consistent application of low airway pressure and low tidal volume ventilation (a lung-protective strategy). Some trials, the largest of which was the lower tidal volume trial conducted by the ARDS Clinical Trials Network,[3] revealed that reducing tidal volume from a typical traditional size of 12 ml per kilogram of *actual* body weight to 6 ml per kilogram of *predicted* body weight reduced mortality by 9%.[3] The remainder of the reduction in overall mortality in modern ICUs has not been clearly explained, but it appears to be attributable to better training of physicians, nurses, respiratory therapy staff, and others involved in the routine care of patients with ARDS. Improved alarm systems, ventilator equipment, and radiologic and laboratory evaluation — the availability of magnetic resonance imaging and computed tomography, for example, as well as point-of-care testing with rapid return of results — have undoubtedly also contributed.

Though the vast improvement in ICU and hospital mortality from ARDS is heartening, the poor subsequent condition of the growing number of survivors is showing us that much more needs to be done. Full recovery in patients who have been hospitalized with ARDS happens very slowly, if at all. At 1 year after discharge, vital capacity and 6-minute walk distance remain significantly reduced from patients' pre-ARDS status. Furthermore, less than half of such patients have been able to return to work.[4] Perhaps most worrisome, as many as 50% of patients who have received mechanical ventilation in an ICU — commonly but not always because of ARDS — have cognitive impairment as long as a year after hospital discharge.[5]

We have come a long way since the middle of the last century with regard to recognition, evaluation, treatment, and long-term follow-up of patients with ARDS. Related mortality has fallen so far that it may be reaching a floor rate dictated more by the underlying diseases associated with ARDS — such as sepsis, severe trauma, or pancreatitis — than by either the syndrome itself or other associated organ dysfunction. Fortunately, such progress allows us to turn a much larger portion of our research and clinical attention to the rapidly growing population of ICU survivors.

Disclosure forms provided by the author are available at NEJM.org.

From Vanderbilt University Medical Center, Nashville.

1. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med 1994;149:818-24.
2. The ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012;307:2526-33.
3. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med 2000;342:1301-8.
4. Herridge MS, Cheung AM, Tansey CM, et al. One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med 2003;348:683-93.
5. Pandharipande PP, Girard TD, Jackson JC, et al. Long-term cognitive impairment after critical illness. N Engl J Med 2013;369:1306-16.

