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Health-Related Motivational and Behavioral Processes Associated With DNA Methylation of the *TNF* Gene

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Objective: Epigenetics has been described as one of the most exciting areas of contemporary biology, and research has begun to explore whether epigenetic modifications are influenced by psychological processes. The present research explored the associations of health-related motivation and behavior with the DNA methylation of tumor necrosis factor (*TNF*) gene. ***Method:*** Participants ($N = 88$) completed questionnaires examining engagement with health-related behavior (i.e., physical activity, diet, and smoking) and health-related motivation from the perspective of self-determination theory. They also provided a capillary blood sample for DNA extraction and analysis of four CpG sites via bisulfite conversion within Exon 1 of *TNF*. ***Results:*** Health-related autonomous motivation was weakly but positively associated with *TNF* methylation ($\beta = .18$, $p = .08$). Indirect effects were identified in a subsequent step; autonomous motivation was positively associated with fruit consumption ($\beta = .29$, $p = .004$), negatively associated with smoking ($\beta = -.22$, $p = .03$), but not associated with physical activity ($\beta = .10$, $p = .34$). Moreover, *TNF* methylation was positively associated with lifetime physical activity ($\beta = .18$, $p = .08$) and negatively associated with smoking ($\beta = -.23$, $p = .03$). Direct effects of autonomous motivation on DNA methylation did not persist when these indirect effects were included ($\beta = .09$, $p = .43$). ***Conclusions:*** Results support the idea that autonomous motivation is associated with DNA methylation, albeit indirectly through tobacco consumption.

Keywords: epigenetic, *TNF*, physical activity, health, self-determination theory

Supplemental materials: http://dx.doi.org/10.1037/hea0000795.supp

Epigenetics has been described as one of the most exciting areas of contemporary biology and has captured the imagination of the press and popular media (Bird, 2007). The scientific term *epigenetics* was first coined in pioneering work exploring the association between the genotype and phenotype (Waddington, 1942). The genotype represents an organism's inherited genetic makeup: its sequence of nucleotide bases in its DNA. The phenotype represents the organism's physical traits and arises from a wide variety of biological mechanisms. Over the last three decades, the term epigenetics has been used to broadly explain how the environment can affect gene expression; however, the precise meaning of epigenetics has undergone several revisions that mirror the evolution of the underlying science (e.g., Holliday, 1994). The generally accepted contemporary definition of epigenetics is "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in the sequence of DNA" (Wu & Morris, 2001, p. 1104). In other words, chemical modifications that do not alter the DNA sequence but alter the expression of genes can be passed through generations of cell division (i.e., mitosis) or through sexual reproduction (i.e., meiosis).

There are several epigenetic mechanisms implicated in the alteration of gene expression, including, but not limited to, histone modification, methylation of messenger RNA, and the most frequently studied, and the focus of the present investigation, DNA methylation (Henikoff & Greally, 2016). DNA methylation is characterized by the addition of a methyl group to a cytosine nucleotide, creating 5-methylcytosine, most commonly at cytosine-phosphate-guanine (CpG) sites. The effect of DNA methylation is most commonly the transcriptional silencing of gene expression around the transcription start site and the first exon. This leads to the gene being "switched off", and as a result expression of the protein associated with the gene is downregulated (Brenet et al., 2011). Genetic modifications that influence gene expression without altering the DNA sequence represent the essence of epigenetics.

Although epigenetic modifications are studied at the genetic level, their influence is often evident as observable physical manifestations. For example, monozygotic (identical) twins retain

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identical DNA throughout their lives; however, exposure to different environments, lifestyles, and experiences leads to epigenetic drift (Teschendorff, West, & Beck, 2013). This effect is exacerbated when monozygotic twins have been raised separately, with observable differences most evident in old age, as the impact of environmental differences accumulates over time (Fraga et al., 2005). The research in this area has tended to focus on the DNA methylation of candidate genes associated with inflammatory and immune functioning because of the implications for developmental and psychopathological processes (Jones, Moore, & Kobor, 2018). For example, a recent review examined the pathogenesis of disease in relation to physical activity and the methylation of inflammation-related epigenetic markers (Horsburgh, Robson-Ansley, Adams, & Smith, 2015). Horsburgh and colleagues (2015) suggested that the inflammatory response to acute physical activity was potentially a regulatorily mechanism for changes in DNA methylation, with the review highlighting implications for healthy and diseased populations. The present research extends this line of investigation by focusing on regulatory motivational process involved in physical activity, health-related behavior, and the methylation of the *TNF* gene promoter associated with the expression of circulatory TNFα.

