Eating Patterns

Two Weeks of Early Time-Restricted Feeding (eTRF) Improves Skeletal Muscle Insulin and Anabolic Sensitivity in Healthy Men


**Significance:** Early time-restricted feeding resulted in improved whole-body insulin sensitivity and increased skeletal muscle glucose and BCAA uptake in free-living conditions.

**Background:** Altering the temporal distribution of energy intake (EI) and introducing periods of intermittent fasting (IF) exert important metabolic effects. Restricting EI to earlier in the day [early time-restricted feeding (eTRF)] is a novel type of IF. **Objectives:** We assessed the chronic effects of eTRF compared with an energy-matched control on whole-body and skeletal muscle insulin and anabolic sensitivity. **Methods:** Sixteen healthy males (aged 23 ± 1 y; BMI 24.0 ± 0.6 kg·m⁻²) were assigned to 2 groups that underwent either 2 wk of eTRF (n = 8) or control/caloric restriction (CON:CR; n = 8) diet. The eTRF diet was consumed ad libitum and the intervention was conducted before the CON:CR, in which the diet was provided to match the reduction in EI and body weight observed in eTRF. During eTRF, daily EI was restricted to between 08:00 and 16:00, which prolonged the overnight fast by ~5 h. The metabolic responses to a carbohydrate/protein drink were assessed pre- and post-interventions following a 12-h overnight fast. **Results:** When compared with CON:CR, eTRF improved whole-body insulin sensitivity [between-group difference (95% CI): 1.89 (0.18, 3.60); P = 0.03; η²p = 0.29] and skeletal muscle uptake of glucose [between-group difference (95% CI): 4266 (261, 8270) μmol·min⁻¹·kg⁻¹·180 min; P = 0.04; η²p = 0.31] and branched-chain amino acids (BCAAs) [between-group difference (95% CI): 266 (77, 455) nmol·min⁻¹·kg⁻¹·180 min; P = 0.01; η²p = 0.44]. eTRF caused a reduction in EI (~400 kcal·d⁻¹) and weight loss (-1.04 ± 0.25 kg; P = 0.01) that was matched in CON:CR (-1.24 ± 0.35 kg; P = 0.01). **Conclusions:** Under free-living conditions, eTRF improves whole-body insulin sensitivity and increases skeletal muscle glucose and BCAA uptake. The metabolic benefits of eTRF are independent of its effects on weight loss and represent chronic adaptations rather than the effect of the last bout of overnight fast.

Low-Calorie Sweeteners

Intake of Sugar-Sweetened and Low-Calorie Sweetened Beverages and Risk of Cardiovascular Disease: A Meta-Analysis and Systematic Review


**Significance:** Regular consumption of sugar-sweetened beverages is associated with a higher risk of cardiovascular disease morbidity and mortality in a dose-response manner. Low-calorie sweetened beverages were also associated with these risks, but reverse causation and residual confounding limits interpretation of these findings.

The long-term associations between the consumption of sugar-sweetened beverages (SSBs) and low-calorie sweetened beverages (LCSBs) with cardiovascular diseases (CVDs) remains inconsistent. To synthesize the evidence, we conducted a meta-analysis of prospective cohort studies published up to 1 December, 2019 on the associations between SSB and LCSB intake and the risk of CVD incidence and mortality. Out of 5301 articles retrieved from our literature search, 11 articles evaluating the consumption of SSBs (16,915 incident CVD cases, 18,042 CVD deaths) and 8 articles evaluating the consumption of LCSBs (18,077 incident CVD cases, 14,114 CVD deaths) were included in the meta-analysis. A 1 serving/d increment of SSBs was associated with an...
8% (RR: 1.08; 95% CI: 1.02, 1.14, I² = 43.0%) and 8% (RR: 1.08; 95% CI: 1.04, 1.13, I² = 40.6%) higher risk of CVD incidence and CVD mortality, respectively. A 1 serving/d increment of LCSBs was associated with a 7% (RR: 1.07; 95% CI: 1.05, 1.10, I² = 0.0%) higher risk of CVD incidence. The association between LCSBs and CVD mortality appeared to be nonlinear (P = 0.003 for nonlinearity) with significant associations observed at high intake levels (>2 servings/d). Under an assumption of causality, the consumption of SSBs may be linked to 9.3% (95% CI: 6.6%, 11.9%) of predicted CVD incidence in the USA from 2015 to 2025, among men and nonpregnant women, who were aged 40-79 y in 2015-2016. The habitual consumption of SSBs was associated with a higher risk of CVD morbidity and mortality in a dose-response manner. LCSBs were also associated with a higher risk of these outcomes, however, the interpretation of these findings may be complicated by reverse causation and residual confounding.

