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Mitochondrial DNA copy number and diabetes: the Atherosclerosis Risk in Communities (ARIC) study

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ABSTRACT

Introduction Mitochondrial DNA copy number (mtDNA-CN) is a measure of mitochondrial dysfunction and is associated with diabetes in experimental models. To explore the temporality of mitochondrial dysfunction and diabetes, we estimated the prevalent and incident association of mtDNA-CN and diabetes.

Research design and methods We assessed the associations of mtDNA-CN measured from buffy coat with prevalent and incident diabetes, stratified by race, in 8954 white and 2444 black participants in the Atherosclerosis Risk in Communities (ARIC) study, an observational cohort study. Follow-up for incident analyses was complete through visit 6, 2016.

Results Mean age at mtDNA-CN measurement was 57 years and 59% were female. Prevalence of diabetes at time of mtDNA-CN measurement was higher in blacks (563/2444, 23%) than whites (855/8954, 10%). The fully adjusted odds of prevalent diabetes for the 10th vs 90th percentile of mtDNA-CN was 1.05 (95% CI 0.74 to 1.49) among black and 1.49 (95% CI 1.20 to 1.85) among white participants. Over a median follow-up time of 19 years (Q1, Q3: 11, 24 years), we observed 617 incident diabetes cases among 1744 black and 2121 cases among 7713 white participants free of diabetes at baseline. The fully adjusted hazard of incident diabetes for the 10th vs 90th percentile of mtDNA-CN was 1.07 (95% CI 0.84 to 1.38) among black and 0.97 (95% CI 0.86 to 1.10) among white participants.

Conclusions Lower mtDNA-CN in buffy coat was associated with prevalent diabetes in white but not black ARIC participants. Lower mtDNA-CN was not associated with incident diabetes over 20 years of follow-up in whites or blacks.

Significance of this study

What is already known about this subject?

- Markers of mitochondrial dysfunction are associated with insulin resistance and diabetes in animal models and small human trials, but this association has not been explored in a large community-based population.
- In this cohort, previous research has demonstrated that peripheral mitochondrial DNA copy number (mtDNA-CN), a marker of mitochondrial function, is associated with chronic kidney disease, heart disease, frailty and all-cause mortality.

What are the new findings?

- Lower mtDNA-CN was associated with prevalent, but not incident, diabetes in this sample.
- Associations differed between white and black participants, although it is unclear if the lack of association in blacks was due to a smaller sample size.

How might these results change the focus of research or clinical practice?

- This research question should be addressed in a more diverse prospective community-based study with multiple mtDNA-CN measurements per person to provide a more definitive answer to the mitochondrial dysfunction and diabetes temporality question.
- Peripheral mtDNA-CN can be used to further explore the role of mitochondrial dysfunction and its metabolic implications in chronic disease, aging and mortality.

INTRODUCTION

Insulin resistance and type 2 diabetes are associated with markers of mitochondrial dysfunction including increased glycolysis and decreased oxidative phosphorylation in muscle,[1–5] decreased mitochondrial size and density,[6–9] decreased oxidative gene expression[10 11] and decreased aerobic capacity.[12–14] The association of mitochondrial dysfunction with insulin resistance and diabetes may be due to a number of possibilities. Genetic forms of mitochondrial dysfunction can cause insulin resistance and diabetes.[15] Mitochondrial dysfunction may also be a key element in the pathway leading to insulin resistance. For example, decreased fitness may lead to mitochondrial dysfunction and subsequent insulin resistance in muscle.[6–9 12 14 16] Similarly, increased adiposity may lead to hypoxia and subsequent mitochondrial dysfunction and insulin resistance in adipose tissue.[17 18] Alternatively, mitochondrial dysfunction may be a consequence, rather than a cause, of insulin resistance. For example, hyperglycemia and lipid toxicity may lead to mitochondrial

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dysfunction in those with diabetes.[19] Whether mitochondrial dysfunction is a cause or consequence of population variation in insulin resistance and diabetes, however, is unknown.

In support of an early role for mitochondrial dysfunction in the development of insulin resistance, we have previously shown that blood lactate, a marker of mitochondrial dysfunction, is strongly associated with incident diabetes, independent of traditional risk factors.[20 21] Blood lactate's association with incident diabetes may be due to primary mitochondrial dysfunction as occurs in genetic forms of insulin resistance. Alternatively, the association may be due to mitochondrial dysfunction in muscle resulting from low fitness or in adipose tissue resulting from hypoxia. One approach to address this question is to determine if mitochondrial dysfunction in tissues other than those affected by low fitness or increased adiposity is associated with insulin resistance and diabetes.

Mitochondrial DNA copy number (mtDNA-CN) represents a marker of mitochondrial dysfunction that can be assessed in large research or clinical populations. mtDNA-CN represents the amount of mtDNA relative to nuclear DNA in a cell[22] and is associated with mitochondrial enzyme activity and ATP production, which are measures of mitochondrial function.[22] mtDNA-CN can be measured in population-based studies using circulating leukocytes and has been associated with diabetes in animal studies and small human studies.[23–25] Previous prospective cohort studies have shown that lower mtDNA-CN is associated with frailty and all-cause mortality,[26] sudden cardiac death,[27] cardiovascular disease[28] and chronic kidney disease.[29] Here, we examined the cross-sectional and incident association of mtDNA-CN with diabetes in middle-aged adults from the Atherosclerosis Risk in Communities (ARIC) study to explore the temporality of mitochondrial dysfunction and diabetes. We hypothesized that mtDNA-CN would be lower among persons with diabetes compared with those without diabetes. Furthermore, we hypothesized that lower mtDNA-CN would be associated with an increased risk of incident diabetes.

