MESENCHYMAL STEM CELLS FROM AFRICAN AMERICANS DISPLAY GREATER GLUCOSE OXIDATION COMPARED TO STEM CELLS FROM CAUCASIAN INFANTS

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ABSTRACT

Background: Type 2 Diabetes and Obesity are more prevalent in African Americans (AA) than Caucasian (C) adults. Associated with this is Metabolic Inflexibility, the inability to switch between substrate usage depending on the physiological demand. Data regarding metabolic differences between racial groups at birth has still not been explored. **PURPOSE:** The purpose was to discover if there are racial differences in substrate metabolism that is evident at birth utilizing mesenchymal stem cell (MSCs) collected from infant umbilical cords. **METHODS:** 12 mother-child dyads were included in this study; mesenchymal stem cells were collected from the umbilical cords of 6 AA and 6 C infants. Radio-labeled tracers were used to test differences in fatty acid and glucose metabolism in the differentiated and myogenically differentiated states (a model of infant skeletal muscle). **RESULTS:** The results from our analysis indicate in myogenic MSCs, the glucose oxidation rate was significantly higher in AA compared to C in both basal ($p \leq 0.05$) and insulin-stimulated ($p \leq 0.05$) states. Interestingly, NOGM production and glucose partitioning were similar between groups in both basal ($p \geq 0.05$) and insulin-stimulated ($p \geq 0.05$)

states. In contrast to the previously described phenotype in adults, similar relative glycogen synthesis rates were observed between groups ($p \geq 0.05$). To assess if there is an inherent "metabolic driver" associated with the observed phenotype, we compared the difference between D0 and D21 MSCs. Accordingly, we observed that upon myogenic differentiation, MSCs from AA infants increase glucose oxidation rate significantly more ($p \leq 0.05$) than MSCs from C infants. Further, AA shifts significantly more towards glucose rather than palmitate oxidation upon myogenic differentiation ($p \leq 0.05$) compared to C infants. **CONCLUSION:** Collectively, these data suggest that there are metabolic differences present at birth between races. Further investigation is needed to address if these differences are influential in the manifestation of metabolic disease disparities observed in adults.

Mesenchymal Stem Cells from African Americans Display Greater Glucose Oxidation Compared to Stem Cells from Caucasian Infants

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Chapter 1: Introduction

Introduction

Skeletal muscle (SkM) insulin resistance (IR), evident by lower SkM glucose uptake upon insulin stimulation, has been associated with a greater risk for the development of metabolic syndrome, type 2 diabetes, and cardiovascular disease. African Americans have a higher prevalence of insulin resistance (Kodama et al., 2013) compared to Caucasians and are seemingly metabolic inflexible (MI) (Berk et al., 2006). MI is the inability to alter metabolism regardless of available fuel and is associated with insulin resistance. This inability to switch between substrates causes the metabolism to predominantly oxidize glucose, which increases lipid accumulation subsequently potentiating IR. To date, it is unclear if African Americans are innately IR and MI. Understanding if African Americans display MI and are IR at birth will help close the gap among racial disparities in health and inform potential intervention during development and early childhood subsequently decreasing the likelihood of cardiometabolic diseases.

Purpose

It has been proposed that insulin resistance is genetically driven, however, little research is done on a non-adult population. There is little literature directly testing this at the early stages of life. Mesenchymal stem cells (MSC) have been proposed as a model to study infant metabolism considering that MSCs reflect the phenotype of an offspring donor. Additionally, MSCs can be differentiated in a multitude of mesenchymal tissue including skeletal muscle, which allows us to understand innate differences in infant metabolism. The primary purpose of this study is to determine if there is a preference for glucose oxidation in infant myogenically

differentiated MSCs between African Americans and Caucasians. The secondary aim was to determine if there is a difference in insulin sensitivity and substrate preference. A nested pilot study design using myogenically differentiated mesenchymal stem cells derived from offspring umbilical cords was used as a model. This model was used to avoid confounding variables of nurture (i.e., diet of infant, breastfeeding), and assessed metabolic differences between races. The study evaluated fatty acid and glucose oxidation in undifferentiated MSCs (D0) and myogenically differentiated stem cells (D21) from the infant of African American and Caucasian women.

Research question

Undifferentiated (D0) and Differentiated (D21) MSC of infants from African American women will demonstrate a preference for glucose oxidation and similar fatty acid oxidation, relative to the Undifferentiated (D0) and Differentiated (D21) MSC of infants from Caucasian women.

Limitations

The study did not account for women outside a Body Mass Index of 18.5-39.9 kg/m^2, which could limit generalizability. This study also did not account for other maternal variables (i.e., diet), which may influence fetal development. Race was self-reported by the participants. The study did not account for the modes of exercise or lifestyle habits of the recruited women that could influence fetal development. There was also the inability to measure gene expression and protein content. The study also did not have cells incubated with more than one substrate, which does not measure the true physiological state of competing substrates present.

Delimitations

This study accounted for premenopausal women 18-40 years old. The study matched participants based on maternal age, pre-pregnancy BMI, gestational weight gain, parity, and mode of delivery, along with fetal sex.

