Contrasting Effects of Western vs. Mediterranean Diets on Monocyte Inflammatory Gene Expression and Social Behavior in a Primate Model

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Abstract

Dietary changes associated with industrialization substantially increase the prevalence of chronic diseases, such as obesity, type II diabetes, and cardiovascular disease, which are major contributors to the public health burden. The high prevalence of these chronic diseases is often attributed to an "evolutionary mismatch," between human physiology and modern nutritional environments. In support of this idea, Western diets enriched with foods that were scarce throughout human evolutionary history (e.g., simple sugars and saturated fats) promote inflammation and disease relative to diets more akin to hunter-gatherer diets, such as a Mediterranean diet; however, the mechanisms linking dietary mismatch to inflammation and chronic disease are poorly understood. We used a macaque model and whole diet manipulations to evaluate one possible mechanism – inflammatory polarization of monocytes – that potentially leads to this evolutionary mismatch. After consuming a Western- or Mediterranean-like diet for 15 months, monocytes from Western diet consumers exhibited a more proinflammatory phenotype, with 40% of their genes differentially expressed (FDR<0.05). Compared to the Mediterranean diet, the Western diet shifted the co-expression of 445 gene pairs, including small RNAs and transcription factors associated with metabolism and adiposity in humans, and dramatically altered animal behavior. For example, Western-fed individuals were more anxious and less socially integrated compared to the Mediterranean-fed subjects. These behavioral changes were also associated with some of the effects of diet on gene expression, suggesting an interaction between diet, central nervous system activity, and monocyte gene expression. The results of this study provide new insights into evolutionary mismatch at the molecular level and uncover new pathways through which Western diets generate inflammation and disease.

Introduction

Modern human diets vary across geography, cultures, and socioeconomic strata and have profound impacts on health, survival, and reproduction. The Western diet, prevalent in high income countries (HICs), has been long associated with adverse effects on health, particularly in relation to chronic diseases of aging (Cordain et al., 2005; Drake et al., 2018; Jacka et al., 2010; Manzel et al., 2014; Pontzer et al., 2018; Smil, 1989; Smyth & Heron, 2006). Western diets are high in simple sugars and saturated and n-6 fatty acids, which increase sympathetic nervous activity, oxidative stress, and levels of inflammatory markers (Drescher et al., 2019; Giugliano et al., 2006; Holt et al., 2009; Lopez-Garcia et al., 2004; Nanri et al., 2007; Nettleton et al., 2006). Consequently, Western diets are associated with increased risk for metabolic syndrome (Drake et al., 2018), type II diabetes (Smyth & Heron, 2006), cardiovascular disease (Drake et al., 2018; Smil, 1989), nonalcoholic hepatosteatosis (Jump et al., 2015), autoimmune disorders (Manzel et al., 2014), depression (Jacka et al., 2010), and premature death (Cordain et al., 2005). From an evolutionary perspective, the negative health effects of Western diets are hypothesized to be driven by a "mismatch" between human physiology – which evolved to subsist on a plant-based diet supplemented with fish and meat but no refined products – and the radically different nutritional environment of many human populations today (Eaton et al., 1988; Lieberman, 2014; Stearns & Koella, 2008).

In contrast to the Western diet, the Mediterranean diet derives most protein and fat from vegetable sources, which are enriched with antioxidants, monounsaturated and n-3 fatty acids. This diet more closely resembles that of modern hunter-gatherer populations and presumed ancestral human populations in macronutrient composition and key dietary components

(Mackenbach, 2007; Pontzer et al., 2018). Interestingly, the Mediterranean diet is also associated with an anti-inflammatory phenotype (O'Keefe et al., 2008), reduced incidence of chronic disease, and increased longevity, relative to a Western diet (Farchi et al., 1994; Osler & Schroll, 1997; Romagnolo & Selmin, 2017; Trichopoulou et al., 1995). At face value, the detrimental health effects associated with Western relative to Mediterranean diets are consistent with evolutionary mismatch. However, the mechanisms through which this mismatch may negatively and causally affect health, and conversely how the Mediterranean diet positively impacts health remains poorly understood. Disentangling these mechanisms is especially difficult in humans, as population shifts toward Western diets may be accompanied by other challenges to health such as reduced physical activity or increased total caloric intake (Snodgrass, 2013; Kraft et al., 2018; Lagranja et al., 2015).

In this study, we used a macaque model and whole diet manipulations (Western versus Mediterranean) to investigate one possible mechanism linking diet to chronic disease risk – polarization of immune cell populations toward a proinflammatory state. Previous attempts to understand how Western versus Mediterranean diets impact the immune system have relied on correlational analyses of self-reported diet or short-term dietary interventions in humans, which are limited in their ability to address causality (Stice & Durant, 2014; Suchanek et al., 2011). Many experimental manipulations have focused on single nutrients in animal models (Hu, 2002; Kimmig & Karalis, 2013; Ohlow et al., 2017; Steinhubl, 2008; Whelton et al., 1992), which cannot address the potentially important synergistic effects of the multiple nutrients that make up human diet patterns. Our study design circumvents these challenges to address the role that a major inflammatory cell type - monocytes - plays in sensing and responding to dietary inputs

(Devêvre et al., 2015; Drescher et al., 2019; Holt et al., 2009; Nanri et al., 2007; Nettleton et al., 2006). Monocytes and monocyte-derived macrophages are innate immune cells that vary phenotypically along a spectrum, which ranges broadly from proinflammatory (M1-like) to regulatory/reparative (M2-like). An appropriate balance of these monocyte phenotypes is essential for a healthy immune system. Classically-activated M1 monocytes respond to proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ by becoming macrophages, which propagate the inflammatory response towards infection (Mosser & Edwards, 2008). In contrast, M2 activated monocytes mobilize the tissue repair processes and release anti-inflammatory cytokines in response to IL-4, IL-13, and transforming growth factor (TGF)-β (Mosser & Edwards, 2008). Thus, dietary constituents or patterns may influence pathologic processes by altering the balance between these proinflammatory and anti-inflammatory monocyte subsets – a hypothesis that has yet to be tested (Devêvre et al., 2015).

In addition to diet, psychosocial stress is also known to impact immune phenotypes. In particular, multiple sources of social adversity, such as low social status and poor social integration, have been shown to increase the expression of inflammatory genes in primary white blood cells in humans and other animals (Cole, 2013, 2019; Cole et al., 2015; Snyder-Mackler et al., 2016; Snyder-Mackler & Lea, 2018; Tung & Gilad, 2013). Given that some food constituents can directly alter social behaviors themselves (Hollis et al., 2018; Kaplan et al., 1991; Kasprowska-Liśkiewicz et al., 2017; Kougias et al., 2018; Warden & Fisler, 2008), it is therefore possible that diet effects on immune cell regulation may, to some degree, be mediated through changes in these behaviors. It is also possible that diet-induced alterations in systemic inflammation may alter behavior. However, because no detailed studies of diet, social behavior,

and immune cell phenotypes have been conducted, it remains unclear how these factors are linked and how, together, they impact health. To address these gaps, we conducted a whole-diet manipulation to directly and simultaneously compare the behavioral and physiological effects of Mediterranean and Western diets, formulated to mimic human diet patterns, in a randomized, preclinical, nonhuman primate trial. The randomized trial design allowed us to identify causal effects of realistic, complex diet patterns. Previous reports from this preclinical trial demonstrate that relative to the Mediterranean diet, the Western diet increased body weight, body fat, insulin resistance, and hepatosteatosis (Shively et al., 2019); exacerbated autonomic and hypothalamic-pituitary-adrenal responses to psychosocial stress (Shively et al., 2020); and altered brain neuroanatomy (Frye et al., 2020). Here, we report the effects of the Mediterranean and Western diet patterns on behavior and monocyte gene expression.

Results

Diet intervention

Adult female cynomolgus macaques were fed either a Western-like (hereafter, "Western") or a Mediterranean-like (hereafter, "Mediterranean") diet for 15 months (approximately equivalent to 4 human years). The experimental diets were formulated to model human diet patterns and have been previously described (Shively et al., 2019). Briefly, the Western diet was designed to mimic the diet typically consumed by middle-aged Americans (Centers for Disease Control and Prevention [CDC], 2014), whereas the Mediterranean diet reflected key aspects of the human Mediterranean diet (Kafatos et al., 2000). The experimental diets were matched on macronutrients and cholesterol content but differed in fatty acids. Fats and proteins were mostly plant based in the Mediterranean diet (Kafatos et al., 2000), and from animal sources in the

Western diet. This resulted in high levels of monounsaturated fats in the Mediterranean diet, and saturated fats in the Western diet (Cordain et al., 2005; Kafatos et al., 2000). The Mediterranean diet was higher in complex carbohydrates and fiber, and had a lower n-6:n-3 fatty acid ratio (similar to a traditional hunter-gatherer type diet (Cordain et al., 2005)), and lower sodium and refined sugars than the Western diet. Key Mediterranean ingredients included English walnut powder and extra-virgin olive oil which were the primary components provided to participants in the PREDIMED trial (Estruch et al., 2018). Macronutrient composition of experimental diets compared to monkey chow and human diet patterns can be found in Table 1, Methods.

Diet induced major shifts in monocyte gene expression

RNA sequencing was employed to measure genome-wide gene expression of purified CD14+ monocytes after 15 months on the experimental diets. Diet had a strong effect on monocyte gene expression: the first principal component of gene expression, which explained 59.2% variance, was significantly associated with diet ($t_{(25.1)} = 4.41$, $p = 1.7 \times 10^{-4}$; Fig. 1A). PC1 score was correlated with expression of known proinflammatory genes such as *IL6* (Pearson's r = 0.77, p = 5.4×10^{-8}), *IL1A* (Pearson's r = 0.69, p = 4.3×10^{-6}), *NFKB1* (Pearson's r = 0.61, p = 1.2×10^{-4}), and *NFKB2* (Pearson's r = 0.72, p = 1.3×10^{-6}). Approximately 40% of the 12,240 expressed genes were significantly differentially expressed genes (DEGs) between the two diets (n = 4,900 genes, FDR < 0.05; for all detected genes and the effect size of diet, see Table S1A; for DEGs sorted by the effect size of diet, see Table S1B). The number of diet-responsive genes was roughly balanced between those that were more highly expressed in monkeys fed the Mediterranean diet (n = 2,664; hereafter "Mediterranean genes") and those that were more highly expressed in monkeys fed the Western diet (n = 2,236; hereafter "Western genes"). While

178 balanced in direction, the effect sizes of diet in Western genes were, on average, 1.6-fold larger
179 than in Mediterranean genes (Mann-Whitney $U = 4.1 \times 10^{6}$, $p = 6.1 \times 10^{-117}$; Fig. 1B). Thus, the
180 strongest effects were observed in genes that were either activated by a Western diet or
181 suppressed by a Mediterranean diet.
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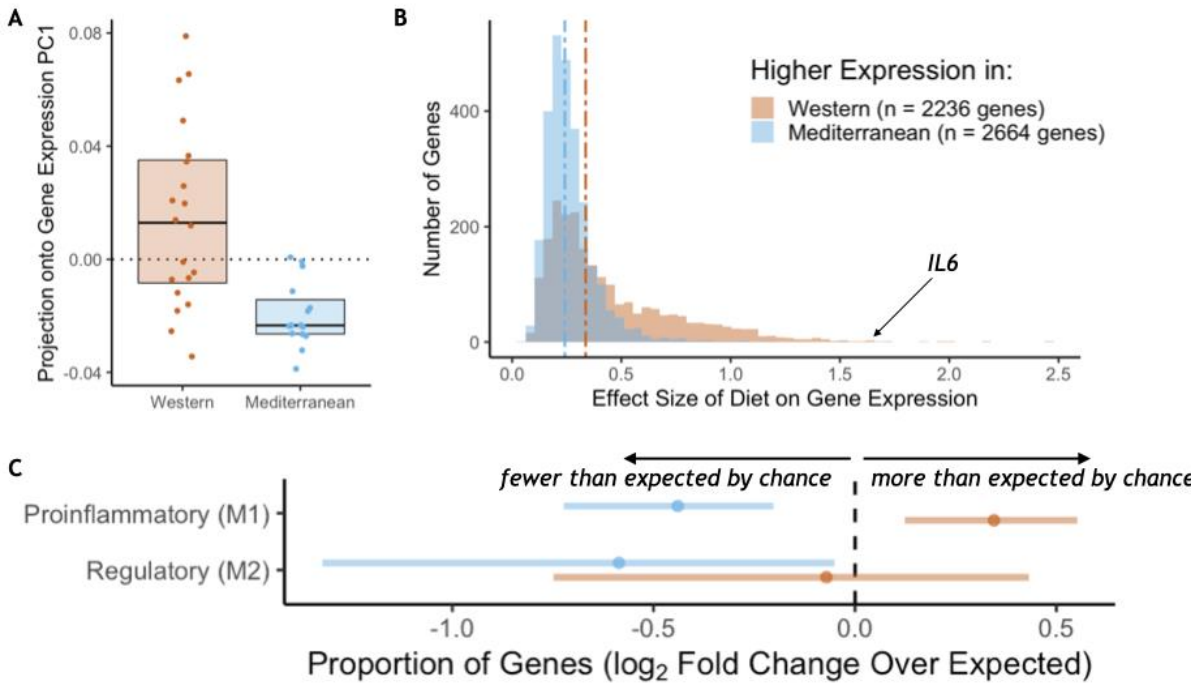