RESEARCH DESIGN AND METHODS

Study population

The ARIC study is an ongoing prospective cohort study of 15 792 individuals aged 45–64 years initially recruited from 4 US communities between 1987 and 1989.[30] Follow-up visits occurred in 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), 2011–2013 (visit 5) and 2016–2017 (visit 6) with annual or semi-annual telephone follow-up in between visits. Additional details regarding the design and methods of the ARIC study are described elsewhere.[30]

Two study populations were constructed: one for cross-sectional analyses and one for incident analyses. The cross-sectional analyses include 8964 white and 2466 black ARIC participants for a total of 11 431 participants with measured mtDNA-CN at visits 1, 2, 3 or 4 (online supplementary table S1). The incident analyses included 7857 white participants and 1835 black participants without diabetes at time of mtDNA-CN measurement.

Measurement of mtDNA-CN

To measure mtDNA-CN, DNA was extracted using the Gentra Puregene Blood Kit (Qiagen) from buffy coat collected at ARIC visits 1 through 4. mtDNA-CN measurement was done based on available buffy coat samples for extraction. Array genotyping data (Affymetrix 6.0 array, genome-wide single nucleotide polymorphism (SNP) array) was available in 13 444 of the 15 792 participants. There were additional sample exclusions based on sample quality, relatedness and not self-identifying as black or white, which have been previously described.[29] Of the 11 431 participants included in these analyses, 480 had mtDNA measured at visit 1, 9093 at visit 2, 1791 at visit 3 and 67 at visit 4. No participants had measurements at more than one visit. For a given participant, the visit at which mtDNA-CN was measured serves as baseline for these analyses. See online supplementary file 1 for a detailed breakdown of mtDNA-CN by visit and race.

Briefly, DNA samples from buffy coat were used to calculate mtDNA-CN from probe intensities of mitochondrial SNPs on Affymetrix Genome-Wide Human SNP Array 6.0 (Genvisis). This mtDNA-CN measure serves as a ratio of mtDNA to nuclear DNA per sample, representing cellular mtDNA content. To correct for batch effects, DNA quality and the beginning DNA sample quantity, surrogate variable analysis was applied to probe intensities of 43 316 autosomal SNPs to adjust for the amount of total DNA hybridized to the array and additional technical artifacts.[31] Standardized residuals were generated using linear regression to adjust for age, sex, collection site, race/ethnicity, white blood cell count and the surrogate variables. These standardized residuals were used as the mtDNA-CN metric for analyses (ie, mean of 0 and SD of 1). A negative mtDNA-CN in these analyses represents lower mtDNA cell content. Of note, white blood cell counts differ by race due to population differences in genetic polymorphisms. As mtDNA-CN was measured in buffy coat, we controlled for potential confounding by white blood cell count by calculating mtDNA-CN quintiles separately by race.

Outcome definition

Diabetes was defined as self-reported diabetes diagnosis by a physician, fasting blood glucose ≥126 mg/dL, non-fasting blood glucose ≥200 mg/dL or use of glucose-lowering medication at mtDNA-CN measurement, with medications verified by study staff at clinic visits. Persons were classified as incident cases if they were free of diabetes at baseline and met the above criteria for diabetes at subsequent study visits or self-reported diagnosis or medication use via annual telephone follow-up calls through visit 6. Participants who did not develop

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diabetes were censored on the date of death, date of last annual follow-up response or ARIC visit, up to 2016. Note that visit 7 was completed in 2019.

Other measurements

Trained personnel collected all data using standardized protocols with extensive quality control measures, as described previously.[3 32] Age, sex, race and parental history of diabetes were assessed via self-report. Prevalent cardiovascular disease was operationalized as any self-report of stroke or coronary heart disease (coronary heart disease or ECG confirmation of previous myocardial infarction) at visit 1. For subsequent visits, adjudicated cardiovascular events occurring between visit 1 and the visit of interest were also included as prevalent cardiovascular disease. Smoking and drinking status were self-reported and categorized as never, former or current. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or use of any antihypertensive medication in the past few weeks. Height, weight, waist circumference at the umbilical level and hip circumference at the top of the iliac crest were measured during physical examinations. High-risk waist-to-hip ratio (WHR) was defined as >0.90 for males and >0.85 for females. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, fasting glucose and fasting insulin were measured in serum. Serum fasting glucose was quantified using a hexokinase method. We calculated the homeostatic model assessment insulin resistance index (HOMA-IR) using serum insulin from visit 1 as ((fasting insulin in μ/mL)×(fasting glucose in mg/dL))/405. Of the 480 mtDNA-CN measures at visit 1 479 had insulin data. See online supplementary table S1 for an overlap of participants with mtDNA-CN measurements and insulin measurements. High-sensitivity C reactive protein (hs-CRP) was measured from blood collected at visit 2 using immunoturbidimetric assay on the Roche Modular P chemistry analyzer.[33] Of the 9093 mtDNA-CN measures at visit 2, 8752 had hs-CRP data also from visit 2. hs-CRP was also measured at visit 4, however mtDNA-CN samples did not overlap with measured hs-CRP from visit 4. Detailed methods on measuring lactate are described elsewhere,[34] but briefly plasma lactate was measured at visit 4 using enzymatic reaction to convert lactate to pyruvate with a Roche Hitachi 911 auto-analyzer. Of the 9093 mtDNA-CN measures at visit 2 649 had lactate data from visit 4. See online supplementary table S1 for breakdown of mtDNA-CN samples by visit and race with overlap of these variables.

