Immune Response Gene Profiles in the Term Placenta Depend Upon Maternal Muscle Mass

Reproductive Sciences
19(10) 1041-1056
© The Author(s) 2012
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719112440051
http://rs.sagepub.com

P. F. O'Tierney, PhD¹, R. M. Lewis, PhD²,
S. K. McWeeney, PhD^{3,4,5,6}, M. A. Hanson, DPhil^{2,7},
H. M. Inskip, PhD⁸, T. K. Morgan, MD⁹, D. J. Barker, MD, PhD¹,
G. Bagby, MD¹⁰, C. Cooper, DM^{7,8}, K. M. Godfrey, PhD^{7,8}, and
Kent L. Thornburg, PhD^{1,11,12}

Abstract

Maternal thinness leads to metabolic challenges in the offspring, but it is unclear whether reduced maternal fat mass or muscle mass drives these metabolic changes. Recently, it has been shown that low maternal muscle mass—as measured by arm muscle area (AMA)—is associated with depressed nutrient transport to the fetus. To determine the role of maternal muscle mass on placental function, we analyzed the gene expression profiles of 30 human placentas over the range of AMA (25.2–90.8 cm²) from uncomplicated term pregnancies from the Southampton Women's Survey cohort. Eighteen percent of the ~60 genes that were highly expressed in less muscular women were related to immune system processes and the interferon- γ (IFNG) signaling pathway in particular. Those transcripts related to the IFNG pathway included *IRF1*, *IFI27*, *IFI30*, and *GBP6*. Placentas from women with low muscularity are, perhaps, more sensitive to the effects of inflammatory cytokines than those from more muscular women.

Keywords

placenta, muscle mass, immune response

Introduction

Adult muscle mass derives from muscle growth during fetal and postnatal life. In men, adult fat-free mass increases 2.2 kg for every 1 kg increase in birth weight.¹ Barker et al proposed that in low-birth-weight babies, muscle development in utero is sacrificed in preference to brain growth in the “brain-sparing” process.¹ Accordingly, people undernourished in the womb will have a relatively low muscle mass for life.² This finding has been validated by Baker et al using data from the National Health and Nutrition Examination Survey.³ They showed that the rate of growth of muscle mass—as measured by arm muscle area (AMA)—remains stunted in people born small, while brain growth is conserved well into childhood. Low muscularity in a pregnant woman may have important implications for her amino acid turnover and glucose metabolism, two processes that are critically important for fetal growth.^{2,4} There is evidence that maternal thinness leads to metabolic challenges in the offspring. For example, low maternal body mass index (BMI) is associated with higher fasting insulin levels in adult progeny independent of birth weight and placental weight.^{5,6} Maternal influences on placental structure and function could explain relationships between maternal body composition and disease in later life. Placental function

¹ Heart Research Center, Oregon Health and Science University, Portland, OR, USA

² Institute of Developmental Sciences, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, UK

³ Division of Bioinformatics & Computational Biology, Medical Informatics & Clinical Epidemiology, Oregon Health and Science University, Portland, OR, USA

⁴ Division of Biostatistics, Public Health & Preventative Medicine, Oregon Health and Science University, Portland, OR, USA

⁵ OHSU Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA

⁶ Oregon Clinical and Translational Research Institute, Oregon Health and Science University, Portland, OR, USA

⁷ Southampton NIHR Biomedical Research Unit in Nutrition, Diet & Lifestyle, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, UK

⁸ MRC Lifecourse Epidemiology Unit, University of Southampton Faculty of Medicine, Southampton General Hospital, Tremona Road, Southampton, UK

⁹ Department of Pathology, Oregon Health and Science University, Portland, OR, USA

¹⁰ Dept of Medicine (Hematology & Medical Oncology), Oregon Health and Science University, Portland, OR, USA

¹¹ Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR, USA

¹² Department of Medicine (Cardiovascular Medicine), Oregon Health and Science University, Portland, OR, USA

Corresponding Author:

Kent L. Thornburg, Heart Research Center, Oregon Health & Science University, 3303 SW Bond Ave, CH15H Portland, Oregon, USA
Email: thornbur@ohsu.edu

is sensitive to maternal BMI as reflected in changes in placental gene expression of inflammatory markers, metabolic pathways, and nutrient transporters that relate to maternal BMI.⁷⁻¹⁰ However, maternal muscle mass, a component of BMI that may also affect placental and fetal growth and function, has received little attention. While BMI is usually used as an indicator of relative fat mass in pregnant women, a high BMI can reflect a large muscle mass-to-height ratio as well. Our lack of understanding of the separate role of muscle mass in regulating placental growth and function leaves an important gap in our knowledge because muscle mass is a key regulator of maternal metabolic capacity¹ and is thus a potential regulator of placental function. To shed light on that issue, Lewis et al recently reported that placental system A amino acid transport activity is reduced in mothers with low AMA,¹¹ suggesting that the placenta is sensitive to maternal muscle mass.

We hypothesized that the quantity of muscle in a mother's body would lead to a unique molecular signature in her term placenta. We sought to determine whether specific groups of genes or functional pathways in the placenta would be differentially regulated according to the levels of maternal muscle mass. To test this hypothesis, we analyzed the gene expression profiles of 30 human placentas from women in three different quintiles of AMA from a cohort of uncomplicated term pregnancies collected during the Southampton Women's Survey.

Materials and Methods

Participants

Fifty women enrolled in the Southampton Women's Survey with healthy term pregnancies were ranked by AMA. For this study, 10 women were selected using random numbers from the highest, middle, and lowest quintiles across the AMA spectrum.¹² Women from the fifth, third, and first quintiles were chosen for further study in order to maximize the ability to detect the expression differences between placentas by focusing on clusters of 10 within the middle of the AMA range and the extremes. Their AMA had been measured before pregnancy using the mid-upper arm circumference and triceps skinfold thicknesses according to the established formulae¹³ [(arm circumference – $\Pi \times$ triceps skinfold thickness)²/4 Π] – 6.5. The trend in maternal characteristics across the AMA groups was assessed using linear regression in Stata version 11.0 (Statacorp, Texas).

Tissue Collection and RNA Extraction

The placenta was collected within 30 minutes of delivery. The umbilical cord and blood clots were removed, membranes were trimmed and placentas weighed. Five villous tissue samples were collected from each placenta using a stratified random method. Villous tissue was snap frozen in liquid nitrogen and stored at –80°C until further analysis. Prior to analysis, samples were pulverized in a frozen press and mixed to generate a representative sample. RNeasy fibrous tissue RNA isolation

mini kit (Qiagen, UK) was used to extract total RNA per manufacturer's instructions.

