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Glucose metabolism derangements in adults with the MELAS m.3243A>G mutation

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Abstract

The m.3243A>G mutation in the mitochondrial gene *MT-TL1* leads to a wide clinical spectrum ranging from asymptomatic carriers to MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) at the severe end. Diabetes mellitus (DM) occurs in mitochondrial diseases, with the m.3243A>G mutation being the most common mutation associated with mitochondrial DM. The pathogenesis of mitochondrial DM remains largely unknown, with previous studies suggesting that impaired insulin secretion is the major factor. In this study we used stable isotope infusion techniques to assess glucose metabolism *in vivo* and under physiological conditions in 5 diabetic and 11 non-diabetic adults with the m.3243A>G mutation and 10 healthy adult controls. Our results revealed increased glucose production due to increased gluconeogenesis in both diabetic and non-diabetic subjects with the m.3243A>G mutation. In addition, diabetic subjects demonstrated insulin resistance and relative insulin deficiency, resulting in an inability to increase glucose oxidation which can explain the development of DM in those subjects. Non-diabetic subjects showed normal insulin sensitivity; and therefore, they were able to increase their glucose oxidation rate. The ability to increase glucose utilization can act as a compensatory mechanism that explains why these subjects do not have DM despite the higher rate of glucose production. These results suggest that increased gluconeogenesis is not enough to cause DM and the occurrence of combined insulin resistance and relative insulin deficiency are needed to develop DM in individuals with the m.3243A>G mutation. Therefore, multiple defects in insulin and glucose metabolism are required for DM to occur in individuals with mitochondrial diseases. The results of this study uncovers previously

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undocumented alterations in glucose metabolism in individuals with the m.3243A>G mutation that contribute significantly to our understanding of the pathogenesis of mitochondrial DM and can have significant implications for its management.

Keywords

Mitochondrial diseases; diabetes; gluconeogenesis; insulin resistance; stable isotope infusion

1. Introduction

Mitochondria are found in all nucleated human cells and perform a variety of essential functions, including the generation of most cellular energy. Mitochondria contain extra-chromosomal DNA (mitochondrial DNA, mtDNA); however, the majority of mitochondrial proteins are encoded by the nuclear DNA (nDNA). Defects in either mtDNA or nDNA-encoded mitochondrial proteins can result in mitochondrial dysfunction leading to mitochondrial diseases. Mitochondrial diseases result from impaired mitochondrial energy production and other cellular perturbations related to mitochondrial dysfunction such as altered calcium homeostasis and signal transduction, dysregulated apoptosis, and uncontrolled reactive oxygen species (ROS) production (Wallace 1999).

MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) is a common mitochondrial disorder, with an estimated prevalence of 16:100,000 (Chinnery and Turnbull 2001). The clinical diagnosis of MELAS is based on three invariant features which are 1) stroke-like episodes, typically before age 40 years, 2) encephalopathy with seizures and/or dementia, and 3) mitochondrial myopathy, evidenced by lactic acidosis and/or ragged red fibers on muscle biopsy (Hirano et al. 1992). Other common features are short stature, sensorineural hearing loss, and diabetes mellitus (DM) (Hirano and Pavlakis 1994). After its initial clinical description, MELAS was found to be associated with a mutation in the *MT-*TL1** gene encoding tRNA^{LEU(UUR)}, changing adenine to guanine at position 3243 of mtDNA (m.3243A>G) (Goto et al. 1990; Kobayashi et al. 1990). Other mutations have subsequently been found to be associated with MELAS; however, the m.3243A>G remains the most common and is known as the MELAS mutation (Sproule and Kaufmann 2008). The m.3243A>G mutation was also found to be associated with other phenotypes that collectively constitute a wide spectrum, ranging from MELAS at the severe end to asymptomatic carrier status. Between these two extremes intermediate phenotypes exist, including multi-organ involvement with various combinations of symptoms (e.g., myopathy, DM, and deafness) and single organ involvement (e.g., cardiomyopathy or DM) (Azevedo et al 2010; Damian et al 1995; Lev et al 2004; Ma et al 2010; Ohkubo et al 2001; van den Ouweland et al 1992; Vionnet et al. 1993). The extreme variability in phenotypes associated with the m.3243A>G mutation is a common observation in many mtDNA-related mitochondrial diseases. Similarly to the majority of mtDNA mutations, the m.3243A>G is a heteroplasmic mutation, i.e. present in some copies of mtDNA, and therefore cells harbor a mixture of mutant and normal mtDNA. During cell division, mutant mtDNAs are distributed randomly among daughter cells. Therefore, the percentage of mutant mtDNAs differs in different tissues and organs within the same individual. These tissues and organs have