Chapter 2: Review of Literature

Prevalence of Insulin Resistance in African Americans

Insulin resistance is described as a condition in which normal or elevated insulin levels produce an attenuated physiological response and lower glucose disposal (Wilcox et al., 2005). A person is classified as insulin resistant when higher circulating levels are needed for cells to uptake glucose from the blood (Peterson & Shulman, 2018). Insulin resistance increases the risk for the development of fatty liver, Type II Diabetes, and Metabolic Syndrome (Centers for Disease Control and Prevention [CDC], 2021). African Americans have a higher prevalence of IR compared to Caucasian populations (Haffner et al., 1996). A study by Haffner et al. showed that African Americans categorized as nondiabetic were hyperinsulinemic and insulin resistant compared to non-Hispanic Whites independent of obesity status; yet a point of causation is still unknown (Hyatt, et al., 2009).

Metabolic Inflexibility Associated with Insulin Resistance

Metabolic Inflexibility is known as an inability to switch between oxidation of the substrate's glucose and fatty acid (Muoio, 2014). In a healthy state, mitochondria switch between glucose and fatty acids freely depending on the physiological state, and the presence of food (Muoio, 2014). Insulin resistance has been associated with inefficient mitochondrial substrate oxidation and the inability to switch between substrates (Muoio, 2014). Greater reliance on glucose oxidation and inability to switch to fat oxidation results in intramyocellular lipid accumulation leading to fat accumulation and insulin resistance (Galgani et al., 2008). Finally, Randle (Randle, 1998) noted that fat oxidation is suppressed proportional to the increase in glucose oxidation, suggesting that greater reliance on glucose could promote lower fat oxidation

African Americans are Metabolically Inflexible

Metabolic Inflexibility was observed in obese and type II diabetics and is demarked by an absence of fatty acid oxidation in skeletal muscle, and the inability to switch substrate use across different physiological states (fed vs fasted) (Kelley et al, 1999). In a healthy population, substrates can be switched based on their availability; however, even within a healthy population, there is variability in the range of metabolic flexibility (Ukropcova et al, 2005). Despite this range, individuals who have mitochondria with decreased ability to switch substrate oxidation based on substrate availability or physiological state will have an increased propensity of developing obesity and/or diabetes.

According to the National Center for Health Statistics, 1981, compared to Caucasian women, African American women have a higher obesity prevalence. African American women also have an increased prevalence of insulin resistance than Caucasian counterparts (Nicklas et al, 2012). A study by Berk et al, in 2006, showed that African American women do not change their fat oxidation, regardless of whether they were on a low- or a high-fat diet, and prefer carbohydrate oxidation compared to Caucasian women. Since glucose oxidation remained high regardless of the greater fatty acid availability after a high-fat diet, Berk et al demonstrated that African American women, regardless of BMI, are metabolically inflexible (Berk et al, 2006). African Americans, regardless of BMI and diet, have an innate preference for glucose oxidation rather than fat oxidation, suggesting that the system is altered in this population (Berk et al, 2006). One of the reasons why African Americans might be affected is due to the differences in phenotype in metabolic pathways (Ama et al, 1986). For example, in skeletal muscle, African Americans display more glycolytic enzymatic activity in comparison to Caucasians (Ama et al, 1986). Furthermore, the enzyme succinate dehydrogenase has lower activity in non-obese sedentary African American women in comparison to Caucasians; however, beta-oxidation along

with beta-hydroxy acyl CoA were similar between the racial groups (Privette et al, 2003). Along with these findings, lower levels of mitochondrial acyl-CoA synthetase in African American relative to Caucasians suggest a lower potential for fat oxidation (Privette et al, 2003). This finding is further supported by the observed lower fat oxidation in muscle homogenates of African Americans compared to Caucasians (Cortright et al, 2006). Even though Cortright et al. observed differences in subjects with obesity, the difference in palmitate oxidation from lean African Americans relative to lean Caucasians followed the same pattern, though was not significant (Cortright et al. 2006). This finding infers that predispositions for African Americans affect fatty acid oxidation specifically and might be influenced by the degree of metabolic stress. Thus, the inability to switch substrate preference, due to decreased metabolic enzymatic activity suggests African Americans have a different metabolic phenotype that predisposes them to become metabolically inflexible.

Mesenchymal Stem Cell Model

Research suggests that mesenchymal stem cells obtained from the umbilical cord represent the phenotype of the infant (Erickson et al, 2021). MSCs can be experimentally differentiated along mesenchymal lineages, including skeletal muscle. Therefore, the MSC model provides the opportunity to directly test human neonatal metabolic function (Erickson et al, 2021). For example, a previous studying using the myogenically MSC model demonstrates that infants born to obese mothers exhibit greater lipid accumulation and lower fatty acid oxidation, correlated with offspring adiposity (Boyle et al, 2017). Together, experiments by Boyle et al and Erickson et al, show that MSC metabolism is consistent with phenotypes observed in infant offspring donors and provide insight into early child metabolism.

Most of the research comparing the substrate metabolism between races focuses on the adult population, where lifestyle and nutritional habits influence their metabolism. By using a MSC model, these factors can be attenuated, allowing us to get a representation of the subject phenotype independent of lifestyle habits, and address innate metabolic differences. Thus, the MSC model can help identify the metabolic phenotype of African Americans related to insulin sensitivity and substrate oxidation.

Summary

Insulin Resistance is associated with metabolic inflexibility and preferential carbohydrate use. It is known that African Americans are metabolically inflexible and have a higher prevalence of Insulin Resistance; however, there is no clear explanation for their metabolic inflexibility. Further, African Americans, regardless of BMI, are more insulin resistant, and have preferential glucose oxidation, regardless of nutrient availability or physiological state. The use of MSC model provides the opportunity to isolate the cellular metabolic phenotype of African Americans and will allow us to see if there is an innate difference in substrate use compared to Caucasians.

