GeroScience (2018) 40:269–278
https://doi.org/10.1007/s11357-018-0024-0

ORIGINAL ARTICLE

Maternal nutrient restriction in baboon programs later-life cellular growth and respiration of cultured skin fibroblasts: a potential model for the study of aging-programming interactions

Adam B. Salmon · Jonathan Dorigatti · Hillary F. Huber · Cun Li · Peter W. Nathanielsz

Received: 19 April 2018 / Accepted: 14 May 2018 / Published online: 25 May 2018

Abstract Compelling data exist for programming of chronic later-life diseases and longevity by perinatal developmental programming challenges. Understanding mechanisms by which life course health trajectory and longevity are set is fundamental to understanding aging. Appropriate approaches are needed to determine programming effects on cellular function. We have developed a baboon model in which control mothers eat *ad libitum* while a second group eat 70% of the global diet fed controls, leading to male and female offspring intrauterine growth restriction (IUGR). We have shown that IUGR suffer from acceleration of several age-related physiological declines. Here, we report on a skin-derived fibroblast model with potential relevance for mechanistic studies on how IUGR impacts aging. Fibroblasts were cultured from the skin biopsies taken from adult baboons from control and IUGR cohorts. IUGR-derived fibroblasts grew in culture less well than controls and those derived from male, but not female, IUGR baboons had a significant reduction in maximum respiration rate compared to control-derived fibroblasts. We also show that relative levels of several mitochondrial protein subunits, including NDUFB8 and cytochrome c oxidase subunit IV, were reduced in IUGR-derived fibroblasts even after serial passaging in culture. The lower levels of electron transport system components provide potential mechanisms for accelerated life course aging in the setting of programmed IUGR. This observation fits with the greater sensitivity of males compared with females to many, but not all, outcomes in response to programming challenges. These approaches will be powerful in the determination of programming-aging interactions.

Keywords Mitochondria · Electron transport · Fibroblasts · Cell culture · Programming · Metabolism

Abbreviations

ECAR	Extracellular acidification rate
FCCP	Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
GH	Growth hormone
IGF1	Insulin-like growth factor 1
IUGR	Intrauterine growth restriction
OCR	Oxygen consumption rate

A. B. Salmon (✉)
Geriatric Research, Education and Clinical Center, South Texas Veterans Health Care System, San Antonio, TX, USA
e-mail: salmona@uthscsa.edu

A. B. Salmon · J. Dorigatti
The Sam and Ann Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

A. B. Salmon
Department of Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

H. F. Huber · C. Li · P. W. Nathanielsz
Department of Animal Science, University of Wyoming, Laramie, WY, USA

H. F. Huber · C. Li · P. W. Nathanielsz
Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, USA

Introduction

There are now compelling data for programming of chronic diseases in later life by challenges during plastic periods of perinatal development including pioneering human epidemiological observations by investigators at Southampton, England, and in the Harvard Nurses Study (Barker 1998; Nathanielsz 1999), as well as more recent well-controlled animal studies. Developmental programming can be defined as responses to challenges in critical plastic time windows in utero or neonatal life that alter development and phenotype in ways that persist postnatally (Nathanielsz et al. 2013). Programming has been shown to have profound implications on the entire life course of health including longevity (Zambrano et al. 2015; Tarry-Adkins and Ozanne 2017). Understanding the mechanisms by which developing organisms can set their life course trajectory of health and longevity is then fundamental to understanding aging and frailty.

Human epidemiological studies are always accompanied by multiple confounds, presenting the need for carefully controlled animal studies. The majority of developmental programming studies have been conducted in rodents and have provided a body of highly significant data. However, there are some fundamental differences in perinatal development between polytocous, altricial rodents and monotocous, precocial primate species that greatly influence developmental programming outcomes. To overcome these limitations and enable precise experimental control, we have developed a baboon (*Papio* sp.) model of intrauterine growth restriction (IUGR) as a means to test effects of developmental programming. Since studies are not permitted in great apes and chimpanzees, the baboon is the nonhuman primate species closest to humans for translational studies to human programming challenges, mechanism, outcomes, and potential development of effective interventions. A strength of baboon studies is that their genetics and genome sequence, physiology, and metabolism are closer to the human than any other experimental nonhuman primate species and thus stronger for translation to humans (Cox et al. 2013). Finally, the baboon fetus is considerably larger than the rhesus fetus providing more tissue for in vitro studies. In our model, control mothers eat *ad libitum* during pregnancy while mothers undergoing maternal nutrient reduction during pregnancy eat only 70% of global food eaten by controls. Both male and female offspring (F1) of undernourished mothers are IUGR with term body weights ~ 12% less than controls (Li et al. 2013a). Studies in the IUGR F1 have demonstrated programming of metabolism (Choi et al. 2011), neuroendocrine (Li et al. 2013b), cardiovascular (Muralimanoharan et al. 2017), brain (Antonow-Schlorke et al. 2011), behavioral (Keenan et al. 2013), and cognitive (Rodriguez et al. 2012) function.

In our studies, we have shown that age-related changes appear earlier in IUGR baboon F1 than the control F1 (Franke et al. 2017). We and others have shown similar acceleration of aging phenotypes in rodent models (Zambrano et al. 2015; Allison et al. 2016). To identify associations with aging in a comprehensive manner, it is necessary to make measurements across as much of the life course as possible rather than at a restricted number of discrete categorical life course time points (i.e., young and old ages) (Zambrano et al. 2015). Multiple life course time point studies can prove challenging in long-lived species, particularly so in nonhuman primates, if they involve recruiting new subjects for each time point for new terminal studies. To remove this limitation, in the studies reported here, we sought to evaluate the potential of a primary fibroblast cell culture model to characterize programmed metabolic changes that would, if validated, enable longitudinal analyses in the same animals across the life course. Successful development of such a model will also contribute to the 3Rs by addressing reduction of animal numbers in investigations.