different thresholds in heteroplasmy percentage before clinical phenotypes manifest thus accounting for the clinical diversity seen in individuals harboring this mutation (Chinnery et al 1997; Jeppesen et al 2006). The m.3243A>G mutation was found to be very common with an estimated prevalence of 1:400 in the general population (Manwaring et al 2007).

DM can occur in mitochondrial diseases. The prevalence of DM in adults with the m.3243A>G mutation has been estimated to be 50% (Frederiksen 2009; de Laat 2012), and it has been suggested that 1-3% of patients with DM harbor the m.3243A>G mutation (Ohkubo 2001; Maassen 2004). Therefore, the m.3243A>G mutation is one of the most common mutations associated with DM, and the majority of causes of mitochondrial DM are due to this mutation. The m.3243A>G-related DM typically manifest at an average age of 40 years and can be type 1 or type 2 in nature (Maassen et al 2004).

The pathogenesis of DM in mitochondrial diseases remains largely unknown, with three proposed mechanisms, including impaired insulin secretion, decreased glucose utilization, and increased glucose production. Normally, pancreatic β -cells respond to increased blood glucose by increasing insulin secretion. An increased circulating insulin concentration suppresses hepatic glucose production and stimulates glucose utilization by muscle and adipose tissue. In pancreatic β -cells, an ATP-sensitive potassium channel is required for insulin release. Decreased ATP synthesis as a result of mitochondrial dysfunction can result in impaired insulin secretion (Maassen et al 2004; Sproule and Kaufmann 2008). Impaired glucose utilization by muscle can occur due to insulin resistance. Increased ROS production due to mitochondrial dysfunction can play a role in impaired insulin responsiveness and the development of insulin resistance (Szendroedi et al 2011). Finally, lactic acidemia in mitochondrial diseases can result in increasing hepatic glucose production because lactate is a major substrate for gluconeogenesis. Lactic acidemia results from an inability of dysfunctional mitochondria to oxidize NADH to generate sufficient ATP, leading to a shift to anaerobic glycolysis (Maassen et al 2004; Sproule and Kaufmann 2008).

Clinical studies evaluating glucose homeostasis in mitochondrial DM are very limited and have not always been associated with consistent results. Impaired insulin secretion, as assessed by hyperglycemic clamp or a glucose tolerance test in diabetic individuals with the m.3243A>G mutation, was found in several studies (Velho 1996; Brändle 2001; Maassen 2004). Whereas insulin sensitivity was found to be normal in one study (Maassen 2004), another study demonstrated insulin resistance in diabetic subjects with the m.3243A>G mutation using an euglycemic-hyperinsulinemic clamp protocol (Velho 1996). Similarly, inconsistent results were reported regarding non-diabetic individuals with the m.3243A>G mutation. While some studies showed no abnormalities in glucose and insulin metabolism suggesting the absence of detectable abnormalities before the development of DM (Maassen 2004), another study demonstrated impaired insulin secretion in non-diabetic subjects with the m.3243A>G mutation (Velho 1996). Overall, these studies suggest that the main mechanism of DM in subjects with the m.3243A>G mutation is impaired insulin secretion rather than insulin resistance. In addition, the results were controversial regarding the presence of glucose metabolism derangements in non-diabetic subjects.

