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REVIEW

Homage to the 'H' in developmental origins of health and disease

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Abundant evidence exists linking maternal and paternal environments from pericopconception through the postnatal period to later risk to offspring diseases. This concept was first articulated by the late Sir David Barker and as such coined the Barker Hypothesis. The term was then mutated to Fetal Origins of Adult Disease and finally broadened to developmental origins of adult health and disease (DOHaD) in recognition that the perinatal environment can shape both health and disease in resulting offspring. Developmental exposure to various factors, including stress, obesity, caloric-rich diets and environmental chemicals can lead to detrimental offspring health outcomes. However, less attention has been paid to date on measures that parents can take to promote the long-term health of their offspring. In essence, have we neglected to consider the 'H' in DOHaD? It is the 'H' component that should be of primary concern to expecting mothers and fathers and those seeking to have children. While it may not be possible to eliminate exposure to all pernicious factors, prevention/remediation strategies may tip the scale to health rather than disease. By understanding disruptive DOHaD mechanisms, it may also illuminate behavioral modifications that parents can adapt before fertilization and throughout the neonatal period to promote the lifelong health of their male and female offspring. Three possibilities will be explored in the current review: parental exercise, probiotic supplementation and breastfeeding in the case of mothers. The 'H' paradigm should be the focus going forward as a healthy start can indeed last a lifetime.

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Introduction

It has been long recognized that the *in utero* stamp can lead to longstanding health consequences for male and female offspring.[1–3] The well-known Cambridge scientists, Robert McCance and Elsie Widdowson, performed pioneering work that showed the early environment, especially nutrition, could impact pre- and post-natal growth and later risk for adult disease.[3] Subsequently, Chow and Lee[1] reported stunted growth in rat offspring born to dams fed an energy restricted diet during gestation. Roeder and Chow[2] then proposed that maternal undernutrition during pregnancy may result in permanent health consequences in resulting offspring. The late Sir David Barker formalized the concept that the genesis of many non-communicable diseases may trace their origins back to the embryonic/perinatal period, resulting in the 'Barker Hypothesis'.[4,5] The term was then changed to fetal origin of adult disease. However, the final agreed upon terminology is developmental origins of health and disease (DOHaD). This phrase introduces the notion that the perinatal environment can for better or worse shape later offspring health and disease outcomes. Thus, the track to a healthy lifespan may also begin during this vulnerable period.

The DOHaD model has gained greater currency due to the escalating data across various disciplines.[4,6–13] It is also increasingly becoming apparent that the environmental state of both the mother and father can underpin later diseases in offspring, even those who appear healthy at birth.[14–26] Such intrinsic and extrinsic factors in animal models and humans include exposure to endocrine disrupting chemicals, such as bisphenol A, phthalates, heavy metals, stress, obesity, high fat/high caloric diets, metabolic status and starvation conditions to provide a few examples. It is also clear that in general, males may be at greater risk for later disorders, including those of the cardiovascular and neurological systems, than females.[27–30]

Overall, less attention has been devoted to maternal and paternal factors that promote the long-term health of males and females. Even though the concept has been extended to include health, the 'H' factor has been for the most part overlooked. Yet, good habits that promote offspring health may be able to mitigate negative perinatal influences. In this review, we will consider three such possibilities: parental exercise, probiotic supplementation and breastfeeding in the case of mothers.

Parental exercise

Maternal exercise and offspring metabolic phenotypes

Maternal exercise either before conception or during gestation might confer later health benefits for male and female offspring and may even be able to compensate for suboptimal

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uterine environments. The effects of maternal exercise on several offspring trajectories will be considered: cardiometabolic, growth, neurobehavioral and other physiological responses.

There are currently conflicting reports as to whether maternal exercise can improve offspring cardiovascular function with most, however, suggesting positive benefits.[31] Table 1 summarizes the recent animal studies, and provides further details on sex differences and variation observed in lean (control) *v*. obese exercised dams, where appropriate. In pigs, maternal exercise during pregnancy results in enhanced endothelium-dependent vaso-relaxation response in the thoracic aorta of newborn female offspring.[32] Adult male and female pigs derived from pregnant sows subjected to exercise during pregnancy show decrease vascular smooth muscle responsiveness when challenged with an exogenous nitric oxide donor.[33] Conversely, maternal voluntary wheel running during pregnancy does not affect vascular function or voluntary activity in Sprague-Dawley male and female rat offspring.[34]

Several rodent studies indicate that maternal exercise before and/or during gestation improves glucose homeostasis,[35,36] and insulin sensitivity[36] and decreases leptin concentrations in offspring.[37] Long lasting changes in the musculoskeletal system and adiposity are observed in Wistar rat offspring, especially in males, of exercised dams.[38] Such changes include lower bone mineral density, increase circulating concentrations of undercarboxylated osteocalcin and greater percentage of total fat but lower percentage of lean fat compared with controls. The beneficial metabolic effects in offspring of exercised dams may be due to gene expression changes in skeletal muscle, such as decrease *Il6* (an inflammatory cytokine secreted by T-lymphocytes and macrophages),[39] increase peptide *Pyy* (a peptide released from the ileum and colon in response to feeding),[39] and decrease hypermethylation/increased expression of *Pgc1a* (a transcriptional co-activator regulating many genes involved in energy metabolism).[40]

Maternal exercise may also be able to alleviate the effects of high fat diet (HFD) feeding to rodent dams during gestation or post-weaned offspring. Male offspring born to dams that exercised during gestation are protected against HFD-induced hepatic steatosis.[41] In female mice, exercise before and during pregnancy combats the effects of maternal HFD on later offspring metabolic parameters, including reversing glucose intolerance, stabilized circulating insulin concentrations, and decreasing adiposity and weight gain.[39,42] Similarly, rat offspring born to dams fed a HFD but allowed to exercise during pregnancy show improved insulin/glucose metabolism with the effects more pronounced in male *v*. female offspring.[35] A maternal low protein diet fed to rats results in decreased resting oxygen consumption and growth rate of offspring, but these effects are attenuated in dams subjected to physical training.[43]

Although the rodent studies suggest that maternal exercise may confer positive benefits on offspring metabolism, it is not clear whether similar outcomes can be replicated in humans. The handful of studies to date has yielded mixed findings. Table 2 summarizes the current human epidemiological studies or meta-analyses examining the effects of maternal exercise, probiotic supplementation and breastfeeding (the latter two are discussed below) on offspring DOHaD outcomes. Male and female offspring of mothers who exercised during pregnancy show lower birth growth rates and reduce umbilical cord concentrations of serum IGF-1 (growth promoting factor, especially in children).[44] In women, vigorous exercise before pregnancy increases endothelial progenitor cells in umbilical cord blood, which could improve offspring cardiovascular function.[45] Lower toddler weights and weight-for-height *z*-scores are associated with mothers who engaged in increased leisure-time physical activity during pregnancy.[46] In contrast, another study reported that muscular fitness of mothers is associated with increase offspring birth weight.[47] Additional studies with larger sample sizes of pregnant women are needed before any definitive conclusions can be drawn. One such ongoing study that might be enlightening is currently underway in Auckland, New Zealand and being called the 'Improving Maternal and Progeny Risks of Obesity Via Exercise' (IMPROVE).[48]

Maternal exercise and offspring behavioral and reproductive phenotypes

In several rodent studies, maternal exercise is associated with increased offspring learning and memory ability[49–52] and reduced anxiety.[53] The cognitive and emotional improvements may be due to maternal exercise-induced increase in neural *Bdnf* (a neurotrophic factor associated with enhanced learning and memory abilities), especially in the hippocampus and prefrontal cortex,[51–53], leptin (*Lep*, a satiety hormone),[49] *Vegf* (an angiogenic protein) in the prefrontal cortex,[53] and *c-Fos* (a proto-oncogene) expression in the perirhinal cortex.[50] Further, maternal exercise may exert positive effects on brain mitochondrial function[51,54] and neuron cell numbers and viability.[49,52,55] Dendritic growth of parietal neurons is blunted in offspring of dams subjected to prenatal restraint stress.[56] However, this neuropathological changed is alleviated in offspring of stressed dams who are allowed to exercise on a voluntary running wheel, further emphasizing the power of maternal exercise to overcome negative periconceptional stimuli. Maternal exercise is insufficient though in fully reversing the brain damage observed in rats subjected to *in utero* hypoxia-ischemia.[57] In humans, mothers who had increased leisure activity during pregnancy gave birth to offspring who demonstrated transient improvement in vocabulary score at 15 months of age.[58]