Several previous studies have now shown that primary fibroblasts isolated from the skin of donor animals can be used to probe fundamental mechanisms of aging in an ex vivo model. For example, in a series of reports, we and others showed that fibroblasts from long-lived genetic mutant mice retain increased cellular resistance to multiple forms of cytotoxic stress and metabolic inhibition, elevated activity of DNA repair mechanisms, increased proteostasis, and resistance to cellular senescence (Murakami et al. 2003; Salmon et al. 2005; Maynard and Miller 2006; Harper et al. 2007; Salmon et al. 2008; Page et al. 2009; Wang and Miller 2012). Importantly, these cellular phenotypes persist even after culturing over weeks while maintained in standardized cell culture conditions. Across mammalian species, fibroblasts isolated from the skin of mammalian species that tend to live a very long time, including nonhuman primates, tend to be much more resilient to cellular stresses, proteostatic challenge, and metabolic inhibition than are cells isolated from species with shorter maximum lifespan (Harper et al. 2007; Pickering et al. 2015a, b). Importantly for this study, there is evidence that

early-life endocrine intervention can significantly impact both longevity and the cellular resilience phenotype in mice (Panici et al. 2010). In this study, we directly test whether the developmental challenges caused by IUGR have similar sustained effects on cellular physiology that can be determined using this fibroblast model.

Methods

Animal care

Animal care details are published in detail (Schlabritz-Loutsevitch et al. 2004; Li et al. 2017). Briefly, baboons (*Papio* species) were housed and maintained in a social environment and fed using an individual feeding system. Healthy gravid female baboons of similar age and weight were randomized to an *ad lib* diet during pregnancy and lactation (control) or maternal nutrient reduction by reducing diet to 70% globally of feed eaten by controls on a weight-adjusted basis from 0.16 gestation to the end of lactation (Li et al. 2017). F1 baboons were fully weaned at 9 months of age and moved to juvenile group housing, and fed *ad lib* with Monkey Diet 5038 (Purina LabDiets, St Louis, MO), containing 13% calories from fat; 18% calories from protein; 69% calories from carbohydrates, mineral, and vitamin additives; and a metabolizable energy content of 3.22 kcal/g.

Biopsy

A 2-mm diameter skin punch biopsy was taken from behind the upper back portion of one ear on each animal during an ancillary procedure in which baboons were anesthetized (ketamine 10 mg/kg IM). F1 control (*n* all = 10, *n* males = 5, *n* females = 5) and IUGR (*n* all = 8, *n* males = 4, *n* females = 4) ranged in age from 7.3–11.7 years at time of biopsy. A rough approximation of age comparison is that 1 baboon year equates to 4 human years with differences at different stages of the life course; i.e., these animals were roughly equivalent to 29–47-year-old humans.

Isolation and growth of cell cultures

Primary fibroblasts were established and cultured using modification of methods previously published (Salmon et al. 2005; Harper et al. 2007). Skin biopsies were sterilized in 70% ethanol then digested with Liberase DH (Roche, Mannheim, Germany) for 150 min at 37 °C as outlined by manufacturer's instructions. Cells were then passed through 100-μM sterile filter, stained by Trypan Blue live-cell exclusion method, and counted. Equal numbers of cells for each culture were plated in 24-well dishes in complete media (DMEM, 10% fetal calf serum, antibiotics) and kept in humidified cell culture incubator at 37 °C, 5% CO_2, and 3% O_2 in air. Upon confluence, cells were split using 0.25% Trypsin-EDTA, stained using Trypan Blue, and counted and subcultured using standard cell culture methods with replacement of complete media every 3 days and trypsin subculturing when cell lines reached > 95% confluence in given plastic ware. Following two subcultures (i.e., passage 3), expanded cell lines were cryopreserved for future assays. For all subsequent cellular assays, cells were studied at a similar state of passage (either passage 3 or 4).

Cellular respiration

A Seahorse Bioscience XF24 Extracellular Flux Analyzer (North Billerica, MA, USA) was used to measure cellular respiration. For each experiment, cells were seeded at a density of 40,000 cells per well. After overnight attachment, cells were washed, and media was replaced with unbuffered DMEM medium + 4.5 g/L glucose and L-glutamine, with 1 mM sodium pyruvate (Invitrogen, Carlsbad, CA). For mitochondrial stress tests, cells were incubated in oligomycin at a final concentration of 1 μM or FCCP at a final concentration of 1 μM. Immediately following each assay, cells were lysed to quantify and normalize respiration to total protein using Pierce BCA.

Protein expression

Passaged cells were plated in a 6-dish and allowed to reach confluence at which time media was aspirated, and cells were washed 3× with ice-cold PBS. Cells were lysed in plasticware using modified RIPA buffer containing protease and phosphatase inhibitors. Protein homogenates were centrifuged with supernatant removed for further analysis by immunoblot. Primary antibodies for electron transport complex units NDUFB8, UQCR2, COX1, and ATP5α were assayed together as part of OXPHOS antibody cocktail (Abcam, Cambridge, MA). Primary antibodies for vinculin and COXIV were sourced also from Abcam. Protein bands were

visualized using alkaline phosphatase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and ECL reagent. All immunoblots were quantified using ImageJ (NIH).

