulin resistance and cardiovascular disease. Hence, it can be indicator of this fact that MNAM could also possible therapeutic option for cardiometabolic risks especially in metabolic disorders such as diabetes, obesity, and PCOS.

3.2. MNAM, NO, and ADMA

It is well known that ECD is one of valuable characteristic of various disorders such as diabetes, and PCOS, which could cause cardiovascular disease ([92–95]. Among studied reasons for ECD, deterioration of NO metabolism is well documented ([94,96–98]. There has been a recent focus on trying to discovery agents with regulate NO metabolism [99]. MNAM is an anti-inflammatory and anti-thrombotic factor, which is known as guardian of cardiovascular system [100]. It has been documented that MNAM improves endothelial function through NO production and by that has beneficial effects on cardiovascular system [101].

It has been reported that MNAM increases plasma levels of arginine/ADMA in the ApoE/LDLR$^{-/-}$ mice [102]. Furthermore, study by jiang et al. indicated that MNAM boosts NO bioavailability and reduces ADMA levels through the regulation of methylation of DDAM and thereby they have proposed epigenetical mechanism for effects of MNAM on ADMA levels.

4. MNAM and PCOS

Until now, there is no report regarding the possible effects of MNAM on PCOS. Only one study has shown higher expression of its producer enzyme, NNMT, in the cumulus cells of women with PCOS [103]. The higher expression of NNMT has two different patterns; for instance, up-regulation of NNMT in WAT is deteriorative and positively correlated to insulin resistance and elevation of its product, MNAM is due to compensatory response as we mentioned that MNAM was able to reduce homocystein secretion more than 50% in adipose tissue [87]. On the other hand, the production of MNAM undergoing NNMT activity is beneficial for tissue function; dominant example of such a tissue is liver, which exerts its NNMT-dependent functions via generation of MNAM as in this tissue, MNAM does not inhibit NNMT activity but increased activity of enzyme is done for production of more MNAM not for its

feedback inhibition [104,105]. Therefore, it is not obvious that higher expression of NNMT in the cumulus cells of PCOS patients is parallel to its activity and also whether pattern of this up-regulation is similar to adipose tissue or liver. We could hypothesize that higher expression of NNMT is similar to its function in adipose tissue and higher MNAM production in cumulus cells of women with PCOS is vital for regulation of NNMT activity. However, more investigations are needed to unravel possible roles of NNMT and its product, MNAM, in PCOS patients.

5. MNAM and cardiometabolic markers in PCOS

In previous sections, we mentioned some cardiometabolic markers in PCOS patients. There are some important studies that indicate possible association of MNAM to these markers in various types of disorders like cardiovascular disease, diabetes, obesity, and so on.

It has been demonstrated that MNAM exerts its anti-thrombotic roles via reduction of PAI-1 [106]. Due to the reduction of PAI-1 by MNAM administration, it is possible that MNAM could be able to regulate thrombosis and insulin resistance at least partially through PAI-1.

Visfatin was another cardiometabolic marker that contributed to PCOS [107]. It has been indicated that NNMT knockout increases visfatin gene expression in adipose tissue of obese mice [108]. So, it is possible that MNAM could be able to modulate visfatin gene expression and by that controls insulin resistance in PCOS. However, possibility of the current comments should be investigated more precisely.

A growing body of literature indicates that higher levels of CRP are contributed to risk of cardiovascular disease via reduction of NO, and elevation of PAI-1[109–111]. Since NO, and PAI-1 are deregulated in PCOS patients and also MNAM modulates their levels [112], so MNAM can affect the CRP levels. However, it has been shown the positive correlation of CRP with MNAM in serum of patients with cardiovascular disease [113]. We believe that such a correlation may be related to compensatory elevation of MNAM following cardiac injury [104,113].

It has been shown that hyperhomocysteinemia and higher ADMA levels in PCOS patients [109]. On the other hand, MNAM reduces homocystein secretion in adipose tissue and circulating ADMA levels [104]. Although possible effect of MNAM on the modulation of these markers has not reported yet, evidence shows that one of possible beneficial roles of MNAM in women with PCOS may be partially through regulation of these factors.

