THE IMPACT OF MATERNAL METHYL-DONOR NUTRIENT SUPPLEMENTATION ON VITAMIN D STATUS AND INFLAMMATION AMONG ADULT OFFSPRING

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
1,25D	1,25-dihydroxy vitamin D
24,25D	1,24,25-trihydroxyvitamin D
25D	25-dihydoxycholecalciferol
BMI	Body mass index
CKD	Chronic kidney disease
CRP	C-reactive protein
DIO	Diet-induced obese
DMRs	Differentially methylated regions
GDM	Gestational diabetes mellitus
HFD	High-fat diet
HFS	High-fat high-sucrose
IBD	Inflammatory bowel disease
LGA	Large-for-gestational age
OCM	One-carbon metabolism
OWO	Overweight and obese
MDD	Methyl-donor nutrients/ MN deficiency

MN	Methyl-donor nutrients
MS	Methyl-donor nutrient supplementation
MWG	Maternal weight gain
SD	Sprague-Dawley
VD	Vitamin D
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
WD	Western diet

ABSTRACT

Maternal overweight and obesity is associated with higher risks of childhood obesity. Low vitamin D (VD) status is often observed among overweight and obese (OWO) individuals, enhancing their risk for the development of secondary complications, such as inflammatory bowel diseases, cardiovascular diseases, and cancers. In addition to its classical role in bone health, the immunomodulatory role of VD has been widely reported. In this study, we aim to investigate the effect of maternal methyl-donor nutrient supplementation (MS) to a high-fat high-sucrose (HFS) diet during pregnancy and lactation on VD status and inflammation among offspring. Our results suggest a critical role of prenatal MS in mediating VD status among offspring and suppressing systemic inflammation in offspring. We further demonstrated that prenatal and postnatal diets differentially regulate renal and colonic VD signaling, as well as the respective local inflammatory signaling. Mechanisms by which prenatal and postnatal diets regulate VD signaling locally remained to be elucidated. Collectively, this thesis project supported the role of prenatal dietary modifications in optimizing VD status as a strategy to prevent the development of metabolic disorders.

I. INTRODUCTION

A meta-analysis involving European, North American, and Australian populations demonstrated a strong association between maternal overweight/obesity and higher risks of childhood OWO, and this effect was slightly enhanced when maternal weight gain (MWG) exceeded the 2009 guidelines from the Institute of Medicine (IOM) of 11 to 25 lbs. in overweight and obese (OWO) women.[1] The Western diet (WD) culture, which is rich in saturated fat and added sugar, contributed to increased risks of metabolic dysfunctions not only to pregnant mothers but also to their offspring. In addition, the disruptions in both central and peripheral immunity among offspring induced by a maternal WD were recently demonstrated by Dunn et al. in a non-human primate study.[2] Low vitamin D (VD) status is often observed in OWO individuals as evidenced by an observational study demonstrating that low serum 25-hydroxycholecalciferol (25D) in Swedish child-bearing aged women of BMI > 30 kg/m^2.[3] Maternal VD status can also impact the health outcomes among their offspring. Specifically, a study has shown that maternal serum 25D levels lower than 37 nmol/L were associated with higher BMI and central adiposity in their 6-year-old offspring, which may subsequently increase their risks of obesity-associated chronic diseases throughout adulthood.[4] Methyl-donor nutrients (MN), which include micronutrients such as folate, methionine, vitamin B_{12}, betaine, choline and zinc, involved in one-carbon metabolism (OCM), have long been known about their effects on epigenetically modified genes involved in glucose and lipid metabolism. It has been shown that maternal MN deficiency (MDD) affects bone health in offspring by decreasing protein vitamin D receptor (VDR) levels.[5] This may suggest the role of MN in modulating VD signaling through epigenetic modifications. Thus, the primary objective of this proposed work is to determine if maternal MS can normalize VD status and inflammation induced by maternal high-fat high-

sucrose (HFS) diet among adult offspring. The central hypothesis is that maternal MS can restore disrupted VD balance and suppress inflammation induced by maternal HFS diet among adult offspring.

II. BACKGROUND AND SIGNIFICANCE

1. Significance of the Proposed Research.

A population study showed that mothers who had a dietary pattern rich in fast food, which is high in saturated fat and sugar, or commonly known as the Western diet (WD) tended to be in the overweight and obese (OWO) category.[6] In addition, this study demonstrated the association between maternal Western dietary pattern and maternal obesity with risks of children being OWO at the age of 4.[7] Childhood BMI has been reported by Ajala et al. as a predictor for being obese and developing pre-diabetes in adulthood.[8] Maternal obesity also is commonly associated with adverse maternal and offspring health outcomes, such as gestational diabetes mellitus (GDM), preterm birth, and babies who are large-for-gestational age (LGA).[9] Neonates born to OWO mothers had lower vitamin D (VD) status and low VD status has been linked to higher inflammation status in *in vivo* mice offspring.[10]

2. Review of Literature

A) Introduction

Maternal dietary pattern has recently been shown to be correlated with fetal head circumference, where dietary patterns rich in meat and meat products had positively correlated with increased head circumference.[11,12] In addition, several *in vivo* studies have shown adverse effects of maternal high-fat diet (HFD) or WD, which is rich in saturated fat and added sugar, on offspring health, such as increased adiposity,[13] liver damage, and impaired cognitive functions.[14,15] Low VD status is often associated in OWO individuals and maternal VD status has been related to dysregulated lipid metabolism and higher inflammation status in offspring.[16] A recent publication investigated the causal relationship between VD deficiency (VDD) and

obesity and found lower methylation status in genes related to VD metabolism in obese families.[17] On the other hand, MS has long been known for its role in supplying methyl groups to DNA,[18] therefore, it may serve as a helpful strategy to suppress the effects of maternal HFS-induced DNA methylation loss.

B) <u>Maternal Dietary Pattern</u>

Pregnant women in the OWO BMI categories have been associated with both maternal complications and adverse health outcomes in offspring. A large cohort study that included over 10,000 pregnant women suggested that being OWO tended to exceed maternal weight gain (MWG) recommended by IOM, which is between 11 to 25 pounds.[19] These OWO pregnant women have a higher prevalence of being hyperinsulinemia, hyperleptinemia, and displaying impaired glucose tolerance and low-grade systemic inflammation,[10,19-21] indicated by increased serum levels of pro-inflammatory markers, TNF-α, IFN-γ, IL-6 and IL-8.[20,21] Adverse outcomes arouse from maternal obesity include preeclampsia, gestational heart diseases, GDM, caesarean section, having preterm (< 37 gestational weeks) and post-term delivery (> 42 gestational weeks). Moreover, pregnant women with an OWO status also increased prevalence of adverse neonatal health outcomes, such as macrosomia (birth weight > 8.2 lbs.), small-for-gestational age, and LGA.[10,22] In addition, risks of adverse maternal and offspring health outcomes increased across maternal pre-pregnancy BMI as reported in Black et al. and Voerman et al.[19,22] OWO status accompanied with excessive MWG further increased adverse offspring health outcomes as evidenced in a few cohort studies.[9,19]

Maternal obesity has been found to mitigate the risks of children being OWO not only during neonatal period, but also continued throughout adulthood. Increased mean birth weight was observed across maternal BMI categories and there were significantly higher proportions of

LGA infants born from OWO mothers as compared to mothers with normal BMI.[9,10,19] Andersen et al. observed the eating pattern in 9-month-old offspring born to OWO mothers and demonstrated that they tended to have less healthy eating patterns, as measured by lower fiber intakes and higher calorie intakes compared to offspring from mothers with normal BMI.[23] Chang et al. also did a follow up with offspring born to OWO mothers with excessive GWG after two years and demonstrated a linear correlation with the rate of children becoming OWO at 2 years old.[9] In addition, several studies have also observed that offspring born to OWO women had a higher prevalence of being OWO throughout late childhood, and this may be due to the low-grade systemic inflammation, which is a common characteristic in a maternal obesogenic diet.[1,20,24]

Corroborate with human studies, offspring born to dams on HFD had higher body weights and larger sizes of both visceral and subcutaneous fat pads.[25,26] The exact mechanism underlying contribution of maternal obesity to risks of being OWO among offspring remained unclear. A possible mechanistic link between maternal obesity and risks of metabolic diseases among offspring is induction of chronic inflammation from a maternal HFD. As shown in placentas collected from C57BL/6J mice, increased mRNA expression of *Tnf,Il6, Il10, Traf6, Nf-kB, Tlr2, Ccl2, Csf1* and *Csf2* suggested maternal HFD promoted TLR-mediated pro-inflammatory signaling and phenotypic changes in macrophages.[27] Wallace et al., in consistent with Gohir et al., also demonstrated that maternal HFD increased placental hypoxia and altered placental vascularization by increasing transcript levels of carbonic anhydrase IX, VEGF, CD31 and several nutrient transporters, but reducing blood vessel maturity.[27,28] Gohir et al. also observed sex-dependent effects on metabolic dysregulations induced by a maternal HFD, where female fetuses were more affected,[28] however, it was contradicted with the findings from Chang

et al., who found larger effects in male offspring.[25] Another possible mechanistic link is suggested from several *in vivo* mice studies which found that maternal HFD containing 60 % of total calorie intakes from fat induced phenotypic changes in genes involved in hepatic lipid metabolism,[26,27] therefore predisposing risk of metabolic dysregulations to offspring. The dysregulated gene expression of hepatic leptin receptor, *Lepr,* and enzymes involved in gluconeogenesis, *Pc* and *Pepck,* may be explained by LXR-mediated suppression where LXR is known as a regulator of adipogenesis.[26,27]

The link between maternal OWO status and DNA methylation status has been extensively studied. An *in vivo* study that involved offspring from diet-induced obese (DIO) Sprague Dawley (SD) rats showed highly enriched genes involved in pattern specification, cell fate commitment and mesenchymal cell development, also significant hypomethylation in key adipogenic transcription factors, C/EBP-B, *Zfp423*, and PPARγ.[29] *Zfp423* has been found to be sequestered by WISP2, which is a target of Wnt signaling, suggesting Wnt signaling may be regulated by maternal obesity to induce epigenetic changes among offspring.[29] Consistent with the results in human studies, Perkins et al. found the odd ratios for DNA hypermethylation at H19 differentially methylated regions (DMRs) was 3.7-fold higher in 1-year-old offspring born to OWO mothers.[30] Also, there were significant changes in cord blood methylome obtained from obese pregnant women diagnosed with GDM as compared to those from non-GDM pregnant women, primarily on genes involved in cell signaling, chromatin remodeling and transcriptional regulation.[31] However, Antoun et al. found that antenatal dietary interventions and lifestyle changes assigned to GDM pregnant women had null effects on DNA methylation changes among offspring from OWO pregnant women, which is similarly observed in a recent study done by Louise et al. who also started interventions in OWO pregnant women during their second

trimester.[31] Therefore, it may be helpful to modify maternal diet as early as preconception and during first trimester to prevent the induction of DNA methylation changes among offspring.

Maternal obesity, mostly due to Western diet culture, also contributed to decreased VD status in offspring as observed in both animal and human studies. A study that involved 45 pairs of mothers and children found that serum 25D levels were significantly reduced among neonates from obese mothers as compared to those from normal-weight mothers.[32] This may be due to modulations in genes involved in VD metabolism by maternal HFD as evidenced by a primate study. Placentas from diet-induced obese (DIO) baboons on a HFD (45 % kcal from fat) exhibited decreased mRNA expression of *Lpr2, Cyp27b1, Vdr* and ratio of *Cyp27b1/Cyp24a1* and protein levels of VDR as compared to placentas from non-obese pregnant baboons, indicating a repressed placental VD metabolism.[33] Yu et al. recently demonstrated associations of single nucleotide polymorphisms in *Cyp27b1* with serum 25D levels, but not with BMI in obese families,[17] indicating VDD may be a causal factor of obesity, but this causal relationship between VDD and obesity remains to be investigated.

Furthermore, children from OWO mothers have been found to have higher odds of displaying developmental delay on communication, fine and gross motor skills, and personal-social skills at the age of 3.[34] These may further indicate that maternal obesity not only predisposed risks of being OWO and inflammation,[35] but also altered neurocognitive function among offspring.[34] A cross-sectional study that followed up with 12-year-old offspring born to mother with a maternal BMI higher than 66 % of mean BMI had higher levels of C-reactive protein (CRP), suggested that offspring from OWO parents are more prone to inflammation.[35] Yang et al. also reported upregulations in mRNA expression of *Tlr4*, *Il6* & *Il8* in placenta collected from obese pregnant women and a positive correlation between placental TLR4

transcript levels and maternal pre-pregnancy BMI, plasma insulin, leptin and CRP levels.[21] TLR4 signaling has been found to activate the innate immune system by stimulating release of cytokines and can be mitigated by VD signaling,[36] therefore, proposing a potential use of VD status to mitigate the risks of inflammation and chronic diseases, which will be further discussed in the next section.

Other than the onset of inflammatory diseases, maternal obesity has been linked to playing a part in contributing to the risk of chronic kidney disease (CKD). CKD, which is associated with progressive loss of neurons, tubulointerstitial fibrosis, glomerular sclerosis, and tubular atrophy, has been found to be more prevalent in offspring born to dams on a HFD in animal studies.[37-39] Two studies involving C57BL/6 mice found that offspring from HFD dams presented higher levels of renal injury, enhanced levels of oxidative stress and DNA oxidative damage, in addition to increased renal lipogenesis, which leading to more lipid accumulation in the kidneys.[37,38] A study involving offspring from Wistar rats on HFD also found similar results, where Zhou et al. observed increased renal damage, oxidative damage and renal inflammation in 12-week-old adult offspring.[39] When offspring from dams on HFD were put on HFD, Jackson et al. and Flynn et al. found synergistic effects from both maternal and postnatal diets in progression of renal injury and inflammation in both male and female offspring.[40,41] Therefore, these studies suggested the potential roles of both prenatal and postnatal diets in determining risks of developing CKD among offspring during adulthood.

C) <u>Vitamin D Metabolism</u>

Vitamin D presents naturally in plant sources, such as mushroom and soy products, as pro-vitamin D_2 and in animal sources, such as fish and dairy products, as 7-dehydrocholesterol (pro-vitamin D_3). In addition, cholesterol can be converted endogenously into 7-

dehydrocholesterol by 7-dehydrocholesterol reductase. UVB rays from sun act as a catalyst to catalyze the conversion of pro-vitamins D_2 and D_3 into cholecalcidiol (vitamin D_2) and cholecalciferol (vitamin D_3) in our skin. Lipid-soluble vitamins D_2 and D_3 are bound to vitamin D binding protein (VDBP) once entering the blood circulation. Dietary sources of VD are incorporated with bile acids into micelles and carried to liver to be converted into chylomicrons, which then being transported to peripheral cells. In liver, VDBP-bound vitamins D_2 and D_3 are being converted into 25D by hepatic 25-hydroxylase (encoded by *Cyp27a1* and *Cyp2r1*), and 25D is further metabolized in the kidney into 1,25-dihydroxy vitamin D (1,25D) by 1α-hydroxylase (encoded by *Cyp27b1*). 1,25D is the active form of VD, which binds to vitamin D receptor (encoded by *Vdr*), to regulate activation of gene transcriptions. Serum VD levels are regulated by the kidney through production of 24-hydroxylase (encoded by *Cyp24a1*) to convert 1,25D into 1,24,25-trihydroxyvitamin D (24,25D), which is a less bioavailable form to be excreted.

Population study has found that VDD is commonly observed among OWO individuals. In addition, Karlsson et al. also found that obese pregnant women had lower VD status in the first trimester of pregnancy than normal-weight women despite having a higher dietary VD intake.[42] Maternal VD status has been found to be associated with risks of being OWO among offspring.[4,43] In a study that involved VD-inadequate, or serum 25D levels equals or lower to 30 ng/mL, OWO mothers, an inverse association has been found between visceral adiposity in neonates, as shown by a greater area of abdominal subcutaneous adipose tissue and maternal VD status.[43] Furthermore, each 10 nmol/L decrease in maternal serum 25D levels was associated with increased risks of childhood OWO, as indicated by BMI standard deviation score, waist circumference and body fat percentage in several follow-up studies.[4,44,45] These may be

explained by the role of maternal VD status in modulating phenotypic changes of offspring VD metabolism, and therefore, the risks of metabolic dysfunctions. Two studies found that neonatal VD status was highly correlated with maternal serum 25D levels and there were positive associations between neonatal serum 25D levels with placental CYP27B1 protein levels, showing a higher maternal VD status is effective in upregulating VD metabolism among offspring.[46,47] In addition, O'Brien et al. examined the link between maternal inflammation status and both mRNA and protein levels of VD metabolism-related enzymes, which demonstrated that maternal serum IL-6 levels were positively associated with placental CYP27B1 protein level and negatively associated with placental *Cyp24a1* mRNA expression.[47] This may indicate an association between VDD and maternal obesity involved in regulating VD status among offspring at both transcriptional and translational levels.