**A Novel Urinary Biomarker Approach Reveals Widespread Exposure to Multiple Low-Calorie Sweeteners in Adults**


**Significance:** Using a biomarker may be the best way to address the discrepancies between actual consumption of low-calorie sweeteners and self-reported data on consumption.

**Background:** Observational investigations into the health impacts of low-calorie sweeteners (LCSSs) in humans fail to adequately identify or fully characterize LCS consumption. **Objectives:** We aimed to utilize a novel biomarker approach to investigate exposure to 5 LCSs and to test whether reported low-calorie sweetened beverage (LCSB) consumption effectively identifies exposure to LCSSs in adults. **Methods:** In this cross-sectional analysis, 2 population studies were conducted in adults. Urinary excretions of 5 LCSSs, namely ascesulfame-K, saccharin, cyclamate, sucralose, and steviol glycosides, were simultaneously determined using LC tandem-MS. In Study 1, previously collected 24-h urine samples (n = 357) were analyzed. In Study 2, previously collected 24-h urine samples (n = 79) were analyzed to compare urinary excretions of LCSSs with self-reported LCSB consumption for identifying LCS exposure. Exposure to LCSSs was characterized using descriptive statistics and chi-square tests were performed to assess associations between age-groups and LCS excretion, and to assess the proportion of individuals identified as LCS consumers using biomarker data or reported LCSB consumption. **Results:** A total of 341 adults (45% men) and 79 adults (39% men) were included in the final analysis of Studies 1 and 2, respectively. In Study 1, >96% of samples contained ≥1 LCS and almost 60% contained ≥3 LCSs. A greater proportion of younger adults (<40 y old) excreted ≥3 LCSs than older adults (39% men) were included in the final analysis of Studies 1 and 2, respectively. In Study 1, >96% of samples contained ≥1 LCS and almost 60% contained ≥3 LCSs. A greater proportion of younger adults (<40 y old) excreted ≥3 LCSs than older adults (>40 y old) (P < 0.001). In Study 2, a much higher prevalence of LCS consumption was observed using biomarker data (92%) than reported LCSB consumption (6%) (P < 0.001). **Conclusions:** This work indicates widespread exposure to LCSSs, suggesting that population-based research to date into LCS exposure and health may be flawed. Therefore, a urinary biomarker approach offers considerable potential for more robust investigations in this area.

**Protein**

**Blood 15N:13C Enrichment Ratios Are Proportional to the Ingested Quantity of Protein with the Dual-Tracer Approach for Determining Amino Acid Bioavailability in Humans**


**Significance:** A dual-stable isotope tracer approach was developed to assess dietary amino acid bioavailability, a measure of protein quality.

**Background:** Assessment of amino acid bioavailability is of key importance for the evaluation of protein quality; however, measuring ileal digestibility of dietary proteins in humans is challenging. Therefore, a less-invasive dual stable isotope tracer approach was developed. **Objective:** We aimed to test the assumption that the 15N:13C enrichment ratio in the blood increases proportionally to the quantity ingested by applying different quantities of 15N test protein. **Methods:** In a crossover design, 10 healthy adults were given a semi-liquid mixed meal containing 25 g (low protein) or 50 g (high protein) of 15N-labeled milk protein concentrate simultaneous with 0.4 g of highly 13C-enriched spirulina. The meal was distributed over multiple small portions, frequently provided every 20 min during a period of 160 min. For several amino acids, the blood 15N-related to 13C-isotopic enrichment ratio was determined at t = 0, 30, 60, 90, 120, 180, 240, 300, and 360 min and differences between the 2 meals were compared using paired analyses. **Results:** No differences in 13C AUC for each of the measured amino acids in serum was observed when ingesting a low- or high-protein meal, whereas 15N AUC of amino acids was ~2 times larger on the high-protein meal (P < 0.001). Doubling the intake of 15N-labeled amino acids increased the 15N:13C ratio by a factor of 2.04 ± 0.445 for lysine and a factor between 1.8 and 2.2 for other analyzed amino acids, with only phenylalanine (2.26), methionine (2.48), and tryptophan (3.02) outside this range. **Conclusions:** The amino acid 15N:13C enrichment ratio in the peripheral circulation increased proportionally to the quantity of 15N-labeled milk protein ingested, especially for lysine, in healthy adults. However, when using 15N-labeled protein, correction for, e.g., α-carbon 15N atom transamination is advised for determination of bioavailability of individual amino acids.
Sodium

Sodium Intake, Health Implications, and the Role of Population-Level Strategies

Significance: This review focuses on current available evidence on regional sodium intake levels, health implications of sodium intake, and population-level strategies implemented worldwide.