Chapter 3: Methods

Participates and Recruitment

For the purposes of this study, we have used the nested design and selected 6 non-Hispanic African American and 6 non-Hispanic Caucasian participants, matched for maternal age, pre-pregnancy BMI, gestational weight gain, parity, age at and mode of delivery, and fetal sex. Healthy pregnant women between 18 and 39 years of age and $\leq$16 weeks' gestation were recruited by flyers placed in local obstetric clinics. Inclusion criteria included a body mass index between 18.5 and 34.9 kg/m^2; singleton pregnancy; free from chronic conditions including diabetes, hypertension, HIV, etc.; absence of tobacco, alcohol, and drug use during pregnancy; and not taking medications that may affect fetal development.

MSC Cell Culture

Isolations

Isolation of human offspring MSCs from umbilical cord explants was completed. Tissue samples for this purpose were collected during a 5-year period (2017-2022). Briefly, a 4-inch section was cleared of all visible blood vessels and adipose tissue, dissected into ~0.5-cm^2 pieces, and placed Wharton's jelly/lumen side-down onto droplets of 30% BSA in 10-cm^2 dishes. The tissue sections were incubated in mesenchymal growth media containing low glucose DMEM (Gibco laboratories, Grand Island, NY) supplemented with 10% MSC-qualified fetal bovine serum, 1x Gentamicin Amphotericin (Life Technologies, Gaithersburg, MD), until cells reached 80% to 100% confluency. Once confluency was obtained, the samples were cryopreserved in liquid nitrogen.

Experimentation

For the metabolic studies, MSCs were seeded onto 12-well plates (Corning, Corning, NY) at a density of 7 x 10^3 cells/cm^2 in mesenchymal growth media and grown to confluency before experimentation in the undifferentiated state (D0). To ensure that stemness and premature differentiation did not confound our D0 measures, we have previously assessed Wnt for myogenic induction. Cells were treated with myogenic induction media (MIM) containing low glucose DMEM, 10% FBS, 5% horse serum, 0.1µM dexamethasone, 50µM hydrocortisone, 0.01x penicillin/streptomycin, for 21 days prior to experimentation (D21). To reduce the potential loss of the donor phenotype and the influence of replicative senescence, all experiments were performed on MSCs in passages 4-6 (Erickson et al., 2021).

Glucose Oxidation

Glucose oxidation was measured as described previously (Chaves et al., 2022; Hinkley et al., 2017; Park et al., 2019; Zou et al., 2020). Following 3 hours of serum starvation, MSCs were incubated with media containing d-[1-^{14}C] glucose (Perkin-Elmer, MA, USA; 1.6 mCi/mL, 5.0 mM glucose) for 2 hours at 37°C. Immediately following incubation, radioactive media was transferred into a customized 48-well trapping plate with fabricated grooves between 2 continuous wells. CO_2 in the media was acid trapped in 1N NaOH via the addition of 70% perchloric acid. The incorporation of radioactive glucose into CO_2 was determined with liquid scintillation counting of the conditioned 1N NaOH to derive a rate of complete glucose oxidation. MSCs were washed with DPBS and solubilized in 0.5% SDS to measure protein concentration and the BCA assay for normalization. Non-oxidized anionic glycolytic metabolite (NOGM) production (i.e., lactate, pyruvate, alanine) was measured as described

previously (Chaves et al., 2022; Zou et al., 2020). Briefly, unacidified media collected during the glucose oxidation experiments was placed onto ion-exchange cellulose paper (anion exchange paper; Macherey-Nagel, Duren, Germany) followed by 30 minutes of drying and 4 washes of 10 minutes each with dH_2O. After washing, ^{14}C-labeled glucose incorporation into NOGMs was determined via liquid scintillation counting of the cellulose paper. Data were normalized to total protein content (BCA assay) and rates were normalized to internal control. Experiments were performed in both D0 and D21 MSCs.

Fatty Acid Metabolism

Following 3 hours of serum starvation, MSCs were washed with DPBS and incubated in ^{14}C-palmitate-containing media (d-[1-^{14}C] palmitate (Perkin-Elmer; 1.0 mCi/mL, 5.0 mM glucose, 200 mM palmitate, 1 mM carnitine) for 2 hours at 37°C. The experimental media were transferred into a customized 48-well trapping plate with fabricated grooves between 2 continuous wells. CO_2 in the media was acid trapped in 1N NaOH via the addition of 70% perchloric acid. Oxidation of ^{14}C-palmitate into CO_2 was determined with liquid scintillation counting of the conditioned 1N NaOH to derive a rate of complete fatty acid oxidation. Acidified media was collected following CO_2 trapping and centrifuged at 12,000*g* at 4°C. An aliquot of the supernatant was measured via scintillation counting to determine levels of 14C-acid-soluble metabolites (i.e., palmitoyl-carnitine, acetyl-carnitine, acetyl-coenzyme A, etc.).