Microarray Hybridization

Microarray assays were performed using Affymetrix HG-U133 Plus 2.0 oligonucleotide arrays designed for analysis of 47 000 transcripts. Hybridizations were done in the Affymetrix Microarray Core of the Oregon Health and Science University (OHSU) Gene Microarray Shared Resource and amplified and labeled target complementary RNA (cRNA) was generated following methods described in the Affymetrix GeneChip Expression Analysis Technical Manual (www.affymetrix.com/support/technical/manual/expression_manual.affx). Messenger RNA (mRNA) was amplified and labeled from 2 μ g of total RNA using the Affymetrix IVT amplification/labeling protocol (Affymetrix, Inc, Santa Clara, CA) for gene expression profiling with GeneChip 3'IVT arrays. Target yield was measured by UV₂₆₀ absorbance. Before hybridization, quality assessment of cRNA was performed on the RNA 6000 Lab-Chip using the 2100 Bioanalyzer (Agilent, Palo Alto, California).

Microarray Analysis

Raw Affymetrix data files (.CEL files) were normalized together using Robust Multiarray Average. It was noted that anomalies in the expression distributions remained after normalization. One sample in the low AMA group had a noticeably distinct interquartile range and was excluded from further analysis. A linear model was fit to each transcript to test for putative differential expression. All *P* values were false discovery rate (FDR) adjusted per Benjamini and Hochberg.¹⁴ Based on FDR adjusted *P* < .10, there were 61 significant transcripts used for high versus low AMA comparison, 123 for high versus mid-AMA comparison, and 15 for mid versus low AMA.

Differentially expressed transcript data (FDR 10%) was imported into GeneSifter software (GeoSpiza, Inc, Seattle, Washington) for annotation and further analysis. Unsupervised hierarchical clustering of data was performed using Euclidean distance. To identify genes whose expression differed between maternal AMA groups, samples were partitioned using K-medoid clustering. Gene ontology (GO) functional terms were used to aid in the interpretation of the biological significance of the gene clusters. GO stat software¹⁵ was used to find statistically overrepresented GO terms within gene clusters. *P* values for GO enrichment were corrected for multiple testing and dependencies.¹⁶ Functional gene networks were identified by uploading the gene lists to the Ingenuity pathway analysis software version 8.8 (Ingenuity Systems; Redwood City, CA <http://ingenuity.com>).

Real-Time PCR

Polymerase chain reaction (PCR) was performed using the Stratagene Mx3005P Thermocycler (Stratagene, Santa Clara,

Table 1. Neonatal and Placental Characteristics Within Maternal Arm Muscle Area Quintiles

Mean $\pm$ SD	High AMA (5th Q)	Mid AMA (3rd Q)	Low AMA (1st Q)	P_{trend}
Maternal AMA, cm ²	55.4 $\pm$ 13.3	36.7 $\pm$ 0.94	27.8 $\pm$ 1.97	<.001
Maternal height, cm	167.1 $\pm$ 9.9	162.1 $\pm$ 7.7	160.1 $\pm$ 5.4	.054
Maternal BMI, kg/m ²	33.4 $\pm$ 5.7	26.0 $\pm$ 3.8	21.9 $\pm$ 2.1	<.001
Maternal age, years	29.1 $\pm$ 4.4	32.0 $\pm$ 5.6	32.7 $\pm$ 4.3	.1
Gestational age, week	40.5 $\pm$ 1.1	39.2 $\pm$ 1.3	39.8 $\pm$ 1.3	.3
Birth weight, g	3786 $\pm$ 534	3311 $\pm$ 316	3526 $\pm$ 415	.2
Placental weight, g	522 $\pm$ 88	439 $\pm$ 67	504 $\pm$ 88	.7
Fetal sex, M/F	6/4	7/3	7/3	

Abbreviations: SD, standard deviation; AMA, arm muscle area; BMI, body mass index.

^a N = 10 per group.

CA). Gene-specific primers were designed using primer designer software (Clone Manager Professional Suite ver.8). β -Actin, a housekeeping gene that is not altered by maternal AMA based on the microarray data and confirmed by real-time PCR analysis, was used as a control. The PCR amplicons were detected by fluorescent detection of SYBR Green (Power SYBR Green Master Mix; Applied Biosystems, Carlsbad, CA). For each primer pair, standard curve samples, no template controls, and unknowns were run in triplicate. Following cycling, the melt curve of the resulting amplicon was analyzed to ensure that a single product was detected. Quantification of mRNA was achieved using the respective standard curves with manufacturer's software (MxPro QPCR; Stratagene). Values were expressed as a ratio of the gene of interest:control in each sample. Significant differences between maternal AMA groups were assessed by 1-way analysis of variance (ANOVA) with post hoc differences determined by Tukey multiple comparison tests. Significance was defined as $P < .05$. Data are expressed as mean $\pm$ standard error of the mean.

Pathological Scoring

Fixed placental tissue was paraffin embedded and sectioned for histologic examination. The chorionic villi and chorionic plate were scored for histologic evidence of acute or chronic inflammation using routine hematoxylin and eosin-stained histologic sections by a board-certified placental pathologist (T.K.M.) blinded to all identifiers.

Immunohistochemistry

Samples of 6 randomly selected placentas from women in each of the 3 AMA groups were studied by immunohistochemistry: a human superoxide dismutase (SOD) 2 antibody (The Binding Site; San Diego, California; Cat. # PC096), CD3 (Dako; Carpinteria, California; Cat. # A0452; Polyclonal Rabbit Anti-Human), CD20 (Dako; Cat. # M0755; Monoclonal Mouse Anti-Human), CD64 (AbD Serotec; Raleigh, North Carolina; Cat. # MCA756GT; Monoclonal Mouse Anti-Human), and CD68 (Dako; Cat. # M0814; Monoclonal Mouse Anti-Human). Staining intensity of SOD2 was scored blindly on a scale of 1 to 3 as *weak focal* (1) to *diffuse strong* (3) using at least 4

representative $\times 10$ objective fields with the most staining per case. For CD3, CD20, CD64, and CD68, the number of positive cells counted per area of tissue analyzed in each objective field was averaged over $\times 10$ objective fields per case. Scores were averaged for cases within each group and compared by 1-way ANOVA with post hoc differences determined by Tukey multiple comparison tests. Statistical significance was defined as $P < .05$.

Results

Characteristics of Maternal Muscle Mass Groups

The physical characteristics of the mothers, babies, and placentas are shown in Table 1. There was a trend across AMA groups by AMA and BMI which was not surprising, and a suggestion of a trend with height, but apart from these variables, there was no apparent difference between the groups in parameters relating to the pregnancy or birth.

Effect of Maternal Muscle Mass on Global Placental Gene Expression Profiles

Of 47 000 genes queried on the HG-U133 Plus 2.0 oligonucleotide array, we observed a statistically significant change in expression (FDR 10%) of 188 probe sets, corresponding to 184 genes, between placentas of women with high, mid, and low AMA. To examine the relationship between groups (H, M, and L), hierarchical clustering of the groups was performed. Placentas from the mid- and low-AMA groups were most similar to each other. K-medoids (Euclidean) were used to supervise the clustering of probe sets into 2 groups. Cluster 1 and cluster 2 represent probe sets that show low (Figure 1A) or high (Figure 1B) levels of gene expression in the lower maternal AMA groups (M and L). The 2 clusters were further analyzed to determine GO terms associated with these different gene expression patterns. We found that the GO significantly overrepresented in cluster 2 (genes with high expression in the lower maternal AMA groups) are involved in immune response ($P = 10^{-7}$) and immune system processes ($P = 10^{-5}$). No GO terms were statistically significantly overrepresented in cluster 1 (transcripts with low expression in the lower maternal AMA placentas).