Several *in vivo* rodent studies have investigated the role of maternal VDD on anthropometric and biochemical measurements in offspring. Belenchia et al. fed weaned VDD offspring either a low-fat diet or a HFD (45% kcal from fat) for 12 weeks and found that maternal VDD induced higher visceral adiposity and a 3.7-fold increase in PPARγ gene expression in perigonadal adipose tissue among adult offspring as compared to offspring from dams on a VD-sufficient diet,[16] indicating maternal VDD altered lipid metabolism in offspring born to dams on a VDD diet. In addition, when offspring from VDD dams were weaned to a control diet (consists of 1.0 IU vitamin D/g), these mice exhibited higher mRNA expression of VDR and PPARγ and elevated levels of serum resistin and IL-2 at 15-week of age from baseline, as compared to offspring born to dams on a control diet.[48] These suggested that VD and inflammation status among offspring were dependent on maternal VD status, and that postnatal supplementation may not be as effective as modulating maternal VD status on maintaining VD

status and suppressing inflammation and maternal VDD-induced metabolic dysregulations among offspring. Moreover, when 1,25D was supplemented to pregnancy induced VDD dams, they had increased litter size and fetal weights as compared to rat models which had sufficient serum VD levels during prengnancy.[49] Villa et al. and Gazquez et al. studied the effects of prenatal and postnatal VD supplementation on offspring health. Both studies found optimizing VD status by supplementing either D2 or D3 to dams during pregnancy was effective in raising neonatal serum 25D levels and protecting their intestinal barriers,[50,51] proving the role of maternal VD status in mitigating risks of diseases among offspring.

Garg et al. used colonic tissues resected from patients with inflammatory bowel disease (IBD) showed upregulations in both gene expression and protein level of VDR, which were inversely correlated with inflammation.[52-54] Apart from the traditional role of VD in maintaining bone mineral density, VD also plays a critical role in both innate and adaptive immune system. VD supplementation has been shown to mitigate symptoms in patients diagnosed with acute or chronic inflammatory diseases, such as IBD, COVID-19, and asthma.[55,56] An *in vitro* study proved that the gene encodes for production of cathelicidin is a direct target of VDR and it was strongly upregulated by treatment of 1,25D, showing VD can mediate inflammatory signaling through production of antimicrobial peptides, cathelicidin.[55,56] A human study involving asthma patients also found that serum VD status showed an inverse correlation with serum cathelicidin levels in patients having asthma attacks. Moreover, an increase in serum 25D levels predicted a decreased risk of asthma attacks.[54] Arikoglu et al. also found a significant positive correlation between serum cathelicidin levels and BMI, further suggesting a possible link of cathelicidin that underlies the protective role of VD in inflammation, and therefore risks of being OWO.[54] Taken

together, these observations strongly suggest a role of VD metabolism in mediating inflammation.

The protective effect of vitamin D in IBD has been proposed to be mediated by cathelicidin through induction of TLR signaling and production of inflammatory cytokines.[36,57,58] An *in vitro* study found treatment of 1,25D upregulated gene expression of cathelicidin,[57,58] while cathelicidin has been observed to inhibit activation of TLR signaling.[36] Liu et al. further investigated the gene expression of *Cyp27b1* and *Cyp24a1* and found that TLR signaling induced upregulation in *Cyp27b1* expression, while treatment of 1,25D upregulated *Cyp24a1* expression.[57] These results suggested that TLR signaling stimulated activation of VD metabolism and its downstream target, cathelicidin, which then confirms the role of VD status in the innate immune system. On the other hand, animal studies found controversial results on supporting maternal VD status to modulate inflammation among offspring. Although an *in vivo* mice study has reported that maternal VD status did not affect systemic inflammation markers, such as IL-6 and TNF-α, in female offspring,[59] but a recent study using C57BL/6JRJ mice fed on VD-depleted diet found a higher adiposity and inflammatory response through activation of NF-κB signaling among offspring fed on HFD.[60] Further investigations are required to investigate the role of maternal VD status in mediating immune system among offspring.

One of the possible mechanisms underlying VD protects against inflammatory diseases will be VD plays a role in epigenetically modifying genes related to growth and metabolic functions. A few human studies have found that DNA methylation status is closely linked to VD metabolism. Meyer et al. found that TLR-induced upregulation of VDR protein levels influenced DNA methylation status positively in blood extracted from South Africans.[61] When they were supplemented with 1,25D, decreased cathelicidin antimicrobial peptide (cAMP) protein levels,

which could be transactivated by VDR, were observed and its mRNA expression inversely impacted DNA methylation status.[61] These results were consistent to findings from Zhu et al., who demonstrated reduced DNA methylation status of *DHCR7* (encodes 7-dehydrocholesterol reductase), *Cyp2r1*, and *Cyp24a1* genes in African American VDD adolescents.[62] The hypomethylated genes found were mostly associated with regulation of metabolic processes, cellular development, cell differentiation and transcriptional factor binding.[62] In addition, a randomized controlled trial done on supplementing vitamin D_3 to pregnant women of 24 to 28 weeks of gestation confirmed the silencing of genes associated with metabolic functions and developmental processes in skeletal, nervous and respiratory systems.[63] These studies suggested the need of optimizing maternal VD status to decrease DNA methylation status, therefore, activating transcriptions of genes involved in VD metabolism and its downstream to induce protective effect against low-grade inflammation among offspring born to OWO mum.

In addition to the role of VD in systemic and colonic inflammation, its role in renal health has been documented. In fact, VD insufficiency has been commonly observed in patients with CKD.[64] When human monocytic cells were cultured in pooled serum from hemodialysis patients, Brito et al. demonstrated reduced expression of inflammatory markers, TLR-4, MCP-1 and cathelicidin upon treatment with 1,25D.[65] This may suggest a potential anti-inflammatory role of 1,25D under a uremic environment. These observations were further suggested by a 12-week intervention study where patients with Stage II and III CKD, when supplemented with VD, showed reduced circulating levels of inflammatory markers compared to non-supplemented patients.[66] In preclinical models, Alkharfy et al. also reported that vitamin D_3 supplementation was capable of reversing severe renal damage induced by a maternal HFD among offspring ,[67] and reduced oxidative stress and inflammation and improved renal histopathological score.[68,69] In

support of these studies, a recent rat study conducted by Bernardo et al. also shown that obese rats with VDD exhibited higher inflammatory status and much severe damage to renal tubulointerstitial components compared to VDD non-obese rats and VD sufficient obese rats, suggesting a possible synergistic effects of obesity and VDD on progression of CKD.[70] While promising data have been shown in both human and animal studies that VD might play a critical role in attenuating renal injury and/or renal inflammation, a gap in knowledge still remains, particularly on how maternal VD status or maternal dietary intake may affect renal health and exacerbate VDD-related complications among offspring.

D) <u>Methyl-donor Nutrients</u>

MN include micronutrients that act either as a co-enzyme or a co-factor in one-carbon metabolism (OCM), such as folate, methionine, betaine, choline, vitamin B_{12} (B_{12}), and zinc. Dietary folate obtained from food such as leafy green vegetables need to be hydrolyzed into folic acid (FA) with the aid of zinc-dependent enzyme upon absorption into enterocytes. In the blood circulation, folate is transported to peripheral cells in the form of FA or 5-methyl tetrahydrofolate (5MTHF). Folate can be absorbed into peripheral cells, such as brain, placenta, and hematopoietic cells, via folate receptors in the form of FA, 5MTHF, or reduced folate, such as dihydrofolate (DHF) and tetrahydrofolate (THF). 5MTHF supplies methyl group through B_{12} to homocysteine to form methionine, catalyzed by methionine synthase. Methionine can then be converted to S-adenosyl methionine (SAM), catalyzed by methionine adenosyl transferase, which is a universal donor of methyl groups. After donating its methyl group to compounds, such as DNA and phosphatidyl-choline, SAH is formed and recycled to form homocysteine to continue the cycle. After phosphatidyl-choline receives a methyl group, choline is formed, which then can be used in synthesis of cell membranes and precursor of neurotransmitter, acetylcholine.

Choline can then be oxidized into betaine, which then be converted into methionine, catalyzed by betaine homocysteine methyltransferase, to maintain the methionine cycle.

DNA methylation from SAM is catalyzed by DNA methyltransferase (DNMT) and hypermethylated DNA is related to gene silencing, which means decreasing transcription of targeted genes, and this can be passed down to the next generation. This is evidenced by Godfrey et al., which followed up with pregnancy women 9 year-postpartum and found a positive association between child's fat mass and methylation status at CpG sites in RXRA gene,[71] suggesting the link between epigenetic modifications and adiposity among offspring throughout late childhood. Although a recent genome-wide study on offspring in France failed to prove the role of maternal dietary pattern in global DNA methylation status among offspring,[72] Cooper et al. found preconception supplementation that includes folate, B_{12} and zinc significantly altered methylation status in genes associated with child growth and development, such as IGF-2.[73] A randomized trial providing daily supplementation of 400 μg folic acid to pregnant women also showed significantly lower DNA methylation status of genes, such as *IGF2*, *BDNF* and LINE-1 in cord blood.[74] These data collectively suggested that epigenetic modifications passed down from OWO mothers rescued by maternal MS.

Moreover, rodent models have been used extensively to investigate the effects of maternal MS on epigenetic modifications and offspring health in either nutrient-deprived or DIO dams. A few studies fed SD rats with either a control or a protein-restricted (PR) diet with or without MS preconception and throughout gestation and lactation.[18,75,76] Collectively, they found MN restored both global and specific DNA methylation status induced by dams in a nutrient-deprived state.[18,76] In addition, there were alterations found in several gene expression associated with fetal growth, such as *Igf2, H19 and Plagl1*.[75] Awazu and Hida further investigated the role

of MN in renal development among offspring in *in vitro* organoid cultures and found that MDD significantly reduced number of ureteric bud tips and surface area of kidneys,[76] suggesting that MN is important in offspring growth and development.

On the other hand, several studies examined the role of MS in mitigating offspring from dams on HFS diet.[77,78] Both studies fed rodents with either a control or a HFD (45% kcal from fat) during lactation,[77,78] while Jiao et al. fed C57BL/6 mice started the diet 1.5 weeks prior to mating.[78] Both studies found that MS, including FA, B_{12}, choline and betaine, reversed insulin resistance and alterations in gene expression of hepatic enzymes involved in lipid metabolism induced by HFS.[77,78] In addition, MS was found to significantly regulate methylation status at the promoter regions in genes involved in lipogenesis and adipogenesis, such as leptin, adiponectin, PPARγ and FAS.[77,78] Cordero et al. also found that offspring born to Wistar rats on HFS had higher body weights, % body fat, liver fat content and plasma triglyceride levels, while MS protected against these effects.[79] Another study that used similar Wistar rat models found a B-12 deficient maternal diet contributed to significant decrease in essential fatty acid levels and reduced global DNA methylation in placenta.[80] Moreover, maternal choline supplementation to mouse model of placental insufficiency was found to decrease apoptosis and suppress gene expression of inflammatory and angiogenic genes, such as *Tnfα*, *Nfκb*, *Il1b*, and *Vegfa*.[81] On the other hand, Cho et al. investigated the role of maternal MS on mammary carcinogenesis among female SD rat offspring and found significant reduced tumor volume and higher survival rate in those from MN-supplemented dams.[82] These results collectively suggested the role of maternal MS in reducing risks of obesity-related complications and chronic diseases among offspring.

In addition, MS has been found to play a role in metabolic functions, cancer, and bone health. A recent publication by Khatiwada fed weaned male SD rats with MN, but not

methionine until 7 months of age and found that MS was able to maintain body weight, adiposity, and metabolic functions, which were not significantly different from rats fed on a control diet.[83] Consistent with Khatiwada et al., another study, which investigated the novel function of dietary choline supplementation to HFD mice on hepatocarcinoma, also found greater expression of genes involved in cholesterol uptake and synthesis and significant decreases in concentrations of circulating immune cells, therefore slowing progression of HFD-induced hepatocarcinoma.[84] The underlying mechanism can be explained by an *in vitro* study done on hepatic carcinoma cancer cells HepG2.[85] This study found higher methionine levels induced proteomic changes and reduced cancer associated phenotypes through increasing phosphorylation of Akt and activating mTOR pathways, suggesting MN may mitigate risk of cancer through Akt/mTOR signaling pathway.[85] On the other hand, Feigerlova et al. fed Wistar rats, a MN-deficient model, with either a control or a diet lack of folate and B_{12} one month before pregnancy throughout gestation and lactation,[5] reduced body weight, tibia length, total bone mineral density was demonstrated in offspring from dams with MDD, accompanied by significant reductions in protein levels of VDR, ER-α and PRMT1.[5] Feigerlova et al. also studied effects of MDD in *in vitro* human osteosarcoma cells and found slower cell proliferation.[5] Moreover, when MN-deficient bone cells were treated with 1,25D, a blunted response was observed and VDR expression, which was expected to increase, was not altered after the treatment.[5] This suggested VD needed to function in a MN-sufficient environment and the possible link between MN and VD metabolism.

A Mexican cross-sectional study published in 2012 aimed to investigate the dietary intakes of micronutrients in dialysis patients and they found less than half of the participants had adequate intake of B vitamins.[86] A more recent retrospective study published in 2017 found that

a higher serum B_{12} level and a lower serum folate level were modestly associated with a higher risk of all-cause mortality in hemodialysis patients.[87] In addition, another cross-sectional study involving Taiwanese hemodialysis patients found that folic acid supplementation reduced all-cause mortality rate by 10%.[88] These clinical studies conclusively suggest that MN, such as folic acid, B_{12} and zinc, may help in mitigating progression of CKD.

There are several animal studies also investigating role of single methyl-donor nutrient in development of renal diseases or acute renal injury. B_{12} supplemented to male mice with acute renal injury found to be effective in ameliorating renal injury, kidney inflammation, tubular fibrosis, and reversing oxidative stress in reductive oxygen species-induced renal injury.[89] Another study utilizing betaine supplemented to rats showed the beneficial effect of betaine in repairing renal dysfunction, tubulointerstitial injury, insulin signaling and reducing renal inflammation and lipid accumulation induced by a high-fructose diet.[90] However, several animal studies have resulted in controversial results. For example, methionine restriction, in contrast to other MN, was shown to be helpful in reducing renal damage by downregulating renal fibrosis, tubular damage, and inflammation.[91,92] Perhaps further investigation should focus on the combined effects of these MN, including folic acid, vitamin B_{12}, zinc, betaine and methionine supplemented to maternal diet on renal health among offspring due to the gap in knowledge on how MN synergistically affects kidneys of offspring when supplemented to maternal diet.

E) Conclusion

In conclusion, a Western diet rich in saturated fat and added sugar has contributed to adverse pregnancy and offspring outcomes. A human study indicated that VDD may be a causal factor of obesity as observed from DNA methylation loss in *Cyp27b1* gene, which encodes enzyme to activate VD. VD status has been found to mitigate risks of inflammatory-related

diseases, such as IBD, CKD, and COVID-19. One proposed mechanism on its role in the immune system is that cathelicidin is a direct downstream target of VDR and presence of active VD, 1,25D upregulated its expression. In an *in vitro* study, the skeletal role of 1,25D has been shown to be blunted in the absence of MN, including folate and B12, indicating MN-sufficient environment is important for VD to function. In addition, MN has been widely known for their role in supplying methyl groups to hypomethylated DNA,[18] therefore, this may be a potential strategy to restore loss in DNA methylation status in genes involved in VD metabolism.

