Peripheral Blood Mitochondrial DNA Content Is Related to Insulin Sensitivity in Offspring of Type 2 Diabetic Patients

JIHYUN SONG, PHD[1]
JEE YOUNG OH, MD[2]
YEON-AH SUNG, MD[3]
YOUNGMI KIM PAK, PHD[1]
KYONG SOO PARK, MD[2]
HONG KYU LEE, MD[1,2]

OBJECTIVE — To investigate whether the peripheral blood mtDNA (pb-mtDNA) content is decreased and linked to insulin resistance in the offspring of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — A total of 82 offspring of type 2 diabetic patients and 52 age-, sex-, and BMI-matched normal subjects from the Mokdong, Korea, population were selected for this study by stratified, randomized sampling. Of the offspring of diabetic patients, 52 had normal glucose tolerance (NGT), 21 had impaired glucose tolerance (IGT), and 9 had newly diagnosed type 2 diabetes. The pb-mtDNA content was measured by real-time polymerase chain reaction with a mitochondria-specific fluorescent probe, normalized by a nuclear DNA, 28S rRNA gene. The associations between pb-mtDNA content and several parameters of insulin resistance were studied.

RESULTS — The pb-mtDNA contents tended to be lower in the 82 offspring of type 2 diabetic patients (1,084.7 ± 62.6 vs. 1,304.0 ± 99.2 in the offspring and control subjects, respectively, $P = 0.051$) and was significantly lower in the combined NGT and IGT offspring group (NGT+IGT, 1,068.0 ± 67.8, $P < 0.05$) than in the control subjects. In NGT+IGT offspring, the pb-mtDNA content was significantly correlated with logarithmically transformed insulin sensitivity ($r = 0.253$, $P < 0.05$) and was the main predictor of insulin sensitivity.

CONCLUSIONS — Quantitative mtDNA status might be a hereditary factor associated with type 2 diabetes and could serve as an indicator for insulin sensitivity.

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The mitochondria are the major site of intracellular respiration and energy metabolism, and their function is intimately related to insulin secretion and possibly insulin action (1,2). Moreover, the mitochondria contain their own genome, with mtDNA coding for some proteins of the respiratory chain. The mutation of mtDNA was believed to cause ~0.5–1% of diabetes (3,4). The content of mtDNA is vital for maintaining the mitochondrial function and the energy demands of the body, but little attention has been paid to the quantitative aspects of mtDNA in diabetes. Several animal and human studies have described variations in mtDNA content. Decreased mtDNA content was found in the pancreatic islets of diabetes-prone Goto-Kakizaki rats (5) and in mitochondrial transcriptional factor A (Tfam)-defective mice (4). Decreased mtDNA content was also found in the skeletal muscle of type 1 and type 2 diabetic patients (6). Although the changes resulting from diabetes might influence the mtDNA content, decreases in peripheral blood mtDNA (pb-mtDNA) were observed before the onset of diabetes (7). Peripheral blood could provide an alternative sample type to skeletal muscle in the diagnosis of mitochondrial pathology because the data on the mitochondrial state in skeletal muscles and peripheral blood lymphocytes were comparable (8). Decreased pb-mtDNA was found to be related to insulin resistance or the development of type 2 diabetes (7).

Although maximum oxygen consumption ($V\text{O}_{2\text{max}}$) was positively related to muscle mtDNA and pb-mtDNA content in healthy subjects (9,10), $V\text{O}_{2\text{max}}$ was negatively related to insulin resistance in healthy first-degree relatives of patients with type 2 diabetes (11). Decreased $V\text{O}_{2\text{max}}$ mtDNA content and increased insulin resistance are expected in the offspring of diabetic patients. Thus, the purpose of our work was to investigate *1*) whether low mtDNA is present in the offspring of the patients with type 2 diabetes and *2*) whether low mtDNA is associated with insulin resistance in this population.

RESEARCH DESIGN AND METHODS

Subjects

Subjects were selected by random cluster sampling at Mokdong, which has a population of ~22,000, located in the southwestern part of Seoul (12). We selected 13 of 124 apartment complexes. Of a total 1,804 eligible apartment residents, 750 underwent a 75-g oral glucose tolerance test and were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or newly diagnosed

From the [1]Department of Biomedical Sciences, Korean National Institute Health; the [2]Department of Internal Medicine and Institute of Endocrinology, Nutrition and Metabolism, Medical Research Center, Seoul National University; and the [3]Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul, Korea.