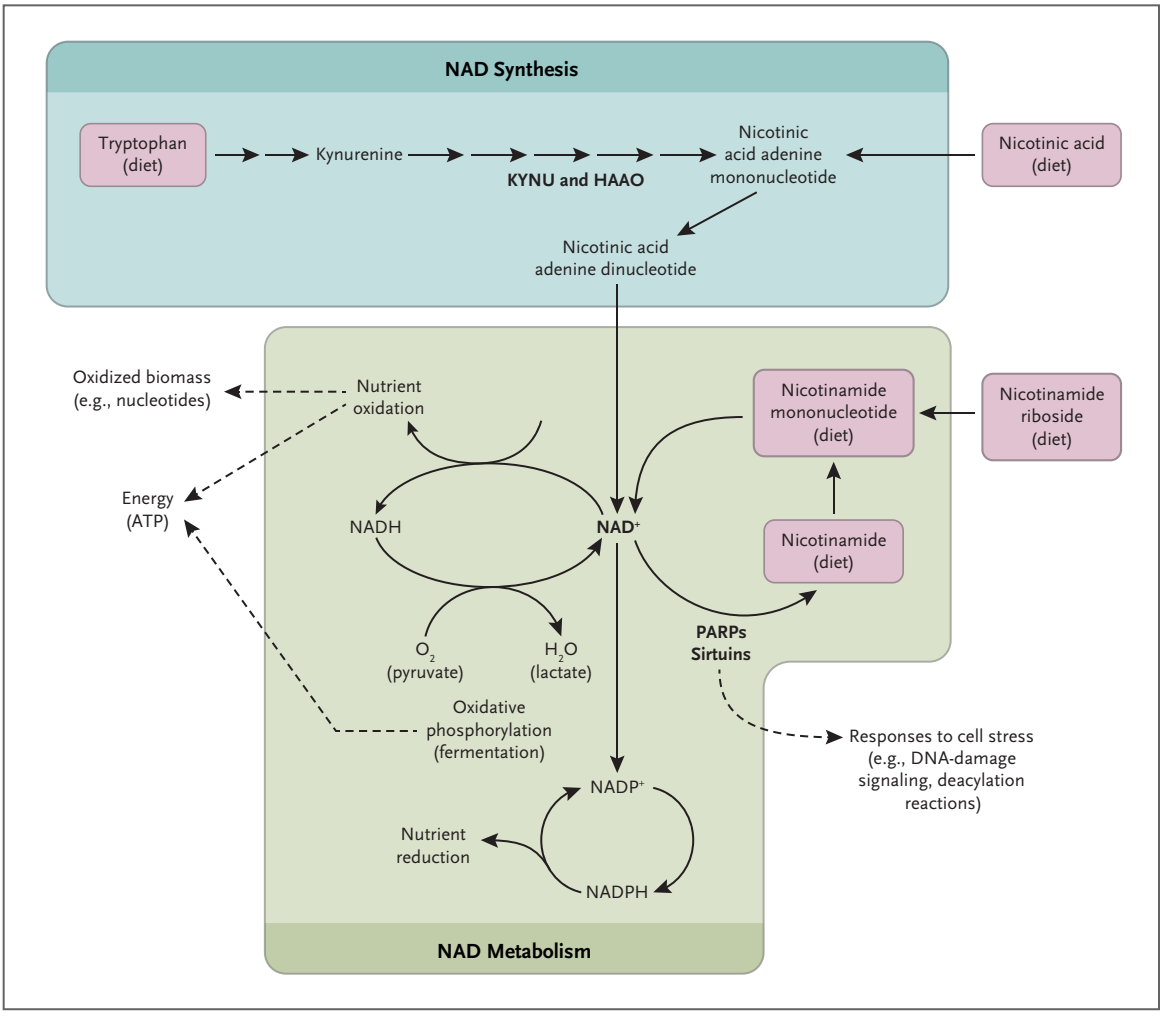
DOI: 10.1056/NEJMp1706595

Metabolism and Congenital Malformations — NAD's Effects on Development

Matthew G. Vander Heiden, M.D., Ph.D.

Related article, page 544

We have long known that metabolism must be tightly controlled to support the needs of individual cells and tissues. Nutrient oxidation is necessary for the production of adenosine triphosphate (ATP), which supports energetically unfavorable processes in cells, and for the metabolic conversion of nutrients to build biomass in support of cell proliferation. So it makes sense that disrupting metabolism can perturb normal development and that specific nutrient deficiencies can cause congenital anomalies. For example, folate deficiency delays neural tube closure, resulting in anencephaly or spina bifida, and fortification of cereal food products and folic acid supplementation in pregnant women have reduced the incidence of these birth defects.[1] Thus, an understanding of the relationship between metabolism and development can guide dietary interventions that reduce the risk of congenital defects. In this issue of the *Journal*, Shi et al. (pages 544–552) report that a deficiency of nicotinamide adenine dinucleotide (NAD) causes congenital malformations, sug-



Nicotinamide Adenine Dinucleotide Metabolism.

The diagram shows the pathway whereby nicotinamide adenine dinucleotide (NAD) is produced from the amino acid tryptophan by means of the kynurenine pathway enzymes kynureninase (KYNU) and 3-hydroxyanthranilic acid 3,4-dioxygenase (HAAO), along with the pathways involved in salvaging dietary precursors for NAD. Dietary precursors include niacin (nicotinic acid), nicotinamide riboside, nicotinamide mononucleotide, and nicotinamide. NAD is used in metabolism and in stress-response pathways involving poly(adenosine diphosphate–ribose) polymerases (PARPs) and sirtuin proteins.

gesting that interventions to raise NAD levels during fetal and early postnatal development might further reduce the incidence of congenital anomalies.

By sequencing the exomes or genomes of members of four unrelated families with offspring born with combinations of congenital defects that meet the criteria for the so-called VACTERL association of vertebral, anal, cardiac, tracheo-esophageal, renal, and limb anomalies, Shi et al. found loss-of-function mutations in two genes, each of which encodes an enzyme involved in the synthesis of NAD. The affected patients had loss-of-function mutations in both copies of the gene encoding 3-hydroxyanthranilic acid 3,4-dioxygenase (HAAO) or of the gene encoding kynureninase (KYNU) (see diagram). These mutations result in the accumulation of upstream intermediates in the kynurenine pathway and depletion of NAD.

Engineering the same enzyme deficiencies in mice resulted in birth defects similar to those found in humans, but only if they were combined with dietary interventions to reduce NAD levels. Deletion of *Haao* or *Kynu* in mice was sufficient to increase levels of upstream pathway intermediates but had no effect on development provided that sufficient NAD levels were maintained through diet. Therefore, the authors could conclude that NAD depletion, rather

than the buildup of the upstream intermediates, was responsible for the congenital malformations.

