Original Article

MOLECULAR METABOLISM

Grandpaternal-induced transgenerational dietary reprogramming of the unfolded protein response in skeletal muscle

Petter S. Alm [1,2,4], Thais de Castro Barbosa [1,2,4], Romain Barrès [3], Anna Krook [1,2], Juleen R. Zierath [1,2,3,*]

ABSTRACT

Objective: Parental nutrition and lifestyle impact the metabolic phenotype of the offspring. We have reported that grandpaternal chronic high-fat diet (HFD) transgenerationally impairs glucose metabolism in subsequent generations. Here we determined whether grandpaternal diet transgenerationally impacts the transcriptome and lipidome in skeletal muscle. Our aim was to identify tissue-specific pathways involved in transgenerational inheritance of environmental-induced phenotypes.

Methods: F0 male Sprague–Dawley rats were fed a HFD or chow for 12 weeks before breeding with chow-fed females to generate the F1 generation. F2 offspring were generated by mating F1 males fed a chow diet with an independent line of chow-fed females. F1 and F2 offspring were fed chow or HFD for 12 weeks. Transcriptomic and LC-MS lipidomic analyses were performed in extensor digitorum longus muscle from F2-females rats. Gene set enrichment analysis (GSEA) was performed to determine pathways reprogrammed by grandpaternal diet.

Results: GSEA revealed an enrichment of the unfolded protein response pathway in skeletal muscle of grand-offspring from HFD-fed grandfathers compared to grand-offspring of chow-fed males. Activation of the stress sensor (ATF6α), may be a pivotal point whereby this pathway is activated. Interestingly, skeletal muscle from F1-offspring was not affected in a similar manner. No major changes were observed in the skeletal muscle lipidome profile due to grandpaternal diet.

Conclusions: Grandpaternal HFD-induced obesity transgenerationally affected the skeletal muscle transcriptome. This finding further highlights the impact of parental exposure to environmental factors on offspring's development and health.

Keywords Skeletal muscle; Epigenetics; Unfolded protein response (UPR); Lipodome; Transcriptome; Activation of the stress sensor (ATF)

1. INTRODUCTION

Obesity is a major world-wide public health problem that is strongly associated with increased risk of other comorbidities, such as insulin resistance, type 2 diabetes, and cardiovascular diseases [1]. While relatively simple energy balance equations have been valuable in advancing the understanding of how energy intake and energy expenditure influence body composition, obesity is now recognized as a chronic progressive disease of complex etiology, resulting from multiple environmental and genetic factors. Clinical studies reveal that parental obesity affects body weight accumulation in children and adolescents, with obesity in one or both of the parents influencing the risk of developing obesity in the offspring later in life [2–4], highlighting a heritable component to obesity. Indeed, genome-wide association studies identify more than 100 different candidate genes linked to obesity, with the majority targeted to neuroendocrine pathways influencing food intake [5]. However, environmental factors such as the nutritional status of the parent at conception or during pregnancy can influence fetal growth and development *in utero*, and may influence the susceptibility to obesity and obesity-associated diseases later in life through epigenetic modifications [6]. While the impact of maternal obesity on the health of subsequent generations is strongly attributed to the adverse intrauterine environment [7–9], paternal obesity can also impact metabolic health of the offspring in later life [10–12].

Grandpaternal exposure to altered food availability during the slow growth period is associated with a greater risk for obesity and cardiovascular disease in grandchildren [10], while parental diabetes at conception is associated with altered birth weight and increased risk of diabetes in the offspring [13]. When phenotypic changes in the first generation are similar or the same as the inducing stressor, this can, in turn, program the phenotype of the second generation by a process known as serial programming [14]. In rodents, paternal chronic exposure to low-protein diet increases the expression of hepatic genes

[1]Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden [2]Department of Molecular Medicine and Surgery, Section of Integrative Physiology, Karolinska Institutet, 171 76 Stockholm, Sweden [3]The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

[4] Petter S. Alm and Thais de Castro Barbosa contributed equally to this work.

*Corresponding author. Section of Integrative Physiology, Department of Molecular Medicine and Surgery, Karolisnka Institutet, Von Eulers väg 4a, 171 77 Stockholm, Sweden. E-mail: Juleen.zierath@ki.se (J.R. Zierath).

Received May 11, 2017 • Accepted May 16, 2017 • Available online 22 May 2017

http://dx.doi.org/10.1016/j.molmet.2017.05.009

Abbreviations

ATF6	Activating transcription factor 6
Cer	Ceramide
Chac1	ChaC Glutathione Specific Gamma-Glutamylcyclotransferase
CHOP	CCAAT enhancer-binding protein (C/EBP) homologous protein
DG	Diacylglyceride
DNAJA4	DnaJ heat shock potein family (Hsp40) member A4 (Dnaja4)
EDL	Extensor Digitorum Longus
EIF2A	Eukaryotic translation initiation factor 2A
Eif4a1	Eukaryotic translation initiation factor 4a1
ER	Endoplasmic reticulum
Gosr2	Golgi SNAP Receptor Complex Member 2
GSEA	Gene set enrichment analysis
GRP78	78-kilodalton glucose-regulated protein
GRP94	94-kilodalton Glucose-Regulated Protein
HFD	High-fat diet
IRE1α	Inositol-requiring enzyme 1 alpha
JNK	c-Jun N-terminal kinases
Nolc1	Nucleolar and coiled-body phosphoprotein 1
O2PLS	Orthogonal 2 projections to latent structures
PC	Phosphatidylcholine
PCA	Principal Component Analysis
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
ROS	Reactive oxygen species
Sec11a	SEC11 homolog A, signal peptidase complex subunit
SM	Sphingomyelin
TG	Triacylglyceride
UPR	Unfolded protein response
Wfs1	Wolframin ER transmembrane glycoprotein
Wipi1	WD Repeat Domain, Phosphoinositide Interacting 1