Address correspondence and reprint requests to Dr. Hong Kyu Lee, Department of Biomedical Sciences, Korean National Institute of Health, Eunpyung-Ku, Nokbun-Dong, #5, Seoul 122-701, Korea. E-mail: hkleemd@plaza.snu.ac.kr.

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J.S. and J.Y.O. contributed equally to this work.

Abbreviations: AIR, acute insulin response to glucose; FAM, 5-carboxyfluorescein; FSIGT, frequently sampled intravenous glucose tolerance test; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; pb-mtDNA, peripheral blood mtDNA; PCR, polymerase chain reaction; S_G, glucose effectiveness; S_I, insulin sensitivity index; TAMRA, 6-carboxy-tetramethyl-rhodamine; Tfam, mitochondrial transcriptional factor A.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

diabetes based on the World Health Organization criteria (13). Among the 750 subjects examined, 82 offspring of type 2 diabetic patients as well as 52 age-, sex-, and BMI-matched NGT subjects without a family history of diabetes were selected by questionnaire. Informed consent was obtained from all subjects. The protocol used was approved by the ethics committee of Ewha Women's University Hospital.

Anthropometric data

In all subjects, blood pressure, height, weight, and waist and hip circumferences were measured. Total body fat content was measured as fat mass (kg) and percent body fat (%) using a bioelectric impedance analyzer. Cross-sectional body fat areas of two sites (subcutaneous abdomen and intra-abdomen) were measured by computed tomography at the umbilical level using an established method (14,15).

Biochemical studies and evaluation for insulin secretion and sensitivity

To determine the insulin sensitivity index (S_I), glucose-dependent glucose elimination (glucose effectiveness [S_G]), and insulin secretion, the insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) was used, as described previously (15,16). Glucose (0.3 g glucose/kg body wt) was administered and insulin (0.0125 U/kg body wt) was infused after glucose. S_I and S_G were calculated using a MINMOD computer program (Version 3.0; University of Southern California, Los Angeles, CA). Acute insulin response to glucose (AIR) was calculated as the mean difference between the insulin level at 2, 3, 4, 5, 6, and 8 min and the basal level. Serum glucose concentrations were determined using the glucose oxidase method. Total cholesterol, triglyceride, and HDL cholesterol levels were determined by enzymatic methods using a Hitachi 7150 autoanalyzer (Hitachi, Tokyo). Nonesterified fatty acid levels were measured by the enzymatic method. Commercial radioimmunoassay kits were used to measure serum insulin, C-peptide (Diagnostic Products, Los Angeles, CA), and serum leptin (Linco Research, St. Charles, MO).

Quantification of mitochondrial DNA using polymerase chain reaction

The total DNA was extracted from peripheral blood leukocytes, and its concentration was measured with a spectrophotometer. The mtDNA content was measured by a real-time polymerase chain reaction (PCR) method using an ABI Prism 7700 (PE Biosystems, CA) as previously described (17). The mtDNA quantity was corrected by simultaneous measurement of the nuclear DNA, 28S rRNA. The mtDNA- and 28S rRNA-specific fluorescent probes were labeled internally using the fluorescent dyes 5-carboxyfluorescein (FAM) on the 5′ end, and 6-carboxy-tetramethyl-rhodamine (TAMRA) on the 3′ end. The primers for mtDNA were mt2981 (5′ACGACCTCGATGTTGGATC3′) and mt3245 (5′GCTCTGCCATCTTAACAAACC3′) and were used together with the mtDNA probe (5′FAM-TTCAGACCGGAGTAATCCAGGTCG-TAMRA3′ made to nt 3071-3095). The primers of 28S rRNA were 28S-7358 (5′-TTAAGGTAGCCAAATGCCTCG3′) and 28S-7460 (5′CCTTGGCTGTGGTTTCGCT3′) and the 28S rRNA probe was 5′-FAM-TGAACGAGATTCCCACTGTCC-CTACCTACTATC-TAMRA3′ made to nt 7408-7440.

The PCR was performed separately for mtDNA and the reference 28S rRNA amplification. The PCR mixture contained the primers (10 pmol each), 200 nmol/l Taqman probe, 200 nmol/l dATP, 200 nmol/l dCTP, 200 nmol/l dGTP, 400 nmol/l dUTP, 4.5 mmol/l $MgCl_2$, 1.25 U AmpliTaqGold polymerase, 0.5 U AmpErase Uracil N-glycosylase, and 1 × PCR buffer A. Amplification was performed under the following conditions: one cycle of 50°C for 2 min, one cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. The amount of starting target in a particular reaction mixture was measured by interpolation from a standard curve of Ct values generated from known concentrations of the standard DNAs.

