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Postprandial glycemic response differed by early-life nutritional exposure in a longitudinal cohort: a single- and multi-biomarker approach

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Abstract

Purpose: Populations malnourished in early life are at increased risk for cardiometabolic diseases. We assessed if improved nutrition predicts cardiometabolic function, as assessed by postprandial biomarker responses.

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Authors' contributions: SH collected laboratory data, conducted statistical analysis, interpreted the data, wrote the paper, and have primary responsibility of final content. NAL designed biomarker data collection procedures, and provided essential materials for laboratory data collection. NAL, MRZ, RM, and KMVK contributed to the interpretation of data, writing, and reviewing of the manuscript. ADS designed the research and guided the overall data analysis, interpretation, and writing of the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of interest: SH, NAL, MRZ, RM, KMVN, and ADS, no conflicts of interest.

Ethics approval: The study was approved by the Institutional Review Board at Emory University and the Ethics Review Committee of INCAP.

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Consent for publication: The publication of this manuscript has been approved by all co-authors, as well as by the responsible authorities at the institute where the work has been carried out.

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Code availability: Statistical codes used to produce the results in the manuscript will be made available upon request, pending approval by the principal investigator.

Methods: Participants had been randomized at the village level to receive one of two nutritional supplements as children. At mean age 44 y (range 37 – 53 y), we obtained plasma samples before and 2h after a mixed-component meal challenge. We assayed biomarkers including lipids, glycemic measurements, and inflammatory cytokines. We compared postprandial biomarker responses among those who received the improved nutrition intervention from conception through to their second birthday (the first 1000 days) to those with other exposure status, including those who received the improved nutrition intervention at other ages, and those who received the less nutritious supplement.

Results: Among 1,027 participants (59.4% female), 22.9% were exposed to improved nutrition in the first 1000 days. Insulin increased the most in response to the meal challenge (over two folds), and non-esterified fatty acids decreased the most (by half). Glucose increased postprandial by 11.4% in the exposed group, compared with 15.7% in the other exposure group ($p < 0.05$), which remained significant after adjusting for confounders (−4.7%; 95% confidence interval: −9.3%, −0.01%). Responses to the prandial challenges for the other biomarkers did not differ by intervention group (all $p > 0.05$).

Conclusion: Early-life exposure to improved nutrition was associated with more favorable postprandial glucose response in this population. We did not observe a difference in overall cardiometabolic responses between the exposure groups.

Keywords

early life; nutritional intervention; cardiometabolic diseases; diabetes; obesity; inflammation

INTRODUCTION

The first 1000 days from conception to the second birthday is a critical window for growth and development [1,2]. During this period of time, balanced maternal diet and optimal infant and young child feeding is essential in supporting growth and developing the psycho-neuro-endocrinological systems [3]. The developmental origins of health and disease (DOHaD) paradigm centers around the lifelong consequences of exposures in early life [4]. An important aspect of DOHaD is identifying mismatch between early-life factors (genetic predisposition, epigenetic modification, and early-life environment) and the environment later in life [5]. The phenomenon of developmental mismatch is observed in populations around the world that are undergoing nutrition transition: a shift in dietary trends, along with changes in activities and body composition, at the population level over long periods of time [6,7]. Parallel to the global nutrition transition is a shift in disease patterns: from the early 1990s till recent years, there has been a 41% decrease in communicable diseases and neonatal disorders worldwide [9]. However, a simultaneous 40% increase in non-communicable diseases was observed, with cardiometabolic diseases being an important subset [9].

Key molecular mechanisms that contribute to the onset and progression of cardiometabolic conditions include inflammation and oxidative stress [10]. Among the many different stress signals that can initiate these biochemical processes is meal consumption, a recurring daily activity that is directly linked to inflammation and oxidative stress [11]. Following the

consumption of a meal containing fat and sugar, acute and transient hyperlipidemia and hyperglycemia are key metabolic perturbations that determine the extent of cardiometabolic insults [11,12]. The impact of postprandial hyperlipidemia and hyperglycemia on inflammation and oxidative stress has been reported to be independent and cumulative [13,14].

The ability to maintain homeostasis, for example in the context of intermittent and recurrent exposure to a high-fat and high-sugar environment, is a measure of overall cardiometabolic health [15]. This ability is referred to as phenotypic flexibility [16]. Both early life factors (e.g., undernutrition) and challenges throughout the life course (e.g., an obesogenic environment) can reduce phenotypic flexibility [15]. The association of early-life nutrition and adulthood cardiometabolic diseases is therefore difficult to ascertain if we focus solely on the overt clinical diagnosis. Pre-clinical cardiometabolic perturbations, such as the cardiometabolic syndrome, may occur well before clinical diagnosis is made [17]. Hence altered or impaired phenotypic flexibility is a more sensitive measure of overall cardiometabolic status than assessments in the fasted state [16]. A meal challenge model that mimics dietary stress is ideal in studying metabolic responses: mixed-component meal challenges can simultaneously test several cardiometabolic pathways, and may provide valuable insights into systemic stress response capacity [18,19].

In Guatemala, a country that is undergoing nutrition transition, we have previously reported (when the participants were approximately 25 years of age) that exposure to improved nutrition in the first 1000 days was associated with reduced fasting glucose concentration [20–22]. We also recently reported divergent cardiometabolic outcomes associated with early-life improved nutrition in the same study population (in their mid-forties), including reduced risk for diabetes but increased risk for obesity [23]. We further investigated this differential association using biomarkers at the fasted state, with a focus on the role of leptin as a mediating factor [24]. We do not, however, have any information on how early-life nutritional improvements may impact phenotypic flexibility. Therefore, using data from this same wave of follow-up [23], we tested the hypothesis that early-life nutritional exposure is associated with meal-induced biomarker responses in a population that is undergoing nutrition transition.

METHODS

Study population

We conducted a follow-up study of the Institute of Nutrition of Central America and Panama (INCAP) Longitudinal Study [25]. Between 1969 and 1977, a total of 2,392 children were randomized at the village level to receive either *atole* (the “improved nutrition” supplement) or *fresco* (the “control” supplement). *Atole* is a dietary supplement in the form of porridge that provides 6.4 g protein, 0.4 g fat, and 90 kcal per 100 mL. *Fresco* is a low-calorie drink (all calories from carbohydrates, 33 kcal per 100 mL) that does not contain protein. The consumption of the supplements was meticulously documented for each participant, and more details were reported elsewhere [25].

In the current analysis, we dichotomized the participants into “exposed group” and “other exposure group”, considering both the *type* and the *timing* of early-life nutritional exposure. We used the first 1000 days as proxy for early life, calculated as 266 days before birth (the average length of pregnancy) till two years after birth. The exposed group included participants who received *atole* during the full first 1000 days. The other exposure group included the rest of the participants, who either received *atole* but not during the entire period of first 1000 days or received *fresco.* In the “other exposure” group, 181 participants were exposed to *atole* in part of the first 1000 days (the “partial exposure” group). On average, the partial exposure group had atole exposure for 499.7 days (range, 12 to 959 days) during the first 1000 days. The partial exposure group was combined with the “no exposure during the first 1000 days” group to improve sample size for subsequent analyses.