Evidence to date suggests that high sodium intake affects health adversely, yet the role of a population-level strategy to reduce sodium intake is often contested. This review focuses on current available evidence on regional sodium intake levels, health implications of sodium intake, and population-level strategies implemented worldwide. The limitations in evidence, the difficulties in implementing population-wide strategies to reduce sodium intake, and the need for such strategies are critically reviewed. Evidence clearly shows that sodium has an adverse effect on blood pressure, cardiovascular disease, and mortality. However, whether reduced sodium intake benefits all individuals or only hypertensive individuals is still unclear. Methodological issues and publication bias in current evidence are other matters of concern in sodium-related research. While it is essential to continue working toward the World Health Organization’s target of 30% reduction in sodium intake, due consideration should be given to improving the quality of research, reducing bias in publications, and reviewing evidence more critically.

Lipids

The Effects of Diets Enriched in Monounsaturated Oleic Acid on the Management and Prevention of Obesity: A Systematic Review of Human Intervention Studies

Significance: This systematic review found that oleic acid-rich diets may regulate food intake, body mass and energy expenditure by stimulating AMP-activated protein kinase signaling.

Obesity is associated with an increased risk of several major noncommunicable diseases and is an important public health concern globally. Dietary fat content is a major contributor to the increase in global obesity rates. Changes in dietary habits, such as the quality of fatty acids in the diet, are proposed to prevent obesity and its metabolic complications. In recent years, a number of studies have found that oleic acid (OA), the most common MUFA in daily nutrition, has protective effects against human disease. Importantly, there is emerging evidence indicating the beneficial effects of OA in regulating body weight. Accordingly, the objective of this systematic review was to investigate the effects of diets enriched in monounsaturated OA on the management and prevention of obesity, emphasizing possible mechanisms of action of OA in energy homeostasis.

Searches were performed in PubMed/MEDLINE, ScienceDirect, Scopus, ProQuest, and Google Scholar databases for clinical trials that examined the effects of diets rich in OA on obesity. Of 821 full-text articles assessed, 28 clinical trials were included in the present study. According to the studies examined in this review, diets enriched in OA can influence fat balance, body weight, and possibly energy expenditure. Importantly, abdominal fat and central obesity can be reduced following consumption of high-OA–containing meals. Mechanistically, OA-rich diets can be involved in the regulation of food intake, body mass, and energy expenditure by stimulating AMP-activated protein kinase signaling. Other proposed mechanisms include the prevention of the nucleotide-binding oligomerization domain-like receptor 3/caspase-1 inflammasome pathway, the induction of oleoylthanolamide synthesis, and possibly the downregulation of stearoyl-CoA desaturase 1 activity. In summary, current findings lend support to advice not restricting consumption of OA-rich meals so as to maintain a healthy body weight.

Carbohydrates

The Carbon Isotope Ratios of Serum Amino Acids in Combination with Participant Characteristics Can Be Used to Estimate Added Sugar Intake in a Controlled Feeding Study of US Postmenopausal Women

Significance: Development of a biomarker for added sugar is complicated by meat intake rates, but a serum carbon isotope ratio of amino acid measures in combination with participant body weight, smoking status and physical activity may serve as a potential biomarker for added sugar intake.
Background: The carbon isotope ratio (CIR) is a proposed biomarker for added sugar (AS) intake in the United States; however, because the CIR is also associated with meat intake in most populations the need for specificity remains. The CIR of amino acids (AAs) has the potential to differentiate sugars from meat intakes, because essential AAs must derive from dietary protein whereas certain nonessential AAs can be synthesized from sugars. Objectives: We tested whether serum CIR-AAs in combination with participant characteristics could meet a prespecified biomarker criterion for AS intake in the Nutrition and Physical Activity Assessment Study Feeding Study (NPAAFS-FS) of the Women’s Health Initiative, a population in which the whole-serum CIR was not associated with AS intake. Methods: Postmenopausal women (n = 145) from Seattle, WA, were provided with individualized diets that approximated their habitual food intakes for 2 wk. Dietary intakes from consumed foods were characterized over the feeding period using the Nutrition Data System for Research. The CIR of 7 AAs-Ala, Gly, Val, Leu, Ile, Pro, and Phe-were measured in fasting serum collected at the end of the 2-wk feeding period, using gas chromatography-combustion isotope ratio mass spectrometry. Biomarker models were evaluated using regression R² ≥ 0.36 as a major biomarker criterion, based on the benchmark R² values of well-established recovery biomarkers in the NPAAFS-FS. Results: AS intake was associated with CIR-Ala (ρ = 0.32; P < 0.0001). A model of AS intake based on CIR-Ala, CIR-Gly, CIR-Ile, smoking, leisure physical activity, and body weight met the biomarker criterion (R² = 0.37). Biomarker-estimated AS intake was not associated with meat or animal protein intake. Conclusions: Results support serum CIR-AAs in combination with participant characteristics as potential biomarkers of AS intake in US populations, including those with low AS intake.