Therefore, we reviewed beneficial effects of MNAM on cardiometabolic risk factors, which are associated to PCOS and try to describe possible mode of action of MNAM in the regulation of these markers.

6. Conclusion and perspectives

It has been documented that MNAM could modulate cardiometabolic markers which involved in PCOS. However, until now, there are no specific therapeutic agents, which have been designed and targeted for cardiometabolic risks in PCOS patients. The potential of MNAM in the regulation of cardiometabolic markers such as PAI-1, visfatin, CRP, and homocystein led us to hypothesize that MNAM could ameliorate endothelial-dependent cardiometabolic problems in PCOS. Since MNAM is considered as a safe food and also unlike nicotinamide, it does not have methylation imbalance problems that can change epigeneome of target cell/tissues; therefore, using this agent especially in clinical studies is required. For future studies, exact mode of actions of MNAM should be studied as these kinds of mechanistic insights could pave the way for designing targeted therapeutic agents for treatment of metabolic disorders like PCOS.

Acknowledgements and funding

The current work was supported by a Grant (Research Project Number:61867) from the Stem Cell Research Center of Tabriz University of Medical Sciences and this article was extracted from the Ph.D. thesis of Hamid Reza Nejabati.

References

[1] R. Azziz, E. Carmina, Z. Chen, A. Dunaif, J.S. Laven, R.S. Legro, et al., Polycystic ovary syndrome, Nat Rev Dis Primers 2 (2016) 16057.
[2] H.F. Escobar-Morreale, Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment, Nat Rev Endocrinol 14 (2018) 270–284.
[3] H.F. Escobar-Morreale, M. Luque-Ramírez, J.L. San Millán, The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome, Endocr. Rev. 26 (2005) 251–282.
[4] Bajuk Studen K, Pfeifer M. Cardiometabolic risk in polycystic ovary syndrome. Endocr Connect. 2018;7:R238-R51.
[5] T.M. Barber, P. Vojtechova, S. Franks, The impact of hyperandrogenism in female obesity and cardiometabolic diseases associated with polycystic ovary syndrome, Horm. Mol. Biol. Clin. Invest. 15 (2013) 91–103.
[6] J.M. Elbers, H. Asscheman, J.C. Seidell, J.A. Megens, L.J. Gooren, Long-term testosterone administration increases visceral fat in female to male transsexuals, J. Clin. Endocrinol. Metab. 82 (1997) 2044–2047.
[7] H.F. Escobar-Morreale, J.L. San Millán, Abdominal adiposity and the polycystic ovary syndrome, Trends in Endocrinology & Metabolism 18 (2007) 266–272.
[8] M.Y. Boudreaux, E.O. Talbott, K.E. Kip, M.M. Brooks, S.F. Witchel, Risk of T2DM and impaired fasting glucose among PCOS subjects: results of an 8-year follow-up, Current Diabetes Reports 6 (2006) 77–83.
[9] K. Alberti, R.H. Eckel, S.M. Grundy, P.Z. Zimmet, J.I. Cleeman, K.A. Donato, et al., Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity, Circulation 120 (2009) 1640–1645.
[10] M. Laakso, J. Kuusisto, Insulin resistance and hyperglycaemia in cardiovascular disease development, Nat. Rev. Endocrinol. 10 (2014) 293.