The participants were followed up in several subsequent study waves to track the growth and development of the children, as well as human capital-related information in their adolescence and adulthood [26]. Attrition was as follows: at the time of data collection, 369 (15.4%) cohort members have died, 249 (10.4%) emigrated, and 113 (4.7%) were lost to follow-up. Out of the 1,661 eligible members, 134 could not be reached, and 366 declined to participate. Of the remaining participants who provided consent, 16 did not attend the scheduled clinical exam, 6 women were pregnant or lactating at the scheduled time for data collection, 27 did not have fasting plasma samples required for this set of analysis, and an additional 85 did not have postprandial samples. Our final sample size for this analysis is 1,027 (42.9% of the original cohort) (Supplemental Figure 1).

Meal challenge procedure

During the 2015-17 data collection, we administered a standardized, mixed-component meal challenge to test the metabolic stress response among the participants. For each study participant who provided informed consent, a trained phlebotomist drew venous blood after confirming fasting status (an overnight fast of eight hours or longer). Following a safety protocol, we did not give participants the meal challenge if their fasting glucose concentration was >180 mg/dL. For all other participants, we administered the meal challenge in the form of a milk shake, which has macronutrient composition within the physiological range. The 259 mL shake was a mixture of 25 g safflower oil, 52 g sugar (sucrose, manufacturer: Azucar Caña Real), 12 g *Incaparina* powder (a soy- and maize-based protein mixture developed by INCAP), and 170 mL lactose-free skim milk. Each 100 g of the meal challenge contained 164.7 calories (31% from fat), 3.4 g protein, 25.2 g carbohydrate, and 5.7 g fatty acids (fatty acids were calculated as triglycerides, including 3.04 g monounsaturated fatty acids, 0.89 g polyunsaturated fatty acids, and 0.5 g saturated fatty acids), as well as 1.8 mg cholesterol. At the two-hour mark after the meal challenge, the phlebotomist drew venous blood a second time. The ingredients used in the meal were locally sourced readily acceptable to the study population. The nutrient composition was determined based on previous experimental studies (data not published) and on established meal challenges, with the rationale to simultaneously challenge multiple cardiometabolic systems [19].

We collected additional relevant data during this study wave, including anthropometry measurements and cardiometabolic status. Body mass index (BMI) was calculated as weight (kg) divided by height-squared (m^2) [27]. All cardiometabolic conditions and risk factors were defined according to established standards, including overweight, obesity, central obesity, metabolic syndrome (MetS), hyperglycemia, and type 2 diabetes [27–29].

Cardiometabolic biomarkers

We assayed 17 biomarkers that represented four cardiometabolic pathways. Lipids included total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), apolipoprotein A-I and B (apoA-I, apoB), and non-esterified fatty acids (NEFA). Glycemic markers included insulin and glucose. Pro-inflammation markers included high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), leptin, resistin, and monocyte chemoattractant protein 1 (MCP-1). Anti-inflammation markers included IL-10, adiponectin, and soluble TNF receptor II (TNFsR).

Fasting and postprandial glucose concentrations were assayed in Guatemala, before the plasma samples were frozen for storage at −80 °C. The samples were shipped in dry ice to a biomarker core laboratory in the US, where a trained lab personnel thawed the samples at 4 °C in 28 batches for all other assays to be performed (one batch at a time). Samples in each batch included pre- and post-challenge samples from the same individuals, balanced on the location of data collection, village of birth during the INCAP trial, and timing of exposure to the nutritional supplements. We presented the details of laboratory methods for each biomarker in Supplemental Table 1.

Statistical methods

We describe selected sociodemographic information, cardiometabolic conditions, and cardiometabolic risk factors in the study population. We compared these characteristics between the two early-life nutritional exposure groups, using Student's *t*-test, chi-squared test, or Mann-Whitney *U* test, where appropriate.

The main outcome variables are meal-induced biomarker responses. For each biomarker, we designated postprandial relative change as %Δ, calculated as: $\frac{Postprandial\ concentartion - Fasting\ concentration}{Fasting\ concentration} \times 100\%$. To ensure visual comparability of the responses, we then calculated standardized Z-scores for each $\%\ \Delta : \frac{\text{Observed}(\%\ \Delta) - Mean(\%\ \Delta)}{\text{Standard Deviation}(\%\ \Delta)}$.

We first analyzed the postprandial responses using single-biomarker approach. We constructed difference-in-difference (DD) models for each %Δ (comparison of raw values) and corresponding Z-scores (visual comparison). In each DD model, the exposure variable of interest is the interaction term between the type of exposure (*atole* versus *fresco*) and the timing of exposure (in the full first 1000 days versus other). We controlled for birth village (since the randomization was at the village level, this controls for the type of supplementation and undocumented characteristics at the village level), timing of exposure, age, sex, and BMI. In models with lipid responses (TC, TG, HDLc, LDLc, apoA-I, apoB,

and NEFA) as outcomes, we also controlled for lipid-lowering medication use. In models with glycemic responses (insulin and glucose), we also controlled for diabetes medication use. We tested sex-specific stratum heterogeneity by adding an interaction term between the DD exposure variable and sex. We reported significant interactions by sex, but the final models did not include this interaction term. In a sensitivity analysis (n = 659), we repeated the analysis restricted to individuals without obesity or diabetes.

We then used multi-biomarker approaches to analyze overall (global) and domain-specific postprandial responses. Prior to conducting the multivariate analyses, we examined the correlation matrix across all biomarker responses. To extract multivariate information and reduce the data dimensionality in postprandial biomarker responses, we conducted two sets of analysis. Multivariate analysis of variance (MANOVA) was used to test mean differences in postprandial biomarker responses between exposure groups, and linear discriminant analysis (LDA) was used to predict exposure group membership based on collective biomarker responses.

We used MANOVA (base R), in combinations with DD modeling (previously described) to compare differences in %Δ between exposure groups. We conducted both domain-specific (separately for lipid, glycemic, pro-inflammatory, and anti-inflammatory responses) and global (all biomarker responses combined) comparisons. We then conducted LDA (package "MASS") to test whether the global biomarker responses adequately predict group separation by early-life nutritional exposure status [30]. We partitioned the data into two random parts, 80% of the data were used to train the LDA models, and the remaining 20% were used to test the established models. For the first set of LDA model, we obtained one linear discriminant that is a combination of the multivariate data of all postprandial %Δ to maximize between-group differences. We removed IL-6 in this model to improve sample size. For the second set of LDA model, we only retained the glycemic domain (insulin and glucose responses) to calculate the linear discriminant to distinguish the two exposure groups.

We used R version 3.6.1 (R Core Team, Foundation for Statistical Computing, Vienna, Austria) for all our analyses. Statistical significance was set *a priori* at p value < 0.05. All p-values were two-sided.

RESULTS

Description of the study population

One fifth of the population were exposed to *atole* during the full first 1000 days (235, 22.9%), and 60.4% and 59.1% were women in the exposed group and other exposure group, respectively (not statistically significant, or NS). Due to the nature of the study design, those in the exposed group were younger than those in the other exposure group (mean ± standard deviation 42.2 ± 1.6 y and 44.6 ± 4.6 y, respectively, p < 0.001) (Table 1). Cardiometabolic conditions and risk factors were similar between the two groups, with slightly over one third of the participants categorized as obese in both groups (NS). Type 2 diabetes affected 5.1% of the exposed and 7.5% of the other exposure group (NS), whereas over half in each group (53.4% of exposed and 56.2% of other) had metabolic syndrome (NS) (Table 1). The fasting

concentrations of the biomarkers were similar between the two early-life nutritional exposure groups (Table 1). Glucose, resistin, adiponectin, and TNFsR concentrations were significantly lower in the exposed group than in the other exposure group ($p = 0.03$, < 0.001, 0.02, and < 0.001, respectively) (Table 1).

