Brain, Behavior, & Immunity - Health 25 (2022) 100519

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Associations of circulating cell-free DNA, C-reactive protein, and cardiometabolic risk among low-active smokers with elevated depressive symptoms

Teresa E. Daniels [a,b,c,*], Emily K. Zitkovsky [a,d], Zachary J. Kunicki [b], Destiny J. Price [e], Abigail L. Peterson [f], Phyllis A. Dennery [f,g], Hung-Teh Kao [b], Lawrence H. Price [a,b], Audrey R. Tyrka [a,b,c,1], Ana M. Abrantes [b,h,1]

[a] Mood Disorders Research Program and Laboratory for Clinical and Translational, Neuroscience, Butler Hospital, 345 Blackstone Boulevard, Providence, RI, 02906, USA
[b] Department of Psychiatry and Human Behavior, Warren Alpert Medical School of Brown University, 345 Blackstone Boulevard, Providence, RI, 02906, USA
[c] Initiative on Stress, Trauma, and Resilience (STAR), Department of Psychiatry and Human Behavior, Warren Alpert Medical School, Brown University, Providence, RI, USA
[d] Warren Alpert Medical School of Brown University, Providence, RI, USA
[e] Department of Psychiatry, New York State Psychiatric Institute and Columbia University Irving Medical Center, 1051 Riverside Dr, New York, NY, 10032, USA
[f] Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI, USA
[g] Department of Pediatrics, Warren Alpert Medical School of Brown University, 593 Eddy St, Providence, RI, 02903, USA
[h] Behavioral Medicine and Addictions Research Department, Butler Hospital, 345 Blackstone Boulevard, Providence, RI, 02906, USA

ARTICLE INFO

Keywords:
Cell-free DNA
Metabolic syndrome
Cardiometabolic risk
C-reactive protein
Inflammation

ABSTRACT

Background and aims: Cell-free DNA (cfDNA) is elevated in several disease states. Metabolic syndrome is a constellation of factors associated with poor cardiometabolic outcomes. This study examined associations of cfDNA from the nucleus (cf-nDNA) and mitochondria (cf-mtDNA), C-reactive protein (CRP), and metabolic syndrome risk, in low-active smokers with depressive symptoms.

Methods: Participants ($N = 109$; mean age 47) self-reported medical history. Physical activity was determined by accelerometry and anthropometrics were measured. Blood was collected and analyzed for cf-nDNA, cf-mtDNA, CRP, triglycerides, high-density lipoprotein, hemoglobin A1c. A continuous metabolic syndrome composite risk score was calculated. Relationships of cf-nDNA, cf-mtDNA, CRP, and cardiometabolic risk were examined with correlations and linear regression.

Results: CRP and cf-nDNA were significantly associated with metabolic syndrome risk ($r = .39$ and $r = .31$, respectively), cf-mtDNA was not ($r = .01$). In a linear regression, CRP and cf-nDNA significantly predicted the metabolic syndrome risk score, findings that remained significant controlling for age, gender, nicotine dependence, and physical activity.

Conclusions: Associations of cf-nDNA with both CRP and metabolic risk suggest a role for cf-nDNA in inflammatory processes associated with metabolic syndrome. The negative findings for cf-mtDNA suggest distinct roles for cf-nDNA and cf-mtDNA in these processes.

Abbreviations: cfDNA, cell-free DNA; cf-nDNA, cell-free nuclear DNA; cf-mtDNA, cell-free mitochondrial DNA; CES-D, Center for Epidemiologic Studies Depression Scale; DAMP, damage-associated molecular pattern; NET, neutrophil extracellular trap; FTND, Fagerström Test for Nicotine Dependence.

* Corresponding author. 1011 Veterans Memorial Parkway, Riverside, RI, 02915, USA.
E-mail addresses: Teresa_Daniels@brown.edu (T.E. Daniels), Emily_Zitkovsky@brown.edu (E.K. Zitkovsky), Zachary_Kunicki@brown.edu (Z.J. Kunicki), Destiny.Price@nyspi.columbia.edu (D.J. Price), Abigail.L.Peterson9@gmail.com (A.L. Peterson), Phyllis_Dennery@brown.edu (P.A. Dennery), Hung-Teh_Kao@brown.edu (H.-T. Kao), Lawrence_Price_MD@brown.edu (L.H. Price), Audrey_Tyrka@brown.edu (A.R. Tyrka), Ana_Abrantes@brown.edu (A.M. Abrantes).
[1] Drs. Tyrka and Abrantes contributed equally to this work.