Figure 1. K-medoids clusters of differentially expressed genes that are coexpressed. K-medoids (Euclidean) were used to cluster probe sets into 2 groups. Clusters 1 and 2 represent probe sets that show a decrease (A) or increase (B), respectively, in gene expression with a decrease in maternal arm muscle area (AMA). H, high maternal AMA; M, mid maternal AMA; L, low maternal AMA.

Heat maps of differentially expressed placental transcripts (limited to those with >0.5 $\log_2$ fold change for display purposes) from clusters 1 and 2 are shown in Figure 2. Placentas from women with high AMA are the most distinct from the other 2 groups, indicated by the highest branch point. Tables 2 and 3 list placental transcripts with significantly lower and higher expression, respectively, in less muscular women (mid-AMA and low-AMA) as compared with the high muscle mass group.

Confirmation of Gene Expression

Real-time PCR analysis was used to confirm the expression patterns of selected genes from clusters 1 (Figure 3C) and 2 (Figure 3A and B). We were able to confirm the microarray expression patterns in 12 of 17 genes analyzed. Superoxide dismutase 2 mRNA ($P < .05$ by ANOVA, Tukey post hoc test) and protein expression (Figure 4) were low in placentas of women with high AMA. Interferon-inducible 27 (IFI27) was higher in women with low AMA ($P < .05$). Placental Ras homolog enriched in brain (RHEB) was lowest in women with mid or low AMA.

Gene Ontology and Ingenuity Pathway Analysis

To further examine the biological significance of placental genes which are differentially expressed between maternal AMA groups, we grouped the transcripts by function using GO annotation. Cluster 1 contained 125 transcripts representing 121 unique genes, 78 of which were annotated. Cluster 2 contained 63 transcripts representing 63 unique genes, 33 of which were annotated. Table 4 shows the distribution of gene ontologies of interest among placental transcripts which are lower or highly expressed in the less muscular women. The only GO categories that significantly differ between the 2 clusters were in the “inflammation and immune response” category. They include the GO categories “immune response” and “immune system process” (Table 5). These GO terms were overrepresented among transcripts that were more highly expressed in cluster 1 than in cluster 2. To investigate the

Figure 2. Differentially expressed genes with ≥ 0.5 $\log_2$ fold change between placentas from women with different arm muscle areas (AMAs). Transcripts were clustered into two groups using K-medoids (Euclidean). Clusters represent probe sets that show a decrease (A) or increase (B), respectively, in gene expression with a decrease in maternal AMA. These clusters are displayed as heat maps of normalized log intensity data. Groups (columns) are arranged from left to right ordered by decreasing maternal AMA. Each gene is annotated with Affymetrix probe set ID and gene symbol. H indicates high maternal AMA; M, mid maternal AMA; L, low maternal AMA.

potential relationships between differentially expressed immune response/inflammatory transcripts, we used Ingenuity pathway analysis software (Figure 5) and found that many of these genes are associated with the interferon- γ (IFNG) signaling pathway.

Histologic Examination

To further explore the observed differences in immune response gene expression, a pathologist blinded to groupings

Table 2. Distribution of Gene Ontologies of Interest Among Differentially Expressed Transcripts (False Discovery Rate 10%) Which Are Higher (Cluster 1) or Lower (Cluster 2) in Placentas From Women With High Arm Muscle Area Compared With Less Muscular Women

Biological process	Cluster 1, %	Cluster 2, %
Metabolic process	8	9
Signal transduction	11	6
Multicellular organismal development	7	3
Cellular differentiation and proliferation	11	6
Inflammation and immune response ^a	1	18
Transcription and translation	14	6
Transport	6	3
Other	5	6
Unassigned	36	44

^a Gene ontologies included in the inflammation and immune response category (immune response and immune system process) were overrepresented in cluster 2 as compared with cluster 1 ($P < .0001$, corrected for multiple comparisons).

reviewed histologic sections of placentas from each AMA group. Histological analysis did not reveal any significant difference in the frequency of chronic inflammation (chronic villitis) or acute inflammation (acute chorioamnionitis) between groups. To increase the sensitivity to detect differences in immune cells, placentas were also immunostained for lineage-specific markers of macrophages (CD68), neutrophils (CD64), T cells (CD3), and B cells (CD20). No differences were identified between groups (Figure 6).

Discussion

This study compared placental transcriptomes across the range of muscularity in 3 quintile groups of mothers. We found that levels of placental immune response gene transcripts were higher in women in the middle and lowest quintiles of prepregnant AMA compared with the upper quintile. Of the ~100 genes with lower expression within the lower AMA groups, no particular functional pathway or gene ontology was over- or underrepresented. In contrast, 18% of the ~60 genes that were highly expressed in less muscular women were involved in immune system processes, and many were related to the IFNG-signaling pathway. While the biological meaning of this finding is uncertain, we speculate that placentas from the lower muscle mass women would be more sensitive to the actions of cytokines and especially to those that stimulate inflammatory processes.

Maternal Muscle Mass and Placental Immune Response

Muscle mass is a key regulator of metabolism,¹ and as maternal metabolic capacity has an important influence on placental function, fetal growth, and development,¹⁷⁻¹⁹ we hypothesized that women with high and low muscle mass would have unique placental gene expression profiles. One key finding of this study was that placental immune response pathways were

stimulated among women with lower muscle mass. Excessive activation of inflammatory molecules within the placenta has been associated with preeclampsia, fetal growth restriction, preterm labor, and fetal death.^{7,19-23} Additionally, maternal obesity and diabetes (conditions of altered metabolic capacity) are associated with placental inflammation.^{7,19}

We found increased expression levels in a number of genes that are regulated by IFNG in placentas of less muscular women. The IFNG is a proinflammatory cytokine that plays an important role in activating cellular immunity and apoptosis. It is important for normal reproduction including successful implantation and establishment of pregnancy in mice.²⁴ We would not characterize the placentas from the lower muscle mass women as “inflamed” because we did not find histologic evidence of acute or chronic inflammation or immune cell infiltration in placentas from any group. We did not expect to find obvious signs of inflammation in these term placentas from uncomplicated pregnancies. Roberts et al found high inflammatory gene expression within placentas of nonpreeclamptic, nondiabetic obese women, without detecting an influx of leukocytes in the chorionic villi.²⁵ We suggest that the high expression of IFNG-regulated genes in placentas of less muscular women indicates the activation of inflammatory pathways that may have significant downstream effects on other placental pathways. At first glance, our observations in placentas of lean, less muscular women seem to contradict previous findings that women with the highest BMI have the highest levels of placental inflammation.^{7,19,25} However, placental inflammatory signatures differ between obese women with and without gestational diabetes,¹⁹ between lean women with and without preeclampsia²⁰ and between lean and severely obese women,^{7,25} suggesting along with our data that different maternal phenotypes and pregnancy conditions are associated with unique placental gene expression patterns. Although recent studies have focused on the poor outcomes associated with maternal obesity, the negative effects associated with maternal thinness, which are supported by our current findings, have also been reported.^{6,26}