[11] T. Apridonidze, P.A. Essah, M.J. Iuorno, J.E. Nestler, Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 90 (2005) 1929–1935.
[12] D.A. Ehrmann, D.R. Liljenquist, K. Kasza, R. Azziz, R.S. Legro, M.N. Ghazzi, et al., Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome, The Journal of Clinical Endocrinology & Metabolism 91 (2006) 48–53.
[13] M. Kravariti, K.K. Naka, S.N. Kalantaridou, N. Kazakos, C.S. Katsouras, A. Makrigiannakis, et al., Predictors of endothelial dysfunction in young women with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 90 (2005) 5088–5095.
[14] F. Orio Jr., S. Palomba, T. Cascella, B. De Simone, S. Di Biase, T. Russo, et al., Early impairment of endothelial structure and function in young normal-weight women with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 89 (2004) 4588–4593.
[15] E.O. Talbott, D.S. Guzick, K. Sutton-Tyrrell, K.P. McHugh-Pemu, J.V. Zborowski, K.E. Remsberg, et al., Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2414–2421.
[16] M.L. Meyer, A.M. Malek, R.A. Wild, M.T. Korytkowski, E.O. Talbott, Carotid artery intima-media thickness in polycystic ovary syndrome: a systematic review and meta-analysis, Hum. Reprod. Update 18 (2012) 112–126.
[17] T. Ding, P.J. Hardiman, I. Petersen, F.F. Wang, F. Qu, G. Baio, The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis, Oncotarget 8 (2017) 96351–96358.
[18] D. Glintborg, K.H. Rubin, M. Nybo, B. Abrahamsen, M. Andersen, Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome, Cardiovasc. Diabetol. 17 (2018) 37.
[19] J. Schmidt, K. Landin-Wilhelmsen, M. Brannstrom, E. Dahlgren, Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-year controlled follow-up study, J. Clin. Endocrinol. Metab. 96 (2011) 3794–3803.
[20] C.G. Solomon, F.B. Hu, A. Dunaif, J.E. Rich-Edwards, M.J. Stampfer, W.C. Willett, et al., Menstrual cycle irregularity and risk for future cardiovascular disease, J. Clin. Endocrinol. Metab. 87 (2002) 2013–2017.
[21] E.T. Wang, P.M. Cirillo, E. Vittinghoff, K. Bibbins-Domingo, B.A. Cohn, M.I. Cedars, Menstrual irregularity and cardiovascular mortality, J. Clin. Endocrinol. Metab. 96 (2011) E114–E118.
[22] S. Wild, T. Pierpoint, H. Jacobs, P. McKeigue, Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study, Human Fertility (Cambridge, England) 3 (2000) 101–105.
[23] B.S. Nayaker, S. Thomas, S. Ramachandran, S. Loganathan, M. Sundari, K. Mala, Polycystic ovarian syndrome-associated cardiovascular complications: an overview of the association between the biochemical markers and potential strategies for their prevention and elimination, Diabetol. Metab. Syndr. 11 (2) (2017) S841–S851.
[24] H.R. Nejabati, A. Mihanfar, M. Pezeshkian, A. Fattahi, Z. Latifi, N. Safaie, et al., N1-methylnicotinamide (MNAM) as a guardian of cardiovascular system, J. Cell. Physiol. 233 (2018) 6386–6394.
[25] R. Cotran, The fine structure of the microvasculature in relation to normal and altered permeability, Physical Bases of Circulatory Transport: Regulation and Exchange, 1967, pp. 249–275.
[26] M.A. Gimbrone, G. García-Cardeña, Endothelial cell dysfunction and the pathobiology of atherosclerosis, Circ. Res. 118 (2016) 620–636.

