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.
182

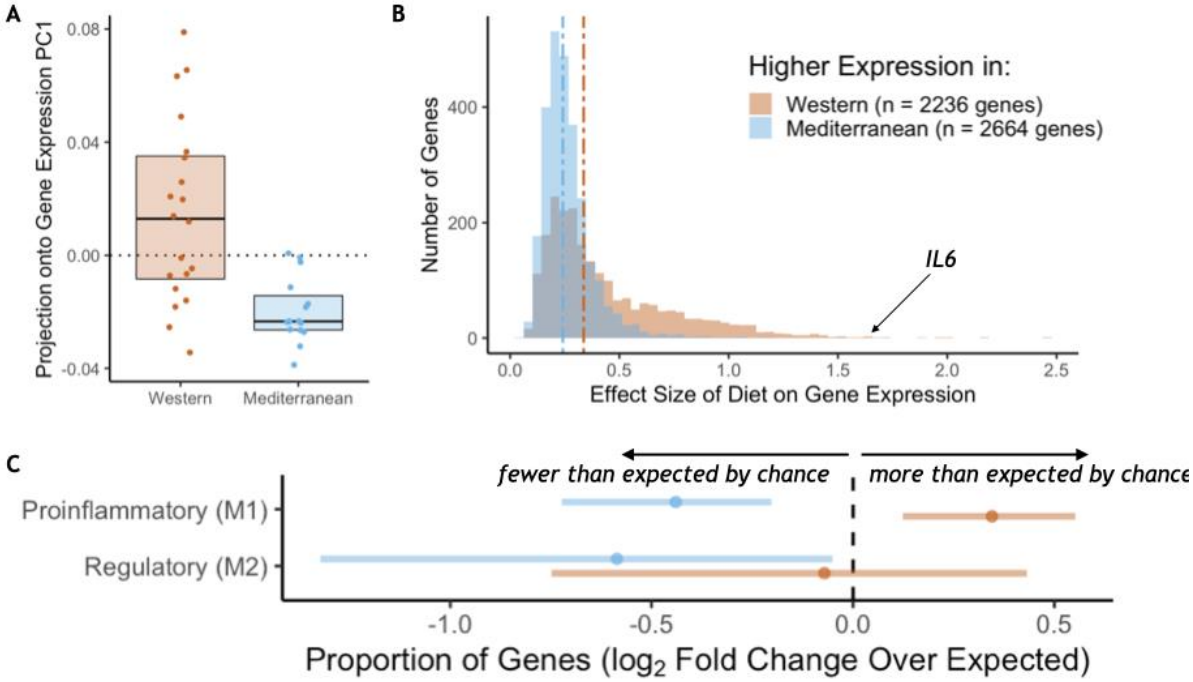

Figure 1. Diet effects on monocyte gene expression. A) Diet was significantly associated with the first principal component of gene expression (59.3% variance explained, $t_{(25.0)} = 4.41$, $p = 1.72 \times 10^{-4}$). **B)** The average effect size of diet on Western genes was 60% stronger than the 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

Monocytes from animals fed the Western diet had higher expression of a number of well-known inflammatory-related genes, including interleukin-6 (β_{diet} = 1.66, FDR = 8.9x10^{-3}; Fig. 1B), interleukin-1α (β_{diet} = 1.22, FDR = 0.033), and two subunits of the NF-κB protein (*NFKB1* β_{diet} = 0.30, FDR = 0.017; *NFKB2* β_{diet} = 0.42, FDR = 0.012). Western genes were more likely to be involved in replication and metabolic cellular processes, including response to growth factor (GO:0070848, weighted Fisher's Exact Test (FET) p = 4.6x10^{-3}) and response to insulin (GO:0032868, weighted FET p = 4.0 x10^{-4}; for all GO terms enriched in Western genes, see Table S2A), suggesting that the Western diet also reprogrammed oxidative metabolic aspects of monocyte gene regulation. Conversely, Mediterranean diet monocyte expression patterns were involved in enhanced oxidation-reduction processes (GO:0055114, weighted FET p = 6.0x10^{-3}; for all GO terms enriched in Mediterranean genes, see Table S2B), a critical function in keeping proinflammatory monocytes in check.