Figure 1. Diet effects on monocyte gene expression. A) Diet was significantly associated with the first principal component of gene expression (59.3% variance explained, $t_{(25.0)} = 4.41$, $p = 1.72 \times 10^{-4}$). **B)** The average effect size of diet on Western genes was 60% stronger than the effect size of diet on Mediterranean genes (Mann-Whitney $U = 4.1 \times 10^{6}$, $p = 6.1 \times 10^{-117}$). **C)** Western genes (orange) contained more M1 genes than expected by chance, indicating that the Western diet induced a shift towards a proinflammatory monocyte phenotype. Western genes were enriched for proinflammatory (M1-like) genes (fold-enrichment = 1.27, *95% CI* = 1.09, 1.46), while Mediterranean genes (blue) were depleted of these same M1-like genes (fold-enrichment = 0.74, *95% CI* = 0.61, 0.88). Regulatory (M2-like) genes were also under-represented in Mediterranean genes (fold-enrichment = 0.67, *95% CI* = 0.40, 0.97), but not in Western genes (fold-enrichment = 0.95, *95% CI* = 0.60, 1.35).

183

184 Monocytes from animals fed the Western diet had higher expression of a number of well-known
185 inflammatory-related genes, including interleukin-6 (β_{diet} = 1.66, FDR = 8.9x10^{-3}; Fig. 1B),
186 interleukin-1α (β_{diet} = 1.22, FDR = 0.033), and two subunits of the NF-κB protein (*NFKB1* β_{diet} =
187 0.30, FDR = 0.017; *NFKB2* β_{diet} = 0.42, FDR = 0.012). Western genes were more likely to be
188 involved in replication and metabolic cellular processes, including response to growth factor
189 (GO:0070848, weighted Fisher's Exact Test (FET) p = 4.6x10^{-3}) and response to insulin
190 (GO:0032868, weighted FET p = 4.0 x10^{-4}; for all GO terms enriched in Western genes, see
191 Table S2A), suggesting that the Western diet also reprogrammed oxidative metabolic aspects of
192 monocyte gene regulation. Conversely, Mediterranean diet monocyte expression patterns were
193 involved in enhanced oxidation-reduction processes (GO:0055114, weighted FET p = 6.0x10^{-3};
194 for all GO terms enriched in Mediterranean genes, see Table S2B), a critical function in keeping
195 proinflammatory monocytes in check.
196
197 We next conducted a more targeted analysis of monocyte polarization by focusing on genes
198 previously shown to be differentially expressed between induced proinflammatory (M1) and
199 regulatory (M2) monocytes (Schmidl et al., 2014) (see Table S1 for polarization categories).
200 Western genes contained more M1-associated genes than expected by chance (n = 162 genes,
201 fold-enrichment = 1.27, *95% CI* = 1.09 - 1.46; Fig. 1C), but not M2-associated genes (n = 24
202 genes, fold-enrichment = 0.95, *95% CI* = 0.60 - 1.35). Conversely, both M1-associated genes (n
203 = 112 genes, fold-enrichment = 0.74, *95% CI* = 0.61 - 0.88) and M2-associated genes (n = 20
204 genes, fold-enrichment = 0.67, *95% CI* = 0.40 - 0.97) were underrepresented among
205 Mediterranean genes.
206

To identify putative upstream gene regulatory mechanisms, we examined whether DEGs were associated with predicted *cis*-regulatory transcription factor binding sites. We identified 34 distinct transcription factor-binding motifs enriched within 2 kilobases of the transcription start sites of Mediterranean genes and one that was enriched near the transcription start sites of Western genes (FDR < 0.05; Fig. 2, for all transcription factor binding motifs enriched in the regulatory regions of either set of diet genes, see Table S3). Diet altered expression of the genes encoding for seven of these 35 transcription factors, including *IRF3*, *IRF8*, *MEF2C*, and *SP1*, which drive monocyte fate and polarization in response to extracellular signals (Chistiakov et al., 2018; Günthner & Anders, 2013; Schuler et al., 2008; Scott et al., 1994; Zhang et al., 1994). Thus, some of the diet-associated changes in monocyte transcriptional profiles may be mediated by changes in the expression and *cis*-regulatory binding of these key transcription factors.

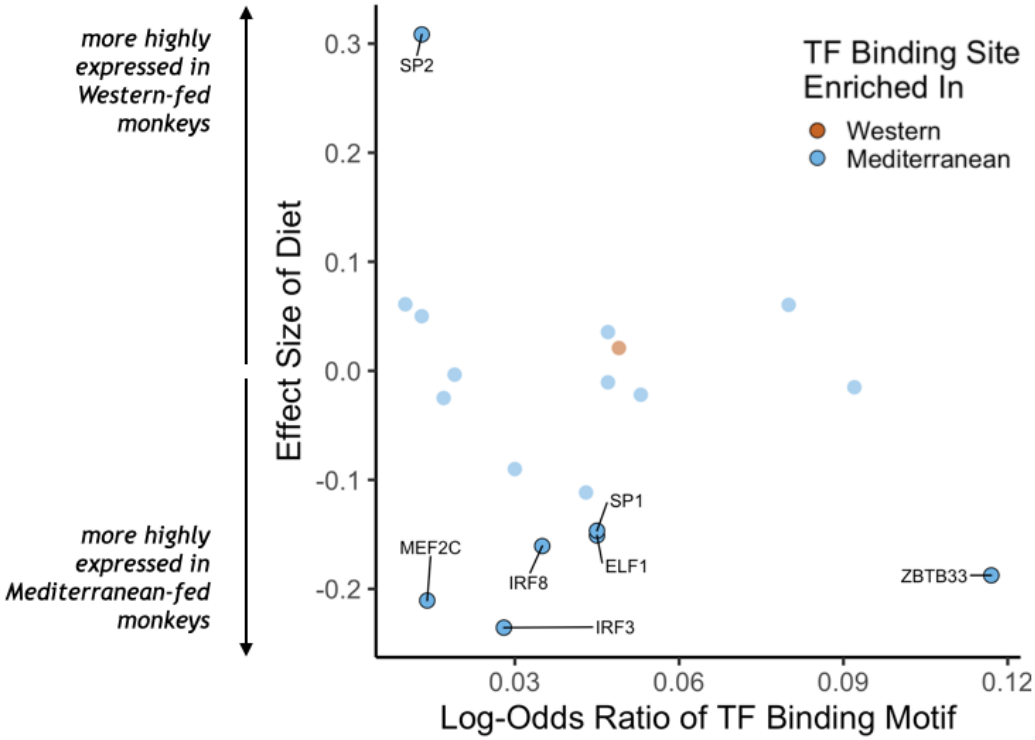

Figure 2. Transcription factor (TF) binding motifs correlated with diet effects on gene expression. The log-odds ratio of TF binding motif enrichment in Western genes (orange) or Mediterranean genes (blue) are depicted on the x-axis. The y-axis shows the effect size of diet on the expression of the gene that encodes for the TF. Only TFs with binding motifs significantly enriched in either gene set and that were detectably expressed in our samples are shown, with those significantly affected by diet (FDR < 0.05) outlined and labeled.

Diet differentially influenced gene co-expression patterns

The effects of diet on the magnitude and direction of pairwise gene expression correlations were assessed for the most strongly diet-affected genes, as such effects could reveal key gene regulatory networks that are altered by diet, that may themselves be regulated by key upstream targets (de la Fuente, 2010; Gaiteri et al., 2014). To reduce the number of tests, we limited our analyses to the pairwise combinations of the top 140 DEGs (n = 9730 combinations). Of these gene pairs, many were significantly associated with each other in both diets, both positively (n = 714) and negatively (n = 332, $p < 0.05$; for all gene pairs tested and their correlations, see Table S4A), suggesting that while diet altered expression of these genes, it did not change their co-expression relationships. Drawing on a newly developed approach, "correlation by individual level product" (CILP) (Lea et al., 2019), we identified 445 other gene pairs that exhibited significant differences (FDR < 0.2) in their correlation between the Mediterranean- and Western-fed monkeys (Table S4A; Fig. 3A), suggesting that one of the experimental diets altered the coherence between the genes (Fig. 3A).

We also identified 16 "hub" genes that exhibited differential correlations with more partner genes than expected by chance (Fig. 3B, for all genes included in one or more differentially correlated gene pairs, see Table S4B). These hub genes were enriched for genes encoding

transcription factors (OR = 7.40, FET $p = 7.0 \times 10^{-3}$), including *SOX4* (essential for normal insulin secretion and glucose tolerance) and *NR4A2* (involved in lipid, carbohydrate, and energy metabolism (Goldsworthy et al., 2008; Pearen & Muscat, 2010)), providing further support for immunological and metabolic reprogramming induced by our diet manipulation. Interestingly, the hub gene involved in the greatest number of differentially-correlated gene pairs was *RF00283, aka SCARNA18,* a non-coding RNA that has been associated with BMI, HDL cholesterol, and aging in human genome-wide association studies (Davis et al., 2017; Dluzen et al., 2018; Kanai et al., 2018; Tachmazidou et al., 2017) (Fig. 3B-D). This small nucleolar RNA is thus a key transcriptional regulator that is altered by diet and has a cascading effect on other genes and pathways.

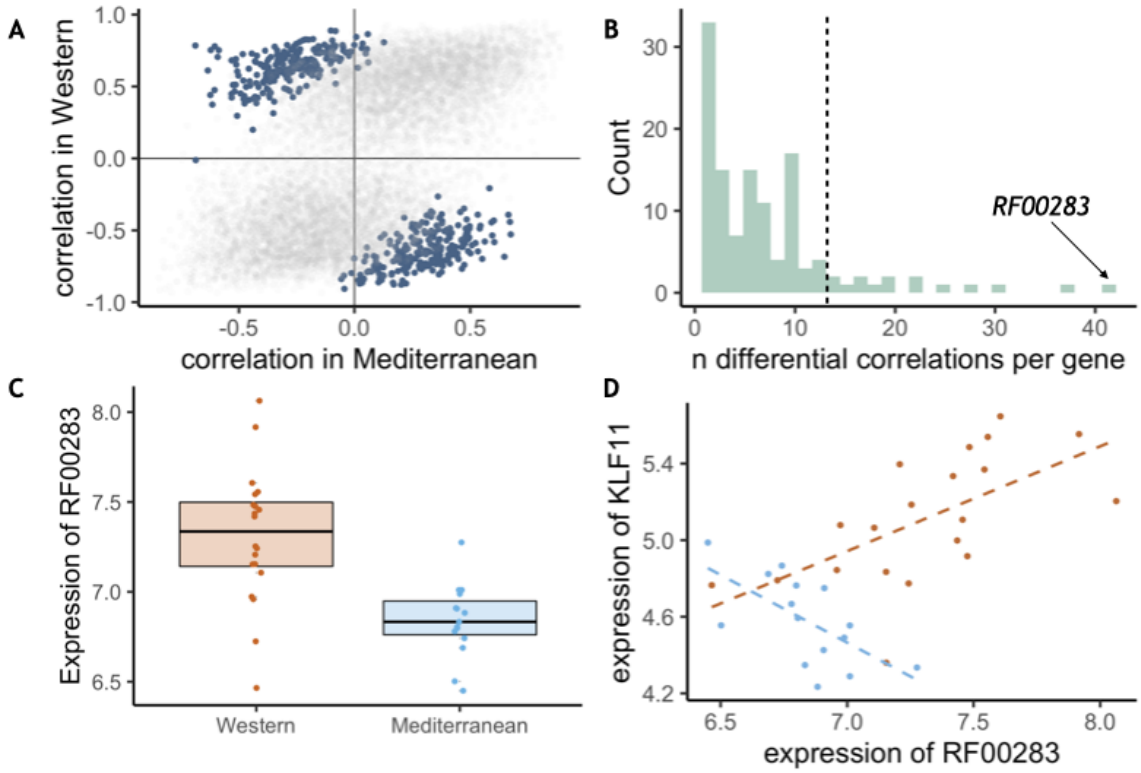

Figure 3. Diet altered monocyte gene co-expression. A) The Pearson correlation between each pair of genes

within each of the experimental diets. Gene pairs that were significantly differently correlated between diets are highlighted in blue (n = 445 significant pairs, FDR < 0.2). **B)** Of the genes involved in significant pairs, some were paired with more genes than expected by chance, called "hub" genes (n = 16 hub genes; dotted black line is the maximum number of significant pairs expected by chance). The strongest hub gene was the non-coding RNA *RF00283*. **C)** Residual normalized expression of *RF00283* is significantly greater in Western- than Mediterranean-fed monkeys (β_{diet} = 0.507, FDR = 2.3 x 10^{-6}). **D)** Example of a differential correlation involving *RF00283*. Residual normalized expression of *RF00283* is plotted against expression of *KLF11*, a differentially-expressed transcription factor that regulates insulin and has been associated with type II diabetes in humans (Neve et al., 2005). The two genes were more highly expressed in Western monocytes, were positively correlated with one another in Western-fed monkeys (Pearson's r = 0.61, p = 4.2 x 10^{-3}), were negatively correlated in Mediterranean-fed monkeys (Pearson's r = -0.63, p = 0.011), and were differentially correlated between the two diets (p = 4.1 x 10^{-5}, FDR = 0.11).

