Published in final edited form as:

Mol Genet Metab. 2016 April ; 117(4): 407–412. doi:10.1016/j.ymgme.2016.01.010.

Impaired nitric oxide production in children with MELAS syndrome and the effect of arginine and citrulline supplementation

Ayman W. El-Hattab^{1,2}, Lisa T Emrick^{1,3}, Jean W. Hsu⁴, Sirisak Chanprasert^{1,3}, Mohammed Almannai^{1,3}, William J. Craigen^{1,3}, Farook Jahoor⁴, and Fernando Scaglia^{1,3}

¹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA.

²Division of Clinical Genetics and Metabolic Disorders, Pediatrics Department, Tawam Hospital, Al-Ain, United Arab Emirates.

³Texas Children's Hospital, Houston, Texas, USA.

⁴USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, Texas, USA.

Abstract

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most frequent maternally inherited mitochondrial disorders. The pathogenesis of this syndrome is not fully understood and believed to result from several interacting mechanisms including impaired mitochondrial energy production, microvasculature angiopathy, and nitric oxide (NO) deficiency. NO deficiency in MELAS syndrome is likely to be multifactorial in origin with the decreased availability of the NO precursors, arginine and citrulline, playing a major role. In this study we used stable isotope infusion techniques to assess NO production in children with MELAS syndrome and healthy pediatric controls. We also assessed the effect of oral arginine and citrulline supplementations on NO production in children with MELAS syndrome. When compared to control subjects, children with MELAS syndrome were found to have lower NO production, arginine flux, plasma arginine, and citrulline flux. In children with MELAS syndrome, arginine supplementation resulted in increased NO production, arginine flux, and arginine concentration. Citrulline supplementation resulted in a greater increase of these parameters. Additionally, citrulline supplementation was associated with a robust increase in citrulline concentration and flux and *de novo* arginine synthesis rate. The greater effect of citrulline in increasing NO production is due to its greater ability to increase arginine availability particularly in the intracellular compartment in which NO synthesis takes place. This study, which is the first one to assess NO metabolism in children with mitochondrial diseases, adds more evidence to the

Corresponding author: Fernando Scaglia, MD, FACMG, Professor, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, MS BCM225, Houston, TX, 77030, U.S.A., Phone: 832-822-4280, Fax: 832-825-4294, fscaglia@bcm.edu.

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notion that NO deficiency occurs in MELAS syndrome, suggests a better effect for citrulline because of its greater role as NO precursor, and indicates that impaired NO production occurs in children as well as adults with MELAS syndrome. Thus, the initiation of treatment with NO precursors may be beneficial earlier in life. Controlled clinical trials to assess the therapeutic effects of arginine and citrulline on clinical complications of MELAS syndrome are needed.

Keywords

stable isotope; mitochondrial diseases; stroke-like; lactic acidosis

1. Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most frequent maternally inherited mitochondrial disorders [1]. The clinical diagnosis of this syndrome is based on the presence of three invariant criteria: stroke-like episodes before age 40 years, encephalopathy characterized by seizures and/or dementia, and mitochondrial myopathy evident by lactic acidosis and/or ragged-red fibers (RRFs) [2]. Other common features include recurrent headaches, hearing impairment, peripheral neuropathy, ataxia, recurrent vomiting, diabetes, and short stature. Childhood is the typical age of onset with about 70% of affected individuals presenting before the age of 20 years [3, 4]. The most common mutation associated with MELAS syndrome is the adenine to guanine transition at position 3243 of mtDNA (m.3243A>G) in the *MT-TL1* gene encoding tRNA^{Leu(UUR)} [5, 6]. The m.3243A>G mutation was subsequently found to be associated with other phenotypes that collectively constitute a wide spectrum ranging from MELAS syndrome at the severe end to asymptomatic carrier status at the other end. This mutation was found to be relatively common with a prevalence of 16-18:100,000 [7, 8].