These previous studies used clamp techniques which are widely accepted as the reference methods for direct insulin assessment. However, an important limitation of hyperinsulinemic clamp is the utilization of steady-state insulin levels that may be supraphysiological. In addition, the intravenous administration of insulin results in a reversal of the normal portal to peripheral insulin gradient. Thus, the use of hyperinsulinemic clamps may not accurately reflect insulin action and glucose dynamics under physiological conditions (Muniyappa 2008). Similarly, the hyperglycemic clamp utilizes steady-state glucose levels that are supraphysiological and may not accurately reflect glucose metabolism under physiological conditions (Elahi 1996). Furthermore, no previous studies have assessed the hepatic glucose production in subjects with mitochondrial diseases.

In this study, we hypothesized that DM develops in individuals harboring the m.3243A>G mutation due to multiple defects in glucose metabolism. We assessed glucose metabolism in diabetic and non-diabetic adults with the m.3243A>G mutation and in control healthy subjects. We used stable isotope infusion techniques to assess glucose metabolism *in vivo* and under physiological overnight fasting conditions without the need for administration of exogenous insulin or significant amounts of glucose.

2. Methods

The study was approved by the Institutional Review Board (IRB) at Baylor College of Medicine (BCM) and the Clinical Research Center (CRC) Scientific Advisory Committee (SAC) at Texas Children's Hospital (TCH). A written informed consent was received from each research subject prior to inclusion in the study. Sixteen subjects with the m.3243A>G mutation and 10 control subjects participated in this study. Glucose metabolism was assessed by the U-¹³C₆ glucose stable isotope infusion technique, which is a well-established method that is used commonly by our group and others (Dwarkanath et al 2009; Thame et al 2010).

Research subjects were admitted to the CRC at TCH for 2 days. In the first day, a DXA (dual-energy X-ray absorptiometry) scan was performed for each subject to assess the lean body mass. Research subjects fasted overnight starting at 8 pm. In the second day, an intravenous catheter was placed in each arm, one for isotope infusion and the other for blood withdrawals. After the 12 hour fast at 8 am (time 0) blood and breath samples were obtained, then the stable isotope infusion was started by the administration of two isotope primes: NaH¹³CO₃ (4 μmol/kg) and U-¹³C₆ glucose (48 μmol/kg) followed by continuous infusion of U-¹³C₆ glucose (48 μmol/kg/hour) for 6 hours. Blood and breath samples were collected at times 4.5, 5, 5.5, and 6 hours. Four hours after starting the infusion (time 4 hr) the CO₂ production was measured by indirect calorimetry for 20 minutes. Three diabetic subjects were on oral hypoglycemic agents, and one subject was on insulin therapy (Humalog and Lantus in the morning and Humalog at night) (Supplementary table 1). The oral hypoglycemic agents and the insulin therapy were withheld starting 12 hours before the initiation of the isotope infusion (during the 12-hour fast) until the end of the isotope infusion.

Plasma glucose, insulin, lactate, and glycosylated hemoglobin (HbA1c) concentrations were measured in the first sample drawn (time 0). Plasma insulin was measured using ARCHITECT insulin assay (Abbott laboratories, IL, USA) which is as a chemiluminescent microparticle immunoassay (CMIA). The isotopic enrichment for plasma glucose and breath CO₂ were measured in all samples (times 0, 4.5, 5, 5.5, and 6 hours). The plasma glucose isotopic enrichment was measured by positive chemical ionization gas chromatography mass spectrometry of its penta-acetate derivative, monitoring ions from m/z 331 to 336 (M1 to M6) as previously described by us (Balasubramanyam et al 1999; Thame et al 2010). The breath samples were analyzed in duplicate for ¹³C abundance in CO₂ by gas-isotope-ratio mass spectrometry, monitoring ions at m/z ratios 44 and 45 as previously described by us (Dwarkanath et al 2009).

Glucose production was calculated by the steady-state equation (Haymond and Suneag 2000) as follows:

$$\text{Glucose production } (\mu\text{mol/kg/min}) = (\text{IE}_{\text{inf}}/\text{IE}_{\text{plateau}}) \times i$$

where i is the infusion rate of U-¹³C₆ glucose, IE_{inf} is isotopic enrichment of the infused glucose (mole%), and $\text{IE}_{\text{plateau}}$ is the isotopic enrichment of plasma glucose isotopomer M6 (mass+6) at steady-state calculated by averaging the M6 isotopic enrichment at times 5, 5.5, and 6 hours.

