Clement A, et al., Arch Clin Biomed Res 2023
DOI:10.26502/acbr.50170351

SHWHFWLRQVRYHDOLHQLWHDWKLQWHDWKLQHYDWHG IRORIROGGFLZKSRRRRFWHDW\WLYHYLGHQW that MTHFR SNPs create a source of abnormal regulation LQDFDEUHFFLQJKLVIHDWXUHKDVDVREHHQFRQUPHG in the testis [21,23,24]. Mitochondria, of maternal origin in the embryo, need to have protection against HHcy, especially as cellular Hcy transport is shared by transporters for most of the classical amino acids. Notably, Hcy competes with methionine for transport in the embryo [1], albeit with a 4.5 WLPHVHZHUDvQLWFGHFUHDWKLHHQDUPHWKLDWLRQ index.

The mechanism of Hcy regulation in women can be explained via two major pathways (Figure 1):

1. Estrogens stimulate BHMT (Betaine Homocysteine Methyl Transferase) activity [42,44], increasing betaine availability by upregulation of Phosphoethanolamine Methyl Transferase (PEMT); this pathway removes Hcy and allows regeneration of methionine.
2. Estrogens also stimulate activity of the CBS (Cystathionine Beta Synthase) pathway: "the homocysteine concentration varies inversely with estradiol concentration" [43] leading to the formation of cysteine and then glutathione from Hcy. This facilitates better protection against oxidative stress IRU HSLJHQHWLF PDUNV EXW FDQ LPSDLUHFW HSLJHQHWLF methyl setting during a critical period of maturation.

Estrogens increase the activity of the CBS (cystathionine beta synthase) and the BHMT (%HWDLQH RPRFVWHLQH 0HWKLRQLQHUDVH) pathways, decreasing the Hcy. In FRQWUDU\WKLV SDWKZD\ is inhibited by androgen [45] SAM synthesis is strongly regulated: it increases also the CBS pathway, allowing an equilibrium between the MS (methionine synthase) and the CBS pathways. But it also regulates negatively the BHMT and the MTHFR activity. Elevated activity of CBS reduces Hcy but may reduce the methylation process as Methionine then SAM synthesis is reduced.

FA (folic acid) has a low capacity to enter the folate FHOO)5 DFWLYLW\LV SRRUO\HIILFLHQW LQ KXPDQ >@WR form THF (tetrahydrofolate). In MTHFR SNP carriers, the capacity to metabolize FA is low: these two bottle necks lead to UMFA (Un metabolized Folic acid) elevation. These 2 metabolic steps are then strongly inhibited by a Michaelis DQG 0HQWHQ HIIHFW 6SDQG F\DUH ERWK VWURQJ LQKLELWRUV methylation processes, not depending of gender

Two secondary systems of regulation are also active: SAM normally exerts negative feedback (retro control) on two enzymatic reactions, MTHFR and BHMT [44]. Low concentrations of SAM, associated with high estrogen levels result in high BHMT and CBS activity, allowing removal of Hcy mainly towards the formation of Cysteine. However, this upheaval has certain limits: the CBS pathway is usually poorly expressed in the oocyte/ early embryo before the stage of zygote genomic activation. There is clearly a risk WKDWKHHDUO\HPEU\RZLOO XIIHUD VKRUWDJH RI PHWKLRQL then SAM, especially in carriers of the MTHFR 6777SNP. This isoform associates with lower steroids concentration ERWK DQGURJHQV DQG HVWURJHQV >@LQ IRO FXOD U IOLG 7KH shortage of SAM, will deregulate the folate cycle. Moreover,