Diet altered social and affective behavior

There were no significant differences in behavior between assigned diet groups during the baseline phase while consuming chow (Fig. S1A, B). However, after 15 months on experimental diets, the two diet groups differed significantly in behavior. The Mediterranean group spent more time in body contact (Mann-Whitney U = 284, Holm-Bonferroni-adjusted p = 1.1 x 10^{-5}) and resting (U = 269, Holm-Bonferroni-adjusted p = 1.6 x 10^{-3}), while those fed the Western diet spent more time alone (U = 255, Holm-Bonferroni-adjusted p = 4.9 x 10^{-3} Fig. 4A; see Fig. S1C, D for behaviors during experimental diet consumption).

Principal component analysis was conducted to identify key behaviors associated with one another (Benito et al., 2018; Seltmann et al., 2018). Behaviors associated with dominance interactions—including aggression, submission, and being groomed—all loaded heavily onto the

first principal component, which explained 32.4% of the overall variance in behavior and did not differ between diets (Welch-Satterthwaite $t_{(30.4)}$= -0.388, p = 0.70; for relationship between dominance rank and PC1, see Fig. S2; for further discussion of social status in these animals, see Note S1; Fig. 6A, B).

The second principal component, which explained 19.2% of the variance in behavior, differed significantly between the two diets ($t_{(26.8)}$= 4.13, p = 3.2 x 10^{-4}; Fig. 4B). No other principal component was significantly correlated with diet, thus PC2 represented a composite of diet-altered behaviors (Fig. 4C; hereafter, DAB). DAB captured socially relevant behaviors. Specifically, percent of time spent in body contact, indicative of social integration, was positively correlated with an individual's PC2 score (Pearson's r = 0.89, Holm-Bonferroni-adjusted p = 1.0 x 10^{-11}; hereafter, DAB score), and higher in Mediterranean-fed animals. Conversely, percent of time spent alone was associated with lower DAB scores (Pearson's r = -0.85, Holm-Bonferroni-adjusted p = 3.0 x 10^{-9}), and was higher in animals fed the Western diet (Fig. 4D). Previous work has validated a behavioral index of anxiety in nonhuman primates (rate of self-grooming and scratching) (Coleman et al., 2011; Maestripieri et al., 1992; Schino et al., 1996; Shively et al., 2015; Troisi et al., 2000; Troisi, 2002), which loaded heavily onto PC2 and is significantly negatively correlated with DAB score (Pearson's r = -0.53, Holm-Bonferroni-adjusted p = 0.019). Thus, PC2 (DAB) captured a measure of social integration associated with consuming a Mediterranean-like diet, and social isolation and anxiety associated with consuming a Western-like diet.

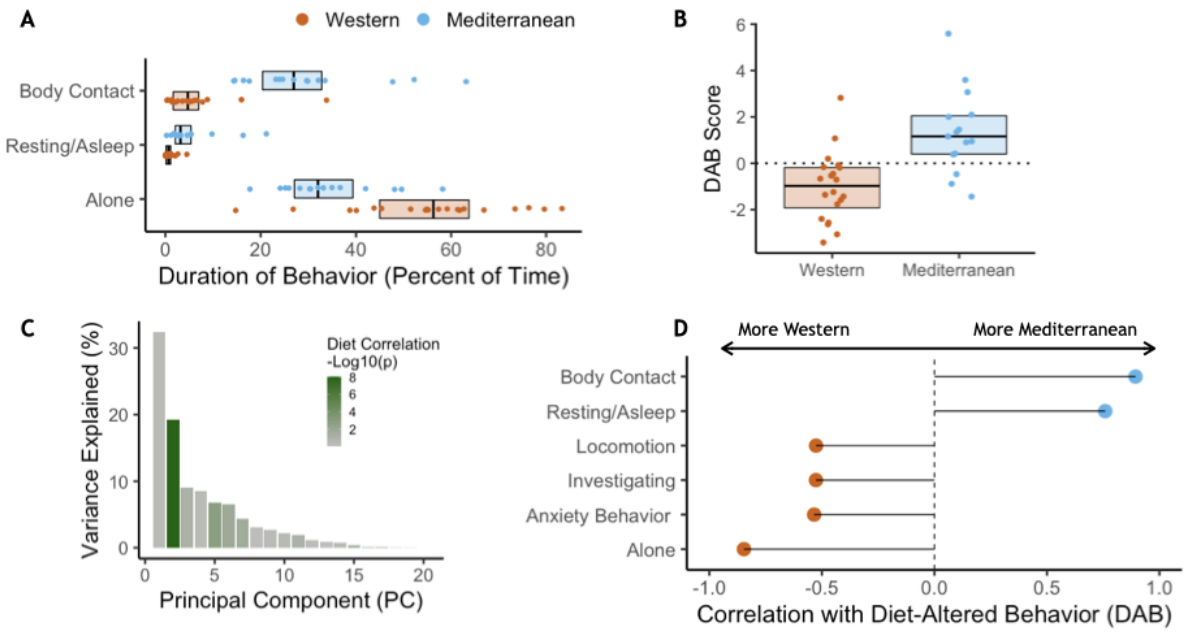

Figure 4. Diet alters behavioral phenotype. A) Three behaviors were significantly different between the two diet groups. Monkeys fed the Mediterranean diet spent more time in body contact (Holm-Bonferroni adjusted p = 1.1 x 10^{-5}) and resting (Holm-Bonferroni adjusted p = 1.6 x 10^{-3}) than Western-fed monkeys. Monkeys eating the Western diet spent more time alone than Mediterranean-fed monkeys (Holm-Bonferroni adjusted p = 4.9 x 10^{-3}). **B)** PC2 represents a composite measure of diet-altered behavior, as individual loadings onto PC2 ("DAB scores"; 19.2% of all variance in behavior) were significantly higher in Mediterranean diet compared to Western diet animals ($t_{(26.8)}$= 4.13, p = 3.2 x 10^{-4}). **C)** Principal component 2 (PC2) explained 19.2% of the variance in behavior and was the only PC significantly correlated with diet. **D)** Six of the 20 behaviors observed are significantly correlated with DAB score (Holm-Bonferroni adjusted $p < 0.05$). Here, significant correlations with DAB score in which behaviors are more frequent in Mediterranean diet or Western diet monkeys are indicated with blue or orange points, respectively.

Diet-altered behaviors and monocyte gene expression as mediators

Given the effects of diet on both behavior and gene expression, we used mediation analyses to explore the potential influences of one on the other. Of the 4,900 DEGs, 29% were also

significantly associated with the DAB score in a univariate model (n = 1,414, FDR < 0.05). Of these, DAB score significantly mediated the effect of diet on the expression of 1199 genes (24% of all DEGs, $p < 0.05$; Fig. 5A). Among these DAB-mediated genes, DAB score mediation accounted for significantly more of the total effect of diet in Western genes (mean = 52.6 %, s.d. = 12.6%), than Mediterranean genes (mean = 45.3 %, s.d. = 10.1%; Mann-Whitney $U = 1.1 \times 10^5$, $p = 6.4 \times 10^{-25}$; Fig. 5B). These DAB-mediated genes were also significantly more likely to be Western genes than Mediterranean genes (n = 712 Western genes, 59%, two-sided binomial test $p = 1.5 \times 10^{-21}$), and were enriched in regulation of inflammatory response (GO:0050727, weighted FET $p = 2.9 \times 10^{-3}$; for all GO terms significantly enriched in DAB-mediated genes, see Tables S5A-C). Together, these observations suggest that the effect of diet on monocyte gene regulation may partially be due to diet-induced changes in key social behaviors.

We also tested the hypothesis that peripheral immune cell gene expression mediated the effects of diet on behavior in the 27% of DEGs for which monocyte gene expression significant predicted DAB in a univariate model (n = 1,324, FDR < 0.05). Gene expression mediated the effect of diet on DAB score in 898 genes (18% of all DEGs, $p < 0.05$; Fig. 5A). Almost all of these genes (99%; 889/898) were in the set of genes for which behavioral changes mediated changes in gene expression. The genes that mediated the effect of diet on DAB score were more likely to be Western genes (n = 523 Western genes, 58%, two-sided binomial test $p = 4.6 \times 10^{-14}$), however the portion of the total effect of diet that was accounted for by gene expression did not vary between Western (mean = 27.1 %, s.d. = 5.2%) and Mediterranean genes (mean = 27.1 %, s.d. = 4.5%; Mann-Whitney $U = 1.0 \times 10^5$, $p = 0.55$; Fig. 5C).

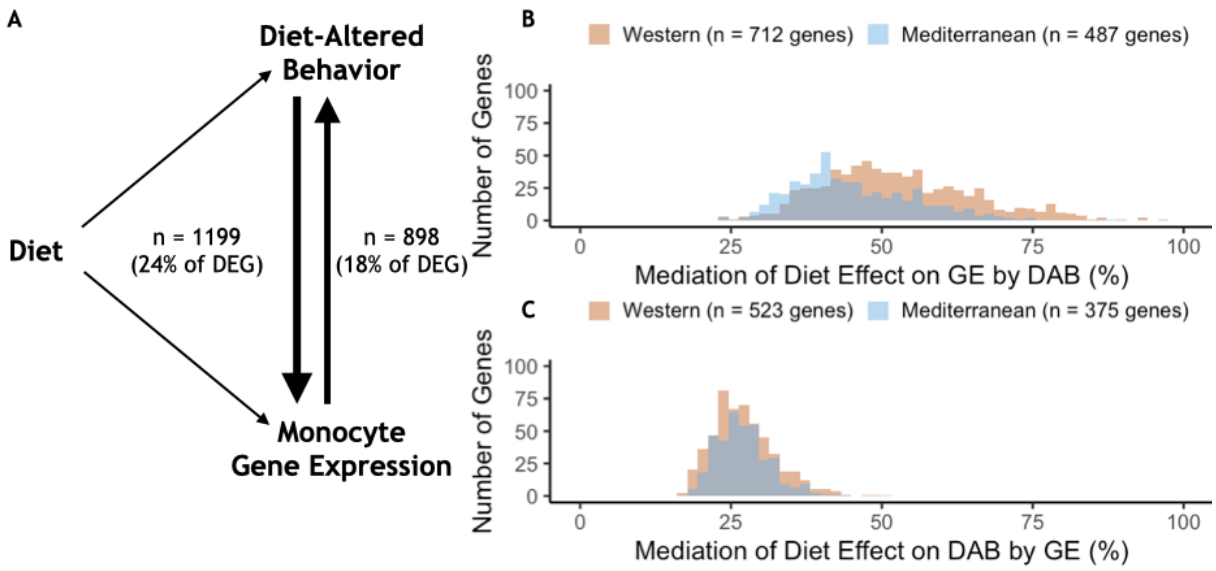

Figure 5. Behavior partially mediates the effect of diet on gene expression for 25% of diet-associated genes. **A)** Diet-altered behavior (DAB) mediated the effect of diet on gene expression for 25% (n = 1220) of genes for which diet had an effect (DEG). For 19% of differentially expressed genes (DEG), gene expression mediated the effect of diet on DAB score. **B)** DAB score mediated 24-97% of the total effect of diet on gene expression in 1220 genes (n = 741 Western genes, orange; n = 479 Mediterranean genes, blue). DAB score mediated a greater number of Western genes than Mediterranean genes ($p = 6.3 \times 10^{-14}$) and accounted for a greater portion of the effect size of diet ($p = 7.5 \times 10^{-23}$) in Western genes. **C)** In gene-by-gene models of DAB score as a function of diet + gene expression, gene expression mediated 15-51% of the total effect of diet on DAB in 940 genes (n = 558 Western genes; n = 382 Mediterranean genes). Gene expression mediated a greater number of Western genes than Mediterranean genes ($p = 1.0 \times 10^{-8}$), although expression of these genes did not account for more of the effect of diet on DAB score than Mediterranean genes (Mann-Whitney $U = 1.1 \times 10^{5}$, $p = 0.75$).