https://doi.org/10.1016/j.bbih.2022.100519
Received 29 July 2022; Received in revised form 13 September 2022; Accepted 18 September 2022
Available online 19 September 2022

1. Introduction

Fragments of genetic material known as circulating cell-free DNA (cfDNA) are present in low levels in the blood of healthy individuals (Aucamp et al., 2018). Extracellular DNA arises from processes of cellular breakdown, such as apoptosis and necrosis, as well as activities of living cells such as vesicular release during cell-signaling (Aucamp et al., 2018). Levels of cfDNA increase dramatically in a number of disease states (Xie et al., 2018; Ranucci, 2019; Chang et al., 2003), presenting broad potential for clinical and research utility as a biomarker and as a mechanistic factor contributing to disease-related cellular processes. Circulating cell-free genetic material can originate from the genome of the nucleus (cf-nDNA) or the mitochondria (cf-mtDNA). These subtypes of cfDNA have distinct and overlapping properties, and levels of each may reflect the mechanism of release and the cell-types involved in pathological processes (Aucamp et al., 2018; Thierry et al., 2016). Both cf-nDNA and cf-mtDNA are actively engaged with the immune system, functioning as damage-associated molecular patterns (DAMPs), or signals of injury that elicit an immune response, and provide defense against pathogens by recruiting additional white blood cells when released by activated immune cells in neutrophil extracellular traps (NETs) (Aucamp et al., 2018). Additional inflammatory properties are attributed to cf-mtDNA, in part due to structural similarities of mitochondrial DNA and bacterial DNA (Aucamp et al., 2018).

A growing body of evidence suggests that cfDNA may be particularly valuable in understanding cardiometabolic disease processes (Nishimoto et al., 2016). Metabolic syndrome is a physiologic state characterized by a constellation of systemic derangements, including elevations in blood pressure, circulating triglycerides, and waist circumference, decreased high-density lipoprotein (HDL), and the development of insulin resistance (Grundy et al., 2005). The systemic cellular changes associated with metabolic syndrome give rise to a chronic low-grade inflammatory state, with well-established associations with inflammatory markers (Weiss et al., 2013), including C-reactive protein (CRP) (Liu et al., 2016). Smoking is associated with high-risk cardiometabolic profiles (Weiss et al., 2013) through a number of proposed mechanisms involving metabolic dysregulation, endothelial dysfunction, and altered coagulation cascades (Kar et al., 2016).

Preclinical reports support an association of cfDNA, inflammation, and insulin resistance, with evidence from mouse models suggesting that obesity-induced cfDNA release directly elicits inflammation (Nishimoto et al., 2016). In a small number of clinical studies, levels of cfDNA are observed to increase following acute myocardial infarction (Xie et al., 2018; Wang et al., 2015), and in individuals with insulin resistance (Bartels et al., 2020) and cardiovascular disease (Nie et al., 2020). In a study of $N = 1337$ Finnish adults ages 46-77 years-old, cfDNA was found to be associated with individual cardiometabolic risk factors, such as hypertension and hyperlipidemia (Jylhava et al., 2014). The observed associations of cfDNA with cardiovascular disease suggest that each may play a role in identifying cardiometabolic risk profiles and provide insight into the underlying disease mechanism.

However, very few studies have examined the independent associations of cf-nDNA and cf-mtDNA with cardiometabolic risk, and there is little evidence focused on populations with significant risk factors for cardiovascular disease, such as those who use tobacco. The present study aims to investigate the relationships of cf-nDNA, cf-mtDNA and the inflammatory marker CRP with a composite metabolic syndrome risk score, in a sample of low-active adults with daily tobacco use and depressive symptoms.

2. Methods

2.1. Participants

A subset of participants enrolled in a smoking cessation exercise intervention study provided baseline data for the current study. The original study enrolled $N = 231$ adult smokers. Participants were adults ages 18–65, recruited using newspaper, internet, and community advertisements seeking adult cigarette smokers (at least 10 cigarettes per day). Participants also had depressive symptoms (Center for Epidemiologic Studies Depression Scale (CES-D) ≥ 6), low levels of physical activity (less than 90 min of moderate-intensity exercise per week for the past 3 months), were able to walk for 1 mile on a treadmill, and were cleared by their physician for participation. Exclusion criteria included current pregnancy, manic episode, suicidality or homicidal thoughts, psychotic symptoms or lifetime psychotic disorder, current substance use disorder or treatment within the past six months, anorexia or bulimia nervosa within the past six months, starting or change in dose of antidepressant medication in the last 3 months. Eligibility of prospective participants was assessed via phone screen. Prior to enrollment, participants were informed about the study and voluntary written informed consent was obtained. The study was approved by the Butler Hospital Institutional Review Board.