involved in lipid and cholesterol metabolism [12]. Paternal chronic high fat diet (HFD) disturbs whole-body glucose metabolism, as well as pancreatic and adipose function, in F1 female offspring [12,15]. Moreover, diet-induced paternal obesity increased adiposity and insulin resistance of two resultant generations has been shown with different degrees of penetrance to subsequent generations [16].

In rodents, high-fat diets impart a transgenerational influence on insulin sensitivity. We have shown that paternal diet-induced obesity affects the metabolic health of both F1 and F2 generations [17]. Glucose tolerance is impaired in female offspring and grand-offspring born to high-fat-fed F0-male rats indicating a paternal-transmitted epigenetic phenotype [17]. Moreover, altered expression of miRNA let-7c in sperm of F0 and F1 founders was passed down to adipose tissue of the offspring, coincident with a transcriptomic shift of the let-7c predicted targets in white adipose tissue [17]. While, adipose tissue is one of the major sites of paternal high caloric intake-induced transgenerational reprogramming [15–19], comparatively less is known regarding the impact of parental diet on skeletal muscle physiology and metabolism. Thus, we determined the transgenerational response of paternal diet-induced obesity on the skeletal muscle transcriptome and lipidome. Our goal was to identify novel tissue-specific pathways involved in paternal obesity-induced epigenetic transmission of disturbed metabolic phenotype to the future generations.

2. METHODS

2.1. Animal care

Sprague–Dawley rats were obtained from Charles River Laboratories (Germany). The breeding strategy and diet treatment has been described previously [17]. F0 male breeders (4 weeks of age) were fed either a HFD (TD.88137/TD.08811, 42/45% energy from fat, Harlan Laboratories; USA) or chow diet (R36, Lactamin, Labfor; Sweden) for 12 weeks. The F1 offspring were generated by housing one F0 male breeder with a 12 week-old female rat. The female rats were fed chow diet during mating, gestation, and lactation. Pups were weaned from mothers at 21 days of age and fed a chow diet. At 10 weeks of age, the F1 litters were further divided into subgroups, and rats were fed either chow or HFD for 12 weeks. Chow-fed F1 males were mated with an independent line of 12 week-old female rats to generate the F2 generation. Pups were weaned to a chow diet at 21 days of age. At week 10, the F2 litters were divided into subgroups and rats were fed chow or HFD for 12 weeks (experimental layout depicted in Figure 1).

Animals were housed in grouped cages throughout the entire experiment. Rats were subjected to food deprivation from 4 h prior to the termination. Animals were anesthetized with sodium pentobarbital (100 mg/kg, ip). Tissues were harvested, snap-frozen in liquid nitrogen and stored at −80 °C until use.

All rats were housed in a temperature-controlled environment and 12:12-h light:dark cycle at the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. Animals had free access to water and food. All experimental procedures were approved by the Stockholm North Ethical Committee on Animal Research (N101/

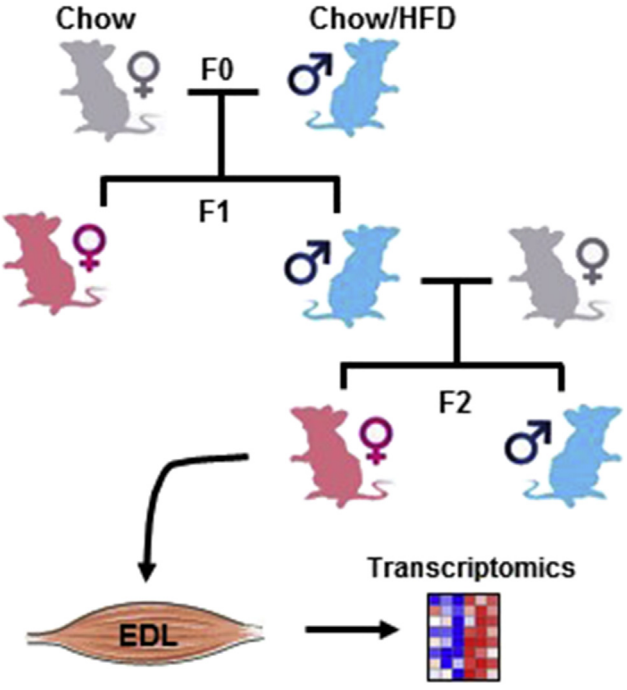

Figure 1: Schematic diagram of breeding and diet strategy. F0 male Sprague–Dawley rats were fed chow or HFD for 12 weeks before breeding with chow-fed females to generate a F1 generation. Chow-fed F1 males were mated with an independent line of chow-fed females to generate F2 offspring. At 10 weeks of age, a subgroup of F1 and F2 offspring were fed HFD for 12 weeks. EDL muscle from F2-females was studied. The muscle drawing was downloaded from Servier's PowerPoint image bank (www.servier.com).