Statistical analyses

We generated mtDNA-CN quintiles separately by race, as mtDNA-CN variance differs by race. We examined baseline characteristics in race-stratified datasets by mean (SD) or per cent (N) across mtDNA-CN quintiles. P value for trend was calculated over the quintiles using Stata *nptrend*, which is an extension of the Wilcoxon rank-sum test. We used logistic regression to estimate the ORs and 95% CIs for the associations between mtDNA-CN and prevalent diabetes. We used a Cox proportional hazards model to estimate the HR and 95% CI for the association between mtDNA-CN and incident diabetes, among participants free of diabetes at baseline visit (when mtDNA-CN was measured). We also compared the cumulative incidence of diabetes by mtDNA-CN quintiles using the Kaplan-Meier survival curve, with time measured from baseline visit (when mtDNA-CN was measured). In both prevalent and incident analyses, we modeled mtDNA-CN in quintiles and as a continuous variable, comparing the 10th with the 90th percentile of mtDNA-CN. Additional methods can be found in the online supplementary file 1.

We used four multivariable models to adjust for confounding with progressive adjustment for both prevalent and incident analyses. Model 1 was adjusted for age at baseline visit (when mtDNA-CN was measured) and sex. Model 2 was adjusted for model 1 variables plus hypertension, parental history of diabetes, prevalent cardiovascular disease, smoking status and drinking status. Model 3 was adjusted for model 2 variables plus BMI, WHR and hs-CRP. Model 4 was adjusted for model 3 variables plus log HDL-C, log LDL-C, log total cholesterol and log triglycerides.

We tested for heterogeneity by sex using a multiplicative interaction term in both prevalent and incident models. We conducted sensitivity analyses by estimating the association between mtDNA-CN and diabetes after excluding participants with prevalent cardiovascular disease at the time of mtDNA-CN measurement. All statistical analyses were performed using Stata IC V.14.1 (StataCorp, College Station, Texas, USA).

RESULTS

Prevalent diabetes

Of the 11 431 participants with mtDNA-CN measured between visit 1 and visit 4, 23% (563/2444) black and 10% (855/8954) white participants had diabetes at the time of mtDNA-CN measurement. The breakdown of prevalence by visit was as follows: for blacks, 25% (29/116) at visit 1, 22% (414/1918) at visit 2, 28% (110/397) at visit 3 and 30% (10/35) at visit 4; for whites, 13% (48/364) at visit 1, 8% (592/7175) at visit 2, 15% (208/1394) at visit 3 and 21% (7/32) at visit 4 (online supplementary table S1). The mean age of black participants was 57 years, 64% were female, 59% had a history of hypertension, 11% had a history of cardiovascular disease and 22% had a parental history of diabetes (table 1). The mean age of white participants was 58 years, 53% were female, 30% had a history of hypertension, 9% had a history of cardiovascular disease and 18% had a parental history of diabetes (table 2).

The crude odds of prevalent diabetes in 2444 black participants was similar across mtDNA-CN quintiles, with an OR of 0.98 (95% CI 0.73 to 1.31) comparing quintile 1 with

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Table 1 Baseline characteristics of 2444 black study participants by quintiles of mtDNA-CN, Atherosclerosis Risk in Communities study, 1987–1998