We next conducted a more targeted analysis of monocyte polarization by focusing on genes previously shown to be differentially expressed between induced proinflammatory (M1) and regulatory (M2) monocytes (Schmidl et al., 2014) (see Table S1 for polarization categories). Western genes contained more M1-associated genes than expected by chance (n = 162 genes, fold-enrichment = 1.27, *95% CI* = 1.09 - 1.46; Fig. 1C), but not M2-associated genes (n = 24 genes, fold-enrichment = 0.95, *95% CI* = 0.60 - 1.35). Conversely, both M1-associated genes (n = 112 genes, fold-enrichment = 0.74, *95% CI* = 0.61 - 0.88) and M2-associated genes (n = 20 genes, fold-enrichment = 0.67, *95% CI* = 0.40 - 0.97) were underrepresented among Mediterranean genes.

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.

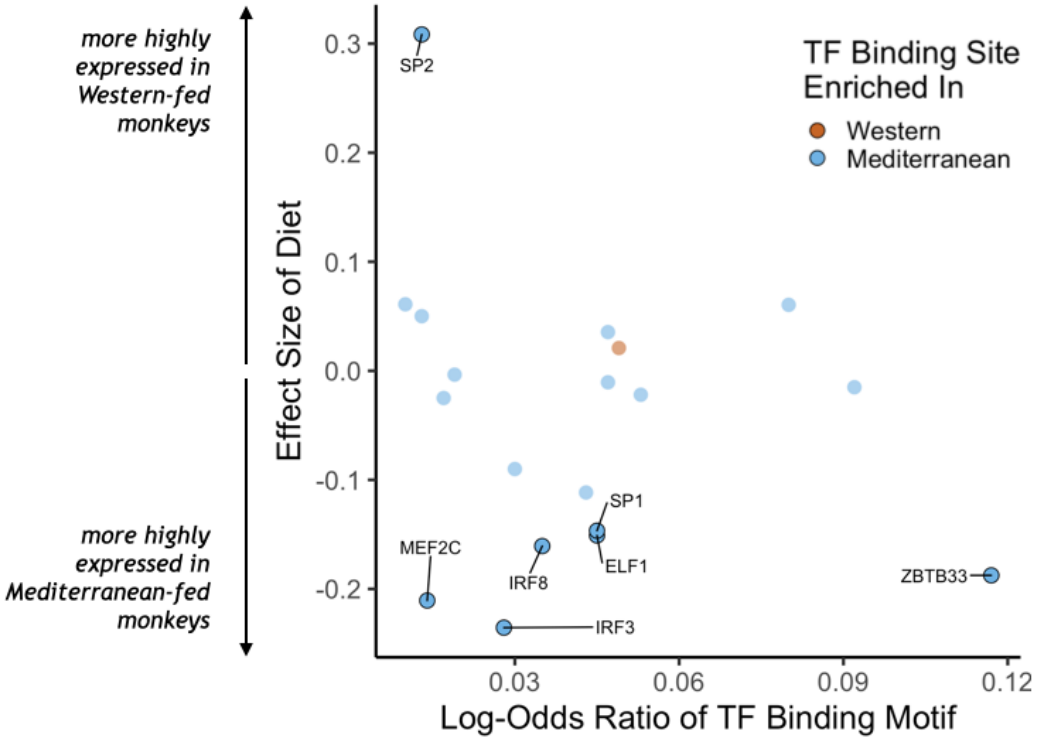

Figure 2. Transcription factor (TF) binding motifs correlated with diet effects on gene expression. The log-odds ratio of TF binding motif enrichment in Western genes (orange) or Mediterranean genes (blue) are depicted on the x-axis. The y-axis shows the effect size of diet on the expression of the gene 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.