TNFα is a proinflammatory cytokine that plays a prominent role in the body's immunomodulatory system. In response to inflammation, TNFα is stimulated and regulates the accumulation of macrophages and neutrophils to kill pathogens at the site of inflammation (Vassalli, 1992). It helps to induce fever and protect against viruses (Tortora & Derrickson, 2017) as well as other important functions in the body, such as assisting the regulation of glucose and fat metabolism (Hotamisligil & Spiegelman, 1994). However, elevated levels of circulatory TNFα have been associated with several noncommunicable diseases, including Type 1 diabetes (Arroyo-Jousse, Garcia-Diaz, Codner, & Pérez-Bravo, 2016), rheumatoid arthritis (Maxwell et al., 2008), and Crohn's disease (Van Deventer, 1997). Furthermore, methylation at the *TNF* gene has been identified as a limiting risk factor in some disease states (e.g., obesity; Campión, Milagro, Goyenechea, & Martínez, 2009; Milagro et al., 2011). In summary, regulating circularity levels of TNFα is important in maintaining the body's ability to deal with inflammation, while not compromising susceptibility to disease. For example, Crohn's disease is often managed using medication that reduces circulatory levels of TNFα; however, this can leave the patient at risk of increased susceptibility to infection (National Health Service, 2018). Therefore, the identification of modifiable factors that can positively influence methylation of the *TNF* gene represents a worthwhile scientific endeavor.

To a small degree, psychological experiences have been investigated as a possible source of epigenetic changes because they offer significant potential to enhance understanding of how everyday states shape human development. For example, subjective experiences of social isolation, but not objective social isolation, are associated with the upregulation of proinflammatory genes and downregulation of antiviral and antibody regulated genes (Cole et al., 2007). Furthermore, stress, posttraumatic stress disorder, and self-control have also been implicated as psychological mechanisms associated with DNA methylation (Lam et al., 2012; Miller, Yu, Chen, & Brody, 2015; Smith et al., 2011). This line of investigation suggests that subjective psychological states may play a prominent role in epigenetic modifications. The focus of the present article is to propose and explore a novel area of psychological influence on DNA methylation of *TNF*, namely, human motivation.

Traditionally, human motivation has been conceptualized as a unidimensional, linear process concerned with the direction and energization of behavior toward positive stimuli (Elliot, 2006). Within this paradigm, motivation has been empirically linked to physiological changes, most notably cardiovascular responses (Richter, Gendolla, & Wright, 2016). For example, increases in cardiovascular reactivity occur as cognitive effort increases, provided that success is deemed possible and effort is worthwhile (e.g., Richter, Friedrich, & Gendolla, 2008). Complementing this perspective, multidimensional motivation theories differentiate not only the amount of motivation but also the quality of motivation. When considering health-related choices, many healthy behaviors are driven by extrinsic motives rather than an inherent love, enjoyment, or interest in the activity. For example, there is little pleasure in refraining from smoking or resisting a large slice of cake, but many people choose to do so because being a healthy person is important or to avoid guilt after succumbing to the temptation. A motivation theory that captures this differentiation is organismic integration theory (OIT; Ryan & Deci, 2017). Grounded in the larger self-determination metatheory, OIT proposes that extrinsic motives vary in self-determination and quality, and can be placed on a continuum with behavior that is pursued for more autonomous, internalized reasons contrasted with behavior that is underpinned by controlled regulation (Ryan & Connell, 1989). When a behavior is fully endorsed and aligned with an individual's core values or beliefs, it is based on integrated regulation. Identified regulation reflects pursuing a behavior because it is personally meaningful although not necessarily fully aligned with all other aspects of the self (Ryan & Deci, 2017). Although integrated and identified motivation are classified as extrinsic in origin, they nonetheless represent behavior that is internalized and therefore autonomously regulated (Ryan & Deci, 2000a). Introjected motivation represents the shift on the continuum from autonomous to controlled regulation and encompasses behavior that is controlled internally, such as a desire to avoid guilt or seek approval (Ryan, 1982). Finally, behavior can be externally regulated, such as contingent rewards or punishments (Ryan & Deci, 2000b). Although many behaviors are externally regulated, such as working in an unsatisfying job for the salary, motivation to engage in healthy behavior is rarely externally regulated for adults and is therefore less relevant in the present context.