In pigs, maternal exercise influences fetal and neonatal ovarian development, as evidenced by increased number of proliferating cells in the cortex, but it is not clear if these early changes alter later fecundity.[59] In addition, maternal exercise may decrease offspring risk of cancer later in life. Rat offspring derived from dams that ran on a running wheel while pregnant demonstrate decreased incidence of developing mammary cancers when later challenged with a carcinogen, *N*-methyl-*N*-nitrosourea.[60]

Table 1. *Animal model studies showing beneficial effects of maternal or paternal changes on offspring DOHaD outcomes*

Publication	Offspring sex(es) and animal model examined	Maternal or paternal intervention	Major findings
		Maternal and paternal exercise	
33	Female pigs (*Sus domesticus)*	Maternal exercise during pregnancy	Enhanced endothelium-dependent vaso-relaxation in the thoracic aorta in newborn offspring derived from exercised sows
34	Male and female pigs	Maternal exercise during pregnancy	Adult offspring derived from exercised sows showed decreased vascular smooth muscle responsiveness when challenged with an exogenous nitric oxide donor
35	Male and female Sprague Dawley rats	Maternal exercise (voluntary wheel running) during pregnancy	Maternal exercise did not affect vascular function or voluntary activity of their offspring
36	Male and female Sprague Dawley rats	Maternal exercise during pregnancy in lean and obese individuals	Male pups from obese, non-exercised dams were lighter Lean exercised dams had lighter male and female pups but no effects were observed on offspring body weight in those derived from obese exercised dams Visceral fat mass was reduced in male pups from lean exercised dams Male offspring from obese, non-exercised dams had elevated blood glucose concentrations. However, male offspring from exercised, obese dams showed reduced glucose concentrations Maternal exercise did not impact blood glucose concentrations in female pups Insulin concentrations were reduced in male pups from exercised lean and obese dams Female pups from obese, non-exercised dams were significantly increased, and maternal exercise did not alter insulin concentrations in female offspring Plasma triglycerides were lower in male pups from lean exercised dams but increased in those from obese exercised dams *Glut4* (glucose transporter) and *Myod1* (moygenesis-related gene) was downregulated in male offspring from non-exercised, obese dams but normalized in those from exercised, obese dams. *Pgc1*α, a transcriptional co-activator regulating many genes involved in energy metabolism, was upregulated in male offspring from exercised dams *Glut4* and Pgc1a were upregulated in retroperitoneal white adipose tissue (WAT) of male and female offspring from lean but not obese exercised dams *Tnf*-α (a marker of inflammation) was elevated in male pups from both lean and obese exercised dams but in females, this gene was only increased in lean exercised dams *Il6*, another inflammatory marker, was elevated in male pups from lean and to a lesser extent obese exercised dams
37	Female Sprague Dawley rats	Maternal exercise (voluntary wheel running) before mating and throughout pregnancy and lactation	Female offspring of exercised dams displayed enhanced glucose turnover during glucose tolerance testing, increased glucose infusion rates, and whole body glucose turnover rates during hyperinsulinemic–euglycemic clamp testing Female offspring from exercised dams had decreased insulin levels and hepatic glucose production during the clamp procedure Insulin infusion resulted in increased glucose uptake in skeletal muscle and decreased heart glucose uptake in offspring of exercised dams

38	Male and female Wistar Albino rats	Maternal exercise before mating and throughout gestation for control and obese dams	Serum corticosterone was increased in neonatal offspring of non-exercised obese dams but decreased in offspring from exercised control and obese dams Insulin was increased in male offspring of non-exercised obese dams Leptin and triglycerides were increased in male offspring of obese non-exercised dams but leptin and partially triglycerides were reduced in those from obese exercised dams Glucose and homeostasis model assessment (HOMA), calculated from HOMA = glucose (mmol/l) × insulin (μU/ml), were decreased in male and female offspring of control exercised dams
39	Male and female Wistar rats	Maternal exercise 5 days before breeding and throughout pregnancy	Male offspring of exercised dams had greater percentage of fat and lower percentage of lean than non-exercised dams The mid-tibial diaphysis had lower volumetric bone mineral density in offspring of exercised dams and bone:muscle relationship was altered in these male offspring Circulating concentrations of undercaroxylated osteocalcin, a protein produced by osteoblasts, were elevated in male offspring of exercised dams, even though there was no change in its gene expression
40	Male and female C57Bl6J mice	Maternal exercise (swim bouts) before and during pregnancy for a total of 6 weeks. Offspring were then placed a control or high fat diet (HFD)	Body weight gain and energy intake was reduced in male and female offspring placed on a HFD but born to dams who exercised with the effects most pronounced in male offspring Male offspring of exercised mothers demonstrated less white adipose tissue (WAT) fat mass and burned more calories during the day Insulin stimulation resulted in a reduced time-dependent decline in plasma glucose in males of non-exercised mothers Decreased Il6 and increased peptide PYY, a peptide released from the ileum and colon in response to feeding, occurred in sera of adult offspring of exercised dams
41	Female C57Bl6J mice	Maternal exercise for 6 weeks before and throughout pregnancy and fed either a control or HFD	Maternal HFD alone resulted in hypermethylation of *Pgc1a* and suppressed gene expression in skeletal muscle of female offspring at birth and up to 12 months of age, but concurrent exercise reversed this epigenetic change and increased expression of *Pgc1a* Maternal exercise also improved age-associated metabolic dysfunction at 9 months of age
42	Male and female Sprague-Dawley rats	Maternal exercise during gestation	Male offspring fed a HFD demonstrated increased hepatic triacylglycerol (TAG) accumulation However, male offspring from exercised dams were protected against developing diet-induced hepatic steatosis No protective effects of maternal exercise were noted in female offspring
43	Male C57Bl6J offspring	Dams were exercised 2 weeks before conception, throughout gestation, or 2 weeks before conception and throughout gestation and fed a chow or HFD	Male offspring of chow-fed dams who exercised before or during gestation showed improved glucose tolerance beginning at 8 weeks of age and throughout the first year of life and at 1 year of age had lower serum insulin concentrations and percentage body fat compared with other groups Male offspring of non-exercised HFD-fed dams had impaired glucose tolerance, increased serum insulin concentrations, and elevated body fat. However, maternal exercise before or during gestation alleviated these metabolic disruptions in male offspring

Table 1. (*Continued*)

Publication	Offspring sex(es) and animal model examined	Maternal or paternal intervention	Major findings
[44]	Male and female Wistar rats	Maternal exercise training for a period of 4 weeks before mating and then reduced once pregnancy confirmed, and dams were fed a low protein or control diet	Growth rate of pups from the low-protein fed dams was reduced by about 50% relative to controls Pups from exercised and low protein diet fed dams showed an increase in body weight by 60 days an onward; whereas, those from non-exercised, low protein diet fed dams had reduced body weight from weaning onwards
[52]	Male Wistar rats	Maternal exercise throughout gestation	Male offspring from exercised dams demonstrated increased expression of *Bdnf*, greater cellularity in the hippocampus but not the cerebral cortex Male offspring of exercised dams showed better cognitive performance in non-associative (habituation) and associative (spatial learning) tests than those from non-exercised dams
[51]	Male and female C57Bl/6J mice	Maternal exercise throughout gestation for varying times (20, 30 or 40 min/day) on a treadmill at 12 m/min, 5 days/week for a total of 3 weeks	Maternal exercise for 40 min/day improved mitochondrial function in resulting pups and increased *Bdnf* expression in the hippocampus
[53]	Male and female Wistar Albino rats	Adolescent male and female rats were subjected to 6 weeks of involuntary or voluntary physical activity	Improved learning abilities were evident in both involuntary and voluntary exercise groups Neuron density in the CA1 region of the hippocampus, dentate gyrus and prefrontal cortex was increased in both exercise groups Exercise decreased anxiety and corticosterone concentrations in females *Bdnf* and *Vegf* expression were greater in the voluntary than involuntary exercise group
[49]	Male and female Wistar Albino rats	Maternal exercise during pregnancy	When tested in a Morris Water Maze that measures spatial learning and memory, offspring from exercised dams learned the platform earlier and spent more time in the correct quadrant. This group also demonstrated decreased thigmotaxis behavior, suggestive of reduced anxiety Increased neurons were evident in the CA1 and C3 regions of the hippocampus and gyrus dentatus region in offspring from exercised dams *Lepr* was increased in prepubertal and adult male and adult female offspring from exercised dams
[50]	Male Long Evans rats	Maternal exercise during pregnancy	Male offspring of exercised dams were able to discriminate between novel and familiar objects, but those from non-exercise dams were not able to do so Increased expression of C-fos (a proto-oncogene) was evident in the perirhinal cortex of male offspring of exercised dams
[54]	Male and female Wistar rats	Female Wistar rats were subjected to five swimming lesson before mating and then trained during pregnancy	Maternal swimming activity resulted in increased reactive oxygen species in the cerebellum, parietal cortex and hippocampus in postnatal day 7 offspring This group also had reduced mitochondrial superoxide in the cerebellum and parietal cortex but increased nitrate levels in the cerebellum, parietal cortex, hippocampus, and striatum Antioxidant activity, catalase, and glutathione-peroxidase was increased in the cerebellum, parietal cortex and hippocampus for the maternal exercise offspring Superoxide dismutase was increased only in the parietal cortex of this offspring group Mitochondrial mass and membrane potential were increased in the cerebellum and parietal cortex of pups from exercised dams