2.2. Procedures

2.2.1. Demographics

Medical history and current medications were obtained by self-report. Participants were considered to have current hypertension, hyperlipemia or diabetes if they endorsed having these disorders on a self-report form or if they were taking medications to treat these conditions.

2.2.2. Psychometrics

Symptoms of depression were measured using the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977).The Fagerström Test for Nicotine Dependence (FTND) assessed nicotine dependence (Meneses-Gaya et al., 2009).

2.2.3. Anthropometrics

Weight and height were measured with a Detecto Scale/Stadiometer and waist circumference was measured using tape measure by an exercise physiologist. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

2.2.4. Accelerometry

Accelerometry data was collected via a wrist-worn accelerometer (Actigraph, Pensacola, FL) for one week prior to the blood draw. Moderate to vigorous physical activity (MVPA) was estimated using the Freedson cutpoint of $\geq$1952 counts/minute (Freedson et al., 1998).

2.2.5. Blood collection, processing

DNA extraction and measurement: Non-fasting blood draw was carried out by a phlebotomist via venipuncture in the antecubital fossa. Blood samples were centrifuged at 1000 RCF (G-force) for 5 min immediately following the blood draw. Plasma was stored at −80 °C until cfDNA was extracted. Upon thawing, plasma was centrifuged again at 1000 RCF (G-force), and cfDNA was extracted using the QIAamp circulating nucleic acid kit (Qiagen, Valencia CA). Quantitative PCR was performed in triplicate reactions at 25 ng per reaction using Platinum SYBR-Green SuperMix (Invitrogen, Carlsbad CA) with primers against the beta-hemoglobin gene to detect cf-nDNA (with forward and reverse primer sequences as follows: GCT TCT GAC ACA ACT GTG TTC ACT AGC and CAC CAA CTT CAT CCA CGT TCA CC) and the mitochondrial D-loop to detect cf-mtDNA (with forward and reverse mitochondrial primer sequences directed toward the D-loop region: CAT CTG GTT CCT ACT TCA GGG and TGA GTG GTT AAT AGG GTG ATA GA) (Bai and Wong, 2005). An initial heating step of 95 °C for 10 min was followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A standard curve of cloned amplicons was used to calculate the copy number of unknown samples in each reaction, which was then normalized to plasma volume.

2.2.6. Metabolic and inflammatory assays

Assays were performed in the Women and Infants Core Clinical Laboratory. Total cholesterol enzymatic assay was performed on the Architect Ci4100 analyzer (Abbott Laboratory, Chicago IL) using the Abbott cholesterol total reagent with a range of detection of 5.0mg/dl-705 mg/dl. HDL cholesterol was assayed via accelerator selective detergent on the Architect Ci4100 using the Abbott HDL Ultra reagent, with a range of detection of 5mg/dl-117.0 mg/dl. Triglyceride level was assayed via glycerol phosphate oxidase on the Architect Ci4100 analyzer (Abbott Laboratory, Chicago IL) using the Abbott triglyceride reagent, with a range of detection of 5.0mg/dl-1226 mg/dl. Hemoglobin A1C % was assayed via HPLC with a boronate column on the Premier Hb9210 analyzer (Trinity Biotech, Kansas City MO) with a range of detection of 3.8%–18.5%. C-reactive protein (CRP) was measured with an immunoturbidimetric method using the Architect Ci4100 analyzer latex immunoassay, with a reference range of 0.5–75.0 mg/L.

2.3. Statistical analyses

Analyses were conducted using R version 4.1.2 (Vienna, Austria). CRP, cf-nDNA, and cf-mtDNA were skewed and therefore were normalized using natural log transformation. A continuous metabolic syndrome composite risk score was calculated using the sum of z-scores for each metabolic syndrome measure (waist circumference, systolic blood pressure, diastolic blood pressure, HDL, triglycerides, and hemoglobin A1C). This approach allows for the assessment of the full range of data relevant to cardiometabolic outcomes and avoids the loss of information that occurs with the use of binary threshold variables when metabolic syndrome is clinically defined with thresholds for each component. A previous study demonstrated non-fasting z-score composites of metabolic syndrome risk reliably and accurately predict cardiometabolic outcomes (DeBoer et al., 2020). In order to further assess the metabolic syndrome composite risk score, the relationship of the risk score with a medical history of cardiovascular and metabolic disorders was examined. A diagnosis score was calculated by summing participants' total number of current diagnoses of hypertension, hyperlipidemia and diabetes. The continuous metabolic syndrome composite risk score and diagnosis score were found to be highly associated ($r = .39$, $p < .0001$).