Several of the immune response genes that were highly expressed in low AMA placentas are known to be regulated by IFNG including *IFI27* (interferon- α inducible protein 27), *IFI35* (interferon-induced protein 35), *GBP6* (guanylate-binding protein family member 6), *IFITM2* (interferon-induced transmembrane protein 2), and *IRF1* (interferon regulatory factor 1). The latter is a transcription factor, activated by IFNG signaling through the JAK/STAT1 pathway,²⁷ that stimulates the transcription of major histocompatibility complex (MHC) class Ia and II molecules as well as *GBP6*.^{24,27} Furthermore, we found that expression of the MHC class II β chain gene (*HLA-DRB1*) and proteasome subunit beta type 8 and 9 (*PSMB8* and *PSMB9*), located in the class II region of MHC, were highest in placentas of women with lower muscle mass. Although MHC class II genes are not expressed by the trophoblast, they are constitutively expressed in leukocytes²⁸; either maternal or fetal immune cells are likely sources of *HLA-DRB1* mRNA. The trophoblast mimics immune cell

Table 3. Transcripts With Lower Expression in Placentas of Less Muscular Women as Compared With Those With High Arm Muscle Area

GO Term	Gene Symbol	Fold Change		Gene Name	Location
		H vs M	H vs L		
Cellular process Cell cycle	UHMKI	1.2	1.5	U2AF homology motif (UHM) kinase 1	Nucleus
	TFDPI	1.1	1.2	Transcription factor Dp-1	Nucleus
	PARD6G	1.1	1.1	Par-6 partitioning defective 6 homolog gamma (<i>Caenorhabditis elegans</i>)	Plasma membrane
Cell growth	PPP2CA	1.2	1.2	Protein phosphatase 2, catalytic subunit, alpha isozyme	Cytoplasm
	FGFRL1	1.2	1.1	Fibroblast growth factor receptor-like 1	Plasma membrane
	TSPAN3	1.2	1.2	Tetraspanin 3	Plasma membrane
Cell motility	ARHGDIA	1.2	1.3	Rho GDP dissociation inhibitor (GDI) alpha	Cytoplasm
	ARPC5	1.2	1.3	Actin related protein 2/3 complex, subunit 5, 16kDa	Cytoplasm
	CNOT8	1.1	1.2	CCR4-NOT transcription complex, subunit 8	Nucleus
Cell proliferation Cytoskeleton organization	MID1	1.2	1.1	Midline 1 (Optiz/BBB syndrome)	Nucleus
	MAP7	1.2	1.2	Microtubule-associated protein 7	Cytoplasm
	NPAS2	1.3	1.2	Neuronal PAS domain protein 2	Nucleus
Developmental process	RAB23	1.3	1.3	RAB23, member RAS oncogene family	Cytoplasm
	PPAP2B	1.3	1.2	Phosphatidic acid phosphatase type 2B	Plasma membrane
	TBX3	1.2	1.1	T-box 3	Nucleus
	NGRN	1.2	1.1	Neugrin, neurite outgrowth associated	Unknown
	SNCA	1.1	-1.1	Synuclein, alpha (non A4 component of amyloid precursor)	Cytoplasm
	TSN22	1.1	1.1	Teashirt zinc finger homeobox 2	Unknown
	DCX	1.1	1.2	Doublecortin	Cytoplasm
	ACRVI	1.1	1.1	Acrosomal vesicle protein 1	Cytoplasm
	CYTH1	1.1	1.2	Cytohesin 1	Cytoplasm
Inflammatory response	MAB2IL2	1.1	1.1	Mab-21-like 2 (<i>C. elegans</i>)	Unknown
	RELA	1.2	1.3	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	Nucleus
	UBFD1	1.4	1.3	Ubiquitin family domain containing 1	Unknown
Metabolic process	MTHFSD	1.3	1.1	Methylenetetrahydrofolate synthetase domain containing	Unknown
	PPAP2B	1.3	1.2	Phosphatidic acid phosphatase type 2B	Plasma membrane
	PPP2CA	1.2	1.2	Protein phosphatase 2, catalytic subunit, alpha isozyme	Cytoplasm
	UHMKI	1.2	1.5	U2AF homology motif (UHM) kinase 1	Nucleus
	FKBP1A	1.1	1.2	FK506-binding protein 1A, 12kDa	Cytoplasm
	ADSS	1.1	1.1	Adenylosuccinate synthase	Cytoplasm
	SNCA	1.1	-1.1	Synuclein, alpha (non-A4 component of amyloid precursor)	Cytoplasm
	UBQLN3	1.1	1.0	Ubiquilin 3	Cytoplasm
	MMP8	1.1	-1.1	Matrix metalloproteinase 8 (neutrophil collagenase)	Extracellular space
	MIPEP	1.1	1.2	Mitochondrial intermediate peptidase	Cytoplasm
	NSMAF	1.0	1.4	Neutral sphingomyelinase (N-SMase) activation associated factor	Cytoplasm
	EXOSC3	1.2	1.3	Exosome component 3	Nucleus
Nucleic acid metabolic process	SERP1	1.2	1.3	SERPINE1 mRNA-binding protein 1	Nucleus
	LSM11	1.1	1.1	LSM11, U7 small nuclear RNA associated	Nucleus

(continued)

Table 3. (continued)