Statistical analysis

Because we have reported IUGR sex-specific effects in various outcomes in control and IUGR F1, we analyzed these data separately for males and females by two-way ANOVA. Statistical significance is reported at p value of 0.05 or below. Post hoc analysis was performed by Holm-Sidak method.

Results

This study tested primary fibroblast cell lines derived from a small skin biopsy taken from the area behind the ear of adult baboon F1 of *ad lib*-fed control mothers or IUGR F1 of mothers fed 70% of global diet of controls during pregnancy and lactation. From weaning, all baboons ate the same normal primate chow *ad lib*. Baseline morphometric data of control and IUGR males and females are shown in Table 1. At time of study (adulthood ages 7.3–11.7 years), body weight as well as body mass indices (BMI) were higher at time of biopsy in male baboons compared to females ($p < 0.001$), a known physiologic difference. At time of biopsy, body weight of control males was significantly higher than in IUGR males; there was no effect of treatment in females. While birth weight did not differ between sexes or treatments in this selection of animals, birthweight was significantly reduced in both females and IUGR animals in the larger cohort of animals from which these subjects were selected (see (Li et al. 2017)). Detailed morphometric and other phenotypic data for juvenile (Li et al. 2017) and adult (Kuo et al. 2017a, b, 2018) IUGR are published elsewhere.

After enzyme-mediated digestion of skin biopsies, we counted and seeded isolated fibroblasts in equal numbers in cell culture flasks and cultured cells using standard protocols. At the first passage of these cells, we found significantly fewer cells in cultures isolated from IUGR F1 donors relative to those isolated from control F1 donors (Fig. 1a). There was no difference between males and females in either control or IUGR F1 groups. Cell counts at subsequent passages (i.e., when cells reached confluence) tended to be lower in IUGR-derived lines though these differences did not reach statistical significance (Fig. 1b). Similarly, the time required to reach confluence tended to be extended in IUGR-derived cultures but did not reach statistical significance by two-way ANOVA (Fig. 1c).

Cellular respiration was measured to assess its potential effect on the differences in growth rate between control- and IUGR-derived cell lines. There was no difference between IUGR and the control cell lines in either basal respiration state or in the presence of oligomycin, which blocks ATP synthase, effectively blocking electron transport chain-mediated respiration (Fig. 2a). However, treatment with the mitochondrial uncoupler FCCP revealed that IUGR-derived cells have a significant reduction in maximum respiration rate compared to control-derived cells (Fig. 2a). For basal respiration, we again found no significant effect of IUGR in cells from either sex (Fig. 2b). However, compared with same sex controls, maximum respiration was significantly reduced in IUGR-derived cells from male, but not female, baboons. Interestingly, maximum

Table 1 Baseline characteristics and vitals of subjects studied (mean ± SEM)

Group	CTL		IUGR		ANOVA
	M	F	M	F	
N	6	5	5	6	
Age (year)	8.4 ± 0.5	9.7 ± 0.6	8.5 ± 0.5	8.7 ± 0.6	NS
Weight (kg)	30.2 ± 1.0	18.1 ± 0.8	26.9 ± 1.1	18.1 ± 0.8	M > F** CTL M > IUGR M*
BMI (kg/m^2)	20.7 ± 0.8	17.2 ± 0.7	21.0 ± 0.7	16.2 ± 0.7	M > F**
Birth weight (kg)	0.97 ± 0.06	0.86 ± 0.04	0.83 ± 0.04	0.77 ± 0.07	NS

ANOVA main effects reported. No significant sex-group interaction was found. *$p < 0.05$; **$p < 0.001$; *NS* not significant

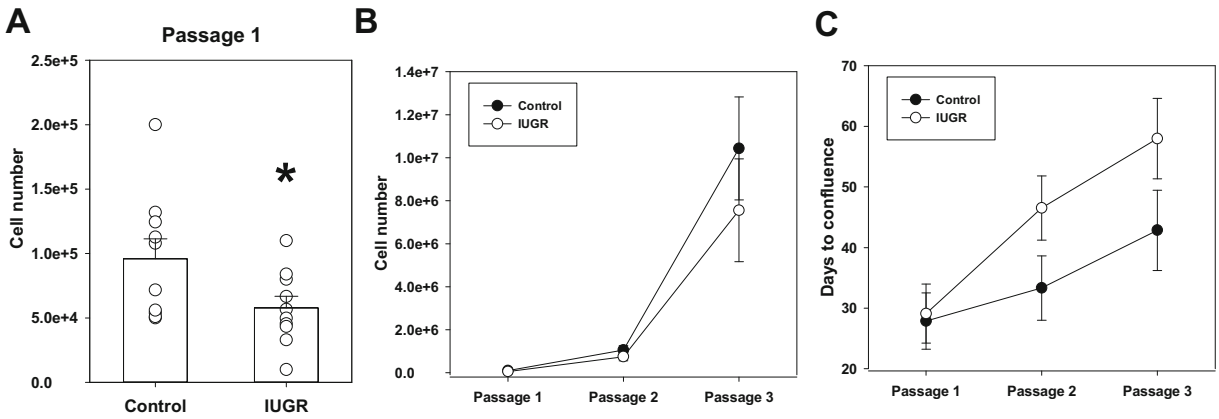

Fig. 1 Cell growth characteristics of baboon cells used in this study. **a** Cell counts from cultures following trypsin digestion upon reaching first confluence after isolation. Open circles indicate counts from each cell line, i.e., fibroblasts derived from an individual animal. Bars represent average for each treatment group ± standard error of mean. **b** Average cell counts at confluence and **c** average days to confluence for fibroblast cultures in this study. Closed (control) and open (IUGR) circles represent average value for each ± standard error of mean. Asterisks indicate p value < 0.05

respiration rate overall (i.e., independent of IUGR) was significantly higher in female-derived compared to male-derived cell lines.