[27] G. Palade, Fine structure of blood capillaries, J. Appl. Phys. 24 (1953) 1424–1448.
[28] M.A. Gimbrone, G. García-Cardeña, Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis, Cardiovasc. Pathol. 22 (2013) 9–15.
[29] G. RossR, The pathogenesis of atherosclerosis (first of two parts), N. Engl. J. Med. 295 (1976) 369–377.
[30] K. Nishida, D. Harrison, J. Navas, A. Fisher, S. Dockery, M. Uematsu, et al., Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase, J. Clin. Invest. 90 (1992) 2092–2096.
[31] P.L. Huang, Lessons learned from nitric oxide synthase knockout animals, Semin. Perinatol. (2000) 87–90 Elsevier.
[32] P.J. Kuhlencordt, R. Gyurko, F. Han, M. Scherrer-Crosbie, T.H. Aretz, R. Hajjar, et al., Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice, Circulation 104 (2001) 448–454.
[33] L.V. d'Uscio, L.A. Smith, Z.S. Katusic, Hypercholesterolemia impairs endothelium-dependent relaxations in common carotid arteries of apolipoprotein E-deficient mice, Stroke 32 (2001) 2658–2664.
[34] U. Förstermann, W.C. Sessa, Nitric oxide synthases: regulation and function, Eur. Heart J. 33 (2011) 829–837.
[35] H. Li, U. Forstermann, Prevention of atherosclerosis by interference with the vascular nitric oxide system, Curr. Pharm. Des. 15 (2009) 3133–3145.
[36] H. Li, U. Förstermann, Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease, Curr. Opin. Pharmacol. 13 (2013) 161–167.
[37] K. Chalupsky, H. Cai, Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9056–9061.
[38] H.R. Nejabati, Z. Latifi, T. Ghasemnejad, A. Fattahi, M. Nouri, Placental growth factor (PlGF) as an angiogenic/inflammatory switcher: lesson from early pregnancy losses, Gynecol. Endocrinol. 33 (2017) 668–674.
[39] J.S. Pober, R.S. Cotran, Cytokines and endothelial cell biology, Physiol. Rev. 70 (1990) 427–451.
[40] J.S. Pober, R.S. Cotran, The role of endothelial cells in inflammation, Transplantation 50 (1990) 537–544.
[41] J.S. Pober, W.C. Sessa, Evolving functions of endothelial cells in inflammation, Nat. Rev. Immunol. 7 (2007) 803.
[42] P. Libby, J.M. Ordovas, K.R. Auger, A. Robbins, L. Birinyi, C. Dinarello, Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells, Am. J. Pathol. 124 (1986) 179.
[43] J.M. Munro, J.S. Pober, R.S. Cotran, Tumor necrosis factor and interferon-gamma induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papio anubis, Am. J. Pathol. 135 (1989) 121.
[44] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685–1695.
[45] G.K. Hansson, P. Libby, The immune response in atherosclerosis: a double-edged sword, Nat. Rev. Immunol. 6 (2006) 508.
[46] P. Libby, P.M. Ridker, A. Maseri, Inflammation and atherosclerosis, Circulation 105 (2002) 1135–1143.
[47] R. Ross, Atherosclerosis—an inflammatory disease, N. Engl. J. Med. 340 (1999) 115–126.
[48] S.A. Fickling, D.P. Holden, J.E. Cartwright, S.S. Nussey, P. Vallance, G.S. Whitley, Regulation of macrophage nitric oxide synthesis by endothelial cells: a role for NG,NG-dimethylarginine, Acta Physiol. Scand. 167 (1999) 145–150.
[49] M.T. Bedford, S.G. Clarke, Protein arginine methylation in mammals: who, what, and why, Mol. Cell 33 (2009) 1–13.
[50] K. Sydow, T. Munzel, ADMA and oxidative stress, Atheroscler. Suppl. 4 (2003) 41–51.
[51] B.O. Pamuk, A.N. Torun, M. Kulaksizoglu, D. Ertugrul, O. Ciftci, S. Kulaksizoglu, et al., Asymmetric dimethylarginine levels and carotid intima-media thickness in obese patients with polycystic ovary syndrome and their relationship to metabolic parameters, Fertil. Steril. 93 (2010) 1227–1233.