Insulin-Stimulated Glycogen Synthesis

Following 3 hours of serum starvation, MSCs were incubated with media containing d-[1-^{14}C] glucose (Perkin-Elmer, MA, USA; 1.6mCi/mL, 5.0 mM glucose) in the presence (100nM) or absence of insulin at 37°C for 2 hours. Cells were then washed twice with Dulbecco's PBS (DPBS) and solubilized with 0.5% SDS. Lysates were combined with carrier glycogen (1 mg)

and denatured at 100°C for 1 hour. The remaining lysate was used to measure protein concentration using the bicinchoninic acid (BCA) assay (Pierce Biotechnology, Rockford, IL, USA). Ice-cold 100% ethanol was added to the denatured lysates, and samples rotated overnight at 4°C for the precipitation of glycogen. Glycogen pellets were centrifuged at 11,100*g* for 15 minutes at 4°C and washed with 70% ethanol followed by centrifugation. The glycogen pellets were resuspended with dH_2O, and the incorporation of radioactive glucose into glycogen was determined with liquid scintillation counting. Insulin-mediated glycogen synthesis rates were determined by comparing a relative rate (fold change over basal) of glycogen synthesis. Experiments were performed in both D0 and D21 MSCs.

Statistical Analysis

An unpaired t-test was used to determine the statistical significance between group means. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 9.3 for Windows (GraphPad Software, San Diego, CA). An unpaired t-test was used to test the differences in participant descriptors, complete and incomplete oxidation, substrate partitioning, and glycogen synthesis rates between the two groups.

Chapter 4: Results

A total of 12 child-mother dyads (African American, n=6; Caucasian, n=6) were included in this study. Maternal and infant characteristics are reported in Table 1. Mothers in both groups were of similar age, pre-pregnancy BMI, gravida, parity, and gestational length and remained free of gestational diabetes. Infants in all groups had similar sex distribution, birth length, weight, BMI, and mode of delivery.

The results from our analysis indicated that D0 basal glucose oxidation rates and NOGM production were similar between groups ($p \geq 0.05$; Figure 1a-b). However, the glucose partitioning ratio, derived from a ratio of NOGM (index of incomplete oxidation of substrate) and complete glucose oxidation to CO_2, was significantly higher in African American compared to the Caucasian group ($p \leq 0.05$; Figure 1c). Finally, insulin-mediated glycogen synthesis rates were determined by comparing the fold change of glycogen synthesis over basal. Upon insulin stimulation there was no difference in relative glycogen synthesis rates between groups ($p \geq 0.05$; Figure 1d).

The palmitate oxidation rate was similar between groups ($p \geq 0.05$; Figure 2a). Further, ASM production with or without glucose was similar between African American and Caucasian ($p \geq 0.05$; Figure 2b-c). Finally, we used a ratio of palmitate to glucose oxidation ratio as a surrogate for substrate preference; however, group outcomes were similar ($p \geq 0.05$; Figure 2d).

In myogenic MSCs, the glucose oxidation rate was significantly higher in African Americans compared to Caucasians in both basal ($p \leq 0.05$; Figure 3a) and insulin-stimulated ($p \leq 0.05$; data not shown) states. Interestingly, NOGM production and glucose partitioning were similar between groups in both basal ($p \geq 0.05$; Figure 3b-c) and insulin-stimulated ($p \geq 0.05$; data

not shown) states. In contrast to the previously described phenotype in adults, similar relative glycogen synthesis rates were observed between groups ($p \geq 0.05$; Figure 3d).

Palmitate oxidation rates were similar between groups ($p \geq 0.05$; Figure 4a). Similarly, when palmitate was the sole substrate oxidized, ASM production was similar between groups ($p \geq 0.05$; Figure 4b). These data suggest no decrements in the fatty acid oxidation potential in the basal state. A significantly higher ratio of palmitate to glucose oxidation rate was observed in Caucasians compared to African Americans, indicative of differential substrate preference ($p < 0.01$; Figure 4d), and greater glucose preference in African Americans.

To assess if there is an inherent "metabolic driver" associated with the observed phenotype, we compared the difference between D0 and D21 MSCs. Accordingly, we observed that upon myogenic differentiation, MSCs from African American infants increase glucose oxidation rate significantly more ($p \leq 0.05$; Figure 5a) than MSCs from Caucasian infants. Further, African Americans shift significantly more towards glucose rather than palmitate oxidation upon myogenic differentiation ($p \leq 0.05$; Figure 5b) compared to Caucasian infants.

Chapter 5: Discussions

In this study, it was hypothesized that Undifferentiated (D0) and Differentiated (D21) MSC of infants from African American women will demonstrate a preference for glucose oxidation and similar fatty acid oxidation in comparison to the MSC of infants from Caucasian women.

The data showed undifferentiated MSCs from African American offspring exhibit similar glucose and fatty acid oxidation rates, as well as insulin action in comparison to Caucasian infants. The experiments were done with a single substrate of glucose or palmitate; therefore, oxidation rates are solely dependent on the function of the associated metabolic pathways. The similarity in fatty acid oxidation and ASM production rates between the two groups infer that there are no innate impairments of fatty acid oxidation from MSC offspring of African American mothers, contrary to previously described findings in African American adults (Hasson et al., 2015). Similar glucose oxidation and NOGM production rates were also found with a greater partitioning of glucose towards NOGM (i.e., lactate) in African American D0 MSCs. Higher levels of lactate and greater incomplete oxidation with NOGM production have been found to be associated with the development of obesity and metabolic syndrome, as observed in myotubes from women with obesity and type 2 diabetes (Park et al., 2019). This shows the effect on the development of Insulin Resistance in African American needs to be further investigated.