By measuring the ratio of oxygen consumption rate (OCR) to extracellular acidification rate (ECAR) within a cell line, an estimate of the ratio of oxidative phosphorylation to glycolysis can be determined (Zhang et al. 2012). We found no difference in OCR/ECAR ratio in our cell lines suggesting the reduced respiration of IUGR-derived lines is not likely due to specific changes in relative ratio of energy substrate preference (Fig. 3). Moreover, we found no difference between sexes in this cellular respiratory marker.

An alternative rationale for differences in cellular respiration might be the content or composition of mitochondria within cells. We tested this idea by immunoblot for key components of the electron transport chain (ETC) that were then normalized to a protein found in the cytosol (vinculin) as a general estimate of mitochondrial content. Protein levels of NDUFB8, a subunit of ETC I, and cytochrome c oxidase subunit IV (COX4), a component of ETC IV, were significantly reduced in cells derived from IUGR donors (Fig. 4). Similarly, we found trends toward lower levels of subunits of ETC III (UQCR2) and an additional subunit of complex IV (COX1) in IUGR donors. Interestingly, we found no difference in levels of complex V (ATP5α) in IUGR donors. We found no significant effect of sex for levels of any ETC protein measured. Together, these data point to persistent reduction of mitochondrial content in cells derived from IUGR donors.

Discussion

There is now considerable interest in the interaction of developmental programming and biological aging mechanisms. To determine the antecedents of aging and frailty, it is necessary to begin investigations early in life (Zambrano et al. 2015). In our baboon model, we have reported persistent developmental programming outcomes in multiple physiological systems in IUGR F1 in early life that relate to antecedents of aging. IUGR baboons had lower birth weights compared to control baboons (Choi et al. 2011; Li et al. 2013a). With respect to metabolic programming and aging, IUGR baboons continued to have lower body weight and showed signs of a pre-diabetic phenotype at 3.5 years of age, which was evident through increased insulin resistance and β-cell responsiveness (Choi et al. 2011). Despite weighing less at 3.5 years of age, female IUGR offspring had caught up in weight to controls by the time of this study (Table 1); male IUGR in this study remained lower in weight than controls for the specific animals used in this study. A rough approximation of age comparison is that 1 baboon year equates to 4 human years with differences at different stages of the life course. By 9 years of life, IUGR F1 baboons manifested a sex-dimorphic increase in circulating low-density lipoproteins and increased pericardial and liver fat compared to values in a normative life-course baboon cohort (Kuo et al. 2018). The increase in male apical pericardial fat shown in this previous study was equivalent to advancing age by 6 years and the increase in female low-density