Under steady-state conditions glucose production equals the rate of disappearance and glucose clearance was calculated as follows (Thame et al 2010; Dwarkanath et al 2009):

$$\text{Glucose clearance (ml/kg/min)} = \text{glucose production} / \text{plasma glucose concentration.}$$

The fractional rate of gluconeogenesis was calculated according to the reciprocal pool model of Haymond and Suneag using the mass isotopomer distribution analysis of plasma glucose (Haymond and Suneag 2000) as follows:

$$\text{Fractional gluconeogenesis} = [\Sigma_{\text{M1-M5}} / \Sigma_{\text{M1-M6}}] \times [\Sigma_{\text{M1-M5}}(^{12}\text{C}) / \Sigma_{\text{M1-M5}}(^{13}\text{C})]$$

where $\Sigma_{\text{M1-M5}}$ represents the sum of all glucose isotopomers labeled with ¹³C on carbons 1–5 which are arising from gluconeogenesis and $\Sigma_{\text{M1-M6}}$ represents those isotopomers with ¹³C on carbons 1–6 which represents all the labeled glucose isotopomers including the infused U-¹³C₆ glucose; so $[\Sigma_{\text{M1-M5}} / \Sigma_{\text{M1-M6}}]$ represents the fraction of labeled glucose isotopomers derived from gluconeogenesis. $[\Sigma_{\text{M1-M5}}(^{12}\text{C}) / \Sigma_{\text{M1-M5}}(^{13}\text{C})]$ represent the ratio of the sum of ¹²C and the sum of ¹³C in the isotopomers derived from gluconeogenesis.

Absolute gluconeogenesis was calculated by multiplying fractional gluconeogenesis by glucose production (Haymond and Suneag 2000) as follows:

$$\text{Absolute gluconeogenesis } (\mu\text{mol/kg/min}) = \text{fractional gluconeogenesis} \times \text{glucose production}$$

Rate of glycogenolysis was calculated by subtracting absolute gluconeogenesis from glucose production (Haymond and Suneag 2000) as follows:

Glycogenolysis rate ($\mu\text{mol/kg/min}$) = glucose production - absolute gluconeogenesis

Glucose oxidation rate was calculated as described by us (Dwarkanath et al 2009) as follows:

$$\text{Glucose oxidation } (\mu\text{mol/kg/min}) = [\text{IE}^{13}\text{CO}_2 \times \text{RaCO}_2] / [\Sigma_{\text{M1-M6}}(^{13}\text{C})]$$

where $\text{IE}^{13}\text{CO}_2$ represents the ^{13}C isotopic enrichment of breath CO_2 at steady-state calculated by averaging the CO_2 isotopic enrichment at times 5, 5.5, and 6 hours, RaCO_2 is the rate of appearance of CO_2 measured by indirect calorimetry, $\Sigma_{\text{M1-M6}}(^{13}\text{C})$ is the sum of ^{13}C enrichment in glucose isotopomers weighted by the number of ^{13}C in each isotopomer

Homeostatic model assessment (HOMA) of β -cell insulin release function (HOMA- β) was calculated as follows (Muniyappa et al 2008; Wallace et al 2004):

$$\text{HOMA-}\beta = [20 \times \text{fasting plasma insulin } (\mu\text{U/ml})] / [\text{fasting plasma glucose (mmol/l)} - 3.5].$$

HOMA of insulin resistance (HOMA-IR) was calculated as follows:

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/l)}] / 22.5$$

Heteroplasmy of the m.3243A>G mutation in urine was measured using the amplification refractory mutation systems quantitative PCR (ARMS-qPCR) method, which provides simultaneous detection and quantification of heteroplasmic mitochondrial DNA point mutations (Bai and Wong 2004).

All results were calculated based on lean body mass obtained from DXA scanning and expressed as means $\pm$ SEMs. The results of control subjects and subjects with the m.3243A>G mutation were compared using the unpaired Student's t test. Correlations were assessed using Pearson Product-Moment Correlation Coefficient. Tests were considered statistically significant if $p < 0.05$.