55	Female transgenic (TG) CRND8 mice, who hemizygously carry the human APP 695 transgene on a hybrid C57Bl/6-C3H/HeJ background and are considered a good mouse model for Alzheimer's disease	Maternal exercise during pregnancy	Maternal exercise reduced β-amyloid (Aβ) plaque burden and amyloidogenic APP processing in TG female offspring Neurovascular function was improved in TG female offspring of exercised dams. This was accompanied by different Ab transporters, increased angiogenesis, reduced inflammation (microgliosis), down-regulation of pro-inflammatory mediators, and reduced oxidative stress Plasticity changes (up-regulation of *reelin*, *synaptophysin*, and *Arc*) were detected in wild-type and TG offspring of exercised dams
56	Male CF1 mice	Dams were divided into three groups: control, restraint stress [which began at gestational day (GD) 14], restraint stress (starting on GD14) voluntary wheel running (GD1–17)	Maternal stress resulted in blunted dendritic growth in parietal neurons but did not alter locomotor behavior in male offspring Maternal voluntary wheel running offset the maternal stress-induced neuronal cell processes changes in offspring
57	Male and female Wistar rats	Maternal exercise 1 week before mating and throughout gestation	Maternal exercise did not prevent oxidative alterations in the brain of offspring who were subjected to hypoxia-ischemia (HI) on PND 7 Maternal exercise resulted in positive expression of antioxidant enzymes in the hippocampus, striatum, and cerebellum of HI pups
59	Yorkshire gilts	Yorkshire gilts were exercised by walking during mid-gestation (day 40–104).	Ovarian weight was greater in light but not heavy neonates from exercised gilts Maternal exercise increased labeling index in heavy compared with light offspring in fetal but not neonatal or adolescent ovaries
60	Female Sprague Dawley rats	Maternal exercise throughout pregnancy	Pups from exercised dams had reduced mammary tumor incident after being placed at weaning on a HFD and exposed at 6 weeks of age to the carcinogen, *N*-methyl-*N*-nitrosourea
61	Male C57Bl6J mice	Male mice in the exercised group were placed on a motor-driven rodent treadmill for 5 days per week for 6 weeks and then mated to a non-exercised female	Paternal treadmill exercise before mating improved spatial learning and memory in resulting male offspring Male offspring of fathers who exercised had increased expression of *Bdnf* and *reelin*
62	Male C57Bl6J mice	Male mice were exercised on a voluntary wheel for 12 weeks before mating	Male offspring of exercised fathers were more vulnerable to the adverse effects of a HFD, as evidenced by increased body weight and adiposity, impaired glucose tolerance and increased insulin levels Several metabolic genes, including *Ogt*, *Oga*, *Pdk4*, *H19*, *Glut4* and *Ptpn* were altered in the skeletal muscle of male offspring of exercised fathers Chronic exercise altered DNA methylation and micro-RNA content in sperm of fathers, which may result in transgenerational effects

Table 1. (*Continued*)

Publication	Offspring sex(es) and animal model examined	Maternal or paternal intervention	Major findings
		Maternal probiotic supplementation	
63	Male and female Swiss Albino mice	Females were provided *Lactobacillus rhamnosus GG* (LGG) beginning at 31 days of age and continued on this supplementation through mating, gestation and lactation. At weaning, pups were either provided the same probiotic or it was discontinued	Prenatal exposure to the probiotic supplement increased serum IgG, intestinal sIgA, and IFNγ in resulting offspring. All of these are considered inflammatory markers Prenatal probiotic supplementation resulted in enhanced antibody response to hepatitis-B surface antigen (HBsAg)
64	Male and female Swiss Albino mice	Probiotic-containing milk (PFM) supplemented with *L. rhamnosus* was provided to dams during lactation and/or to their offspring after weaning	PFM-exposed mice had greater leukocyte functionality, as shown by increased release of lysosomal enzymes (β-galactosidase and β-glucuronidase) and nitric oxide production Reduced levels of inflammatory markers (TNF-α, monocytic chemotactic protein-1) and allergic antibodies (total and milk specific IgE) were observed in offspring supplemented with PFM or where it was provided to the dams Other inflammatory markers (IFNγ and IL-4) were increased in offspring exposed to PFM
65	Male and female BALB/c mice	Females received on an every other day basis LGG 10 days before conception and throughout gestation and lactation	Intestinal colonization with LGG was evident in the dams but not their pups Decreased expression of TNF-α, IFN-γ, Il-5, and Il-10 occurred in offspring of LGG-supplemented dams Allergic airway, peribronchial inflammation and goblet cell hyperplasia were reduced in offspring of LGG-supplemented mothers TNF-α was increased in the placenta of offspring in LGG-supplemented dams
66	Male and female Wistar rats	Dams were treated with *Bifidobacterium animalis subsp lactis* (BB-12) and *Propionibacterium jensenii* 702 through the water from 10 days before conception through PND 22 (weaning). Offspring were subjected to maternal separation (MS) from PND 2 or 14 or left undisturbed. At 83–85 days of age, offspring were either subjected to restraint stress (RS) or left undisturbed	Maternal probiotic supplementation resulted in decreased IFN-γ levels in resulting offspring Offspring subjected to MS and derived from dams supplemented with probiotic supplementation had increased Il-6 levels Stressed and non-stressed offspring from probiotic-treated dams showed decrease haptoglobin levels Maternal probiotic treatment downregulated ileal gene expression of *Muc2* in male offspring also subjected to MS but this gene was upregulated in adult males subjected to MS or RS
67	Male and female Wistar rats	Dams were treated with *Bifidobacterium animalis subsp lactis* (BB-12) and *Propionibacterium jensenii* 702 through the water from 10 days before conception through PND 22 (weaning). Offspring were subjected to maternal separation (MS) from PND 2 or 14 or left undisturbed. At 83–85 days of age, offspring were either subjected to restraint stress (RS) or left undisturbed	MS alone increased ACTH levels and neonatal fecal counts of aerobic and anaerobic bacteria, *E. coli*, Enterococci, and *Clostridia* but reduced circulating IgA levels RS alone offspring had elevated ACTH and corticosterone levels, decreased aerobic bacteria, *Bifidobacteria* but increased *Bacteroides* and *E.coli* counts MS + RS decreased anaerobes and Clostridia in resulting offspring Maternal probiotic supplementation increased neonatal corticosterone levels that persisted until 12 weeks in female offspring, resulted in elevated adult ACTH levels and altered neonatal microflora that was a s similar signature pattern to MS However, maternal probiotic supplementation improved plasma IgA response, increased Enterococci, and *Clostridia* in adult offspring that were also subjected to MS Maternal probiotic supplementation also increased IgA levels, restored anaerobes, *Bifidobacteria*, and *E. coli* to normal in RS adult offspring