Comparison between exposure groups at the single-biomarker level

Early-life exposure to *atole* was associated with attenuated postprandial glucose response. The exposed group had 4.7% (95%CI: −9.3% to −0.01%) smaller magnitude of postprandial glucose increase than the other exposure group (the %Δ was 11.4 ± 17.3 in exposed group, compared with 15.7 ± 21.2 in the other exposure group, $p < 0.05$). The remaining biomarker responses were non-differential between the two exposure groups (Table 2, Figure 1). The most pronounced differences between the two groups was in glycemic responses and pro- and anti-inflammatory responses, but no clear pattern was observed in terms of inflammatory responses (Figure 1).

Biomarkers that had postprandial increase in both exposure groups: Insulin increased the most after the meal challenge in both exposure groups, and the %Δ was 232.4 ± 163.5 in the exposed group, compared with 237.7 ± 175.5 in the other exposure group (NS). TG and IF-10 increased by approximately 15% in both exposure groups. The %Δ of TG was slightly higher in the exposed group (16.0 ± 19.2) than in the other exposure group (15.3 ± 18.5, NS), whereas the %Δ of IF-10 was slightly lower in the exposed group (14.8 ± 84.3) than in the other exposure group (15.8 ± 116.9). hsCRP increased more in the exposed group (1.6 ± 14.9) than in the other exposure group (0.8 ± 14.3, NS) (Table 2, Figure 1).

Biomarkers that had postprandial decrease in both exposure groups: NEFA decreased the most in both exposure groups, with %Δ of −45.1 ± 31.9 in exposed group and −49.3 ± 29.3 in other exposure group (NS). Postprandial leptin reduction was milder in the exposed group (−13.5 ±31.7) than in the other exposure group (−15.3 ± 26.2, NS). TNFsR also decreased less in the exposed (−8.1 ± 16.9) than in the other exposure group (−9.6 ± 14.2, NS) (Table 2, Figure 1).

Biomarkers that had mixed postprandial changes in two exposure groups: In addition, IL-6 decreased in the exposed group (−1.4 ± 68.8) but increased in the other exposure group (9.7 ± 81.3, NS). MCP-1 increased in the exposed group (1.1 ± 37.1) but decreased in the other exposure group (−3.3 ± 37.5, NS). Adiponectin increased by 11.4% in the exposed group but decreased by 1.4% in the other exposure group (NS) (Table 2, Figure 1).

We did not observe any stratum heterogeneity by sex except for MCP-1 ($p = 0.04$ for the interaction between exposure variable and sex) (Table 2).

The sensitivity analysis that included participants without obesity or diabetes showed similar results for all biomarkers. The difference between the two exposure groups (156 exposed, 503 other exposure) in glucose response was −5.6% (95% CI: −8.8, −2.4%; $p < 0.001$) (Supplemental Table 2).

Comparison between exposure groups at the multi-biomarker level

MANOVA results indicated that the multivariate mean of glycemic biomarker responses differed by early-life nutritional exposure ($p = 0.03$). Apart from a borderline significant difference observed in the lipid response domain ($p = 0.06$), we did not observe any other domain-specific or global difference between the two groups ($p > 0.05$ for all) (Table 3). LDA results confirmed that neither the global multivariate biomarker responses nor responses in the glycemic domain alone predicted exposure group. Figure 2 indicated that the center and spread of the two groups significantly overlapped in both sets of LDA models.

DISCUSSION

Our study is the first of its kind in linking early-life nutrition to long-term cardiometabolic status through a combination of single- and multi-biomarker approaches. Testing biomarker responses to meal challenge can help gauge the phenotypic flexibility of the cardiometabolic system. Cumulative disturbances throughout the life course, similar to the meal challenge in this study, eventually lead to cardiometabolic diseases such as type 2 diabetes and cardiovascular diseases. Therefore, it is important to assess whether and how early-life exposure to improved nutrition can attenuate such disturbances later in life. Regarding our main hypothesis, we did not observe an overall difference in postprandial biomarker responses between those who were exposed to improved nutrition in the first 1000 days and those who had other exposure status. We observed a modest reduction in the glucose response in the exposed group, compared with the other exposure group. This finding was consistent at both the single-biomarker and the multi-biomarker levels.

The one favorable difference that we observed is worth noting – the exposed group differed in the domain of glycemic responses from the other exposure group. At the individual biomarker level, the exposed group had a smaller magnitude of postprandial glucose response. In previous studies within the same population, researchers reported that early-life exposure to improved nutrition was associated with lower fasting glucose concentration [21,23]. We consider it equally, if not more, important that postprandial glucose response was also attenuated among participants with improved nutrition in the first 1000 days. The smaller magnitude in postprandial glucose response indicated improved capacity of the metabolic system to regulate glucose within a tight range. This finding further substantiated the euglycemic effect of having improved early-life nutrition in our study population. Mechanisms that link early-life nutritional exposure with long-term glycemic regulation is not yet fully elucidated. We postulate that potential mechanisms include positive effects of improved nutrition on ontogeny, especially on the development of metabolically active tissues [2,31–33]. Based on results from animal studies, pancreas may be the key metabolic organ in this linkage [34,35].

Overall, participants who were exposed to improved nutrition in the first 1000 days did not differ significantly from those with other exposure status in phenotypic flexibility, as assessed by collective biomarker responses in our study. Chronic undernutrition was prevalent in the study population as children [36]. The improved protein and calorie content provided in the form of *atole* was hypothesized to attenuate the mismatch between undernutrition signals in early life and the increasingly obesogenic environment that they

later encountered. The fact that we did not observe significant improvements in terms of adulthood cardiometabolic health among the exposed group may be a result of multiple factors. First, we know that *atole* improved early growth and development, and the benefits extended to human capital gains and intellectual capacities in early adulthood [2]. The lack of benefit in cardiometabolic health may be due to different pathways that additional protein and energy affect growth and development. Gruszfeld *et al.* reported that high-protein (versus low-protein) infant formula was associated with increased body mass in childhood, perhaps due to increasing availability of long-chain amino acids, which stimulates IGF-1 and thus promotes fat distribution [37]. Higher protein and energy may improve early linear growth, cognitive development, but may not directly benefit the metabolic tissues. In addition, cardiometabolic perturbations are a result of both early-life nutrition and cumulative exposures in subsequent years, with the latter being more indicative of current status. Therefore, it is possible that early protective effects of improved nutrition in the first 1000 days is insufficient to fend off long-term negative impact due to lifestyle factors and obesogenic environment.

Insulin increased two hours after the meal challenge by more than two folds, but the increase did not differ significantly between the two exposure groups. Nevertheless, glucose response was attenuated in the exposed group. This is interesting because insulin is the major glycemic regulating hormone that rapidly respond to postprandial glucose signals [38]. It is possible that in our study population, glycemic regulation was improved by early nutrition through other mechanisms, such as the leptin-mediated glucose-lowering pathways [24,39]. Since glucose concentration is strictly regulated under physiological conditions, it is possible that the cardiometabolic system mobilized other compensatory pathways to improve glycemic regulation [40]. Despite the potential of nutritional improvements in early life to promote the growth and development of endocrine pancreas, its effect on insulin production and secretion may be limited in the long term. On the other hand, our observation may have been limited by the availability of only two data points surrounding the meal challenge. Postprandial insulin response varies greatly and can be influenced by numerous factors [38]. It is possible that insulin reached peak reaction sooner than the two-hour time point, hence helped reduce glucose concentration. But our assessment did not capture the highest level of such response. This postulation warrants further investigation, preferably through trajectory analysis with multiple data points following the meal challenge.