III. SUPPLEMENTATION OF METHYL-DONOR NUTRIENTS TO A HIGH-FAT HIGH-SUCROSE DIET DURING PREGNANCY AND LACTATION NORMALIZES VITAMIN D STATUS AND SYSTEMIC INFLAMMATION AMONG OFFSPRING

1. Introduction

In a recent analysis conducted in the United States, the prevalence of overweight and obesity (OWO) among children had increased from 2% to 5% in 20 years (from 1999 to 2018).[93] This may be due to the recently discovered association between maternal dietary pattern with growth trajectories and risks of childhood obesity, whereby 4-year-old children possessed higher risks of being OWO if their mothers had higher intakes of fast food, characterized by high saturated fat, and added sugar, commonly known as the Western diet.[94] Several *in vivo* animal studies have linked the consumption of a high-fat diet, HFD (60% kcal from fat) to increased inflammation and metabolic dysfunctions among offspring.[27,28] In addition, a higher maternal healthy eating index (HEI) had sex-specific associations with phenotypic changes in inflammation and growth factor signaling.[95]

Vitamin D (VD) presents naturally either in the form of cholecalciferol (vitamin D_3) from sunlight and animal sources or in ergocalciferol (vitamin D_2) from plant sources. VD is absorbed in the form of D_3 and binds to vitamin D binding protein (VDBP) to be transported in blood circulation to the liver. In the liver, vitamin D_3 is converted into 25-hydroxyvitamin D (25D), which is then further hydroxylated into its active form, 1α,25-dihydroxy vitamin D (1,25D) by the 1α-hydroxylase enzyme (encoded by *Cyp27b1* gene) in the kidneys. Homeostasis of 1,25D levels can be maintained by the expression of VD receptor (VDR) through upregulation or downregulation of the 1α-hydroxylase enzyme and the 24-hydroxylase enzyme (encoded by

Cyp24a1 gene), which catabolizes 1,25D into 1-24,25-trihydroxyvitamin D3 to be excreted out of the body, respectively.

Low VD status and changes in genes related to VD metabolism are commonly observed among offspring born to dams on HFD.[32,33] Moreover, suboptimal VD status (serum 25D < 30 ng/mL) is strongly correlated with inflammatory diseases, cancers and autoimmune diseases.[52,96] Furthermore, VD supplementation has been shown to protect against intestinal inflammation. One proposed underlying mechanism is the production of antimicrobial peptides, such as cathelicidin.[97] Gene expression of cathelicidin has been found to be directly regulated by VDR upon binding to 1,25D, a common ligand for VDR.[55] Furthermore, emerging evidence has demonstrated the ability of cathelicidin to attenuate the activation of toll-like receptor 4 (TLR4), thus suppressing the downstream inflammatory response.[36,98]

A recent European cohort study reported that a diet rich in methyl-donor nutrients (MN), such as folate, vitamin B_{12}, choline, methionine, and betaine, was associated with global methylation changes,[72] which were linked to risks of cardiometabolic diseases among offspring.[31] Previously, an *in vivo* rat study showed that maternal MN deficiency downregulated gene expression of *VDR* and reduced the bioavailability of active VD.[5] However, the role of MN in modulating methylation status of VD-related genes under an obesogenic environment remains unclear. In this study, we hypothesized that MN supplementation (MS) during pregnancy and lactation could improve VD status and alleviate maternal high-fat, high-sucrose (HFS) diet-induced inflammation among offspring.

2. Materials and Methods

2.1 Animal Design

The animal study was completed in Feb 2022 at Texas State University (Approved IACUC protocol # 7616). A brief animal study design is described below and shown in **Figure 1**.

Female Sprague Dawley (SD) rats aged 8 weeks were purchased from Charles River (Wilmington, MA) and mated at 10 weeks of age with sires fed a control diet. After confirmation of pregnancy through detection of spermatozoa in vagina, the dams were randomly assigned into 4 groups of 10 rats each (n = 10). One group of rats (CON) consumed a control diet containing adequate levels of MS (AIN-93G, Research Diets, Inc.). The second group of rats (CON-MS) consumed the control diet supplemented with MN as in previous studies,[78,99-101] and included vitamin B12 (1.5 mg/kg diet), folic acid (15 mg/kg diet), betaine (15 mg/kg diet), choline (11.2 mg/kg diet), L-methionine (7.5 mg/kg diet), and zinc (34.1 mg/kg diet). The third group of rats (HFS) consumed a 45 % fat diet on kcal basis with added sucrose and adequate levels of MN (D2011906, Research Diets, Inc.). The last group of rats consumed this HFS diet supplemented with MN (HFS-MS). Pups were weaned at 3 weeks of age to either a CON or a HFS diet until sacrifice (15-weeks-old). The diets were stored frozen and fresh diets were provided twice weekly. All rats had *ad libitum* access to feed and water. At euthanization, blood and colonic samples were collected for further analyses.

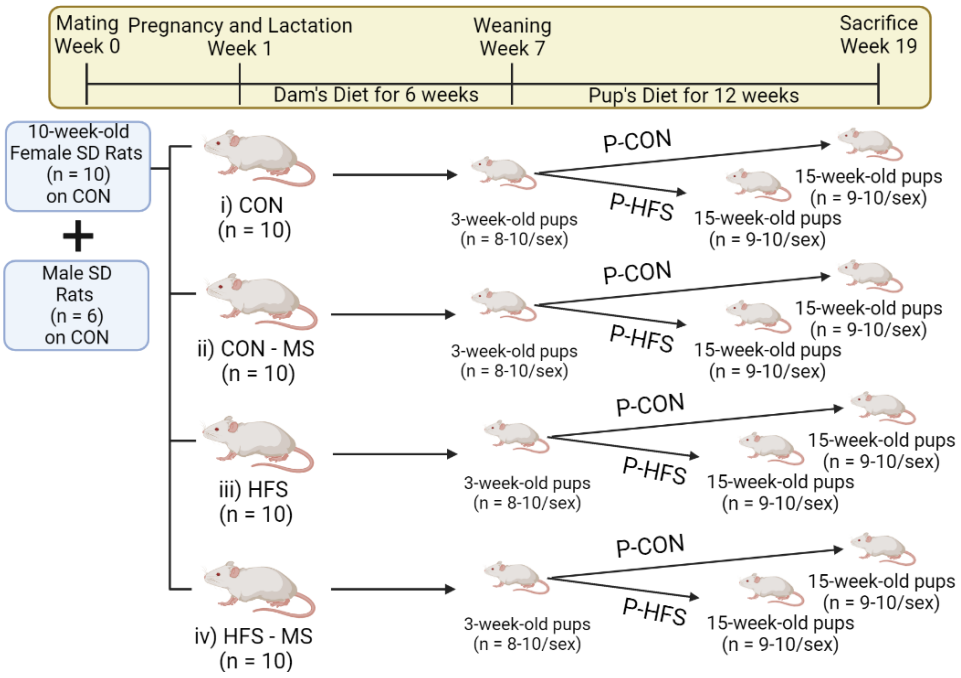

Figure 1. Animal study design. After mating with male Sprague Dawley (SD) rats, dams were randomly assigned to control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS) during pregnancy and lactation for 6 weeks (n = 10/group). Three-week-old pups were weaned into either a control or a HFS diet for 12 weeks until euthanasia at 15 weeks of age (n = 9 -10/sex/group). p-CON, postnatal control diet; p-HFS, postnatal high-fat high-sucrose diet.

2.2 Analysis of Vitamin D Status among Offspring

To examine the effect of maternal HFS on VD status among offspring, blood collected from the rats via cardiac puncture was centrifuged at 1,500 × *g* for 15 minutes to collect serum. Serum 25D levels were determined using a commercially available ELISA kit (Crystal Chem).

2.3 Analysis of Colonic Vitamin D Signaling among Offspring

To examine the effect of maternal diet on colonic VD signaling, colonic tissues collected were fixed in formalin before vertical dissection. Glasss microscopic slides were used to scrap off colonic mucosa which was frozen at -80℃ for further analysis. RNA was extracted from lysed

colonic tissues and quantified using a Nanodrop. cDNA was synthesized using a SuperScript IV cDNA Reverse Transcriptase kit (Invitrogen). Real-time PCR reactions were used to determine the expression of vitamin D receptor (VDR), Cyp27b1, which encodes VD-activating enzyme, 1α-hydroxylase, and cathelicidin, a downstream target of VD metabolism. The primer sets specific for each target were presented in Table 1.

Table 1. List of primers set for RT-PCR examined in this study.

Primer	Forward (5' – 3')	Reverse (5' – 3')
Gapdh	*GCA CAG TCA AGG CTG AGA AT*	*TGA AGA CGC CAG TAG ACT CC*
Vdr	*CCA TTC AGG ACC GCC TAT CC*	*GTC GGC CAG TTT CTG GAT CA*
Cyp27b1	*AAA GGT GTC TGT CCA GTC CA*	*CTC ATA GAG TGC CCA GGA GA*
Cathelicidin	*GGG TTG CCT CTA GCC GTT T*	*TGA AGT CAT CCA CAG CAG CAC GG*
Il1β	*GCA CAG TTC CCC AAC TGG TA*	*ACA CGG GTT CCA TGG TGA AG*
Il6	*GTT GCC TTC TTG GGA CTG ATG*	*ATA CTG GTC TGT TGT GGG TGG T*
Tlr4	*ACA GGG CAC AAG GAA GTA GC*	*GTT CTC ACT GGG CCT TAG CC*

2.4 Analysis of Colonic and Systemic Inflammation among Offspring

Real-time PCR was also used to determine the expression of pro-inflammatory markers, such as IL-1β, IL-6 and TLR4 in colonic tissue as above. The primer sets specific for each target were presented in Table 1. To examine the effect of maternal diet on systemic inflammation among offspring, blood collected from the rats (via cardiac puncture) was centrifuged at 1,500 × *g* for 15 minutes to collect plasma. Circulating levels of pro-inflammatory cytokines IL-1β and IL-6 were measured using a commercially available ELISA kit (Crystal Chem).

2.5 Statistical Analysis

All statistical analyses were performed by using SigmaPlot version 14.5 (Palo Alto, CA, USA). Data from adult offspring were analyzed by using two-way ANOVA. All data were followed by

Tukey's post hoc analyses or Dunn's multiple comparison for unequal groups. All data were normally distributed and correlations of colonic VDR mRNA expression and colonic proinflammatory markers mRNA expression were determined by Pearson's correlation. For mRNA expression data, statistical analyses were conducted by using data from ΔCt, and relative expression values are reported as indicated. *p*-values < 0.05 were considered significant. All data are presented as mean ± standard error, unless otherwise specified. The data presented in this study are pooled from both male and female pups, as no sex interaction was found.

3. Results

3.1 MS during Pregnancy and Lactation Normalizes Vitamin D Status among Adult Offspring Induced by Maternal HFS Diet

Among adult offspring, no significant effect was observed from postnatal diet on VD status within each dam's diet (Figure 2). Adult pups born to HFS dams exhibited 20% less circulating 25D compared to pups born to CON dams (*P = 0.013*). On the other hand, MS to HFS dams during pregnancy and lactation reversed this trend, in which VD status in their offspring was increased by 25% (*P = 0.043*), resulting in levels similar to those observed in pups born to CON dams.

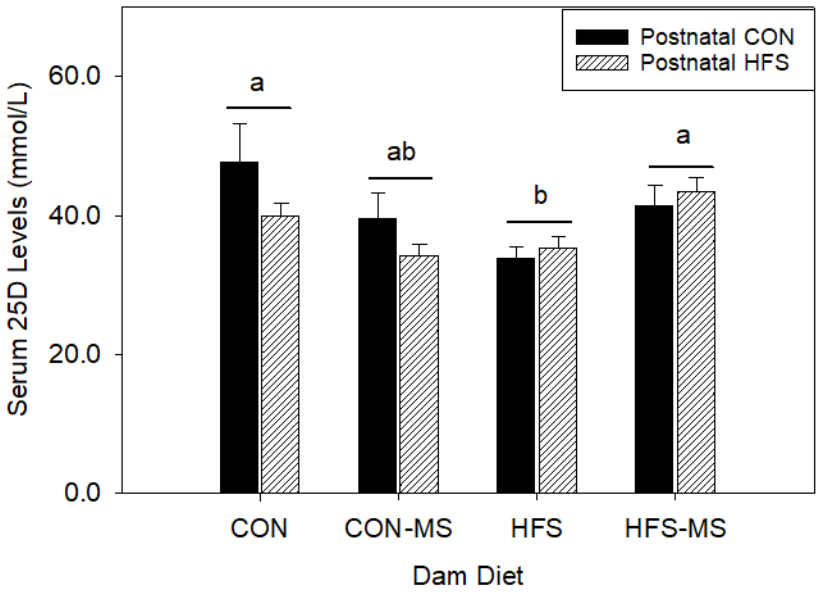

Figure 2. Circulating 25-hydroxycholecalciferol (25D) levels among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Different letters indicate significant differences between maternal diets at $P < 0.05$. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

3.2 MS during Pregnancy and Lactation Attenuates Maternal HFS-Induced Inflammation in Adult Pups

To further evaluate the inflammatory status in the adult pups, we determined the circulating levels of proinflammatory cytokines, IL-1β and IL6, by using commercially available ELISA kits. Plasma IL-1β levels in adult pups born to dams that consumed the HFS diet were higher than those born to dams that consumed the CON diet ($p = 0.01$) regardless of the diet that the pups received after weaning (Figure 3). Compared to pups born to HFS dams, circulating IL-1β concentrations were 24% lower in pups of HFS-MS dams ($p = 0.038$). Circulating IL-1β concentrations did not differ between pups born to HFS-MS and CON dams. Similarly, no difference was observed between pups of CON dams and pups of CON-MS dams. Circulating IL-6 concentrations were also investigated; however, plasma IL-6 levels were too low to be detected in these pups.

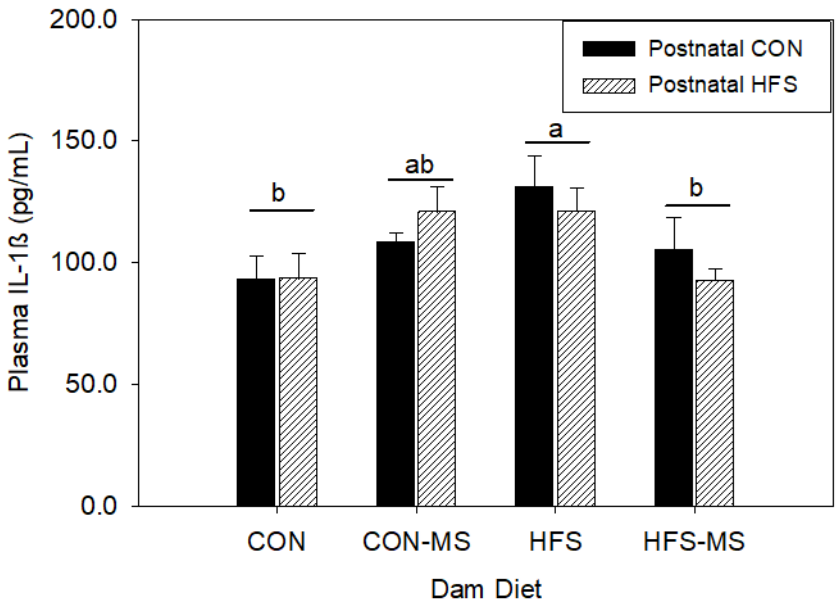

Figure 3. Circulating IL-1β levels among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Different letters indicate significant differences between maternal diets at $P < 0.05$. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

3.3 Colonic Vitamin D Signaling among Adult Offspring Was Not Affected by Pre- or Postnatal Diets

At 15 weeks of age, the mRNA expression of colonic *VDR* was numerically lower in pups born to HFS dams than in pups born to CON dams, but this effect failed to reach significance ($p = 0.42$) (Figure 4). However, mRNA expression of colonic cathelicidin in pups born to HFS dams tended to be lower compared to those of CON dams ($p = 0.09$) (Figure 5). To determine if maternal or pup diets may have altered the synthesis of active VD (1,25-dihydroxycholecalciferol) in the colon, we further investigated the expression of the mRNA encoding the *Cyp27b1* enzyme. However, no difference was detected between all groups at either weaning or adulthood (Figure 6).