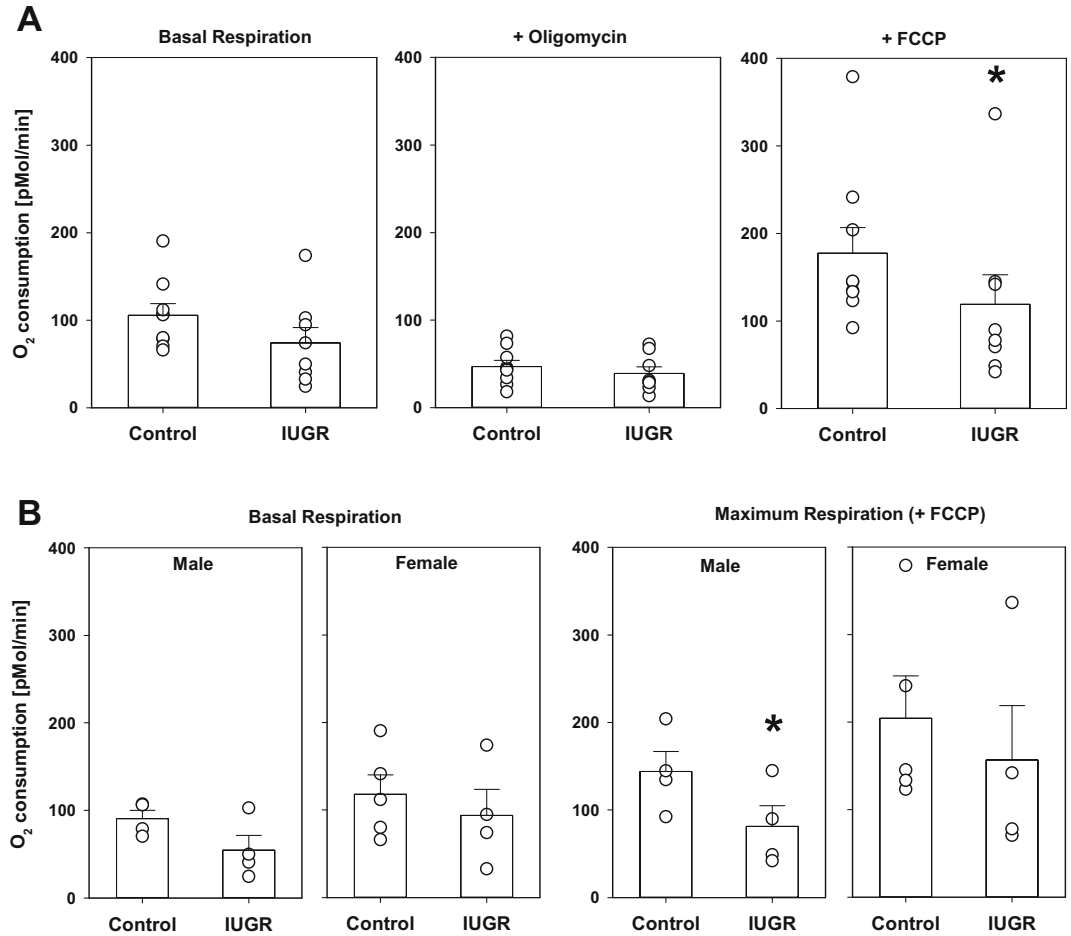

Fig. 2 Decreased oxygen consumption in fibroblasts from IUGR baboons. **a** Oxygen consumption rates of fibroblasts from indicated group under basal conditions (left), in the presence of oligomycin (center), or in the presence of FCCP (right). **b** Oxygen consumption rates from (A) separated by sex of donor under the indicated conditions. For all, open circles indicate average rates derived from each cell line, *i.e.*, fibroblasts derived from an individual animal. For each line, average rate is calculated from respiration rates from 4 to 5 replicate samples. Bars represent average for each treatment group ± standard error of mean. Asterisks indicate p value < 0.05

lipoprotein to an increase of 3 years (Kuo et al. 2018). In addition, liver metabolism is altered by IUGR beginning even at the fetal stage with evidence that protein abundance for PEPCK, the rate-limiting enzyme in gluconeogenesis, is elevated, and PCK1 promotor methylation reduced in IUGR fetuses (Nijland et al. 2010).

We have provided evidence to show that IUGR significantly alters the programming of endocrine systems. Previous reports show that the F1 fetal hypothalamic-pituitary-adrenal axis is activated in IUGR baboons (Li et al. 2013a). In this regard, it is interesting that we have previously reported a strong association between reduction in GH/IGF1 signaling and enhanced cellular resilience in long-lived mutant mice (Murakami et al. 2003; Salmon et al. 2005, 2008). Perhaps most importantly, GH supplementation during early-life developmental stages significantly reduces the lifespan of these long-lived mice as well as ablates the cellular resilience phenotype (Panici et al. 2010). A recent report also showed that early-life (perinatal) nutrient restriction also reduces the long lifespan of GH/IGF1 mutant mice (Fang et al. 2017). The precise mechanisms for this relationship are not clear, but suggest that early life changes in endocrine signaling can have profound effects on lifelong health. While we recognize that changes in fibroblast resiliency are unlikely to be the driving force for changes in aging in the animal overall, this model system provides a useful platform for assessing

Fig. 3 IUGR does not influence primary fibroblast energy substrate preference. Ratios of oxygen consumption rate (OCR) to extracellular acidification rate (ECAR) of fibroblasts under basal respiration. Data are presented for cell lines from all donors (left), males only (center), and females only (right). For all, open circles indicate average rates derived from each cell line, *i.e.*, fibroblasts derived from an individual animal. For each line, average rate is calculated from respiration rates from four to five replicate samples. Bars represent average for each treatment group ± standard error of mean