Statistical analysis

All results are expressed as means ± SEM. Statistical analysis was performed using SPSS software for Windows (SPSS, Chicago, IL). Results of the control subjects and the offspring were analyzed using Students' *t* test and analysis of variance. Pearson's correlation and multiple linear regression analysis were used to evaluate the relationships among mtDNA content and the indexes of insulin secretion and sensitivity and the parameters of insulin resistance syndrome. Results were considered significant when the corresponding *P* value was <0.05.

RESULTS

Clinical characteristics of subjects

According to the criteria (13), 52 of the 82 offspring of type 2 diabetic patients had NGT, 21 had IGT, and 9 had newly diagnosed type 2 diabetes. The clinical characteristics of the subjects are shown in Table 1. Age, sex distribution, BMIs, and lipid profiles were not significantly different for the control subjects and the NGT offspring. In diabetic offspring, age, BMI, waist-to-hip ratio, and fasting glucose, fasting insulin, and cholesterol levels were significantly higher than in the control NGT and IGT offspring. Whereas visceral fat areas and visceral-to-subcutaneous fat areas increased, the insulin sensitivity index was significantly lower in diabetic subjects than in the other groups. AIR was significantly lower in NGT and IGT offspring of type 2 diabetic patients than in the control subjects, and it was lowest in the offspring with diabetes (Table 2).

pb-mtDNA content in control subjects and offspring of diabetic parents

The pb-mtDNA/28S rRNA content was ~17% lower in the offspring of type 2 diabetic subjects (1,084.7 ± 62.6, $n = 82$) than in the control subjects (1,304.0 ± 99.2, $n = 52$) at $P = 0.051$. When the offspring group was divided into three groups, control and NGT subjects were found to have significantly different mtDNA levels ($P < 0.05$) (Table 1). Interestingly, pb-mtDNA levels tended to increase in those in whom glucose intolerance or diabetes developed. Because the insulin resistance parameters were not significantly different for IGT and NGT offspring, the two groups were combined and compared with the control subjects in terms of mtDNA content; the difference between the control and NGT+IGT subjects was still statistically significant ($P < 0.05$) (Fig. 1).

Table 1—*Clinical characteristics of subjects*

Variable	Control subjects (n = 52)	Offspring of diabetic patients		
		NGT (n = 52)	IGT (n = 21)	Diabetes (n = 9)
Age (years)	41.8 ± 0.9	41.7 ± 0.9	45.1 ± 1.3	50.6 ± 1.9*
Sex (male/female)	17/35	18/34	11/10	4/5
mtDNA	1,304.0 ± 99.2	1,053.7 ± 74.8‡	1,103.3 ± 149.2	1,220.5 ± 150.1
BMI (kg/m^2)	24.0 ± 0.3	23.7 ± 0.4	24.2 ± 0.7	25.8 ± 0.7*
Waist-to-hip ratio	0.82 ± 0.0	0.82 ± 0.0	0.84 ± 0.0	0.87 ± 0.0*
Fasting glucose (mmol/l)	4.8 ± 0.1	5.0 ± 0.1	5.3 ± 0.2†	7.7 ± 0.8*
Fasting insulin (pmol/l)	60.0 ± 5.2	46.9 ± 2.8	60.0 ± 9.7	87.3 ± 16.8*
Fasting C-peptide (ng/ml)	1.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.0
Systolic blood pressure (mmHg)	117.2 ± 1.9	116.9 ± 1.8	121.0 ± 2.8	121.1 ± 3.5
Diastolic blood pressure (mmHg)	76.5 ± 1.3	75.7 ± 1.3	80.0 ± 2.3	76.7 ± 3.7
Cholesterol (mmol/l)	4.8 ± 0.1	4.9 ± 0.1	5.5 ± 0.2	5.8 ± 0.4*
Triglyceride (mmol/l)	1.4 ± 0.1	1.6 ± 0.2	2.4 ± 0.5	1.8 ± 0.2
HDL (mmol/l)	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.1
Fatty acid (g/l)	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.1

Values are means ± SEM; mtDNA represents mtDNA copy number/28S rRNA copy number. *$P < 0.05$ versus control, NGT, and IGT; †$P < 0.05$ versus control and NGT; ‡$P < 0.05$ versus control.