Most lipid responses were modest or null, except a 15% increase in TG and a 50% in NEFA. There were no statistically significant differences the lipid domain between two exposure groups. After a fat-containing meal, such as the meal challenge in this study, postprandial TG usually elevate within an hour, and can remain elevated for 5 to 8 hours [41,42]. Postprandial increase in TG is a predictor of future cardiovascular diseases [43]. In physiological state, NEFA is excepted to fall rapidly after a mix-component meal due to the suppression of fat mobilization by insulin [44]. We indeed observed this sharp decrease of NEFA. In both of the exposure groups, NEFA reduced to approximately half of the fasting concentration two hours after the meal challenge, which adequately reflected the insulin-driven suppression of postprandial release of free fatty acids from adipocyte [44].

Leptin and adiponectin showed interesting patterns in this study. At the fasted state, leptin was lower in the exposed group than in the other exposure group (NS), whereas adiponectin showed the opposite between-group difference (NS). Two hours after the meal challenge, we observed that adiponectin increased more in the exposed than the other exposure group, but the decrease of leptin was abated in the exposed group. Evidence is mounting that, apart from their main functions of regulating feeding behaviors, these two adipokines have important role in inflammation and insulin resistance [45]. Leptin and adiponectin have opposing effects, with leptin being pro-inflammatory and adiponectin being anti-inflammatory [46]. Leptin-to-adiponectin ratio is positively associated with metabolic syndrome and other cardiometabolic disturbances. Therefore, we postulate that early-life exposure to improved nutrition may help reduce leptin-to-adiponectin ratio, both at the fasted state and through dynamic assessment. In addition, leptin has complex biological functions beyond appetite control and pro-inflammation, therefore the attenuation in postprandial leptin response in the exposed group is not necessarily an unfavorable observation. More research is needed to elucidate the mechanisms behind this observation.

We tested the sex-specific stratum heterogeneity in postprandial responses and concluded that the association of early nutrition and adulthood cardiometabolic responses did not differ by sex, except for MCP-1. This is notable because at the fasted state, concentrations of many cardiometabolic biomarkers differ between the two sexes at physiological state [47]. Leptin, for example, is significantly higher in women than in men due to hormonal regulation of the production and secretion of leptin [48]. These findings, if replicable in other populations, may have clinical implications: despite the sex-specific differences of cardiometabolic mechanisms and outcomes, early nutrition-associated long term phenotypic flexibility may be non-differential between the two sexes [49].

We note several limitations of the study. Despite the innovative challenge model, we only had two time points available. We did not observe any pattern in the postprandial responses in pro- and anti-inflammation markers. It is possible that we did not observe the peak action of these biomarkers at exactly two hours after the meal challenge. The carbohydrate component of our meal challenge was sucrose, which induces a lower glucose response (measured as area under the curve) than does glucose [50]. This difference may impact inflammatory responses as well, with sucrose triggering smaller inflammatory responses. There is limited information in the literature to indicate whether there is great inter- and intra-person variability throughout the day regarding postprandial biomarker responses. In our study, we tested the responses in all participants following an overnight fast as an attempt to attenuate inter-person variability. Another limitation is the varying degrees of missingness across the selected biomarkers. Because of limited funds, certain assays were discontinued when interim analysis failed to show significant changes between fasting and postprandial samples within the same individuals. Nevertheless, the samples were processed in randomly grouped batches, and the missingness was batch-based. Therefore, the overall results should be representative of the study population despite the missingness.

The meal challenge we used is novel, and is designed to elicit the response to a mixed meal. There is no generally accepted standard for such a challenge, because each meal challenge is designed to test a specific research hypothesis. For instance, the mixed meal tolerance test

(MMTT) can be used to test pancreatic β-cell function, whereas a high fat diet has been used to test metabolic risks [51, 52]. In addition, we need to pay attention to potential nutrient-nutrient interaction in the study. Despite gaining popularity as a dynamic metabolic challenge model, mixed-component meals are more complex to analyze. For instance, glucose response may be slower after a mixed-component meal than following a standardized oral glucose tolerance test [53]. Nevertheless, a mixed-component meal challenge is likely more representative of how nutrients interact in the context of normal diets. Our multi-biomarker approach also served to investigating cardiometabolic responses collectively. Another potential confounding factor that added to the complexity of the current analysis is the current diets of study participants. We did not control for dietary information in our analysis based on the assumptions of balanced intervention arms in a randomized controlled trial. It is, however, plausible that study participants' recent diets may have an impact on their responses to our meal challenge. This factor needs to be taken into consideration when interpreting the results.

Because of our focus on the metabolic processes and mechanistic investigation, we did not examine the role of economic and sociocultural factors in the association between early-life nutrition and long-term health outcomes. Previous INCAP studies have reported favorable outcomes in human capital and economic productivity in those who were exposed to *atole* in early life [2,54]. Socioeconomic changes inevitably alter lifestyle choices that have health implications, which may have influenced the outcomes we observed in this study [55,56]. We also caution against over-generalization of our findings because this study involved only one test within a single (and relatively homogeneous) population. More studies in other populations are needed to further evaluate or confirm the findings.

This is a longitudinal study with a randomized controlled trial as the starting point, which enables us to provide strong measurements of association between the exposure and the outcomes. We also further distinguished four cardiometabolic pathways, including lipid response, glycemic response, as well as pro- and anti-inflammatory responses [18]. Previously, researchers have suggested that multiple biomarkers should be used in concordance for each chronic disease, and even for different stages of the same disease [16,18]. We therefore tested the stress responses of the whole cardiometabolic system using key biomarkers, instead of focusing on clinically diagnosed disease status. At the multivariate level, we summarized the information of biomarker responses from two mutually compensatory aspects: we first compared between-group differences in the overall responses (MANOVA), then used the multivariate information to predict group membership (LDA).

Our study population were undernourished in childhood and have been undergoing the nutrition transition. Despite the benefits of exposure to *atole* that have been observed in terms of physical growth, cognitive development, and human capital gains, a recent study reported mixed association between early-life nutritional exposure and cardiometabolic risk factors [2,23]. We considered it important to test the DOHaD hypothesis from a biochemical perspective in our study population. We combined single- and multi-biomarker approaches to investigate the phenotypic flexibility associated with early-life nutritional exposure. We observed a modest euglycemic effect following exposure to improved nutrition in the first