219

220 *Diet differentially influenced gene co-expression patterns*

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

234

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

238 transcription factors (OR = 7.40, FET $p = 7.0 \times 10^{-3}$), including *SOX4* (essential for normal
239 insulin secretion and glucose tolerance) and *NR4A2* (involved in lipid, carbohydrate, and energy
240 metabolism (Goldsworthy et al., 2008; Pearen & Muscat, 2010)), providing further support for
241 immunological and metabolic reprogramming induced by our diet manipulation. Interestingly,
242 the hub gene involved in the greatest number of differentially-correlated gene pairs was
243 *RF00283, aka SCARNA18,* a non-coding RNA that has been associated with BMI, HDL
244 cholesterol, and aging in human genome-wide association studies (Davis et al., 2017; Dluzen et
245 al., 2018; Kanai et al., 2018; Tachmazidou et al., 2017) (Fig. 3B-D). This small nucleolar RNA is
246 thus a key transcriptional regulator that is altered by diet and has a cascading effect on other
247 genes and pathways.
248

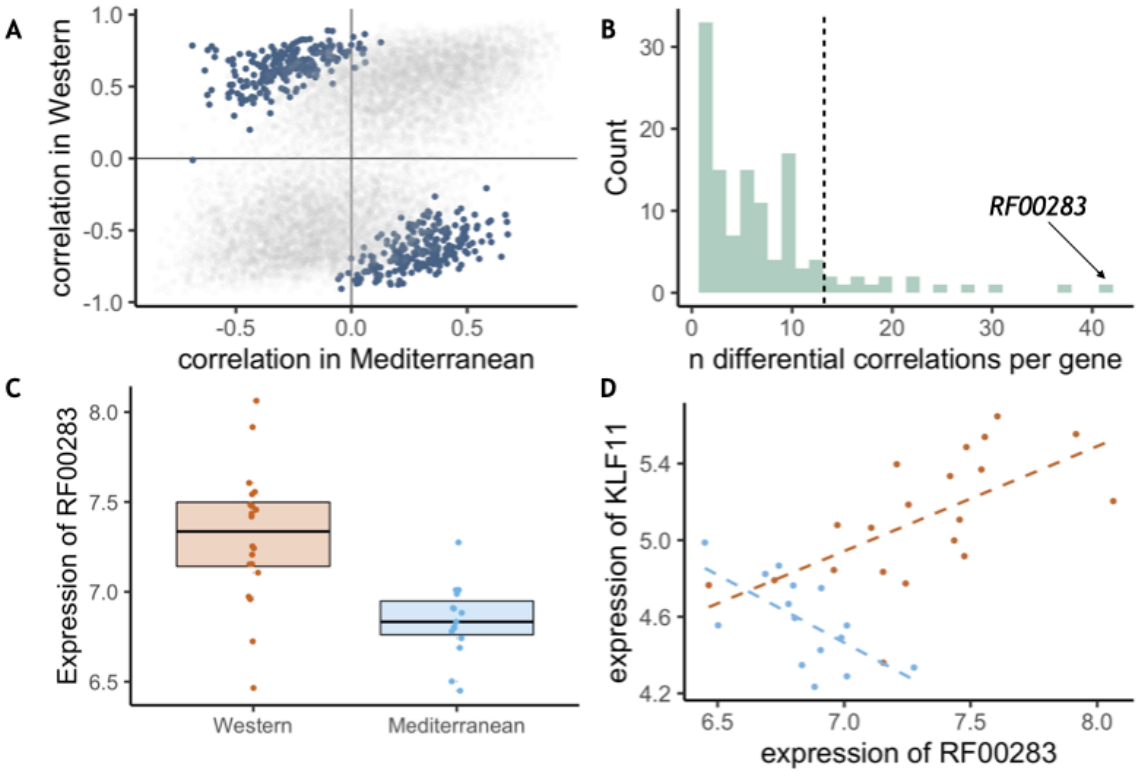

Figure 3. Diet altered monocyte gene co-expression. A) The Pearson correlation between each pair of genes

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.