VD plays a critical role in immune regulation. However, no significant differences among each prenatal and postnatal diet groups were observed in mRNA expression of pro-inflammatory markers, including toll-like receptor 4 (TLR4), IL-1β and IL-6 (Figure 7,8 & 9). We also investigated the relationship between colonic VDR and these pro-inflammatory markers. Positive correlations between the mRNA expression of colonic *VDR* and *Tlr4*, as well as with *Il-1β* and *Il6*, were observed in 15-week-old adult pups (Table 2).

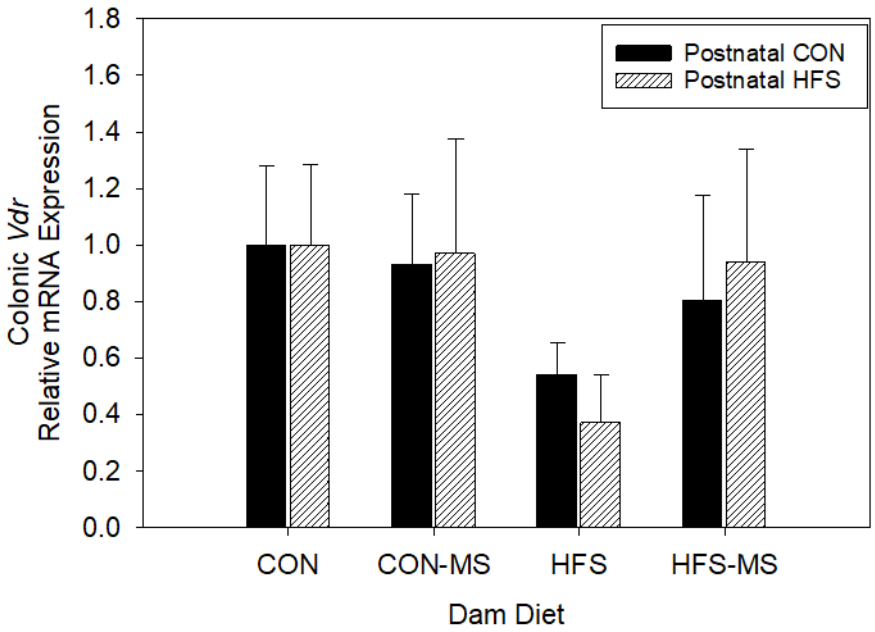

Figure 4. Relative mRNA expression of colonic *Vdr* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

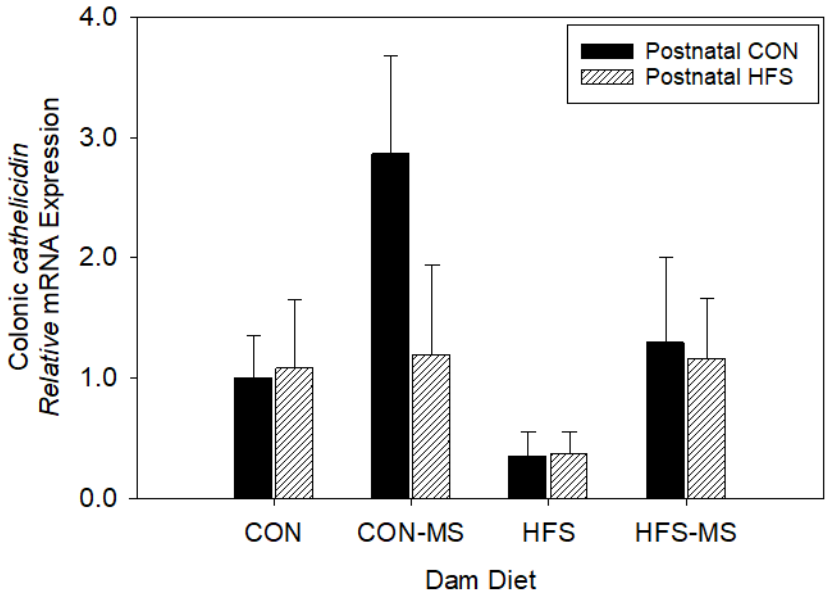

Figure 5. Relative mRNA expression of colonic *cathelicidin* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

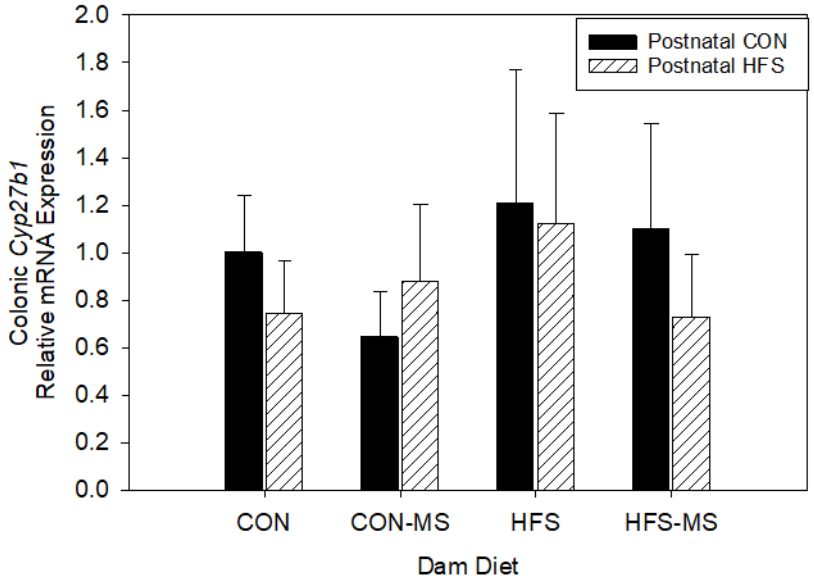

Figure 6. Relative mRNA expression of colonic *Cyp27b1* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

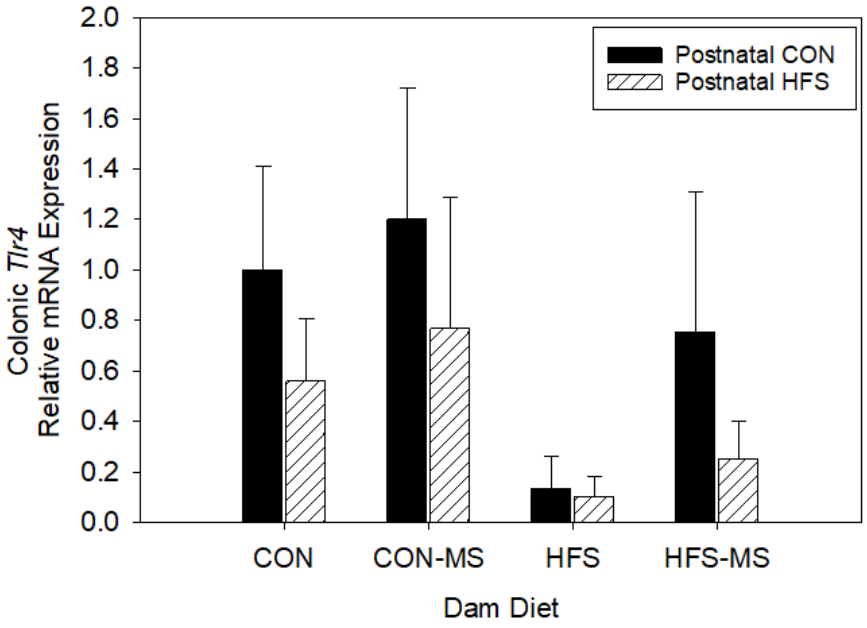

Figure 7. Relative mRNA expression of colonic *Tlr4* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

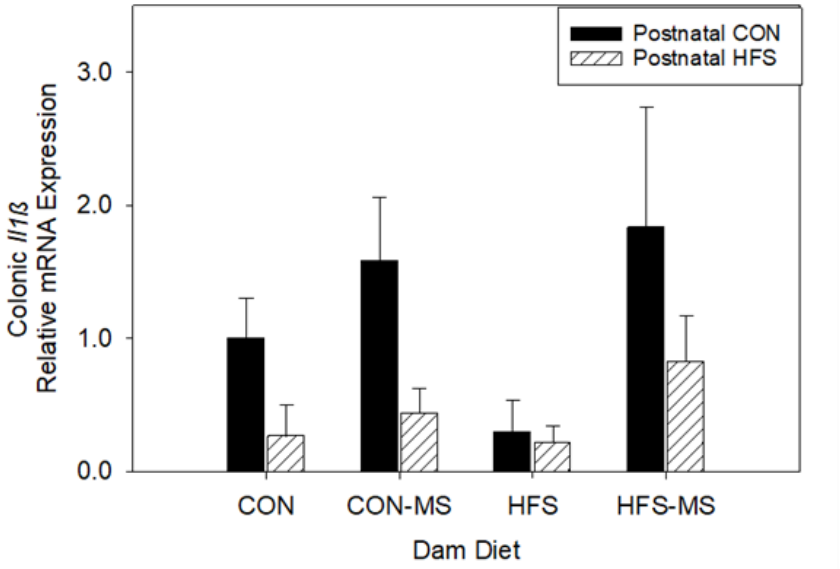

Figure 8. Relative mRNA expression of colonic *Il1β* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

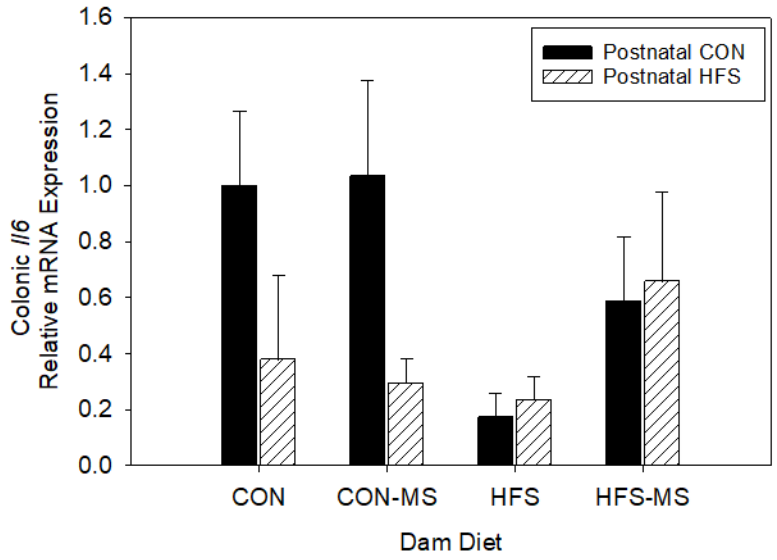

Figure 9. Relative mRNA expression of colonic *Il6* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

Table 2. Pearson's correlation between mRNA expression of *Vdr* and proinflammatory markers in offspring colonic mucosa.

mRNA species	Pearson's correlation coefficient (*r*)	*p*- value
Tlr4	0.419	<0.01
Il1β	0.323	<0.01
Il6	0.294	<0.01

4. Discussion

Our results present the novel role of MS to a maternal HFS diet in regulating systemic inflammation and VD status among adult offspring. Although we previously found that MS to a maternal HFS diet did not alter the VD status among weanling pups born from dams,[102] after 12 weeks of postnatal diet intervention, in the current study we show that MN supplemented to a maternal HFS diet improved VD status among adult offspring compared to those born to dams on a HFS diet. Nonetheless, no significant changes were observed in colonic VD signaling

among adult pups. On the other hand, maternal HFS-induced inflammation was attenuated among adult offspring, as indicated by reduced circulating IL-1β levels. Our results suggest that prenatal diet, but not postnatal diet, may determine the systemic changes in VD status and inflammation observed in this study.

MN are crucial in one-carbon metabolism, particularly supplying methyl groups to DNA to induce epigenetic changes. Maternal high-fat diet has been shown to alter DNA methylation status, which could further contribute to impaired glucose tolerance and increased inflammation among offspring.[26,31] However, there are conflicting results in animal and human studies on the associations between MN, such as folate, choline and methionine, and risk of chronic disease.[85,103] These may be attributed to the fact that most studies focus solely on one methyl-donor nutrient and not the interactions among these nutrients. To date, limited information is available on the synergistic effect of these nutrients as a dietary pattern. Our study utilized a combination of several MN with the goal of optimizing one-carbon metabolism and correct the epigenetic changes typically associated with high-fat high-sucrose intake, such as VD deficiency, impaired glucose tolerance, and chronic low-grade inflammation.

The transgenerational effects of VD in prevention and management of diseases commonly used VD-deficient model or supplementation during pregnancy, lactation, or both.[104,105] We showed that excessive intake of fat and sucrose during pregnancy and lactation disrupted VD status among adult offspring, and that it was independent from VD status among the dams. With prenatal MS, serum 25D levels were enhanced in offspring from HFS-MS dams, which demonstrated the possible role of MS in reversing epigenetic changes in VD metabolism. Studies have shown that maternal VD status altered *VDR* gene expression and VD status among fetal and adult offspring.[48,50] We did not observe significant changes in VD status among dams,

indicating that VD metabolism among offspring may be mediated by other factors, such as maternal inflammatory status. Future research will focus on elucidating the relationship between maternal inflammation and VD status among offspring.

The functional role of VD beyond bone health is well recognized. In the past two decades, the role of VD in other phenomena, including cardiovascular health, autoimmune diseases, and various cancers, has been elucidated.[106] The immunomodulatory role of VD has further demonstrated in infectious diseases and metabolic diseases.[107,108] Low VD status was often observed in obese individuals and weight loss was associated with increased levels of 25D as shown in clinical studies.[42,109] This is further supported by a meta-analysis showing that supplementation of cholecalciferol reduces BMI and waist circumferences among obese and overweight individuals, and so may exert a potential weight loss effect.[110] Hence, optimization of VD status is critical in preventing the development or progression of obesity, which may serve as a viable strategy to prevent morbidity or mortality associated with obesity.

It is believed that greater risk for developing gastrointestinal diseases, such as colorectal cancer and IBD, among the obese could be driven by low-grade inflammation in the gut.[28] We hypothesized that optimization of VD status can attenuate the obesity-induced colonic inflammation through the activation of VDR in the gut. In support of this concept, we demonstrated a positive correlation between colonic *VDR* and other proinflammatory cytokines (i.e., *Tlr4*, *Il6*, and *Il1β*) in the gut, confirming an immunomodulatory role of VD signaling in the gut. Furthermore, cathelicidin has been shown to activate TLR4 signaling and modulate inflammatory status *in vivo*.[111]

Though it is still unclear how MN mediated the restorative effect of VD status among offspring, folate and vitamin B_{12} deficiencies have been shown to suppress VDR protein levels in

bone cells *in vitro*.[5] This may indicate a critical role of MN in mediating VD signaling and subsequent inflammatory responses among the offspring. It is interesting that the mRNA expression of colonic *Vdr* and cathelicidin were upregulated in weanling offspring born to HFS dams as demonstrated in our previous study;[102] yet, at 15 weeks postnatal, mRNA expression of colonic *Vdr* and cathelicidin appear to be lower in pups born to HFS dams compared to those of CON dams. Because we observed an increased levels of circulating IL-1β in adult pups born to HFS dams compared to those of CON dams, the reduced expression of colonic *Vdr* and proinflammatory cytokines in these pups may suggest a compensatory mechanism to suppress inflammation induced by a maternal HFS diet among the adult offspring. It is also important to note that mRNA expression of colonic *Vdr* is not dependent on serum 25D circulating levels. Because no changes in colonic 1α- hydroxylase (*Cyp27b1*) were observed, we postulated that regulation of VDR in the colon could be mediated by other factors, such as inflammatory cytokines.

5. Conclusion

A maternal Western diet, characterized by high fat and high sugar intakes, predisposes risk of obesity-related chronic diseases among offspring, including impaired glucose and lipid metabolism, higher inflammation status, and low VD status, possibly via epigenetic modifications. Our study concludes the beneficial effect of MN supplemented to a HFS diet during pregnancy and lactation for the maintenance of VD signaling and regulation of inflammation among offspring. Most importantly, our data demonstrate the role of prenatal diet in modulating VD status and inflammation among offspring, suggesting a potential dietary intervention to maternal diet in promoting offspring health. These may further fill the gap regarding the efficacy of VD supplementation in the prevention of chronic diseases. It is well

recognized that VD insufficiency is a public health concern as it enhances risks for metabolic comorbidities. Hence, our findings shed new light for chronic disease prevention by optimizing VD status through prenatal dietary modifications.