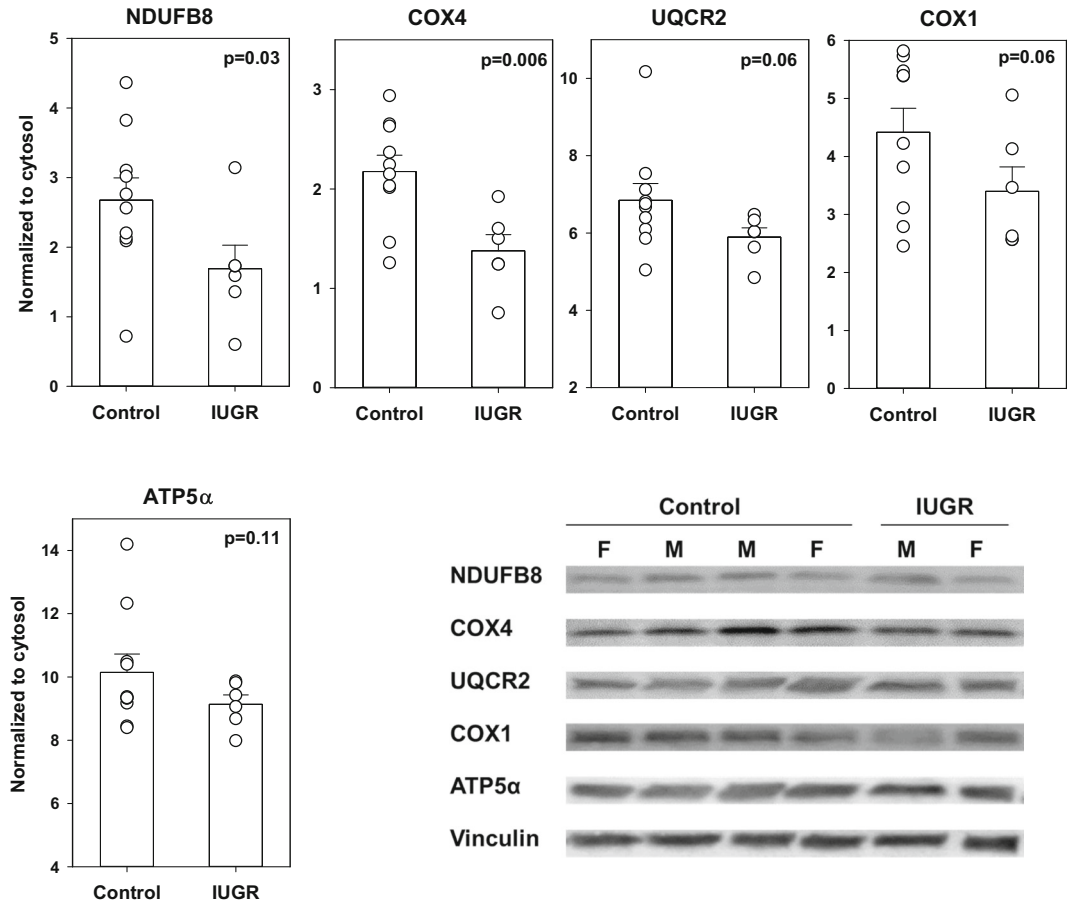

Fig. 4 Fibroblasts from IUGR donors have reduced levels of mitochondrial subunits. Bar graphs present average normalized level of indicated mitochondrial subunit protein expression ± standard error of mean. Samples include both cell lines derived from both male and female donors. Sample sizes for control (n = 10; 5M/5F) and IUGR (n = 6; 3M/3F). Blot is representative of each indicated protein and presents samples from both control- and IUGR-derived cell lines. M = male donor, F = female donor

persistent changes in specific functions—as here with energy metabolism—due to such programming events.

As discussed above, we have growing evidence that IUGR drives aging across multiple organ systems that might be regulated by metabolic function. For example, age-dependent cardiovascular programming also occurs in this model. In an MRI study, IUGR baboons showed impaired diastolic and systolic cardiac function and right ventricular changes suggestive of pulmonary hypertension (Kuo et al. 2017b, 2018). IUGR male and female baboons at approximately 9 years of age showed a decrease in distensibility in iliac but not carotid arteries and increased carotid arterial blood flow velocity during late systole and diastole was present in the IUGR group in the iliac but not the carotid arteries. The pattern of alteration observed suggests vascular redistribution efforts to preserve blood flow to the brain and heart, organs critical to postnatal survival, initiated in fetal life in response to hypoxic and metabolic challenges in the perinatal period that may persist into adulthood (Kuo et al. 2017a). We have identified biomarkers of programmed cardiovascular dysfunction in the breath metabolite signature in IUGR F1 at 4.8 years (Bishop et al. 2018). It is unknown, but of extreme interest, to determine whether the fibroblast model might similarly predict physiological outcomes in these animals at both the population and individual level. Future studies will be required to validate the translatability of these findings to programming of human systems.

In contrast to the accelerated aging phenotype of IUGR, there is evidence from rodent models that restricting nutrient intake during the perinatal period promotes longevity. Ozanne and Hales first showed that nutrient restriction during weaning by means of restricting protein available to lactating mothers extended the lifespan of male mice relative to control mice weaned normally (Ozanne and Hales 2004). Later studies from the Miller laboratory used a different experimental paradigm to show that restricting nutrients during weaning by artificially increasing litter size could similarly increase longevity (Sun et al. 2009). Follow-up studies using this model show persistent effects on glucose metabolism, mTOR signaling and protein translation, hypothalamic regulation, and xenobiotic response in these crowded litter mice (Steinbaugh et al. 2012; Drake et al. 2014; Sadagurski et al. 2014, 2015). However, there are important distinctions between these lines of study and the IUGR baboon model discussed here. The design of our IUGR baboon study includes calorie/nutrient restriction during *both* developmental and weaning periods of the offspring rather than only during weaning. In the seminal study from Ozanne and Hales, they showed that protein restriction of mothers during development, i.e., in the womb, significantly shortens the life of these offspring in contrast to the longevity-promoting effect of nutrient restriction during weaning (Ozanne and Hales 2004). Thus, the acceleration of aging phenotypes in our IUGR baboon cohort and effects on cellular respiration shown here are in line with these studies. While it would be interesting to identify the effects of calorie restriction limited to the weaning period on baboon aging, further resources will be required to address this question.

As we have reported, the functional outcome of IUGR in our baboon cohorts has been acceleration of age-related declines across multiple different systems including metabolism (Choi et al. 2011), neuroendocrine (Li et al. 2013b), cardiovascular (Muralimanoharan et al. 2017), brain (Antonow-Schlorke et al. 2011), behavioral (Keenan et al. 2013), and cognitive (Rodriguez et al. 2012). These multiple phenotype changes show the value of maintaining these baboon cohorts for life course studies. To this end, the minimally invasive approaches described here can be repeated at relatively short time intervals as well as longitudinally across the lifespan of an individual animal. The ability to assess metabolic endpoints such as mitochondrial function as well as cellular signaling pathways and responses to drugs are powerful tools.

In summary, we have demonstrated the ability to obtain fibroblast cultures from programmed IUGR baboons which (1) grew in culture less well than cultures from controls; (2) had a reduced maximum respiration rate compared to control-derived cells; and (3) showed differences in impairment of maximum respiration in IUGR-derived cells from male, but not female, baboons. This observation fits with the greater sensitivity of males compared with females to many, but not all, outcomes in response to programming challenges. We have shown fetal sex-specific responses to this level of maternal nutrient reduction in fetal baboon liver and adipose tissue (Guo et al. 2013). Interestingly, maximum respiration rate overall (i.e., independent of IUGR) was higher in female-derived compared to male-derived cell lines, an observation that may provide a mechanistic explanation for studies showing that female offspring are less susceptible to many programming challenges. In one such study, in children of obese mothers at 2–6 years of age, increased body fat was noted in males but not

females (Andres et al. 2015). The lower levels of electron transport system components provide potential mechanisms for accelerated life course aging in the setting of programmed IUGR. These approaches will be powerful in the determination of programming-aging interactions.