The degree to which behavioral regulation is internalized has important implications for health and well-being. Behavior that is underpinned by higher quality integrated and identified reasons is associated with improved outcomes in health care and health promotion settings (e.g., Ng et al., 2012). Conversely, when behavior is undertaken for lower quality controlled reasons, it not only yields less likelihood of long-term adherence but also may lead to psychological dysregulation (Ryan & Deci, 2017). Furthermore, there is emerging evidence to suggest that this motivational distinction can help predict downstream physiological responses. For example, environments that facilitate greater autonomous functioning are linked to adaptive social stress responses in the endocrine system (Reeve & Tseng, 2011). In addition, high-quality intrinsic motivation is associated with adaptive dopaminergic processes, such as greater dopamine D2-receptor availability (de

Manzano et al., 2013; see also Di Domenico & Ryan, 2017). We aimed to extend this broad motivation-physiology paradigm to epigenetic modifications.

Based on the above review, this study adopts an exploratory approach to investigate integrated, identified, and introjected motivations for healthy behavior as potential correlates of *TNF* methylation. Exploratory research represents an important part of most research programs, yielding the opportunity to uncover serendipitous occurrences and construct complicated multivariate structures (Ledgerwood, Soderberg, & Sparks, 2017). Furthermore, we aim to establish whether any observed associations persist after accounting for indirect effects via lifestyle factors, specifically physical activity, diet, alcohol consumption, and smoking. These lifestyle choices have been linked to global DNA methylation (e.g., Breitling, Yang, Korn, Burwinkel, & Brenner, 2011) and methylation at the *TNF* promoter (e.g., Bollati et al., 2014; Shaw et al., 2014), and could therefore plausibly explain any observed motivational effects (i.e., indirect effects exist). Alternatively, motivation may have direct associations with epigenetic modifications that exist over and above energizing behavioral choice. The former "indirect effects" conclusion would provide evidence for a motivational emphasis in attempts to modify healthy behavior and subsequent epigenetic states. The latter "direct effects" conclusion would suggest the need to investigate currently unknown motivational processes influencing gene expression.

Method

Participants

Ethical clearance was granted by the Loughborough University Approvals (Human Participants) Sub-Committee. A power analysis to detect a coefficient of determination greater than 5% suggested that it would require 88 participants to detect this size of effect ($\alpha = .10$, $\beta = .80$, $r^2 > .05$). We adopted a generous alpha level given the exploratory nature of the study. Data were subsequently collected from 54 women and 34 men recruited from a university campus opportunity sample between May and August 2016, with participants receiving no compensation for their time. The sample was recruited via advertising on a university website as well as posters in the local area. All participants provided written informed consent prior to taking part. Socioeconomic status of the cohort was relatively affluent (range: 1 = *least deprived* to 10 = *most deprived*; $M = 3.90$, $SD = 2.25$). The participants' ages ranged between 19 and 74 years ($M_{age} = 32.28$, $SD = 14.01$). The population was healthy in several measurable aspects, including a mean body mass index (BMI) of 24.60 ($SD = 4.24$); mean current weekly leisure time physical activity (LTPA) of 5.63 hr ($SD = 5.32$), and a mean Dietary Quality Score (DQS) of 9.68 ($SD = 1.47$) out of a maximum score of 12. In addition, 64 participants reported never having smoked, with 78 participants consuming an average of two units of alcohol or less per day.

Measures

Motivation for healthy behavior. A hybrid motivation questionnaire grounded in OIT was designed to measure the degree to which healthy behaviors were underpinned by different motivational regulations. Introjected and identified motivation were measured using six items from the Exercise Self-Regulation Questionnaire (Ryan & Connell, 1989). This questionnaire does not include a subscale to measure integrated motivation; hence, this type of motivation was measured using four items drawn from the Behavioral Regulation in Exercise Questionnaire (Version 3; Markland & Tobin, 2004). As the items contained in both questionnaires were originally developed for measuring exercise behavior, they were adapted to measure the broader construct of health behavior. For example, the original item "I consider exercise a fundamental part of who I am" was changed to "I consider healthy behavior a fundamental part of who I am." Responses were measured on a 5-point Likert scale from 0 (*not true for me*) to 4 (*very true for me*). The subscales of Introjected, Identified, and Integrated Motivation have all demonstrated acceptable reliability in previous research ($\alpha > .73$; González-Cutre et al., 2014; Silva et al., 2011).