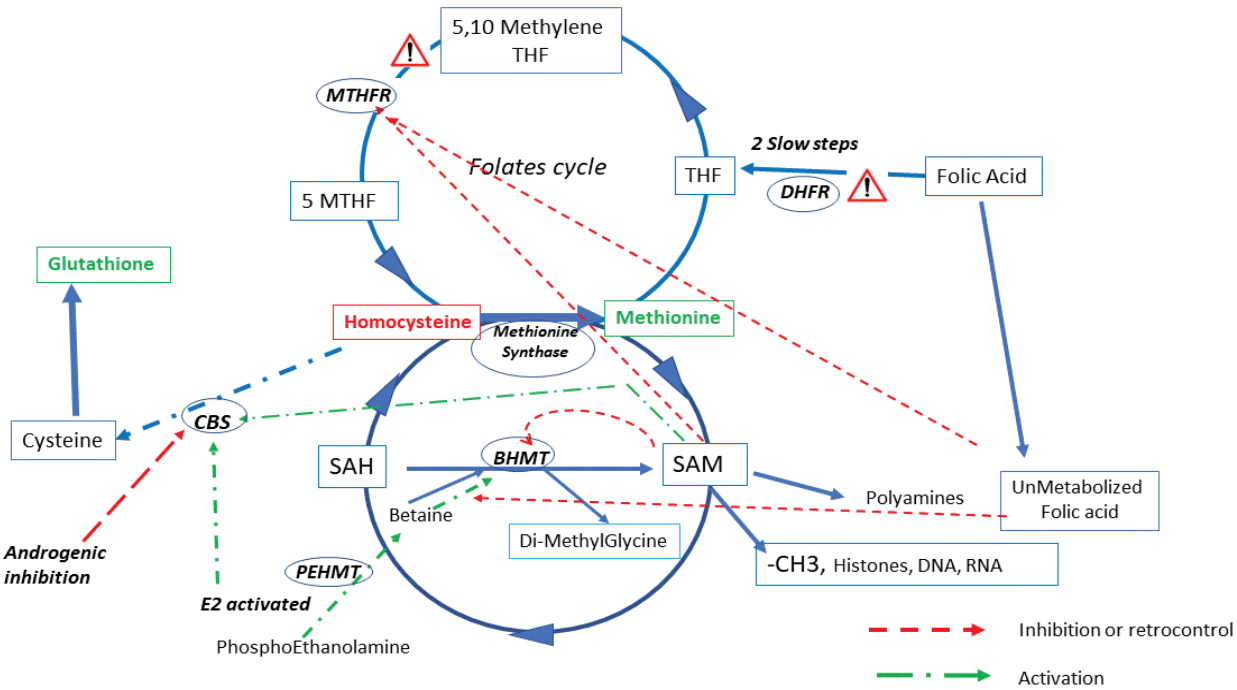

Figure 1: 2HFDERQQIRDWHFFHWKHHuFWRIHPRORJLFDO

Citation: Arthur Clement, Silvia Alvarez, Laetitia Jacquesson-Fournols, Edouard Amar, Charles Brami, Dominique Cornet, Marc Cohen, Patrice Clement, Edouard Servy, Kay Elder & Yves Menezo. Women are More Protected than Men against HHcy (Hyperhomocysteinemia) 8QWKHL5HSHZLYH/LIH+LVWRULHVHODWHGGIIHUHQHVL2Q&DERQRGW &FOHV0HWDEROLVP 6KLYHVRI&OLQLFDO DQG Biomedical Research. 7 (2023): 376-383.

one has to consider that estrogens are strong inducers/ regulators of epigenetically related gene expression [47].

Dosage of Hcy, at the age over 2 (age 2 to 7) in 20 of the children "at risk", based on the MTHFR SNP background of the parents and their circulating Hcy, shows a mean value of 6.2 (SD: 1.7) micromolar with no difference between boys (9) and girls (11). This level is low and similar to the one described in literature [48]. Before puberty circulating Hcy remains low, but may fluctuate according to MTHFR SNPs; our data are in line with an OCC dependence of the sexual hormones.

In men, the combination of Hcy/MTHFR 677TT has a noticeable impact on sperm quality, through a significant effect of this mutation on sperm DNA methylation [21,22]. It is also clear that Hcy is involved in sperm DNA degradation, as cause and a consequence of oxidative stress [41]. Oxidative stress impairs methylation and affects the "methylation" setting via formation of 5-OH Cytosine; 5,6-diOH Cytosine; Cytosine glycol and uracil (deamination). Hcy also induces thrombophilia and circulatory system problems, known to affect spermatogenesis.