3. Results and Discussion

Sixteen adults with the m.3243A>G mutation participated in this study. Eleven of them were non-diabetic and five were diabetic. The diagnosis of DM was based on the identification of fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l) with fasting being defined as no caloric intake for at least 8 hours (American Diabetes Association 2009). Three of the diabetic subjects were on oral hypoglycemic agents, one was on insulin therapy, and one was only on dietary treatment. Ten of the subjects with the m.3243A>G mutation were diagnosed to have MELAS based on the presence of the three invariant criteria, whereas, three individuals had clinical manifestations related to multiple organ dysfunction but without fulfilling the clinical diagnostic criteria for MELAS, and three subjects were asymptomatic. The m.3243A>G mutation heteroplasmy in urinary tract cells ranged from 3-99%. A detailed clinical description is presented in Supplementary Table 1. Ten control adults who did not have DM or any other chronic illness were also studied. When compared to the control

cohort, subjects with the m.3243A>G mutation had a lower mean body weight, height, and body mass index, but no significant differences in age or gender (Table 1).

Glucose metabolism parameters were compared among diabetic subjects with the m.3243A>G mutation (n=5), non-diabetic subjects with the m.3243A>G mutation (n=11), and control subjects (n=10). Two individuals with the m.3243A>G mutation showed low fasting plasma insulin (insulin level < 1mU/L, normal 3-25 mU/L). HOMA values were not calculated for these two individuals because HOMA does not give appropriate results when plasma insulin is very low (Muniyappa et al 2008).

The mean fasting plasma glucose and HbA1c values for the control cohort were 86.1 ± 1.2 mg/dl and $5.2 \pm 0.1\%$, respectively. Non-diabetic subjects with m.3243A>G showed values that are not different from those of the control subjects (fasting plasma glucose of 85.3 ± 3.0 mg/dl and HbA1c of $5.1 \pm 0.1\%$), whereas diabetic subjects with m.3243A>G had significantly higher fasting plasma glucose (162.8 ± 28.7 mg/dl) and HbA1c ($7.2 \pm 0.4\%$) values when compared to control cohort (Fig. 1A, B). These findings ensure that our classification of diabetic and nondiabetic groups was accurate.

To evaluate glucose production, the studies were performed in the fasting state, when there is no enteral carbohydrate being absorbed and the only sources for glucose in the body are gluconeogenesis and glycogenolysis. All data were expressed per kg of lean body mass. Control subjects had a glucose production rate of 13.5 ± 0.7 $\mu\text{mol/kg/min}$, with gluconeogenesis contributing 8.5 ± 0.8 $\mu\text{mol/kg/min}$ and glycogenolysis contributing 5.0 ± 0.5 $\mu\text{mol/kg/min}$, hence 62% of glucose production was derived from gluconeogenesis. Both diabetic and non-diabetic subjects with the m.3243A>G mutation demonstrated a significant increase in glucose production (20.4 ± 1.7 and 18.4 ± 0.8 $\mu\text{mol/kg/min}$, respectively), with elevated gluconeogenesis rates (17.1 ± 2.2 and 14.7 ± 0.7 $\mu\text{mol/kg/min}$, respectively) but glycogenolysis rates that were not significantly different from the control cohort. These results indicate that the increased glucose production was driven by increased gluconeogenesis, which accounted for 82% of glucose production in the diabetic and 80% in the non-diabetic groups (Fig. 2A-D).

To evaluate glucose utilization, we measured glucose oxidation and clearance rates, and evaluated insulin resistance using HOMA-IR and insulin release using HOMA- β . Control subjects oxidized glucose at a rate of 8.3 ± 0.6 $\mu\text{mol/kg/min}$, which was 56% of glucose production. The glucose clearance rate was 3.1 ± 0.2 ml/kg/min, the insulin level was 5.7 ± 0.7 mU/L, the HOMA- β was 92.2 ± 13.3 , and the HOMA-IR was 1.2 ± 0.2 . When compared to control subjects, diabetic subjects with the m.3243A>G mutation showed no statistically significant differences in insulin level or HOMA- β ; however, HOMA-IR was significantly higher (3.9 ± 1.3). Additionally, glucose clearance and oxidation rates in this group were not significantly different from the control group; however, the fraction of glucose production that was oxidized was significantly lower at 44%. Similar to diabetic subjects with the m.3243A>G mutation, nondiabetic subjects did not show significant differences in insulin level or HOMA- β when compared to control subjects. In contrast to diabetic subjects, non-diabetic subjects with the m.3243A>G mutation exhibited higher rates of glucose clearance (4.2 ± 0.2 ml/kg/min) and oxidation (10.6 ± 0.7 $\mu\text{mol/kg/min}$), with no significant alteration in