The pathogenesis of MELAS syndrome is not fully understood and believed to result from several interacting mechanisms including impaired mitochondrial energy production, microvasculature angiopathy, and nitric oxide (NO) deficiency [9]. The impaired mitochondrial translation due to the m.3243A>G mutation results in decreased mitochondrial protein synthesis including the electron transport chain (ETC) complex subunits leading to decreased mitochondrial energy production [10, 11]. The inability of dysfunctional mitochondria to generate sufficient ATP to meet the energy needs of various organs is a major player in the multi-organ dysfunction observed in MELAS syndrome. Energy deficiency can induce a compensatory mitochondrial proliferation in various tissues. Mitochondrial proliferation in smooth muscle and endothelial cells of small blood vessels can result in angiopathy and impaired blood perfusion in the microvasculature contributing significantly to the complications observed in MELAS syndrome particularly the stroke-like episodes [12, 13].

In addition to energy depletion there has been growing evidence that NO deficiency occurs in MELAS syndrome and can contribute significantly to its complications [14]. NO produced by the vascular endothelium plays a major role in vascular smooth muscle relaxation that is needed to maintain the patency of small blood vessels [15, 16]. Therefore,

NO deficiency in MELAS syndrome can result in impaired blood perfusion in the microvasculature of different organs that can contribute to the pathogenesis of several complications including the stroke-like episodes. In stroke-like episodes the affected areas in neuroimaging do not correspond to classic vascular distribution (hence called “stroke-like”) [17]. During early stages of these episodes, SPECT (single photon emission computed tomography) scanning studies demonstrated hypoperfusion in the affected regions, indicating that these episodes are due to ischemic insults [18]. It is believed that these ischemic insults result from impaired perfusion in cerebral microvasculature due to the angiopathy and NO deficiency that occur in MELAS syndrome [17, 18]. NO deficiency can also contribute to other manifestations observed in MELAS syndrome including myopathy, lactic acidosis, and diabetes [9, 19]

NO is formed from arginine via the enzyme nitric oxide synthase (NOS), which catalyzes the conversion of arginine to citrulline. Citrulline can be converted to arginine via argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). Therefore, both citrulline and arginine are NO precursors [20]. NO deficiency in MELAS syndrome is believed to be multifactorial in origin. Mitochondrial proliferation in vascular endothelial cells can result in impaired normal endothelial function (endothelial dysfunction), and impaired endothelial NO synthesis can reflect one aspect of endothelial dysfunction [9]. Decreased availability of NO precursors, arginine and citrulline, may have a major contribution in impaired NO production. Low plasma citrulline may result from decreased citrulline synthesis in the mitochondria of enterocytes due to mitochondrial dysfunction [21-23]. Arginine is synthesized *de novo* from citrulline, and most of the citrulline flux is directed toward arginine synthesis. Therefore, lower citrulline availability can result in decreased *de novo* arginine synthesis and lower intracellular arginine availability [21]. Decreased NO production can also result from impaired NOS activity due to oxidative stress resulting from mitochondrial dysfunction [24, 25]. Oxidative stress may also result in increased asymmetric dimethylarginine (ADMA), which is an endogenous inhibitor of NOS [21]. In addition to impaired NO production, postproduction NO sequestration by cytochrome C oxidase in the proliferating mitochondria and NO shunting into reactive nitrogen species driven by oxidative stress can contribute to the NO deficiency in MELAS syndrome [17, 24, 26].

There is no specific consensus approach for treating individuals with MELAS syndrome whose management remains largely not evidence based. Several supplementations, including antioxidants and cofactors, are being used in MELAS syndrome based on limited clinical trials [27]. Uncontrolled studies have reported that L-arginine therapy can be beneficial in stroke-like episodes treatment and prevention [18, 28]. The therapeutic effect of arginine in stroke-like episodes in MELAS syndrome is proposed to be due to increased NO availability leading to improving intracerebral vasodilation and blood flow. This potential mechanism has been supported by the demonstration that arginine supplementation in subjects with MELAS syndrome results in increased NO production and improved flow-mediated dilation (FMD), which is considered a measure of NO synthesized by endothelial cells in response to re-perfusion [21, 29]. Although the clinical effects of citrulline administration in MELAS syndrome have not been studied, it was demonstrated that citrulline supplementation can induce a greater increase in the NO synthesis rate than that associated with arginine

supplementation, indicating that citrulline is a more effective NO precursor than arginine. Therefore, citrulline may have better therapeutic effect than arginine [21].

