C-reactive protein were unchanged. Of note, there was a nonlinear dose response between FRC and FMD, with the maximum effect achieved with the 500-mg daily dose. This is the first meta-analysis to evaluate the effect of FRC on all CVD risk factors and a dose–response relationship, and its limitations include the trials being short term (2 to 18 weeks) and not having access to patient-level data, said Dr. Ding. In addition, another meta-analysis of cocoa flavonoids performed by researchers from Harvard also found benefits for lowering insulin and improving insulin resistance [Hooper L et al. *Am J Clin Nutr* 2008].

The Zutphen Elderly Study, a prospective cohort study of 470 men in the Netherlands who were followed for 15 years, was among the high-quality studies that showed an association between cocoa intake and reductions in CVD and total mortality [Buijsse B et al. *Arch Intern Med* 2006]. Subject who had the highest amount of cocoa intake were associated with reduced risk of CV death (adjusted relative risk, 0.50; 95% CI, 0.32 to 0.78; p = .004) and all-cause death at 15 years (adjusted relative risk, 0.53; 95% CI, 0.39 to 0.72; p < .001) as compared to subjects with the least amount of ingestion.

A systematic review and meta-analysis of 7 nonrandomized studies showed that higher versus lower levels of chocolate consumption were associated with a reduced risk of any CVD (relative risk, 0.63; 95% CI, 0.44 to 0.90) [Buitrago-Lopez A et al. *BMJ* 2011]. Further evidence for an association between chocolate consumption and reduction in stroke came from the Cohort of Swedish Men study of 37,103 men that found a 17% relative risk reduction in the highest (62.9 g/week) versus the lowest (0 g) quartiles of consumption [Larsson S et al. *Neurology* 2012] and a meta-analysis of 5 studies that showed a relative risk reduction of 0.81 for stroke (95% CI, 0.73 to 0.90) [Larsson S et al. *Neurology* 2012].

The evidence of benefit with FRC and chocolate from preclinical, prospective cohort, and short-term clinical studies to prevent CVD has provided the framework for long-term RCTs with hard CVD endpoints. Planning is underway for the Cocoa Supplement and Multivitamins Outcomes Study [COSMOS], led by researchers from Brigham and Women's Hospital and Harvard Medical School, that will begin in 2015 and enroll 18,000 men and women who are free of CVD to determine the effect of high-quality cocoa flavanol supplementation (750 mg daily) and also a multivitamin in a double-blind, placebo-controlled, 2×2 factorial study.

HMO Effective Against Rotavirus Diarrhea in Experimental Studies

Written by Mary Mosley

Rotavirus infection is reduced by breastfeeding in the first year of life (Panda S et al. *Epidemiol Infect* 2014). Human milk oligosaccharides (HMO) are thought to contribute to this protection, in part by binding to some rotavirus strains and interrupting virus binding to host cell glycoconjugate receptors.

The burden of rotavirus infection is significant. In 2008, based on global surveillance by the World Health Organization, rotavirus was estimated to cause 25 million outpatient visits, >2 million hospitalizations [http://www.who.int/biologicals/areas/vaccines/rotavirus/background/en/], and >453,000 deaths in children aged <5 years [http://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/en/]. The greatest disease burden is in developing countries where the availability of rotavirus vaccines is limited and also seems to be less effective [Patel MM et al. *Pediatr Infect Dis J* 2011].

Sharon M. Donovan, PhD, RD, University of Illinois, Urbana, Illinois, USA, reviewed work by her group that tested the hypothesis that HMO, particularly those forms containing sialic acid, would decrease rotavirus infectivity by reducing binding and modulating gut microflora.

In a set of experiments to screen for rotavirus inhibitory activity *in vitro*, they found that sialyllactose-containing HMO and HMO isolated from preterm human milk (iHMO) inhibited infectivity and binding of sialic acid-dependent rotavirus at those concentrations present in human milk [Hester SN et al. *Br J Nutr* 2013]. They also found dose-dependent effects for binding and infectivity, which were more effective at a lower concentrations with 6'-sialyllactose than with 3'-sialyllactose; the groups believes that this distinction may be related to differences in the structures of the cellular binding sites. Dr. Donovan stated these results support sialic acid-dependent rotavirus binding by sialic acid-containing HMO as a primary mechanism of action.