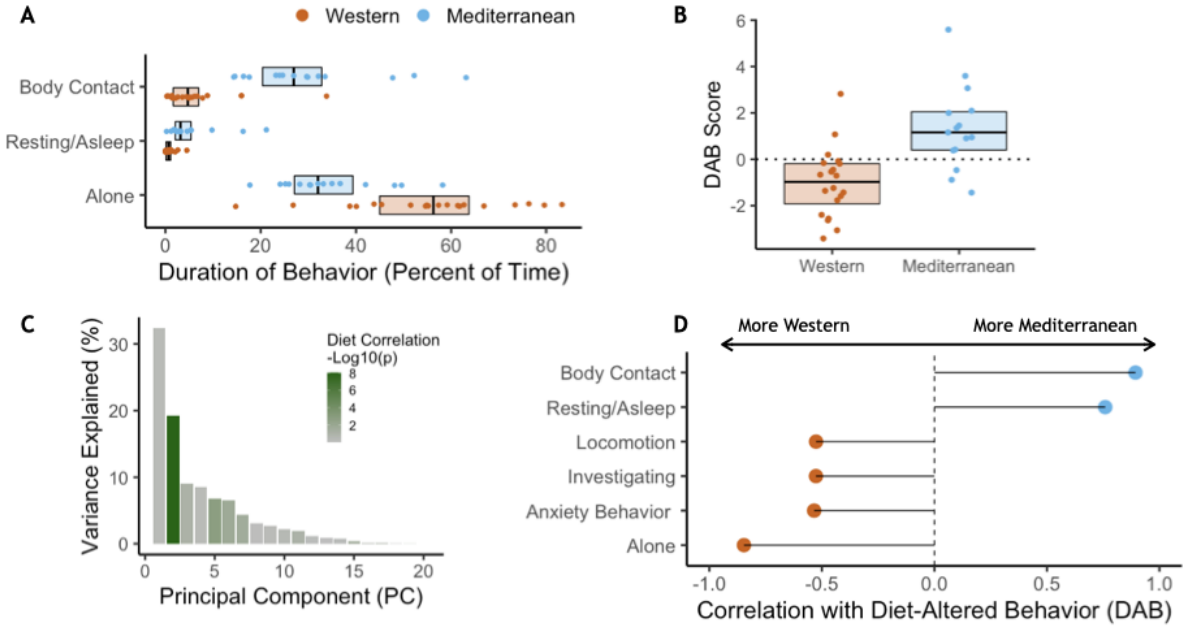

Figure 4. Diet alters behavioral phenotype. A) Three behaviors were significantly different between the two diet groups. Monkeys fed the Mediterranean diet spent more time in body contact (Holm-Bonferroni adjusted p = 1.1 x 10^{-5}) and resting (Holm-Bonferroni adjusted p = 1.6 x 10^{-3}) than Western-fed 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.

284

285 *Diet-altered behaviors and monocyte gene expression as mediators*

286 Given the effects of diet on both behavior and gene expression, we used mediation analyses to

287 explore the potential influences of one on the other. Of the 4,900 DEGs, 29% were also