1000 days, despite an overall null effect. This study is the first step in population-based research to further discern the key cardiometabolic pathways that may be influenced by early-life environment. Replicability in other populations, especially in populations that are undergoing nutrition transition, will be of value to further test the hypotheses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS, Maternal, Child Undernutrition Study G (2008) Maternal and child undernutrition: consequences for adult health and human capital. Lancet 371 (9609):340–357. doi:10.1016/S0140-6736(07)61692-4 [PubMed: 18206223]
2. Martorell R (2017) Improved nutrition in the first 1000 days and adult human capital and health. Am J Hum Biol 29(2). doi:10.1002/ajhb.22952
3. Adair LS (2014) Long-term consequences of nutrition and growth in early childhood and possible preventive interventions. Nestle Nutr Inst Workshop Ser 78:111–120. doi:10.1159/000354949 [PubMed: 24504211]
4. Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1 (8489):1077–1081. doi:10.1016/s0140-6736(86)91340-1 [PubMed: 2871345]
5. Suzuki K (2018) The developing world of DOHaD. J Dev Orig Health Dis 9 (3):266–269. doi:10.1017/s2040174417000691 [PubMed: 28870276]
6. Popkin BM, Adair LS, Ng SW (2012) Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev 70 (1):3–21. doi:10.1111/j.1753-4887.2011.00456.x [PubMed: 22221213]
7. Popkin BM (2015) Nutrition Transition and the Global Diabetes Epidemic. Curr Diab Rep 15 (9):64. doi:10.1007/s11892-015-0631-4 [PubMed: 26209940]
8. Popkin BM (1998) The nutrition transition and its health implications in lower-income countries. Public Health Nutr 1 (1):5–21 [PubMed: 10555527]
9. Findings from the Global Burden of Disease Study 2017 (2018). Institute for Health Metrics and Evaluation (IHME), Seattle, WA
10. Ndisang JF, Rastogi S (2013) Cardiometabolic diseases and related complications: current status and future perspective. Biomed Res Int 2013:467682. doi:10.1155/2013/467682 [PubMed: 24224165]
11. Klop B, Proctor SD, Mamo JC, Botham KM, Castro Cabezas M (2012) Understanding postprandial inflammation and its relationship to lifestyle behaviour and metabolic diseases. Int J Vasc Med 2012:947417. doi:10.1155/2012/947417 [PubMed: 21961070]
12. Ceriello A, Genovese S (2016) Atherogenicity of postprandial hyperglycemia and lipotoxicity. Rev Endocr Metab Disord 17 (1):111–116. doi:10.1007/s11154-016-9341-8 [PubMed: 26880302]
13. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, Motz E (2002) Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation 106 (10):1211–1218 [PubMed: 12208795]

14. de Vries MA, Klop B, Janssen HW, Njo TL, Westerman EM, Castro Cabezas M (2014) Postprandial inflammation: targeting glucose and lipids. Adv Exp Med Biol 824:161–170. doi:10.1007/978-3-319-07320-0_12 [PubMed: 25038999]
15. Miranda JJ, Barrientos-Gutierrez T, Corvalan C, Hyder AA, Lazo-Porras M, Oni T, Wells JCK (2019) Understanding the rise of cardiometabolic diseases in low- and middle-income countries. Nat Med 25 (11):1667–1679. doi:10.1038/s41591-019-0644-7 [PubMed: 31700182]
16. van Ommen B, van der Greef J, Ordovas JM, Daniel H (2014) Phenotypic flexibility as key factor in the human nutrition and health relationship. Genes Nutr 9 (5):423. doi:10.1007/s12263-014-0423-5 [PubMed: 25106484]
17. Castro JP, El-Atat FA, McFarlane SI, Aneja A, Sowers JR (2003) Cardiometabolic syndrome: pathophysiology and treatment. Curr Hypertens Rep 5 (5):393–401. doi:10.1007/s11906-003-0085-y [PubMed: 12948432]
18. van Ommen B, Keijer J, Heil SG, Kaput J (2009) Challenging homeostasis to define biomarkers for nutrition related health. Mol Nutr Food Res 53 (7):795–804. doi:10.1002/mnfr.200800390 [PubMed: 19517455]
19. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, Holgate ST, Jonsson LS, Latulippe ME, Marcos A, Moreines J, M'Rini C, Muller M, Pawelec G, van Neerven RJ, Watzl B, Zhao J (2013) A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr 109 Suppl 1:S1–34. doi:10.1017/S0007114512005119
20. Popkin BM, Corvalan C, Grummer-Strawn LM (2020) Dynamics of the double burden of malnutrition and the changing nutrition reality. Lancet 395 (10217):65–74. doi:10.1016/S0140-6736(19)32497-3 [PubMed: 31852602]
21. Conlisk AJ, Barnhart HX, Martorell R, Grajeda R, Stein AD (2004) Maternal and child nutritional supplementation are inversely associated with fasting plasma glucose concentration in young Guatemalan adults. J Nutr 134 (4):890–897 [PubMed: 15051843]
22. Stein AD, Wang M, Ramirez-Zea M, Flores R, Grajeda R, Melgar P, Ramakrishnan U, Martorell R (2006) Exposure to a nutrition supplementation intervention in early childhood and risk factors for cardiovascular disease in adulthood: evidence from Guatemala. Am J Epidemiol 164 (12):1160–1170. doi:10.1093/aje/kwj328 [PubMed: 17018700]
23. Ford ND, Behrman JR, Hoddinott JF, Maluccio JA, Martorell R, Ramirez-Zea M, Stein AD (2018) Exposure to improved nutrition from conception to age 2 years and adult cardiometabolic disease risk: a modelling study. Lancet Glob Health 6 (8):e875–e884. doi:10.1016/S2214-109X(18)30231-6 [PubMed: 30012268]
24. He S, Le NA, Ramirez-Zea M, Martorell R, Narayan KMV, Stein AD (2020) Leptin partially mediates the association between early-life nutritional supplementation and long-term glycemic status among women in a Guatemalan longitudinal cohort. Am J Clin Nutr. doi:10.1093/ajcn/nqaa001
25. Martorell R, Habicht JP, Rivera JA (1995) History and design of the INCAP longitudinal study (1969-77) and its follow-up (1988-89). J Nutr 125 (4 Suppl):1027S–1041S
26. Stein AD, Melgar P, Hoddinott J, Martorell R (2008) Cohort Profile: the Institute of Nutrition of Central America and Panama (INCAP) Nutrition Trial Cohort Study. Int J Epidemiol 37 (4):716–720. doi:10.1093/ije/dyn028 [PubMed: 18285366]
27. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 7 (4):284–294 [PubMed: 22715120]
28. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 106 (25):3143–3421 [PubMed: 12485966]
29. American Diabetes Association (2018) 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care 41 (Suppl 1):S13–s27. doi:10.2337/dc18-S002 [PubMed: 29222373]
30. Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth edition. Springer