With skeletal muscle having a crucial role in glucose disposal, the MSCs were also differentiated myogenically and then assessed for metabolic differences between the two races. This is the first study demonstrating that glycolytic substrate preference is inherent to the myogenic cell lineage, and independent of the postnatal environment (i.e., diet, activity). All subject pairs had a BMI ranging from healthy to obese and demonstrated greater glucose oxidation, which is indicative of a greater glycolytic capacity. These results are similar to the

previously described phenotype in African American adults, in which African American women have a higher carbohydrate preference in the postprandial state regardless of obesity status (Chatterjee et al., 2014). It is noted that racial differences in skeletal muscle enzymatic activities have been observed with African Americans having higher enzymatic activities for a glycolytic pathway predominance (Zou et al., 2020). While this project did not assess enzymatic activities across groups, future studies should be done to use this data to help support the findings. Interestingly, higher complete glucose oxidation rates with similar NOGM production did not result in improved glucose partitioning. These findings require further verification.

As previously mentioned, fatty acid oxidation decrements in African American adults have been observed mostly in the presence of competing, rather than in the isolation of a single substrate (Muoio, 2014). No differences were found in complete (CO2) and incomplete (ASM) palmitate oxidation when palmitate was used as the sole substrate in either undifferentiated or myogenically differentiated MSCs. These data suggest that African Americans do not have innate decrements in fatty acid oxidation. However, considering that the preference for glucose could inhibit fatty acid oxidation, as substrates are mutually exclusive, lowering the dietary glycemic index could result in greater fatty acid oxidation and subsequently lower adiposity. As shown from studies by Gower et al. African Americans have comparatively greater decreases in adiposity and improvements in metabolic outcomes following a low carbohydrate diet.

Lastly, the ratio of palmitate to glucose oxidation was used as a proxy for substrate metabolic pathway dominance. In line with the original hypothesis and previous reports, we found a lower palmitate-to-glucose oxidation ratio in myogenically differentiated MSCs from African American compared to Caucasian infants. It is also worth noting that the differentiation of MSCs to the myogenic lineage demonstrated a ~40% increase in glucose oxidation in African

Americans, and a ~14% decrease in the Caucasian group (Figure 5a). This increase in glucose oxidation upon myogenic differentiation of MSCs elicited a significantly greater preference for glucose in African American, but not in Caucasian infant MSCs (Figure 5b). Collectively, these data suggest that there is a differential prenatal programming of the metabolic phenotype between races which calls for further studies in association with different metabolic diseases and insulin resistance. Further studies in additional cell lines such as adipogenic differentiation and hepatic tissue should be performed to observe if these metabolic differences are tissue specific.

Tables & Figures

Table 1. Maternal and Infant Characteristics			
Maternal Characteristics	**AA (n=6)**	**C (n=6)**	**p-value**
Age	28.67±6.02	31.00±4.10	0.43
Pre-pregnancy BMI	28.46±5.95	27.97±5.89	0.89
Gravida*	2 (1, 5)	2 (1, 3)	0.51
Parity*	0.50 (0, 4)	0.50 (0, 1)	0.36
Gestational length (weeks)	39.30±0.61	38.75±1.44	0.41
Mode of delivery (Spontaneous vaginal delivery/Cesarean-section)	3/3	4/2	0.64
Neonate Characteristics	**AA (n=6)**	**C (n=6)**	**p-value**
Fetal sex (F/M)	2/4	1/5	0.59
Birth weight (kg)	3.37±0.42	3.56±0.72	0.89
Birth length (m)	0.49±0.01	0.49±0.04	0.95
Birth BMI	14.06±1.29	14.72±1.99	0.51
* Mann-Whitney U test was performed, and data is expressed as median (minimum, maximum). All other data is expressed as mean ± SD, and t-test was performed with $p \leq 0.05$.			