Missing data diagnostics showed 6% of the data were missing across all variables used in the analyses. Missing data were imputed using multiple imputation with $m = 10$ imputations. Imputation was done using the *mice* package (Buuren and Groothuis-Oudshoorn, 2010). Bivariate relationships were examined with Pearson correlations. A linear regression model was used to examine unique contributions of cf-nDNA and CRP to prediction of the metabolic syndrome risk score, controlling for covariates that have substantial evidence supporting associations with increased risk for metabolic syndrome (age, gender, nicotine dependence, and physical activity). Sensitivity analyses were conducted to rule out confounding effects of cancer history and depression by including these as covariates in the model predicting metabolic syndrome composite risk score.

3. Results

3.1. Sample characteristics

Table 1 presents the sample characteristics. The sample ($N = 109$) was predominantly female, with a mean age of 47 ($SD = 10$). Participants identified race as White ($n = 92$, 84.4%), Black/African-American ($n = 9$, 8.3%), American Indian/Alaska Native ($n = 1$, 0.9%), Asian ($n = 1$, 0.9%), Hawaiian/Pacific Islander ($n = 1$, 0.9%), more than one race ($n = 3$, 2.8%), or other ($n = 3$, 2.8%), and 7.3% of participants identified ethnicity as Hispanic/Latinx ($n = 8$). The mean FTND score, assessing nicotine dependence, was 5.2 ($SD = 2.1$). The mean daily moderate to vigorous physical activity was 25.9 min ($SD = 26.3$). Participants identified as single ($n = 58$, 53.2%), married/cohabitating ($n = 47$, 43.2%), or divorced/separated ($n = 3$, 2.8%). Completion of high school or a GED was reported by 95.5% of participants ($n = 103$), and 28.4% ($n = 31$) had a college degree. Forty-seven participants (43.1%) were employed full-time, while 33.0% of participants ($n = 36$) reported unemployment.

Table 1
Sample characteristics.

Characteristic (N = 109)	M (SD) or N (%)
Age	46.6 (10.1)
Gender (*N*, % women)	76 (70%)
Racial Identity	
White	92 (84%)
Black or African American	9 (8%)
Asian	1 (1%)
American Indian or Alaska Native	1 (1%)
Native Hawaiian or Other Pacific Islander	0 (0%)
Multiracial	3 (3%)
Chose not to respond	3 (3%)
Ethnicity	
Hispanic/Latinx	8 (7%)
Non-Hispanic/Latinx	101 (93%)
Fagerström Test for Nicotine Dependence Score	5.2 (2.1)
Cancer history	7 (6.4%)
Cancer, current	0 (0.0%)
Hypertension	28 (26%)
Diabetes	12 (11%)
Hyperlipidemia	22 (20%)
Diagnosis score	
0	66 (61%)
1	21 (19%)
2	14 (13%)
3	4 (4%)
Metabolic syndrome composite risk score	0.0 (3.7)
Cell-free nuclear DNA (cf-nDNA, copies/ml)	12.0 (.8)
Cell-free mitochondrial DNA (cf-mtDNA, copies/ml)	18.5 (1.6)
Center for Epidemiology Depression Scale (CES-D)	15.1 (11.1)

3.2. Bivariate associations

Levels of cf-nDNA were highly correlated with cf-mtDNA ($r(107) = .43$, $p < .0001$) and significantly associated with CRP ($r(107) = .26$, $p = .008$). There was also a trend negative association of cf-nDNA with nicotine dependence ($r(107) = -.16$, $p = .089$). The metabolic syndrome composite risk score was significantly associated with cf-nDNA ($r(107) = .31$, $p = .001$) and CRP ($r(107) = .39$, $p < .0001$), but not cf-mtDNA ($r(107) = .04$, $p = .868$). Lower metabolic syndrome composite risk score was associated with female gender ($r(107) = -.26$, $p = .009$) and nicotine dependence ($r(107) = -.25$, $p = .013$). Greater metabolic syndrome risk score was diagnosis score ($r(107) = .44$, $p < .0001$). cf-nDNA was also weakly associated with the diagnosis score ($r(107) = .18$, $p = .077$).

Less physical activity was associated with CRP ($r(107) = -.24$, $p = .023$) and female gender ($r(107) = -.36$, $p = .0004$). Lastly, female gender was significantly associated with CRP ($r(107) = .22$, $p = .006$).