31. Lopez-Lopez J, Lopez-Jaramillo P, Camacho PA, Gomez-Arbelaez D, Cohen DD (2015) The Link between Fetal Programming, Inflammation, Muscular Strength, and Blood Pressure. Mediat Inflamm 710613. doi: 10.1155/2015/710613.
32. Yan X, Zhu MJ, Dodson MV, Du M (2013) Developmental programming of fetal skeletal muscle and adipose tissue development. J Genomics 1:29–38. doi:10.7150/jgen.3930 [PubMed: 25031653]
33. Symonds ME, Pope M, Sharkey D, Budge H (2012) Adipose tissue and fetal programming. Diabetologia 55 (6):1597–1606. doi:10.1007/s00125-012-2505-5 [PubMed: 22402988]
34. Waterland RA, Garza C (2002) Early postnatal nutrition determines adult pancreatic glucose-responsive insulin secretion and islet gene expression in rats. J Nutr 132 (3):357–364. doi:10.1093/jn/132.3.357 [PubMed: 11880555]
35. Holness MJ, Langdown ML, Sugden MC (2000) Early-life programming of susceptibility to dysregulation of glucose metabolism and the development of Type 2 diabetes mellitus. Biochem J 349 Pt 3(Pt 3):657–65. doi: 10.1042/bj3490657. [PubMed: 10903125]
36. Martorell R (1995) Results and implications of the INCAP follow-up study. J Nutr 125 (4 Suppl):1127S–1138S [PubMed: 7536833]
37. Gruszfeld D, Weber M, Gradowska K, Socha P, Grote V, Xhonneux A, Dain E, Verduci E, Riva E, Closa-Monasterolo R, Escribano J, Koletzko B (2016) Association of early protein intake and pre-peritoneal fat at five years of age: follow-up of a randomized clinical trial. Nutr Metab Cardiovasc Dis 26 (9):824–832. doi:10.1016/j.numecd.2016.04.005 [PubMed: 27212617]
38. Wilcox G (2005) Insulin and insulin resistance. Clin Biochem Rev 26 (2):19–39 [PubMed: 16278749]
39. Meek TH, Morton GJ (2016) The role of leptin in diabetes: metabolic effects. Diabetologia 59 (5):928–932. doi:10.1007/s00125-016-3898-3 [PubMed: 26969486]
40. Scarlett JM, Schwartz MW (2015) Gut-brain mechanisms controlling glucose homeostasis. F1000Prime Rep 7:12. doi:10.12703/p7-12 [PubMed: 25705395]
41. Roche HM, Gibney MJ (1995) Postprandial triacylglycerolaemia--nutritional implications. Prog Lipid Res 34 (3):249–266 [PubMed: 8685241]
42. Langsted A, Freiberg JJ, Nordestgaard BG (2008) Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 118 (20):2047–2056. doi:10.1161/CIRCULATIONAHA.108.804146 [PubMed: 18955664]
43. O'Keefe JH, Bell DS (2007) Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. Am J Cardiol 100 (5):899–904 [PubMed: 17719342]
44. Miles JM, Isley WL, Harris WS (2005) Postprandial free fatty acid metabolism in normal, dyslipidemic and type 2 diabetic subjects: a relationship to hepatic triglyceride metabolism? J Investig Med 53 (1):S106–S106. doi:10.2310/6650.2005.00005.162
45. Kwon H, Pessin JE (2013) Adipokines mediate inflammation and insulin resistance. Front Endocrinol (Lausanne) 4:71. doi:10.3389/fendo.2013.00071 [PubMed: 23781214]
46. Lopez-Jaramillo P, Gomez-Arbelaez D, Lopez-Lopez J, Lopez-Lopez C, Martinez-Ortega J, Gomez-Rodriguez A, Triana-Cubillos S (2014) The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm Mol Biol Clin Investig 18 (1):37–45. doi:10.1515/hmbci-2013-0053
47. Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, Gore MO, Neeland I, Berry JD, Khera A, Rohatgi A, de Lemos JA (2017) Sex-Based Differences in Cardiometabolic Biomarkers. Circulation 135 (6):544–555. doi:10.1161/circulationaha.116.023005 [PubMed: 28153991]
48. Chen GC, Qin LQ, Ye JK (2014) Leptin levels and risk of type 2 diabetes: gender-specific meta-analysis. Obes Rev 15 (2):134–142. doi:10.1111/obr.12088 [PubMed: 24102863]
49. Kautzky-Willer A, Handisurya A (2009) Metabolic diseases and associated complications: sex and gender matter! Eur J Clin Invest 39 (8):631–648 [PubMed: 19496803]
50. Lee BM, Wolver TM (1998) Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread. Enr J Clin Nutr 52(12):924–8.
51. Besser REJ, Shields BM, Casas R, Hattersley AT, Ludvigsson J (2013) Lessons from the Mixed-Meal Tolerance Test. Diabetes Care 36:195–201. [PubMed: 23111058]

52. Van Dijk S, Mensink M, Esser D, Feskens EJM, Müller M, Afman LA (2012). Response to high-fat challenges varying in fat type in subjects with different metabolic risk phenotypes: a randomized trial. PlosOne 7(7):e41388.

53. Westphal S, Leodolter A, Kahl S, Dierkes J, Malfertheiner P, Luley C (2002) Addition of glucose to a fatty meal delays chylomicrons and suppresses VLDL in healthy subjects. Eur J Clin Invest 32 (5):322–327. doi:10.1046/j.1365-2362.2002.00978.x [PubMed: 12027871]

54. Martorell R, Melgar P, Maluccio JA, Stein AD, Rivera JA (2010) The nutrition intervention improved adult human capital and economic productivity. J Nutr 140 (2):411–414. doi:10.3945/jn.109.114504 [PubMed: 20032473]

55. Kemper H, Post G, Twisk J, Van Mechelen W (1999) Lifestyle and obesity in adolescence and young adulthood: results from the Amsterdam Growth And Health Longitudinal Study (AGAHLS). Int J Obes Relat Metab Disord 23 (3):S34–S40 [PubMed: 10368000]

56. McLaren L (2007) Socioeconomic Status and Obesity. Epidemiol Rev 29 (1):29–48. doi:10.1093/epirev/mxm001 [PubMed: 17478442]