	Mitochondrial DNA copy number quintiles					
	Overall	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Blacks (N)	2444	489	490	488	488	489
mtDNA-CN	0 (1)	−1.48 (0.76)	−0.39 (0.16)	0.09 (0.13)	0.55 (0.14)	1.25 (0.37)
Age, years	56.7 (6.0)	56.8 (6.1)	56.9 (6.1)	56.7 (5.9)	56.6 (5.9)	56.7 (5.9)
Female	64% (1554)	62% (308)	63% (310)	63% (311)	64% (315)	63% (310)
Center						
Forsyth County, North Carolina	12% (303)	11% (56)	14% (71)	11% (55)	13% (63)	12% (58)
Jackson, Mississippi	88% (2163)	89% (438)	86% (422)	89% (438)	87% (430)	88% (435)
Minneapolis, Minnesota	–	–	–	–	–	–
Washington County, Maryland	–	–	–	–	–	–
Hypertension	59% (1460)	62% (305)	60% (295)	56% (277)	57% (282)	61% (301
Cardiovascular disease	11% (258)	15% (73)	10% (48)	10% (49)	8% (40)	10% (48)
Parental history of diabetes	22% (531)	23% (112)	20% (100)	21% (103)	21% (104)	23% (112)
Diabetes	23% (563)	23% (115)	20% (99)	23% (113)	24% (119)	24% (117)
BMI, kg/m^2	30.1 (6.3)	30.0 (6.4)	29.5 (5.8)	30.6 (6.5)	30.2 (6.6)	30.1 (6.3)
High-risk waist-to-hip ratio						
Males (>0.90)	83% (761)	82% (153)	85% (155)	82% (149)	84% (149)	85% (155)
Females (>0.85)	81% (1252)	82% (253)	80% (249)	83% (258)	78% (245)	80% (247)
HDL-C, mg/dL						
Male	49 (17.5)	51 (19.0)	46 (15.4)	49 (14.8)	50 (20.0)	49 (17.5)
Female	56 (16.8)	55 (15.5)	57 (17.4)	56 (16.7)	56 (18.1)	55 (16.0)
Total cholesterol, mg/dL	211 (41.8)	210 (43.5)	210 (40.9)	210 (38.9)	212 (42.8)	214 (42.8)
LDL-C, mg/dL	134 (39.7)	133 (40.3)	134 (38.6)	133 (36.5)	134 (41.8)	137 (41.2)
Triglycerides, mg/dL	119 (76.5)	122 (99.9)	117 (73.2)	117 (72.3)	118 (67.2)	119.8 (64.7)
Smoking status						
Never	43% (1063)	39% (194)	42% (208)	46% (229)	45% (224)	42% (208)
Former	29% (723)	30% (148)	28% (137)	30% (147)	28% (140)	31% (151)
Current	27% (658)	30% (148)	29% (142)	23% (115)	25% (123)	26% (130)
Alcohol drinker						
Never	34% (840)	32% (160)	31% (154)	38% (187)	32% (158)	37% (181)
Former	32% (787)	34% (169)	33% (162)	31% (151)	32% (157)	30% (148)
Current	33% (817)	33% (161)	35% (172)	31% (153)	35% (172)	32% (159)
Hemoglobin A1C, %	6.4 (1.7)	6.4 (1.7)	6.3 (1.7)	6.4 (1.8)	6.3 (1.6)	6.4 (1.8)
Hemoglobin A1C, mmol/mol	46 (18.9)	46 (18.7)	45 (18.4)	46 (20.0)	46 (17.9)	47 (19.6)
Lactate, mmol/L†	9.5 (3.9)	9.8 (4.4)	9.2 (3.5)	9.4 (3.7)	9.9 (4.1)	9.4 (3.8)
Fasting glucose, mg/dL*	130 (66.8)	132 (70.6)	128 (68.3)	129 (67.6)	131 (66.5)	129 (61.1)
HOMA-IR, U	0.06 (0.03)	0.06 (0.14)	0.08 (0.15)	0.06 (0.08)	0.06 (0.08)	0.04 (0.03)
hs-CRP, mg/L†	6.1 (8.2)	5.6 (8.2)	6.4 (8.9)	6.2 (7.1)	6.5 (8.3)	5.9 (8.3)

Continued

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Table 1 Continued

	Mitochondrial DNA copy number quintiles					
	Overall	Q1	Q2	Q3	Q4	Q5

Mean (SD) or % (N). Hypertension defined as diastolic blood pressure ≥90 mm Hg or systolic blood pressure ≥140 mm Hg or use of any antihypertensive medication in the past 2 weeks. Cardiovascular disease defined as self-reported history of stroke or coronary heart disease (previous myocardial infarction, coronary heart disease or ECG confirmation of previous myocardial infarction). Diabetes defined as self-report of doctor diagnosis, use of glucose-lowering medication, fasting blood glucose ≥126 mg/dL or non-fasting blood glucose ≥200 mg/dL. To convert lipids from mg/dL to mmol/L, divide by 38.67 for total cholesterol, HDL-C and LDL-C, and by 88.57 for triglycerides.
*Glucose (mg/dL)/405, with insulin measured at visit 1.
†Lactate measured at visit 4, n=259 blacks (224 without diabetes, 32 with diabetes) and n=390 whites (357 without diabetes, 32 with diabetes); CRP measured at visit 2, n=1838 blacks (1431 without diabetes, 392 with diabetes) and n=6914 whites (6356 without diabetes, 554 with diabetes).
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance (insulin (U/L); hs-CRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; mtDNA-CN, mitochondrial DNA copy number.

quintile 5 (table 3, p-trend=0.39). Adjustment for demographic factors, comorbidities, anthropometrics and lipids did not change the association (Q1 vs Q5 OR 0.89 (95% CI 0.56 to 1.41) in fully adjusted model). Comparing the 10th with the 90th mtDNA-CN percentile, the OR for diabetes was 0.95 (95% CI 0.77 to 1.21) in the unadjusted model vs 1.05 (95% CI 0.74 to 1.49) in the fully adjusted model (online supplementary figure S1). Tests for heterogeneity did not reveal a significant interaction between mtDNA-CN quintile and sex (online supplementary table S2).

The crude odds of prevalent diabetes in the 8954 white participants significantly differed by quintile (online supplementary figure S1). Those in Q1 had 1.87 times the odds of prevalent diabetes than those in the Q5 (95% CI 1.49 to 2.33, p-trend<0.0001). Adjustment for age and sex did not appreciably change this association. Adjustment for comorbidities, anthropometrics and lipids slightly attenuated the association (table 3, model 4) but the linear trend remained significant. The OR for diabetes in the 10th vs the 90th mtDNA-CN percentile ranged from 1.79 (95% CI 1.53 to 2.10) in the unadjusted model to 1.49 (95% CI 1.20 to 1.85) in the fully adjusted model.