GO Term	Gene Symbol	Fold Change		Gene Name	Location
		H vs M	H vs L		
Signal transduction	GTF2I	1.4	1.5	General transcription factor Ili	Nucleus
	RAB1A	1.3	1.4	RAB1A, member RAS oncogene family	Cytoplasm
	NIPAS2	1.3	1.2	Neuronal PAS domain protein 2	Nucleus
	SMAD6	1.3	1.2	SMAD family member 6	Nucleus
	RHEB	1.3	1.2	Ras homolog enriched in brain	Plasma membrane
	RAB23	1.3	1.3	RAB23, member RAS oncogene family	Cytoplasm
	OR7E156P	1.3	1.2	Olfactory receptor, family 7, subfamily E, member 156 pseudogene	Unknown
	PPAP2B	1.3	1.2	Phosphatidic acid phosphatase type 2B	Plasma membrane
	GPR126	1.2	1.2	G protein-coupled receptor 126	Plasma membrane
	MLL14	1.2	1.2	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	Nucleus
	RASSF8	1.2	1.5	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	Unknown
	MED17	1.2	1.1	Mediator complex subunit 17	Nucleus
	FKBP1A	1.1	1.2	FK506-binding protein 1A, 12kDa	Cytoplasm
	TFG	1.1	1.1	TRK-fused gene	Cytoplasm
	DCX	1.1	1.2	Doublecortin	Cytoplasm
	NSMAF	1.0	1.4	Neutral sphingomyelinase (N-SMase) activation associated factor	Plasma membrane
	CNKSR2	1.0	1.1	Connector enhancer of kinase suppressor of Ras 2	Plasma membrane
	CDC42SE2	1.0	1.2	CDC42 small effector 2	Plasma membrane
	GTF2I	1.4	1.5	General transcription factor Ili	Nucleus
	NIPAS2	1.3	1.2	Neuronal PAS domain protein 2	Nucleus
	RBM15	1.3	1.2	RNA-binding motif protein 15	Nucleus
	SMAD6	1.3	1.2	SMAD family member 6	Nucleus
	ZNF200	1.3	1.2	Zinc finger protein 200	Nucleus
	PPP2CA	1.2	1.2	Protein phosphatase 2, catalytic subunit, alpha isozyme	Cytoplasm
	RELA	1.2	1.3	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	Nucleus
	MED17	1.2	1.1	Mediator complex subunit 17	Nucleus
	GTF2F2	1.2	1.2	General transcription factor IIF, polypeptide 2, 30kDa	Nucleus
	TMPO	1.1	1.3	Thymopoietin	Nucleus
	CNOT8	1.1	1.2	CCR4-NOT transcription complex, subunit 8	Nucleus
	TFDPI	1.1	1.2	Transcription factor Dp-1	Nucleus
Transport	TSHZ2	1.1	1.1	Teashirt zinc finger homeobox 2	Unknown
	TSC22D1	1.1	1.1	TSC22 domain family, member 1	Nucleus
	ZNF582	1.1	1.2	Zinc finger protein 582	Unknown
	IRF5	1.0	1.1	Interferon regulatory factor 5	Nucleus
	STX16	1.4	1.5	Syntaxin 16	Cytoplasm
	RAB1A	1.3	1.4	RAB1A, member RAS oncogene family	Cytoplasm
	RAB23	1.3	1.3	RAB23, member RAS oncogene family	Cytoplasm
	VPS26A	1.1	1.1	Vacuolar protein sorting 26 homolog A (<i>Schizosaccharomyces pombe</i>)	Cytoplasm
	COL4A4	1.1	1.0	Collagen, type IV, alpha 4	Extracellular space
	APIG1	1.1	1.2	Adaptor-related protein complex 1, gamma 1 subunit	Cytoplasm

(continued)

Table 3. (continued)

GO Term	Gene Symbol	Fold Change		Gene Name	Location
		H vs M	H vs L		
Unassigned	TM9SF3	1.6	1.7	Transmembrane 9 superfamily member 3	Cytoplasm
	LTBP3	1.5	1.1	Latent transforming growth factor β -binding protein 3	Extracellular space
	AKIRIN1	1.4	1.5	Akirin 1	Cytoplasm
	IMPAD1	1.3	1.3	Inositol monophosphatase domain containing 1	Unknown
	RLIM	1.3	1.2	Ring finger protein, LIM domain interacting	Nucleus
	RCN2	1.3	1.3	Reticulocalbin 2, EF-hand calcium binding domain	Cytoplasm
	LCA5	1.2	1.1	Leber congenital amaurosis 5	Unknown
	C6ORF57	1.2	1.4	Chromosome 6 open reading frame 57	Unknown
	SRSF9	1.2	1.2	Serine/arginine-rich splicing factor 9	Nucleus
	SPPL3	1.2	1.1	Signal peptide peptidase 3	Plasma membrane
	ZNF609	1.2	1.0	Zinc finger protein 609	Unknown
	PPPDE2	1.2	1.2	PPPDE peptidase domain containing 2	Unknown
	LOC404266	1.2	1.1	Hypothetical LOC404266	Unknown
	RNF32	1.2	1.1	Ring finger protein 32	Cytoplasm
	UQCC	1.1	1.1	Ubiquinol-cytochrome c reductase complex chaperone	Cytoplasm
	FAM135B	1.1	1.1	Family with sequence similarity 135, member B	Unknown
	C6ORF204	1.1	1.1	Chromosome 6 open reading frame 204	Unknown
	PSMD5	1.1	1.1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 5	Cytoplasm
	C2ORF83	1.1	1.1	Chromosome 2 open reading frame 83	Unknown
	C19ORF69	1.1	1.1	Chromosome 19 open reading frame 69	Unknown
	LOC100287147	1.1	1.0	Hypothetical protein LOC100287147	Unknown
	RAB11FIP3	1.1	1.1	RAB11 family interacting protein 3 (class II)	Cytoplasm
	C2ORF49	1.1	1.4	Chromosome 2 open reading frame 49	Unknown
	RBM33	1.1	1.3	RNA-binding motif protein 33	Unknown
	RNF38	1.1	1.3	Ring finger protein 38	Unknown
	PPPDE1	1.1	1.2	PPPDE peptidase domain containing 1	Unknown
	ACAP2	1.1	1.2	ArtGAP with coiled-coil, ankyrin repeat and PH domains 2	Nucleus
	TMEM67	1.0	1.1	Transmembrane protein 67	Plasma membrane
	DPPA4	1.0	1.3	Developmental pluripotency associated 4	Nucleus

Abbreviation: GO: gene ontology

Figure 3. Markers of T cells (CD3; A&E), B cells (CD20; B&F), macrophages (CD68; C&G), and neutrophils (CD64; D&H) immunostaining in term placental sections from women with high, average, or low arm muscle area (AMA). Immune cells staining positive for their respective markers (E-H: representative photomicrographs showing positive staining, arrows) within villous tissue were counted and averaged to villous area (A-D: histograms of positive cells/area). Photomicrograph using Leica microscope and $\times 40$ objective. Stain is brown and sections are counter-stained with hematoxylin. N = 6 per group; H, high maternal AMA; M, mid maternal AMA; L, low maternal AMA.

behavior by monitoring the surrounding environment and communicating with local leukocytes to protect the fetus from infection.^{24,29} Although not associated with an influx of immune cells, the high levels of IFNG-responsive genes detected in placentas of lower muscle mass women may signify an increased sensitivity of maternal or fetal leukocytes, trophoblast, or endothelial cells to inflammatory cytokines.

Three immune response genes were lower in placentas of lower AMA women compared with those with high AMA: the

NF-kappaB subunit *RELA* (transcription factor p65),³⁰ *IRF5* (interferon responsive factor 5)³¹ a protein known to influence inflammatory macrophage polarization,³² and CD40³³ required for activation of antigen-presenting cells. Expression of all 3 of these genes is responsive to IFNG. Despite seeing numerous changes in IFNG-responsive genes, we did not detect differences in the expression of the *IFNG* gene itself in the placentas of women with lower AMA groups compared with the high-AMA group. We speculate that many of the highly expressed

Figure 4. Gene expression confirmation using real-time PCR. Genes were chosen from cluster 1 (A-B) and 2 (C) for further analysis by PCR. Gene-of-interest expression was normalized to β -actin levels which were not significantly different between groups by microarray analysis or PCR. * $P < .05$ versus high muscle mass by ANOVA. N = 10 per group; H, high maternal AMA; M, mid maternal AMA; L, low maternal AMA; PCR, polymerase chain reaction; ANOVA, analysis of variance.