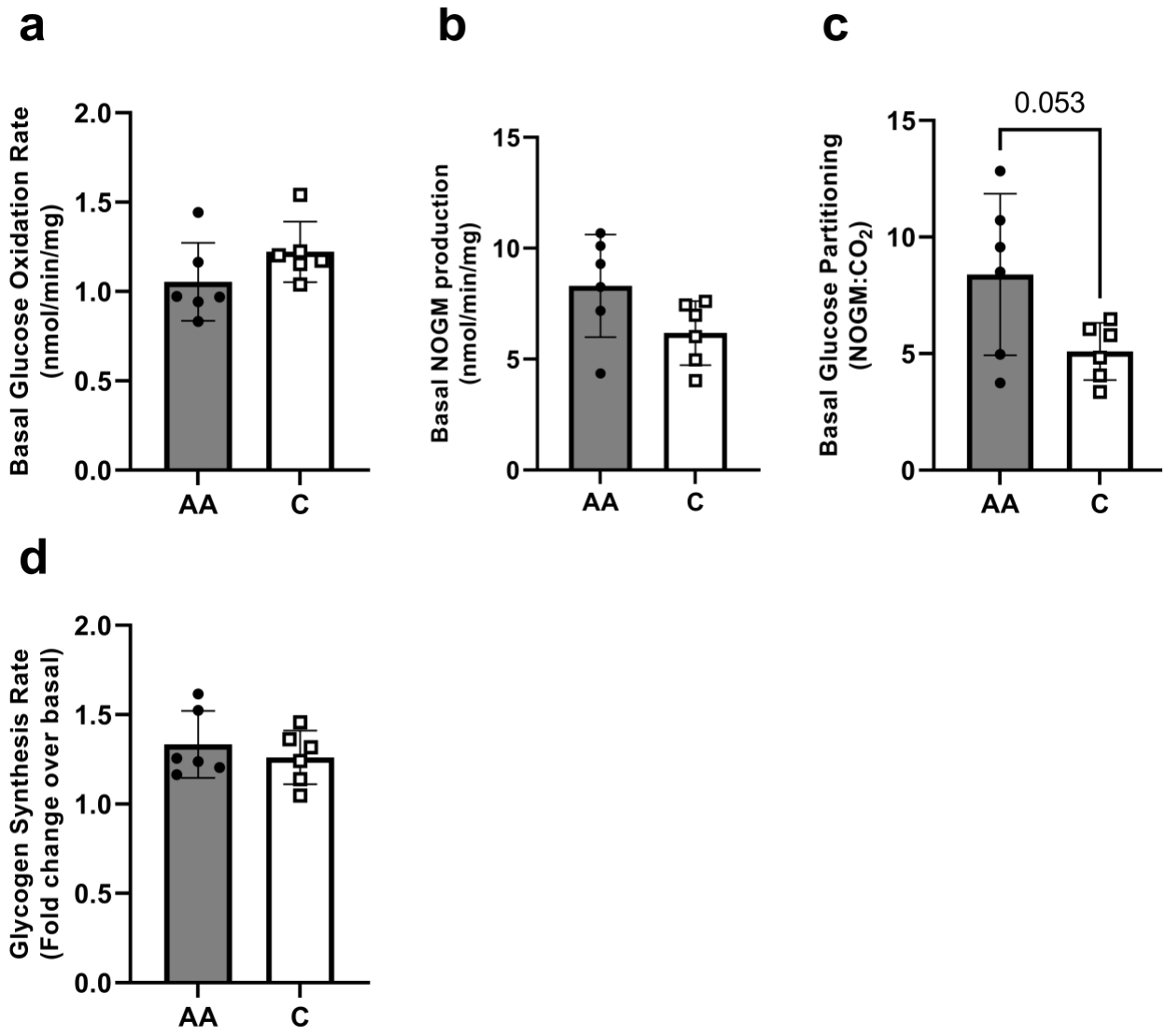

Figure 1. Undifferentiated D0 MSCs from African American (AA) and Caucasian (C) infants display similar glucose oxidation (a) and non-oxidized glucose metabolite (NOGM) production (b), yet MSCs from offspring of AA mothers have a greater glucose partitioning towards NOGM than complete oxidation (CO_2) (c). Both groups display similar insulin action measured by the relative rate of glycogen synthesis (d). * $p \leq 0.05$.

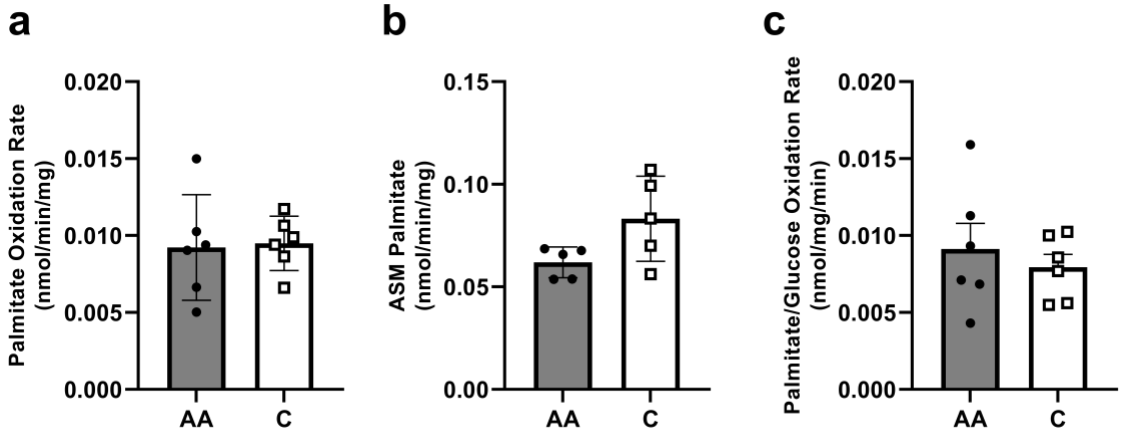

Figure 2. Undifferentiated D0 MSCs from African American (AA) and Caucasian (C) infants have similar rates of palmitate oxidation (a) and acid soluble metabolite (ASM) production in both palmitate-only conditions (b). Both groups display a similar ratio of palmitate to glucose oxidation rate (c).