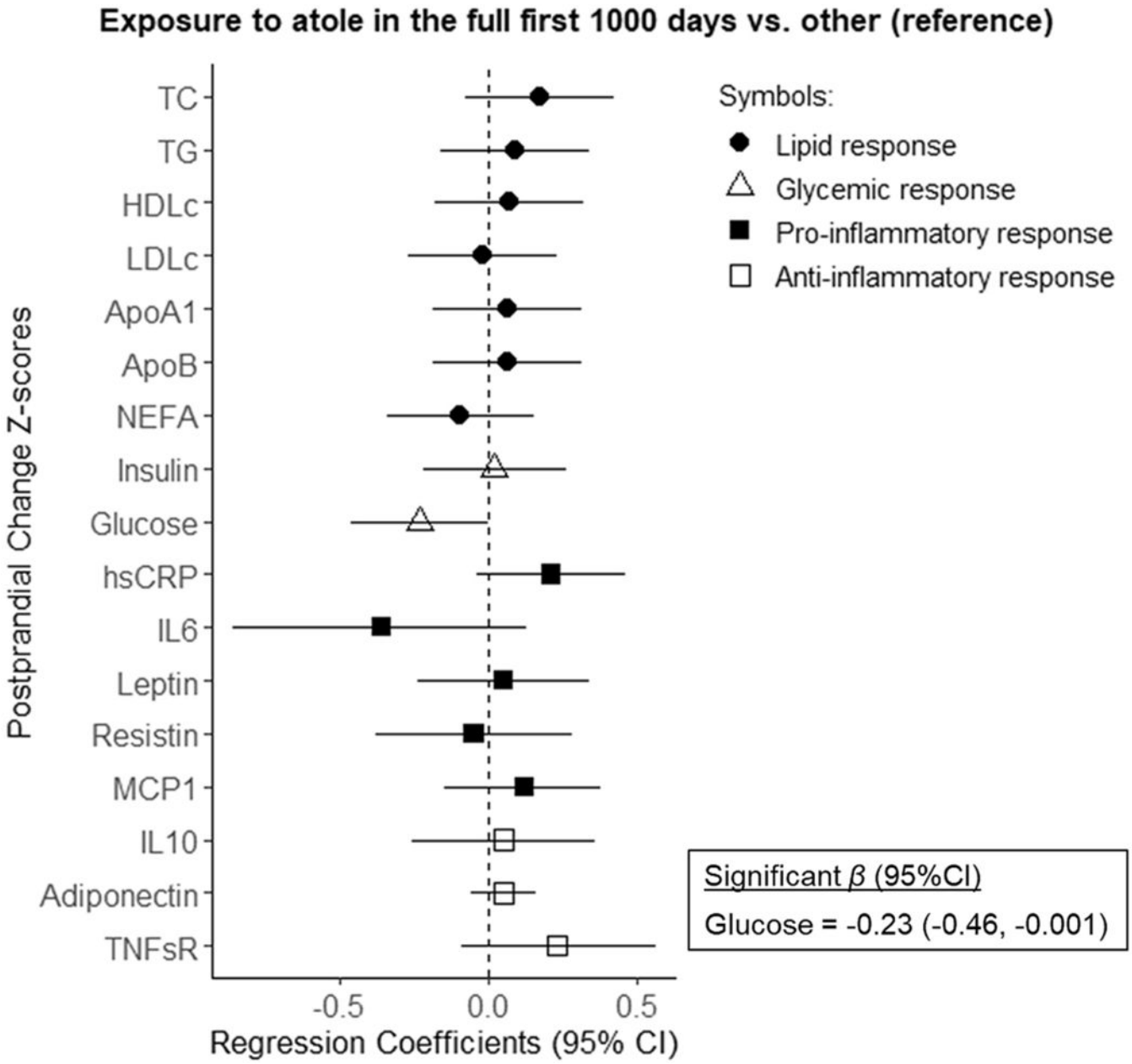

Figure 1. Standardized regression results of postprandial biomarker responses, between participants exposed to *atole* in the full first 1000 days versus other exposure status

1. This figure presents linear regression results to compare postprandial biomarker responses between the participants who were exposed to *atole* in the full first 1000 days (n=235) versus other (n=792). Each regression model had one biomarker response z-score as the dependent variable. The independent (exposure) variable is an interaction term between the type (*atole* or *fresco*) and the timing (full first 1000 days versus other) of exposure, and adjusted for age, body mass index, village of birth (in place of type of exposure), and timing of exposure. We also adjusted for lipid-lowering medication in the lipid models, and adjusted for anti-diabetic medication in the glycemic models.

2. Postprandial change (%Δ) equals the difference between postprandial and fasting biomarker concentrations, divided by fasting concentration. Standardized Z-scores were calculated as %Δ minus the mean and divided by the standard deviation for each biomarker.
3. Figure created using R.
Abbreviations: TC, total cholesterol; TG, triglycerides; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; apo, apolipoprotein; NEFA, non-esterified fatty acid; hsCRP, high sensitivity C-reactive protein; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; TNFsR, soluble TNF receptor II.

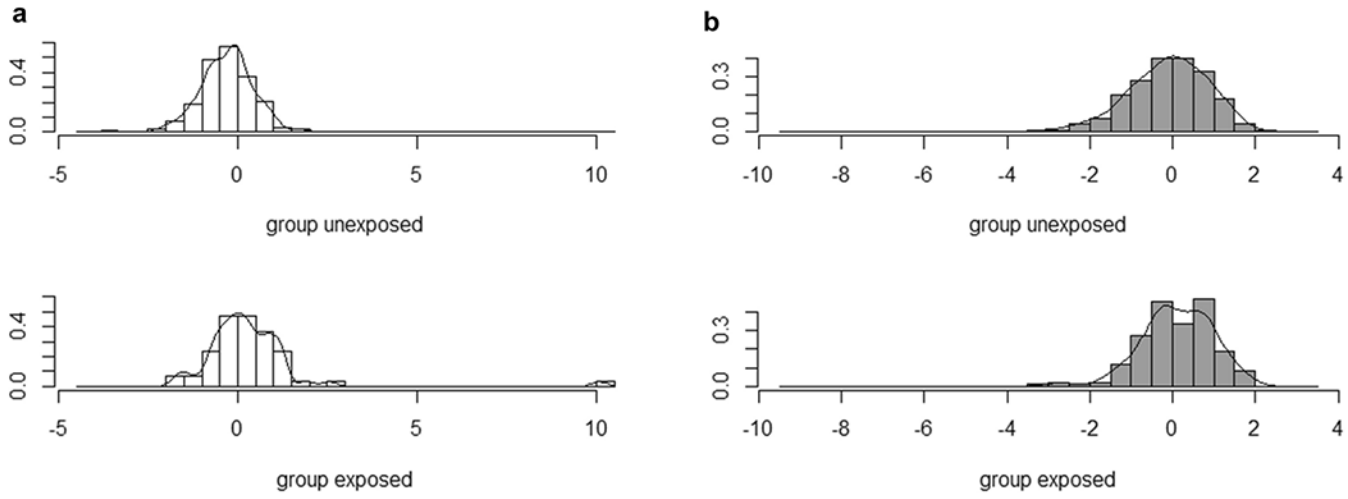

Figure 2. Linear discriminant analysis based on postprandial biomarker responses to predict group membership

1. The graphs were based on linear discriminant analysis to predict group membership by early-life nutrtional exposure status (exposed to *atole* in the full first 1000 days versus other exposure status)

2. The histograms represent linear combination of the multivariate biomarker responses that allow for the greatest separation between groups. The x-axis shows distribution of the linear combination by each group, and the y-axis represents density.

3. Figure 2a) postprandial responses in all markers: coefficients of linear discriminant (LD) for the prediction of early-life nutritional exposure = 0.49*TC − 0.38*TG − 0.11 *HDLc + 0.47*LDLc − 0.85*ApoAl − 0.26*ApoB + 0.23*NEFA + 0.16*Insulin − 0.33*Glucose + 0.10*hsCRP − 0.29*Leptin − 0.08*Resistin + 0.73*MCP1 − 0.18*IL10 + 0.14*Adiponectin + 0.02*TNFsR

4. Figure 2b) glycemic domain (glucose and insulin responses): LD = −0.21 *Insulin − 0.90* Glucose

Table 1:

Selected characteristics of the study population, between participants exposed to *atole* in the full first 1000 days versus other exposure status

Characteristics [1,2]	Exposed to *atole* in the full first 1000 days (n = 235)	Other exposure status (n = 792)	*p* value [3]
Age (years), Mean (SD)	42.2 (1.6)	44.6 (4.6)	< 0.001
Female, %	60.4	59.1	0.77
Body mass index (kg/m^2), Mean (SD)	28.3 (4.6)	28.2 (5.2)	0.76
Obesity, %	31.9	32.6	0.92
Type 2 diabetes, %	5.1	7.5	0.27
Metabolic syndrome, %	53.4	56.2	0.50
Fasting lipids, Median (IQR)			
TC (mmol/L)	4.6 (3.9, 5.3)	4.6 (3.9, 5.2)	0.43
TG (mmol/L)	1.7 (1.2, 2.4)	17 (1.2, 2.4)	0.72
HDLc (mmol/L)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	0.88
LDLc (mmol/L)	2.8 (2.2, 3.5)	2.8 (2.3, 3.4)	0.84
ApoA-I (g/L)	1.1 (0.9, 1.3)	17 (0.9, 1.2)	0.14
ApoB (g/L)	0.8 (0.7, 1.0)	0.8 (0.6, 0.84)	0.17
NEFA (mEq/L)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.44
Fasting glycemic markers, Median (IQR)			
Insulin (pmol/L)	78.5 (49.3, 133.7)	88.2 (54.2, 139.6)	0.09
Glucose (mmol/L)	5.4 (5.0, 5.7)	5.5 (5.2, 5.8)	0.03
Fasting pro-inflammation markers, Median (IQR)			
hsCRP (mg/L)	1.8 (0.9, 3.7)	1.9 (0.9, 4.0)	0.34
IL-6 (pg/mL)	5.5 (2.1, 12.2)	5.1 (2.4, 13.0)	0.99
Leptin (ng/mL)	11.6 (3.2, 19.8)	9.8 (3.3, 18.5)	0.62
Resistin (ng/mL)	1.3 (0.8, 2.1)	17 (1.0, 2.7)	< 0.001
MCP-1 (pg/mL)	82.0 (54.8, 126.3)	80.2 (51.1, 120.6)	0.22
Fasting anti-inflammation markers, Median (IQR)			
IL-10 (pg/mL)	14.2 (4.6, 56.3)	22.6 (6.7, 67.6)	0.10
Adiponectin (μg/mL)	7.9 (4.7, 12.0)	9.0 (4.9, 15.9)	0.02
TNFsR (ng/mL)	2.2 (1.6, 3.0)	2.6 (2.0, 3.3)	<0.001