Funding information During the preparation of this manuscript, ABS received support from grants from the National Institute of Health (R01 AG050797 and R01 AG057431), the San Antonio Nathan Shock Center and Claude A. Pepper Center, and from the Geriatric Research, Education and Clinical Center of the South Texas Veterans Health Care System. This material is the result of work supported with resources and the use of facilities at South Texas Veterans Health Care System, San Antonio, TX. The contents do not represent the views of the US Department of Veterans Affairs or the US Government. Baboons in this study were generated under 1 R24 RR021367-01 (PWN).

References

Allison BJ, Kaandorp JJ, Kane AD, Camm EJ, Lusby C, Cross CM, Nevin-Dolan R, Thakor AS, Derks JB, Tarry-Adkins JL, Ozanne SE, Giussani DA (2016) Divergence of mechanistic pathways mediating cardiovascular aging and developmental programming of cardiovascular disease. FASEB J 30: 1968–1975

Andres A, Hull HR, Shankar K, Casey PH, Cleves MA, Badger TM (2015) Longitudinal body composition of children born to mothers with normal weight, overweight, and obesity. Obesity 23:1252–1258

Antonow-Schlorke I, Schwab M, Cox LA, Li C, Stuchlik K, Witte OW, Nathanielsz PW, McDonald TJ (2011) Vulnerability of the fetal primate brain to moderate reduction in maternal global nutrient availability. Proc Natl Acad Sci U S A 108: 3011–3016

Barker DJP (1998) Mothers, babies and diseases in later life. Churchill Livingstone, Edinburgh

Bishop AC, Libardoni M, Choudary A, Misra BB, Lange K, Bernal J, Nijland M, Li C, Olivier M, Nathanielsz PW, Cox LA (2018) Nonhuman primate breath volatile organic compounds associate with developmental programming and cardio-metabolic status. J Breath Res 12(3):036016. https://doi.org/10.1088/1752-7163/aaba84

Choi J, Li C, McDonald TJ, Comuzzie A, Mattern V, Nathanielsz PW (2011) Emergence of insulin resistance in juvenile baboon offspring of mothers exposed to moderate maternal nutrient reduction. Am J Physiol Regul Integr Comp Physiol 301:R757–R762

Cox LA, Comuzzie AG, Havill LM, Karere GM, Spradling KD, Mahaney MC, Nathanielsz PW, Nicolella DP, Shade RE, Voruganti S, Vande Berg JL (2013) Baboons as a model to study genetics and epigenetics of human disease. ILAR J 54: 106–121

Drake JC, Bruns DR, Peelor FF 3rd, Biela LM, Miller RA, Hamilton KL, Miller BF (2014) Long-lived crowded-litter mice have an age-dependent increase in protein synthesis to DNA synthesis ratio and mTORC1 substrate phosphorylation. Am J Physiol Endocrinol Metab 307:E813–E821

Fang Y, McFadden S, Darcy J, Hill CM, Huber JA, Verhulst S, Kopchick JJ, Miller RA, Sun LY, Bartke A (2017) Differential effects of early-life nutrient restriction in long-lived GHR-KO and normal mice. GeroScience 39:347–356

Franke K, Clarke GD, Dahnke R, Gaser C, Kuo AH, Li C, Schwab M, Nathanielsz PW (2017) Premature brain aging in baboons resulting from moderate fetal undernutrition. Front Aging Neurosci 9:92

Guo C, Li C, Myatt L, Nathanielsz PW, Sun K (2013) Sexually dimorphic effects of maternal nutrient reduction on expression of genes regulating cortisol metabolism in fetal baboon adipose and liver tissues. Diabetes 62:1175–1185

Harper JM, Salmon AB, Leiser SF, Galecki AT, Miller RA (2007) Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. Aging Cell 6:1–13

Keenan K, Bartlett TQ, Nijland M, Rodriguez JS, Nathanielsz PW, Zurcher NR (2013) Poor nutrition during pregnancy and lactation negatively affects neurodevelopment of the offspring: evidence from a translational primate model. Am J Clin Nutr 98:396–402

Kuo AH, Li C, Huber HF, Clarke GD, Nathanielsz PW (2017a) Intrauterine growth restriction results in persistent vascular mismatch in adulthood. J Physiol. https://doi.org/10.1113/JP275139

Kuo AH, Li C, Huber HF, Schwab M, Nathanielsz PW, Clarke GD (2017b) Maternal nutrient restriction during pregnancy and lactation leads to impaired right ventricular function in young adult baboons. J Physiol 595:4245–4260

Kuo AH, Li C, Mattern V, Huber HF, Comuzzie A, Cox L, Schwab M, Nathanielsz PW, Clarke GD (2018) Sex-dimorphic acceleration of pericardial, subcutaneous, and plasma lipid increase in offspring of poorly nourished baboons. Int J Obes. https://doi.org/10.1038/s41366-018-0008-2

Li C, McDonald TJ, Wu G, Nijland MJ, Nathanielsz PW (2013a) Intrauterine growth restriction alters term fetal baboon hypothalamic appetitive peptide balance. J Endocrinol 217:275–282