Diet. The DQS (Toft, Kristoffersen, Lau, Borch-Johnsen, & Jørgensen, 2007) is a four-item questionnaire designed as a brief instrument to assess diet. Higher DQS scores are negatively associated with cholesterol, triglyceride, low-density lipoprotein-cholesterol, homocysteine, and risk of heart disease, and positively correlated with a longer dietary assessment (i.e., 198-item Food Frequency Questionnaire; Toft et al., 2007). The four items measure intake of vegetables, fruit, fish and fat, respectively, with a range of 1 to 3 for each item. For example, the item assessing fruit intake was followed by the response choice of either *3 pieces/day*, *more than 3 pieces/week and less than 2 pieces/day*, and *less than 2 pieces/week*, with a score of 3, 2, or 1 attributed, respectively. Scores were then summed to give a range of 4 to 12, with a higher score indicating a higher quality diet.

Physical activity. LTPA and occupational physical activity was assessed using the Modifiable Activity Questionnaire (MAQ; Kriska et al., 1990). The questionnaire provides formulae for calculating the average number of hours engaged in leisure time and occupational activity during past year and lifetime, with leisure-time physical activity also recorded over the previous week. The MAQ provides protocols for the conversion of moderate and hard occupational physical activity to metabolic equivalents (METs; total hours multiplied by 4 and 7, respectively). For consistency, the LTPA scores were also converted to METS using established MET activity equivalents (Ainsworth et al., 2000). Test–retest reliability of the MAQ for historical physical activity is good, with past-year ($r = .89$) and lifetime ($r = .94$) strongly correlated with measures taken up to 3 weeks previously. The MAQ also has good validity between activity monitor counts and weekly physical activity ($r = .66$; Kriska et al., 1990), and compares favorably with alternative questionnaires measuring short- and long-term physical activity (De Vera, 2006).

Tobacco and alcohol consumption. Participants were asked about their level of alcohol and tobacco consumption using a questionnaire based on an assessment tool used by the U.K. National Health Service (NHS, n.d.). Tobacco consumption was measured on an 8-point scale ranging from 1 (*never ever smoked*), to 8 (*more than 40 a day*). Alcohol consumption was measured in a 6-point scale ranging from 1 (*I never drink alcohol*) to 6 (*I drink more than 9 units a day*).

Procedure

Participants were asked to provide written informed consent and then completed the self-report questionnaires. In accordance with the standard instructions, the MAQ questions were read aloud for the participant and, when applicable, probes for further information were made. Once the MAQ had been completed, the participant's height and weight were recorded. They were then escorted to the laboratory, where a capillary blood sample was collected.

Capillary blood sampling was administered by the lead researcher in accordance with the university's standard operating procedure. Capillary puncture was administered using commercially available lancets, and blood was collected in ethylenediaminetetraacetic acid coated microvette tubes. To preserve the quality of the blood samples, they were immediately placed in an ice-filled container. A blood cell count was subsequently obtained for each sample on a Yumizen H500 OT Coulter counter (Horiba Medical, 2015) and stored at −80 °C prior to analysis.

DNA was extracted from capillary blood and bisulfite converted using the EpiTect Fast LyseAll Bisulfite conversion kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Briefly, the blood sample was lysed, proteins denatured, and the resulting pellet was resuspended in phosphate-buffered saline and added directly into the bisulfite reaction. The DNA was sodium bisulfite treated and subjected to two cycles of denaturing at 95 °C for 5 min, and incubation at 60 °C for 20 min. Bisulfite converted DNA was desulfonated and purified using MinElute spin columns.

The methylation of four CpG sites within Exon 1 of the *TNF* promoter were measured using pyrosequencing on the PyroMark Q48 Autoprep System (Qiagen). The assay covered four methylation sites: +197 +202, +214 and +222 base pairs from the transcription start site of *TNF*. Bisulfite converted DNA was used as a template for polymerase chain reaction (PCR) amplification using the PyroMark PCR kit (Qiagen) and a Veriti thermocycler (Applied Biosystems, Waltham, MA). Using 10uL of the PCR product, CpG quantification was completed using PyroMark Q48 Advanced CpG Reagents (Qiagen) in accordance with the manufacturer's protocol. The percentage methylation of the four CpG sites was calculated using PyroMark Q48 Autoprep 2.4.1 Software (Qiagen). The pyrosequencing primers and assay design for the four methylation sites can be found in the online supplemental materials.

Mean *TNF* methylation profiles were adjusted for leukocyte counts (Jones, Islam, Edgar, & Kobor, 2015). Adjustment is important, as individual differences in cell type composition of leukocytes (e.g., age-related reductions; Gowers et al., 2011) represents a potential confound when examining *TNF* methylation. Adjustment for cell type composition reduces the likelihood of false positives, or masking of true effects (Jones et al., 2015). In the present study, leukocyte counts were not available for the first three participants recruited into the study due to insufficient blood draw; therefore, their *TNF* measurements were classified as missing data.