Diet differentially induced expression of the conserved transcriptional response to adversity (CTRA) genes

Additional analyses focused on expression of a well-studied set of social adversity-responsive genes known as the "conserved transcriptional response to adversity" (CTRA) (Cole et al., 2015)

in the Western- and Mediterranean-fed animals in our study. Animals fed a Western diet exhibited significantly higher expression of pro-inflammatory genes included in the CTRA (Mann-Whitney $U = 222$, $p = 0.016$) and lower expression of antiviral- and antibody-related CTRA genes (Mann-Whitney $U = 82$, $p = 0.023$; Fig. S3; for categorization of CTRA genes, see Table S1).

Western diet induced a mosaic response

Western diet induced substantial variation in multiple phenotypes, including body weight, gene expression, and behavior; consistent with previous studies demonstrating that some individuals may be more resistant (or susceptible) to the effects of a Western diet (Shively et al., 2009), presumably due to genetic variation or past environmental exposures. However, we were unable to identify consistencies in individual responsiveness across the phenotypes (Fig. S4A-C). For instance, monkeys that exhibited a strong gene regulatory response to the Western diet did not necessarily exhibit a large increase in body weight or a strong negative DAB score (all $p > 0.2$). Furthermore, change in body weight did not significantly predict gene expression in monocytes (FDR < 0.2). Western diet fed individuals thus exhibited a mosaic response to diet across multiple phenotypes, presumably involving interactions between diet, stress, behavior, environment, microbiome, and genome/epigenome.

Discussion

This study shows, for the first time, that a whole-diet manipulation exerted profound effects on monocyte polarization and social behavior in primates. Forty percent of expressed genes were differentially expressed between monkeys fed Western or Mediterranean diets, indicating that

diet dramatically altered monocyte programming. Relative to monocytes from Mediterranean-fed subjects, monocytes from Western diet consumers exhibited increased expression of proinflammatory and monocyte polarization regulatory genes. Our findings extend previous studies, such as a randomized human cross-over trial that demonstrated changes in monocyte proinflammatory genes in elderly individuals consuming a Mediterranean like diet enriched in olive oil versus a diet more enriched in saturated fat (butter) (Camargo et al., 2012).

We identified a putative molecular mechanism, altered monocyte polarization, that may contribute to the established links between changes in human diets associated with industrialization and increases in chronic disease (Cordain et al., 2005; Drake et al., 2018; Jacka et al., 2010; Lea et al., 2020; Manzel et al., 2014; Pontzer et al., 2018; Smil, 1989; Smyth & Heron, 2006). Comparative studies of human health across different modern populations – namely those consuming traditional hunter-gatherer, forager-horticulturalist, or pastoralist diets versus modern, Western-like diets – lend support for the evolutionary mismatch hypothesis (Eaton et al., 1988; H. Kaplan et al., 2017; Lea et al., 2020; Pontzer et al., 2018). In particular, this work has found that traditional populations have much lower rates of non-communicable diseases, especially cardiometabolic diseases, relative to Western societies; however, because so many lifestyle factors differ between traditional and Western, HICs, it has been difficult to understand the role of diet specifically in driving health variation or to address causality (Snodgrass, 2013; Kraft et al., 2018; Lagranja et al., 2015). Our preclinical randomized study design allows us to draw causal inferences about the role of Western diets in the development of chronic diseases of aging, and provides important data about cellular and molecular mechanisms that may contribute to evolutionary mismatch. These data support future studies to compare the

transcriptional response to diet in our preclinical study with the gene regulatory variation observed between traditional and more market-integrated or Western-like human groups.

Beyond alterations in gene expression, we also identified differences in gene co-expression and enrichment of transcription factor binding motifs, suggesting that diet exerts differential effects on gene regulatory networks. Numerous transcription factors appear to be involved in diet-regulated gene expression. Members of the E26 transformation-specific (ETS), specificity protein (Sp)/Krüppel-like family (KLF), myocyte-specific enhancer factor (MEF), and interferon-regulatory factor (IRF) families of transcription factors, which have all been linked to myeloid differentiation (Chistiakov et al., 2018; Schuler et al., 2008; Scott et al., 1994; Zhang et al., 1994), were overrepresented in regulatory regions of genes with higher expression in the Mediterranean diet group ("Mediterranean genes). Three IRF family transcription factors had binding motifs enriched in Mediterranean genes: IRF-1 and IRF-8 are both linked to M1 monocyte polarization, while IRF-3 is associated with M2 polarization. The sole transcription factor with binding sites enriched in Western diet-associated genes, ATF2, is a key mediator of inflammatory pathways and diseases, including response to bacterial endotoxin, atherosclerosis, and obesity (Fledderus et al., 2007; Miyata et al., 2012; Reimold et al., 2001). Western genes were enriched for activation of the MAPKK pathway, which lies upstream of ATF2 (Herlaar & Brown, 1999), supporting a role in monocyte polarization. Transcription factors were also overrepresented in the pairs of differentially co-expressed genes, further indicating that diet alters regulatory networks and monocyte differentiation and polarization.

It is also worth pointing out that changes in gene co-expression and network connectivity have

been previously proposed as a response to novel or challenging environmental conditions, including Western diets. In particular, work on decanalization has hypothesized that gene regulatory networks evolve over many generations of stabilizing selection, and that novel environmental challenges (such as Western diets and lifestyles) may disrupt these fine-tuned connections leading to dysregulation, a breakdown in co-expression, and ultimately disease (Careau et al., 2014; Gibson, 2009a, 2009b; Hu et al., 2016; Lea et al., 2019). In support of this idea, we found diet-induced changes in the co-expression of transcription factors involved in insulin secretion and glucose tolerance (SOX4), lipid, carbohydrate, and energy metabolism (NR4A2), and BMI, HDL, and aging (*RF00283*) (Davis et al., 2017; Dluzen et al., 2018; Goldsworthy et al., 2008; Kanai et al., 2018; Pearen & Muscat, 2010). We also observed that the transcription factor *MEF2D*, which has previously been implicate in the transcriptomic response to insulin signaling (Samson & Wong, 2002; Solomon et al., 2008), is a hub gene identified in 22 differentially-correlated gene pairs. Hub genes like MEF2D may pinpoint optimized systems that break down as a result of mismatch and are thus intriguing targets for future analyses.

It is worth noting that the dichotomous M1/M2 paradigm of monocyte polarization is an oversimplification of the more complex heterogeneity of monocytes. (Martinez & Gordon, 2014; Nahrendorf & Swirski, 2016) For example, there are at least three classes of monocytes in the circulation–classical, intermediate, and non-classical. We did not assess the relative abundance of these subsets, thus the observed gene expression patterns could reflect either changes in the relative proportions of these subsets and/or shifts in monocyte polarization within subsets (Michalson et al., 2019; Wolf et al., 2017).

The diets also altered key behaviors. Monkeys consuming the Western diet exhibited more behaviors related to anxiety and social isolation, a phenotype remarkably similar to that observed in juvenile Japanese macaques born to mothers consuming a high-fat Western diet (Thompson et al., 2018). In that study, offspring behavior was associated with maternal levels of macrophage-derived chemokine (MDC), which showed higher expression in Western-diet fed animals in our study (β_{diet} = 0.243, FDR = 0.059). Our findings suggest that a Western diet may also exert similar behavioral effects when consumed during adulthood.

There are myriad pathways through which diet may affect behavior. Diet may induce changes in the central nervous system by altering gut microbiota which alters vagal input to the brain (Bonaz et al., 2018). Previous results from our study demonstrated a strong diet effect on the gut microbiome (Nagpal et al., 2018), and lower parasympathetic (vagal) activity in the Western diet group at the time the monocyte transcriptome was assessed (Shively et al., 2020). Taken together these observations suggest that diet-induced changes in vagal tone in the gut-brain axis may be one pathway through which diet impacted brain function, potentially affecting behavior.

Diet-altered behaviors were linked to changes in monocyte gene expression. For a subset (24%) of genes, the DAB score mediated the effect of diet on monocyte gene expression. Monocytes have been shown to be responsive to social isolation (Cole, 2019) and anxiety (Cole et al., 2015). Social isolation and anxiety, produced by Western diet consumption, may be accompanied by increased sympathetic outflow and increased hypothalamic-pituitary adrenal production of cortisol, both of which modulate monocyte intracellular processes governing inflammatory molecule production (Cacioppo et al., 2015; Holwerda et al., 2018; Juruena et al., 2020).

Supporting the involvement of these systems, we previously reported that the Western diet group had increased sympathetic activity, and increased cortisol concentrations (Shively et al., 2020).Western diet may contribute to inflammation by producing a more socially isolated or anxious animal with increased sympathetic and hypothalamic pituitary adrenal activity, which in turn alters monocyte function. Higher expression of genes in the conserved transcriptional response to adversity support this pathway (Cole, 2019; Cole et al., 2015) (Fig. S3). Behavior is a functional assay for the central nervous system. Thus, this observation suggests that diet may alter central nervous system function, which may in turn alter circulating monocyte gene expression.

In a somewhat smaller and overlapping subset of genes (18%), diet-induced differences in monocyte gene expression significantly mediated the effect of diet on behavior (DAB). This observation suggests that diet alters monocyte gene expression, which in turn may affect central nervous system function. There are a variety of mechanisms through which diets differentially influence the nervous system. Western diet may disrupt the blood-brain barrier, increasing infiltration of Western-diet induced cytokines, chemokines, and myeloid cells from the periphery (Raison et al., 2006; Yang et al., 2019). Once in the brain these molecules can alter BDNF production, neurotransmitter systems, and hypothalamic-pituitary-adrenal function (Raison et al., 2006). Western diet induced inflammatory molecules also may affect the brain through direct effects on the afferent vagus nerve (Maier & Watkins, 1998), activation of glial cells (Graham et al., 2016), and alter neuronal membrane lipid composition affecting neurotransmission (Du et al., 2016), whereas a Mediterranean diet may have direct anti-inflammatory actions by increasing n-3 fatty acids in the brain (Layé et al., 2018).

Together, these results support both mediation pathways, suggesting that multiple mechanistic pathways may contribute to these observations; however, we are unable to conclusively state that one mediation pathway is supported over the other. As each gene is modeled independently in the mediation analyses, it is possible that the expression of a subset of genes in monocytes alters central nervous system function and induces behavioral change, while expression of another subset of genes is responsive to behavioral phenotypes and the central nervous system function driving them. These two possibilities present an intriguing possibility for future experiments.

In summary, we found that diet significantly alters monocyte polarization and gene expression, and to a lesser extent behavior. The Western diet promoted a proinflammatory monocyte phenotype relative to a Mediterranean diet, which supports the role of monocyte polarization in diet-associated chronic inflammatory diseases. Thus, altered monocyte programming represents a putative proximate mechanism underlying the evolutionary mismatch between our past and current diets. This suggests that avoiding a Western-style diet and/or consuming a Mediterranean-style diet could be beneficial in preventing or treating chronic inflammation and disease. The majority of the effects of diet are presumably mediated through direct or combined actions of saturated/polyunsaturated fats, n-6:n-3 ratios, pro- and anti-antioxidant characteristics, and other features of the Western diet inconsistent with the nutritional environment in which humans and nonhuman primates evolved. Ongoing and future work will address interactions between social behavior (e.g., social status) and diet to further understand how environmental stressors may impact inflammation in the periphery and in the central nervous system.