Among blacks, 17.9% (101/563) of those with prevalent diabetes at baseline also had prevalent cardiovascular disease compared with 8.2% (155/1881) of those without prevalent diabetes (χ^2 p<0.0001). Among whites, 21.8% (185/855) of those with prevalent diabetes at baseline also had prevalent cardiovascular disease compared with 7.7% (627/8099) of those without prevalent diabetes (χ^2 p<0.0001). In our sensitivity analyses, after excluding participants with prevalent cardiovascular disease at baseline, the association between mtDNA-CN and prevalent diabetes was stronger (online supplementary table S3), with an adjusted OR of 1.13 (95% CI 0.77 to 1.66) vs 1.05 (95% CI 0.74 to 1.49) in original analyses in blacks (online supplementary figure S2) and an adjusted OR of 1.64 (95% CI 1.29 to 2.09) vs 1.49 (95% CI 1.20 to 1.85) in original analyses in whites (online supplementary figure S3).

Incident diabetes

Among 1744 black participants without diabetes at mtDNA-CN measurement, 617 developed diabetes over a median follow-up of 16 years (Q1, Q3: 9, 23 years). Among 7713 white participants without diabetes at mtDNA-CN measurement, 2121 developed diabetes over a median follow-up of 20 years (Q1, Q3: 12, 24 years). The mean age of black participants without diabetes at mtDNA-CN measurement was 56 years, 62% were female, 54% had a history of hypertension, 8% had a history of cardiovascular disease and 19% had a parental history of diabetes (online supplementary table S4). The mean age of white participants without diabetes at mtDNA-CN measurement was 57 years, 55% were female, 27% had a history of hypertension, 7% had a history of cardiovascular disease and 17% had a parental history of diabetes (online supplementary table S5). Further details on follow-up time by race, incident diabetes status and baseline mtDNA-CN measurement visit can be found in online supplementary table S6 and online supplementary table S7.

mtDNA-CN measured between visits 1 and 4 was not associated with incident diabetes among blacks or whites in these analyses (table 4, online supplementary figure S4). Compared with quintile 5, the hazard of incident diabetes in quintile 1 was 1.20 (95% CI 0.95 to 1.52) in the unadjusted model and 1.19 (95% CI 0.88 to 1.62) in the fully adjusted model among blacks, and 1.00 (95% CI 0.88 to 1.13) to 1.05 (95% CI 0.91 to 1.22) among whites. Fully adjusted HR for the 10th vs the 90th percentile of mtDNA-CN was 1.10 (95% CI 0.88 to 1.37) among blacks and 1.02 (95% CI 0.90 to 1.14) among whites. A multiplicative interaction term between mtDNA-CN quintile and sex among whites was statistically significant (p=0.03) after adjusting for age alone, but no longer significant after adjusting for additional covariates (online supplementary table S8).

Regarding sensitivity analyses excluding prevalent cardiovascular disease, we found that among blacks, 8.5% (51/700) of those who developed incident diabetes also had prevalent cardiovascular disease at baseline, compared with 7.3% (92/1080) of those who did not develop incident diabetes (χ^2 p=0.35) (online supplementary figure S2). Among whites, 8.3% (190/2303) of those who developed incident diabetes over follow-up also had prevalent cardiovascular disease compared with 7.1% (385/5434) who did not (χ^2 p=0.07) (online supplementary figure S3). Our findings after excluding those with prevalent cardiovascular disease at baseline were not substantively different than our main findings (online supplementary table S9).

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Table 2 Baseline characteristics of 8954 white study participants by quintiles of mtDNA-CN, Atherosclerosis Risk in Communities study, 1987–1998