Results

Preliminary Analysis

Means, standard deviations, and bivariate correlations of the independent variables and adjusted *TNF* CpG scores are shown in Table 1. Prior to constructing our substantive models, we employed path analysis using observed variables to explore what facets of healthy lifestyles might predict *TNF* methylation and warrant inclusion. Nonsignificant predictors were not included in our substantive models, enabling us to adopt the most parsimonious model. All statistical analysis was conducted using Mplus (Version 8.0; Muthén & Muthén, 1998–2017) software.

Smoking. Smoking was significantly negatively associated with *TNF* methylation ($\beta = -.23$, $p = .03$), explaining 5.0% of the variance.

Alcohol use. Consumption of alcohol was not significantly associated with *TNF* methylation ($\beta = -.09$, $p = .39$).

Diet. The overall score for diet was not a significant predictor of adjusted *TNF* methylation ($\beta = .08$, $p = .30$). However, exploration of individual food components revealed that fruit consumption emerged (albeit weakly) as a positive influence ($\beta = .18$, $p = .08$), explaining 3.3% of the variance in *TNF* methylation. The remaining separate diet score variables of vegetables, fish, and fat were nonsignificant ($\beta = -.05$, $p = .64$; $\beta = .10$, $p = .35$; and $\beta = -.03$, $p = .81$, respectively).

Physical activity. For occupational physical activity, neither lifetime nor previous year were associated with *TNF* methylation ($\beta = -.12$, $p = .28$, and $\beta = -.05$, $p = .61$, respectively). The values for LTPA over the previous week and the previous year had nonsignificant relationships with *TNF* methylation ($\beta = .00$, $p = .95$ and $\beta = .12$, $p = .27$, respectively). However, lifetime LTPA was positively associated with *TNF* methylation ($\beta = .19$, $p = .08$), explaining 3.4% of the variance. When LTPA was adjusted for METs, the pattern of associations was similar, but slightly stronger, for lifetime LTPA ($\beta = .19$, $p = .07$); however, weekly and yearly LTPA scores remained nonsignificant ($\beta = .02$, $p = .89$; $\beta = .11$, $p = .32$, respectively). We therefore included lifetime LTPA adjusted for METs in the path analysis.

Path Analysis

Preliminary analysis of identified and integrated motivation revealed a strong correlation between the two variables. Following the precedent set by previous work (e.g., Taylor, 2017), integrated and identified motivation were collapsed into one variable for further analysis (hereafter referred to as *autonomous motivation*). Introjected motivation was not significantly associated with *TNF* methylation ($\beta = .13$, $p = .23$), and therefore we did not test the indirect effects involving this type of regulation. Two models were constructed to explore the relationship between autonomous motivation, healthy behaviors, and *TNF* methylation (see Figure 1). The first model explored direct associations between autonomous motivation and *TNF* methylation. Model fit indices demonstrated acceptable model fit to the data, Satorra-Bentler (S-B) $\chi^2(3) = 2.86$, $p = .09$, standardized root mean square residual (SRMR) = .00, comparative fit index (CFI) = 1.00, root mean square error of approximation (RMSEA) = .00, 90% confidence interval [CI] [.00, .00] (Hu & Bentler, 1999). Autonomous motivation was a positive predictor of *TNF* methylation ($\beta = .18$, $p = .08$). Next, the healthy behaviors identified as associated with *TNF* methylation in the preliminary analysis were included in the model as indirect effects. That is, autonomous motivation was hypothesized to predict lifestyle behaviors, which, in turn, predicted *TNF* methylation. Model fit indices demonstrated acceptable model fit to the

Table 1
Means, Standard Deviations, and Bivariate Correlations of the Independent Variables and DNA Methylation of TNF CpG Mean Scores (Adjusted for White Blood Cell Counts)