3.3. Linear models predicting metabolic syndrome composite risk score

Table 2 displays the results of the linear regression predicting the metabolic syndrome composite risk score with cf-nDNA and CRP, controlling for age, gender, nicotine dependence, and physical activity. Both cf-nDNA ($\beta = 0.98$, $SE = 0.37$, $p = .009$) and CRP ($\beta = 0.24$, $SE = 0.05$, $p < .0001$) were significant independent predictors of the metabolic syndrome composite risk score after controlling for the covariates. Gender also emerged as a significant covariate ($\beta = -3.68$, $SE = 0.72$, $p < .0001$). While physical activity was significantly correlated with the metabolic syndrome composite risk score, in the model it was not significant after controlling for the other variables ($p = .086$). Nicotine dependence was a trend predictor ($p = .059$) and age was not significant

Table 2
Linear regression models predicting metabolic syndrome composite risk score (N = 109).

Variable	β (Std. Error)	95% Confidence Interval	*p*-value
Model 1: cf-nDNA R^2 = 0.41, 95% CI 0.26, 0.55			
Intercept	−6.11 (5.18)	−16.41, 4.18	.241
CRP	0.24 (0.05)	0.13, 0.35	**< .0001**
cf-nDNA	0.98 (0.37)	0.24, 1.73	**.009**
Age	0.03 (0.03)	−0.03, 0.10	.285
Gender	−3.68 (0.72)	−5.13, −2.23	**< .0001**
Nicotine dependence	−0.28 (.15)	−0.57, 0.01	.059
Physical activity	−0.02 (0.01)	−0.05, 0.00	.086
Model 2: cf-mtDNA R^2 = 0.37, 95% CI 0.22, 0.51			
Intercept	7.05 (4.54)	−1.99, 16.08	.124
CRP	0.27 (0.06)	0.16, 0.38	**< .0001**
cf-mtDNA	−0.06 (0.20)	−0.45, 0.32	.742
Age	0.03 (0.03)	−0.03, 0.10	.322
Gender	−3.57 (0.76)	−5.07, −2.06	**< .0001**
Nicotine dependence	−0.34 (0.15)	−0.64, −0.04	**.024**
Physical activity	−0.03 (0.01)	−0.06, 0.00	.054
Model 3: Sensitivity Analysis of Model 1 R^2 = 0.42, 95% CI 0.27, 0.55			
Intercept	−6.91 (5.26)	−17.35, 3.54	.193
CRP	0.24 (0.05)	0.13, 0.35	**< .0001**
cf-nDNA	1.03 (0.37)	0.29, 1.78	**.007**
Age	0.04 (0.03)	−0.02, 0.11	.211
Gender	−3.89 (0.74)	−5.35, −2.43	**< .0001**
Nicotine dependence	−0.30 (0.15)	−0.60, −0.002	**.048**
Physical activity	−0.02 (0.01)	−0.05, 0.01	.142
Cancer	−1.18 (1.34)	−3.86, 1.50	.381
Depression	0.06 (0.08)	−0.10, .22	.460

Notes: Gender coded as 1 = male, 2 = female. Physical activity defined as moderate to vigorous physical activity. CRP = C-reactive protein. cf-nDNA = Cell-free nuclear DNA.

(p = .285). The overall model R^2 was 0.41, 95% CI [0.26, 0.55].

A second linear regression model predicting metabolic syndrome composite risk score included cf-mtDNA and CRP as predictors and the same covariates reported in the previous model. In this model, cf-mtDNA was not a significant predictor of metabolic syndrome composite risk score ($\beta = -0.06$, $SE = 0.20$, $p = .742$), but the relationship of CRP and metabolic risk remained significant ($\beta = 0.27$, $SE = 0.06$, $p <$ .0001). The overall model R^2 was 0.37, 95% CI [0.22, 0.51].

We then conducted a sensitivity analysis to examine the effects of cancer history and depressive symptoms, as these conditions may each affect cf-nDNA levels, cardiometabolic indicators, and CRP. The relationship of cf-nDNA and CRP with the metabolic syndrome composite remained significant after controlling for these variables. The results for each of these three models are displayed in Table 2.

4. Discussion

This is the first study to examine the relationships between cf-nDNA, cf-mtDNA, CRP, and a metabolic syndrome composite risk score in adults. This study was carried out in a sample of adult smokers with depressive symptoms and low levels of physical activity, and also assessed additional contributions of nicotine dependence and physical activity. In the results presented here, cf-nDNA, but not cf-mtDNA, was significantly associated with metabolic risk. Additionally, cf-nDNA and CRP were independent significant predictors of the metabolic syndrome composite risk score in a linear model controlling for relevant covariates including age, gender, nicotine dependence, and physical activity.