	Mitochondrial DNA copy number quintiles					
	Overall	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
N	8954	1791	1791	1790	1791	1791
mtDNA-CN	0 (1.0)	−1.44 (0.72)	−0.44 (0.16)	0.06 (0.13)	0.53 (0.14)	1.30 (0.45)
Age, years	57.6 (5.9)	57.7 (6.0)	57.5 (6.0)	57.6 (6.0)	57.5 (5.9)	57.6 (5.8)
Female	53% (4766)	54% (960)	54% (968)	52% (939)	53% (958)	52% (941)
Center						
Forsyth County, North Carolina	30% (2733)	31% (556)	31% (549)	30% (534)	29% (525)	32% (569)
Jackson, Mississippi	–	–	–	–	–	–
Minneapolis, Minnesota	37% (3316)	36% (644)	37% (667)	39% (706)	39% (692)	34% (607)
Washington County, Maryland	33% (2916)	33% (593)	32% (577)	31% (553)	32% (576)	34% (617
Hypertension	30% (2728)	33% (597)	31% (552)	30% (536)	29% (512)	30% (531)
Cardiovascular disease	9% (816)	12% (217)	9% (9160)	9% (159)	7% (131)	8% (149)
Parental history of diabetes	18% (1651)	19% (336)	19% (333)	17% (307)	17% (302)	21% (373)
Diabetes	10% (855)	13% (237)	10% (185)	9% (165)	7% (133)	8% (135)
BMI, kg/m^2	27 (5.0)	28 (5.2)	27 (5.0)	28 (5.0)	27 (4.9)	28 (5.2)
High-risk waist-to-hip ratio						
Males (>0.90)	91% (3839)	93% (775)	93% (767)	90% (767)	91% (760)	90% (770)
Females (>0.85)	68% (3242)	68% (652)	66% (640)	72% (672)	65% (622)	70% (656)
HDL-C, mg/dL						
Male	42 (12.9)	40 (12.2)	42 (13.4)	42 (12.1)	43 (13.5)	42.5 (13.2)
Female	56 (17.2)	54 (17.2)	56 (16.7)	56 (17.4)	57 (17.0)	56 (17.8)
Total cholesterol, mg/dL	210 (39.1)	209 (41.2)	209 (39.0)	210 (37.6)	209 (37.5)	211 (39.9)
LDL-C, mg/dL	132 (36.1)	132 (37.3)	131 (35.8)	132 (35.1)	132 (35.6)	134 (36.7)
Triglycerides, mg/dL	143 (94.7)	140 (115.4)	142 (90.2)	142 (83.5)	137 (86.1)	142 (94.6)
Smoking status						
Never	37% (3318)	35% (636)	39% (695)	37% (655)	37% (659)	38% (673)
Former	41% (3674)	39% (708)	39% (696)	42% (745)	43% (770)	42% (755)
Current	22% (1969)	25% (449)	22% (502)	22% (392)	20% (363)	20% (363)
Alcohol drinker						
Never	18% (1606)	20% (363)	18% (322)	17% (310)	16% (286)	18% (325)
Former	18% (1650)	20% (367)	17% (305)	20% (352)	17% (296)	18% (330)
Current	64% (5704)	59% (1063)	65% (1166)	63% (1130)	67% (1210)	63% (1135)
Hemoglobin A1C, %	5.6 (0.9)	5.7 (1.1)	5.6 (1.0)	5.6 (0.8)	5.5 (0.8)	5.6 (0.8)
Hemoglobin A1C, mmol/mol	38 (10.1)	39 (12.3)	38 (10.6)	37 (9.1)	37 (9.0)	38 (9.1)
Lactate, mmol/L†	8.5 (3.6)	8.0 (2.6)	8.3 (3.2)	8.8 (3.3)	8.2 (3.6)	9.0 (4.7)
Fasting glucose, mg/dL*	109 (34.4)	114 (42.3)	109 (35.0)	108 (32.4)	107 (30.5)	108 (30.3)
HOMA-IR, U	0.04 (0.07)	0.04 (0.08)	0.03 (0.03)	0.03 (0.05)	0.03 (0.03)	0.06 (0.12)
hs-CRP, mg/L†	3.9 (7.1)	4.2 (6.4)	3.6 (5.4)	4.0 (8.2)	3.5 (5.6)	4.2 (8.9)

Mean (SD) or % (N). Hypertension defined as diastolic blood pressure ≥90 mm Hg or systolic blood pressure ≥140 mm Hg or use of any antihypertensive medication in the past 2 weeks. Cardiovascular disease defined as self-reported history of stroke or coronary heart disease (previous myocardial infarction, coronary heart disease or ECG confirmation of previous myocardial infarction). Diabetes defined as self-report of doctor diagnosis, use of glucose-lowering medication, fasting blood glucose ≥126 mg/dL or non-fasting blood glucose ≥200 mg/dL. To convert lipids from mg/dL to mmol/L, divide by 38.67 for total cholesterol, HDL-C and LDL-C, and by 88.57 for triglycerides.
*Glucose (mg/dL)/405, with insulin measured at visit 1.
†Lactate measured at visit 4, n=390 whites (357 without diabetes, 32 with diabetes); CRP measured at visit 2, n=6914 whites (6356 without diabetes, 554 with diabetes).
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance (insulin (U/L); hs-CRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; mtDNA-CN, mitochondrial DNA copy number.

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Table 3 ORs (95% CIs) for the association of mtDNA-CN quintile and 10th vs 90th percentile with prevalent diabetes among 2444 black and 8954 white participants, visits 1–4, Atherosclerosis Risk in Communities study, 1987–1998

mtDNA-CN quintile	Unadjusted	Model 1	Model 2	Model 3	Model 4
Black (N)	2444	2444	1510	1438	1419
Q1	0.98 (0.73 to 1.31)	0.97 (0.72 to 1.31)	0.84 (0.56 to 1.25)	0.87 (0.56 to 1.34)	0.89 (0.56 to 1.41)
Q2	0.81 (0.60 to 1.09)	0.79 (0.59 to 1.08)	0.71 (0.47 to 1.08)	0.79 (0.50 to 1.22)	0.83 (0.52 to 1.32)
Q3	0.96 (0.71 to 1.29)	0.95 (0.71 to 1.29)	0.90 (0.61 to 1.34)	0.88 (0.57 to 1.34)	0.89 (0.57 to 1.39)
Q4	1.03 (0.76 to 1.37)	1.04 (0.77 to 1.39)	0.83 (0.56 to 1.25)	0.93 (0.60 to 1.43)	0.98 (0.62 to 1.55)
Q5	Ref	Ref	Ref	Ref	Ref
P for trend	0.39	0.35	0.27	0.37	0.45
10th vs 90th percentile	0.96 (0.77 to 1.21)	0.95 (0.76 to 1.20)	1.00 (0.74 to 1.37)	1.03 (0.73 to 1.44)	1.05 (0.74 to 1.49)
White (N)	8954	8954	6681	6437	6320
Q1	1.87 (1.49 to 2.33)	1.87 (1.49 to 2.34)	1.69 (1.28 to 2.23)	1.77 (1.32 to 2.39)	1.73 (1.27 to 2.36)
Q2	1.41 (1.12 to 1.78)	1.43 (1.13 to 1.80)	1.34 (1.00 to 1.80)	1.52 (1.11 to 2.08)	1.57 (1.13 to 2.17)
Q3	1.24 (0.98 to 1.55)	1.24 (0.97 to 1.57)	1.06 (0.78 to 1.44)	1.13 (0.81 to 1.57)	1.16 (0.83 to 1.63)
Q4	0.98 (0.77 to 1.26)	0.99 (0.77 to 1.27)	1.11 (0.82 to 1.51)	1.22 (0.88 to 1.69)	1.23 (0.88 to 1.73)
Q5	Ref	Ref	Ref	Ref	Ref
P for trend	<0.0001	0.0001	0.0001	0.0002	0.0002
10th vs 90th percentile	1.79 (1.53 to 2.10)	1.79 (1.53 to 2.10)	1.53 (1.25 to 1.86)	1.55 (1.25 to 1.91)	1.49 (1.20 to 1.85)