NAD has a central role in cell metabolism. It is an electron carrier for redox reactions in cells (see diagram). Oxidized NAD (NAD^+) can accept an electron pair as a cofactor in oxidation reactions, including those involved in glycolysis and the tricarboxylic acid cycle that are important for ATP production. NAD^+ is also an electron acceptor in biosynthetic pathways, including pathways for the synthesis of purine and pyrimidine nucleotides — fundamental building blocks of RNA and DNA. When NAD^+ accepts electrons, it is reduced to NADH. Because the pool of NAD^+ is small relative to the flux through metabolic pathways involving oxidation reactions, NAD^+ must be continually regenerated by transferring the electrons from NADH to another substrate, such as the terminal electron acceptors oxygen or pyruvate. Thus, the continued shuttling of electrons between NADH and NAD^+ is critical for many cellular processes, including the generation of ATP and the synthesis of nucleotides. NAD^+ can also be phosphorylated to generate $NADP^+$ as a second cofactor pool that when reduced to NADPH can provide electrons for reductive biosynthesis and maintenance of a reduced intracellular redox state.

NAD^+ is also a substrate for enzymes, such as the poly(adenosine diphosphate[ADP]–ribose) polymerases (PARPs), that signal the presence of DNA damage and other types of cell stress. PARPs cleave nicotinamide from the ADP-ribose portion of NAD^+ and then catalyze the transfer of ADP-ribose to (and thereby activate) proteins involved in DNA repair, telomere maintenance, and other proteins that participate in cell-stress responses.[2] Another group of stress responders are members of the sirtuin family of proteins,[3] which use NAD^+ as a cofactor and also release free nicotinamide.

The nicotinamide generated through the action of the PARPs and sirtuins can be salvaged to regenerate NAD. NAD can also be synthesized from the amino acid tryptophan through the kynurenine pathway or generated from dietary nicotinic acid (also known as niacin, part of the vitamin B_3 complex), as well as from dietary nicotinamide (also part of the vitamin B_3 complex), nicotinamide riboside, or nicotinamide mononucleotide. Because these molecules permit NAD synthesis using enzymes that bypass the kynurenine pathway, supplementation of any of them in pregnant women or newborns with kynurenine pathway mutations should restore NAD levels. Furthermore, because supplementation is sufficient to prevent congenital malformations in mice with kynurenine pathway mutations, ensuring adequate NAD levels during development may reduce the risk of congenital malformations in humans and may also prevent cognitive impairment in persons with VACTERL association.

How NAD deficiency causes congenital malformations is not known. Metabolism is coordinated with tissue differentiation to allow normal development.[4] The fact that congenital defects similar to those found with NAD deficiency are observed in persons with Fanconi anemia (caused by loss-of-function variants in the *FANC* genes) suggests that the two disorders have related underlying causes. Although it is also unclear how *FANC* variants cause congenital abnormalities, it is known that the FANC proteins guard against faulty DNA replication and repair, processes that are relevant to those of NAD metabolism: DNA-damage sensing and nucleotide production.

Regardless of how NAD depletion leads to congenital malformations (whether by compromising the detection of DNA damage by PARP proteins, reducing the supply of nucleotides, or both), dietary supplementation with NAD precursors merits further study. At high doses, niacin can cause flushing and gastrointestinal symptoms, but it has few side effects at lower doses. Nicotinamide mononucleotide, nicotinamide riboside, and nicotinamide itself are better tolerated than niacin and are generally considered to be safe as dietary supplements, but the doses of NAD precursors required to reduce the risk of congenital malformations in humans are not known. Also unknown is the extent to which raising dietary levels of NAD would limit cognitive impairment in infants with congenital malformations.

Disclosure forms provided by the author are available at NEJM.org.

From the Koch Institute for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; and the Dana–Farber Cancer Institute, Boston.

1. De Wals P, Tairou F, Van Allen MI, et al. Reduction in neural-tube defects after folic acid fortification in Canada. N Engl J Med 2007;357:135-42.
2. Kraus WL. PARPs and ADP-ribosylation: 50 years . . . and counting. Mol Cell 2015; 58:902-10.
3. Wagner GR, Hirschey MD. Nonenzymatic protein acylation as a carbon stress regulated by sirtuin deacylases. Mol Cell 2014;54:5-16.
4. Naganathan SR, Oates AC. The sweetness of embryonic elongation and differentiation. Dev Cell 2017;40:323-4.

DOI: 10.1056/NEJMp1707487