IV. EFFECTS OF PRENATAL AND POSTNATAL DIETS ON SYSTEMIC VITAMIN D SIGNALING AND RENAL INFLAMMATION AMONG ADULT OFFSPRING

1. Introduction

Maternal obesity has been shown to increase the risk for developing chronic kidney disease (CKD) among offspring. CKD is characterized by loss of nephron in number and function, tubulointerstitial injury, glomerular fibrosis, and tubular atrophy. Several rodent studies found greater risks of renal damage among adult offspring due to increased levels of inflammation and oxidative stress induced by a maternal high-fat diet, HFD (60% kcal from fat).[37,38] In addition, when offspring were weaned to a HFD postnatally, greater progression of renal injury and higher inflammatory status in both male and female offspring were demonstrated, suggesting a combined effect from both prenatal and postnatal diets on modifying renal health among offspring.[40,41]

Maternal vitamin D (VD) status has been found to be associated with renal outcomes in children at the age of 6.[112] Vitamin D (VD) presents naturally either in the form of cholecalciferol (vitamin D_3) from sunlight and animal sources or in ergocalciferol (vitamin D_2) from plant sources. Upon binding to vitamin D binding protein (VDBP), VD undergoes first hydroxylation in the liver to yield 25-hydroxycholecalciferol (25D), which is then further hydroxylated into its active form, 1α,25-dihydroxy vitamin D (1,25D) by 1α-hydroxylase enzyme (encoded by *Cyp27b1* gene) in the kidneys. The genomic actions of 1,25D are regulated by VD receptor expression. In addition to activating 25D into 1,25D, homeostasis of 1,25D levels can be maintained by catabolizing 25D into 1-24,25-trihydroxyvitamin D3, a reaction catalyzed by the 24-hydroxylase enzyme (encoded by *Cyp24a1* gene), for excretion.

Lower maternal 25D levels, a common indicator of VD status, tended to be associated with larger renal volume, indicating increased urinary output, and higher eGFR score in their children.[112] Additionally, VD insufficiency is commonly observed in patients with CKD, especially among those overweight and obese (OWO) individuals.[64] VD deficiency in obese rats with CKD further damaged renal tubulointerstitial component and induced higher inflammatory status compared to VD-deficient non-obese and VD-sufficient obese rats.[70] A double-blinded randomized controlled trial involving Stage II and III CKD patients reported that that VD supplementation in the form of cholecalciferol or VD analogue, paricalcitol, was beneficial in reducing serum levels of inflammatory marker, monocyte chemoattractant protein-1 (MCP-1).[66,113] Consistent with the reported clinical studies, several rodent studies further supported the beneficial role of VD in protecting against progression of CKD by reducing oxidative stress and inflammation in the kidneys.[67,68] Though the cause and effect were still uncertain, the role of VD signaling in CKD may be attribute to the fact that kidney is the main regulation site of VD activation, as it has been shown that restoration of renal health normalized low VD status in type 2 diabetic animal model.[114]

We previously demonstrated that supplementation of methyl-donor nutrients (MN) to a high-fat high-sucrose (HFS) diet during pregnancy and lactation normalized VD status and suppressed inflammation in adult offspring,[102] and activation of VD is manly regulated in the kidneys. MN, such as folate, methionine, and betaine, play a critical role in one-carbon metabolism. To date, there is a lack of information regarding the role of MN on renal VD signaling and subsequent renal outcomes. Studies have thus far reported mixed results. A recent retrospective study conducted in hemodialysis patients demonstrated that higher serum vitamin B_{12} levels and lower serum folate levels were modestly associated with higher risk of all-cause

mortality.[87] In contrast, others have reported a beneficial effect of folic acid supplementation in reducing mortality rate by 10%.[88] Animal studies also generated inconclusive results on the effect of methyl-donor nutrient supplementation (MS) on renal outcomes.[90,91] Because most of the studies only utilized single methyl-donor nutrient, and the synergistic effects, particularly the transgenerational effects, of MN in renal health and VD signaling, have not been researched, which may partially explain the discrepancy of data from clinical and preclinical studies. The objective of this study was to investigate the effect of MS during pregnancy and lactation on renal VD signaling and its impact on renal inflammation among their offspring. We hypothesized that maternal HFS diet attenuates renal vitamin D signaling and promotes renal inflammation among adult offspring, and that supplementation with MN during pregnancy and lactation can reverse such trend.

2. Materials and Methods

2.1 Animal Design

The study was conducted using tissues and samples generated from previous study (Approved IACUC protocol #7616). Study design was described in Chapter III (Section III, Page 23; Figure 1). In brief, supplementation of MN to a maternal CON and HFS was given during pregnancy and lactation. Pups were weaned at 3-week of age and randomized into 2 groups receiving either a CON or HFS diets. After 12 weeks of dietary intervention, 15-week-old pups were sacrificed and euthanized to collect serum and kidney samples for further analysis.

2.2 Analysis of Circulating Levels of 1,25D among Offspring

To examine the effect of maternal HFS on serum 1,25D levels among offspring, blood collected from the rats (via cardiac puncture) was centrifuged at 1,500 × *g* for 15 minutes to collect serum.

Concentrations of 1,25D in the serum were determined using a commercially available ELISA kit (Crystal Chem).

2.3 Analysis of Renal Vitamin D Signaling and Renal Inflammation among Offspring

To determine the effect of maternal diet on renal VD metabolism and renal inflammation among adult offspring, frozen kidney tissues were lysed for RNA extraction and quantification using a Nanodrop. cDNA was synthesized using a SuperScript IV cDNA Reverse Transcriptase kit (Invitrogen). Real-time PCR reactions were used to determine gene expression of VDR, *Cyp27b1* and *Cyp24a1*, as well we gene expression of pro-inflammatory markers, IL-1β, IL-6 and TLR4. The primer sets specific for each target were presented in Table 3. The mRNA expression of specific gene target were normalized to *Gapdh* (housekeeping gene) and expressed in relative to offspring fed a CON diet born to CON dams.

Table 3. List of primers set for RT-PCR examined in this study.

Primer	**Forward (5' – 3')**	**Reverse (5' – 3')**
Gapdh	*GCA CAG TCA AGG CTG AGA AT*	*TGA AGA CGC CAG TAG ACT CC*
Vdr	*CCA TTC AGG ACC GCC TAT CC*	*GTC GGC CAG TTT CTG GAT CA*
Cyp27b1	*AAA GGT GTC TGT CCA GTC CA*	*CTC ATA GAG TGC CCA GGA GA*
Cyp24a1	*AGC CCG GGG CAG ATT TCC TCT G*	*CAT ATT CCT CAG GTC TTC CGC*
Il1β	*GCA CAG TTC CCC AAC TGG TA*	*ACA CGG GTT CCA TGG TGA AG*
Il6	*GTT GCC TTC TTG GGA CTG ATG*	*ATA CTG GTC TGT TGT GGG TGG T*
Tlr4	*ACA GGG CAC AAG GAA GTA GC*	*GTT CTC ACT GGG CCT TAG CC*

2.4 Analysis of DNA Methylation Status of Vitamin D Receptor (VDR)

To investigate the changes in DNA methylation status of genes involved in VD metabolism, genomic DNA (gDNA) was extracted from homogenized kidney tissues using a commercially available kit (EZ Quick DNA Miniprep Plus Kit, Zymo Research). gDNA extracted from kidney

tissues underwent bisulfite conversion using EZ DNA methylation Lightning Kit (Zymo Research). Bisulfite-converted DNA were amplified using methylation-specific qRT-PCR (MSP) to determine DNA methylation status in promoter regions on *Vdr* gene using bisulfite sequencing technique. The primer sets specific for *Vdr* gene were presented in Table 4. The percentage of DNA methylation in the promoter regions were calculated relative to DNA methylation status of adult offspring born to CON dams.

Table 4. List of primers set for MSP examined in this study.

Primer	Forward (5' – 3')	Reverse (5' – 3')
Vdr (unmethylated)	*TTG AGT GTT TTG TAG GAG AAA GTT GT*	*TAT CAC CCA TAC CTA AAT TAA CCC A*
Vdr (methylated)	*TGA GCG TTT TGT AGG AGA AAG TC*	*CCG TAC CTA AAT TAA CCC GAA*

2.5 Statistical Analysis

All statistical analyses were performed by using SigmaPlot version 14.5 (Palo Alto, CA, USA). Data from serum 1,25D were analyzed using two-way ANOVA followed by Tukey's post hoc analyses. All data were normally distributed. Correlations of renal *Vdr* mRNA expression and renal *Tlr4* mRNA expression were determined by Pearson's correlation coefficient. For mRNA expression data, statistical analyses were conducted by using data from ΔCt, and relative expression values are reported as indicated. p-values < 0.05 were considered significant. All data are presented as mean ± standard error, unless otherwise specified. The data presented in this study are pooled from both male and female pups, as no sex interaction was found.

3. Results

3.1 Circulating 1,25D levels were higher in offspring born to HFS dams regardless of maternal MS

Adult offspring born to dams on HFS diet exhibited 39% higher levels of circulating 1,25D compared to those born to dams on CON diet, regardless of the diet the pups consumed (Figure 10). Maternal MS did not affect serum 1,25D levels among offspring born to dams of CON or HFS diets compared to offspring from non-supplemented CON or HFS dams, respectively. No significant interactions between pup's diets on serum 1,25D levels were observed.

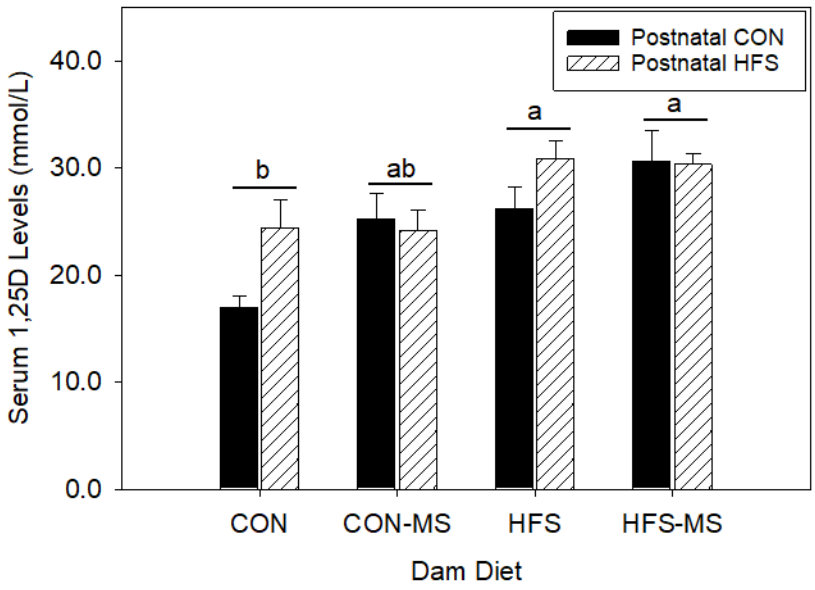

Figure 10. Circulating 1,25-dihydroxy vitamin D (1,25D) levels among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Different letters indicate significant differences between maternal diets at $P < 0.05$. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

3.2 Maternal HFS Suppresses Systemic Vitamin D Signaling in Adult Offspring Rats

The mRNA expression of *Vdr* contrasted with serum 1,25D levels. Compared to offspring born to CON dams, mRNA expression of renal *Vdr* was 50% lower in offspring born to HFS dams, but MS to a prenatal CON or HFS diets did not alter the expression of renal *Vdr* in adult offspring (Figure 11). There were no significant effects due to postnatal diet nor significant interactions between maternal and postnatal diets observed.

The mRNA levels of enzymes regulating VD metabolism, *Cyp27b1* and *Cyp24a1* were not impacted by both prenatal and postnatal diets (Figure12 & 13).

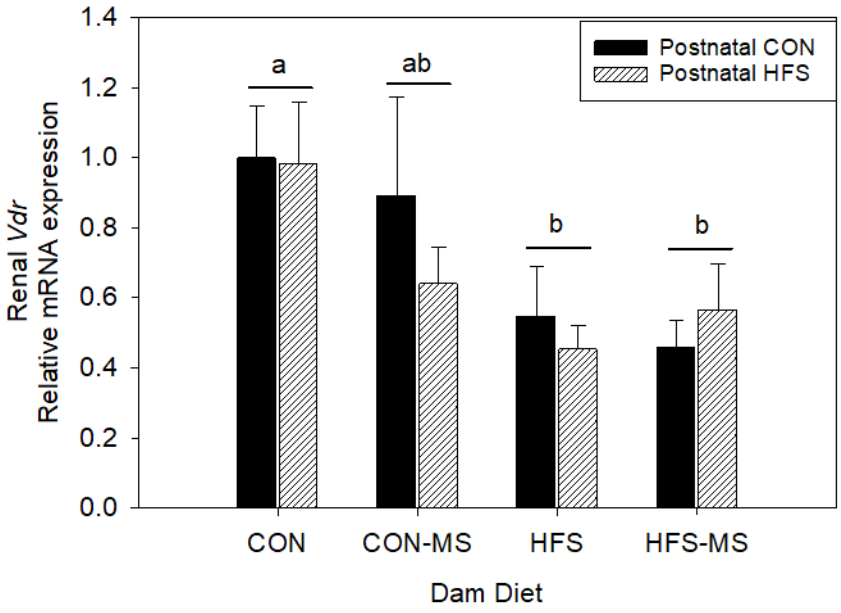

Figure 11. Relative mRNA expression of renal *Vdr* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Different letters indicate significant differences between maternal diets at $P < 0.05$. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

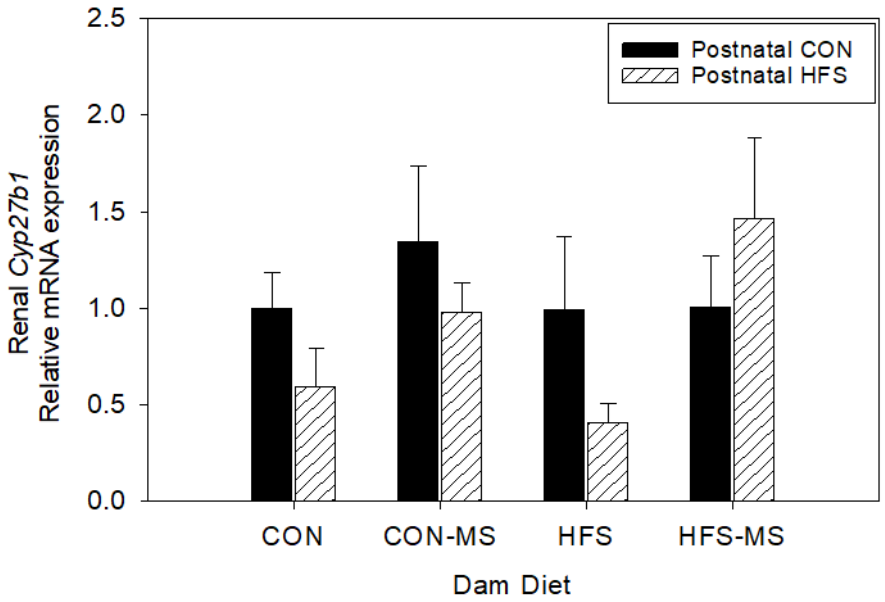

Figure 12. Relative mRNA expression of renal *Cyp27b1* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