From a therapeutic point of view, the metabolism of folic acid (FA), pteroyl glutamic acid is crucial, especially with regard to its capacity to enter the folates cycle. It is usually prescribed at more or less high doses at the beginning of pregnancy and even before (preparation to IVF treatments in women). The first two steps of the cycle are driven by dihydrofolate reductase (DHFR), and these steps are very slow and poorly efficient [49]. This leads to the accumulation of unmetabolized folic acid, UMFA. UMFA prevents entry of natural folate, probably by saturating the receptors FolRα and SLC 19A1 [50]. The situation is more severe in carriers of the MTHFR SNPs, for whom the formation of 5-Methyl tetrahydrofolate (5-MTHF), the active form that is required for recycling Hcy to Methionine by the methionine synthase enzyme. An excess of unmetabolized substrate provokes a succession of inhibitory Michaelis and Menten reactions. El Aarabi et al. [23,24] observed that high doses of folic acid administered to men carriers of the 677TT mutation clearly have a damaging effect on sperm methylation profiles. The use of 5-MTHF instead of FA is preferable, and thus far has yielded promising results [31-33, ,51]. Based on the consequence of these features on embryo development [14,52,53], when both partners of a couple are affected by the MTHFR mutation; 5-MTHF treatment is mandatory for both.

In conclusion, investigating carrier status for the two main MTHFR SNPs (677TT and 677CT/1298AC), together with evaluation of circulating Hcy is a relevant strategy for patients suffering long-duration/idiopathic infertility. The paternal effect, which is often neglected, should not be overlooked [51,53] especially since Hcy levels are higher in men and can reach easily pathological values. Our data are in line with a major role of Estrogens and androgens on the impact of circulating homocysteine. They highlight the interference with the MTHFR SNPS, as it has been demonstrated in menopausal women [54-57]. Concerns regarding elevated circulating Hcy and its negative impact in conceptus quality and fertility have recently led the "Rotterdam periconception cohort" to recommend the "routine analysis of homocysteine levels in preconceptional and pregnant women and their partners" [58]. Patients must be informed of these pleiotropic medical implications for their own health, as well as for the health of future children, especially boys [59], Hcy can be high in MTHFR carrier infants. The potential impact of elevated homocysteine on psychiatric diseases, and multiple other diseases must also be considered [59]. Information about the parental genetic background and the risk of transmitting 677TT and 677CT/1298AC to male children may have important implications [59-60]. However, our preliminary observations do not show any noticeable Hcy increase in the young children, in agreement with the marked impact of sexual hormones on the CBS pathway. Since treatment with 5-MTHF decreases Hcy down to baseline levels, with no UMFA accumulation, this therapeutic option should be considered especially for male carriers of the MTHFR SNPs at puberty and before, in order to fulfill the ubiquitous needs for methylation [31-33,59,60] as this compound has proven its safety in infant and children [61].

Conflict of interest: None to declare

Funding: No funding

References

1. Menezo Y, Khatchadourian C, Gharib A, et al. Regulation of S-adenosyl methionine synthesis in the mouse embryo. Life Sci 44 (1989):1601-9.
2. Zhang H P, Wang YH, Ma SC, et al. Homocysteine inhibits endothelial progenitor cells proliferation via DNMT1-mediated hypomethylation of Cyclin A. Exp Cell Res 362 (2018): 217-226.
3. Yi P, Melnyk S, Pogribna M, et al. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. J. Biol. Chem 275 (2000): 29318–29323.
4. James SJ, Melnyk S, Pogribna M, et al. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J Nutr 132 (2002): 2361S-2366.
5. Menezo YJ, Silvestris E, Dale B, et al. Oxidative stress and alterations in DNA methylation: Two sides of the same coin in reproduction. Reprod. Biomed. Online 33 (2016): 668–683.

6. Boxmeer JC, Macklon NS, Lindemans J, et al. IVF outcomes are associated with biomarkers of the homocysteine pathway in monofollicular fluid. Hum. Reprod 24 (2009): 1059–1066.

7. Szymański W, Kazdepka-Ziemińska A. Effect of homocysteine concentration in follicular fluid on a degree of oocyte maturity. Ginekol. Pol 74 (2003): 1392–1396.