[52] D. Heutling, H. Schulz, I. Nickel, J. Kleinstein, P. Kaltwasser, S. Westphal, et al., Asymmetrical dimethylarginine, inflammatory and metabolic parameters in women with polycystic ovary syndrome before and after metformin treatment, J. Clin. Endocrinol. Metab. 93 (2008) 82–90.
[53] E. Paffen, H.L. Vos, R.M. Bertina, C-reactive protein does not directly induce tissue factor in human monocytes, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 975–981.
[54] W.B. Kannel, Overview of hemostatic factors involved in atherosclerotic cardiovascular disease, Lipids 40 (2005) 1215–1220.
[55] U. Singh, S. Devaraj, I. Jialal, C-reactive protein decreases tissue plasminogen activator activity in human aortic endothelial cells: evidence that C-reactive protein is a procoagulant, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2216–2221.
[56] O. Yousuf, B.D. Mohanty, S.S. Martin, P.H. Joshi, M.J. Blaha, K. Nasir, et al., High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? J. Am. Coll. Cardiol. 62 (2013) 397–408.
[57] K.S. McCully, R.B. Wilson, Homocysteine theory of arteriosclerosis, Atherosclerosis 22 (1975) 215–227.
[58] K.A. Toulis, D.G. Goulis, G. Mintziori, E. Kintiraki, E. Eukarpidis, S.A. Mouratoglou, et al., Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome, Hum. Reprod. Update 17 (2011) 741–760.
[59] M.C. Stuhlinger, O. Stanger, Asymmetric dimethyl-L-arginine (ADMA): a possible link between homocyst(e)ine and endothelial dysfunction, Curr. Drug Metab. 6 (2005) 3–14.
[60] H. Kaji, Adipose tissue-derived plasminogen activator inhibitor-1 function and regulation, Comprehensive Physiology 6 (2011) 1873–1896.
[61] M. Iglesias-Osma, M. Torres, M. García-Barrado, J. Moratinos, Adipocytokines: implications in the prognosis and drug treatment of cardiovascular diseases, Revista Clinica Espanola 208 (2008) 239–246.
[62] I. Mertens, L.V. Gaal, Obesity, haemostasis and the fibrinolytic system, Obes. Rev. 3 (2002) 85–101.
[63] P.M. Spritzer, S.B. Lecke, F. Satler, D.M. Morsch, Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome, Reproduction 149 (2015) R219–R227.
[64] C.E. Pepene, Evidence for visfatin as an independent predictor of endothelial dysfunction in polycystic ovary syndrome, Clin. Endocrinol. 76 (2012) 119–125.
[65] K.-M. Seow, J.-L. Hwang, P.-H. Wang, L.-T. Ho, C.-C. Juan, Expression of visfatin mRNA in peripheral blood mononuclear cells is not correlated with visfatin mRNA in omental adipose tissue in women with polycystic ovary syndrome, Hum. Reprod. 26 (2011) 2869–2873.
[66] O. Koulouri, G.S. Conway, A systematic review of commonly used medical treatments for hirsutism in women, Clin. Endocrinol. 68 (2008) 800–805.
[67] P. Moghetti, F. Tosi, A. Tosti, C. Negri, C. Misciali, F. Perrone, et al., Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial, The Journal of Clinical Endocrinology & Metabolism 85 (2000) 89–94.
[68] R. Mathur, O. Levin, R. Azziz, Use of ethinylestradiol/drospirenone combination in patients with the polycystic ovary syndrome, Ther. Clin. Risk Manag. 4 (2008) 487.
[69] R.S. Legro, R.G. Brzyski, M.P. Diamond, C. Coutifaris, W.D. Schlaff, P. Casson, et al., Letrozole versus clomiphene for infertility in the polycystic ovary syndrome, N. Engl. J. Med. 371 (2014) 119–129.
[70] D. Polson, H. Mason, M. Saldahna, S. Franks, Ovulation of a single dominant follicle during treatment with low-dose pulsatile follicle stimulating hormone in women with polycystic ovary syndrome, Clin. Endocrinol. 26 (1987) 205–212.