IFNG-related genes in the lower muscle mass women have high expression in response to circulating IFNG or the stimuli from unknown maternal chemical cues related to low maternal muscularity.¹¹ Alternatively, the placenta, maternal, or fetal immune cells may be sensitive to altered metabolism (glucose homeostasis) in women with lower muscle mass leading to the stimulation of IFNG-related genes, as found in other tissues.³⁴ Although altered glucose homeostasis is typically studied in the setting of obesity, several studies have found an association between altered glucose metabolism, thinness, and low muscle mass.^{1,6,26,35}

Nutrient-Sensitive Signaling Pathways

Lewis et al found that maternal muscle mass was positively correlated with placental system A amino acid transport activity.¹¹ The authors proposed that placentas of muscular women “sense” a metabolic or endocrine factor released by maternal skeletal muscle that leads to enhanced amino acid transport. Amino acid transport activity is known to be regulated by mammalian target of rapamycin (mTOR).³⁶ The mTOR signaling pathway is responsive to glucose, insulin, and amino acid levels and is important in regulating nutrient uptake and transport.³⁶⁻³⁸ mTOR is potently activated by the binding of the guanosine triphosphatase (GTPase) RHEB.³⁹ High levels of RHEB stimulate the mTOR pathway.³⁹ We found that placental RHEB mRNA expression was 20-fold higher in women with high muscle mass as compared with less muscular women. Thus, increases in RHEB may underlie the positive relationship between placental system A amino acid transport activity and high muscle mass in pregnant women and vice versa.¹¹

We did not detect differences in system A amino acid transporter (SNATs) expression between maternal AMA groups. This is consistent with the findings of Lewis et al that placental SNAT mRNA expression did not mirror activity levels.¹¹ Their findings suggest that the placental transport activity for system A amino acids is sensitive to maternal muscularity; this may be driven by changes in the mTOR signaling pathway, independent of concomitant alterations in SNAT gene expression.

Placental Antioxidant Expression is Related to Maternal Muscle Mass

Lower levels of the antioxidant SOD-2 were found in placentas of women with high AMA as compared with less muscular women. This dismutase binds the superoxide anion, a natural product of oxidative phosphorylation, and catalyzes the conversion to hydrogen peroxide and diatomic oxygen which protects the cell from damage from reactive oxygen species.⁴⁰ The interpretation of this finding is unclear. While decreased antioxidant levels may indicate a response to chronic oxidative stress reviewed by Myatt and Cui,⁴¹ others have shown that genes for antioxidants, such as SOD2, are upregulated in response to increases in pro-oxidant molecules and that low levels indicate a low demand for antioxidant action.⁴² Further experiments are needed to determine the relationship between maternal muscularity and placental oxidant/antioxidant status.

Limitations

Arm muscle area is a validated measure of muscle mass,¹³ but we cannot exclude the possibility that other maternal factors which are also different among AMA groups could be contributory. Based on anthropometric measures, it is not possible to cleanly separate different aspects of maternal body composition, and future studies may confirm these findings through dual energy x-ray absorptiometry in women before or after pregnancy. Groups of 10 women with the highest, lowest, and mid quintiles of AMA were chosen for analysis. Information may have been lost by grouping the data in this manner rather than analyzing AMA as a continuous variable against gene expression. Although the differences in SOD2 expression were confirmed using immunohistochemical techniques, protein levels of the remaining differentially expressed genes were not investigated in this study.

Summary

We have shown that placentas from women with high and average/low prepregnancy maternal muscle masses are distinguished by their unique gene expression signatures. High

Table 4. Transcripts High Expressed in Placentas of Women With Lower Arm Muscle Area as Compared With Those With High Arm Muscle Area

GO Term	Gene Symbol	Fold Change		Gene Name	Location
		H vs M	H vs L		
Immune response	CD40	-1.4	-1.2	CD40 molecule, TNF receptor superfamily member 5	Plasma membrane
	GBP6	-1.1	-1.1	Guanylate-binding protein family, member 6	Unknown
	HLA-DRB1	-2.5	-1.6	Major histocompatibility complex, class II, DR beta 1	Plasma membrane
	ICOS	-1.2	-1.1	Inducible T-cell co-stimulator	Plasma Membrane
	IFI27	-1.9	-1.9	Interferon, alpha-inducible protein 27	Cytoplasm
	IFI35	-1.4	-1.3	Interferon-induced protein 35	Nucleus
	LST1	-1.2	-1.1	Leukocyte specific transcript 1	Plasma membrane
	LY86	-1.3	-1.1	Lymphocyte antigen 86	Plasma membrane
	PSMB8	-1.8	-1.4	Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	Cytoplasm
	PSMB9	-2.0	-1.6	Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	Cytoplasm
Metabolic process	FYB	-1.2	-1.1	FYN-binding protein	Nucleus
	CIQA	-1.6	-1.4	Complement component 1, q subcomponent, A chain	Extracellular space
	CIQB	-1.4	-1.5	Complement component 1, q subcomponent, B chain	Extracellular space
	AKRIC1	-1.1	-1.1	Aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	Cytoplasm
	APOL6	-1.3	-1.1	Apolipoprotein L, 6	Extracellular space
Response to stress	FYB	-1.2	-1.1	FYN-binding protein	Nucleus
	MMAB	-1.2	-1.1	Methylmalonic aciduria (cobalamin deficiency) cblB type	Cytoplasm
	PADI1	-1.1	-1.0	Peptidyl arginine deiminase, type 1	Cytoplasm
Signal transduction	ANXA8L2	-1.3	-1.1	Annexin A8-like 2	Unknown
	SOD2	-1.7	-1.4	Superoxide dismutase 2, mitochondrial	Cytoplasm
	CD40	-1.4	-1.2	CD40 molecule, TNF receptor superfamily member 5	Plasma membrane
	LY86	-1.3	-1.1	Lymphocyte antigen 86	Plasma membrane
Transcription	APOL6	-1.3	-1.1	Apolipoprotein L, 6	Extracellular space
	GIT2	-1.1	-1.2	G protein-coupled receptor kinase interacting ArfGAP 2	Nucleus
	IFITM1	-1.3	-1.2	Interferon induced transmembrane protein 1 (9-27)	Plasma membrane
	PLEK	-1.5	-1.2	Pleckstrin	Cytoplasm
	IRF1	-1.3	-1.2	Interferon regulatory factor 1	Nucleus
	ISY1	-1.2	-1.1	ISY1 splicing factor homolog (<i>Saccharomyces cerevisiae</i>)	Nucleus
	POLR3C	-1.3	-1.2	Polymerase (RNA) III (DNA directed) polypeptide C (62kD)	Nucleus
Transport	SPI00	-1.2	-1.2	SPI00 nuclear antigen	Nucleus
	SOD2	-1.7	-1.4	Superoxide dismutase 2, mitochondrial	Cytoplasm
	CIQA	-1.6	-1.4	Complement component 1, q subcomponent, A chain	Extracellular space
	CIQB	-1.4	-1.5	Complement component 1, q subcomponent, B chain	Extracellular space
	AKRIC1	-1.1	-1.1	Aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	Cytoplasm
	APOL6	-1.3	-1.1	Apolipoprotein L, 6	Extracellular space
	APOL6	-1.3	-1.1	Apolipoprotein L, 6	Extracellular space