68	Male and female Sprague Dawley rats	Pregnant and lactating dams were provided *L. plantarum* 299v (Lp299v) in the drinking water	Lp299v colonized the intestines of the dam and resulting offspring In offspring of Lp299v dams, the small intestine, pancreas, and liver weighed more at PND 14 than control pups
69	Male and female BALB/c mice	Females were treated daily with *Lactobacillus paracasei* in the drinking water from the last week of gestation through lactation. Offspring were sensitized to recombinant Bet v 1 followed by aerosol challenge with birch pollen extract	Maternal probiotic supplementation prevented airway inflammation (reduced eosinophil migration into the lungs, decreased Il-5 in brochoalveolar lavage, and lung and mediastinal lymph node cell cultures, and reduced peribrochial inflammatory infiltrate and mucus hypersecretion) in resulting offspring Il-4 and Il-5 production by splenic cell cultures was decreased in offspring of probiotic-treated dams
70	Male and female C3H/HeJ mice	Lactating dams with suckling pups were *ad libitum* provided a probiotic supplemented diet (Primalac 454 Feed Grade Microbials), and this diet was then provided to weaned pup until they were 10 weeks of age. Weaned mice with challenged with peanut extract, saline, or adjuvant	Offspring of dams provided the probiotic and who themselves received it had enhanced probiotic fecal counts These offspring also had greater amounts of T regulatory cell populations and reduced splenic gene expression of allergic mediator Il-13 compared with controls
		Breastfeeding	
89,90	Male and female polymeric Ig receptor (*Pigr*)-sufficient (*Pigr*$^{+/-}$) or -deficient (*Pigr*$^{-/-}$) mice	Maternal transfer of sIgA in the milk	Maternal sIgA stimulated in WT offspring gut microbiome changes that persisted were amplified at adulthood and blocked translocation of aerobic bacteria, including *Ochrobactrum antropi*, across the intestines and into the draining lymph nodes Maternal sIgA in WT offspring altered the gene expression profile, such as transcripts linked with enteritis in humans, and alleviated dextran-induced colonic mucosal damage

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Table 2. *Human epidemiological studies or meta-analyses showing beneficial effects of maternal changes on offspring DOHaD outcomes*

Publication	Cohort population or meta-analyses	Maternal intervention	Major findings
	Maternal exercise		
45	84 healthy nulliparous women (mean ± SD, age = 30 ± 4 years, body mass index (BMI) = 25.5 ± 4 kg/m^2 and their male and female offspring from Auckland, New Zealand	Maternal exercise during pregnancy	Male and female offspring of exercised mothers showed lower birth growth rates and reduce umbilical cord concentrations of serum IGF-1
32	373 singleton full-term pregnancies where maternal exercise during pregnancy was determined by questionnaires and umbilical cord blood samples was assayed in their male and female babies	Maternal exercise during pregnancy	Vigorous exercise before pregnancy increased endothelial progenitor cells in umbilical cord blood, which could improve offspring cardiovascular function
46	300 pregnant women who reported on type, duration and frequency of trimester-specific leisure time physical activity (LTPA) and reported on their toddler's current LTPA level and a subset (n = 23) volunteered to have measurements done on maternal and toddler height, weight, and body fat	Mothers who engaged in increased leisure time activities	These mothers gave birth to toddlers with lower weights and weight for height z-scores
47	65 healthy pregnant women who were assessed at 16 weeks of gestation and their male and female offspring	At 16 weeks of gestation, maternal anthropometry (BMI and skin-folds), physical activity, cardiorespiratory fitness (VO_2 peak) and muscular fitness (handgrip strength) and offspring birth weight was determined at delivery	Mothers with increased muscular fitness had offspring with increased birth weight
58	At 18 weeks of gestation, women reported on hours per week they engaged in 11 leisure-time physical activities and hours spent on general physical activity. Parents completed a modified MacArthur Infant Communication scale at 15 months, and a verbal IA test was performed at 8 years of age. Number of participants for these various analyses ranged from 4529 to 7162	Increased maternal leisure time physical activity	Mothers who had increased leisure activity during pregnancy gave birth to offspring who demonstrated transient improvement in vocabulary score at 15 months of age
	Probiotic supplementation		
71,72	Pregnant women from Trondheim, Norway (n = 415) were randomized to either receive probiotic (*Lactobacillus rhamnosos GG*, *L. acidophilus La-5* and *Bifidobacterium animalis subs. Lactis Bb-12*) or placebo milk in a double-blinded trial that spanned from 36 weeks of gestation through 3 months post-partum. At 6 years of age, their children were re-examined for atopic dermatitis, atopic sensitization, asthma, an allergic rhinoconjunctivitis (ARC).[71] The follow-up study used the same groups and experimental design as listed in.[71] In this study, stool samples were collected from mothers at 30 and 36 weeks of gestation and 3 months after birth and from their children at 10 days, 3 months, 1 year and 2 years of age. The bacteria were isolated and analyzed by quantitative PCR. Stool samples from 3 month and 2-year-old children were also analyzed with 16S ribosomal RNA gene deep sequencing[72]	Maternal probiotic supplementation during pregnancy and the post-partum period	Children of mothers who received probiotic supplementation while pregnant showed reduced incidence of atopic dermatitis However, maternal probiotic administration did not alter the gut microbial composition in their offspring

	Breastfeeding		
75	Meta-analyses of 23 individual high quality reports with a total sampling size of 1500 participants	Breastfeeding for varying lengths of time	13% pooled reduction in the prevalence of overweight or obesity in breastfed offspring
80	Cross-sectional data from a cohort at the 18-month visit (n = 179) who were enrolled in the Peri/postanatal Epigenetics Twin Study (PETS) to assess duration of breastfeeding and infant size at this age. Inclusion criteria included birth weight of more than 2000 g and infants had to be breastfed for <1 month, 1–3 months or 4–6 months	Breastfeeding for 4–6 months of age compared with 1–3 months of age or supplementation with non-breast milk before 4 months of age	Mean BMI decreased from 65 to 85% when infants were breastfed for 4–6 months of age Supplementation with non-breast milk before 4 months of age correlated with an increased BMI, arm circumference, and abdominal circumference in offspring at 18 months of age
81	125 women of varying body weight, including those who were overweight/obese, with 81 diagnosed with type 1 diabetes and 44 with type 2 diabetes and mean age of male and female offspring was 4.5 years	Breastfeeding by mothers who experienced pre-gestational diabetes	Breastfeeding prevented obesity in offspring of mothers who experienced pre-gestational diabetes, and this was independent of maternal BMI and type of diabetes
82	A cross-sectional multiethnic study in the United States with 1387 mothers and their male and female children (0–5.9 years of age).	Breastfeeding by mothers who had excessive gestational weight gain	Breastfeeding attenuated the risk of obesity in offspring of mothers who had excessive gestational weight gain
83	Meta-analyses of 11 high quality studies identified through MEDLINE, LILACS, SCIELO and Web of Science databases that included a total of 113 male and female offspring	Breastfeeding for varying lengths of time	26% overall reduction of breast-fed offspring becoming overweight or obese regardless of parental income status A positive association of breastfeeding and reduced odds of offspring developing type 2 diabetes
84	Breastfeeding history was determined by questionnaire of mothers enrolled in the Framingham Offspring Study. For the young- to middle aged adult children or third generation participants int (n = 962, mean age = 41 years, and 54% women). Of these individuals, 26% of their mothers reported they were breastfed	Breastfeeding of varying lengths	Breastfeeding inversely associated with offspring BMI but positively associated with high-density lipoprotein cholesterol, which is a beneficial from
85	Male and female offspring born to 2900 pregnant Australian women and who were assessed from 18 weeks of gestation to 17 years of age	Breastfeeding of varying lengths	Breastfeeding reduced later offspring cardiometabolic diseases
86	Fitness was examined in 1025 male and female children (ages 9.5 ± 0.4 years of age) and 971 male and female adolescents (15.5 ± 0.5 years of age) from Estonia and Sweden	Breastfeeding of varying lengths	Breastfeeding appeared to positively influence cardiorespiratory fitness in children and adolescents
91,92	Ultrasound assessment of thymic index at birth and 4 months of age in 47 healthy male and female infants with 21 exclusively breastfed, 13 partially breastfed, 13 exclusively formula fed.[91] A cohort of 50 male and female infants and all partially breastfed when recruited at 8 months of age. Ultrasound assessment of thymic index was performed at 8 and 10 months of age[92]	Breastfeeding either early or later in infancy	Thymic enlargement in offspring breastfed earlier or later in infancy
93	4-month examination, infants were divided into three groups according to breastfeeding status Exclusively breastfed (n = 17) Partially breastfed (n = 12) Exclusively formula fed (n = 12)	Breastfeeding during infancy	Breastfed infants had greater number of circulating CD8 + lymphocytes