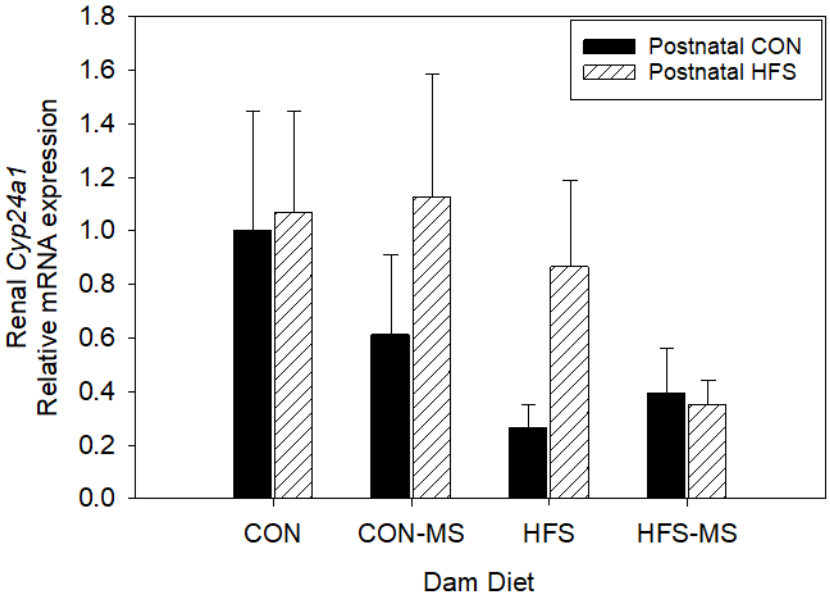

Figure 13. Relative mRNA expression of renal *Cyp24a1* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

3.3. Prenatal and Postnatal Diet Differentially Regulate Renal Inflammation among Adult Offspring

To assess renal inflammatory status, we measured the mRNA expression of pro-inflammatory cytokines, *Il6* and *Il1β*, and the pro-inflammatory receptor, toll-like receptor 4 (*Tlr4*) in the kidney. While no effect from prenatal diets was detected with renal *Il1β* mRNA expression among all groups, postnatal diets, specifically the HFS diet, downregulated the mRNA expression of renal *Il1β* by 50% in adult pups born to HFS-MS dams when compared to pups born to the same groups of dams but fed a postnatal CON diet (Fig. 14). However, we did not observe similar effects of postnatal diets on other pups born to CON, CON-MS, or HFS dams. Similarly, we did not observe an effect of prenatal diets on renal *Il6* mRNA expression. However, a significant interaction was observed between prenatal and postnatal diets (P= 0.011) with regards to renal *Il6* mRNA expression. Within offspring born to HFS dams, postnatal HFS diets upregulated the expression of renal *Il6* by 2.8-fold (P = 0.021) when compared to those on CON diet. Inversely, postnatal HFS diets attenuated renal *Il6* expression by 6.25-fold in pups born to HFS-MS dams compared to those born to HFS dams and fed a postnatal HFS diet (P = 0.023). We also observed an 88% increase in renal *Il6* expression between CON-fed pups born to CON and HFS dams (Fig. 15). *Tlr4* activation is known to mediate the production of pro-inflammatory cytokines. We further evaluated the renal *Tlr4* mRNA expression in these rats. As expected, prenatal diet did not affect the expression of *Tlr4* among offspring. Yet, postnatal HFS, but not postnatal CON, attenuated the expression of *Tlr4* by 60% (P = 0.018) and 40% (P = 0.039), respectively, in pups born to HFS-MS when compared to pups of CON dams and pups of HFS dams. Within HFS-MS dams, the mRNA expression of *Tlr4* were decreased by 50% when pups were fed a HFS diets compared to those of CON diets (P = 0.008; Fig 16).

As we have previously demonstrated a positive correlation between mRNA expression of colonic *Vdr* and several pro-inflammatory markers, such as IL-1β, IL-6 and TLR4, we ran Pearson's correlation test to examine the relationships between mRNA expression of renal *Vdr* and pro-inflammatory markers. However, null result was generated from the tests.

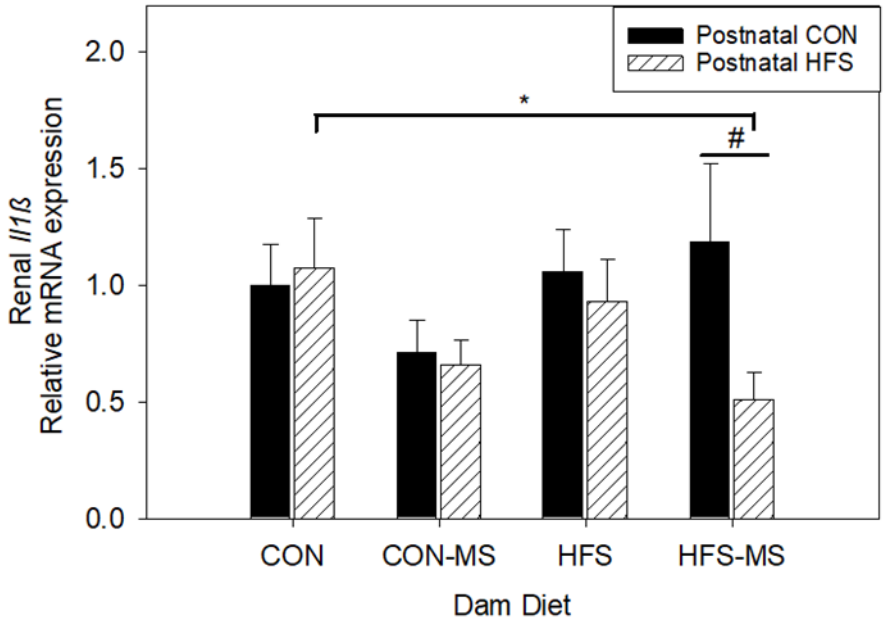

Figure 14. Relative mRNA expression of renal *Il1β* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). * Indicates statistically significant difference between dam's diet within offspring fed on postnatal HFS diet. # indicates statistically significant difference between postnatal diet within offspring from same group of dams. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

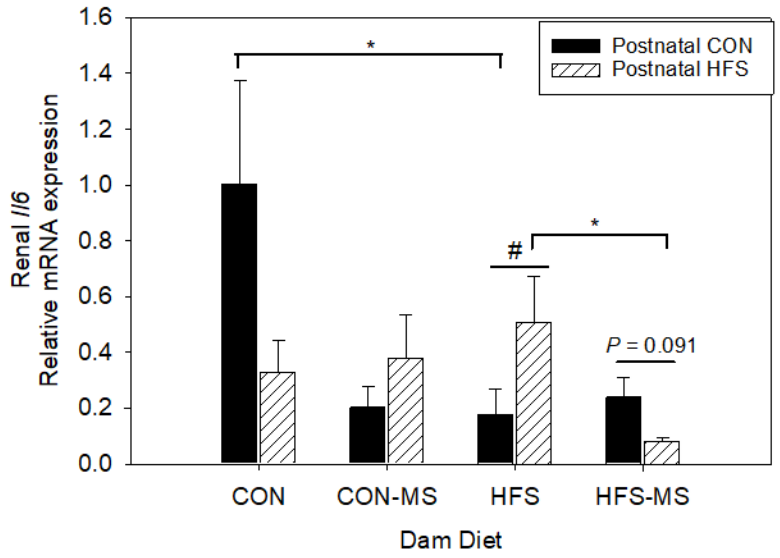

Figure 15. Relative mRNA expression of renal *Il6* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). * Indicates statistically significant difference between dam's diet within offspring fed on postnatal HFS diet. # indicates statistically significant difference between postnatal diet within offspring from same group of dams. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

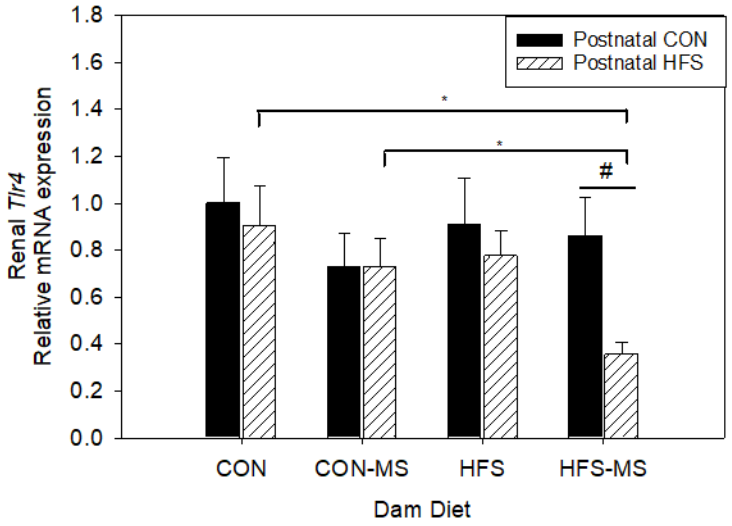

Figure 16. Relative mRNA expression of renal *Tlr4* gene among adult offspring born to dams fed a control (CON) or high-fat high-sucrose (HFS) diet with or without methyl-donor nutrient supplementations (MS). * Indicates statistically significant difference between dam's diet within offspring fed on postnatal HFS diet. # indicates statistically significant difference between postnatal diet within offspring from same group of dams. Data are expressed as mean ± SEM (n = 10/ group). Postnatal CON, control postnatal diet; Postnatal HFS, high-fat high-sucrose postnatal diet.

3.4 Null Effects on DNA Methylation Status in Vitamin D Metabolism-Related Genes

Methylation-specific PCR (MSP) analysis on VDR was performed. Unmethylated VDR was detected in all samples. However, out of 48 samples, only 4 methylated VDR were detected in samples via MSP analysis. Three of the detected samples were from HFS dams fed on CON diet postnatally, while one sample was from pup born to HFS-MS dams on postnatal HFS diet. As most of methylated samples generated null results, therefore, did not run further analysis on genes encoding enzymes regulating vitamin D metabolism (i.e., *Cyp27b1* and *Cyp24a1*).

4. Discussion

Our previous study demonstrated that consumption of a HFS diet, which is similar to the Western diet, during pregnancy and lactation by dams resulting in adverse health outcomes, such as declined VD status and elevated inflammation, among both weanling and adult pups.[102] The supplementation of MS was capable of attenuating maternal HFS-induced changes in VD status, colonic VD signaling, and both colonic and systemic inflammation.[102] In our current study, we have demonstrated increased serum levels of active VD, 1,25D in offspring born to HFS dams, regardless of MS status. In contrast to serum 1,25D, systemic VD signaling, as characterized by VDR activation, which is regulated mainly in the kidneys, was suppressed by maternal HFS compared to those born to dams on CON diet. Like serum 1,25D, renal *VDR* mRNA expression was not attenuated by maternal MS. In addition, our results showed differential regulations of maternal and postnatal diet interventions in modulating renal inflammation.

Similar to a non-human primate study done by Mata-Greenwood et al,[33] our study demonstrated a decreased mRNA expression of VDR in offspring born to mothers on a HFS diet. Recent years, there have been studies researching the immunomodulatory of VD in several

diseases, such as inflammatory bowel disease (IBD),[115] COVID-19,[116] autoimmune disease,[117] and cancer.[96] In a recent review paper, TLR4 has been identified as a therapeutic target for renal injury due to its role in inducing production of inflammatory cytokines.[118] On the other hand, TLR signaling have been found to mediate production of cathelicidin, which is an antimicrobial peptide and a direct downstream target of VDR, and it was also correlated with upregulation of *Cyp27b1* mRNA expression,[36,57] an observation that is consistent with our results where numerically lower relative expression of *Cyp27b1* was observed in HFS offspring with lower renal *Tlr4* gene expression. We previously demonstrated a positive correlation between mRNA expression of colonic *Vdr* and pro-inflammatory markers, IL-1β, IL-6 and TLR4, however, no correlation was found between mRNA expression of renal *Vdr* and pro-inflammatory markers, IL-1β, IL-6 and TLR4 in current study. This may suggest renal inflammatory signaling may be independent from renal VD signaling.

Previously, we have demonstrated that prenatal, but not postnatal diet, modulate colonic VD signaling, and subsequently, colonic, and systemic inflammation among offspring rats.[102] In this study, we further supported the fact that maternal diet plays a critical role in modulating VD signaling and renal inflammation among offspring. Yet, differential effects were observed between colonic and renal VD signaling within the same dietary groups, particularly, we did not observe significant changes with renal VD signaling and inflammation in offspring born to dams of MS compared to that of colon.[102] This may suggest a different regulatory system between colon and kidney pertaining to VD metabolism and regulation of downstream inflammatory signals. Regardless, our results presented a novel interaction between prenatal and postnatal diet in renal inflammatory response in offspring, where significant differences were mainly observed within offspring born to HFS-MS dams. Though the mechanisms remain uncertain, it is possible

that prenatal MSs to a HFS diets may have altered the metabolic regulation of lipid and subsequent inflammatory responses among offspring, as renal inflammatory status has shown to be improved in HFS-fed pups born to HFS-MS dams, compared to pups born to HFS dams.

Because serum 1,25D concentrations have been found to be correlated with methylation levels of VDR,[119] we proposed that MS may epigenetically modulate the mRNA expression of VD metabolism-related genes through induction of DNA methylation changes. Although our study did not generate significant results on DNA methylation changes in VDR, 3 of the 4 methylated samples were pups from HFS dams fed on CON diet. These hypermethylated samples were consistent with our hypothesized mechanism, where maternal HFS induces higher DNA methylation status, causing lower *Vdr* gene expression in the kidneys of offspring. This is in line with our observations on *V*dr gene expression, where 50% significantly lower relative gene expression was observed among offspring from HFS dams. While the results on *Vdr* methylation are still at preliminary stage, we suspect that the null results observed in most of the samples (44 out of 48) may be due to low concentrations of DNA retrieved from bisulfite conversion. In addition, a study by Wang et al. found that betaine, one of the MS included in this study, decreased methylation status in hepatic PPARγ, the regulator of liver lipid metabolism, to correct triglyceride accumulation in classical mice model of dyslipidemia.[120] Therefore, we have not ruled out the possibility that hypomethylated DNA in *Vdr* may be a protective mechanism among offspring from HFS-MS dams. Further validation studies will be required to confirm the methylation status of *Vdr* and VD metabolism-related genes.

5. Conclusion

Collectively, our results demonstrated that maternal HFS diet improved serum 1,25D levels but reduced renal VD signaling among adult offspring with no effect observed from

prenatal MS. In contrast to our previous study, maternal MS to either a CON or HFS diet did not alter VD signaling nor inflammatory status in the kidney in their offspring, suggesting that maternal caloric intake, but not MS, during pregnancy and lactation exerted much significant impact on renal health among offspring. The mechanisms underlying the interactions between pre- and postnatal diets on renal inflammation as well as the role of VD in such events remained to be investigated.

V. SUMMARY AND FUTURE DIRECTIONS OF RESEARCH

To our knowledge, this is the first time the synergistic effect of methyl-donor nutrients (MN) supplementation (MS) to a high-fat high-sucrose (HFS) diet during pregnancy and lactation on reversing phenotypic changes in VD status and colonic pro-inflammatory markers have been demonstrated. This may warrant further investigation on the combined effects of several MN, including folate, methionine, vitamin B_{12}, choline, betaine, and zinc, on risks of developing other chronic diseases, such as cardiometabolic events and cancer, among adult offspring.

Results generated from this thesis project indicate distinct regulators for colonic and renal Vitamin D (VD) signaling, where colonic VD signaling and inflammation may be modified by maternal MS. However, renal VD signaling and inflammation are mainly regulated by pre- and postnatal diet modifications. Collectively, our studies support the role of both maternal and postnatal diets in mediating VD signaling and therefore, inflammation both locally in colon and systematically among offspring. Renal inflammation may be regulated by prenatal and postnatal dietary modifications, independent of renal VD signaling. Further, based on our studies, we speculated that the result discrepancy from VD supplementations could be partially explained by maternal dietary intake. Hence, strategies to optimize VD status, and subsequently colon health, may focus on modifications of maternal diet via MN. Furthermore, as we have observed in our study that postnatal diet modifications were effective in modulating renal inflammation, perhaps a healthy diet during childhood would be important to prevent risks of renal diseases later in life.

Future research should focus on evaluating the underlying mechanisms on how MS normalizes VD signaling and attenuates inflammation among offspring as MN have been known to induce epigenetic modifications and have a transgenerational effect on offspring health.

Additionally, modifications in hepatic VD metabolism-related genes, such as *Vdr* and *Cyp2r1*, which encodes enzyme to convert vitamin D_3 into 25D, will need further investigations as previous research has reported a correlation between methylation level of CYP2R1 and serum 1,25D concentrations in patients with pulmonary infection.[119] Thus far, our study provides future directions in research to focus on maternal supplementation in modifying risks of chronic diseases via optimization of VD status among offspring in animal studies, or even further into clinical studies.