[1]Definitions: Obesity: BMI ≥ 30 kg/m^2; Metabolic syndrome: having three or more of the following five components: 1) abdominal obesity (waist circumference ≥ 88 cm for women; ≥; 102 cm for men); 2) fasting glucose ≥ 110 mg/dL or diabetic medication use; 3) triglycerides ≥ 150 mg/dL or statin use; 4) HDL-cholesterol < 40 mg/dL in men or < 50 mg/dL in women, and; 5) systolic blood pressure (SBP) > 130 mmHg, diastolic blood pressure (DBP) > 85 mmHg, and/or hypertension medication use. Hyperglycemia: fasting plasma glucose of 100–125 mg/dL or two-hour post-challenge glucose of 140–199 mg/dL among participants not reporting use of diabetes medication. Type 2 diabetes: fasting plasma glucose > 125 mg/dL, or two-hour post-challenge glucose ≥ 200 mg/dL, or reporting use of diabetes medication

[2]Missingness (> 5.0%): 70.1% of IL-6, 36.1% of TNFsR, 25.8% of IL-10, 12.8% of MCP-1, 7.3% of resistin, and 7.2% of adiponectin.

[3]3 P-values based on Student's *t*-test (continuous variables with normal distribution), Mann-Whitney *U* test (continuous variables with skewed distribution), or chi-squared test (categorical variables)

Abbreviations: Apo, apolipoprotein; HDLc, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; IL, interleukin; IQR, interquartile range; LDLc, low density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein 1; NEFA, non-esterified fatty acid; SD, standard deviation; TC, total cholesterol; TG, triglycerides; TNFsR, soluble TNF receptor II.

Table 2.

Comparison of postprandial changes in biomarkers between participants exposed to *atole* in the full first 1000 days versus other exposure status

Postprandial biomarker responses (%Δ) [1,2]	**Exposed to atole in the full first 1000 days (n=235) Mean (SD)**	**Other exposure status (n = 792) Mean (SD)**	**β (95% CI)** [3,4]
Lipid responses			
TC	0.6 (8.7)	0.2 (6.2)	1.2 (−0.6, 2.9)
TG	16.0 (19.2)	15.3 (18.5)	1.7 (−2.9, 6.4)
HDLc	1.7 (6.9)	0.9 (6.5)	0.4 (−1.2, 2.1)
LDLc	−0.1 (9.9)	0.4 (9.2)	−0.2 (−2.6, 2.1)
ApoA-I	1.0 (7.6)	0.6 (7.2)	0.5 (−1.4, 2.3)
ApoB	−0.8 (10.1)	−0.1 (8.7)	0.6 (−1.7, 2.8)
NEFA	−45.1 (31.9)	−49.3 (29.3)	−2.9 (−10.2, 4.4)
Glycemic responses			
Insulin	232.4 (163.5)	237.7 (175.5)	3.6 (−38.4, 45.5)
Glucose	11.4 (17.3)	15.7 (21.2)	−4.7 (−9.3, −0.01) *
Pro-inflammatory responses			
hsCRP	1.6 (14.9)	0.8 (14.3)	3.1 (−0.5, 6.7)
IL-6	−1.4 (68.8)	9.7 (81.3)	−28.7 (−67.7, 10.4)
Leptin	−13.5 (31.7)	−15.3 (26.2)	1.4 (−6.5, 9.2)
Resistin	−4.0 (21.1)	−3.1 (20.9)	−1.1 (−7.9, 5.8)
MCP-1	1.1 (37.1)	−3.3 (37.5)	4.4 (−5.6, 14.4)
Anti-inflammatory responses			
IL-10	14.8 (84.3)	15.8 (116.9)	5.3 (−28.9, 39.5)
Adiponectin	11.4 (149.3)	−1.4 (23.5)	3.6 (−4.1, 11.4)
TNFsR	−8.1 (16.9)	−9.6 (14.2)	3.5 (−1.4, 8.3)

[1] Postprandial change (%Δ) equals the difference between postprandial and fasting biomarker concentrations, divided by fasting concentrations, presented as percentages.

[2] Missingness (> 5.0%): 70.1% of IL-6, 36.1% of TNFsR, 25.8% of IL-10, 12.8% of MCP-1, 7.3% of resistm, and 7.2% of adiponectin.

[3] Exposed to atole in the full first 1000 days vs. other exposure status (reference group). Each model had the %Δ of one biomarker as dependent variable, and the independent variable is an interaction term between the type (*atole* or *fresco*) and the timing (full first 1000 days versus other) of exposure, and adjusted for age, sex, body mass index, type of exposure (used birth village instead of the binary variable), and timing of exposure. We also adjusted for usage of lipid-lowering medication in the lipid response models, and adjusted for usage of anti-diabetic medication in the glycemic response models.

[4] To test stratum heterogeneity by sex, we added the interaction term between sex and the exposure variable (sex * type of exposure * timing of exposure) to the model. Only significant for MCP-1 ($p = 0.04$).

* $p < 0.05$

Abbreviations: TC, total cholesterol; TG, triglycerides; EtDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; apo, apolipoprotein; NEFA, non-esterified fatty acid; hsCRP, high sensitivity C-reactive protein; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; TNFsR, soluble TNF receptor II.

Table 3.

Multivariate analysis of variance (MANOVA) of postprandial biomarker responses, between participants exposed to *atole* in the full first 1000 days versus other exposure status

Type of comparison [1]	Hotelling-Lawley Trace	*F* statistic	*df*	*df* error	*p* value
Domain-specific comparisons					
Lipid responses	0.02	1.93	7	612	0.06
Glycemic responses	0.01	3.49	2	614	0.03
Pro-inflammatory responses	0.05	1.34	5	121	0.25
Anti-inflammatory responses	0.01	1.81	3	458	0.14
Global comparison [2]	0.22	1.38	17	108	0.16

[1] Each MANOVA model had early-life nutritional exposure variable (the product between the type of exposure and timing of exposure), age, sex, type of exposure, timing of exposure, body mass index, and smoking status.

[2] Global comparisons referred to an overall comparison across all four domains (lipid, glycemic, and pro- and anti-inflammatory biomarker responses)