Dependent variable	Actual range	*M*	*SD*	Pearson correlation
Age	18–74	32.28	14.01	−.233**
Socioeconomic status	1–9[a]	3.90	2.25	−.046
BMI	17.9–41.3	24.60	4.24	−.018
Gender (0 = male, 1 = female)	0–1	.66	.48	.025
Alcohol	0–4	1.40	.86	−.094
Smoking	0–4	.61	1.11	−.225**
Diet				
Total diet score	4–12	9.68	1.47	.113
Vegetables	1–3	2.78	.42	−.051
Fruit	1–3	2.31	.65	.182*
Fish	1–3	2.40	.92	.101
Fat	1–3	2.18	.47	−.026
Motivation				
Introjected	1–12	6.92	2.75	.129
Identified	4–12	9.97	2.25	.169
Integrated	2–16	11.80	3.81	.181*
Physical activity				
Occupational last 12 months	0–46.2	4.22	9.69	−.056
Occupational lifetime	0–48.2	8.27	11.76	−.115
Leisure last week	0–25.5	5.63	5.32	−.007
Leisure last year	0–39.0	8.50	8.53	.117
Leisure lifetime	0–47.3	11.32	8.03	.185*
Leisure last week METS	0–151.5	33.83	32.95	.015
Leisure last year METS	0–240.2	51.38	50.25	.107
Leisure lifetime METS	0–227.2	69.85	49.79	.191*

Note. DNA = deoxyribonucleic acid; *TNF* = Tumor necrosis factor; CpG = cytosine-phosphate-guanine; BMI = body mass index; METS = metabolic equivalents.
[a] 1 = *least derived*, 9 = *most deprived*.
* $p < .10$. ** $p < .05$.

data, S-B$\chi^2(3) = 1.87$, $p = .60$, SRMR = .03, CFI = 1.00, RMSEA = .00, 90% CI [.00, .15]. Autonomous motivation was positively associated with fruit consumption ($\beta = .29$, $p = .004$) and negatively associated with smoking ($\beta = -.22$, $p = .03$) but not lifetime LTPA ($\beta = .10$, $p = .34$). In turn, lifetime LTPA was positively associated with, and smoking negatively associated with, *TNF* methylation ($\beta = .18$, $p = .08$, and $\beta = -.23$, $p = .03$, respectively). Fruit consumption did not remain significantly associated with *TNF* methylation when included in this model ($\beta = .13$, $p = .21$). Direct effects of autonomous motivation on DNA methylation did not persist when these indirect effects were included ($\beta = .09$, $p = .43$). Due to age being associated with adjusted *TNF* methylation scores (Gowers et al., 2011), we repeated the indirect effects model, controlling for this relationship. However, model fit indices suggested that this model was a poor fit for the data, S-B$\chi^2(6) = 15.85$, $p = .01$, SRMR = .08, CFI = .68, RMSEA = .14, 90% CI [.06, .22].

Discussion

The influence of psychological experiences in epigenetic processes have become an increasing area of interest (Jones et al., 2018), with evidence suggesting that the subjective, rather than objective, experiences of the individual may be most important in explaining epigenetic modifications (Cole et al., 2007). The present research represents the first investigation of the relationship between health-related motivation and *TNF* methylation. We also examined the potential role of diet, alcohol, smoking, and physical activity in this epigenetic process, something that has received very little attention.

Our hypotheses proposed two possible roles for motivation in epigenetic processes. First, we aimed to test the direct effect of motivation on DNA methylation of exon 1 of the cytokine encoding gene *TNF*. Second, we aimed to test whether this effect persisted when including indirect effects via healthy behavior. We found some support for our first hypothesis that motivational processes are associated with methylation of the *TNF* exon 1. Autonomous motivation to engage in healthy behavior, but not introjected regulation, was positively associated with *TNF* methylation. These results support a burgeoning area of literature that suggests that the quality of motivation is an important factor in predicting downstream physiological mechanisms (Di Domenico & Ryan, 2017). Furthermore, it provides support for the implication that psychological phenomena are an important factor in epigenetic processes.

Inclusion of various healthy lifestyle behaviors rendered the direct effect of motivation nonsignificant. In particular, the association between autonomous motivation and *TNF* methylation was explained by participants' smoking habit. This finding may have significant implications for health if the processes are found to be causal. The only previous study that explored the relationship between smoking and *TNF* methylation found no direct relationship (Beach et al., 2017). However, this study was limited to a young adult African American population, and therefore is not directly comparable with the present research. Previous studies