8. Berker B, Kaya C, Aytac R, et al. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. Hum. Reprod 24 (2009): 2293–2302.

9. Razi Y, Eftekhar M, Fesahat, F, et al. Concentrations of homocysteine in follicular fluid and embryo quality and oocyte maturity in infertile women: A prospective cohort. J. Obstet. Gynaecol 41 (2021):588–593.

10. Kaplan P, Tatarkova Z, Sivonova MK, et al. Homocysteine and Mitochondria in Cardiovascular and Cerebrovascular Systems. J.Int J Mol Sci 21 (2020):7698

11. Chen L, Xu T, Qiu Y, et al. Homocysteine induced a calcium-mediated disruption of mitochondrial function and dynamics in endothelial cells. J. Biochem. Mol. Toxicol 35 (2021): e22737.

12. Zhang T, Huang D, Hou J, et al. High concentration homocysteine inhibits mitochondrial respiration function and production of reactive oxygen species in neuron cells. Cerebrovasc Dis 29 (2020):105109.

13. Lu S, Hoestje SM, Choo E, et al. Induction of caspase-dependent and -independent apoptosis in response to methionine restriction. Int J Oncol 22 (2003): 415-20.

14. Enciso M, Sarasa J, Xanthopoulou L, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. Hum. Genet 135 (2016): 555–568.

15. Zeng H, Liu Z Zhang L, et al. MTHFR 677TT is associated with decreased number of embryos and cumulative live birth rate in patients undergoing GnRHa short protocol: a retrospective study. BMC Pregnancy Childbirth 22 (2022): 170.

16. D'Elia PQ, dos Santos AA, Bianco B, et al. MTHFR polymorphisms C677T and A1298C and associations with IVF outcomes in Brazilian women. Reprod BioMed Online 28 (2014):733–8.

17. Sun H, Kang J, Su J, et al. Methionine adenosyltransferase 2A regulates mouse zygotic genome activation and morula to blastocyst transition. Biol. Reprod 100 (2019): 601–617.

18. Ishitani H, Ikeda S, Egashira K, et al. Embryonic MTHFR contributes to blastocyst development. J Assist Reprod Genet 37 (2020): 1807-1814.

19. Mu H, Zhang, T, Yang Y, et al. METTL3-mediated mRNA N6-methyladenosine is required for oocyte and follicle development in mice. Cell Death Dis 12 (2021): 989.

20. Sui X, Hu, Y Ren C, et al. METTL3-mediated m6A is required for murine oocyte maturation and maternal-to-zygotic transition. Cell Cycle 19 (2020): 391–404.

21. Aitken RJ, Flanagan HM, Connaughton H, et al. Involvement of homocysteine, homocysteine thiolactone, and paraoxonase type 1 (PON-1) in the aetiology of defective human sperm function. Andrology 4 (2016): 345–60.

22. Gong M, Dong W, He T, et al. MTHFR 677C>T polymorphism increases the male infertility risk: a meta-analysis involving 26 studies. PLoS One 10 (2015): e0121147.

23. Aarabi M, San Gabriel M C, Chan D, et al. . High-dose folic acid supplementation alters the human sperm methylome and is influenced by the MTHFR C677T polymorphism. J. Hum. Mol. Genet 24 (2022): 6301–6313.

24. Aarabi M, Christensen KE, Chan D, et al. Testicular MTHFR deficiency may ex-plain sperm DNA hypomethylation associated with high dose folic acid supplementation. J. Hum. Mol. Genet 27 (2018): 1123–1135.

25. Duthie S, Narayanan S, Brand G, et al. Impact of folate deficiency on DNA stability. J. Nutr 132 (2002): 2444S–2449S.

26. Håberg SE, Page CM, Lee Y, et al. DNA methylation in newborns conceived by assisted reproductive technology. Nat. Commun 13 (2022): 1896.

27. Hattori H, Hiura H, Kitamura A, et al. Association of four imprinting disorders and ART. Clin. Epigenetics 11 (2009): 21.

28. Hiura H, Okae H, Miyauchi N, et al. Characterization of DNA methylation errors in patients with disorders conceived by assisted reproduction technologies. Hum. Reprod 27 (2012): 2541–2548.