288 significantly associated with the DAB score in a univariate model (n = 1,414, FDR < 0.05). Of
289 these, DAB score significantly mediated the effect of diet on the expression of 1199 genes (24%
290 of all DEGs, $p < 0.05$; Fig. 5A). Among these DAB-mediated genes, DAB score mediation
291 accounted for significantly more of the total effect of diet in Western genes (mean = 52.6 %, s.d.
292 = 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
294 be Western genes than Mediterranean genes (n = 712 Western genes, 59%, two-sided binomial
295 test $p = 1.5 \times 10^{-21}$), and were enriched in regulation of inflammatory response (GO:0050727,
296 weighted FET $p = 2.9 \times 10^{-3}$; for all GO terms significantly enriched in DAB-mediated genes,
297 see Tables S5A-C). Together, these observations suggest that the effect of diet on monocyte gene
298 regulation may partially be due to diet-induced changes in key social behaviors.
299
300 We also tested the hypothesis that peripheral immune cell gene expression mediated the effects
301 of diet on behavior in the 27% of DEGs for which monocyte gene expression significant
302 predicted DAB in a univariate model (n = 1,324, FDR < 0.05). Gene expression mediated the
303 effect of diet on DAB score in 898 genes (18% of all DEGs, $p < 0.05$; Fig. 5A). Almost all of
304 these genes (99%; 889/898) were in the set of genes for which behavioral changes mediated
305 changes in gene expression. The genes that mediated the effect of diet on DAB score were more
306 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
308 not vary between Western (mean = 27.1 %, s.d. = 5.2%) and Mediterranean genes (mean = 27.1
309 %, s.d. = 4.5%; Mann-Whitney $U = 1.0 \times 10^5$, $p = 0.55$; Fig. 5C).
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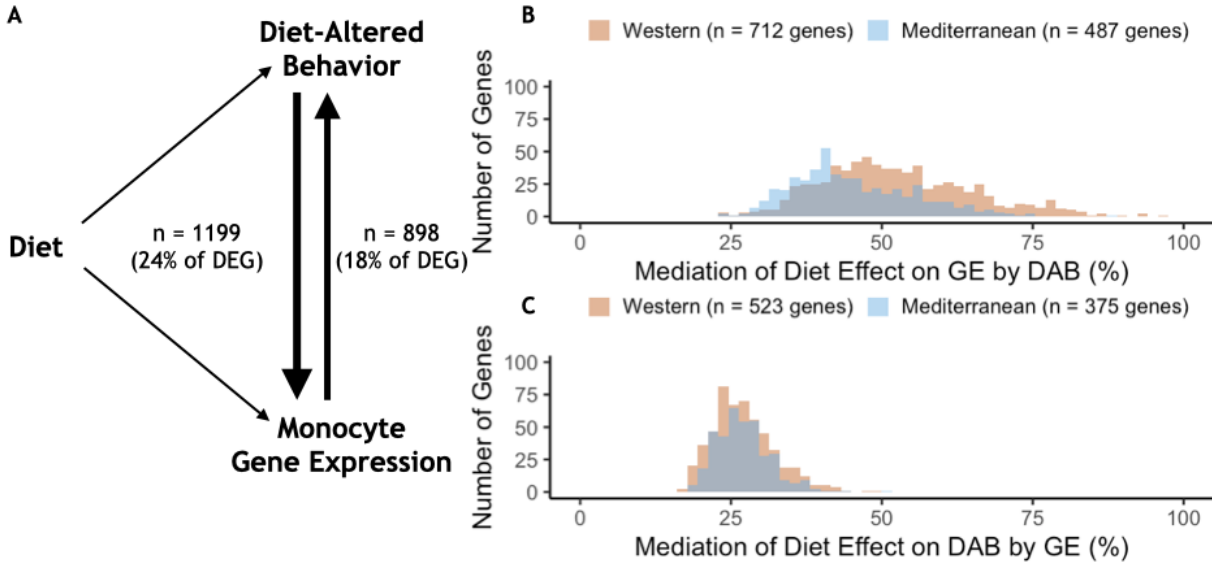

Figure 5. Behavior partially mediates the effect of diet on gene expression for 25% of diet-associated genes. **A)** Diet-altered behavior (DAB) mediated the effect of diet on gene expression for 25% (n = 1220) of genes for which diet had an effect (DEG). For 19% of differentially expressed genes (DEG), 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 $U = 1.1 \times 10^{5}$, $p = 0.75$).

311

312 *Diet differentially induced expression of the conserved transcriptional response to adversity*

313 *(CTRA) genes*

314 Additional analyses focused on expression of a well-studied set of social adversity-responsive

315 genes known as the "conserved transcriptional response to adversity" (CTRA) (Cole et al., 2015)

316 in the Western- and Mediterranean-fed animals in our study. Animals fed a Western diet
317 exhibited significantly higher expression of pro-inflammatory genes included in the CTRA
318 (Mann-Whitney $U = 222$, $p = 0.016$) and lower expression of antiviral- and antibody-related
319 CTRA genes (Mann-Whitney $U = 82$, $p = 0.023$; Fig. S3; for categorization of CTRA genes, see
320 Table S1).
321
322 *Western diet induced a mosaic response*
323 Western diet induced substantial variation in multiple phenotypes, including body weight, gene
324 expression, and behavior; consistent with previous studies demonstrating that some individuals
325 may be more resistant (or susceptible) to the effects of a Western diet (Shively et al., 2009),
326 presumably due to genetic variation or past environmental exposures. However, we were unable
327 to identify consistencies in individual responsiveness across the phenotypes (Fig. S4A-C). For
328 instance, monkeys that exhibited a strong gene regulatory response to the Western diet did not
329 necessarily exhibit a large increase in body weight or a strong negative DAB score (all $p > 0.2$).
330 Furthermore, change in body weight did not significantly predict gene expression in monocytes
331 (FDR < 0.2). Western diet fed individuals thus exhibited a mosaic response to diet across
332 multiple phenotypes, presumably involving interactions between diet, stress, behavior,
333 environment, microbiome, and genome/epigenome.
334
335 **Discussion**
336 This study shows, for the first time, that a whole-diet manipulation exerted profound effects on
337 monocyte polarization and social behavior in primates. Forty percent of expressed genes were
338 differentially expressed between monkeys fed Western or Mediterranean diets, indicating that