These findings add to a small number of existing studies demonstrating associations among levels of cfDNA, cardiometabolic risk, and other indicators of inflammation (Bartels et al., 2020; Jylhava et al., 2014; Polina et al., 2020). In these studies, total cfDNA was found to be associated with independent metabolic risk factors such as waist circumference, cholesterol (Jylhava et al., 2014), and insulin resistance (Bartels et al., 2020), as well as inflammatory markers including CRP (Bartels et al., 2020; Jylhava et al., 2014). In the present study, we found that cf-nDNA, but not cf-mtDNA, was associated with cardiometabolic risk and CRP. It is notable that cf-nDNA, but not cf-mtDNA, is associated with CRP here, as CRP reflects acute inflammatory processes that have a well-established association with cardiovascular and metabolic disease processes (Jylhava et al., 2014; Libby, 2006; Libby et al., 2002). Different relationships of cf-nDNA and cf-mtDNA with cardiometabolic risk and CRP may be due to distinct mechanisms of release (Aucamp et al., 2018). Factors related to the study sample, such as smoking and underlying medical problems may also influence inflammatory environments (Hosseinzadeh et al., 2016), though no significant relationships with cf-mtDNA and these variables were observed. While the mechanism that underlies these distinct associations remains unclear, it is hypothesized that levels of total cfDNA are related to cardiometabolic risk via greater cellular turnover in central adiposity, providing greater release of cfDNA into circulation (Haghiac et al., 2012). Emerging pre-clinical evidence suggests that increased total cfDNA may contribute to low-grade inflammation present in metabolic syndrome (Nie et al., 2020). In rodent models, cfDNA released from apoptotic adipose cells promotes accumulation of macrophages in adipose tissue via toll-like receptor 9 (Nishimoto et al., 2016), suggesting the DAMP function of cfDNA may contribute to a chronic, low-grade inflammatory state, and potentially accelerating the development of poor cardiovascular outcomes (Nie et al., 2020; McCarthy et al., 2014).

While both cf-nDNA and cf-mtDNA function as DAMPs and can elicit an immune response, cf-mtDNA has molecular elements similar to bacteria which stimulate the innate immune system (Aucamp et al., 2018). Results of pre-clinical work suggest that mitochondrial DNA may function as a mediator in the development of cardiovascular diseases due to its unique inflammatory profile (Nakayama and Otsu, 2018).The findings presented here highlight the importance of future studies examining how cf-mtDNA and cf-nDNA might provide distinct insights into inflammatory pathways within cardiometabolic disease risk.

The present study is an important contribution to the literature in part due to its focus on a sample of individuals that use tobacco and have sedentary lifestyles, each prominent risk factors for cardiometabolic disorders. In our sample, severity of nicotine dependence was negatively associated with composite metabolic syndrome risk score, likely due to inhibitory effects of nicotine on appetite and body mass (Chao et al., 2019), that may have outweighed potential effects on blood pressure and other components of the composite risk score. Nicotine effects did not persist in the model with cf-nDNA, CRP, and covariates, and while some studies have suggested an association of nicotine and cfDNA and other inflammatory processes (Hosseinzadeh et al., 2016), our findings did not indicate a link. Very little is published about the specific relationship of smoking and cfDNA and previous work in this area is limited to samples of pregnant women, with mixed findings indicating both increased cfDNA (Poon et al., 2013) and no difference in cfDNA (Lapaire et al., 2007). The low levels of physical activity in the sample added another dimension to the study because physical activity is hypothesized to alter cfDNA clearance mechanisms (Kustanovich et al., 2019), decreasing cfDNA levels after high-intensity physical exercise (Velders et al., 2014). As such, higher levels of cfDNA would be expected in participants with lower levels of physical activity and higher cardiometabolic risk scores. However, we did not observe a relationship of cfDNA and our measure of physical activity.

We also observed significant contributions of gender to cardiometabolic risk. This is expected due to established differences in cardiometabolic profiles leading to different cut offs for metabolic syndrome criteria between men and women (Grundy et al., 2005). Previous studies have had mixed findings related to associations of cf-nDNA and gender. There is no strong evidence to suggest differences in cf-nDNA or cf-mtDNA levels by gender, but there is some limited evidence to suggest menopause may impact cfDNA levels in women (Jylhava et al., 2012). Only one study has previously examined the relationship of cfDNA to ethnicity or race. In a sample of pregnant women, higher levels of cf-nDNA were found in women reporting Hispanic/Latinx ethnicity and

Black race compared to non-Hispanic/Latinx and White women, respectively (Silver et al., 2017).