29. Choux C, Binquet C, Carmignac V, et al. The epigenetic control of transposable elements and imprinted genes in newborns is affected by the mode of conception: ART versus spontaneous conception without underlying infertility. Hum. Reprod 33 (2018): 331–340.

30. Song S, Ghosh J, Mainigi, M, et al. . DNA methylation

differences between in vitro and in vivo conceived children are associated with ART procedures rather than infertility. Clin. Epigenetics 7 (2015): 41–51.

31. Servy EJ, Jacquesson-Fournols L, Cohen M, et al. MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs folic acid: a key to pregnancy outcome: a case series. J Assist Reprod Genet 35 (2018): 1431-1435

32. Servy E & Menezo Y. The Methylene Tetrahydrofolate Reductase (MTHFR) isoform challenge. High doses of folic acid are not a suitable option compared to 5 Methyltetrahydrofolate treatment Clin Obstet Gynecol Reprod Med 3(2017): 1-5

33. Goyco Ortiz LE, Servy EJ, Menezo YJR. A successful treatment with 5 methyltetrahydrofolate of a 677 TT MTHFR woman suffering premature ovarian insufficiency post a NHL (non-Hodgkin's lymphoma) and RPL (repeat pregnancy losses). J Assist Reprod Genet 36 (2019): 65-67.

34. Alnabbat KI, Fardous A M, Cabelof DC, et al. Excessive Folic Acid Mimics Folate Deficiency in Human Lymphocytes. Curr Issues Mol Biol 44 (2022): 1452-1462.

35. Page R, Wong A, Arbuckle TE, et al. The MTHFR 677C>T polymorphism is associated with unmetabolized folic acid in breast milk in a cohort of Canadian women. Am J Clin Nutr 110 (2019): 401-409

36. Clément A, Menezo Y, Cohen M, et al. 5-Methyltetrahydrofolate reduces blood homocysteine level significantly in C677T methyltetrahydrofolate reductase single nucleotide polymorphism carriers consulting for infertility. Gynecol Obstet Hum Reprod 49 (2020): 101622.

37. Clément A, Chouteau J, Clément P, et al. Importance of the determination of MTHFR SNPs (Methylene Tetrahydrofolate Reductase Single Nucleotide Polymorphisms) in couple infertility. Gynecol Obstet Fertil Senol 48 (2020): 422-27.

38. Škovierová H, Vidomanová E, Mahmood S, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci 17 (2016): E1733.

39. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 346 (2002): 476–83.

40. Friso S, Choi SW, Girelli D, et al. Common mutation in the 5,10 methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. J.Proc Natl Acad Sci U S A 99 (2002): 5606-11.

41. Hoffman M. Hypothesis: hyperhomocysteinemia is an indicator of oxidant stress. Med Hypotheses 77 (2011): 1088-93.

42. Estienne A, Portela VM, Choi Y, et al. The endogenous hydrogen sulfide generating system regulates ovulation. Free Radic Biol Med 138 (2019): 43-52.

43. Kim R, Nijhout HF, Reed MC. One-carbon metabolism during the menstrual cycle and pregnancy. PLoS Comput Biol 17 (2021): e1009708.

44. Pey AL, Majtan T, Sanchez-Ruiz JM, et al. Human cystathionine beta-synthase (CBS) contains two classes of binding sites for S-adenosylmethionine (SAM): complex regulation of CBS activity and stability by SAM. Biochem J 449 (2013):109-21.

45. Giltay EJ, Hoogeveen EK, Elbers JM, et al. Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females. J Clin Endocrinol Metab 83 (1998): 550-3.

46. Pavlik R, Hecht S, Noss U, et al. Reduced Steroid Synthesis in the Follicular Fluid of MTHFR 677TT Mutation Carriers: Effects of Increased Folic Acid Administration. Geburtshilfe Frauenheilkd 82 (2022): 1074-1081.

47. Kovács T, Szabó-Meleg E, Ábrahám IM. Oestradiol-Induced Epigenetically Mediated Mechanisms and Regulation of Gene Expression. Int J Mol Sci 2 (2022): 3177.