HOMA-IR and the fraction of glucose production that was oxidized when compared to the control group (Fig. 3A-F).

These results demonstrate that all subjects with the m.3243A>G mutation exhibit overt alterations in glucose metabolism, including those who were not diabetic. Both diabetic and nondiabetic subjects exhibit increased gluconeogenesis rates resulting in an increase in overall glucose production. Diabetic subjects exhibit high insulin resistance (HOMA-IR) with unaltered insulin release (HOMA- β) and plasma insulin levels when compared to the control cohort. The failure to increase insulin in the context of insulin resistance and hyperglycemia indicates a relative insulin deficiency in these subjects. The combination of insulin resistance and relative insulin deficiency results in a failure to increase glucose oxidation to match the higher rate of glucose production, leading to a relative reduction of glucose utilization. This failure to increase glucose utilization in the context of increased glucose production can explain the development of DM in these subjects. Non-diabetic subjects with the m.3243A>G mutation have normal insulin secretion, plasma insulin levels, and insulin sensitivity; therefore they are able to appropriately increase glucose oxidation rate. The ability to increase glucose utilization acts as a compensatory mechanism that explains why these subjects do not have DM despite the higher rate of glucose production. Two of the 16 individuals with the m.3243A>G mutation had fasting insulinopenia, one of whom had DM while the other did not. Interestingly, both of them had comparable increased glucose production rates; however, the fraction of glucose production that was oxidized in the diabetic subject was about half of that of the non-diabetic subject (33% versus 62%) indicating the higher ability of the non-diabetic subject to increase glucose utilization despite the low insulin level and explaining the absence of clinical diabetes in this subject.

While no previous clinical trial evaluated glucose production in mitochondrial DM, previous studies suggested that the main mechanism of DM in individuals with the m.3243A>G mutation is impaired insulin secretion rather than resistance (Velho 1996; Brändle 2001; Maassen 2004). Our results indicate that low fasting plasma insulin occurs in only a minority of individuals harboring the m.3243A>G mutation, as only 2 of the 16 individuals had fasting insulinopenia. Our results also suggest that relative insulin deficiency occurs in diabetic subjects. However, insulin deficiency is not the only factor contributing to the development of mitochondrial DM and insulin resistance along with increased gluconeogenesis also play major roles. Gluconeogenesis is increased in all subjects with the m.3243A>G mutation; however, this derangement is not enough to cause DM and the occurrence of combined insulin resistance and relative insulin deficiency is needed to develop DM. Therefore, multiple defects in insulin and glucose metabolism are needed for DM to occur in individuals with mitochondrial diseases. These results reveal previously undocumented alterations in glucose metabolism in individuals with the m.3243A>G mutation, and contribute significantly to our understanding of the pathogenesis of mitochondrial DM.

Two factors may contribute to increasing gluconeogenesis in subjects with the m.3243A>G mutation: insulin resistance, which results in reduced insulin inhibition of hepatic gluconeogenesis, and lactic acidemia, which can fuel gluconeogenesis in the liver. The plasma lactate level in the control cohort was 0.9 ± 0.1 mmol/L, whereas the levels were

significantly higher in both diabetic and non-diabetic subjects with the m.3243A>G mutation (2.5 ± 0.2 and 1.9 ± 0.4 mmol/L, respectively) (Supplementary Fig. 1A). There is a trend of higher gluconeogenesis rates in the diabetic cohort when compared to the non-diabetic group (Fig. 2B). This finding could be due to the observation that non-diabetic subjects have increased lactate levels, whereas diabetic subjects have both increased lactate levels and insulin resistance. Performing correlation analysis between the lactate level and gluconeogenesis and HOMA-IR and gluconeogenesis resulted in positive correlation coefficient values of 0.27 and 0.44, respectively; however these values did not reach statistically significant levels (Supplementary Fig. 1B and 1C).