The current study measured circulating cfDNA, which has emerged as an increasingly important tool in understanding cellular activity in a range of disease processes. The protocols carried out here reflect current standards for isolating and extraction of genetic material from plasma. The genetic material quantified is considered to be circulating systemically prior to processing, but it is possible that these methods might also quantify intracellular DNA. Even if this was the case, as the same procedure was applied to all participant samples, the effects reported here relating cf-nDNA to metabolic syndrome composite risk score are not likely to be spurious.

The following limitations provide direction for future investigation. The metabolic syndrome composite risk measure used in this study is sample-specific and contains non-fasting measures of triglycerides and uses A1C in place of glucose. A recent epidemiological survey suggests that non-fasting and fasting measures were similarly linked to risk of diabetes and congestive heart disease (CHD) (DeBoer et al., 2020). In that study, the metabolic syndrome measure was a sample-derived z-score sum of each component of metabolic syndrome, which reduces the bias introduced by non-fasting bloodwork. We also found that a diagnosis score calculated by summating the number of cardiometabolic disorder diagnoses (hypertension, hyperlipidemia, and diabetes) reported by participants was strongly associated with the cardiometabolic risk score and weakly associated with cf-nDNA. The metabolic syndrome composite risk score may better reflect the current health status of participants, as the diagnosis score is limited by participant self-report and variable levels of treatment.

4.1. Conclusions

In conclusion, these results demonstrate that cf-nDNA, but not cf-mtDNA, is a predictor of both CRP and a cardiometabolic risk score. Furthermore, cf-nDNA is independent of CRP in predicting metabolic syndrome composite risk score. These findings offer further evidence of a role for cf-nDNA in inflammation and cardiometabolic risk. Cf-mtDNA, which has been shown to activate the immune system in other work, represents a distinct pathway not implicated in cardiometabolic risk in this study. Future work should examine the role of cf-nDNA in pathophysiological processes underlying cardiovascular and metabolic disease.

CRediT author contribution statement

A.M.A. and A.R.T. conceived of the study. T.E.D., E.K.Z., Z.J.K., D.J.P., L.H.P., A.M.A. and A.R.T. were responsible for the study design. A.L.P., P.A.D., and H.T.K. were responsible for performing assays. T.E.D., E.Z.K. and Z.J.K. conducted the data analyses. T.E.D. and E.Z.K. drafted the manuscript. All authors reviewed and edited the manuscript. All authors discussed and approved the final version of the manuscript, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This research was supported by National Institute of Mental Health (NIMH) grant MH101107 (ART), National Cancer Institute (NCI) grant CA173551 (AMA). Dr. Teresa Daniels and Dr. Destiny Price received support from the NIMH grant MH101076 (ART), and Dr. Daniels also received support from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) grant HD101392 (ART).