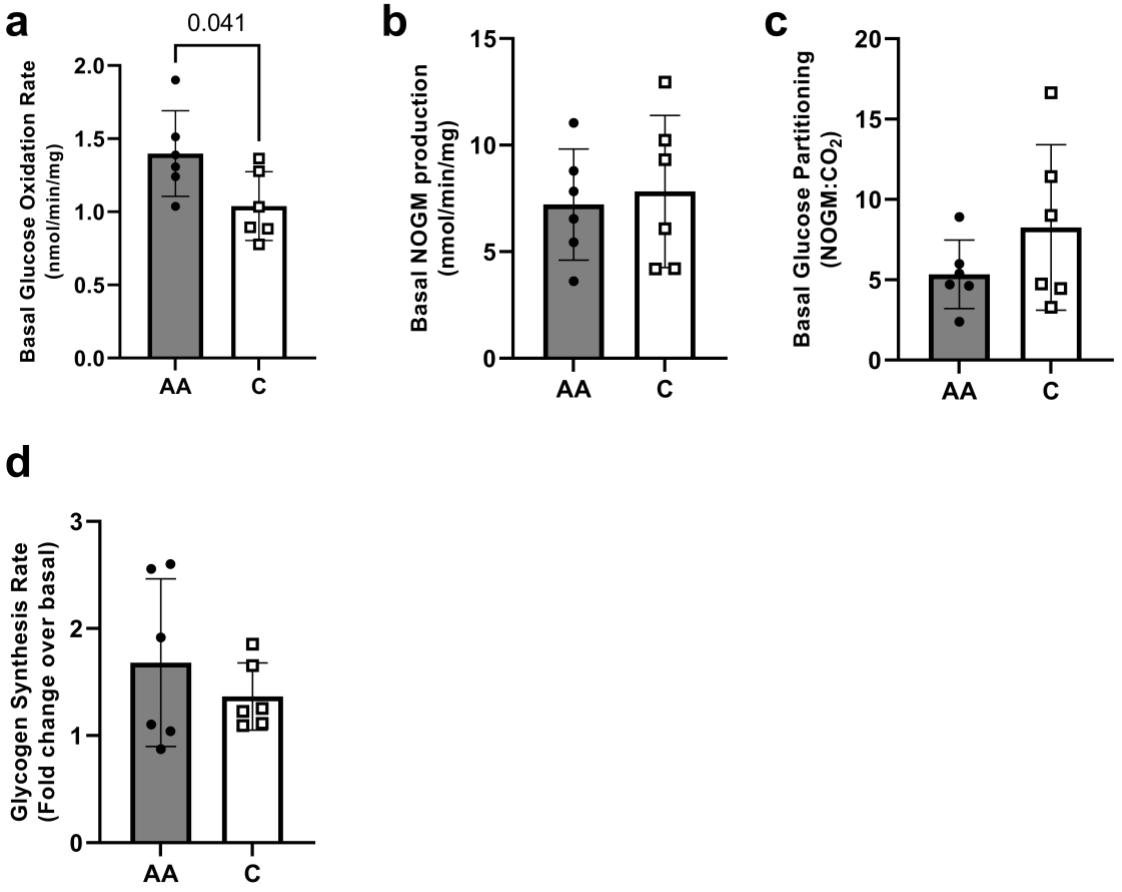

Figure 3. African American (AA) compared to Caucasian (C) myogenically differentiated D21 MSCs have higher basal glucose oxidation rate (a), without any difference in non-oxidized

glucose metabolite (NOGM) production (b). Though myogenically differentiated MSCs from AA display significantly higher glucose oxidation, they have similar basal glucose partitioning (c) relative to MSCs from C infants. Both groups exhibit similar glycogen synthesis rates (d).

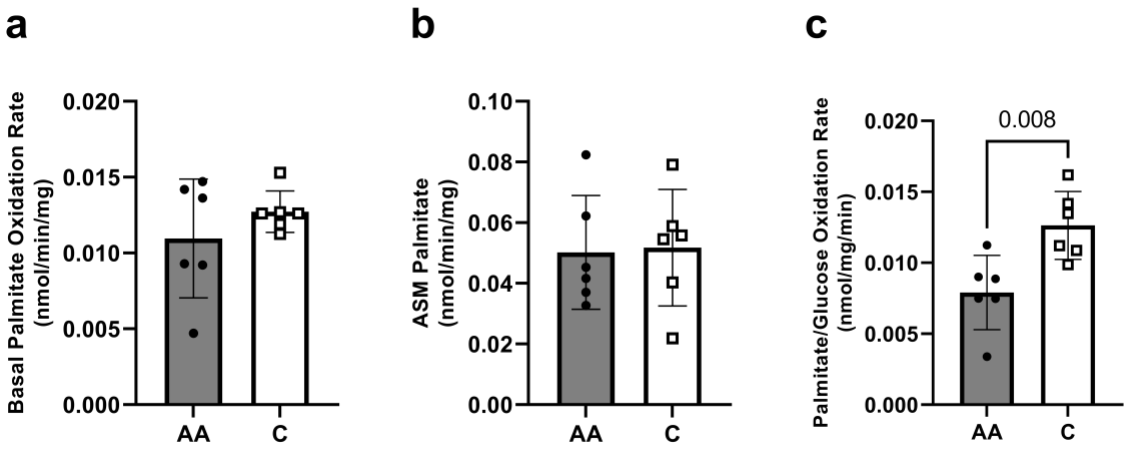

Figure 4. Myogenically differentiated MSCs from African American (AA) and Caucasian (C) infants exhibit similar palmitate oxidation rates (a). Acid-soluble metabolite production was similar between groups when palmitate was the sole substrate (b). C have significantly higher palmitate to glucose oxidation rates suggestive of greater fatty acid substrate preference when compared to AA infant MSCs (c).