Next, they studied the ability of HMO to inhibit rotavirus activity *in situ* using a piglet model. They showed that both sialic acid-containing HMO and a neutral HMO (lacto-N-neotetraose, LNnT) decreased rotavirus infectivity in isolated loops of intestine [Hester SN et al. *Br J Nutr* 2013], suggesting other possible mechanisms for the effect of HMO. Dr. Donovan noted that this study was also important for establishing a model that will allow for the screening of different HMO fractions, such as neutral and acidic, in order to determine which are most

Table 1. Improvements in Rotavirus Infection With Human Milk Oligosaccharides Added to Formula

Hour Post-Infection	FF	HMO	p Value
Onset of diarrhea	35.1 ± 3.8	38.3 ± 2.3	NS
Initial infection			
Recovery	81.4 ± 10.5	76.5 ± 9.0	NS
Duration	46.3 ± 9.4	38.3 ± 7.6	NS
Re-infection			
Recovery	115.7 ± 2.8[a]	87 ± 10.6[b]	.045
Duration	80.6 ± 4.5[a]	48.8 ± 9.8[b]	.038

FF=formula only; HMO=human milk oligosaccharides; NS=not significant.

Mean ± standard error of measurement; different superscript letters indicate differences at the level of significance ($p \leq .05$).

Source: Li M et al. *ISME J* 2014.

efficacious before testing in rotavirus infection studies *in vivo*, which would require large quantities of HMO.

Finally, Dr. Donovan showed the results of an *in vivo* study of rotavirus infection in colostrum-deprived piglets, which sought to determine the efficacy of formula supplemented with HMO (4 g/L), compared with formula alone. Dietary HMO reduced the duration of rotavirus infection by 30 hours, primarily by reducing the second wave of infection [Li M et al. *ISME J* 2014]. The effect of HMO on diarrhea and on the rates of initial and reinfection are detailed in Table 1. HMO increased serum rotavirus-specific immunoglobulin M (IgM) and increased interferon gamma (IFN-γ) and interleukin-10 (IL-10) levels in the ileum, which suggests the effects of HMO on both systemic and mucosal immunity, said Dr. Donovan. Furthermore, rotavirus infection significantly modified the microflora at the level of the phyla, family, and genus in the ascending colon, and that HMO promoted the growth of Lachnospiraceae.

Global Project Examining Link Between Dietary Intake and Breast Cancer

Written by Mary Mosley

The International Breast Cancer and Nutrition (IBCN) Project was launched in 2010 and is the first global effort to elucidate the relation between diet, genetics, and the development of breast cancer. It will also serve as a model for the study of primary prevention of breast cancer and other noncommunicable diseases. Ailsa Welch, PhD, University of East Anglia, Norwich, United Kingdom, reviewed new research approaches for the IBCN and some preliminary data from Phase 1 of the study.

The IBCN is managed by researchers at Purdue University, West Lafayette, Indiana, USA, and participation is voluntary. Currently, 11 countries are participating in the IBCN (including China, Lebanon, France, Ghana, Qatar, the United Kingdom, the United States, and Uruguay), which provides a diverse range of dietary exposure and patterns.

Multidisciplinary teams at the participating centers are collecting diet information, breast tissue, and blood samples. Novel approaches are being used to more accurately capture dietary intake, diet composites, and measurement of biomarkers and nutrient status that are indicators of nutritional exposure. The goal is to better understand the relationship between diet and genetics through the study of tissue samples from patients with breast cancer.

The experience from the European Prospective Investigation Into Cancer and Nutrition [EPIC], which began in 1993 and included 10 countries and 450,000 participants, gave investigators important experience and has helped to overcome some of the methodological challenges being experienced in IBCN. These solutions include the development of standardized nutrient databases, a biomarker validation program, and dietary calibration that included the development of a standardized computer program for 24-hour dietary recall interviews. Prof. Welch noted that these solutions within EPIC were highly labor and resource intensive.