(continued)

Table 4. (continued)

GO Term	Gene Symbol	Fold Change		Gene Name	Location
		H vs M	H vs L		
Unassigned	C10ORF12	-1.2	-1.1	Chromosome 10 open reading frame 12	Unknown
	CLEC12B	-1.0	1.0	C-type lectin domain family 12, member B	Plasma membrane
	CLMN	-1.4	-1.7	Calmin (calponin-like, transmembrane)	Cytoplasm
	FGFR1OP2	-1.3	-1.1	FGFR1 oncogene partner 2	Cytoplasm
	IDO1	-1.7	-1.3	Indoleamine 2,3-dioxygenase 1	Cytoplasm
	INGX	-1.0	-1.2	Inhibitor of growth family, X-linked, pseudogene	Unknown
	LOC286370	-1.1	1.0	Hypothetical LOC286370	Unknown
	MTPAP	-1.2	-1.2	Mitochondrial poly(A) polymerase	Unknown
	MUTED	-1.1	-0.8	Muted homolog (mouse)	Unknown
	NKG7	-1.4	-1.2	Natural killer cell group 7 sequence	Plasma membrane
	SLC43A3	-1.2	-1.2	Solute carrier family 43, member 3	Extracellular space
	TMPRSS15	-1.1	-1.0	Transmembrane protease, serine 15	Extracellular space
	WDR37	-1.2	-1.1	WD repeat domain 37	Unknown

Abbreviation: GO: gene ontology

Table 5. Overrepresented GO Terms Among Placental Transcripts Highly Expressed in Less Muscular Women Versus Women With High Arm Muscle Area

GO Term	Gene Symbol	Fold Change		Gene Name	P Value ^a
		H vs M	H vs L		
Immune system process	HLA-DRB1	−2.5	−1.6	Major histocompatibility complex, class II, DR beta 1	.00001
	PSMB9	−2.0	−1.6	Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	
	PSMB8	−1.8	−1.4	Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	
	CIQA	−1.6	−1.4	Complement component 1, q subcomponent, A chain	
	IFI35	−1.4	−1.3	Interferon-induced protein 35	
	CD40	−1.4	−1.2	CD40 molecule, TNF receptor superfamily member 5	
	CIQB	−1.4	−1.5	Complement component 1, q subcomponent, B chain	
	LY86	−1.3	−1.1	Lymphocyte antigen 86	
	IFITN1	−1.3	−1.2	Interferon-induced transmembrane protein 1	
	FYB	−1.2	−1.1	FYN-binding protein	
	LST1	−1.2	−1.1	Leukocyte specific transcript 1	
	ICOS	−1.2	−1.1	Inducible T-cell costimulator	
	GBP6	−1.1	−1.1	Guanylate-binding protein family, member 6	
Immune response	HLA-DRB1	−2.5	−1.6	Major histocompatibility complex, class II, DR beta 1	<.00001
	PSMB9	−2.0	−1.6	Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	
	PSMB8	−1.8	−1.4	Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	
	CIQA	−1.6	−1.4	Complement component 1, q subcomponent, A chain	
	IFI35	−1.4	−1.3	Interferon-induced protein 35	
	CD40	−1.4	−1.2	CD40 molecule, TNF receptor superfamily member 5	
	CIQB	−1.4	−1.5	Complement component 1, q subcomponent, B chain	
	LY86	−1.3	−1.1	Lymphocyte antigen 86	
	IFITN1	−1.3	−1.2	Interferon-induced transmembrane protein 1	
	FYB	−1.2	−1.1	FYN-binding protein	
	LST1	−1.2	−1.1	Leukocyte specific transcript 1	
	ICOS	−1.2	−1.1	Inducible T-cell costimulator	
	GBP6	−1.1	−1.1	Guanylate-binding protein family, member 6	

Abbreviation: GO: gene ontology.

^a P value is corrected for multiple comparisons and dependencies among GO terms (Yekutieli correction).

Figure 5. Superoxide dismutase (SOD)-2 immunostaining of term placental sections from women with high, average, or low arm muscle area (AMA). SOD-2 localizes to villous trophoblasts (arrow). Photomicrograph using Leica microscope and $\times 10$ objective. Stain is brown and sections are counterstained with hematoxylin. $*P < .05$ versus high muscle mass by Student *t* test. *N* = 5-7 per group; H, high maternal AMA; M, mid maternal AMA; L, low maternal AMA.

maternal muscularity is associated with placental gene expression profiles that promote amino acid transport. Our findings suggest that placentas of less muscular women have gene

expression patterns that suggest cytokine stimulation and that these placentas would be more vulnerable to the effects of proinflammatory cytokines, such as IFNG, compared with mothers with high muscle mass.

Acknowledgments

The authors would like to thank Kristina Vartanian, Michelle Garred, and Chris Harrington of the OHSU Gene Microarray Shared Resource for valuable advice regarding microarrays. In addition, we thank Cara Poage and Carolyn Gendron of OHSU Histopathology Shared Resource for technical assistance in preparation of samples for histologic analysis.

Author's Note

KT, KG, RL, DB, GB, and MH designed and initiated the study. PFO, TKM, and SM were involved in data analysis. KG, CC, HMI, and the Southampton Women's Survey study group designed and/or implemented aspects of the Southampton Women's Survey from which the tissues were collected and prepregnancy measurements were made. RML collected the tissues and performed the RNA extraction. All authors were involved in the writing of the manuscript.

Figure 6. Ingenuity pathway analysis (IPA) displaying regulatory relationships among highly expressed (green) and lower (red) placental transcripts in less muscular women as compared with women with high arm muscle area. Symbol shapes denote the type of protein encoded by the transcript. Uncolored symbols represent transcripts relevant to the pathway but not differentially expressed according to microarray analysis.

Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: PFO is supported by the National Institute of Child Health & Development (1K99HD062841-01). KG is supported by the National Institute for Health Research through the Southampton NIHR Nutrition, Diet & Lifestyle Biomedical Research Unit. SKM is supported by the National Institutes of Health, National Center for Research Resources (5UL1RR024140) and National Institutes of Health, National Cancer Institute (5 P30 CA069533-13). TKM is supported in part by the Office of Research on Women's Health and the National Institute of Child Health and Human Development, Oregon (BIRCWH HD043488-08). This work was also supported by the National Heart Lung and Blood Institute (HL048546 [GB]), National Cancer Institute

(CA138237 [GB]), Department of Veterans Affairs (GB), Edwards Endowment (KLT), British Heart Foundation (MH), Medical Research Council (CC, HI, KG) and Arthritis Research UK (CC).