48. Caldeira-Araújo H, Ramos R, Florindo C, et al. Homocysteine Metabolism in Children and Adolescents: Influence of Age on Plasma Biomarkers and Correspondent Genotype Interactions. . Nutrients 11 (2019): 646.

49. Bailey SW & Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. Proc Natl Acad Sci U S A 106 (2009):15424-9.

50. Menezo Y, Elder K, Clement P, et al. Biochemical Hazards during Three Phases of Assisted Reproductive Technology: Repercussions Associated with Epigenesis and Imprinting. Int J Mol Sci 23 (2022): 8916.

51. Jacquesson-Fournols L, Alvarez S, Cohen M, et al. A paternal effect of MTHFR SNPs on gametes and embryos should not be overlooked: case reports. J Assist Reprod Genet 36 (2019): 1351-1353.

52. Williamson JM, Arthurs AL; Smith MD, et al. Perturbed One-Carbon Metabolism and Gestational Diabetes Mellitus. Nutrients 14 (2022): 3930.

53. Tara SS, Ghaemimanesh F, Zarei S, et al. Methylenetetra hydrofolate Reductase C677T and A1298C

Citation: Arthur Clement, Silvia Alvarez, Laetitia Jacquesson-Fournols, Edouard Amar, Charles Brami, Dominique Cornet, Marc Cohen, Patrice Clement, Edouard Servy, Kay Elder & Yves Menezo. Women are More Protected than Men against HHcy (Hyperhomocysteinemia) during their Reproductive Life: Gender-Related Differences in One-Carbon and Folate Cycles Metabolism. Archives of Clinical and Biomedical Research. 7 (2023): 376-383.

Polymorphisms in Male Partners of Recurrent Miscarriage Couples. J Reprod Infertil 16 (2015): 193-8.

54. Lambrinoudaki I, Papadimitriou D, Kaparos G, et al. MTHFR C677T polymorphism modifies the effect of HRT on metabolic parameters in postmenopausal women. Climacteric 16 (2013): 568-75.

55. Somekawa Y, Kobayashi K, Tomura S, et al. Effects of hormone replacement therapy and methylenetetrahydrofolate reductase polymorphism on plasma folate and homocysteine levels in postmenopausal Japanese women. Fertil Steril 77 (2002): 481-6.

56. Mijatovic V, van der Mooren MJ. Homocysteine in postmenopausal women and the importance of hormone replacement therapy. Clin Chem Lab Med 39 (2001): 764-7.

57. Mijatovic V, Kenemans P, Jakobs C, et al. A randomized controlled study of the effects of 17beta-estradiol-dydrogesterone on plasma homocysteine in postmenopausal women. Obstet Gynecol 91 (1998): 432-436.

58. Rubini E, Snoek KM, Schoenmakers S, et al. First Trimester Maternal Homocysteine and Embryonic and Fetal Growth: The Rotterdam Periconception Cohort. Nutrients 14 (2022): 1129.

59. Yverneau M, Leroux S, Imbard A, et al. Influence of early identification and therapy on long-term outcomes in early-onset MTHFR deficiency. J Inherit Metab Dis 45 (2022): 848-861.

60. Koklesova L, Mazurakova A, Samec M, et al. Homocysteine metabolism as the target for predictive medical approach, disease prevention, prognosis, and treatments tailored to the person. EPMA J 12 (2021): 477-505

61. Troesch B, Demmelmair J, Gimpfl M, et al. MEFOLIN Study Group. Suitability and safety of L-5- methyltetrahydrofolate as a folate source in infant formula: A randomized-controlled trial. PLoS ONE 14 (2019): e0216790.

Citation: Arthur Clement, Silvia Alvarez, Laetitia Jacquesson-Fournols, Edouard Amar, Charles Brami, Dominique Cornet, Marc Cohen, Patrice Clement, Edouard Servy, Kay Elder & Yves Menezo. Women are More Protected than Men against HHcy (Hyperhomocysteinemia) during their Reproductive Life: Gender-Related Differences in One-Carbon and Folate Cycles Metabolism. Archives of Clinical and Biomedical Research. 7 (2023): 376-383.