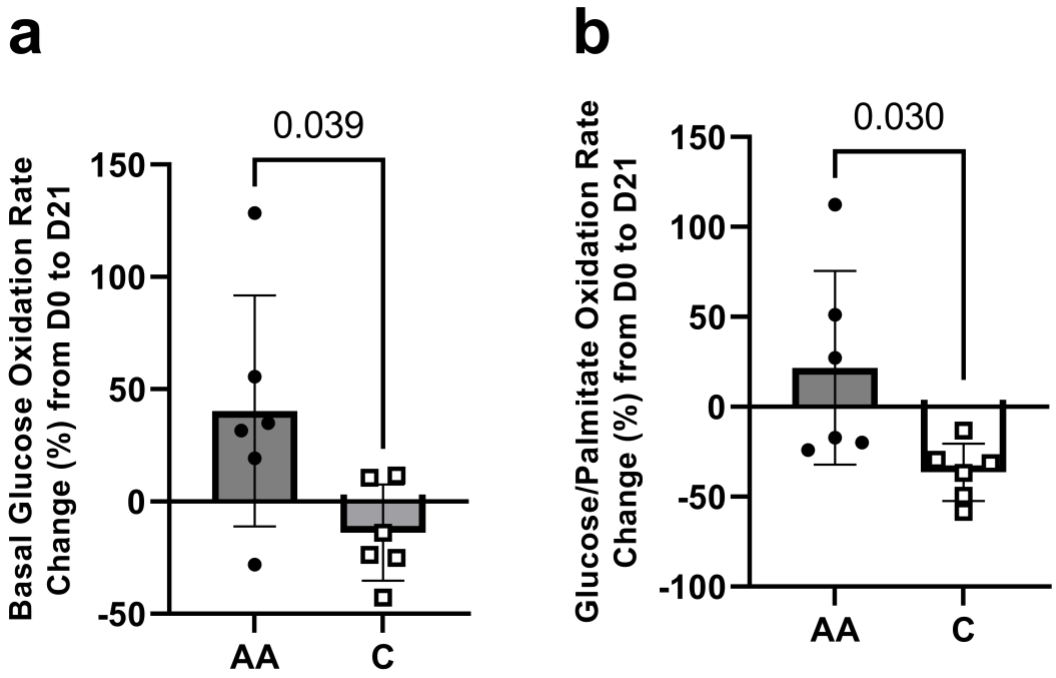

Figure 5. Transition from undifferentiated (D0) to myogenically differentiated (D21) state results in an increase in glucose oxidation (a) in African American (AA) compared to Caucasian (C) MSCs. MSC differentiation into the myogenic lineage potentiates the increase in glucose to palmitate oxidation rates (b) indicative of preferential glucose oxidation in AA infants.

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APPENDIX: IRB APPROVAL LETTER

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office 252-744-2914 · Fax 252-744-2284 · rede.ecu.edu/umcirb/

Notification of Continuing Review Approval: Expedited

From: Biomedical IRB
To: Linda May
CC: Lindsey Rossa
Date: 11/30/2022
Re: CR00009943
UMCIRB 12-002524
ENHANCED by Mom

The continuing review of your expedited study was approved. Approval of the study and any consent form(s) is for the period of 11/29/2022 to 11/28/2023. This research study is eligible for review under expedited categories # 2,5,7. The Chairperson (or designee) deemed this study no more than minimal risk.

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are only initiated with UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Applying for continuing review and receive approval of continuation of the study prior to the study's current expiration date. Application for continuing review should be submitted no less than 30 days prior to the expiration date. Lapses in approval (i.e. study expiration) should be avoided to protect the safety and welfare of enrolled participants and liability to the University; and

5. Submission of a final report when the study meets the UMCIRB criteria for closure. Study approval should not be allowed to expire simply because the study is completed, rather the UMCIRB should be formally notified of study completion via the final report process.

The approval includes the following items:

Document	Description
Aerobic Home Exercise Sheet(0.01)	Additional Items
Circuit Home Exercise Sheet(0.01)	Additional Items
Dietary Assessment(0.01)	Surveys and Questionnaires
ENAHNCED Protocol COVID 19 Addendum (0.01)	Study Protocol or Grant Application
ENHANCED 12-002524-protocol Amendment 40-HIGHLIGHTED 1.26.2020(0.15)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 40-CLEAN-1.26.20(0.22)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 52 - Clean Version 7.6.21_.docx(0.01)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 52 - Highlighted Version 7.6.21_.docx(0.01)	Study Protocol or Grant Application
ENHANCED%20Timeline-2014.pdf(0.01)	Additional Items
Exercise Motivations Inventory(0.01)	Surveys and Questionnaires
Exercise Session Log Sheets(0.01)	Additional Items
International Physical Activity Questionnaire(0.01)	Surveys and Questionnaires
IRB Agreement - ECU-Campbell University 03.30.17(0.01)	Additional Items
Maternal Measurements Form(0.01)	Additional Items
Mindfulness Home Exercise Sheet1.docx(0.01)	Additional Items
Modified Balke Protocol.docx(0.01)	Standardized/Non-Standardized Instruments/Measures
Modified Physical Activity Questionnaire020817(0.05)	Surveys and Questionnaires
Nutrition Powerpoint(0.01)	Additional Items
Psychological Need Satisfaction in Exercise Scale(0.01)	Surveys and Questionnaires
Resistance Home Exercise Sheet(0.01)	Additional Items
Sheffield FFQ.doc(0.02)	Surveys and Questionnaires
Stretching Home Exercise Sheet(0.02)	Additional Items

For research studies where a waiver or alteration of HIPAA Authorization has been approved, the IRB states that each of the waiver criteria in 45 CFR 164.512(i)(1)(i)(A) and (2)(i) through (v) have been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.