[71] M.A. Sagle, D. Hamilton-Fairley, D.S. Kiddy, S. Franks, A comparative, randomized study of low-dose human menopausal gonadotropin and follicle-stimulating hormone in women with polycystic ovarian syndrome, Fertil. Steril. 55 (1991) 56–60.
[72] R. Homburg, M.-L. Hendriks, T.E. König, R.A. Anderson, A.H. Balen, M. Brincat, et al., Clomifene citrate or low-dose FSH for the first-line treatment of infertile women with anovulation associated with polycystic ovary syndrome: a prospective randomized multinational study, Hum. Reprod. 27 (2011) 468–473.
[73] M. Nahuis, N. Kose, N. Bayram, H. Van Dessel, D. Braat, C. Hamilton, et al., Long-term outcomes in women with polycystic ovary syndrome initially randomized to receive laparoscopic electrocautery of the ovaries or ovulation induction with gonadotrophins, Hum. Reprod. 26 (2011) 1899–1904.
[74] K.Y. Cha, H.M. Chung, D.R. Lee, H. Kwon, M.K. Chung, L.S. Park, et al., Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization–embryo transfer, Fertil. Steril. 83 (2005) 1461–1465.
[75] C. Siristatidis, T.N. Sergentanis, P. Vogiatzi, P. Kanavidis, C. Chrelias, N. Papantoniou, et al., In vitro maturation in women with vs. without polycystic ovarian syndrome: a systematic review and meta-analysis, PLoS One 10 (2015) e0134696.
[76] M. Walls, T. Hunter, J. Ryan, J. Keelan, E. Nathan, R. Hart, In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes, Hum. Reprod. 30 (2014) 88–96.
[77] Y. Shi, D. Wei, X. Liang, Y. Sun, J. Liu, Y. Cao, et al., Live birth after fresh embryo transfer vs elective embryo cryopreservation/frozen embryo transfer in women with polycystic ovary syndrome undergoing IVF (FreFro-PCOS): study protocol for a multicenter, prospective, randomized controlled clinical trial, Trials 15 (2014) 154.
[78] E. Carmina, PCOS: metabolic impact and long-term management, Minerva Ginecol. 64 (2012) 501–505.
[79] L.J. Moran, M.L. Misso, R.A. Wild, R.J. Norman, Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis, Hum. Reprod. Update 16 (2010) 347–363.
[80] C.L. Harrison, C.B. Lombard, L.J. Moran, H.J. Teede, Exercise therapy in polycystic ovary syndrome: a systematic review, Hum. Reprod. Update 17 (2010) 171–183.
[81] D. Skubleny, N.J. Switzer, R.S. Gill, M. Dykstra, X. Shi, M.A. Sagle, et al., The impact of bariatric surgery on polycystic ovary syndrome: a systematic review and meta-analysis, Obes. Surg. 26 (2016) 169–176.
[82] T. Tang, J.M. Lord, R.J. Norman, E. Yasmin, A.H. Balen, Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility, Cochrane Database Syst. Rev. 11 (2017).
[83] N. Naderpoor, S. Shorakae, B. de Courten, M.L. Misso, L.J. Moran, H.J. Teede, Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis, Hum. Reprod. Update 21 (2015) 560–574.
[84] S. Aksoy, C.L. Szumlanski, R.M. Weinshilboum, Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization, J. Biol. Chem. 269 (1994) 14835–14840.
[85] K. Palanichamy, S. Kanji, N. Gordon, K. Thirumoorthy, J.R. Jacob, K.T. Litzenberg, et al., NNMT silencing activates tumor suppressor PP2A, inactivates oncogenic STKs, and inhibits tumor forming ability, Clin. Cancer Res. 23 (2017) 2325–2334.
[86] O.A. Ulanovskaya, A.M. Zuhl, B.F. Cravatt, NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink, Nat. Chem. Biol. 9 (2013) 300–306.
[87] M. Riederer, W. Erwa, R. Zimmermann, S. Frank, R. Zechner, Adipose tissue as a source of nicotinamide N-methyltransferase and homocysteine, Atherosclerosis 204 (2009) 412–417.
[88] H. Sperber, J. Mathieu, Y. Wang, A. Ferreccio, J. Hesson, Z. Xu, et al., The metabolome regulates the epigenetic landscape during naive-to-primed human