Relation of mtDNA content to indexes of insulin sensitivity and age

When pb-mtDNA content was plotted against either clinical parameters or indexes of insulin resistance, significant correlations were observed between mtDNA content and either the logarithmically transformed insulin sensitivity index ($r = 0.286$, $P < 0.05$) or the fasting C-peptide concentration ($r = -0.248$, $P < 0.05$) in all subjects. Correlation between mtDNA content and the visceral fat area showed marginal significance ($r = -0.198$, $P = 0.057$). On the other hand, BMI, waist-to-hip ratio, fasting glucose, lipid levels and body fat content, fasting insulin, and AIR did not correlate with pb-mtDNA content. In offspring of type 2 diabetic patients, diastolic blood pressure correlated negatively with mtDNA content ($r = -0.209$, $P = 0.061$). Because pb-mtDNA is believed to be influenced by the disease state, data analysis was performed separately on the control subjects and the offspring. The pb-mtDNA content correlated negatively with age ($r = -0.316$, $P < 0.05$) in the control subjects, whereas the correlation was blurred in offspring of type 2 diabetic patients.

Pb-mtDNA of the offspring correlated positively with logarithmically transformed insulin sensitivity ($r = 0.244$, $P < 0.05$) (Table 3); the linear relationship was maintained in offspring with NGT and IGT ($r = 0.253$, $P < 0.05$) (Table 3) but not in offspring with diabetes. Stepwise regression analysis selected visceral fat area and mtDNA content as the main predictors of insulin sensitivity in the combined group of offspring with NGT and IGT (Table 4).

Table 2—*Patterns of body fat distribution and indexes of insulin secretion and resistance of subjects*

Variable	Control subjects (n = 52)	Offspring of diabetic patients		
		NGT (n = 52)	IGT (n = 21)	Diabetes (n = 9)
Fat mass (kg)	17.6 ± 1.3	16.9 ± 0.6	16.9 ± 1.1	18.3 ± 1.2
Percent body fat (%)	27.9 ± 1.3	26.7 ± 0.6	25.5 ± 1.2	26.6 ± 1.2
Visceral fat area (cm^2)	61.9 ± 8.5	75.0 ± 4.7	87.8 ± 9.1	132.8 ± 13.5*
Visceral-to-subcutaneous ratio	0.40 ± 0.1	0.49 ± 0.0	0.57 ± 0.1	0.73 ± 0.0*
Leptin (ng/ml)	5.8 ± 1.2	7.1 ± 1.1	8.2 ± 2.0	7.1 ± 0.8
S_I ($\times 10^{-4}$ min^{-1}/[μU/ml])	5.88 ± 0.6	5.04 ± 0.6	4.98 ± 1.2	2.04 ± 0.6*
S_G (10^{-2}/min)	2.29 ± 0.0	2.18 ± 0.0	1.94 ± 0.0	2.52 ± 0.1
AIR (μU/ml)	53.2 ± 7.7	38.5 ± 5.0‡	26.3 ± 5.8†	4.9 ± 2.6*

Values are means ± SEM. *$P < 0.05$ versus control, NGT, and IGT; †$P < 0.05$ versus control and NGT; ‡$P < 0.05$ versus control.

CONCLUSIONS — This study shows for the first time that pb-mtDNA content is lower in the offspring of type 2 diabetic patients than in age-, sex-, and BMI-matched control subjects without a family history of diabetes. Along with our previous finding that decreased pb-mtDNA content precedes the onset of diabetes (7), the present data support the notion that quantitative decreases of pb-mtDNA are a determinant of type 2 diabetic development. Although pb-mtDNA content shows a tendency of increasing as glucose me-

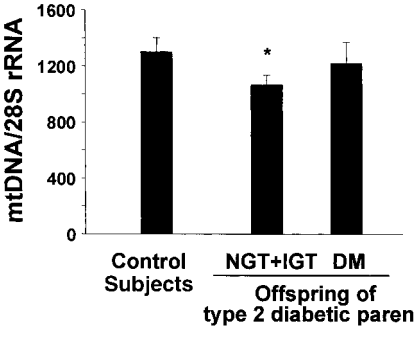

Figure 1—*The pb-mtDNA/28S rRNA content in the offspring of type 2 diabetic patients and control subjects. DM, diabetes mellitus. *Significantly different from the control subjects at P < 0.05*