We previously studied NO metabolism in adults with MELAS syndrome [21]. In this current study we used stable isotope infusion techniques to assess NO production rate and the flux of arginine and citrulline in children with MELAS syndrome and healthy pediatric controls. We also assessed the effect of oral arginine and citrulline supplementations on these variables in children with MELAS syndrome. This study is the first clinical assessment of NO metabolism in children with mitochondrial diseases and its results provide more evidence for the impairment of NO production and its causes in children with MELAS syndrome.

2. Subjects and methods

Five children with MELAS syndrome and five control children participated in this study. Children with MELAS syndrome were diagnosed clinically and harbored the m.3243A>G mutation (Supplementary table 1). 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 at Texas Children's Hospital (TCH).

Children with MELAS syndrome were admitted twice to the CRC at TCH. During the hospital stay, they were placed on a low-nitrite and nitrate, protein-controlled diet containing 0.8 g/kg/day protein. After 48 hours on the diet, the first stable isotope infusion was administered as described below. Following the first infusion, oral L-arginine supplementation was initiated at a dose of 10 g/m² body surface area/day if weight ≥ 20 kg and 500 mg/kg/dose if weight < 20 kg divided every four hours for 48 hours. A second stable isotope infusion was then performed. After an interval of at least one week, the subjects with MELAS syndrome were admitted for a second time, where a similar procedure was conducted, but rather than L-arginine, oral L-citrulline was given at the same dose and frequency. Children who were on regular arginine supplementation before the study were instructed to stop taking arginine at least two weeks before their participation. Control subjects were admitted once and had one stable isotope infusion after being on the controlled diet for 48 hours, but without supplementation with either arginine or citrulline.

Solutions of ¹⁵N₂-guanidino arginine (98%), 5-¹³C-,²H₄-citrulline (99%), and ¹⁵N-citrulline (98%) (Cambridge Isotope Laboratories, Woburn, MA) were prepared in normal saline at the TCH investigational pharmacy. Two intravenous catheters were established in opposite arms, with one catheter used for isotope infusion and the other for blood sampling. The isotope infusion was initiated by the administration of a bolus containing ¹⁵N₂-arginine (5 μmol/kg), ¹³C-,²H₄-citrulline (1 μmol/kg), and ¹⁵N-citrulline (0.16 μmol/kg); followed by a continuous infusion of ¹⁵N₂-arginine (5 μmol/kg/h) and ¹³C-,²H₄-citrulline (1 μmol/kg/h) for six hours. Blood samples were drawn before and hourly during the stable isotope infusion.

The blood samples were drawn into heparinized tubes and centrifuged immediately. The plasma was transferred and stored at -70 °C for later analyses. The plasma arginine and citrulline isotopic enrichments were measured by liquid chromatography-tandem mass

spectroscopy (LC-MS/MS) after conversion to their DANS [5-(dimethylamino)-1-naphthalene sulfonamide] derivatives as previously described [30].

The isotopic enrichments of arginine and citrulline typically reach plateau in plasma during the final 3 hours of the infusion. The enrichment plateau was established during the final three hours in the research subjects and therefore the rate of appearance or flux (Q) of arginine and citrulline were calculated from the steady-state equation:

$$Q (\mu\text{mol/kg/h}) = [(IE_{inf}/IE_{plateau}) - 1] \times i$$

where IE_{inf} is the isotopic enrichment of infused arginine or citrulline, $IE_{plateau}$ is the isotopic enrichment of arginine or citrulline in plasma at the steady state, and i is the infusion rate of $^{15}\text{N}_2$ -arginine or ^{13}C -, $^2\text{H}_4$ -citrulline.

De novo arginine synthesis rate was calculated from the rate of conversion of citrulline to arginine as previously described [31]:

De novo arginine synthesis rate ($\mu\text{mol/kg/h}$)

$$=Q_{cit \rightarrow arg} = Q_{arg} \times (IE_{arg}/IE_{cit}) \times [Q_{cit}/(i_{cit} + Q_{cit})]$$

where IE_{arg} is the plateau plasma enrichment of the M+5 isotopomer of arginine (^{13}C -, $^2\text{H}_4$ -arginine derived from ^{13}C -, $^2\text{H}_4$ -citrulline), IE_{cit} is the plateau plasma enrichment of the M+5 isotopomer of citrulline (^{13}C -, $^2\text{H}_4$ -citrulline), and i_{cit} is the infusion rate of ^{13}C -, $^2\text{H}_4$ -citrulline.