References

1. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Size at birth, fat-free mass and resting metabolic rate in adult life. *Horm Metab Res.* 2002;34(2):72-76.
2. Barker DJ, Hales CN, Fall CH, et al. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia.* 1993; 36(1):62-67.
3. Baker J, Workman M, Bedrick E, Frey MA, Hurtado M, Pearson O. Brains versus brawn: an empirical test of Barker's brain sparing model. *Am J Hum Biol.* 2010;22(2):206-215.
4. Jansson N, Pettersson J, Haafiz A, et al. Down-regulation of placental transport of amino acids precedes the development of

- intrauterine growth restriction in rats fed a low protein diet. *J Physiol.* 2006;576(Pt 3):935-946.
5. Shiell AW, Campbell DM, Hall MH, Barker DJ. Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later. *BJOG.* 2000;107(7):890-895.
6. Mi J, Law C, Zhang KL, Osmond C, Stein C, Barker D. Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. *Ann Intern Med.* 2000;132(4):253-260.
7. Challier JC, Basu S, Bintein T, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta.* 2008;29(3):274-281.
8. Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J.* 2009;23(1):271-278.
9. Roberts VH, Smith J, McLea SA, Heizer AB, Richardson JL, Myatt L. Effect of increasing maternal body mass index on oxidative and nitrative stress in the human placenta. *Placenta.* 2009;30(2):169-175.
10. Stewart FM, Freeman DJ, Ramsay JE, Greer IA, Caslake M, Ferrell WR. Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. *J Clin Endocrinol Metab.* 2007;92(3):969-975.
11. Lewis RM, Greenwood SL, Cleal JK, et al. Maternal muscle mass may influence system A activity in human placenta. *Placenta.* 2010;31(5):418-422.
12. Inskip HM, Godfrey KM, Robinson SM, et al. Cohort profile: The Southampton Women's Survey. *Int J Epidemiol.* 2006;35(1):42-48.
13. Heymsfield SB, McManus C, Smith J, Stevens V, Nixon DW. Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. *Am J Clin Nutr.* 1982;36(4):680-690.
14. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B.* 1995;57(1):289-300.
15. Beissbarth T, Speed TP. Gostat: find statistically overrepresented gene ontologies within a group of genes. *Bioinformatics.* 2004;20(9):1464-1465.
16. Reiner A, Yekutieli D, Benjamini Y. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics.* 2003;19(3):368-375.
17. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics.* 2005;115(3):e290-e296.
18. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care.* 2009;32(6):1076-1080.
19. Radaelli T, Varastehpour A, Catalano P, Hauguel-de Mouzon S. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes.* 2003;52(12):2951-2958.
20. Sitras V, Paulssen RH, Gronaas H, et al. Differential placental gene expression in severe preeclampsia. *Placenta.* 2009;30(5):424-433.
21. Mestan K, Yu Y, Matoba N, et al. Placental inflammatory response is associated with poor neonatal growth: preterm birth cohort study. *Pediatrics.* 2010;125(4):e891-e898.
22. Salafia CM, Ernst LM, Pezzullo JC, Wolf EJ, Rosenkrantz TS, Vintzileos AM. The very low birthweight infant: maternal complications leading to preterm birth, placental lesions, and intrauterine growth. *Am J Perinatol.* 1995;12(2):106-110.
23. Tayade C, Fang Y, Croy BA. A review of gene expression in porcine endometrial lymphocytes, endothelium and trophoblast during pregnancy success and failure. *J Reprod Dev.* 2007;53(3):455-463.
24. Murphy SP, Tayade C, Ashkar AA, Hatta K, Zhang J, Croy BA. Interferon gamma in successful pregnancies. *Biol Reprod.* 2009;80(5):848-859.
25. Roberts KA, Riley SC, Reynolds RM, et al. Placental structure and inflammation in pregnancies associated with obesity. *Placenta.* 2011;32(3):247-254.
26. Ravelli AC, van der Meulen JH, Michels RP, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet.* 1998;351(9097):173-177.
27. Kroger A, Koster M, Schroeder K, et al. Activities of IRF-1. *J Interferon Cytokine Res.* 2002;22(1):5-14.
28. Murphy SP, Choi JC, Holtz R. Regulation of major histocompatibility complex class II gene expression in trophoblast cells. *Reprod Biol Endocrinol.* 2004;2(5):52.
29. Mor G. Inflammation and pregnancy: the role of toll-like receptors in trophoblast-immune interaction. *Ann N Y Acad Sci.* 2008;1127(1):121-128.
30. Chen FE, Huang DB, Chen YQ, Ghosh G. Crystal structure of p50/p65 heterodimer of transcription factor NF-kappaB bound to DNA. *Nature.* 1998;391(6665):410-413.
31. Takaoka A, Yanai H, Kondo S, et al. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature.* 2005;434(7030):243-249.
32. Krausgruber T, Blazek K, Smallie T, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol.* 2011;12(3):231-238.
33. Nguyen VT, Benveniste EN. Involvement of STAT-1 and its family members in interferon-gamma induction of CD40 transcription in microglia/macrophages. *J Biol Chem.* 2000;275(31):23674-23684.
34. Nareika A, Sundararaj KP, Im YB, Game BA, Lopes-Virella MF, Huang Y. High glucose and interferon gamma synergistically stimulate MMP-1 expression in U937 macrophages by increasing transcription factor STAT1 activity. *Atherosclerosis.* 2009;202(2):363-371.
35. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third national health and nutrition examination survey. *J Clin Endocrinol Metab.* 2011;96(9):2898-2903.
36. Roos S, Jansson N, Palmberg I, et al. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J Physiol.* 2007;582(pt 1):449-459.
37. Roos S, Powell TL, Jansson T. Placental mTOR links maternal nutrient availability to fetal growth. *Biochem Soc Trans.* 2009;37(pt 1):295-298.

38. Roos S, Kanai Y, Prasad PD, Powell TL, Jansson T. Regulation of placental amino acid transporter activity by mammalian target of rapamycin. *Am J Physiol Cell Physiol.* 2009;296(1):C142-C150.
39. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.* 2003;17(15):1829-1834.
40. Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. *Free Radic Biol Med.* 2009;47(4):344-356.
41. Myatt L, Cui X. Oxidative stress in the placenta. *Histochem Cell Biol.* 2004;122(4):369-382.
42. Lyall F, Gibson JL, Greer IA, Brockman DE, Eis AL, Myatt L. Increased nitrotyrosine in the diabetic placenta: evidence for oxidative stress. *Diabetes Care.* 1998;21(10):1753-1758.