Nondigestible Carbohydrates Affect Metabolic Health and Gut Microbiota in Overweight Adults after Weight Loss


Significance: Inclusion of resistant starch in the WM diet altered gut microbiota composition positively and resulted in lower fasting glucose compared with the control, with no apparent change in appetite.

Background: The composition of diets consumed following weight loss (WL) can have a significant impact on satiety and metabolic health. Objective: This study was designed to test the effects of including a nondigestible carbohydrate to achieve weight maintenance (WM) following a period of WL. Methods: Nineteen volunteers [11 females and 8 males, aged 20-62 y; BMI (kg/m²): 27-42] consumed a 3-d maintenance diet (15%:30%:55%), followed by a 21-d WL diet (WL; 30%:30%:40%), followed by 2 randomized 10-d WM diets (20%:30%:50% of energy from protein:fat:carbohydrate) containing either resistant starch type 3 (RS-WM; 22 or 26 g/d for females and males, respectively) or no RS (C-WM) in a within-subject crossover design without washout periods. The primary outcome, WM after WL, was analyzed by body weight. Secondary outcomes of fecal microbiota composition and microbial metabolite concentrations and gut hormones were analyzed in fecal samples and blood plasma, respectively. All outcomes were assessed at the end of each dietary period. Results: Body weight was similar after the RS-WM and C-WM diets (90.7 and 90.8 kg, respectively), with no difference in subjectively rated appetite. During the WL diet period plasma ghrelin increased by 36% (P < 0.001), glucose-dependent insulinotropic polypeptide (GIP) decreased by 33% (P < 0.001), and insulin decreased by 46% (P < 0.001), but no significant differences were observed during the RS-WM and C-WM diet periods. Fasting blood glucose was lower after the RS-WM diet (5.59 ± 0.31 mmol/L) than after the C-WM diet (5.75 ± 0.49 mmol/L; P = 0.015; standard error of the difference between the means (SED): 0.09]. Dietary treatments influenced the fecal microbiota composition (R² = 0.054, P = 0.031) but not diversity. Conclusions: The metabolic benefits, for overweight adults, from WL were maintained through a subsequent WM diet with higher total carbohydrate intake. Inclusion of resistant starch in the WM diet altered gut microbiota composition positively and resulted in lower fasting glucose compared with the control, with no apparent change in appetite.

Contrasting Effects of Viscous and Particulate Fibers on Colonic Fermentation In Vitro and In Vivo, and Their Impact on Intestinal Water Studied by MRI in a Randomized Trial


Significance: Both viscous and particulate fibers are equally effective at increasing colonic T1 over the period of 24 hours.

Background: Wheat bran, nopal, and psyllium are examples of particulate, viscous and particulate, and viscous fibers, respectively, with laxative properties yet contrasting fermentability. Objectives: We assessed the fermentability of these fibers in vitro and their effects on intestinal function relevant to laxation in vivo using MRI. Methods: Each fiber was predigested prior to measuring gas production in vitro during 48-h anaerobic incubation with healthy fecal samples. We performed a randomized, 3-way crossover trial in 14 healthy volunteers who ingested 7.5 g fiber twice on the day prior to study initiation and once with the study test meal. Serial MRI scans obtained after fasting and hourly for 4 h following meal ingestion were used to assess small bowel water content (SBWC), colonic volumes, and T1 of the ascending colon (T1AC) as measures of colonic water. Breath samples for hydrogen analysis were obtained while patients were in the fasted state and every 30 min for 4 h following meal ingestion. Results: In vitro, the onset of gas production was significantly delayed with psyllium (mean ± SD: 14 ± 5 h) compared
with wheat bran (6 ± 2 h, P = 0.003) and was associated with a smaller total gas volume (P = 0.01). Prefeeding all 3 fibers for 24 h was associated with an increased fasting T1AC (>75% of values >90th centile of the normal range). There was a further rise during the 4 h after psyllium (0.3 ± 0.3 s P = 0.009), a fall with wheat bran (-0.2 ± 0.2 s; P = 0.02), but no change with nopal (0.0 ± 0.1 s, P = 0.2). SBWC increased for all fibers; nopal stimulated more water than wheat bran (AUC mean (95% CI) difference: 7.1 (0.6, 13.8) L/min, P = 0.03). Breath hydrogen rose significantly after wheat bran and nopal but not after psyllium (P < 0.0001). Conclusion: Both viscous and particulate fibers are equally effective at increasing colonic T1 over a period of 24 h. Mechanisms include water trapping in the small bowel by viscous fibers and delivery of substrates to the colonic microbiota by more fermentable particulate fiber.