(N) indicates number observations included in model. P for linear trend using Stata *contrast*. Model 1 adjusted for age and sex. Model 2 adjusted for model 1+hypertension, parental history of diabetes, previous cardiovascular disease, smoking status and drinking status (current, former, never). Model 3 adjusted for model 2+body mass index, waist-to-hip ratio and hs-CRP. Model 4 adjusted for model 3+log triglycerides, log HDL-C, log LDL-C and log total cholesterol. mtDNA-CN quintile cut-offs (SD), black: –1.48 (0.76), –0.39 (0.16), 0.09 (0.13), 0.55 (0.14), 1.25 (0.37); white: –1.44 (0.72), –0.44 (0.16), 0.06 (0.13), 0.53 (0.14), 1.30 (0.45).
HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; mtDNA-CN, mitochondrial DNA copy number.

Table 4 HRs (95% CIs) for the association of mtDNA-CN quintile and 10th vs 90th percentile with incident diabetes among 1774 black and 7713 white participants without diabetes at baseline, Atherosclerosis Risk in Communities study, 1987–2016

mtDNA-CN quintile	Unadjusted	Model 1	Model 2	Model 3	Model 4
Black, N (events)	1744 (617)	1744 (617)	1061 (372)	1015 (358)	1005 (351)
Q1	1.20 (0.94 to 1.53)	1.21 (0.95 to 1.55)	1.10 (0.80 to 1.51)	1.11 (0.80 to 1.55)	1.14 (0.82 to 1.59)
Q2	0.95 (0.73 to 1.22)	0.95 (0.73 to 1.22)	0.92 (0.66 to 1.28)	0.92 (0.66 to 1.28)	0.94 (0.67 to 1.32)
Q3	0.90 (0.69 to 1.17)	0.90 (0.69 to 1.17)	0.84 (0.60 to 1.17)	0.82 (0.48 to 1.16)	0.84 (0.60 to 1.19)
Q4	0.93 (0.72 to 1.19)	0.93 (0.72 to 1.20)	0.82 (0.69 to 1.14)	0.89 (0.63 to 1.25)	0.90 (0.64 to 1.28)
Q5	Ref	Ref	Ref	Ref	Ref
P for trend	0.17	0.16	0.41	0.51	0.41
10th vs 90th percentile	1.14 (0.95 to 1.38)	1.15 (0.95 to 1.38)	1.06 (0.82 to 1.35)	1.07 (0.83 to 1.37)	1.07 (0.84 to 1.38)
White, N (events)	7713 (2121)	7713 (2121)	5737 (1639)	5557 (1596)	5487 (1563)
Q1	0.97 (0.85 to 1.10)	0.97 (0.85 to 1.11)	0.96 (0.82 to 1.11)	1.00 (0.86 to 1.17)	1.01 (0.86 to 1.17)
Q2	0.84 (0.74 to 0.97)	0.85 (0.74 to 0.97)	0.82 (0.70 to 0.96)	0.85 (0.72 to 0.99)	0.83 (0.71 to 0.98)
Q3	0.97 (0.85 to 1.10)	0.97 (0.85 to 1.11)	1.00 (0.86 to 1.16)	1.00 (0.85 to 1.17)	0.98 (0.84 to 1.15)
Q4	0.88 (0.77 to 1.01)	0.89 (0.78 to 1.01)	0.95 (0.82 to 1.10)	0.98 (0.84 to 1.14)	0.98 (0.84 to 1.14)
Q5	Ref	Ref	Ref	Ref	Ref
P for trend	0.45	0.49	0.18	0.44	0.39
10th vs 90th percentile	1.00 (0.89 to 1.12)	1.00 (0.90 to 1.12)	0.95 (0.84 to 1.07)	0.98 (0.87 to 1.11)	0.97 (0.86 to 1.10)

(N) indicates number observations included in model. P for linear trend using Stata *contrast*. Model 1 adjusted for age and sex. Model 2 adjusted for model 1+hypertension, parental history of diabetes, prevalent cardiovascular disease, smoking status (current, former, never) and alcohol drink. Model 3 adjusted for model 2+body mass index, waist-to-hip ratio and hs-CRP. Model 4 adjusted for model 3+log triglycerides, log HDL-C, log LDL-C and log total cholesterol.
HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C reactive protein; LDL-C, low-density lipoprotein cholesterol; mtDNA-CN, mitochondrial DNA copy number.