Materials and Methods

Subjects

Forty-three adult (age: mean = 9.0, range = 8.2-10.4 years, estimated by dentition), female cynomolgus macaques (*Macaca fascicularis*), were obtained (Shin Nippon Biomedical Laboratories, USA SRC, Alice, TX) and housed at the Wake Forest School of Medicine Primate Center (Winston-Salem, NC) (Shively et al., 2019). Briefly, the monkeys were socially housed in groups of 3-4 and consumed standard monkey chow (Table 1) during an eight-month baseline phase, after which pens were assigned to receive either the Western (5 groups, $n = 21$) or Mediterranean (6 groups, $n = 22$) diet, balanced on pretreatment characteristics that reflected overall health, including body weight, body mass index, and plasma triglyceride concentrations (Shively et al., 2019). Two monkeys did not tolerate the experimental diet, and were switched to standard monkey chow, three animals died during the course of the study, and three samples were removed for insufficient CD14 purification (see "Removal of Batch Effects" below), resulting in a final sample size of 35 animals (Western $n = 20$, Mediterranean $n = 15$). All animal manipulations were performed according to the guidelines of state and federal laws, the US Department of Health and Human Services, and the Animal Care and Use Committee of Wake Forest School of Medicine.

Experimental Diets

Experimental diets (Table 1) were formulated to be isocaloric with respect to protein, fat, and carbohydrates, and identical in cholesterol content (~ 320mg / 2000 kilocalories (Cals)/day) as previously described (Shively et al., 2019).

Table 1. Nutritional Contents of Human and Nonhuman Primate Diets

Diet Composition	Human		Nonhuman Primate		
	Western	Mediterranean	Western	Mediterranean	Chow [#]
% of Calories					
Protein	15[a]	17[b]	16[a]	16[b]	18
Carbohydrate[†]	51[a]	51[b]	54[a]	54[b]	69
Fat	33[a]	32[b]	31[a]	31[b]	13
% of Total Fats					
Saturated	33[a]	21[b]	36[a]	21[b]	26
Monounsaturated	36[a]	56[b]	36[a]	57[b]	28
Polyunsaturated	24[a]	15[b]	26[a]	20[b]	32
Other Nutrients					
ω6:ω3 Fatty Acids	15:1[c]	2.1-3:1[d]	14.8:1[c]	2.9:1[d]	12:01
Cholesterol mg/Cal	0.13[a]	0.16[b]	0.16[a]	0.15[b]	trace
Fiber g/Cal	0.01[a]	0.03[e]	0.02[a]	0.04[e]	0.01
Sodium mg/Cal	1.7[a,f]	1.3[b,e]	1.7[a,f]	1.0[b,e]	0.25

[#] LabDiet Chemical Composition Diet 5037/8. Type of fat known in 86% of total fat. Omega-6 from corn and pork fat.

[†] Human carbohydrate calories include alcohol.

[a] (CDC, 2014)

[b] (Bédard et al., 2012)

[c] (Simopoulos, 2006)

[d] (Cordain et al., 2005)

[e] (Kafatos et al., 2000)

[f] (Powles et al., 2013)

reprinted from Shively et al. *2019* Obesity *with permission* (Shively et al., 2019)

Behavioral Characterization

Behavioral data were collected weekly during two 10-minute focal observations, balanced for time of day, for 6 weeks during the baseline phase (2 hours/monkey total) and for 14 months during the experimental phase (17.7 hours/monkey total). Behaviors recorded included the frequency of aggressive and submissive behaviors, time spent in positive social interactions such as sitting in body contact and grooming or alone, and anxious behavior defined as self-directed

behaviors including self-grooming and scratching (Maestripieri et al., 1992; Schino et al., 1996; Shively et al., 2015; Troisi et al., 2000; Troisi, 2002). Behaviors were collected as previously described (Shively, 1998), and combined into summary behaviors (e.g., "aggression" was a combination of all total, noncontact, contact aggressive events). No significant differences in behavior were observed between the diet groups which consuming the baseline standard monkey chow diet (Fig. S1A, B). In order to quantify the overall impact of diet on behavior, we conducted a principal component analysis using the R package *FactoMineR* (Lê et al., 2008). We corrected for multiple hypothesis tests using the Holm-Bonferroni adjusted p-values.

Blood Sample Collection

The monkeys were trained to run out of their social groups on voice command. Blood was drawn via venipuncture within 9 minutes of entering the building. Blood was collected into EDTA-containing tubes, mixed with an equal amount of PBS without calcium or magnesium, and overlaid on a 90% Ficoll-Paque Plus/10% PBS solution in LeucoSep tubes followed by centrifugation at 800 x g for 20 min. Isolated PBMCs were then immediately used for the collection of CD14+ monocytes by positive selection using a Miltenyi bead-based protocol following manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). After assessing cell viability and numbers, CD14+ monocytes were stored in 85% FBS, 15% DMSO sterile freezing media at -80°C and transferred to liquid nitrogen for storage until RNA extraction.

RNA extraction and sequencing

RNA was extracted from monocytes using the AllPrep DNA/RNA Mini Kit (Qiagen, Inc.,

529 Hilden, Germany), and quantified using a NanoDrop spectrophotometer and Agilent 2100
530 Bioanalyzer with RNA 6000 Nano chips (Agilent Technology, Inc., Santa Clara, CA). RNA
531 libraries were prepared for sequencing by the Cancer Genomics Shared Resource (Wake Forest
532 School of Medicine, Winston-Salem, NC) using the TruSeq-stranded total RNA kit (Illumina),
533 which includes a ribosomal depletion step. The RNA-seq libraries were then sequenced using
534 single-end 76-bp reads on an Illumina NextSeq 500 to an average read depth of 34.5 million
535 reads per sample (range 25.9 – 41.6 million reads). Reads were mapped to the *Macaca*
536 *fascicularis* reference genome (Macaca_fascicularis_5.0, v 93, Ensembl) (Kersey et al., 2018;
537 Kinsella et al., 2011) using HiSat2 (Kim et al., 2015) and then converted to a sample-by-gene
538 read count matrix using featureCounts (Liao et al., 2014) (median = 38.0%; range 24.5 - 50.4%
539 of reads mapped to exons).
540
541 *Read Count Normalization and Removal of Batch Effects*
542 First, we removed genes with low expression (median reads per kilobase per million reads
543 mapped < 1), which resulted in 12,240 genes for downstream analyses. We normalized read
544 counts using the *voom* function of the R package *limma* (Ritchie et al., 2015). While
545 investigating monocyte purity, three samples differed in CD3 gene expression from the rest by
546 several orders of magnitude. We concluded that these samples were contaminated with CD3+
547 cells (i.e., inefficient CD14 purification, see Fig. S5) and excluded them from all analyses,
548 leaving a final sample size of 35 monkeys (n = 20 fed the Western diet, n = 15 Mediterranean
549 diet). To control for batch effects related to RNA quality and monocyte purity, we calculated the
550 residual gene expression from a model of normalized gene expression as a function of CD14
551 expression, CD3 expression, RNA integrity, and RNA concentration. These residual gene

expression values were used for all subsequent analyses.

Modeling Effect of Diet on Gene Expression

In order to determine which genes were significantly affected by diet, we modeled the residual expression of each gene as a function of diet using a linear mixed effects model controlling for relatedness among monkeys using the R package *EMMREML* (Akdemir & Godfrey, 2015). Relatedness was estimated using the ngsRelate program (Hanghøj et al., 2019) with SNP genotypes inferred from the RNA-seq reads using bcftools mpileup (Li et al., 2009). We calculated an empirical false discovery rate (FDR) for each gene using a permutation-based approach (Snyder-Mackler et al., 2016, p.), and report genes that passed at FDR < 0.05. To examine global patterns of variation in gene expression, we conducted principal component analysis on the correlation matrix of normalized residual gene expression using the *prcomp* function in R.

Enrichment analyses

Gene ontology (GO) enrichment analyses were conducted using Fisher's Exact Tests and the *weight01* algorithm to test for enrichment implemented in the R package *topGO* (Alexa & Rahnenfuhrer, 2019). For a more targeted analysis of M1 and M2 specific genes, we identified a set of DEGs in our data set that were previously found to be involved in monocyte polarization (Schmidl et al., 2014) (638 proinflammatory and 138 regulatory), which we used to explore monocyte polarization in the current study. We calculated the proportion of genes more highly expressed in the Mediterranean- and Western-fed animals in each polarization category and tested for significance using a permutation test (n = 100,000 permutations).

Transcription Factor Binding Site Analysis

We tested for enrichment of transcription factor binding motifs within 2 kb (upstream or downstream) of the transcription start sites of differentially expressed "Western genes" or "Mediterranean genes" (FDR < 0.05) using the program HOMER (Heinz et al., 2010) and equivalent regions around the transcription start sites of all genes expressed in these data as the background set for enrichment testing. We searched for known vertebrate transcription factor binding motifs and report the TF motifs passing a threshold of FDR < 0.05.

Gene-gene co-expression analysis

In addition to testing whether diet led to mean differences in gene expression between Western and Mediterranean animals, we also tested whether diet impacted the correlation structure among expressed genes (i.e., gene co-expression). Specifically, we employed 'correlation by individual level product' (CILP) (Lea et al., 2019) analyses to test whether diet affected the magnitude or direction of pairwise gene expression correlations among the top 140 DEGs (n = 9730 gene-gene pairs tested). To test whether a given pair of genes was differentially co-expressed as a function of diet, we first obtained a vector of products for each gene pair by multiplying the normalized gene expression values for two genes together. Normalization was performed by scaling expression values to mean 0 and unit variance within Mediterranean and Western subsets of the data respectively, to ensure that distributional differences between sample groups did not bias our results, following the CILP authors' recommendations (Lea et al., 2019). Each of these vectors of products were used as the outcome variable in a linear mixed effects model implemented in the R package *EMMREML* (Akdemir & Godfrey, 2015), which included a fixed

effect of diet and a random effect to control for genetic relatedness. To assess significance, we extracted the p-value associated with the diet effect for all 9730 gene pairs. We then repeated each linear mixed effects model 100 times after permuting diet, extracted the p-value associated with the diet effect, and used these values to calculate an empirical FDR distribution (Snyder-Mackler et al., 2016).

Using this approach, we identified 445 gene pairs that were significantly differentially co-expressed as a function of diet at a 20% empirical FDR. Next, we performed two follow up analyses to understand their biological import. First, we tested for the existence of 'hub genes', defined as genes that displayed differential co-expression to their tested partner genes more so than expected by chance. To define the null distribution for identifying hub genes, we randomly sampled 445 gene pairs from the set of all 9730 tested gene pairs 1000 times and calculated the number of partners a focal gene had in each sample; we considered a gene to be a significant 'hub gene' if it fell outside the 95th percentile of this distribution, which was equivalent to a focal gene that displayed significant differential co-expression with 13 or more of its tested partner genes. Second, we asked whether the set of 'hub genes' we identified were enriched for transcription factors, relative to the background set of all 140 genes tested for differential co-expression. We performed this analysis because many of the proposed mechanisms to generate large scale changes in gene co-expression patterns involve changes in transcription factor function or activity (de la Fuente, 2010; Gaiteri et al., 2014). To implement the enrichment analysis, we used the TRRUST database of known mammalian transcription factors for annotation (Han et al., 2018) paired with hypergeometric tests.

Mediation analysis

To explore relationships between DAB score and differential gene expression, we conducted mediation analyses using a bootstrapping approach involving 10,000 bootstrap iterations of two models: (Model 1) the expression of each gene as a function of diet, and (Model 2) the expression of each gene as a function of diet and DAB score(Preacher & Hayes, 2004). For each bootstrap iteration, we then calculated the mediation effect (i.e., the indirect effect) of DAB score as the difference between the effect size of diet in Model 1 (β_{diet}) and Model 2 (β'_{diet}). We considered there to be a mediation effect when the 90% confidence interval for the indirect effect (β_{diet}-β'_{diet}) did not include zero.

A similar method was used to calculate the mediation of gene expression on DAB, testing the difference between the effect size of diet in two models: (Model 3) DAB as a function of diet, and (Model 4) DAB as a function of diet and the expression of each gene.

Supplementary Materials

Fig. S1. Diet manipulation altered behavior.

Fig. S2. The first PC of all behavioral data captures dominance rank.

Fig. S3. Expression of genes in the conserved transcriptional response to adversity (CTRA (Cole et al., 2015)) indicate inflammatory effects of a Western diet that parallel the effects of social adversity.

Fig. S4. Greater phenotypic variability in Western diet fed monkeys does not show consistency in individual responsiveness across phenotypes.

Fig. S5. Quality control of cell purity by CD14 and CD3 expression levels: three samples were

excluded due to lower CD14 and high CD3 – possible T cell contamination.