Bioactives

Effect of Dietary and Supplemental Lycopene on Cardiovascular Risk Factors: A Systematic Review and Meta-Analysis

Significance: This systematic review and meta-analysis found no significant differences between lycopene intervention and control groups for blood pressure and lipids.

Cardiovascular disease (CVD) is the leading cause of death globally and the presence of ≥1 cardiovascular risk factors elevates total risk. Lycopene, a carotenoid with high antioxidant capacity, may be protective. The aim of this systematic review and meta-analyses is to determine the efficacy of consuming dietary and/or supplemental lycopene on cardiovascular risk factors. Using the PRISMA guidelines, 4 databases were systematically searched from inception: Medline, Cinahl, Proquest, and Scopus. Intervention trials assessing dietary or supplemental lycopene on CVD outcomes were included. The Cochrane Risk-of-Bias tool was used to assess the quality of the included papers. Pooled analysis was conducted using outcomes with available data. Forty-three studies were included. Lycopene interventions were highly variable (supplement with or without food, based as tomato juice/paste/raw product, or combined with olive oil), the dose ranged from 1.44 to 75 mg lycopene/d and was not reported in 11 of 43 included studies. Studies reported conflicting findings for the effect of lycopene on cardiovascular risk factors. This was supported by meta-analyses where there were no significant differences between lycopene intervention and control groups for blood pressure and lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides). This was observed for overall groups and in subgroup analyses for individuals with elevated risk factor concentrations at baseline. Lycopene interventions for cardiovascular risk factors were highly variable across studies in both the dosage provided and the mode of delivery (supplement or food based). As such, there are conflicting findings regarding the efficacy of lycopene to improve cardiovascular risk factors.

Gut Microbiome

The Effect of Routine Probiotics Supplementation on Preterm Newborn Health: A Regression Discontinuity Analysis

Significance: Study findings do not support the routine use of probiotics for improving growth or preventing late-onset sepsis in moderately preterm neonates.

Background: Despite ongoing debate about the health impact of probiotics, rigorous evidence assessing the use of probiotics in routine preterm newborn care is lacking. Objectives: We aimed to estimate the causal effect of routine probiotics supplementation on moderately preterm newborns’ anthropometric development (weight-for-age and height-for-age z scores) and risk of late-onset sepsis. Methods: This study used a regression discontinuity analysis based on hospital guidelines that recommended routine probiotics supplementation for neonates born before 34 completed weeks of gestation. Data for this study came from electronic medical records of a level III neonatal care center in Germany and were collected between 2013 and 2019. Newborns born between 30 to 38 completed weeks of gestation without severe congenital defects were eligible for inclusion. Outcomes were weight-for-age and height-for-age z scores at discharge as well as late-onset sepsis. Results: Study participants included 1734 preterm neonates. The results showed no significant intention-to-treat effect on weight-for-age (effect: -0.033 SD; 95% CI: -0.220, 0.155), length-for-age (-0.133 SD; 95% CI: -0.380, 0.114), or late-onset sepsis probability (-1.175 percentage points; 95% CI: -6.556, 4.205). There was no evidence for significant effects of probiotics for any of the study’s endpoints on those complying with the hospital guidelines (local average treatment effect). Conclusions: Routine treatment of moderately preterm newborns with probiotics is unlikely to improve anthropometric outcomes. Complier-level analysis suggested that this finding was not simply driven by a lack of physician compliance with hospital guidelines but by an overall absence of large health effects from the treatment itself. Moreover, overall sepsis risk was low and did not change significantly as a result of probiotics supplementation. The findings of this study therefore do not support the routine use of probiotics for improving growth or preventing late-onset sepsis in moderately preterm neonates.