Although our results indicate that insulin resistance and relative insulin deficiency are major factors in the development of DM in individuals with the m.3243A>G mutation, the underlying mechanism for insulin resistance in mitochondrial DM remains unknown. The etiology of insulin resistance in mitochondrial DM can be multifactorial. First, decreased glucose oxidative capacity due to the mitochondrial dysfunction can result in impaired insulin responsiveness. Therefore, decreased glucose oxidative capacity can have roles in the pathogenesis of both lactic acidemia and increased insulin resistance. Second, through enhancing endothelial nitric oxide (NO) synthase (eNOS) activity, insulin can induce vasodilation that results in an adequate delivery of glucose and insulin to muscle tissue (Jonk et al 2007). We and others reported that NO deficiency occurs in MELAS (El-Hattab et al 2012a, 2012b, 2014; Koga et al 2005). Therefore, NO deficiency in MELAS may lead to impaired insulin-mediated vasodilation, resulting in impaired insulin delivery and contributing to insulin resistance. Finally, increased ROS production due to mitochondrial dysfunction can play a role in impaired insulin responsiveness and the development of insulin resistance (Szendroedi et al 2011).

The results of this study uncover previously undocumented alterations in glucose metabolism that can have significant implications in clinical management of mitochondrial DM. Our findings support the concept that insulin resistance is a major factor in the development of DM in individuals with the m.3243A>G mutation. Therefore, thiazolidinediones (TZDs), which are potent insulin sensitizers and are used as oral medications for DM (Ahmadian et al 2013), may be more effective in treating mitochondrial DM. However, the safety and efficacy of TZDs have not been established in mitochondrial DM, and clinical trials are needed to evaluate the use of TZDs in this group of diabetic subjects. If NO deficiency plays a significant role in insulin resistance, another approach to ameliorate insulin resistance could be the use of the NO precursors arginine and citrulline, which have been shown to increase NO production in MELAS (El-Hattab et al 2012a, 2012b, 2014). Clinical trials are needed to evaluate optimal therapy for mitochondrial DM.

An important limitation of our study is the fact that the evaluations were performed after an overnight fast. Therefore, our results reflect alterations in glucose and insulin metabolism occurring only during the fasting state and no conclusions can be made regarding the fed state. Secondly, the number of studied subjects with the m.3243A>G mutation is relatively small and the subjects showed considerable variability in their clinical phenotype. Finally, one of the diabetic subjects was on insulin therapy (Humalog and Lantus in the morning and Humalog at night) which can affect the assessment of glucose and insulin metabolism in this

subject. To minimize the effect of exogenous insulin, the insulin therapy was withheld starting 12 hours before the initiation of the isotope infusion, so the last Humalog and Lantus the subject received were 12 and 24 hours, respectively, before the initiation of the study.