REFERENCES

1. Voerman E, Santos S, Patro Golab B, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Med*. Feb 2019;16(2):e1002744. doi:10.1371/journal.pmed.1002744

2. Dunn GA, Mitchell AJ, Selby M, Fair DA, Gustafsson HC, Sullivan EL. Maternal diet and obesity shape offspring central and peripheral inflammatory outcomes in juvenile non-human primates. *Brain Behav Immun*. May 2022;102:224-236. doi:10.1016/j.bbi.2022.02.024

3. Karlsson T, Osmancevic A, Jansson N, Hulthen L, Holmang A, Larsson I. Increased vitamin D-binding protein and decreased free 25(OH)D in obese women of reproductive age. *Eur J Nutr*. Feb 2014;53(1):259-67. doi:10.1007/s00394-013-0524-8

4. Daraki V, Roumeliotaki T, Chalkiadaki G, et al. Low maternal vitamin D status in pregnancy increases the risk of childhood obesity. *Pediatr Obes*. Aug 2018;13(8):467-475. doi:10.1111/ijpo.12267

5. Feigerlova E, Demarquet L, Melhem H, et al. Methyl donor deficiency impairs bone development via peroxisome proliferator-activated receptor-gamma coactivator-1alpha-dependent vitamin D receptor pathway. *FASEB J*. Oct 2016;30(10):3598-3612. doi:10.1096/fj.201600332R

6. Aarestrup J, Bjerregaard LG, Gamborg M, et al. Tracking of body mass index from 7 to 69 years of age. *Int J Obes (Lond)*. Sep 2016;40(9):1376-83. doi:10.1038/ijo.2016.88

7. Collaborators GBDO, Afshin A, Forouzanfar MH, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*. Jul 6 2017;377(1):13-27. doi:10.1056/NEJMoa1614362

8. Ajala O, Mold F, Boughton C, Cooke D, Whyte M. Childhood predictors of cardiovascular disease in adulthood. A systematic review and meta-analysis. *Obes Rev*. Sep 2017;18(9):1061-1070. doi:10.1111/obr.12561

9. Chang R, Mei H, Zhang Y, Xu K, Yang S, Zhang J. Early childhood body mass index trajectory and overweight/obesity risk differed by maternal weight status. *Eur J Clin Nutr*. Mar 2022;76(3):450-455. doi:10.1038/s41430-021-00975-6

10. Xie D, Yang W, Wang A, et al. Effects of pre-pregnancy body mass index on pregnancy and perinatal outcomes in women based on a retrospective cohort. *Sci Rep*. Oct 6 2021;11(1):19863. doi:10.1038/s41598-021-98892-y

11. Colon-Ramos U, Racette SB, Ganiban J, et al. Association between dietary patterns during pregnancy and birth size measures in a diverse population in Southern US. *Nutrients*. Feb 16 2015;7(2):1318-32. doi:10.3390/nu7021318

12. Qin R, Ding Y, Lu Q, et al. Associations of maternal dietary patterns during pregnancy and fetal intrauterine development. *Front Nutr*. 2022;9:985665. doi:10.3389/fnut.2022.985665

13. Frihauf JB, Fekete EM, Nagy TR, Levin BE, Zorrilla EP. Maternal Western diet increases adiposity even in male offspring of obesity-resistant rat dams: early endocrine risk markers. *Am J Physiol Regul Integr Comp Physiol*. Dec 1 2016;311(6):R1045-R1059. doi:10.1152/ajpregu.00023.2016

14. Guibourdenche M, El Khayat El Sabbouri H, Bonnet F, et al. Perinatal exposure to chlorpyrifos and/or a high-fat diet is associated with liver damage in male rat offspring. *Cells Dev*. Jun 2021;166:203678. doi:10.1016/j.cdev.2021.203678

15. Ke X, Fu Q, Sterrett J, Hillard CJ, Lane RH, Majnik A. Adverse maternal environment and western diet impairs cognitive function and alters hippocampal glucocorticoid receptor promoter methylation in male mice. *Physiol Rep*. Apr 2020;8(8):e14407. doi:10.14814/phy2.14407

16. Belenchia AM, Johnson SA, Ellersieck MR, Rosenfeld CS, Peterson CA. In utero vitamin D deficiency predisposes offspring to long-term adverse adipose tissue effects. *J Endocrinol*. Sep 2017;234(3):301-313. doi:10.1530/JOE-17-0015

17. Yu S, Feng Y, Qu C, et al. CYP27B1 as an instrument gene to investigate the causal relationship between vitamin D deficiency and obesity: a family-based study. *Eur J Clin Nutr*. May 2020;74(5):806-810. doi:10.1038/s41430-020-0594-7

18. Giudicelli F, Brabant AL, Grit I, Parnet P, Amarger V. Excess of methyl donor in the perinatal period reduces postnatal leptin secretion in rat and interacts with the effect of protein content in diet. *PLoS One*. 2013;8(7):e68268. doi:10.1371/journal.pone.0068268

19. Black MH, Sacks DA, Xiang AH, Lawrence JM. The relative contribution of prepregnancy overweight and obesity, gestational weight gain, and IADPSG-defined gestational diabetes mellitus to fetal overgrowth. *Diabetes Care*. Jan 2013;36(1):56-62. doi:10.2337/dc12-0741

20. Maguire RL, House JS, Lloyd DT, et al. Associations between maternal obesity, gestational cytokine levels and child obesity in the NEST cohort. *Pediatr Obes*. Jul 2021;16(7):e12763. doi:10.1111/ijpo.12763

21. Yang X, Li M, Haghiac M, Catalano PM, O'Tierney-Ginn P, Hauguel-de Mouzon S. Causal relationship between obesity-related traits and TLR4-driven responses at the maternal-fetal interface. *Diabetologia*. Nov 2016;59(11):2459-2466. doi:10.1007/s00125-016-4073-6

22. LifeCycle Project-Maternal O, Childhood Outcomes Study G, Voerman E, et al. Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *JAMA*. May 7 2019;321(17):1702-1715. doi:10.1001/jama.2019.3820

23. Andersen LB, Pipper CB, Trolle E, et al. Maternal obesity and offspring dietary patterns at 9 months of age. *Eur J Clin Nutr*. Jun 2015;69(6):668-75. doi:10.1038/ejcn.2014.258

24. Stevens DR, Neelon B, Roberts JR, et al. Mediation of the association between maternal pre-pregnancy overweight/obesity and childhood overweight/obesity by birth anthropometry. *J Dev Orig Health Dis*. Feb 2021;12(1):71-78. doi:10.1017/S2040174420000033

25. Chang E, Hafner H, Varghese M, et al. Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. *Sci Rep*. Nov 5 2019;9(1):16027. doi:10.1038/s41598-019-52583-x

26. Keleher MR, Zaidi R, Shah S, et al. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. *PLoS One*. 2018;13(2):e0192606. doi:10.1371/journal.pone.0192606

27. Wallace JG, Bellissimo CJ, Yeo E, et al. Obesity during pregnancy results in maternal intestinal inflammation, placental hypoxia, and alters fetal glucose metabolism at mid-gestation. *Sci Rep*. Nov 26 2019;9(1):17621. doi:10.1038/s41598-019-54098-x

28. Gohir W, Kennedy KM, Wallace JG, et al. High-fat diet intake modulates maternal intestinal adaptations to pregnancy and results in placental hypoxia, as well as altered fetal gut barrier proteins and immune markers. *J Physiol*. Jun 2019;597(12):3029-3051. doi:10.1113/JP277353

29. Borengasser SJ, Zhong Y, Kang P, et al. Maternal obesity enhances white adipose tissue differentiation and alters genome-scale DNA methylation in male rat offspring. *Endocrinology*. Nov 2013;154(11):4113-25. doi:10.1210/en.2012-2255

30. Perkins E, Murphy SK, Murtha AP, et al. Insulin-like growth factor 2/H19 methylation at birth and risk of overweight and obesity in children. *J Pediatr*. Jul 2012;161(1):31-9. doi:10.1016/j.jpeds.2012.01.015

31. Antoun E, Kitaba NT, Titcombe P, et al. Maternal dysglycaemia, changes in the infant's epigenome modified with a diet and physical activity intervention in pregnancy: Secondary analysis of a randomised control trial. *PLoS Med*. Nov 2020;17(11):e1003229. doi:10.1371/journal.pmed.1003229

32. Josefson JL, Feinglass J, Rademaker AW, et al. Maternal obesity and vitamin D sufficiency are associated with cord blood vitamin D insufficiency. *J Clin Endocrinol Metab*. Jan 2013;98(1):114-9. doi:10.1210/jc.2012-2882

33. Mata-Greenwood E, Huber HF, Li C, Nathanielsz PW. Role of pregnancy and obesity on vitamin D status, transport, and metabolism in baboons. *Am J Physiol Endocrinol Metab*. Jan 1 2019;316(1):E63-E72. doi:10.1152/ajpendo.00208.2018

34. Girchenko P, Tuovinen S, Lahti-Pulkkinen M, et al. Maternal early pregnancy obesity and related pregnancy and pre-pregnancy disorders: associations with child developmental milestones in the prospective PREDO Study. *Int J Obes (Lond)*. Jun 2018;42(5):995-1007. doi:10.1038/s41366-018-0061-x

35. Leibowitz KL, Moore RH, Ahima RS, et al. Maternal obesity associated with inflammation in their children. *World J Pediatr*. Feb 2012;8(1):76-9. doi:10.1007/s12519-011-0292-6

36. Coorens M, Schneider VAF, de Groot AM, et al. Cathelicidins Inhibit Escherichia coli-Induced TLR2 and TLR4 Activation in a Viability-Dependent Manner. *J Immunol*. Aug 15 2017;199(4):1418-1428. doi:10.4049/jimmunol.1602164

37. Glastras SJ, Tsang M, Teh R, et al. Maternal Obesity Promotes Diabetic Nephropathy in Rodent Offspring. *Sci Rep*. Jun 9 2016;6:27769. doi:10.1038/srep27769

38. Nguyen LT, Mak CH, Chen H, et al. SIRT1 Attenuates Kidney Disorders in Male Offspring Due to Maternal High-Fat Diet. *Nutrients*. Jan 11 2019;11(1)doi:10.3390/nu11010146

39. Zhou P, Guan H, Guo Y, Zhu L, Liu X. Maternal High-Fat Diet Programs Renal Peroxisomes and Activates NLRP3 Inflammasome-Mediated Pyroptosis in the Rat Fetus. *J Inflamm Res*. 2021;14:5095-5110. doi:10.2147/JIR.S329972

40. Flynn ER, Alexander BT, Lee J, Hutchens ZM, Jr., Maric-Bilkan C. High-fat/fructose feeding during prenatal and postnatal development in female rats increases susceptibility to renal and metabolic injury later in life. *Am J Physiol Regul Integr Comp Physiol*. Feb 15 2013;304(4):R278-85. doi:10.1152/ajpregu.00433.2012

41. Jackson CM, Alexander BT, Roach L, et al. Exposure to maternal overnutrition and a high-fat diet during early postnatal development increases susceptibility to renal and metabolic injury later in life. *Am J Physiol Renal Physiol*. Mar 15 2012;302(6):F774-83. doi:10.1152/ajprenal.00491.2011

42. Karlsson T, Andersson L, Hussain A, et al. Lower vitamin D status in obese compared with normal-weight women despite higher vitamin D intake in early pregnancy. *Clin Nutr*. Oct 2015;34(5):892-8. doi:10.1016/j.clnu.2014.09.012

43. Tint MT, Chong MF, Aris IM, et al. Association between maternal mid-gestation vitamin D status and neonatal abdominal adiposity. *Int J Obes (Lond)*. Jul 2018;42(7):1296-1305. doi:10.1038/s41366-018-0032-2

44. Boyle VT, Thorstensen EB, Thompson JMD, et al. The relationship between maternal 25-hydroxyvitamin D status in pregnancy and childhood adiposity and allergy: an observational study. *Int J Obes (Lond)*. Dec 2017;41(12):1755-1760. doi:10.1038/ijo.2017.182

45. Morales E, Rodriguez A, Valvi D, et al. Deficit of vitamin D in pregnancy and growth and overweight in the offspring. *Int J Obes (Lond)*. Jan 2015;39(1):61-8. doi:10.1038/ijo.2014.165

46. Young BE, Cooper EM, McIntyre AW, et al. Placental vitamin D receptor (VDR) expression is related to neonatal vitamin D status, placental calcium transfer, and fetal bone length in pregnant adolescents. *FASEB J*. May 2014;28(5):2029-37. doi:10.1096/fj.13-246736

47. O'Brien KO, Li S, Cao C, et al. Placental CYP27B1 and CYP24A1 expression in human placental tissue and their association with maternal and neonatal calcitropic hormones. *J Clin Endocrinol Metab*. Apr 2014;99(4):1348-56. doi:10.1210/jc.2013-1366

48. Belenchia AM, Jones KL, Will M, et al. Maternal vitamin D deficiency during pregnancy affects expression of adipogenic-regulating genes peroxisome proliferator-activated receptor gamma (PPARgamma) and vitamin D receptor (VDR) in lean male mice offspring. *Eur J Nutr*. Mar 2018;57(2):723-730. doi:10.1007/s00394-016-1359-x

49. Goyal R, Zhang L, Blood AB, et al. Characterization of an animal model of pregnancy-induced vitamin D deficiency due to metabolic gene dysregulation. *Am J Physiol Endocrinol Metab*. Feb 2014;306(3):E256-66. doi:10.1152/ajpendo.00528.2013

50. Gazquez A, Sanchez-Campillo M, Barranco A, et al. Calcifediol During Pregnancy Improves Maternal and Fetal Availability of Vitamin D Compared to Vitamin D3 in Rats and Modifies Fetal Metabolism. *Front Nutr*. 2022;9:871632. doi:10.3389/fnut.2022.871632

51. Villa CR, Chen J, Wen B, et al. Maternal vitamin D beneficially programs metabolic, gut and bone health of mouse male offspring in an obesogenic environment. *Int J Obes (Lond)*. Dec 2016;40(12):1875-1883. doi:10.1038/ijo.2016.177

52. Garg M, Hendy P, Ding JN, Shaw S, Hold G, Hart A. The Effect of Vitamin D on Intestinal Inflammation and Faecal Microbiota in Patients with Ulcerative Colitis. *J Crohns Colitis*. Jul 30 2018;12(8):963-972. doi:10.1093/ecco-jcc/jjy052

53. Murai IH, Fernandes AL, Sales LP, et al. Effect of a Single High Dose of Vitamin D3 on Hospital Length of Stay in Patients With Moderate to Severe COVID-19: A Randomized Clinical Trial. *JAMA*. Mar 16 2021;325(11):1053-1060. doi:10.1001/jama.2020.26848

54. Arikoglu T, Kuyucu S, Karaismailoglu E, Batmaz SB, Balci S. The association of vitamin D, cathelicidin, and vitamin D binding protein with acute asthma attacks in children. *Allergy Asthma Proc*. Jul-Aug 2015;36(4):51-8. doi:10.2500/aap.2015.36.3848

55. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J*. Jul 2005;19(9):1067-77. doi:10.1096/fj.04-3284com

56. Lee WJ, Cha HW, Sohn MY, Lee SJ, Kim DW. Vitamin D increases expression of cathelicidin in cultured sebocytes. *Arch Dermatol Res*. Oct 2012;304(8):627-32. doi:10.1007/s00403-012-1255-z

57. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. Mar 24 2006;311(5768):1770-3. doi:10.1126/science.1123933

58. Gubatan J, Mehigan GA, Villegas F, et al. Cathelicidin Mediates a Protective Role of Vitamin D in Ulcerative Colitis and Human Colonic Epithelial Cells. *Inflamm Bowel Dis*. May 12 2020;26(6):885-897. doi:10.1093/ibd/izz330

59. Villa CR, Chen J, Wen B, et al. Maternal Dietary Vitamin D Does Not Program Systemic Inflammation and Bone Health in Adult Female Mice Fed an Obesogenic Diet. *Nutrients*. Oct 26 2016;8(11)doi:10.3390/nu8110675

60. Haroun N, Bennour I, Seipelt E, et al. Maternal Vitamin D Deficiency in Mice Increases White Adipose Tissue Inflammation in Offspring. *Cells*. Jun 25 2022;11(13)doi:10.3390/cells11132024

61. Meyer V, Saccone DS, Tugizimana F, Asani FF, Jeffery TJ, Bornman L. Methylation of the Vitamin D Receptor (VDR) Gene, Together with Genetic Variation, Race, and Environment Influence the Signaling Efficacy of the Toll-Like Receptor 2/1-VDR Pathway. *Front Immunol*. 2017;8:1048. doi:10.3389/fimmu.2017.01048

62. Zhu H, Wang X, Shi H, et al. A genome-wide methylation study of severe vitamin D deficiency in African American adolescents. *J Pediatr*. May 2013;162(5):1004-9 e1. doi:10.1016/j.jpeds.2012.10.059

63. Anderson CM, Gillespie SL, Thiele DK, Ralph JL, Ohm JE. Effects of Maternal Vitamin D Supplementation on the Maternal and Infant Epigenome. *Breastfeed Med*. Jun 2018;13(5):371-380. doi:10.1089/bfm.2017.0231

64. Jean G, Souberbielle JC, Chazot C. Vitamin D in Chronic Kidney Disease and Dialysis Patients. *Nutrients*. Mar 25 2017;9(4)doi:10.3390/nu9040328

65. Brito RBO, Rebello JF, Grabulosa CC, et al. 25-vitamin D reduces inflammation in uremic environment. *Sci Rep*. Jan 10 2020;10(1):128. doi:10.1038/s41598-019-56874-1

66. Alvarez JA, Zughaier SM, Law J, et al. Effects of high-dose cholecalciferol on serum markers of inflammation and immunity in patients with early chronic kidney disease. *Eur J Clin Nutr*. Mar 2013;67(3):264-9. doi:10.1038/ejcn.2012.217

67. Alkharfy KM, Ahmed M, Yakout SM, Al-Daghri NM. Effects of calcitriol on structural changes of kidney in C57BL/6J mouse model. *Int J Clin Exp Med*. 2015;8(8):12390-6.