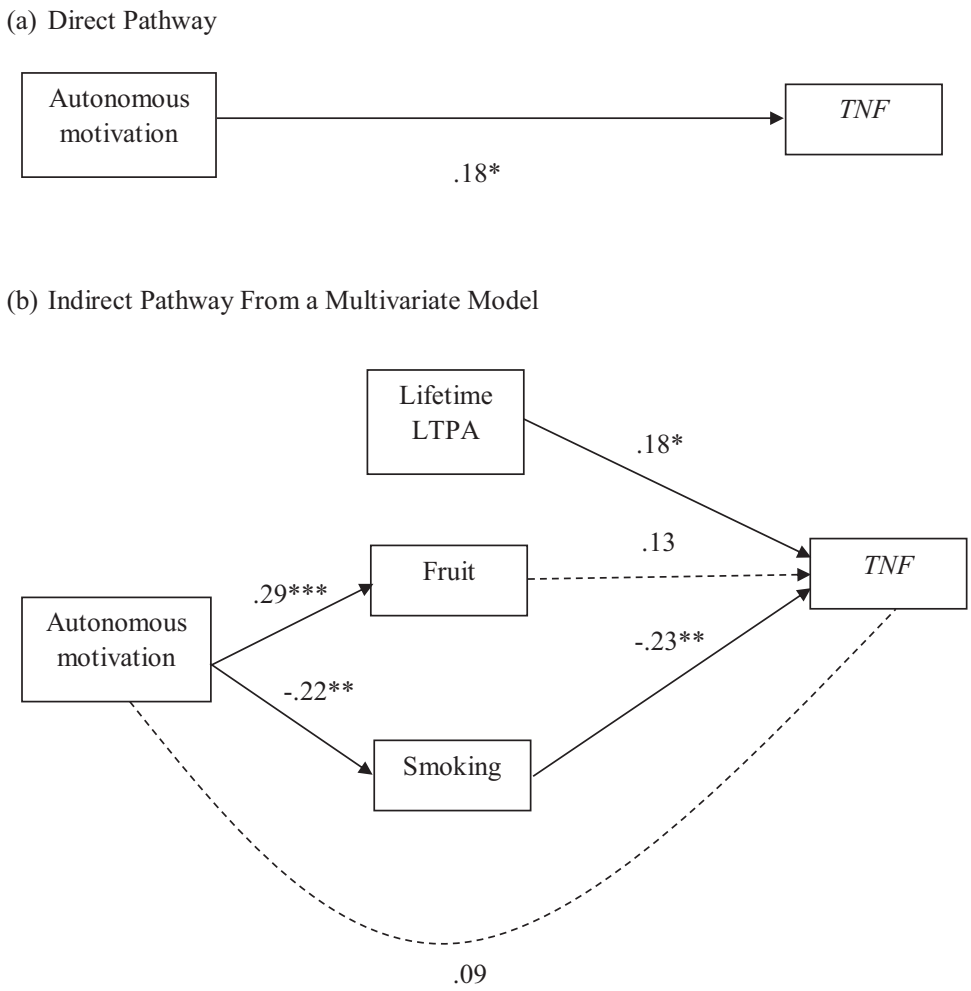

Figure 1. Relationships between motivation to engage in healthy behavior, healthy behavior, and *TNF* methylation. * $p < .10$. ** $p < .05$. *** $p < .01$.

have suggested that higher tobacco consumption is positively associated with circulatory TNFα protein levels (e.g., Arnson, Shoenfeld, & Amital, 2010), which are associated with attenuated methylation of the *TNF* gene promoter and exon 1 regions (Hermsdorff et al., 2013). Understanding the causal processes involving smoking, *TNF* methylation and circulatory TNFα could have important implications for health and disease risk, given the association of increased levels of TNFα with noncommunicable diseases, including Crohn's disease and rheumatoid arthritis (Maxwell et al., 2008; Van Deventer, 1997), and noncommunicable risk factors such as obesity (Campión et al., 2009; Milagro et al., 2011).

Within the wider literature, the association between autonomous motivation and healthy behavior, such as smoking abstinence, physical activity, and a healthy diet, is robust (Ng et al., 2012). In the present study, autonomous motivation was not associated with lifetime LTPA; however, to some extent, this can be explained. The questionnaire measuring motivation toward healthy behavior measured current motivation, and physical activity participation is strongly associated with the motivational regulation at the time of engagement (Ryan & Deci, 2017). Lifetime LTPA by design measures historical physical activity; hence, the chronological mismatch means that it is not surprising to find no association between current motivation toward healthy behavior and historical measures of physical activity. Although lifetime LTPA was not implicated in the motivational-epigenetic processes, this was the first study to demonstrate the association of physical activity and *TNF* methylation across a wide range of ages, with previous research limited to a cohort study within an elderly population (Shaw et al., 2014). The results of the present research supported the findings of Shaw and colleagues (2014): Higher levels of long-term physical activity were associated with methylation of the *TNF* exon 1. Nonsignificant relationships between *TNF* methylation, and weekly and yearly levels of physical activity suggest that adherence to a long-term physical activity program is required to affect *TNF* methylation, although it would be premature to disregard acute physical activity as a regulatory mechanism of DNA methylation of inflammatory-related sites (Robson-Ansley et

al., 2014), and TNFα independent of genetic modifications (e.g., Dufaux & Order, 1989; although see Gökbel et al., 2012). Nonetheless, adherence to long-term physical activity may be beneficial in increasing methylation of the *TNF* gene and may be a potential intervention for inflammatory-related diseases. Indeed, a recent meta-analysis found that physical activity combined with *TNF* inhibitor medication demonstrated reduced disease activity in patients with ankylosing spondylitis (Liang et al., 2015).