The NO synthesis rate was calculated from the rate of conversion of arginine to citrulline as previously described [31]:

NO synthesis rate ($\mu\text{mol/kg/h}$) = $Q_{arg \rightarrow cit} = Q_{cit} \times (IE_{cit}/IE_{arg}) \times [Q_{arg}/(i_{arg} + Q_{arg})]$ where IE_{cit} is the plateau plasma enrichment of the M+1 isotopomer of citrulline (^{15}N -citrulline derived from $^{15}\text{N}_2$ -arginine), IE_{arg} is the plateau plasma enrichment of the M+2 isotopomer of arginine ($^{15}\text{N}_2$ -arginine) and i_{arg} is the rate of infusion of $^{15}\text{N}_2$ -arginine.

Plasma amino acids concentrations were measured by standard high-pressure liquid chromatography (HPLC). Plasma ADMA concentrations were measured by *in vitro* isotope dilution by adding $^2\text{H}_7$ -ADMA to plasma samples, converting into its DANS derivative, and measuring the isotopic enrichment of the ADMA as previously described [30].

Results were expressed as means $\pm$ SEMs. The results of the two initial infusions administered during the first (before arginine) and second (before citrulline) admissions for children with MELAS syndrome were averaged and compared to the values of the control children using the unpaired Student's t test. Differences in children with MELAS syndrome before and after arginine or citrulline supplementation were assessed by the paired Student's t test. Tests were considered statistically significant if $p < 0.05$.

3. Results

The age of children with MELAS syndrome ranged from 4 to 16 years (mean 9.4 years) and of the control group from 7 to 16 years (mean 12.1 years). Children with MELAS had lower body weight (Table 1). When compared to control subjects, children with MELAS syndrome were found to have lower NO production (0.13 ± 0.02 vs 0.19 ± 0.03 $\mu\text{mol/kg/h}$, $p < 0.05$), arginine flux (67 ± 3 vs 159 ± 12 $\mu\text{mol/kg/h}$, $p < 0.001$), plasma arginine concentration (61 ± 5 vs 151 ± 18 $\mu\text{mol/L}$, $p < 0.001$), and citrulline flux (8.0 ± 0.3 vs 9.1 ± 0.4 $\mu\text{mol/kg/h}$, $p < 0.05$). Plasma citrulline concentration showed a trend to be higher (22 ± 7 vs 16 ± 2 $\mu\text{mol/L}$) and *de novo* arginine synthesis showed a trend to be lower in children with MELAS syndrome (5.3 ± 0.5 vs 5.9 ± 0.5 $\mu\text{mol/kg/h}$); however these changes were not statistically significant (Figure 1). ADMA was lower in children with MELAS syndrome (0.66 ± 0.04 vs 0.81 ± 0.05 $\mu\text{mol/L}$, $p < 0.05$).

NO production rate increased with arginine in children with MELAS syndrome ($0.13 \pm 0.02 \rightarrow 0.23 \pm 0.03$ $\mu\text{mol/kg/h}$, $p < 0.05$) and with citrulline ($0.13 \pm 0.03 \rightarrow 0.41 \pm 0.06$ $\mu\text{mol/kg/h}$, $p < 0.001$) supplementations. Arginine flux also increased with arginine ($66 \pm 4 \rightarrow 145 \pm 17$ $\mu\text{mol/kg/h}$, $p < 0.005$) and citrulline ($67 \pm 3 \rightarrow 186 \pm 11$ $\mu\text{mol/kg/h}$, $p < 0.001$) supplementations. Plasma arginine concentration increased with arginine ($59 \pm 5 \rightarrow 184 \pm 14$ $\mu\text{mol/L}$, $p < 0.001$) and citrulline ($64 \pm 5.7 \rightarrow 257 \pm 21$ $\mu\text{mol/L}$, $p < 0.001$) supplementations as well. Plasma citrulline concentration did not change with arginine ($22 \pm 7 \rightarrow 21 \pm 7$ $\mu\text{mol/L}$), but increased with citrulline ($21 \pm 7 \rightarrow 156 \pm 38$ $\mu\text{mol/L}$, $p < 0.05$) supplementation. Citrulline flux and *de novo* arginine synthesis rates increased with arginine supplementation; however, these increments did not reach statistical significance ($7.6 \pm 0.4 \rightarrow 9.3 \pm 1.5$ $\mu\text{mol/kg/h}$ and $5.3 \pm 0.6 \rightarrow 6.4 \pm 1.4$ $\mu\text{mol/kg/h}$, respectively). On the other hand, citrulline supplementation resulted in significant increases in citrulline flux ($8.4 \pm 0.4 \rightarrow 61.3 \pm 3.8$ $\mu\text{mol/kg/h}$, $p < 0.001$) and *de novo* arginine synthesis ($5.3 \pm 0.4 \rightarrow 36.3 \pm 3.8$ $\mu\text{mol/kg/h}$, $p < 0.001$). Compared to the effect of arginine supplementation, citrulline supplementation resulted in greater increases in NO production rate, flux of arginine and citrulline, plasma arginine and citrulline concentrations, and *de novo* arginine synthesis (Figure 2).