Fig. S6. RNA Integrity was correlated with both uncorrected gene expression and relative rank.

Table S1. Effects of Diet on Gene Expression

Table S2A. Biological Processes Enriched in Western Genes Compared to Other Measured Genes

Table S2B. Biological Processes Enriched in Mediterranean Genes Compared to Other Measured Genes

Table S3. Transcription Factor Binding Site Motif Enrichment

Table S4A. Gene Pair Correlations Across and Within Diet Groups

Table S4B. Differentially Correlated Genes

Table S5A. Biological Processes Enriched in Behavior-Mediated Differentially-Expressed Genes (DEG)

Table S5B. Biological Processes Enriched in Behavior-Mediated Western Genes

Table S5C. Biological Processes Enriched in Behavior-Mediated Mediterranean Genes

Note S1. Regarding rank and RNA integrity (RIN).

References

Akdemir, D., & Godfrey, O. U. (2015). *EMMREML: Fitting Mixed Models with Known Covariance Structures* (R package version 3.1) [Computer software]. https://CRAN.R-project.org/package=EMMREML

Alexa, A., & Rahnenfuhrer, J. (2019). *TopGO: Enrichment Analysis for Gene Ontology.* (R package version 2.36.0) [Computer software].

Bédard, A., Riverin, M., Dodin, S., Corneau, L., & Lemieux, S. (2012). Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. *The British Journal of Nutrition*, *108*(8), 1428–1434. https://doi.org/10.1017/S0007114511006969

Benito, X., Fritz, S. C., Steinitz-Kannan, M., Vélez, M. I., & McGlue, M. M. (2018). Lake regionalization and diatom metacommunity structuring in tropical South America. *Ecology and Evolution*, *8*(16), 7865–7878. https://doi.org/10.1002/ece3.4305

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. *Frontiers in Neuroscience*, *12*. https://doi.org/10.3389/fnins.2018.00049

Cacioppo, J. T., Cacioppo, S., Capitanio, J. P., & Cole, S. W. (2015). The neuroendocrinology of social isolation. *Annual Review of Psychology*, *66*, 733–767. https://doi.org/10.1146/annurev-psych-010814-015240

Camargo, A., Delgado-Lista, J., Garcia-Rios, A., Cruz-Teno, C., Yubero-Serrano, E. M., Perez-Martinez, P., Gutierrez-Mariscal, F. M., Lora-Aguilar, P., Rodriguez-Cantalejo, F., Fuentes-Jimenez, F., Tinahones, F. J., Malagon, M. M., Perez-Jimenez, F., & Lopez-Miranda, J. (2012). Expression of proinflammatory, proatherogenic genes is reduced by the Mediterranean diet in elderly people. *The British Journal of Nutrition*, *108*(3), 500–508. https://doi.org/10.1017/S0007114511005812

Careau, V., Buttemer, W. A., & Buchanan, K. L. (2014). Early-Developmental Stress, Repeatability, and Canalization in a Suite of Physiological and Behavioral Traits in Female Zebra Finches. *Integrative and Comparative Biology*, *54*(4), 539–554. https://doi.org/10.1093/icb/icu095

Chistiakov, D. A., Myasoedova, V. A., Revin, V. V., Orekhov, A. N., & Bobryshev, Y. V. (2018). The impact of interferon-regulatory factors to macrophage differentiation and

polarization into M1 and M2. *Immunobiology*, *223*(1), 101–111. https://doi.org/10.1016/j.imbio.2017.10.005

Cole, S. W. (2013). Social regulation of human gene expression: Mechanisms and implications for public health. *American Journal of Public Health*, *103 Suppl 1*, S84-92. https://doi.org/10.2105/AJPH.2012.301183

Cole, S. W. (2019). The Conserved Transcriptional Response to Adversity. *Current Opinion in Behavioral Sciences*, *28*, 31–37. https://doi.org/10.1016/j.cobeha.2019.01.008

Cole, S. W., Levine, M. E., Arevalo, J. M. G., Ma, J., Weir, D. R., & Crimmins, E. M. (2015). Loneliness, eudaimonia, and the human conserved transcriptional response to adversity. *Psychoneuroendocrinology*, *62*, 11–17. https://doi.org/10.1016/j.psyneuen.2015.07.001

Coleman, K., Robertson, N. D., & Bethea, C. L. (2011). Long-term ovariectomy alters social and anxious behaviors in semi-free ranging Japanese macaques. *Behavioural Brain Research*, *225*(1), 317–327. https://doi.org/10.1016/j.bbr.2011.07.046

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., O'Keefe, J. H., & Brand-Miller, J. (2005). Origins and evolution of the Western diet: Health implications for the 21st century. *The American Journal of Clinical Nutrition*, *81*(2), 341–354. https://doi.org/10.1093/ajcn.81.2.341

Davis, J. P., Huyghe, J. R., Locke, A. E., Jackson, A. U., Sim, X., Stringham, H. M., Teslovich, T. M., Welch, R. P., Fuchsberger, C., Narisu, N., Chines, P. S., Kangas, A. J., Soininen, P., Ala-Korpela, M., Kuusisto, J., Collins, F. S., Laakso, M., Boehnke, M., & Mohlke, K. L. (2017). Common, low-frequency, and rare genetic variants associated with lipoprotein subclasses and triglyceride measures in Finnish men from the METSIM study. *PLOS Genetics*, *13*(10), e1007079. https://doi.org/10.1371/journal.pgen.1007079

de la Fuente, A. (2010). From “differential expression” to ’differential networking’—Identification of dysfunctional regulatory networks in diseases. *Trends in Genetics: TIG*, *26*(7), 326–333. https://doi.org/10.1016/j.tig.2010.05.001

Devêvre, E. F., Renovato-Martins, M., Clément, K., Sautès-Fridman, C., Cremer, I., & Poitou, C. (2015). Profiling of the Three Circulating Monocyte Subpopulations in Human Obesity. *The Journal of Immunology*, *194*(8), 3917–3923. https://doi.org/10.4049/jimmunol.1402655

Dluzen, D. F., Noren Hooten, N., De, S., Wood, W. H., Zhang, Y., Becker, K. G., Zonderman, A. B., Tanaka, T., Ferrucci, L., & Evans, M. K. (2018). Extracellular RNA profiles with human age. *Aging Cell*, *17*(4), e12785. https://doi.org/10.1111/acel.12785

Drake, I., Sonestedt, E., Ericson, U., Wallström, P., & Orho-Melander, M. (2018). A Western dietary pattern is prospectively associated with cardio-metabolic traits and incidence of the metabolic syndrome. *The British Journal of Nutrition*, *119*(10), 1168–1176. https://doi.org/10.1017/S000711451800079X

Drescher, H. K., Weiskirchen, R., Fülöp, A., Hopf, C., de San Román, E. G., Huesgen, P. F., de Bruin, A., Bongiovanni, L., Christ, A., Tolba, R., Trautwein, C., & Kroy, D. C. (2019). The Influence of Different Fat Sources on Steatohepatitis and Fibrosis Development in the Western Diet Mouse Model of Non-alcoholic Steatohepatitis (NASH). *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.00770

Du, J., Zhu, M., Bao, H., Li, B., Dong, Y., Xiao, C., Zhang, G. Y., Henter, I., Rudorfer, M., & Vitiello, B. (2016). The Role of Nutrients in Protecting Mitochondrial Function and Neurotransmitter Signaling: Implications for the Treatment of Depression, PTSD, and

Suicidal Behaviors. *Critical Reviews in Food Science and Nutrition*, *56*(15), 2560–2578. https://doi.org/10.1080/10408398.2013.876960

Eaton, S. B., Konner, M., & Shostak, M. (1988). Stone agers in the fast lane: Chronic degenerative diseases in evolutionary perspective. *The American Journal of Medicine*, *84*(4), 739–749. https://doi.org/10.1016/0002-9343(88)90113-1

Energy Intakes: Percentages of Energy from Protein, Carbohydrate, Fat, and Alcohol, by Gender and Age, What We Eat in America, NHANES 2011-2012 (National Health and Nutrition Examination Survey Data). (2014). U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). https://www.ars.usda.gov/research/project/?accnNo=426312&fy=2014

Estruch, R., Ros, E., Salas-Salvadó, J., Covas, M.-I., Corella, D., Arós, F., Gómez-Gracia, E., Ruiz-Gutiérrez, V., Fiol, M., Lapetra, J., Lamuela-Raventos, R. M., Serra-Majem, L., Pintó, X., Basora, J., Muñoz, M. A., Sorlí, J. V., Martínez, J. A., Fitó, M., Gea, A., … Martínez-González, M. A. (2018). Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *New England Journal of Medicine*, *378*(25), e34. https://doi.org/10.1056/NEJMoa1800389

Farchi, G., Fidanza, F., Mariotti, S., & Menotti, A. (1994). Is diet an independent risk factor for mortality? 20 year mortality in the Italian rural cohorts of the Seven Countries Study. *European Journal of Clinical Nutrition*, *48*(1), 19–29.

Fledderus, J. O., van Thienen, J. V., Boon, R. A., Dekker, R. J., Rohlena, J., Volger, O. L., Bijnens, A.-P. J. J., Daemen, M. J. A. P., Kuiper, J., van Berkel, T. J. C., Pannekoek, H., & Horrevoets, A. J. G. (2007). Prolonged shear stress and KLF2 suppress constitutive

proinflammatory transcription through inhibition of ATF2. *Blood*, *109*(10), 4249–4257. https://doi.org/10.1182/blood-2006-07-036020

Frye, B. M., Craft, S., Register, T. C., Andrews, R. N., Appt, S. E., Vitolins, M. Z., Uberseder, B., Silverstein-Metzler, M. G., Chen, H., Whitlow, C. T., Kim, J., Barcus, R. A., Lockhart, S. N., Hoscheidt, S., Say, B. M., Corbitt, S. E., & Shively, C. A. (2020). Diet, psychosocial stress, and Alzheimer's disease–related neuroanatomy in female nonhuman primates. *Alzheimer's & Dementia*. https://doi.org/10.1002/alz.12232

Gaiteri, C., Ding, Y., French, B., Tseng, G. C., & Sibille, E. (2014). Beyond modules and hubs: The potential of gene coexpression networks for investigating molecular mechanisms of complex brain disorders: Beyond modules and hubs. *Genes, Brain and Behavior*, *13*(1), 13–24. https://doi.org/10.1111/gbb.12106

Gibson, G. (2009a). *It Takes a Genome: How a Clash Between Our Genes and Modern Life Is Making Us Sick*. FT Press Science. http://hdl.handle.net/10822/548498

Gibson, G. (2009b). Decanalization and the origin of complex disease. *Nature Reviews Genetics*, *10*(2), 134–140. https://doi.org/10.1038/nrg2502

Giugliano, D., Ceriello, A., & Esposito, K. (2006). The effects of diet on inflammation: Emphasis on the metabolic syndrome. *Journal of the American College of Cardiology*, *48*(4), 677–685. https://doi.org/10.1016/j.jacc.2006.03.052

Goldsworthy, M., Hugill, A., Freeman, H., Horner, E., Shimomura, K., Bogani, D., Pieles, G., Mijat, V., Arkell, R., Bhattacharya, S., Ashcroft, F. M., & Cox, R. D. (2008). Role of the Transcription Factor Sox4 in Insulin Secretion and Impaired Glucose Tolerance. *Diabetes*, *57*(8), 2234–2244. https://doi.org/10.2337/db07-0337

Graham, L. C., Harder, J. M., Soto, I., de Vries, W. N., John, S. W. M., & Howell, G. R. (2016). Chronic consumption of a western diet induces robust glial activation in aging mice and in a mouse model of Alzheimer's disease. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep21568

Günthner, R., & Anders, H.-J. (2013). Interferon-Regulatory Factors Determine Macrophage Phenotype Polarization. *Mediators of Inflammation*, *2013*, 1–8. https://doi.org/10.1155/2013/731023

Han, H., Cho, J.-W., Lee, S., Yun, A., Kim, H., Bae, D., Yang, S., Kim, C. Y., Lee, M., Kim, E., Lee, S., Kang, B., Jeong, D., Kim, Y., Jeon, H.-N., Jung, H., Nam, S., Chung, M., Kim, J.-H., & Lee, I. (2018). TRRUST v2: An expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Research*, *46*(D1), D380–D386. https://doi.org/10.1093/nar/gkx1013

Hanghøj, K., Moltke, I., Andersen, P. A., Manica, A., & Korneliussen, T. S. (2019). Fast and accurate relatedness estimation from high-throughput sequencing data in the presence of inbreeding. *GigaScience*, *8*(5). https://doi.org/10.1093/gigascience/giz034

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., Cheng, J. X., Murre, C., Singh, H., & Glass, C. K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Molecular Cell*, *38*(4), 576–589. https://doi.org/10.1016/j.molcel.2010.05.004

Herlaar, E., & Brown, Z. (1999). P38 MAPK signalling cascades in inflammatory disease. *Molecular Medicine Today*, *5*(10), 439–447. https://doi.org/10.1016/S1357-4310(99)01544-0

Hollis, F., Mitchell, E. S., Canto, C., Wang, D., & Sandi, C. (2018). Medium chain triglyceride diet reduces anxiety-like behaviors and enhances social competitiveness in rats. *Neuropharmacology*, *138*, 245–256. https://doi.org/10.1016/j.neuropharm.2018.06.017

Holt, E. M., Steffen, L. M., Moran, A., Basu, S., Steinberger, J., Ross, J. A., Hong, C.-P., & Sinaiko, A. R. (2009). Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. *Journal of the American Dietetic Association*, *109*(3), 414–421. https://doi.org/10.1016/j.jada.2008.11.036

Holwerda, S. W., Luehrs, R. E., Gremaud, A. L., Wooldridge, N. A., Stroud, A. K., Fiedorowicz, J. G., Abboud, F. M., & Pierce, G. L. (2018). Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety. *Journal of Neurophysiology*, *120*(1), 11–22. https://doi.org/10.1152/jn.00064.2018

Hu, F. B. (2002). Dietary pattern analysis: A new direction in nutritional epidemiology. *Current Opinion in Lipidology*, *13*(1), 3–9.