Table 2. (*Continued*)

Publication	Cohort population or meta-analyses	Maternal intervention	Major findings
	At 8-, 10- and 12- month examinations, male and female infants were divided into two groups according to breastfeeding status Breastfeeding once or more times per day (n = 10, 8 and 5, respectively, based on month examined) No breastfeeding (n = 25, 25 and 30, respectively, based on month examined)		
94	Study included 32 healthy term male and female infants who were matched for gestational age. 20 were randomly assigned to one of two isocaloric formulas (68 kcal/100 ml) containing either 1.1 or 1.5 g/100 ml (1.6 or 2.2 g/100 kcal) of protein with a 50:50 mixture of bovine whey proteins and caseins. The other 12 infants were considered a control group breastfed throughout the 4-month period	Breastfeeding of varying lengths	Breastfed children elicited a greater immunological response against certain vaccinations
95–98	Case–control study over a 6-year period from 1987 to 1992 before general *Haemophilus influenza* (HI) vaccination introduced in Sweden. 54 cases (males and females) with invasive HI infection and 139 matched controls (males and females)[95] Retrospective analyses of HI cases from 1956 to 1992 in the population (270,000 male and female individuals) of Örebro County in south central Sweden[96] A cohort of male and female infants (n = 674) with later data on respiratory illness available for 545 children (mean age 7.3 years), height for 410 children, weight and BMI for 412 children, body composition for 405 children and blood pressure for 301 children (mean age = 7.2 years)[97] Initially, 256 newborn male and female babies with a birth weight of at least 3 kg who were born at Helsinki University Central Hospital during the first 3 months of 1975. Of these, 237 completed the first-year follow-up and 178 were re-examined at 3 years of age to determine whether breastfeeding was associated with a reduced incidence of otitis media[98]	Breastfeeding of varying lengths	Breastfed children demonstrated protection for several years against diseases such as HI type B, respiratory tract infections, otitis media, and diarrhea
99–102	A comprehensive review of 132 studies with 56 being deemed conclusive[99] A comprehensive review of various articles[100] A meta-analyses of 12 prospective articles identified via the 1966–1999 MEDLINE database[101] A meta-analyses of 89 articles from PubMed, CINAHL and EMBASE databases that included a search string of breastfeeding and allergic outcomes[102]	Breastfeeding of varying lengths	Breastfeeding appeared to against allergic diseases, such as asthma
105,106	A parallel, double-blind placebo-controlled trial of 241 mother–infant (males and females) pairs where 205 infants completed the follow-up studies and were included in the final analyses[105] Study included 1828 school age children (males and females) ranging from 9 to 12 years of age[106]	Breastfeeding and maternal probiotic supplementation	Breastfeeding interacted with genetic background and maternal probiotic supplementation to reduce atopic diseases in offspring
108–112	Study included 14 case–control studies that examined the effect of short term (⩽6 months) and long-term (>6 months) breastfeeding on the risk of childhood acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) in males and females[108]	Breastfeeding of varying lengths	Breastfeeding seemingly reduced the incidence of leukemia, including ALL, Hodkin's Lymphoma and AML

	300 male and female patients with childhood cancer with 73 diagnosed with leukemia, 82 with lymphoma and 146 with solid tumors (brain tumors, neuroblastoma, soft tissue sarcomas, germ cell tumors, renal tumor, bone tumor, retinoblastoma, hepatoblastoma and others)[109] Two nationwide case–control studies were performed and included male and female individuals ranging from 0 to 14 years of age who were identified through Australian pediatric oncology units between 2003 and 2007 for ALL and 2005–2010 for childhood brain tumors (CBT) and case controls. Infant feeding up to 2 years of age was determined by surveying the mothers. Data represents 322 ALL cases, 679 ALL controls, 299 CBT cases and 733 CBT controls[110] A case–control study with 137 male and female patients aged 1–16 years of age with ALL, AML, Hodgkin's lymphoma (HL) or non-Hodgkin's lymphoma (NHL) and 146 controls matched for age and sex[111] 169 male and female patients with ALL, HL and NHL age ⩽ 15 years of age and 169 healthy controls matched by age and sex. Mothers of study participants were surveyed to determine the history of breastfeeding and viral infections[112]		
109	300 male and female patients with childhood cancer with 73 diagnosed with leukemia, 82 with lymphoma, and 146 with solid tumors (brain tumors, neuroblastoma, soft tissue sarcomas, germ cell tumors, renal tumor, bone tumor, retinoblastoma, hepatoblastoma and others)	Breastfeeding of varying lengths	Breastfeeding reduce the incidence of brain, germ cell, bone, retinoblastoma and hepatic tumors in offspring
110,113	Two nationwide case–control studies were performed and included male and female individuals ranging from 0 to 14 years of age who were identified through Australian pediatric oncology units between 2003 and 2007 for ALL and 2005–2010 for CBT and case controls. Infant feeding up to 2 years of age was determined by surveying the mothers. Data represents 322 ALL cases, 679 ALL controls, 299 CBT cases and 733 CBT controls[110] Infant breastfeeding *v.* non-breastfeeding were examined in relation to incidence of childhood central nervous system tumors that included 633 male and female cases enrolled in the UK Childhood Cancer Study (UKCCS)[113]	Breastfeeding of varying lengths	No effect of breastfeeding on reducing the risk of childhood central nervous system tumors
114	1267 Chinese male and female children as part of the China Jintan Child Cohort Study and who were 6 years old when assessed, along with their parents	Mothers breastfed and actively engaged their children	Breastfeeding and increased maternal interaction decreased the risk for later internalizing problems in children
115	6841 individuals from the Copenhagen perinatal Cohort with 1671 breastfed for 2 weeks or less (early weaning), 5140 breastfed for longer than 2 weeks	Breastfeeding of varying lengths	Breastfeeding likely provided some protection against later development of offspring schizophrenia
116	A prenatal cross-fostering design with a sample size of 870 families with a child (male or female) 4–11 years of age when assessed	Breastfeeding of varying lengths	Breastfeeding later risk for conduct problems in middle aged children
117	Case–control study with data from the Autism Internet Research Survey representing 861 autism spectrum disorder and 123 control children	Breastfeeding of varying lengths	Children who were not breastfed or provided infant formula containing docosohexaenoic acid for the >6 months had a greater odds of developing autism

Table 2. (*Continued*)