In conclusion, derangements in glucose metabolism occur in both diabetic and nondiabetic subjects with the m.3243A>G mutation. Fasting insulinopenia is found in a minority of subjects, whereas increased glucose production due to increased gluconeogenesis occurs in both diabetic and non-diabetic subjects with the m.3243A>G mutation. Diabetic subjects have insulin resistance and relative insulin deficiency, resulting in an inability to increase glucose oxidation which can explain the development of DM in those subjects. Non-diabetic subjects have normal insulin sensitivity and therefore are able to increase their glucose oxidation rate. The ability to increase glucose utilization can act as a compensatory mechanism that explains why these subjects do not have DM despite the higher rate of glucose production. Lactic acidemia and insulin resistance can both contribute to increasing gluconeogenesis in subjects with the m.3243A>G mutation. The etiology of insulin resistance in mitochondrial DM can be multifactorial with the decreased glucose oxidative capacity due to the mitochondrial dysfunction contributing to the pathogenesis of both lactic acidemia and increased insulin resistance. This study assesses *in vivo* glucose metabolism under physiological conditions and sheds light on previously undocumented alterations in glucose metabolism that can have significant implications for the clinical management of mitochondrial DM. However, clinical trials are still needed to evaluate optimal therapy for mitochondrial DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- The mitochondrial m.3243A>G mutation is common and leads to a wide clinical spectrum ranging from asymptomatic carriers to MELAS.
- Diabetes mellitus occurs in mitochondrial diseases, with the m.3243A>G mutation being the most common mutation associated with mitochondrial DM.
- The pathogenesis of mitochondrial DM remains largely unknown.
- In this study we used stable isotope infusion techniques to assess glucose metabolism in diabetic and non-diabetic adults with the m.3243A>G mutation.
- Our results revealed increased glucose production due to increased gluconeogenesis in both diabetic and non-diabetic subjects with the m.3243A>G mutation.
- Diabetic subjects also demonstrated insulin resistance and relative insulin deficiency, resulting in an inability to increase glucose oxidation.
- Non-diabetic subjects showed normal insulin sensitivity and level; therefore, they were able to increase their glucose oxidation rate.
- Our results suggest that multiple defects in insulin and glucose metabolism are required for DM to occur in individuals with mitochondrial diseases.

Figure 1.

A: Fasting plasma glucose for control subjects, non-diabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plot with means and SEMs. Excluding the apparent outlier 178 mg/dl from the diabetic group results in changing the mean to 134.5 ± 5.7 mg/dl, but the difference between this group and the control group remains statistically significant at the same p value ($p < 0.001$). **B:** HbA1c for control subjects, nondiabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plot with means and SEMs

Figure 2.

A: Glucose production rate, **B:** gluconeogenesis rate, **C:** percentage of gluconeogenesis out of glucose production, and **D:** glycogenolysis rate for control subjects, nondiabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plots with means and SEMs.

Figure 3.

A: Glucose oxidation rate, **B:** percentage of glucose oxidation out of glucose production, and **C:** glucose clearance rate for control subjects, non-diabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plots with means and SEMs. **D:** Fasting plasma insulin for control subjects, non-diabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plot with means and SEMs. Excluding the apparent outliers 26.5 mU/L from the non-diabetic group and 26 mU/L from the diabetic group results in changes the means to 6.1 ± 1.8 and 7.0 ± 1.4 mU/L, respectively. However, excluding these apparent outliers does not affect the overall results with no statistically significant differences between the control and either the non-diabetic or diabetic groups. **E:** HOMA-β for control subjects, non-diabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plot with means and SEMs. Excluding the apparent outliers 162 from the diabetic group results in changing the mean to 38.4 ± 8.4 , but still no statistically significant difference is reached between this group and the control group. **F:** HOMA-IR for control subjects, non-diabetic subjects with m.3243A>G, and diabetic subjects with m.3243A>G presented as dot plot with means and SEMs. Excluding the apparent outliers 7.0 from the nondiabetic group and 7.8 from the diabetic group results in changes the means to 2.7 ± 0.2 and 1.6 ± 0.3 mU/L, respectively. However, excluding these apparent outliers does not affect the overall results with no statistically significant

differences between the control and the nondiabetic group, and statistically significant differences between the control and the diabetic group with $p < 0.001$.

Table 1

Characteristics of the research subjects, presented as mean $\pm$ SD.

Parameter	Control subjects (n=10)	Subjects with m.3243A > G (n=16)	P
Age (years)	30.6 $\pm$ 8.7	34.8 $\pm$ 12.7	NS
Gender (male/female)	4/6	4/12	NS
Lean body mass (kg)	48.4 $\pm$ 10.6	35.1 $\pm$ 7.7	<0.05
Weight (kg)	66.2 $\pm$ 11.3	52.7 $\pm$ 10.8	<0.05
Height (cm)	168.9 $\pm$ 7.2	160.9 $\pm$ 8.4	<0.05
BMI (kg/m ²)	23.1 $\pm$ 2.9	20.3 $\pm$ 3.4	<0.05

(BMI: body mass index, NS: not statistically significant)