4. Discussion

This study evaluates the effect of arginine and citrulline supplementation on NO production in children with MELAS syndrome. It is the first study to assess NO production in children with mitochondrial diseases. The results provide evidence for NO deficiency in children with MELAS syndrome and the potential beneficial effects of the NO donors, arginine and citrulline, in treating children with MELAS syndrome.

The results demonstrated lower NO production rate, flux of arginine and citrulline, and plasma concentration of arginine in children with MELAS syndrome when compared to control children. Although the etiology of NO deficiency in MELAS is believed to be multifactorial due to decreased NO synthesis or post production sequestration, this study provides more insight about the role of decreased NO precursors in the etiology of NO deficiency. Both plasma arginine concentration and flux were lower in children with MELAS syndrome. Therefore, decreased arginine availability acts as a major factor in

limiting NO production. The main source of citrulline, which is a non-protein amino acid, is the *de novo* synthesis in the small intestine through a number of mitochondrial enzymes [32, 33]. Therefore, the observed decreased citrulline flux in children with MELAS syndrome can be a consequence of mitochondrial dysfunction. The majority of citrulline released by the intestine is metabolized within the kidney, where it is converted into arginine [31]. Although, the exact etiology for lower arginine flux and plasma arginine concentration in children with MELAS syndrome is not known, the reduced citrulline flux can contribute to the observed reduced arginine availability as arginine is derived from the diet, as a result of protein turnover, and from endogenous (*de novo*) synthesis from citrulline [34].

Both citrulline and arginine supplementation resulted in increased NO production rate. Arginine supplementation resulted in ~2-fold increase in NO production along with ~2-fold increase in arginine flux and ~3-fold increase in arginine concentration. The effect of citrulline supplementation was greater with ~3-fold increase in NO production, ~3-fold increase in arginine flux, ~4-fold increase in arginine concentration, and ~7-fold increase in citrulline flux, citrulline concentration and *de novo* arginine synthesis rate. Therefore, citrulline supplementation had a greater effect than arginine supplementation in increasing NO production, fluxes and concentrations of arginine and citrulline, and *de novo* arginine synthesis rate.

The ability of oral citrulline supplementation to increase plasma arginine levels more than supplementation of the same dose of arginine may be due to higher intestinal absorption of citrulline relative to arginine due to the action of the intestinal enzyme arginase II on ingested arginine [35]. Another potential cause of this finding is the fact that citrulline bypasses the liver, whereas arginine is converted to ornithine in the liver through the action of arginase I [32]. This finding is further supported by pharmacokinetics studies demonstrating that citrulline has better absorption and systemic bioavailability than arginine [36-38].

The increment in NO production associated with arginine and citrulline supplementation was accompanied with increments in both arginine flux and concentration indicating that the increment of NO production is driven by increased availability of arginine. The greater ability of citrulline to increase arginine flux and arginine concentration explains part of its greater effect in increasing NO production due to its ability to make arginine more available for NO production. However, the more important feature of citrulline is its greater ability to increase the intracellular arginine pool in the subcellular compartment where NO is synthesized. First, arginine transport across the cell membrane is tightly regulated by the cationic amino acid transporter (CAT), whereas citrulline does not have a specific transporter but rather uses generic amino acid transporters [32]. Secondly, part of the intracellular arginine may be utilized by arginase whereas citrulline acts as a direct precursor for intracellular arginine synthesis [14, 35]. Citrulline concentration, citrulline flux, and *de novo* arginine synthesis increased remarkably during citrulline supplementation indicating the superiority of citrulline in increasing the intracellular arginine pool that contributes to the greater ability of citrulline to increase NO production. Third, citrulline has not only a greater ability to increase intracellular arginine, but it has been suggested that citrulline also increases arginine in the subcellular compartments where NO synthesis takes place [21, 22].