Publication	Cohort population or meta-analyses	Maternal intervention	Major findings
120	Retrospective analysis of children 6–12 years of age who were diagnosed at the Schneider's Children Medical Center (Petach Tikva, Israel) with attention deficit hyperactivity disorder (ADHD) between 2008 and 2009 and compared with control children. The ADHD population included 56 individuals, ADHD sibling group had 52 individuals and the control group consisted of 51 individuals	Breastfeeding of varying lengths	Male and female offspring who were breastfed were less likely to develop ADHD
74,124	A review of various papers examining how breastfeeding may affect growth, immune-related effects, mental development and non-communicable diseases[74] Male and female children ($n = 550$) were enrolled at birth in the Auckland Birth weight Collaborative Study who were then assessed again at 3.5 years of age. Half of the original population was considered small for gestation age (SGA ⩽ 10th percentile) and other half were appropriate for gestational age (AGA >10th percentile). Maternal interview was used to determine the duration of breastfeeding and intelligence of the children was determined with the Stanford Binet Intelligence Scale[124]	Breastfeeding in pre-term infants, those born after normal gestational length, and those small for gestational age	Breastfeeding associated with an increase IQ especially in pre-term infants and those born small for gestational age
125	Participants were enrolled in the Dunedin Multidisciplinary Health and Development Study that includes a birth cohort of 1037 male and female children, and individuals were then assessed at 3 years of age where 91% of consecutive births between April 1972 and March 1973 in Dunedin, New Zealand were enrolled. IQ was measured in children were performed at 7, 9, 11 and 13 years of age with the Wechsler Intelligence Scale for Children-Revised. The four IQ score assessments were combined to form an overall score	Breastfeeding of varying lengths	Breastfeeding appeared to interact with genetic status for the *FADS2* gene to increase offspring IQ status
126	Meta-analyses study with 11 individual reports with male and female children ranging from 6 months to 16 years of age	Breastfeeding of varying lengths	The collective studies suggest that breastfeeding positively correlated with an increase IQ by 3.2 points even after controlling for covariant variables, including maternal intelligence
127	Meta-analyses of 17 individual studies with 18 estimates of the relationship between breastfeeding and later performance in IQ tests for male and female children. The studies were identified by two separate searches of MEDLINE, LILACS, SIELO and Web of Science databases	Breastfeeding of varying lengths	Breastfeeding was associated with an increase of 3.4 IQ points in children and adolescents
128	Prospective longitudinal birth cohort with a sample of 973 men and 2280 women who were born in Copenhagen, Denmark between October 1959 and December 1961. Intelligence was assessed using the Adult Intelligence Scale (WAIS) at a mean age of 27.2 years in the mixed-sex sample and the Børge Priens Prøve (BPP) test at a mean age of 18.7 years in the all-male sample	Breastfeeding up to 9 months of age	Breastfeeding during this time was linked with higher adult intelligence, as assessed with two different intelligence tests and two independent samples of young adults
129	Sibling pairs representing 2734 individuals from the National Longitudinal study of Adolescent Health, and they were examined for the relationship of breastfeeding history and 15 indicators of physical and emotional health and cognitive ability	Breastfeeding of varying lengths	When assessed with the Peabody Vocabulary Test, breastfed infants scored 1.7 and 2.4 higher intelligence points within and across families with each month of breastfeeding raising IQ by 0.2 points per month

130	Two consecutive generations of British children enrolled in the National Child Developmental Study. The study included all male and female babies ($n = 17{,}419$) born in Great Britain (England, Wales and Scotland) during 1 week (March 3–9, 1958) and individuals were then interviewed at 7 years of age (1965, $n = 15{,}946$), 11 years of age (1969, $n = 18{,}285$), 16 years of age (1974, $n = 14{,}469$), 23 year of age (1981 $n = 12{,}537$), 33 years of age (1991, $n = 11{,}469$), 41–42 years of age (1999–2000, $n = 11{,}419$), 46–47 years of age (2004–2005, $n = 9534$), and 50–51 years of age (2008–2009, $n = 9790$)	Breastfeeding of varying lengths	Positive association on later IQ scores for each month offspring were breastfed
131	Analysis based on data from Krakow, Poland with a 7-year follow-up of 468 term male and female babies (>36 weeks of gestation) born to non-smoking mothers who were participating in an ongoing prospective cohort study. Cognitive function of children was assessed with psychometric tests performed five times at regular intervals from infancy through preschool age. Neurodevelopment assessments of 443 child participants were evaluated at least twice	Breastfeeding of varying lengths	Breastfeeding alone led to positive association for increase IQ in toddlers by 1 year of age and this link persisted through the preschool period
132	U.S. prospective pre-birth cohort study (Project Viva) that enrolled members from April 22, 1999 to July 31, 2002 and followed the children's progress at three and 7 years of age and totaled 1312 mothers and children. Children's receptive language ability was analyzed with the Peabody Picture Vocabulary Test at 3 years of age, Wide Range Assessment of Visual Motor Abilities at ages 3 and 7 years, and Kaufman Brief Intelligence Test and Wide Range Assessment of Memory and Learning at 7 years of age	Breastfeeding of varying lengths	There was a causal relationship of breastfeeding duration and receptive language and verbal and non-verbal intelligence in these school-age children
133	A prospective cohort study with a random sample of male and female infants born to hospitals at medium-size cities, who were monitored at 30, 90 and 180 days postnatally and then reexamined at 8 years of age. At 8 years of age, 560 children were analyzed with the Raven's Colored Progressive Matrices test	Breastfeeding for 6 months or more	8-year olds who were breastfed for 6 months or more performed better in a general intelligence assessment test
134	Cluster-randomized trial that occurred at 31 Belarussian maternal hospitals and affiliated polyclinics and encompassed initially 17,046 breastfeeding infants who were enrolled from June 17, 1996 to December 31, 1997 and then 13,889 were re-examined at 6.5 years of age (December 21, 2002 to April 27, 2005). Children at this age were assessed with the Wechsler Abbreviated Scales of Intelligence measures and by teacher's academic ratings in reading and writing	Breastfeeding of varying lengths	Breastfed 6.5-year-old children achieved elevated scores in verbal, performance, and full scale IQ tests and were ranked higher in teacher's elevations for academic performance in reading, writing, and mathematics
135	Two individual cohort studies totaling 6000 male and female individuals. The first cohort was the British Avon Longitudinal Study of Parents and Children (ALSPAC, $n \approx 5000$) and the second was the Brazilian Pelotas 1993 cohort ($n \approx 1000$). Analyses were then extended to include results from a meta-analysis of five low- or middle-income countries (LMIC, $n \approx 10{,}000$ male and female individuals). Relationship of breastfeeding and BMI, blood pressure, and IQ were examined	Breastfeeding of varying lengths	Causal effects of breastfeeding on IQ but not blood pressure and BMI were observed

Paternal exercise and offspring phenotypes

To the author's knowledge, only two papers to date have considered whether paternal exercise before fertilization impacts offspring outcomes. One report with mice suggests that paternal treadmill exercise improves spatial learning and memory ability of male offspring and upregulates expression of *Bdnf* and *Reelin* (a gene that mediates neuronal migration in the developing brain and synaptic plasticity in adults), indicating that this paternal behavior can improve male offspring neurobehavioral function.[61] In contrast, another mouse study found that males who exercised 12 weeks before mating gave rise to offspring more vulnerable to deleterious effects induced by a HFD, as exemplified by increased in body weight, adiposity, impaired glucose tolerance and elevated insulin levels.[62] Further, several metabolic genes (*Ogt*, *Oga*, *Pdk4*, *H19*, *Glut4* and *Ptpn1*) are up-regulated in the skeletal muscle of offspring whose fathers underwent long-term exercise. The sperm of exercised fathers harbors DNA methylation and micro-RNA changes, which may lead to transgenerational propagation of the above metabolic changes. Ostensibly, additional studies are needed to determine whether paternal exercise has positive or negative effects on the next generation. The conflicting results so far may be reconciled in part by the duration and type of exercise regimen, whether the activity is voluntary or involuntary, and offspring parameters analyzed.

Probiotic supplementation

Our understanding of how the gut microbiome influences health and disease outcomes has been addressed in some depth in this past decade. The data increasingly reveal that early disruptions in the gut microbiome can affect various systems, including the gastrointestinal, immunological, nervous, metabolic and cardiovascular. With this in mind, there is an interest as to whether pre- and post-natal supplementation through the maternal diet can attenuate later diseases and promote offspring health. The primary focus of most studies to date has been to determine whether maternal probiotic supplementation with select microbes alters offspring immunological gene expression and immunophenotypes. Current animal findings are summarized in Table 1. Rodent dams and pregnant mothers used in the studies detailed below were not exposed to any other extrinsic factors, besides probiotic supplementation, and were not considered overweight or obese.