embryonic stem cell transition, Nat. Cell Biol. 17 (2015) 1523–1535.
[89] H.C. Kim, M. Mofarrahi, T. Vassilakopoulos, F. Maltais, I. Sigala, R. Debigare, et al., Expression and functional significance of nicotinamide N-methyl transferase in skeletal muscles of patients with chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 181 (2010) 797–805.
[90] A. Kannt, A. Pfenninger, L. Teichert, A. Tönjes, A. Dietrich, M.R. Schön, et al., Association of nicotinamide-N-methyltransferase mRNA expression in human adipose tissue and the plasma concentration of its product, 1-methylnicotinamide, with insulin resistance, Diabetologia 58 (2015) 799–808.
[91] M. Liu, L. Li, J. Chu, B. Zhu, Q. Zhang, X. Yin, et al., Serum N 1-methylnicotinamide is associated with obesity and diabetes in Chinese, The Journal of Clinical Endocrinology & Metabolism 100 (2015) 3112–3117.
[92] Austin MA. Plasma triglyceride as a risk factor for cardiovascular disease. The Canadian journal of cardiology. 1998;14:14B-7B.
[93] A.M. Gotto, Triglyceride as a risk factor for coronary artery disease, Am. J. Cardiol. 82 (1998) 22–25.
[94] P. KaŸmierczak, C. Watala, E.M. S3ominska, R.T. Smoleński, P.M. Pisulewski, J. Adamus, et al., 1-Methylnicotinamide (MNA) prevents endothelial dysfunction in hypertriglyceridemic and diabetic rats, Pharmacol. Rep. 60 (2008) 127–138.
[95] J.H. O'Keefe, D.S. Bell, Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor, Am. J. Cardiol. 100 (2007) 899–904.
[96] P.O. Bonetti, L.O. Lerman, A. Lerman, Endothelial dysfunction: a marker of atherosclerotic risk, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 168–175.
[97] S. Chlopicki, R.J. Gryglewski, Angiotensin converting enzyme (ACE) and HydroxyMethylGlutaryl-CoA (HMG-CoA) reductase inhibitors in the forefront of pharmacology of endothelium, Pharmacol. Rep. 57 (2005) 86.
[98] S. Chlopicki, J. Swies, A. Mogielnicki, W. Buczko, M. Bartus, M. Lomnicka, et al., 1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway, Br. J. Pharmacol. 152 (2007) 230–239.
[99] A.-M. Kampoli, D. Tousoulis, C. Tentolouris, C. Stefanadis, Novel agents targeting nitric oxide, Curr. Vasc. Pharmacol. 10 (2012) 61–76.
[100] P. Pissios, Nicotinamide N-Methyltransferase: More Than a Vitamin B3 Clearance Enzyme, Trends Endocrinol. Metab. 28 (5) (2017) 340–353.
[101] C. Watała, P. Kaźmierczak, M. Dobaczewski, T. Przygodzki, M. Bartuś, M. Łomnicka, et al., Anti-diabetic effects of 1-methylnicotinamide (MNA) in streptozocin-induced diabetes in rats, Pharmacol. Rep. 61 (2009) 86–98.
[102] A. Bar, M. Olkowicz, U. Tyrankiewicz, E. Kus, K. Jasinski, R.T. Smolenski, et al., Functional and biochemical endothelial profiling in vivo in a murine model of endothelial dysfunction; comparison of effects of 1-methylnicotinamide and angiotensin-converting enzyme inhibitor, Front. Pharmacol. 8 (2017).
[103] S. Kenigsberg, Y. Bentov, V. Chalifa-Caspi, G. Potashnik, R. Ofir, O.S. Birk, Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients, Mol. Hum. Reprod. 15 (2009) 89–103.
[104] H.R. Nejabati, A. Mihanfar, M. Pezeshkian, A. Fattahi, Z. latifi, N. Safaie, et al., N1-methylnicotinamide (MNAM) as a guardian of cardiovascular system, J. Cell. Physiol. 233 (10) (2018) 6386–6394.
[105] P. Pissios, Nicotinamide N-methyltransferase: more than a vitamin B3 clearance enzyme, Trends Endocrinol. Metab. 28 (2017) 340–353.
[106] A. Mogielnicki, K. Kramkowski, L. Pietrzak, W. Buczko, N-methylnicotinamide inhibits arterial, J. Physiol. Pharmacol. 58 (2007) 515–527.
[107] Spritzer PM, Lecke SB, Satler F, Morsch DM. Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome. Reproduction. 2015b;149:R219-R27.
[108] D. Kraus, Q. Yang, D. Kong, A.S. Banks, L. Zhang, J.T. Rodgers, et al., Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity, Nature 508 (2014) 258.
[109] M. Jacewicz-Swiecka, I. Kowalska, Polycystic ovary syndrome and the risk of cardiometabolic complications in longitudinal studies, Diabetes Metab. Res. Rev. 34 (2018) e3054.
[110] J. Steffel, T.F. Lüscher, Predicting the Development of Atherosclerosis, Am Heart Assoc, 2009.
[111] H. Teoh, A. Quan, F. Lovren, G. Wang, S. Tirgari, P.E. Szmitko, et al., Impaired endothelial function in C-reactive protein overexpressing mice, Atherosclerosis 201 (2008) 318–325.
[112] H. Hauner, Secretory factors from human adipose tissue and their functional role, Proc. Nutr. Soc. 64 (2005) 163–169.
[113] M. Liu, J. Chu, Y. Gu, H. Shi, R. Zhang, L. Wang, et al., Serum N 1-methylnicotinamide is associated with coronary artery disease in Chinese patients, J. Am. Heart Assoc. 6 (2017) e004328.