In accordance with previous evidence (e.g., Mata et al., 2009), a relationship between autonomous motivation and fruit consumption was observed; however, this did not, in turn, associate with *TNF* methylation. Diet and *TNF* methylation have a complex relationship (Bollati et al., 2014); however, the relatively homogeneous healthy profile of the participants in the present research may explain this finding. Authors of the DQS recommend a healthy dietary habit score above 7 as an indicator of a good diet. Almost all (86 of 88) participants met this criterion, with 54 participants scoring higher than 10 on the scale. Furthermore, 54 participants also had a BMI below 25. Evidence for the associations between diet and *TNF* methylation are predominately limited to populations with a higher BMI (Campión et al., 2009; Cordero et al., 2011; Milagro et al., 2011), and therefore the relatively healthy profile of the participants may explain the lack of any association in the present study.

A nonsignificant relationship between introjected motivation and *TNF* methylation was observed, and therefore we did not pursue the indirect effects of lower quality motivation through healthy behavior. Introjected regulation can motivate individuals toward their goals; however, as a more controlled form of motivation, this effect is often short-lived and unreliable in predicting sustained engagement with healthy behavior (Teixeira, Carraça, Markland, Silva, & Ryan, 2012). It is likely that this lower quality motivation does not lead to consistent behavioral choices, which create an adaptive *TNF* methylation profile. Moreover, it highlights the difference between high- and low-quality motivation. High-quality motivation is theoretically and empirically associated with improved well-being, and the implications of the present research further extends this association to epigenetic modifications.

The present study was largely exploratory in nature; hence, it is necessary for future research to build on these findings with confirmatory and replication work. That said, continued exploration of associations with other genetic loci that are potentially responsive to motivation and healthy behavior is recommended. Furthermore, exploration of genome-wide global DNA methylation, which can then be used to estimate chronological versus biological age (e.g., Horvath, 2013), may provide a novel way to extend the present research to more general indicators of health and well-being. For example, the need to examine motivational processes for their potential protective effects against epigenetic aging for individuals exposed to less supportive environments has been highlighted (Brody, Miller, Yu, Beach, & Chen, 2016). Investigation of whether increasing autonomous motivation for healthy behavior could be used as an intervention for improving health outcomes for populations susceptible to increased TNFα levels appears an obvious line of enquiry.

Furthermore, it is currently unknown whether the higher epigenetic plasticity characterized in early developmental periods is particularly sensitive to the increased physical activity levels during preadolescence. In other words, is physical activity when young epigenetically more important than during later periods? Although this potential relationship is persuasive, there is counterevidence of a positive association between physical activity in elderly populations and methylation of the *TNF* gene (Shaw et al., 2014). As a result, the role of physical activity on *TNF* methylation throughout the life span is worthy of further exploration.

Finally, there are methodological limitations of the present research that warrant discussion. First, this study was cross-sectional in design. Although we built a statistical model that implied causality, the limitations of cross-sectional research prevent the determination of a cause–effect relationship between autonomous motivation, healthy behavior, and *TNF* methylation. Additionally, the population was recruited from a university campus, and participants were therefore relatively young, with a lower-than-average BMI, higher-than-average physical activity, and a healthy diet. This precludes generalizing the results of this study to the general population or to populations in which increased circulatory TNFα is linked with diseases such as obesity, Crohn's disease, or Type 1 diabetes (Arroyo-Jousse et al., 2016; Van Deventer, 1997; Ye, 2008).

Conclusion

The goal of the present research was to examine associations between human motivation, healthy behaviors, and *TNF* methylation. We used the data to construct a novel exploratory model showing that the autonomous motivation to engage in healthy behavior was associated with tobacco consumption, which, in turn, was associated with *TNF* methylation. In addition, adherence to long-term physical activity was associated with *TNF* methylation. The present research extends motivational research to epigenetic processes and supports the importance of higher quality motivation for improved health and well-being.

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