Supplementation of Swiss albino female mice before conception and throughout lactation to the probiotic *Lactobacillus rhamnosus* GG (LGG) results in increased serum IgG, intestinal sIgA, IFN-γ (type II interferon produced by T-lymphocytes) concentration, and enhanced antibody response against hepatitis-B surface antigen in offspring.[63] Provisioning of mouse mothers and offspring with a probiotic fermented milk (PFM) comprised of *Lactobacillus rhamnosus* (MTCC: 5897) leads to several beneficial offspring immunological changes.[64] These include decrease inflammatory markers (TNF-α and MCP-1- in the C-C chemokine family and recruits inflammatory cells to a site of injury/inflammation) and IgE but elevations in serum IgA and IFNγ/IL-4 (stimulates development of Th2 lymphocytes and in turn is produced by this cell type). An earlier study with mice perinatally exposed to LGG yielded some similar but also notable cytokine differences.[65] In this report, TNF-α, IFN-γ, Il-5 (produced by Th2 cells and stimulates development of B-lymphocytes and increases immunoglobulin secretion) and Il10 (an anti-inflammatory cytokine) are decreased in offspring derived from dams provided LGG. Another rat study suggests that maternal probiotic intervention decreases IFN-γ levels, upregulates *Muc2* (produced by goblet cells and protein product helps contribute to mucous barrier overlying the intestinal epithelial cells) ileal gene expression in male offspring subjected to maternal separation but increases Il-6 in maternally separated pups.[66] Maternal probiotic supplementation improves immunological responses and partially alleviates gut dysbiosis in offspring subjected to maternal separation.[67] Gestational and lactation administration of *Lactobacillus plantarum 299v* (Lp299v) results in this bacterium colonizing the intestinal tract of the dam and offspring.[68] The small intestines, pancreas, liver and spleen are larger in day 14 offspring exposed to this maternal probiotic supplement, and this group shows reduced gut permeability, which serves an important barrier in preventing systemic spread of pathogenic organisms. In all, the findings suggest that the maternal probiotic supplementation can affect the gastrointestinal and lymphatic systems. Based on the potential immunomodulatory role, select studies have considered whether maternal probiotic supplementation might be a useful adjuvant to prevent various allergic reactions.

Perinatal exposure of mice to *Lactobacillus paracasei* NCC2461 through the dam's drinking water prevents later airway inflammation (decreases eosinophils, reduces levels of Il-5 in respiratory and lymph node samples, and suppresses Il-4 and Il-5 production by cultured splenic cells) when offspring are sensitized with recombinant Bet v 1 followed by aerosol challenge with birch pollen extract.[69] Supplementation of PFM to mouse mothers during lactation or their weanling offspring reduces allergic signs to ovalbumin, increases the number of intestinal goblet and IgA+ cells, reduces antibodies (IgE and IgG) against ovalbumin, and decreases levels of Il-4 and IFN-γ.[64] Probiotic supplementation to dams from gestation through lactation and weanling mouse offspring increases probiotic fecal counts in offspring by 3 weeks of age.[70] The allergic pathway mediator, Il-13, is decreased but T regulatory cell populations are enhanced with the net effect that this group is protected against hypersensitivity reaction due to peanut allergen exposure. In humans, children of women who received probiotic supplementation while pregnant show reduced incidence of atopic dermatitis,[71] although a follow-up study by this same research group suggested that maternal probiotic administration does not alter the gut microbial composition in children (Table 2).[72] However, a study with pregnant mice, rats and pigs indicates that maternal probiotic administration of *Lactobacillus acidophilus* and *Bifidobacterium lactis* 7 days before vaginal delivery results in transfer of this bacterium to neonates (sex of pups not provided) and at least transiently affects the offspring gut microbial colonies.[73]

Breastfeeding

Breastfeeding is associated with several potential health benefits for the mother and her offspring[74,75]. Besides strengthening the maternal-infant bond, the positive offspring benefits of breastfeeding are likely due to the fact that breast milk contains various nutrients, immune-protective cells and factors (discussed below), hormones and other cytokines. Consequently, breastfeeding may be a primary means to prevent many non-communicable offspring diseases, in particular obesity, and even improve human intelligence, which may thereby reduce poverty and social inequalities.[75] We will consider the potential long-term benefits of breastfeeding on reducing offspring obesity and cardiometabolic disorders, improving the gut-microbiome axis, enhancing immunologic functions, decreasing cancer incidence, and strengthening neurobehavioral responses (summarized in Table 2).

Obesity and cardiometabolic disorders

In the past 30 years, childhood obesity rates have doubled in children and quadrupled in adolescents with current estimates approximating 1/3 of children and adolescents are overweight or obese.[76–78] Accordingly, there is a great deal of interest in early factors that may prevent childhood obesity and metabolic disorders. Breastfeeding may provide some protection against later development of these offspring disorders. In support of this notion, the World Health Organization (WHO) published a report suggesting that breastfeeding may yield a small protective effect against childhood obesity.[79] Additional confirmatory studies with even larger cohort populations have been published since this initial WHO report.

A meta-analyses of 23 high-quality studies encompassing a total sample size of 1500 participants reported breastfeeding is associated with a 13% pooled reduction in the prevalence of overweight or obesity in progeny.[75] A recent study concluded that the mean body mass index (BMI) decreases from 85 to 65% when infants are breastfed for 4–6 months as opposed to 1–3 months and supplementation with non-breast milk before 4 months of age correlates with an increased BMI, arm circumference, and abdominal circumference at 18 months of age.[80] Breastfeeding may even confer protective effects on offspring obesity in children whose mothers experienced pre-gestational diabetes.[81] Longer duration of breastfeeding may attenuate the risk of obesity in offspring whose mothers experienced excessive gestational weight gain.[82]

A meta-analyses representing 113 individual studies concluded that increased duration of breastfeeding is associated with a 26% reduction in the likelihood of offspring becoming overweight or obese regardless of parental income status.[83] This report also showed a positive association of breastfeeding and reduced odds of children developing type 2 diabetes. Infant breastfeeding has been inversely associated with adult BMI but positively associated with high-density lipoprotein or the good cholesterol form.[84] A cohort study from Australia with 2900 pregnant women also suggests breastfeeding reduces later offspring cardiometabolic diseases.[85] In addition, breastfeeding may positively influence cardiorespiratory fitness in children and adolescents.[86] Taken together, breastfeeding may confer protective effects against cardiometabolic diseases in offspring of non-obese and obese mothers.

Gut microbiome and immune effects

Breast milk contains abundant immune-related factors, including agranulocytes (B and T lymphocytes and macrophages), granulocytes (neutrophils), antibodies, especially sIgA, lysozyme (an enzyme that catalyzes the destruction of bacterial cell walls), lactoferrin (antimicrobial factor), IFN-γ, oligosaccharides and other compounds.[87,88] By testing mice either expressing or devoid of Ig receptor mice, it has been shown that maternal sIgA in breast milk stimulates offspring gut microbiota changes that persist and are amplified at adulthood, and blocks translocation of aerobic bacteria, including the pathogen *Ochrobactrum antropi*, across the intestines and into draining lymph nodes (Table 1).[89,90] In addition, maternal sIgA alters the intestinal gene expression profile, especially those transcripts associated with enteritis in humans, and alleviates dextran-induced colonic mucosal damage.

Breast milk may stimulate offspring immune system development. The thymus is larger in infants either breastfed earlier or later in infancy compared with those who received formula.[91,92] Thymic enlargement in breastfed infants is associated with increased number of circulating CD8+ (cytotoxic) T-lymphocytes.[93] Breastfeeding appears to strengthen a child's immunological response against certain vaccinations.[94] Breastfed children demonstrate protection for several years afterwards against various pathogens, including *Haemophilus influenza* type B, respiratory tract infections, otitis media and diarrhea.[95–98] Select reports indicate that breastfeeding might provide some protection against allergic diseases, such as asthma[99–102] but to a lesser extent for eczema and allergic rhinitis.[102] However, other cohort studies suggest that breastfeeding does not reduce the incidence of later offspring allergic diseases.[103,104] Additional studies report breastfeeding interacts with other factors, such as genetic background and maternal probiotic supplementation, to reduce atopic diseases.[105,106] Any beneficial effects of breastfeeding on offspring immune responses may be due to stimulation of helper T (Th) sub-type-1 development, which produce more Il2 and IFN-γ than Th2 cells, cells who instead are characterized by greater production of IL4, Il10 and Il13.[107]

Offspring cancer

Several cohort and meta-analysis studies provide support for the notion that breastfeeding reduces the incidence of leukemia, especially acute lymphoblastic leukemia, Hodgkin's Lymphoma, and acute myeloblastic leukemia.[108–112] The potential beneficial effects of breastfeeding on preventing offspring cancer are less clear with one study indicating this practice diminishes the incidence of brain, germ cell, bone, retinoblastoma and hepatic tumors,[109] whereas, two other studies found no evidence that breastfeeding decreases the incidence of childhood central nervous system tumors.[110,113]

Offspring neurobehavioral development

A cohort study with 1267 Chinese children found that those who were breastfed and whose mothers actively engaged with them demonstrate a reduced risk for later internalizing problems.[114] A Copenhagen perinatal cohort suggests that breastfeeding may provide some protective affects against later development of offspring schizophrenia.[115] Conduct problems are reduced in middle aged children who were breastfed.[116]