Hu, J. X., Thomas, C. E., & Brunak, S. (2016). Network biology concepts in complex disease comorbidities. *Nature Reviews Genetics*, *17*(10), 615–629. https://doi.org/10.1038/nrg.2016.87

Jacka, F. N., Pasco, J. A., Mykletun, A., Williams, L. J., Hodge, A. M., O'Reilly, S. L., Nicholson, G. C., Kotowicz, M. A., & Berk, M. (2010). Association of Western and traditional diets with depression and anxiety in women. *The American Journal of Psychiatry*, *167*(3), 305–311. https://doi.org/10.1176/appi.ajp.2009.09060881

Josh Snodgrass, J. (2013). Health of Indigenous Circumpolar Populations. *Annual Review of Anthropology*, *42*(1), 69–87. https://doi.org/10.1146/annurev-anthro-092412-155517

Jump, D. B., Depner, C. M., Tripathy, S., & Lytle, K. A. (2015). Potential for Dietary ω-3 Fatty Acids to Prevent Nonalcoholic Fatty Liver Disease and Reduce the Risk of Primary Liver Cancer. *Advances in Nutrition*, *6*(6), 694–702. https://doi.org/10.3945/an.115.009423

Juruena, M. F., Eror, F., Cleare, A. J., & Young, A. H. (2020). The Role of Early Life Stress in HPA Axis and Anxiety. *Advances in Experimental Medicine and Biology*, *1191*, 141–153. https://doi.org/10.1007/978-981-32-9705-0_9

Kafatos, A., Verhagen, H., Moschandreas, J., Apostolaki, I., & Van Westerop, J. J. (2000). Mediterranean diet of Crete: Foods and nutrient content. *Journal of the American Dietetic Association*, *100*(12), 1487–1493. https://doi.org/10.1016/s0002-8223(00)00416-8

Kanai, M., Akiyama, M., Takahashi, A., Matoba, N., Momozawa, Y., Ikeda, M., Iwata, N., Ikegawa, S., Hirata, M., Matsuda, K., Kubo, M., Okada, Y., & Kamatani, Y. (2018). Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nature Genetics*, *50*(3), 390–400. https://doi.org/10.1038/s41588-018-0047-6

Kaplan, H., Thompson, R. C., Trumble, B. C., Wann, L. S., Allam, A. H., Beheim, B., Frohlich, B., Sutherland, M. L., Sutherland, J. D., Stieglitz, J., Rodriguez, D. E., Michalik, D. E., Rowan, C. J., Lombardi, G. P., Bedi, R., Garcia, A. R., Min, J. K., Narula, J., Finch, C. E., … Thomas, G. S. (2017). Coronary atherosclerosis in indigenous South American Tsimane: A cross-sectional cohort study. *The Lancet*, *389*(10080), 1730–1739. https://doi.org/10.1016/S0140-6736(17)30752-3

Kaplan, J. R., Manuck, S. B., & Shively, C. (1991). The effects of fat and cholesterol on social behavior in monkeys.: *Psychosomatic Medicine*, *53*(6), 634–642. https://doi.org/10.1097/00006842-199111000-00005

Kasprowska-Liśkiewicz, D., Liśkiewicz, A. D., Nowacka-Chmielewska, M. M., Nowicka, J., Małecki, A., & Barski, J. J. (2017). The ketogenic diet affects the social behavior of young male rats. *Physiology & Behavior*, *179*, 168–177. https://doi.org/10.1016/j.physbeh.2017.06.007

Kersey, P. J., Allen, J. E., Allot, A., Barba, M., Boddu, S., Bolt, B. J., Carvalho-Silva, D., Christensen, M., Davis, P., Grabmueller, C., Kumar, N., Liu, Z., Maurel, T., Moore, B., McDowall, M. D., Maheswari, U., Naamati, G., Newman, V., Ong, C. K., … Yates, A. (2018). Ensembl Genomes 2018: An integrated omics infrastructure for non-vertebrate species. *Nucleic Acids Research*, *46*(D1), D802–D808. https://doi.org/10.1093/nar/gkx1011

Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, *12*(4), 357–360. https://doi.org/10.1038/nmeth.3317

Kimmig, L. M., & Karalis, D. G. (2013). Do Omega-3 Polyunsaturated Fatty Acids Prevent Cardiovascular Disease? A Review of the Randomized Clinical Trials. *Lipid Insights*, *6*, LPI.S10846. https://doi.org/10.4137/LPI.S10846

Kinsella, R. J., Kähäri, A., Haider, S., Zamora, J., Proctor, G., Spudich, G., Almeida-King, J., Staines, D., Derwent, P., Kerhornou, A., Kersey, P., & Flicek, P. (2011). Ensembl BioMarts: A hub for data retrieval across taxonomic space. *Database: The Journal of Biological Databases and Curation*, *2011*, bar030. https://doi.org/10.1093/database/bar030

Kougias, D. G., Cortes, L. R., Moody, L., Rhoads, S., Pan, Y.-X., & Juraska, J. M. (2018). Effects of Perinatal Exposure to Phthalates and a High-Fat Diet on Maternal Behavior

and Pup Development and Social Play. *Endocrinology*, *159*(2), 1088–1105. https://doi.org/10.1210/en.2017-03047

Kraft, T. S., Stieglitz, J., Trumble, B. C., Martin, M., Kaplan, H., & Gurven, M. (2018). Nutrition transition in 2 lowland Bolivian subsistence populations. *The American Journal of Clinical Nutrition*, *108*(6), 1183–1195. https://doi.org/10.1093/ajcn/nqy250

Lagranja, E. S., Phojanakong, P., Navarro, A., & Valeggia, C. R. (2015). Indigenous populations in transition: An evaluation of metabolic syndrome and its associated factors among the Toba of northern Argentina. *Annals of Human Biology*, *42*(1), 84–90. https://doi.org/10.3109/03014460.2014.932008

Layé, S., Nadjar, A., Joffre, C., & Bazinet, R. P. (2018). Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology. *Pharmacological Reviews*, *70*(1), 12–38. https://doi.org/10.1124/pr.117.014092

Lê, S., Josse, J., & Husson, F. (2008). **FactoMineR**: An *R* Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1). https://doi.org/10.18637/jss.v025.i01

Lea, A. J., Martins, D., Kamau, J., Gurven, M., & Ayroles, J. F. (2020). Urbanization and market integration have strong, nonlinear effects on cardiometabolic health in the Turkana. *Science Advances*, *6*(43), eabb1430. https://doi.org/10.1126/sciadv.abb1430

Lea, A., Subramaniam, M., Ko, A., Lehtimäki, T., Raitoharju, E., Kähönen, M., Seppälä, I., Mononen, N., Raitakari, O. T., Ala-Korpela, M., Pajukanta, P., Zaitlen, N., & Ayroles, J. F. (2019). Genetic and environmental perturbations lead to regulatory decoherence. *ELife*, *8*. https://doi.org/10.7554/eLife.40538

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence

Alignment/Map format and SAMtools. *Bioinformatics (Oxford, England)*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics (Oxford, England)*, *30*(7), 923–930. https://doi.org/10.1093/bioinformatics/btt656

Lieberman, D. & Vintage Books (Nowy Jork). (2014). *The story of the human body: Evolution, health and disease*. Vintage Books.

Lopez-Garcia, E., Schulze, M. B., Fung, T. T., Meigs, J. B., Rifai, N., Manson, J. E., & Hu, F. B. (2004). Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *The American Journal of Clinical Nutrition*, *80*(4), 1029–1035. https://doi.org/10.1093/ajcn/80.4.1029

Mackenbach, J. P. (2007). The Mediterranean diet story illustrates that "why" questions are as important as "how" questions in disease explanation. *Journal of Clinical Epidemiology*, *60*(2), 105–109. https://doi.org/10.1016/j.jclinepi.2006.05.001

Maestripieri, D., Schino, G., Aureli, F., & Troisi, A. (1992). A modest proposal: Displacement activities as an indicator of emotions in primates. *Animal Behaviour*, *44*(5), 967–979. https://doi.org/10.1016/S0003-3472(05)80592-5

Maier, S. F., & Watkins, L. R. (1998). Cytokines for psychologists: Implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological Review*, *105*(1), 83–107. https://doi.org/10.1037/0033-295X.105.1.83

Manzel, A., Muller, D. N., Hafler, D. A., Erdman, S. E., Linker, R. A., & Kleinewietfeld, M. (2014). Role of "Western diet" in inflammatory autoimmune diseases. *Current Allergy and Asthma Reports*, *14*(1), 404. https://doi.org/10.1007/s11882-013-0404-6

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: Time for reassessment. *F1000Prime Reports*, *6*. https://doi.org/10.12703/P6-13

Michalson, K. T., Macintyre, A. N., Sempowski, G. D., Bourland, J. D., Howard, T. D., Hawkins, G. A., Dugan, G. O., Cline, J. M., & Register, T. C. (2019). Monocyte Polarization is Altered by Total-Body Irradiation in Male Rhesus Macaques: Implications for Delayed Effects of Acute Radiation Exposure. *Radiation Research*, *192*(2), 121–134. https://doi.org/10.1667/RR15310.1

Miyata, Y., Fukuhara, A., Otsuki, M., & Shimomura, I. (2012). Expression of Activating Transcription Factor 2 in Inflammatory Macrophages in Obese Adipose Tissue. *Obesity*. https://doi.org/10.1038/oby.2012.154

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology*, *8*(12), 958–969. https://doi.org/10.1038/nri2448

Nagpal, R., Shively, C. A., Appt, S. A., Register, T. C., Michalson, K. T., Vitolins, M. Z., & Yadav, H. (2018). Gut Microbiome Composition in Non-human Primates Consuming a Western or Mediterranean Diet. *Frontiers in Nutrition*, *5*, 28. https://doi.org/10.3389/fnut.2018.00028

Nahrendorf, M., & Swirski, F. K. (2016). Abandoning M1/M2 for a Network Model of Macrophage Function. *Circulation Research*, *119*(3), 414–417. https://doi.org/10.1161/CIRCRESAHA.116.309194

Nanri, A., Moore, M. A., & Kono, S. (2007). Impact of C-reactive protein on disease risk and its relation to dietary factors. *Asian Pacific Journal of Cancer Prevention: APJCP*, *8*(2), 167–177.