The three enzymes responsible for recycling citrulline to produce NO (ASS, ASL, and NOS) have an interesting relationship. It has been demonstrated that ASS and ASL are transcriptionally co-induced with NOS in various cell types [35, 39]. Furthermore, ASS and ASL interact and co-localize with the different NOS isoforms, suggesting that these proteins work as a complex [40-42]. The loss of ASL was shown to result in decreased abundance of the ASL-ASS-NOS complex and NO synthesis, suggesting that the formation of this complex is needed for NO production and may function in the cellular compartmentalization of NO synthesis [40]. Therefore, the *de novo* arginine synthesis occurs in the sub-cellular compartments that contain NOS, ASS and ASL where arginine can be a direct substrate of NOS to produce NO. In contrast, plasma arginine needs to be transported into the cell and escape arginase degradation to finally reach the sub-cellular compartment that contains NOS [21, 22, 35]. Therefore, the *de novo*-synthesized arginine has been suggested to play a more important role in driving NO synthesis than plasma arginine.

In a previous study we used stable isotope infusion techniques to assess NO production and arginine and citrulline fluxes in control adults and adults with MELAS syndrome [21]. NO production was also lower in adults with MELAS syndrome when compared to the control adults (0.07 ± 0.01 vs 0.10 ± 0.01 $\mu\text{mol/kg/h}$). It is interesting to notice in this study that control children have almost double the rate of NO production compared to control adults (0.19 ± 0.03 vs 0.10 ± 0.01 $\mu\text{mol/kg/h}$). Similarly, children with MELAS syndrome have almost double the rate of NO production compared to adults with MELAS syndrome (0.13 ± 0.02 vs 0.07 $\mu\text{mol/kg/h}$). Arginine fluxes in adults with MELAS and control adults were 50 ± 3 and 56 ± 3 $\mu\text{mol/kg/h}$, respectively. Children with MELAS and control children had higher arginine fluxes (67 ± 3 and 159 ± 12 $\mu\text{mol/kg/h}$, respectively) than adults. Citrulline fluxes in adults with MELAS and control adults were 5.7 ± 0.4 and 8.2 ± 0.6 $\mu\text{mol/kg/h}$, respectively; whereas citrulline fluxes in children with MELAS and control children were 8.0 ± 0.3 and 9.1 ± 0.4 $\mu\text{mol/kg/h}$, respectively [21]. The higher flux values in children are associated with higher metabolism rate during childhood comparing to adulthood. Therefore, is extremely important not to use adults control when studying metabolic flux in children. If the NO production in children with MELAS syndrome were compared to control adults' values, the difference would not have been identified due to comparing children with impaired NO production to adults who physiologically have lower NO production rates. It is worth mentioning that this study is the first study that assesses NO production and flux of arginine and citrulline in healthy children by using stable isotopes.

In our previous study we found that adults with MELAS syndrome have higher ADMA concentration than control adults (0.53 ± 0.04 vs 0.38 ± 0.02 $\mu\text{mol/L}$) [21]. However, in this study ADMA was not higher in children with MELAS syndrome when compared to control children (0.66 ± 0.04 vs 0.81 ± 0.05 $\mu\text{mol/L}$). These finding suggests that ADMA, which is a NOS endogenous inhibitor, does not contribute to the NO deficiency in children with MELAS syndrome. Increased oxidative stress due to mitochondrial dysfunction can inhibit the activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme metabolizing ADMA, and increase proteolysis releasing more methylated arginine residues [21]. The observed higher ADMA in adults with MELAS can be due to increased oxidative stress; whereas children with MELAS syndrome may not have such excessive oxidative stress to generate more ADMA than control children.

Our previous study showed that adults with MELAS syndrome had impaired NO production that improved with arginine and, to greater extent, with citrulline supplementations [21]. This study demonstrated similar results (lower NO production and greater effect for citrulline in restoring NO production) in children with MELAS syndrome. Furthermore, it adds more evidence to the initial findings of NO deficiency in MELAS syndrome. Moreover, it indicates that impaired NO production occurs since childhood in MELAS syndrome and therefore the initiation of the NO precursor treatment may be beneficial earlier in life.