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CONCLUSIONS

This report represents the first cohort study focused on the association of mtDNA-CN with diabetes in a community-based population. Among 11 431 ARIC participants, lower mtDNA-CN in circulating leukocytes was associated with prevalent type 2 diabetes independent of other risk factors among white but not black participants. Among the 9692 participants without diabetes at baseline, mtDNA-CN in circulating leukocytes was not associated with risk of developing diabetes among white or black participants.

A substantial body of work links mitochondrial dysfunction measured directly in tissues responsible for glucose utilization with type 2 diabetes. Mitochondrial dysfunction in skeletal muscle for example is strongly and consistently associated with type 2 diabetes. In biopsy-based and nuclear magnetic resonance-based studies of the muscle of patients with insulin resistance, there is increased glycolysis and decreased oxidative phosphorylation,[1–5 35] lower mitochondrial size and number[6–9] and decreased oxidative gene expression in most,[10 11] but not all studies.[36] There are exceptions, however. In a study of South Asians with type 2 diabetes, for example, mitochondrial dysfunction was not present in muscle.[37]

Mitochondrial function in adipose tissue and pancreatic beta cells may also be impaired. Adipose mitochondrial content, including mtDNA-CN, is lower in individuals with obesity.[38] Expression of genes involved in oxidative phosphorylation is reduced in visceral adipose tissue from patients with type 2 diabetes.[39] Finally, Mendelian forms of mitochondrial dysfunction can cause type 2 diabetes through their impact on beta-cell function, at least in part.[15 40]

Despite the causal role in mitochondrial forms of diabetes, it is unknown whether mitochondrial dysfunction is a cause or consequence of insulin resistance and diabetes. To explore this question, our group has previously found that resting blood lactate, a marker of increased glycolysis and mitochondrial dysfunction, was strongly associated with incident diabetes 10 years before onset and independent of other risk factors.[20 21] This work suggests that mitochondrial dysfunction, as measured by blood lactate, occurs early in the pathway leading to diabetes.

To explore this question further, we examined the cross-sectional and incident association of mtDNA-CN from buffy coat with diabetes. We chose mitochondria from buffy coat due to their availability in large cohort studies in contrast to mitochondria in skeletal muscle, adipose tissue or pancreatic beta cells. Among the white participants, our cross-sectional analysis agrees with previous small cross-sectional studies which found that lower mtDNA-CN from buffy coat was associated with prevalent diabetes.[23 24] However, mtDNA-CN was not associated with prevalent diabetes among the black participants. In our incident analysis, mtDNA-CN from buffy coat was not associated with development of diabetes. Although ours is the first incident analysis to our knowledge, prior studies have examined the association of mtDNA-CN and type 2 diabetes among offspring of those with type 2 diabetes, finding mixed results.[23 25]

The association of lower mtDNA-CN in circulating leukocytes with diabetes in white participants supports the idea that at least portion of mitochondrial dysfunction is related to diabetes. The lack of association of mtDNA-CN in circulating leukocytes with incident type 2 diabetes suggests that mitochondrial dysfunction may not be a cause of type 2 diabetes. This finding, however, does not rule out mitochondrial dysfunction in other tissues as a cause of type 2 diabetes. Nor does this finding rule out an intrinsic defect in mitochondrial function, since intrinsic defects may not be reflected in mtDNA-CN.[5 41]

The strengths of our study include the relatively large sample size, the availability of prevalent and incident data on diabetes and the rigorous assessment of potential confounding factors. Covariates were added progressively in substantive groupings, by demographics, health behaviors, cardiovascular risk factors, anthropometric and metabolic factors and lab values in order to evaluate the potential confounding effect of these variables on mtDNA-CN and diabetes. There are several limitations that should be addressed in future research examining the link between mtDNA-CN and diabetes. First, mtDNA-CN in circulating leukocytes may not reflect mtDNA-CN in tissues responsible for glucose utilization. Second, mtDNA-CN is not a direct measure of mitochondrial dysfunction. Prior studies suggest, however, that mtDNA-CN and mitochondrial dysfunction are closely correlated, with downregulation of mtDNA-CN contributing to decreased oxidative phosphorylation activity in mice,[22] and lower mtDNA content found in patients with type 2 diabetes and/or obesity compared with lean controls,[8 9] and this lower mtDNA content associated with and electron transport chain activity[8] and decreased in respiration.[9] Third, we only had mtDNA-CN measurements for each participant at a single point in time, which limits the precision of the measure. Finally, the smaller sample size of black compared with white participants may have limited power for these analyses.

In conclusion, our results show that lower mtDNA-CN in circulating leukocytes is associated with diabetes among white participants in ARIC. mtDNA-CN was not associated with the risk of developing diabetes over an average of 17 years. Whether mitochondrial dysfunction in tissues responsible for glucose utilization is a cause of insulin resistance and diabetes remains unknown. This question can be further addressed with prospective studies analyzing mitochondrial dysfunction in different tissues responsible for glucose utilization and at different time points.

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Author note At the time of study concept and statistical analyses, BD was affiliated with the Johns Hopkins University Bloomberg School of Public Health. Currently and at the time of manuscript composition, reviewing and submission, BD has been associated with the University of North Carolina at Chapel Hill.

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