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

References

Aucamp, J., et al., 2018. The diverse origins of circulating cell-free DNA in the human body: a critical re-evaluation of the literature. Biol. Rev. Camb. Phil. Soc. 93 (3), 1649–1683.
Bai, R.K., Wong, L.J., 2005. Simultaneous detection and quantification of mitochondrial DNA deletion(s), depletion, and over-replication in patients with mitochondrial disease. J. Mol. Diagn. 7 (5), 613–622.
Bartels, M.S., et al., 2020. Increased circulating cell-free DNA in insulin resistance. Diabetes Metab. 46 (3), 249–252.
Buuren, S.v., Groothuis-Oudshoorn, K., 2010. mice: multivariate imputation by chained equations in R. J. Stat. Software 1–68.
Chang, C.P.Y., et al., 2003. Elevated cell-free serum DNA detected in patients with myocardial infarction. Clin. Chim. Acta 327 (1), 95–101.
Chao, A.M., et al., 2019. Tobacco smoking, eating behaviors, and body weight: a review. Curr Addict Rep 6, 191–199.
DeBoer, M.D., Filipp, S.L., Gurka, M.J., 2020. Associations of a metabolic syndrome severity score with coronary heart disease and diabetes in fasting vs. non-fasting individuals. Nutr. Metabol. Cardiovasc. Dis. 30 (1), 92–98.
Freedson, P.S., Melanson, E., Sirard, J., 1998. Calibration of the computer science and applications, inc. accelerometer. Med. Sci. Sports Exerc. 30 (5), 777–781.
Grundy, S.M., et al., 2005. Diagnosis and management of the metabolic syndrome: an American heart association/national heart, lung, and blood Institute scientific statement. Circulation 112 (17), 2735–2752.
Haghiac, M., et al., 2012. Increased death of adipose cells, a path to release cell-free DNA into systemic circulation of obese women. Obesity 20 (11), 2213–2219.
Hosseinzadeh, A., et al., 2016. Nicotine induces neutrophil extracellular traps. J. Leukoc. Biol. 100 (5), 1105–1112.
Jylhava, J., et al., 2012. Circulating cell-free DNA is associated with mortality and inflammatory markers in nonagenarians: the Vitality 90+ Study. Exp. Gerontol. 47 (5), 372–378.
Jylhava, J., et al., 2014. Circulating cell-free DNA is associated with cardiometabolic risk factors: the Health 2000 Survey. Atherosclerosis 233 (1), 268–271.
Kar, D., et al., 2016. Relationship of cardiometabolic parameters in non-smokers, current smokers, and quitters in diabetes: a systematic review and meta-analysis. Cardiovasc. Diabetol. 15 (1), 158.
Kustanovich, A., et al., 2019. Life and death of circulating cell-free DNA. Cancer Biol. Ther. 20 (8), 1057–1067.
Lapaire, O., et al., 2007. Maternal smoking: effect on circulating cell-free fetal and total DNA levels in maternal plasma from the second trimester. Obstet. Gynecol. 110 (6), 1358–1363.
Libby, P., 2006. Inflammation and cardiovascular disease mechanisms. Am. J. Clin. Nutr. 83 (2), 456S–460S.
Libby, P., Ridker, P.M., Maseri, A., 2002. Inflammation and atherosclerosis. Circulation 105 (9), 1135–1143.
Liu, C., et al., 2016. Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: a systematic review and meta-analysis. Cytokine 86, 100–109.
McCarthy, C.G., et al., 2014. Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension. Am. J. Physiol. Heart Circ. Physiol. 306 (2), H184–H196.
Meneses-Gaya, I.C., et al., 2009. Psychometric properties of the fagerstrom test for nicotine dependence. J. Bras. Pneumol. 35 (1), 73–82.
Nakayama, H., Otsu, K., 2018. Mitochondrial DNA as an inflammatory mediator in cardiovascular diseases. Biochem. J. 475 (5), 839–852.
Nie, S., et al., 2020. Pro-inflammatory role of cell-free mitochondrial DNA in cardiovascular diseases. IUBMB Life 72 (9), 1879–1890.
Nishimoto, S., et al., 2016. Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. Sci. Adv. 2 (3), e1501332.
Polina, I.A., Ilatovskaya, D.V., DeLeon-Pennell, K.Y., 2020. Cell free DNA as a diagnostic and prognostic marker for cardiovascular diseases. Clin. Chim. Acta 503, 145–150.
Poon, L.C., et al., 2013. Maternal plasma cell-free fetal and maternal DNA at 11-13 weeks' gestation: relation to fetal and maternal characteristics and pregnancy outcomes. Fetal Diagn. Ther. 33 (4), 215–223.
Radloff, L.S., 1977. The CES-D Scale:A self-report depression Scale for research in the general population. Appl. Psychol. Meas. 1 (3), 385–401.
Ranucci, R., 2019. Cell-free DNA: applications in different diseases. Methods Mol. Biol. 1909, 3–12.
Silver, R.M., et al., 2017. Cell-free total and fetal DNA in first trimester maternal serum and subsequent development of preeclampsia. Am. J. Perinatol. 34 (2), 191–198.
Thierry, A.R., et al., 2016. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev. 35 (3), 347–376.
Velders, M., et al., 2014. Exercise is a potent stimulus for enhancing circulating DNase activity. Clin. Biochem. 47 (6), 471–474.

Wang, L., et al., 2015. Plasma nuclear and mitochondrial DNA levels in acute myocardial infarction patients. Coron. Artery Dis. 26 (4), 296–300.
Weiss, T.W., Arnesen, H., Seljeflot, I., 2013. Components of the interleukin-6 transsignalling system are associated with the metabolic syndrome, endothelial dysfunction and arterial stiffness. Metabolism 62 (7), 1008–1013.
Xie, J., Yang, J., Hu, P., 2018. Correlations of circulating cell-free DNA with clinical manifestations in acute myocardial infarction. Am. J. Med. Sci. 356 (2), 121–129.