339 diet dramatically altered monocyte programming. Relative to monocytes from Mediterranean-fed
340 subjects, monocytes from Western diet consumers exhibited increased expression of
341 proinflammatory and monocyte polarization regulatory genes. Our findings extend previous
342 studies, such as a randomized human cross-over trial that demonstrated changes in monocyte
343 proinflammatory genes in elderly individuals consuming a Mediterranean like diet enriched in
344 olive oil versus a diet more enriched in saturated fat (butter) (Camargo et al., 2012).
345
346 We identified a putative molecular mechanism, altered monocyte polarization, that may
347 contribute to the established links between changes in human diets associated with
348 industrialization and increases in chronic disease (Cordain et al., 2005; Drake et al., 2018; Jacka
349 et al., 2010; Lea et al., 2020; Manzel et al., 2014; Pontzer et al., 2018; Smil, 1989; Smyth &
350 Heron, 2006). Comparative studies of human health across different modern populations –
351 namely those consuming traditional hunter-gatherer, forager-horticulturalist, or pastoralist diets
352 versus modern, Western-like diets – lend support for the evolutionary mismatch hypothesis
353 (Eaton et al., 1988; H. Kaplan et al., 2017; Lea et al., 2020; Pontzer et al., 2018). In particular,
354 this work has found that traditional populations have much lower rates of non-communicable
355 diseases, especially cardiometabolic diseases, relative to Western societies; however, because so
356 many lifestyle factors differ between traditional and Western, HICs, it has been difficult to
357 understand the role of diet specifically in driving health variation or to address causality
358 (Snodgrass, 2013; Kraft et al., 2018; Lagranja et al., 2015). Our preclinical randomized study
359 design allows us to draw causal inferences about the role of Western diets in the development of
360 chronic diseases of aging, and provides important data about cellular and molecular mechanisms
361 that may contribute to evolutionary mismatch. These data support future studies to compare the

362 transcriptional response to diet in our preclinical study with the gene regulatory variation
363 observed between traditional and more market-integrated or Western-like human groups.
364
365 Beyond alterations in gene expression, we also identified differences in gene co-expression and
366 enrichment of transcription factor binding motifs, suggesting that diet exerts differential effects
367 on gene regulatory networks. Numerous transcription factors appear to be involved in diet-
368 regulated gene expression. Members of the E26 transformation-specific (ETS), specificity
369 protein (Sp)/Krüppel-like family (KLF), myocyte-specific enhancer factor (MEF), and
370 interferon-regulatory factor (IRF) families of transcription factors, which have all been linked to
371 myeloid differentiation (Chistiakov et al., 2018; Schuler et al., 2008; Scott et al., 1994; Zhang et
372 al., 1994), were overrepresented in regulatory regions of genes with higher expression in the
373 Mediterranean diet group ("Mediterranean genes). Three IRF family transcription factors had
374 binding motifs enriched in Mediterranean genes: IRF-1 and IRF-8 are both linked to M1
375 monocyte polarization, while IRF-3 is associated with M2 polarization. The sole transcription
376 factor with binding sites enriched in Western diet-associated genes, ATF2, is a key mediator of
377 inflammatory pathways and diseases, including response to bacterial endotoxin, atherosclerosis,
378 and obesity (Fledderus et al., 2007; Miyata et al., 2012; Reimold et al., 2001). Western genes
379 were enriched for activation of the MAPKK pathway, which lies upstream of ATF2 (Herlaar &
380 Brown, 1999), supporting a role in monocyte polarization. Transcription factors were also
381 overrepresented in the pairs of differentially co-expressed genes, further indicating that diet
382 alters regulatory networks and monocyte differentiation and polarization.
383
384 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)