While there is a great deal of interest as to whether breastfeeding can reduce the risk or severity for complex neurobehavioral disorders, such as autism spectrum disorders (ASD) and attention deficit hyperactivity disorder (ADHD), robust and unequivocal data are lacking. One case–control study with data obtained from the Autism Internet Research Survey representing 861 children with ASD and 123 control children reported those who were not breastfed or provided infant formula lacking docosohexaenoic acid and arachidonic acid for more than 6 months have a greater odds of developing autism.[117] It has been postulated that certain autistic conditions might be related to newborn IGF production deficiencies and the presence of this growth factor in breast milk may compensate for this shortage.[118,119] A retrospective analysis of children 6–12 years of age who were diagnosed with ADHD relative to those without this disorder indicated breastfeeding may protect male and female offspring from developing this disorder.[120] Although such studies provide hints that breastfeeding may combat against developing complex neurological diseases, the findings may also be due to other confounding factors. These include genetics, environmental background, nutritional, overall health, socioeconomic, nutritional and metabolic status of both parents to list a few examples. Thus, larger cohort studies spanning genetically related and unrelated children of varying ages and backgrounds are needed before any firm conclusions can be drawn.

Several studies have examined whether breastfeeding improves later offspring cognitive abilities and IQ status, as measured by several indices. The general consensus is that breastfeeding can improve both of these parameters to a certain extent[74,75], although there are conflicting data[121–123]. Those reporting a positive association also suggest that the effects of breastfeeding on IQ score become more pronounced with increased breastfeeding duration and are enhanced in preterm infants or those small for gestational age.[74,75,124] Genetic variation in the *FADS2* gene, which regulates fatty acid pathways, may modulate breastfeeding effects on IQ status.[125] While it is beyond the scope of the current article to discuss all studies to date, representative individual and meta-analyses reports will be considered.

A meta-analyses study of 11 individual reports with children ranging from 6 months to 16 years of age concluded that breastfeeding positively correlated with an increase in IQ by 3.2 points, even after controlling for covariant variables, namely maternal intelligence.[126] Another meta-analyses representing 17 individual studies also found an increase of 3.4 IQ points in breastfed children and adolescents.[127] A prospective longitudinal birth cohort with a sample of 973 men and 2280 women who were born in Copenhagen, Denmark between October 1959 and December 1961 determined that breastfeeding up to 9 months of age is linked with higher adult intelligence.[128]

Intelligence as measured by the Peabody Vocabulary Test in sibling pairs ($n = 2734$) from the National Longitudinal Study of Adolescent Health showed breastfed infants scored 1.7 and 2.4 higher intelligence points within and across families, respectively, with each month of breastfeeding raising IQ by 0.2 points per month.[129] Similar positive benefits for each month of being breastfed and later IQ scores were demonstrated in two consecutive generations of British children enrolled in the National Child Development Study.[130] A Krakow prospective birth cohort study revealed that breastfeeding alone positively associates with an increase in IQ of toddlers by 1 year of age and this effect persists through the preschool period.[131] A causal relationship of breastfeeding duration and receptive language and verbal and nonverbal intelligence in school age children (3 and 7 years of age) was established in a U.S. cohort representing 1312 mothers and children.[132] Eight-year-old children who were breastfed for 6 months or more performed better in a general intellectual assessment test.[133] Children (6.5-year old, $n = 13{,}889$) from a Belarussian study who were breastfed achieve elevated scores in verbal, performance, and full scale IQ tests, and are ranked higher in teacher's evaluations for academic performance in reading, writing and mathematics.[134]

Causal effects of breastfeeding on IQ but not blood pressure and BMI are suggested based on two cohort children studies (British Avon Longitudinal Study of Parents and Children, ALSPAC and Brazilian Pelotas 1993 cohort) representing ~6000 male and female individuals and a follow-up meta-analysis results from five low- or middle-income countries (LMIC, $n \approx 10{,}000$).[135] A separate meta-analyses concluded breastfeeding may increase intelligence, protect against child infections and malocclusion, and reduce the incidence of obesity and diabetes.[75]

Ostensibly, the beneficial effects of breastfeeding on neurobehavioral development are multi-faceted. One possibility is that breastfeeding may directly shape brain development. In males, breastfeeding is associated with an increase in IQ and enhancing brain white matter growth, presumably due to an increase in neuronal cell processes or dendritic arborization.[136] Nutrients within the milk may stimulate neural programming. Two primary ones that have received considerable attention are essential and nonessential long-chain (LC) polyunsaturated fatty acids (PUFA) and the n-3 fatty acid, docosahexaenoic acid (DHA).[74] Supplementation of DHA to lactating mothers is associated with improved psychomotor indices in 30-month-old children, but this treatment did not affect visual acuity or neurocognitive development.[137] LC-PUFA in colostrum may boost mental development in children who are breastfed for a prolonged duration.[138] Maternal hormones within the milk may also affect later offspring neurobehavioral functions. For instance, greater amount of cortisol in human milk appears to impact infant temperament, as evidenced by a positive association with negative affectivity or emotions in girls.[139]

Conclusions

Parental factors resulting in disease outcomes in offspring have received considerable attention. Howevever, less so are the steps parents can take to promote the lifelong health of male and female offspring. The current data suggest that parental exercise, probiotic supplementation and breastfeeding may abate the risk of various disorders and even foster beneficial effects. However, there are still important unanswered questions and concerns to be addressed.

One of the primary questions is whether beneficial parental habits can mitigate negative perinatal influences. For instance, breastfeeding ameliorates the deleterious metabolic effects in children born to mothers who endured pre-gestational diabetes or excessive weight gain.[81,82]

In rodents, maternal exercise abolishes the deleterious effects of developmental exposure to a high fat or protein restricted diet.[35,39,41–43] Additional studies are needed though to examine whether breastfeeding, exercise, and probiotic administration can combat other negative extrinsic influences, such as stress and environmental chemicals. It is becoming increasingly apparent that gut microbial populations and their by-products can affect various systems ranging from cardiometabolism to the central nervous system. Thus, early colonization with beneficial microbes might protect neonates against harmful stimuli. Yet, there are currently no studies examining concurrent exposure to maternal or paternal obesity, exposure to environmental chemicals, stress or other harmful factors and probiotic supplementation.

Moreover, it would be of interest to determine the collective DOHaD effects of all three presumably beneficial factors, especially in sub-optimal perinatal conditions. Exercise and breastfeeding may alter the gut microbiome of parents and their offspring. As indicated above, breast milk induces long-term offspring gut microbiome changes that can have dramatic health consequences.[89,90] In adult rodents, exercise changes the gut microbial populations.[140–143] In turn, the composition of the gut microbiota may influence exercise performance of mice through anti-oxidant enzyme production.[144] In mice, exercise attenuates gut dysbiosis due to exposure to polychlorinated biphenyls.[145] It is likely that similar and possibly even more complex interactions among the gut microbiome, parental exercise and breastfeeding occur in developing neonates.

There are several published studies exploring the effects of maternal exercise and probiotic supplementation on offspring DOHaD outcomes. However, reports examining these effects on the father and their progeny are sparse. In the case of paternal exercise, the two current studies yield conflicting data with one reporting positive effects on cognitive function[61] but another suggesting this paternal activity results in offspring metabolic disruptions.[62] Clearly, additional studies are needed to sort out the potential offspring DOHaD outcomes due to paternal exercise and whether there can be transgenerational transmission due to epigenetic changes in the spermatozoa.[62] While fathers cannot provide direct nourishment to the young after birth, additional attention should be paid to whether a healthy diet of the father, and the mother, before conception can lead to long-term beneficial offspring consequences. It is increasingly becoming apparent that sub-optimal paternal diets, those high in fat or protein restricted, can result in negative offspring sequelae,[14–21, 146–149] but the impacts of a healthy diet have been largely ignored. In conclusion, much work remains on identifying various steps that both parents can adapt to ensure the lifelong health of their offspring and potentially even triumph over negative influences that the conceptus or neonate may encounter. The current work though offers hope that indeed good habits on behalf of parents can place their children on the path to a healthy lifespan.

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