Nettleton, J. A., Steffen, L. M., Mayer-Davis, E. J., Jenny, N. S., Jiang, R., Herrington, D. M., & Jacobs, D. R. (2006). Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). *The American Journal of Clinical Nutrition*, *83*(6), 1369–1379. https://doi.org/10.1093/ajcn/83.6.1369

Neve, B., Fernandez-Zapico, M. E., Ashkenazi-Katalan, V., Dina, C., Hamid, Y. H., Joly, E., Vaillant, E., Benmezroua, Y., Durand, E., Bakaher, N., Delannoy, V., Vaxillaire, M., Cook, T., Dallinga-Thie, G. M., Jansen, H., Charles, M.-A., Clément, K., Galan, P., Hercberg, S., … Froguel, P. (2005). Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(13), 4807–4812. https://doi.org/10.1073/pnas.0409177102

Ohlow, M. J., Sohre, S., Granold, M., Schreckenberger, M., & Moosmann, B. (2017). Why Have Clinical Trials of Antioxidants to Prevent Neurodegeneration Failed? - A Cellular Investigation of Novel Phenothiazine-Type Antioxidants Reveals Competing Objectives for Pharmaceutical Neuroprotection. *Pharmaceutical Research*, *34*(2), 378–393. https://doi.org/10.1007/s11095-016-2068-0

O'Keefe, J. H., Gheewala, N. M., & O'Keefe, J. O. (2008). Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. *Journal of the American College of Cardiology*, *51*(3), 249–255. https://doi.org/10.1016/j.jacc.2007.10.016

Osler, M., & Schroll, M. (1997). Diet and mortality in a cohort of elderly people in a north European community. *International Journal of Epidemiology*, *26*(1), 155–159. https://doi.org/10.1093/ije/26.1.155

Pearen, M. A., & Muscat, G. E. O. (2010). Minireview: Nuclear Hormone Receptor 4A Signaling: Implications for Metabolic Disease. *Molecular Endocrinology*, *24*(10), 1891–1903. https://doi.org/10.1210/me.2010-0015

Pontzer, H., Wood, B. M., & Raichlen, D. A. (2018). Hunter-gatherers as models in public health: Hunter-gatherer health and lifestyle. *Obesity Reviews*, *19*, 24–35. https://doi.org/10.1111/obr.12785

Powles, J., Fahimi, S., Micha, R., Khatibzadeh, S., Shi, P., Ezzati, M., Engell, R. E., Lim, S. S., Danaei, G., Mozaffarian, D., & on behalf of the Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCoDE). (2013). Global, regional and national sodium intakes in 1990 and 2010: A systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open*, *3*(12), e003733. https://doi.org/10.1136/bmjopen-2013-003733

Preacher, K. J., & Hayes, A. F. (2004). SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behavior Research Methods, Instruments, & Computers: A Journal of the Psychonomic Society, Inc*, *36*(4), 717–731. https://doi.org/10.3758/bf03206553

Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: Inflammation and the pathogenesis of depression. *Trends in Immunology*, *27*(1), 24–31. https://doi.org/10.1016/j.it.2005.11.006

Reimold, A. M., Kim, J., Finberg, R., & Glimcher, L. H. (2001). Decreased immediate inflammatory gene induction in activating transcription factor-2 mutant mice. *International Immunology*, *13*(2), 241–248. https://doi.org/10.1093/intimm/13.2.241

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Romagnolo, D. F., & Selmin, O. I. (2017). Mediterranean Diet and Prevention of Chronic Diseases. *Nutrition Today*, *52*(5), 208–222. https://doi.org/10.1097/NT.0000000000000228

Samson, S., & Wong, N. (2002). Role of Sp1 in insulin regulation of gene expression. *Journal of Molecular Endocrinology*, *29*(3), 265–279. https://doi.org/10.1677/jme.0.0290265

Schino, G., Perretta, G., Taglioni, A. M., Monaco, V., & Troisi, A. (1996). Primate displacement activities as an ethopharmacological model of anxiety. *Anxiety*, *2*(4), 186–191. https://doi.org/10.1002/(SICI)1522-7154(1996)2:4<186::AID-ANXI5>3.0.CO;2-M

Schmidl, C., Renner, K., Peter, K., Eder, R., Lassmann, T., Balwierz, P. J., Itoh, M., Nagao-Sato, S., Kawaji, H., Carninci, P., Suzuki, H., Hayashizaki, Y., Andreesen, R., Hume, D. A., Hoffmann, P., Forrest, A. R. R., Kreutz, M. P., Edinger, M., Rehli, M., & FANTOM consortium. (2014). Transcription and enhancer profiling in human monocyte subsets. *Blood*, *123*(17), e90-99. https://doi.org/10.1182/blood-2013-02-484188

Schuler, A., Schwieger, M., Engelmann, A., Weber, K., Horn, S., Muller, U., Arnold, M. A., Olson, E. N., & Stocking, C. (2008). The MADS transcription factor Mef2c is a pivotal modulator of myeloid cell fate. *Blood*, *111*(9), 4532–4541. https://doi.org/10.1182/blood-2007-10-116343

Scott, E., Simon, M., Anastasi, J., & Singh, H. (1994). Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science*, *265*(5178), 1573–1577. https://doi.org/10.1126/science.8079170

Seltmann, M. W., Helle, S., Adams, M. J., Mar, K. U., & Lahdenperä, M. (2018). Evaluating the personality structure of semi-captive Asian elephants living in their natural habitat. *Royal Society Open Science*, *5*(2), 172026. https://doi.org/10.1098/rsos.172026

Shively, C. A. (1998). Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biological Psychiatry*, *44*(9), 882–891.

Shively, Carol A., Appt, S. E., Chen, H., Day, S. M., Frye, B. M., Shaltout, H. A., Silverstein-Metzler, M. G., Snyder-Mackler, N., Uberseder, B., Vitolins, M. Z., & Register, T. C. (2020). Mediterranean diet, stress resilience, and aging in nonhuman primates. *Neurobiology of Stress*, *13*, 100254. https://doi.org/10.1016/j.ynstr.2020.100254

Shively, Carol A., Appt, S. E., Vitolins, M. Z., Uberseder, B., Michalson, K. T., Silverstein-Metzler, M. G., & Register, T. C. (2019). Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. *Obesity (Silver Spring, Md.)*, *27*(5), 777–784. https://doi.org/10.1002/oby.22436

Shively, Carol A., Register, T. C., Appt, S. E., & Clarkson, T. B. (2015). Effects of long-term sertraline treatment and depression on coronary artery atherosclerosis in premenopausal female primates. *Psychosomatic Medicine*, *77*(3), 267–278. https://doi.org/10.1097/PSY.0000000000000163

Shively, Carol A., Register, T. C., & Clarkson, T. B. (2009). Social Stress, Visceral Obesity, and Coronary Artery Atherosclerosis in Female Primates. *Obesity*, *17*(8), 1513–1520. https://doi.org/10.1038/oby.2009.74

Simopoulos, A. P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *60*(9), 502–507. https://doi.org/10.1016/j.biopha.2006.07.080

Smil, V. (1989). Coronary Heart Disease, Diet, and Western Mortality. *Population and Development Review*, *15*(3), 399. https://doi.org/10.2307/1972440

Smyth, S., & Heron, A. (2006). Diabetes and obesity: The twin epidemics. *Nature Medicine*, *12*(1), 75–80. https://doi.org/10.1038/nm0106-75

Snyder-Mackler, N., & Lea, A. J. (2018). Functional genomic insights into the environmental determinants of mammalian fitness. *Current Opinion in Genetics & Development*, *53*, 105–112. https://doi.org/10.1016/j.gde.2018.08.001

Snyder-Mackler, N., Sanz, J., Kohn, J. N., Brinkworth, J. F., Morrow, S., Shaver, A. O., Grenier, J.-C., Pique-Regi, R., Johnson, Z. P., Wilson, M. E., Barreiro, L. B., & Tung, J. (2016). Social status alters immune regulation and response to infection in macaques. *Science*, *354*(6315), 1041–1045. https://doi.org/10.1126/science.aah3580

Solomon, S. S., Majumdar, G., Martinez-Hernandez, A., & Raghow, R. (2008). A critical role of Sp1 transcription factor in regulating gene expression in response to insulin and other hormones. *Life Sciences*, *83*(9–10), 305–312. https://doi.org/10.1016/j.lfs.2008.06.024

Stearns, S. C., & Koella, J. C. (Eds.). (2008). *Evolution in health and disease* (2nd ed). Oxford University Press.

Steinhubl, S. R. (2008). Why Have Antioxidants Failed in Clinical Trials? *The American Journal of Cardiology*, *101*(10), S14–S19. https://doi.org/10.1016/j.amjcard.2008.02.003

Stice, E., & Durant, S. (2014). Elevated objectively measured but not self-reported energy intake predicts future weight gain in adolescents. *Appetite*, *81*, 84–88. https://doi.org/10.1016/j.appet.2014.06.012

Suchanek, P., Poledne, R., & Hubacek, J. A. (2011). Dietary intake reports fidelity—Fact or fiction? *Neuro Endocrinology Letters*, *32 Suppl 2*, 29–31.

Tachmazidou, I., Süveges, D., Min, J. L., Ritchie, G. R. S., Steinberg, J., Walter, K., Iotchkova, V., Schwartzentruber, J., Huang, J., Memari, Y., McCarthy, S., Crawford, A. A., Bombieri, C., Cocca, M., Farmaki, A.-E., Gaunt, T. R., Jousilahti, P., Kooijman, M. N., Lehne, B., … Zeggini, E. (2017). Whole-Genome Sequencing Coupled to Imputation Discovers Genetic Signals for Anthropometric Traits. *The American Journal of Human Genetics*, *100*(6), 865–884. https://doi.org/10.1016/j.ajhg.2017.04.014

Thompson, J. R., Gustafsson, H. C., DeCapo, M., Takahashi, D. L., Bagley, J. L., Dean, T. A., Kievit, P., Fair, D. A., & Sullivan, E. L. (2018). Maternal Diet, Metabolic State, and Inflammatory Response Exert Unique and Long-Lasting Influences on Offspring Behavior in Non-Human Primates. *Frontiers in Endocrinology*, *9*. https://doi.org/10.3389/fendo.2018.00161

Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M. L., Gnardellis, C., Lagiou, P., Polychronopoulos, E., Vassilakou, T., Lipworth, L., & Trichopoulos, D. (1995). Diet and overall survival in elderly people. *BMJ (Clinical Research Ed.)*, *311*(7018), 1457–1460. https://doi.org/10.1136/bmj.311.7018.1457

Troisi, A., Belsanti, S., Bucci, A. R., Mosco, C., Sinti, F., & Verucci, M. (2000). Affect regulation in alexithymia: An ethological study of displacement behavior during

psychiatric interviews. *The Journal of Nervous and Mental Disease*, *188*(1), 13–18. https://doi.org/10.1097/00005053-200001000-00003

Troisi, Alfonso. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress (Amsterdam, Netherlands)*, *5*(1), 47–54. https://doi.org/10.1080/102538902900012378

Tung, J., & Gilad, Y. (2013). Social environmental effects on gene regulation. *Cellular and Molecular Life Sciences: CMLS*, *70*(22), 4323–4339. https://doi.org/10.1007/s00018-013-1357-6

Warden, C. H., & Fisler, J. S. (2008). Comparisons of Diets Used in Animal Models of High-Fat Feeding. *Cell Metabolism*, *7*(4), 277. https://doi.org/10.1016/j.cmet.2008.03.014

Whelton, P. K., Appel, L., Charleston, J., Dalcin, A. T., Ewart, C., Fried, L., Kaidy, D., Klag, M. J., Kumanyika, S., Steffen, L., Walker, W. G., Oberman, A., Counts, K., Hataway, H., Raczynski, J., Rappaport, N., Weinsier, R., Borhani, N. O., Bernauer, E., … Blethen, E. (1992). The Effects of Nonpharmacologic Interventions on Blood Pressure of Persons With High Normal Levels: Results of the Trials of Hypertension Prevention, Phase I. *JAMA*, *267*(9), 1213–1220. https://doi.org/10.1001/jama.1992.03480090061028

Wolf, S. A., Boddeke, H. W. G. M., & Kettenmann, H. (2017). Microglia in Physiology and Disease. *Annual Review of Physiology*, *79*(1), 619–643. https://doi.org/10.1146/annurev-physiol-022516-034406

Yang, H., Graham, L. C., Reagan, A. M., Grabowska, W. A., Schott, W. H., & Howell, G. R. (2019). Transcriptome profiling of brain myeloid cells revealed activation of Itgal, Trem1, and Spp1 in western diet-induced obesity. *Journal of Neuroinflammation*, *16*(1). https://doi.org/10.1186/s12974-019-1527-z

Zhang, D. E., Hetherington, C. J., Tan, S., Dziennis, S. E., Gonzalez, D. A., Chen, H. M., & Tenen, D. G. (1994). Sp1 is a critical factor for the monocytic specific expression of human CD14. *The Journal of Biological Chemistry*, *269*(15), 11425–11434.

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Data Availability

All data and code used to complete these analyses can be found at https://github.com/cscjohns/diet_behavior_immunity. The raw data can be accessed from the gene expression omnibus repository from accession # GSE144314.