Li C, Ramahi E, Nijland MJ, Choi J, Myers DA, Nathanielsz PW, McDonald TJ (2013b) Up-regulation of the fetal baboon hypothalamo-pituitary-adrenal axis in intrauterine growth restriction: coincidence with hypothalamic glucocorticoid receptor insensitivity and leptin receptor down-regulation. Endocrinology 154:2365–2373

Li C, Jenkins S, Mattern V, Comuzzie AG, Cox LA, Huber HF, Nathanielsz PW (2017) Effect of moderate, 30 percent global maternal nutrient reduction on fetal and postnatal baboon phenotype. J Med Primatol 46:293–303

Maynard SP, Miller RA (2006) Fibroblasts from long-lived Snell dwarf mice are resistant to oxygen-induced in vitro growth arrest. Aging Cell 5:89–96

Murakami S, Salmon A, Miller RA (2003) Multiplex stress resistance in cells from long-lived dwarf mice. FASEB J 17:1565–1566

Muralimanoharan S, Li C, Nakayasu ES, Casey CP, Metz TO, Nathanielsz PW, Maloyan A (2017) Sexual dimorphism in

the fetal cardiac response to maternal nutrient restriction. J Mol Cell Cardiol 108:181–193

Nathanielsz PW (1999) Life in the womb: the origin of health and disease. Promethean Press, Ithaca

Nathanielsz PW, Ford SP, Long NM, Vega CC, Reyes-Castro LA, Zambrano E (2013) Interventions to prevent adverse fetal programming due to maternal obesity during pregnancy. Nutr Rev 71(Suppl 1):S78–S87

Nijland MJ, Mitsuya K, Li C, Ford S, McDonald TJ, Nathanielsz PW, Cox LA (2010) Epigenetic modification of fetal baboon hepatic phosphoenolpyruvate carboxykinase following exposure to moderately reduced nutrient availability. J Physiol 588:1349–1359

Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. Nature 427:411–412

Page MM, Salmon AB, Leiser SF, Robb EL, Brown MF, Miller RA, Stuart JA (2009) Mechanisms of stress resistance in Snell dwarf mouse fibroblasts: enhanced antioxidant and DNA base excision repair capacity, but no differences in mitochondrial metabolism. Free Radic Biol Med 46:1109–1118

Panici JA, Harper JM, Miller RA, Bartke A, Spong A, Masternak MM (2010) Early life growth hormone treatment shortens longevity and decreases cellular stress resistance in long-lived mutant mice. FASEB J 24:5073–5079

Pickering AM, Lehr M, Kohler WJ, Han ML, Miller RA (2015a) Fibroblasts from longer-lived species of primates, rodents, bats, carnivores, and birds resist protein damage. J Gerontol A Biol Sci Med Sci 70:791–799

Pickering AM, Lehr M, Miller RA (2015b) Lifespan of mice and primates correlates with immunoproteasome expression. J Clin Invest 125:2059–2068

Rodriguez JS, Bartlett TQ, Keenan KE, Nathanielsz PW, Nijland MJ (2012) Sex-dependent cognitive performance in baboon offspring following maternal caloric restriction in pregnancy and lactation. Reprod Sci 19:493–504

Sadagurski M, Landeryou T, Blandino-Rosano M, Cady G, Elghazi L, Meister D, See L, Bartke A, Bernal-Mizrachi E, Miller RA (2014) Long-lived crowded-litter mice exhibit lasting effects on insulin sensitivity and energy homeostasis. Am J Physiol Endocrinol Metab 306:E1305–E1314

Sadagurski M, Landeryou T, Cady G, Bartke A, Bernal-Mizrachi E, Miller RA (2015) Transient early food restriction leads to hypothalamic changes in the long-lived crowded litter female mice. Phys Rep 3(4). https://doi.org/10.14814/phy2.12379

Salmon AB, Murakami S, Bartke A, Kopchick J, Yasumura K, Miller RA (2005) Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. Am J Physiol Endocrinol Metab 289:E23–E29

Salmon AB, Ljungman M, Miller RA (2008) Cells from long-lived mutant mice exhibit enhanced repair of ultraviolet lesions. J Gerontol A Biol Sci Med Sci 63:219–231

Schlabritz-Loutsevitch NE, Howell K, Rice K, Glover EJ, Nevill CH, Jenkins SL, Bill Cummins L, Frost PA, McDonald TJ, Nathanielsz PW (2004) Development of a system for individual feeding of baboons maintained in an outdoor group social environment. J Med Primatol 33:117–126

Steinbaugh MJ, Sun LY, Bartke A, Miller RA (2012) Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. Am J Physiol Endocrinol Metab 303:E488–E495

Sun L, Sadighi Akha AA, Miller RA, Harper JM (2009) Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. J Gerontol A Biol Sci Med Sci 64:711–722

Tarry-Adkins JL, Ozanne SE (2017) Nutrition in early life and age-associated diseases. Ageing Res Rev 39:96–105

Wang M, Miller RA (2012) Fibroblasts from long-lived mutant mice exhibit increased autophagy and lower TOR activity after nutrient deprivation or oxidative stress. Aging Cell 11:668–674

Zambrano E, Reyes-Castro LA, Nathanielsz PW (2015) Aging, glucocorticoids and developmental programming. Age 37:9774

Zhang J, Nuebel E, Wisidagama DR, Setoguchi K, Hong JS, Van Horn CM, Imam SS, Vergnes L, Malone CS, Koehler CM, Teitell MA (2012) Measuring energy metabolism in cultured cells, including human pluripotent stem cells and differentiated cells. Nat Protoc 7:1068–1085