68. Abo El-Magd NF, Eraky SM. The molecular mechanism underlining the preventive effect of vitamin D against hepatic and renal acute toxicity through the NrF2/ BACH1/ HO-1 pathway. *Life Sci*. Mar 1 2020;244:117331. doi:10.1016/j.lfs.2020.117331

69. Arfian N, Budiharjo S, Wibisono DP, et al. Vitamin D Ameliorates Kidney Ischemia Reperfusion Injury via Reduction of Inflammation and Myofibroblast Expansion. *Kobe J Med Sci*. Mar 9 2020;65(4):E138-E143.

70. Bernardo DRD, Canale D, Nascimento MM, et al. The association between obesity and vitamin D deficiency modifies the progression of kidney disease after ischemia/reperfusion injury. *Front Nutr*. 2022;9:952028. doi:10.3389/fnut.2022.952028

71. Godfrey KM, Sheppard A, Gluckman PD, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*. May 2011;60(5):1528-34. doi:10.2337/db10-0979

72. Lecorguille M, Charles MA, Lepeule J, et al. Association between dietary patterns reflecting one-carbon metabolism nutrients intake before pregnancy and placental DNA methylation. *Epigenetics*. Jun-Jul 2022;17(7):715-730. doi:10.1080/15592294.2021.1957575

73. Cooper WN, Khulan B, Owens S, et al. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. *FASEB J*. May 2012;26(5):1782-90. doi:10.1096/fj.11-192708

74. Caffrey A, Irwin RE, McNulty H, et al. Gene-specific DNA methylation in newborns in response to folic acid supplementation during the second and third trimesters of pregnancy: epigenetic analysis from a randomized controlled trial. *Am J Clin Nutr*. Apr 1 2018;107(4):566-575. doi:10.1093/ajcn/nqx069

75. Amarger V, Giudicelli F, Pagniez A, Parnet P. Perinatal high methyl donor alters gene expression in IGF system in male offspring without altering DNA methylation. *Future Sci OA*. Mar 2017;3(1):FSO164. doi:10.4155/fsoa-2016-0077

76. Awazu M, Hida M. Folic acid supplementation alleviates reduced ureteric branching, nephrogenesis, and global DNA methylation induced by maternal nutrient restriction in rat embryonic kidney. *PLoS One*. 2020;15(4):e0230289. doi:10.1371/journal.pone.0230289

77. Cordero P, Gomez-Uriz AM, Campion J, Milagro FI, Martinez JA. Dietary supplementation with methyl donors reduces fatty liver and modifies the fatty acid synthase DNA methylation profile in rats fed an obesogenic diet. *Genes Nutr*. Jan 2013;8(1):105-13. doi:10.1007/s12263-012-0300-z

78. Jiao F, Yan X, Yu Y, et al. Protective effects of maternal methyl donor supplementation on adult offspring of high fat diet-fed dams. *J Nutr Biochem*. Aug 2016;34:42-51. doi:10.1016/j.jnutbio.2016.04.005

79. Cordero P, Campion J, Milagro FI, Martinez JA. Transcriptomic and epigenetic changes in early liver steatosis associated to obesity: effect of dietary methyl donor supplementation. *Mol Genet Metab*. Nov 2013;110(3):388-95. doi:10.1016/j.ymgme.2013.08.022

80. Kulkarni A, Dangat K, Kale A, Sable P, Chavan-Gautam P, Joshi S. Effects of altered maternal folic acid, vitamin B12 and docosahexaenoic acid on placental global DNA methylation patterns in Wistar rats. *PLoS One*. Mar 10 2011;6(3):e17706. doi:10.1371/journal.pone.0017706

81. King JH, Kwan STC, Yan J, et al. Maternal Choline Supplementation Modulates Placental Markers of Inflammation, Angiogenesis, and Apoptosis in a Mouse Model of Placental Insufficiency. *Nutrients*. Feb 12 2019;11(2)doi:10.3390/nu11020374

82. Cho K, Mabasa L, Bae S, Walters MW, Park CS. Maternal high-methyl diet suppresses mammary carcinogenesis in female rat offspring. *Carcinogenesis*. May 2012;33(5):1106-12. doi:10.1093/carcin/bgs125

83. Khatiwada S, Lecomte V, Fenech MF, Morris MJ, Maloney CA. Effects of Micronutrient Supplementation on Glucose and Hepatic Lipid Metabolism in a Rat Model of Diet Induced Obesity. *Cells*. Jul 11 2021;10(7)doi:10.3390/cells10071751

84. Brown AL, Conrad K, Allende DS, et al. Dietary Choline Supplementation Attenuates High-Fat-Diet-Induced Hepatocellular Carcinoma in Mice. *J Nutr*. Apr 1 2020;150(4):775-783. doi:10.1093/jn/nxz315

85. Tripodi F, Badone B, Vescovi M, et al. Methionine Supplementation Affects Metabolism and Reduces Tumor Aggressiveness in Liver Cancer Cells. *Cells*. Nov 16 2020;9(11)doi:10.3390/cells9112491

86. Martin-del-Campo F, Batis-Ruvalcaba C, Gonzalez-Espinoza L, et al. Dietary micronutrient intake in peritoneal dialysis patients: relationship with nutrition and inflammation status. *Perit Dial Int*. Mar-Apr 2012;32(2):183-91. doi:10.3747/pdi.2010.00245

87. Soohoo M, Ahmadi SF, Qader H, et al. Association of serum vitamin B12 and folate with mortality in incident hemodialysis patients. *Nephrol Dial Transplant*. Jun 1 2017;32(6):1024-1032. doi:10.1093/ndt/gfw090

88. Chien SC, Li SY, Chen YT, et al. Folic acid supplementation in end-stage renal disease patients reduces total mortality rate. *J Nephrol*. Nov-Dec 2013;26(6):1097-104. doi:10.5301/jn.5000276

89. Li F, Bahnson EM, Wilder J, et al. Oral high dose vitamin B12 decreases renal superoxide and post-ischemia/reperfusion injury in mice. *Redox Biol*. May 2020;32:101504. doi:10.1016/j.redox.2020.101504

90. Fan CY, Wang MX, Ge CX, Wang X, Li JM, Kong LD. Betaine supplementation protects against high-fructose-induced renal injury in rats. *J Nutr Biochem*. Mar 2014;25(3):353-62. doi:10.1016/j.jnutbio.2013.11.010

91. Cooke D, Ouattara A, Ables GP. Dietary methionine restriction modulates renal response and attenuates kidney injury in mice. *FASEB J*. Feb 2018;32(2):693-702. doi:10.1096/fj.201700419R

92. Kitada M, Ogura Y, Monno I, Xu J, Koya D. Methionine abrogates the renoprotective effect of a low-protein diet against diabetic kidney disease in obese rats with type 2 diabetes. *Aging (Albany NY)*. Mar 6 2020;12(5):4489-4505. doi:10.18632/aging.102902

93. Tsoi MF, Li HL, Feng Q, Cheung CL, Cheung TT, Cheung BMY. Prevalence of Childhood Obesity in the United States in 1999-2018: A 20-Year Analysis. *Obes Facts*. 2022;15(4):560-569. doi:10.1159/000524261

94. Hu Z, Tylavsky FA, Kocak M, et al. Effects of Maternal Dietary Patterns during Pregnancy on Early Childhood Growth Trajectories and Obesity Risk: The CANDLE Study. *Nutrients*. Feb 13 2020;12(2)doi:10.3390/nu12020465

95. Francis EC, Dabelea D, Boyle KE, Jansson T, Perng W. Maternal Diet Quality Is Associated with Placental Proteins in the Placental Insulin/Growth Factor, Environmental Stress, Inflammation, and mTOR Signaling Pathways: The Healthy Start ECHO Cohort. *J Nutr*. Mar 3 2022;152(3):816-825. doi:10.1093/jn/nxab403

96. Larriba MJ, Ordonez-Moran P, Chicote I, et al. Vitamin D receptor deficiency enhances Wnt/beta-catenin signaling and tumor burden in colon cancer. *PLoS One*. 2011;6(8):e23524. doi:10.1371/journal.pone.0023524

97. Wu S, Yoon S, Zhang YG, et al. Vitamin D receptor pathway is required for probiotic protection in colitis. *Am J Physiol Gastrointest Liver Physiol*. Sep 1 2015;309(5):G341-9. doi:10.1152/ajpgi.00105.2015

98. Scheenstra MR, van Harten RM, Veldhuizen EJA, Haagsman HP, Coorens M. Cathelicidins Modulate TLR-Activation and Inflammation. *Front Immunol*. 2020;11:1137. doi:10.3389/fimmu.2020.01137

99. Carlin J, George R, Reyes TM. Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology. *PLoS One*. 2013;8(5):e63549. doi:10.1371/journal.pone.0063549

100. McKee SE, Zhang S, Chen L, Rabinowitz JD, Reyes TM. Perinatal high fat diet and early life methyl donor supplementation alter one carbon metabolism and DNA methylation in the brain. *J Neurochem*. Jun 2018;145(5):362-373. doi:10.1111/jnc.14319

101. Ryan DP, Henzel KS, Pearson BL, et al. A paternal methyl donor-rich diet altered cognitive and neural functions in offspring mice. *Mol Psychiatry*. May 2018;23(5):1345-1355. doi:10.1038/mp.2017.53

102. Teoh CM, Cooper A, Renteria KM, Lane M, Zhu J, Koh GY. Supplementation of Methyl-Donor Nutrients to a High-Fat, High-Sucrose Diet during Pregnancy and Lactation Normalizes Circulating 25-Dihydroxycholecalciferol Levels and Alleviates Inflammation in Offspring. *Metabolites*. Dec 12 2022;12(12)doi:10.3390/metabo12121252

103. Elizabeth KE, Praveen SL, Preethi NR, Jissa VT, Pillai MR. Folate, vitamin B12, homocysteine and polymorphisms in folate metabolizing genes in children with congenital heart disease and their mothers. *Eur J Clin Nutr*. Dec 2017;71(12):1437-1441. doi:10.1038/ejcn.2017.135

104. Cooper C, Harvey NC, Bishop NJ, et al. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Diabetes Endocrinol*. May 2016;4(5):393-402. doi:10.1016/S2213-8587(16)00044-9

105. Wolsk HM, Harshfield BJ, Laranjo N, et al. Vitamin D supplementation in pregnancy, prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring: Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. *J Allergy Clin Immunol*. Nov 2017;140(5):1423-1429 e5. doi:10.1016/j.jaci.2017.01.013

106. Marino R, Misra M. Extra-Skeletal Effects of Vitamin D. *Nutrients*. Jun 27 2019;11(7)doi:10.3390/nu11071460

107. Sabico S, Enani MA, Sheshah E, et al. Effects of a 2-Week 5000 IU versus 1000 IU Vitamin D3 Supplementation on Recovery of Symptoms in Patients with Mild to Moderate Covid-19: A Randomized Clinical Trial. *Nutrients*. Jun 24 2021;13(7)doi:10.3390/nu13072170

108. Shah MS, Fogelman DR, Raghav KP, et al. Joint prognostic effect of obesity and chronic systemic inflammation in patients with metastatic colorectal cancer. *Cancer*. Sep 1 2015;121(17):2968-75. doi:10.1002/cncr.29440

109. Rock CL, Emond JA, Flatt SW, et al. Weight loss is associated with increased serum 25-hydroxyvitamin D in overweight or obese women. *Obesity (Silver Spring)*. Nov 2012;20(11):2296-301. doi:10.1038/oby.2012.57

110. Perna S. Is Vitamin D Supplementation Useful for Weight Loss Programs? A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicina (Kaunas)*. Jul 12 2019;55(7)doi:10.3390/medicina55070368

111. Lowry MB, Guo C, Zhang Y, et al. A mouse model for vitamin D-induced human cathelicidin antimicrobial peptide gene expression. *J Steroid Biochem Mol Biol*. Apr 2020;198:105552. doi:10.1016/j.jsbmb.2019.105552

112. Miliku K, Voortman T, Franco OH, et al. Vitamin D status during fetal life and childhood kidney outcomes. *Eur J Clin Nutr*. May 2016;70(5):629-34. doi:10.1038/ejcn.2015.216

113. Verouti SN, Tsoupras AB, Alevizopoulou F, Demopoulos CA, Iatrou C. Paricalcitol effects on activities and metabolism of platelet activating factor and on inflammatory cytokines in hemodialysis patients. *Int J Artif Organs*. Feb 2013;36(2):87-96. doi:10.5301/ijao.5000187

114. Koh GY, Whitley EM, Mancosky K, et al. Dietary resistant starch prevents urinary excretion of vitamin D metabolites and maintains circulating 25-hydroxycholecalciferol concentrations in Zucker diabetic fatty rats. *J Nutr*. Nov 2014;144(11):1667-73. doi:10.3945/jn.114.198200

115. Garg M, Royce SG, Tikellis C, et al. The intestinal vitamin D receptor in inflammatory bowel disease: inverse correlation with inflammation but no relationship with circulating vitamin D status. *Therap Adv Gastroenterol*. 2019;12:1756284818822566. doi:10.1177/1756284818822566

116. Mercola J, Grant WB, Wagner CL. Evidence Regarding Vitamin D and Risk of COVID-19 and Its Severity. *Nutrients*. Oct 31 2020;12(11)doi:10.3390/nu12113361

117. Harrison SR, Li D, Jeffery LE, Raza K, Hewison M. Vitamin D, Autoimmune Disease and Rheumatoid Arthritis. *Calcif Tissue Int*. Jan 2020;106(1):58-75. doi:10.1007/s00223-019-00577-2

118. Jha AK, Gairola S, Kundu S, et al. Toll-like receptor 4: An attractive therapeutic target for acute kidney injury. *Life Sci*. Apr 15 2021;271:119155. doi:10.1016/j.lfs.2021.119155

119. Wang M, Kong W, He B, et al. Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. *Clin Epigenetics*. Sep 12 2018;10(1):118. doi:10.1186/s13148-018-0552-6

120. Wang L, Chen L, Tan Y, et al. Betaine supplement alleviates hepatic triglyceride accumulation of apolipoprotein E deficient mice via reducing methylation of peroxisomal proliferator-activated receptor alpha promoter. *Lipids Health Dis*. Mar 13 2013;12:34. doi:10.1186/1476-511X-12-34