Increasing NO availability with arginine or citrulline supplementation can potentially improve perfusion in all microvasculature compartments. Therefore, the effect of arginine and citrulline supplementation may not be limited to improving stroke-like episodes, but may also lead to improvements in other clinical features of MELAS syndrome, including muscle weakness, exercise intolerance, and lactic acidosis [9]. Interestingly, arginine and citrulline supplementation has been reported to result in a reduction in plasma alanine and lactate concentrations, suggesting that such supplementation may improve lactic acidemia in MELAS syndrome by increasing NO production and improving perfusion and oxygen delivery [21, 19]. Additional clinical studies assessing the clinical effects of citrulline and arginine supplementations on different manifestations of MELAS syndrome are needed to determine their potential therapeutic utility in this syndrome. This study and our previous study assessing the effect of arginine and citrulline in adults with MELAS syndrome [21] support the concept that citrulline leads to higher NO production and suggest that citrulline may have a better therapeutic effect than arginine. However, no clinical studies have yet been performed to assess the long term clinical utility of citrulline in ameliorating the complications associated with MELAS syndrome.

Although we have used standardized stable isotope infusion techniques and limited the effect of diet on amino acid flux and NO by providing a low-nitrite and nitrate, protein-controlled diet, the limited number of research subjects remains a potential weakness of this study. Recruiting children for clinical studies is typically more challenging than recruiting adults particularly when the study includes complex protocols like ours.

5. Conclusion

This study showed that children with MELAS syndrome had lower NO production, arginine flux, plasma arginine concentration, and citrulline flux. Citrulline supplementation was more effective than arginine supplementation in increasing NO production rate, arginine and citrulline fluxes and concentrations, and *de novo* arginine synthesis rate. This study is the first one to assess NO metabolism in children with mitochondrial diseases and it adds more evidence to the initial finding of NO deficiency in MELAS syndrome. It also suggests that impaired NO production occurs since childhood in MELAS syndrome and therefore the initiation of the NO donor treatment may be beneficial earlier in life. Citrulline supplementation may have a better therapeutic effect but this will have to be determined by future controlled clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This study was funded by the Society for Inherited Metabolic Disorders (SIMD) through the SIMD/Hyperion fellowship award that Ayman El-Hattab has received in 2009. We also acknowledge the patients and their families for their participation, the United Mitochondrial Disease Foundation for their referral of patients, the National Institutes of Health for funding the Clinical Research Center (M01-RR0188), and the Clinical Research Center staff at Texas Children's Hospital.

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Highlights

- Using stable isotope infusion we assessed NO metabolism in children with MELAS.
- Children with MELAS had lower NO synthesis rate.
- Arginine and citrulline supplementation increased NO production in MELAS children.
- Citrulline had greater effect in increasing NO production.
- Citrulline may have a better therapeutic effect than arginine.

Figure 1.

NO production, arginine flux, citrulline flux, plasma arginine concentration, plasma citrulline concentration, and *de novo* arginine synthesis rate in control children and children with MELAS syndrome. The bars represent the mean ($n=5$) and the vertical lines above the bar represent the positive SEM (the negative SEM is not shown).

Figure 2.

The effect of arginine and citrulline supplementations on NO production, arginine flux, citrulline flux, plasma arginine concentration, plasma citrulline concentration, and *de novo* arginine synthesis rate in children with MELAS syndrome. The bars represent the mean ($n=5$) and the vertical lines above the bar represent the positive SEM (the negative SEM is not shown).

Table 1

Characteristics of the research subjects, presented as mean $\pm$ 1SD.

Parameter	Control children (n=5)	Children with MELAS (n=5)	P
Age (years)	12.1 $\pm$ 3.6	9.4 $\pm$ 4.8	NS
Gender (male/female)	2/3	4/1	NS
Weight (kg)	44.8 $\pm$ 16.0	20.5 $\pm$ 7.3	<0.05
Height (cm)	153.5 $\pm$ 22.3	123.9 $\pm$ 23.5	NS
BMI (kg/m ²)	18.3 $\pm$ 2.0	13.1 $\pm$ 1.0	<0.05

(NS: not statistically significant)