499

500 *Behavioral Characterization*

501 Behavioral data were collected weekly during two 10-minute focal observations, balanced for
502 time of day, for 6 weeks during the baseline phase (2 hours/monkey total) and for 14 months
503 during the experimental phase (17.7 hours/monkey total). Behaviors recorded included the
504 frequency of aggressive and submissive behaviors, time spent in positive social interactions such
505 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.,

Hilden, Germany), and quantified using a NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer with RNA 6000 Nano chips (Agilent Technology, Inc., Santa Clara, CA). RNA libraries were prepared for sequencing by the Cancer Genomics Shared Resource (Wake Forest School of Medicine, Winston-Salem, NC) using the TruSeq-stranded total RNA kit (Illumina), which includes a ribosomal depletion step. The RNA-seq libraries were then sequenced using single-end 76-bp reads on an Illumina NextSeq 500 to an average read depth of 34.5 million reads per sample (range 25.9 – 41.6 million reads). Reads were mapped to the *Macaca fascicularis* reference genome (Macaca_fascicularis_5.0, v 93, Ensembl) (Kersey et al., 2018; Kinsella et al., 2011) using HiSat2 (Kim et al., 2015) and then converted to a sample-by-gene read count matrix using featureCounts (Liao et al., 2014) (median = 38.0%; range 24.5 - 50.4% of reads mapped to exons).

Read Count Normalization and Removal of Batch Effects

First, we removed genes with low expression (median reads per kilobase per million reads mapped < 1), which resulted in 12,240 genes for downstream analyses. We normalized read counts using the *voom* function of the R package *limma* (Ritchie et al., 2015). While investigating monocyte purity, three samples differed in CD3 gene expression from the rest by several orders of magnitude. We concluded that these samples were contaminated with CD3+ cells (i.e., inefficient CD14 purification, see Fig. S5) and excluded them from all analyses, leaving a final sample size of 35 monkeys (n = 20 fed the Western diet, n = 15 Mediterranean diet). To control for batch effects related to RNA quality and monocyte purity, we calculated the residual gene expression from a model of normalized gene expression as a function of CD14 expression, CD3 expression, RNA integrity, and RNA concentration. These residual gene

552 expression values were used for all subsequent analyses.

553

554 *Modeling Effect of Diet on Gene Expression*

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

565

566 *Enrichment analyses*

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

575

576 *Transcription Factor Binding Site Analysis*

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

583

584 *Gene-gene co-expression analysis*

585 In addition to testing whether diet led to mean differences in gene expression between Western
586 and Mediterranean animals, we also tested whether diet impacted the correlation structure among
587 expressed genes (i.e., gene co-expression). Specifically, we employed 'correlation by individual
588 level product' (CILP) (Lea et al., 2019) analyses to test whether diet affected the magnitude or
589 direction of pairwise gene expression correlations among the top 140 DEGs (n = 9730 gene-gene
590 pairs tested). To test whether a given pair of genes was differentially co-expressed as a function
591 of diet, we first obtained a vector of products for each gene pair by multiplying the normalized
592 gene expression values for two genes together. Normalization was performed by scaling
593 expression values to mean 0 and unit variance within Mediterranean and Western subsets of the
594 data respectively, to ensure that distributional differences between sample groups did not bias
595 our results, following the CILP authors' recommendations (Lea et al., 2019). Each of these
596 vectors of products were used as the outcome variable in a linear mixed effects model
597 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

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

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

646 Table S1. Effects of Diet on Gene Expression

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

648 Genes

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

650 Genes

651 Table S3. Transcription Factor Binding Site Motif Enrichment

652 Table S4A. Gene Pair Correlations Across and Within Diet Groups

653 Table S4B. Differentially Correlated Genes

654 Table S5A. Biological Processes Enriched in Behavior-Mediated Differentially-Expressed Genes

655 (DEG)

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

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

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

659

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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.