Table 3—*Bivariate correlations of mtDNA content with clinical data in subjects*

	All subjects		Control subjects		Offspring					
					NGT+IGT+ Diabetes*		NGT+IGT		Diabetes	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Log S_I	0.286	0.005*	0.316	NS	0.244	0.035*	0.253	0.036*	0.306	NS
Age (years)	−0.142	0.103	−0.316	0.023*	0.022	NS	−0.014	NS	0.046	NS
Diastolic blood pressure (mmHg)	−0.049	NS	0.226	NS	−0.209	0.061	−0.154	NS	−0.787	0.012*
Visceral fat area (cm^2)	−0.198	0.057	−0.211	NS	−0.142	NS	−0.149	0.081	−0.094	NS
Fasting C-peptide (ng/ml)	−0.248	0.032*	−0.350	NS	−0.227	0.067	−0.239	0.055	ND	ND

*NS, $P > 0.1$; ND, not determined.

tabolism deteriorates, this was without statistical significance. The fact that pb-mtDNA content is lower in the offspring of diabetic patients suggests that mtDNA copy number must be inherited. A previous study showed that cord blood mtDNA correlates positively with maternal pb-mtDNA content (18), which also supports the inheritance of mtDNA copy number. Considered together, these findings suggest that there is a heritable factor controlling mtDNA level.

Aging is an another factor known to influence the mtDNA content of tissues (7,19–21). Aging is known to be associated with deletions and mutations of mtDNA resulting from the combined effects of intense oxidative damage (20) and the low efficiency of the mtDNA repair system (21). Age-dependent decline of pb-mtDNA was observed in the present study as well as in our previous study (7).

Although mtDNA content is supposed to be related to age and be under genetic control, the mechanisms regarding regulation of mtDNA content are not entirely clear. Tfam is known to play a role in setting the mtDNA status quantitatively (by interacting with mitochondrial single-stranded binding protein and DNA polymerase γ) and in maintaining the normal glucose level (4). Therefore, the quantitative and qualitative aspects of Tfam must be investigated to clarify the mechanism that controls mtDNA content.

The increase of mtDNA content between offspring with NGT and offspring with diabetes is quite interesting because previous studies have reported that the mtDNA content in muscle (6) and peripheral blood (7) of diabetic patients is reduced. It is possible that the low level of mtDNA causes development of diabetes and that they try to compensate for their lack of mtDNA by enhancing mitochondrial biogenesis. We also have unpublished observations that subjects with diabetic complications show atypical increases in mtDNA copy number. Further studies are needed to clarify the regulation of mtDNA content in response to the state and duration of diabetes.

Although the depletion of mtDNA could impair mitochondrial and pancreatic β-cell function, mitochondrial function can be measured only in the tissues of patients or animals (4,6) and cannot be measured easily in noninvasive samples or in nondiseased subjects. It is believed, therefore, that the pb-mtDNA content could provide an alternative index. An interesting finding of the present study is that the pb-mtDNA content correlates with the insulin sensitivity of the offspring. Fasting serum C-peptide levels correlated negatively with the pb-mtDNA content of the subjects, suggesting that decreased mtDNA content is associated with insulin resistance in offspring. However, mtDNA content did not correlate with either fasting blood glucose or waist-to-hip ratio, and the pb-mtDNA content correlated negatively with diastolic blood pressure, as reported previously in a population-based study (7). The relationship between pb-mtDNA content and the energy utilization pattern of the whole body (22) also suggests that the quantitative status of mtDNA could be an indicator of insulin sensitivity. In summary, we have observed that the pb-mtDNA content decreased in the offspring of type 2 diabetic patients and, furthermore, that the pb-mtDNA content is correlated with insulin sensitivity. These findings suggest that the quantitative mtDNA status might be an early genetic marker for type 2 diabetes and possibly for insulin resistance syndrome.

Table 4—*Stepwise multiple regression analysis for influencing factors of insulin sensitivity*

Group	Variable	R^2	β	*F* value	*P*
Control		0.812			
	Visceral fat area		−0.913	39.844	<0.001
NGT+IGT		0.456			
	Visceral fat area		−0.624	28.340	<0.001
	mtDNA		0.271	18.621	0.022
Diabetes		0.590			
	Visceral-to-subcutaneous ratio		−0.820	8.192	0.046

Regression analysis included parameters, such as age, sex, BMI, diastolic blood pressure, systolic blood pressure, fat mass, percent body fat, waist-to-hip ratio, visceral fat areas, subcutaneous fat area, visceral-to-subcutaneous ratio, fasting plasma glucose, postprandial plasma glucose, free fatty acid, total cholesterol, HDL cholesterol, and triglyceride. Log-transformed insulin sensitivity index was used as a dependent variable.

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